

REPUBLIQUE DU CAMEROUN

Paix – Travail – Patrie

REPUBLIC OF CAMEROON

Peace – Work - Fatherland

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

MINISTRY OF HIGHER EDUCATION

UNIVERSITE DE NGAOUNDERE

INSTITUT UNIVERSITAIRE DE
TECHNOLOGIE

THE UNIVERSITY OF NGAOUNDERE

UNIVERSITY INSTITUTE OF
TECHNOLOGY



DEPARTEMENT DE GENIE ALIMENTAIRE ET CONTROLE QUALITE
DEPARTMENT OF FOOD PROCESSING AND QUALITY CONTROL

**DOSSIER PRESENTE AU COMITE CONSULTATIF
DES INSTITUTIONS UNIVERSITAIRES (CCIU) EN
VUE DE LA PROMOTION AU GRADE DE
PROFESSEUR**

COMMISSION VI : SCIENCES DE L'INGENIEUR

Section 2/Sous-section 2 : Biologie et Biochimie Appliquées

Soumis par :

Desobgo Zangué Steve Carly

Maître de Conférences

Session: Novembre 2024

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I) IDENTIFICATION

**I.1) Demande de changement de grade
adressée au MINESUP S/C Monsieur le
Recteur**

Pr. Desobgo Zangué Steve Carly

Département Génie Alimentaire et Contrôle Qualité

Institut Universitaire de Technologie

Université de Ngaoundéré

BP 455 Ngaoundéré, Cameroun

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Site internet : www.pr-desobgo.com



A

Monsieur le Ministre d'Etat, Ministre de l'Enseignement Supérieur
S/C Madame Le Recteur de l'Université de Ngaoundéré
S/C Monsieur le Directeur de l'IUT de Ngaoundéré

Objet : Demande de changement de grade
de **Maître de Conférences à Professeur**

Monsieur le Ministre d'Etat,

J'ai l'honneur de venir auprès de votre haute bienveillance solliciter un changement de grade de **Maître de Conférences à Professeur** dans la spécialité Biochimie et Biologie Appliquées.

En effet, je suis enseignant au Département de Génie Alimentaire et Contrôle Qualité (GACQ) à l'Institut Universitaire de Technologie (IUT) de l'Université de Ngaoundéré, depuis le 16 avril 2008, date de ma prise de service. Titulaire d'un Doctorat Ph.D, j'ai été admis au grade de Chargé de Cours au cours du Comité Consultatif des Institutions Universitaires (CCIU) du 27 au 30 Novembre 2012, confirmé respectivement par l'arrêté N° 12/0675/MINESUP/SP-CCIU du 17 Décembre 2012 portant liste d'aptitude aux grades de Chargé de Cours, Maître de Conférences et Professeur dans les Institutions Universitaires puis, par l'arrêté N° 014/0910/MINESUP/SG/DDES/CEPE/CEA1 du 11 Décembre 2014 portant ma promotion à ce grade. Par la suite, j'ai été admis au grade de Maître de Conférences au cours du Comité Consultatif des Institutions Universitaires (CCIU) du 28 au 30 Novembre 2019, confirmé respectivement par l'arrêté N° 20/00019/MINESUP/SP-CCIU du 07 Janvier 2020 portant liste d'aptitude aux grades de Chargé de Cours, Maître de Conférences et Professeur dans les Institutions Universitaires puis, par l'arrêté N° 20/00748/MINESUP/SG/DDES/CEPE/CEA2 du 25 Août 2020 portant ma promotion à ce grade.

C'est fort de ce qui précède que je sollicite votre approbation et celle de mes pairs enseignant-chercheurs pour accéder au grade de Professeur.

Dans l'attente d'une suite favorable à ma demande, je vous prie d'agréer, Monsieur le Ministre d'Etat, l'expression de mes sentiments distingués.

Pièces jointes : Sept exemplaires du dossier de candidature

Pr Desobgo Zangué Steve Carly

I.2) Fiche de synthèse de candidature

1. ETABLISSEMENT: Institut Universitaire de Technologie
2. DEPARTEMENT: Génie Alimentaire et Contrôle Qualité
3. GRADE ACTUEL: Chargé de Cours Depuis : 2012

CONSEIL CONSULTATIF DES INSTITUTIONS UNIVERSITAIRES
SESSION DE NOVEMBRE 2019

FICHE DE SYNTHESE DE CANDIDATURE
UNIVERSITE : Université de Ngaoundéré

- 4- GRADE POSTULE: PROFESSEUR
5- SOUS-SECTION: 2
6- SECTION: 2
7- COMMISSION : Sciences de l'Ingénieur

Identification du candidat	Cursus et Titres Universitaires	Publications	Avis motivé du département	Avis de la Section
Nom : DESOBGO ZANGUE Prénom : Steve Carly Né le : 02 Janvier 1977 A : Douala Matière : 652117-M Pièces fournies Sept exemplaires de dossier de changement de grade dont 1 original comprenant chacun : 1. Demande timbrée adressée au Ministre d'Etat, Ministre de l'Enseignement Supérieur, Président du CCU 2. Copie certifiée conforme de l'acte de naissance 3. Curriculum Vitae 4. Liste des enseignements dispensés 5. Travaux scientifiques, technologiques et ou artistiques publiés et/ou réalisés dans le grade de Maître de Conférences 6. Note de présentation 7. Acte de promotion au grade de Maître de Conférences 8. Liste des rapports, mémoires et thèses dirigés ou codirigés 9. Autres 10. Rapport pédagogique 11. Rapport administratif 11. Rapport administratif (1)	1996 : Baccalauréat « D » (Institut Privé Polyvalent de Bonamoussadi, Douala) 1998 : DUT en Génie Agro- Industriel (Institut Universitaire de Technologie, Université de Ngaoundéré) 2001 : Ingénieur des Industries Agricoles et Alimentaires (Ecole Nationale Supérieure des Sciences Agro-Industrielles, Université de Ngaoundéré) 2003 : DEA en Génie des Procédés (Ecole Nationale Supérieure des Sciences Agro- Industrielles, Université de Ngaoundéré) 2012 : Doctorat/Ph.D en Génie des Procédés (Ecole Nationale Supérieure des Sciences Agro- Industrielles, Université de Ngaoundéré) Expérience Professionnelle Depuis 2020 : Maître de Conférence à l'UT de Ngaoundéré Depuis 2012 : Chargé de Cours à l'UT de Ngaoundéré 2008-2012 : Assistant à l'UT de Ngaoundéré Depuis 2019 : Editorial Board member de SCIREA Journal of Food. Depuis 2019 : Editorial Board member de Journal of Food Stability Depuis 2017 : Expert du CNDT, commission Biotechnologie du MINRESI Depuis 2017 : Membre de American Society of Brewing Chemists Depuis 2014 : Reviewer à: Journal of Food and Bioprocess Technologies; Journal of Food Science and Technology; Journal of Biologically Active Products from Nature; African Journal of Science, Technology, Innovation and Development Depuis 2014 : Editorial Board Member de African Journal of Biotechnology 2013/2014 et 2015/2016: Postdoc à l'Université de Johannesburg, Afrique du Sud	1. Linegne D. G. K., Desobgo Z. S.C.* , & Emmanuel Jong Nso. 2024. Potential of β -Amylase from Sweet Potato (<i>Ipomoea batatas</i> Lam) Extract on the Mashing of <i>Sorghum</i> . <i>Journal of Food Processing and Preservation</i> , 2024, 1-13. 2. Kadlezir, F., Mohagir, A. M., & Desobgo Z. S.C.* . 2023. Extracting juice from dates (<i>Phoenix dactylifera</i> L.) using response surface methodology: Effect on pH, vitamin c, titratable acidity, free amino nitrogen (FAN) and polyphenols. <i>Applied Food Research</i> , 100375. 3. Brai, O., Ambindei, W. A., Ndasi, N. P., Wingang, M. C., Muala, W. C. B., Desobgo, Z. S. C. , & Nso, E. J. 2023. Production and Characterization of a Probiotic Sorghum Beverage Fermented with Lactic Acid Bacteria (<i>Lactobacillus fermentum</i> and <i>Bifidobacterium bifidum</i>) and Bil-bil. <i>American Journal of Food Science and Technology</i> , 11(3), 86-95. 4. Kadlezir, F., Mohagir, A.M. Desobgo Z. S.C.* . 2023. Application of response surface methodology in date (<i>Phoenix dactylifera</i> L.) juice extraction: Effect of process parameters on Brix, color and sugar/acid ratio. <i>Journal of Food. Stability</i> , 6(2), 66-83. 5. Nguemogne A. C., Desobgo Z. S.C.* , Nso E. J. 2023. Partial purification and characterization of limit dextrinase from <i>Safran</i> sorghum malt. <i>Journal of Food. Stability</i> , 6(1), 34-50. 6. Man-Ikri B., Desobgo, Z.S.C., 2022. <i>Grewia mollis</i> bark powder impact on the clarification of <i>Mbayeri</i> sorghum wort. <i>Applied Food Research</i> , 3(2023) 100243. 7. Ninga, K.A., Desobgo Z.S.C.* , Nso, E.J., Kayem, J., 2022. White-flesh guava juice clarification by a fixed-angle conical rotor centrifuge laboratory and characterization of continuous disk stack centrifuges. <i>Heligon</i> , 8(2022) e11606 8. Ninga, K.A., Desobgo, Z.S.C.* , Nso, E.J., De, S., 2021. Pectinase hydrolysis of guava pulp: effect on the physicochemical characteristics of its juice. <i>Heligon</i> , 7(10). e08141. 9. Acha Anna Afek, Desobgo, Z.S.C., Nso E.J., 2021. A Field Survey to Assess the Consumption of Nkang for Standardization and Valorization in the North-West Region of Cameroon. <i>Green and Sustainable Chemistry</i> , 11, 107-123 10. Muala, W.C.B., Desobgo, Z.S.C.* , Nso, E.J., 2021. Optimization of extraction conditions of phenolic compounds from <i>Cynbopogon citratus</i> and evaluation of phenolics and aroma profiles of extract. <i>Heligon</i> , 7(4). e06744. 11. Mbarga, M.J.A., Desobgo, Z.S.C. , Tatsadjieu, L.N., Kavhiza, N., Kalisa, L., 2021. Antagonistic effects of raffia sap with probiotics against pathogenic microorganisms. <i>Foods Raw Mater</i> , 9(1). 24-31. 12. Makebe, C.W., Desobgo, Z.S.C. , Ambindei, W.A., Billu A., Nso, E.J., Nisha, P., 2020. Optimization of pectinase-assisted extraction of <i>Ammona muricata</i> L. juice and the effect of liquefaction on its pectin structure. <i>Journal of the science of food and agriculture</i> , 100(15). 5487-5497. 13. Nguemogne A. C., Desobgo Z. S.C.* , Nso E. J. 2020. Optimization of the limit dextrinase extraction of the Cameroonian sorghum variety <i>Safran</i> . <i>Journal of Food. Stability</i> , 3 (2). 9-26. 14. Desobgo Z. S. C.* , Nso, E.J., 2021. Winemaking: Control, Bioreactor and Modeling of Process. In Joshi V.K. & Ramesh C. Ray (Eds.), Wine making: Basics and applied aspects. (pp. 495-519). CRC Press, Taylor & Francis Group.	Avis motivé de l'Etablissement	Avis motivé de la Commission
Avis de la Sous-Section		Avis motivé du Comité		

I.3) Curriculum vitae

Curriculum Vitae



Pr. DESOBGO ZANGUE Steve Carly (Associate Professor at the University Institute of Technology of the University of Ngaoundere, Cameroon)

Associate Professor, University of Ngaoundere, Cameroon
Postdoctoral fellow, University of Johannesburg, Doorfontein Campus
Ph.D in Process Engineering, University of Ngaoundere
Master of Science in Process Engineering, University of Ngaoundere
Master of Engineering in Agricultural and Food Industries, University of Ngaoundere

Personal Informations

Name (s)	DESOBGO ZANGUE Steve Carly
Adress	PO. Box 455 University Institute of Technology (IUT), Ngaoundéré, Cameroun
Phone	(+237) 697160004 / 672790222 / 656858527 / 222251650
Email	desobgo.zangue@gmail.com
Nationality	Cameroonian
Date and place of birth	January 02, 1977 in Douala, Cameroon
Sex	Male
Marital status	Married and father of 4 children

Field of competence

Food Sciences, Food Technology, Biotechnology, Process Engineering, Chemical Engineering, Development of new products, Environment (biochemistry comprehension of waste transformation)

Scientific Activities

Domain	Process Engineering, Chemical Engineering, Development of new products, Environment, Food Technology and Biotechnology, Environment (biochemistry comprehension of waste transformation)
Direction of activities	- Food Technology, Biotechnology, Beverages, Enzymology

Publications in the activity

- Modeling (Response Surface Methodology)
- Environmental science (biochemistry comprehension of waste transformation)
- **41 papers** in refereed journals, **4 books chapters**
- 1 Conference on Scientific and technological innovations: catalysts for growth and sustainable development. From 11 to 13 May 2022, University Institute of Technology, University of Douala, Cameroon
- 1 communication to the GP3A, UCL Belgium.
- Communications in Annual Conference of the Cameroon Biosciences Society
- 1 communication to the Johannesburg Winter Beverage Symposium of the University of Johannesburg at Sedibeng brewery, South Africa
- **5 Ph.D completed**
- **Many Masters completed (12 masters students)**
- **Many engineers (BAC+5), Licences (BAC+3) and DUT students (BAC+2) completed**

Supervision of students

Professional experience

Dates	December 2023
Name of employer	ANRPEC (Association nationale des réseaux pour la promotion de l'entrepreneuriat au Cameroun)
Location	Baré Bakem, Cameroun
Activity sector	Agriculture
Position occupied	Consultant
Activities and responsibilities	Organisateur des premières journées nationales de mise en réseau des acteurs de la chaîne de valeur agroalimentaire
Dates	October 2023
Name of employer	ANOR (Agence camerounaise de normalisation et de qualité)
Location	Douala, Cameroun
Activity sector	Qualité et normalisation
Position occupied	Consultant
Activities and responsibilities	Séminaire sur la normalisation : Les défis de la normalisation dans la production locale de boissons et de spiritueux
Dates	October 2023
Name of employer	APME (Small and Medium-sized Enterprises Promotion Agency)
Location	Douala, Cameroon
Activity sector	Program to promote and process agricultural and agri-food products financed by the French Development Agency
Position occupied	Consultant

Activities and responsibilities	- Inaugural lesson: Impact of food quality on the performance of small and medium-sized agrifood enterprises and their contribution to import-substitution industrialization and food security in Cameroon
Dates	Août 2023
Name of employer	UNIVERSITE DE NGAOUNDERE / ENSAI
Location	Ngaoundéré, Cameroun
Activity sector	Enseignement Supérieur
Position occupied	Examineur
Activities and responsibilities	Participation à la soutenance de thèse de Doctorat Ph.D en Génie des Procédés du candidat Zomegni Gaston. Thème : Potentiel technologique des amandes de mangue (<i>Mangifera indica</i> L.) en panification pour la réduction du rassissement du pain.
Dates	Since June 2023
Name of employer	GIE-MADIKA
Location	Yaoundé, Cameroon
Activity sector	Agri-food
Position occupied	Consultant/Researcher
Activities and responsibilities	- New product development - Research
Dates	Mai 2023
Name of employer	UNIVERSITE DE DSCHANG / DSCHANG SCHOOL OF SCIENCE AND TECHNOLOGY
Location	Dschang, Cameroun
Activity sector	Enseignement Supérieur
Position occupied	Examineur
Activities and responsibilities	- Participation à la soutenance de thèse de Doctorat Ph.D en Nutrition et Sécurité Alimentaire du candidat Tchamani Piame Laverdure. Thème : Caractérisation techno-fonctionnelle des levures constitutives des levains du <i>Sha'a</i> (boisson fermentée traditionnelle à base de maïs) et mise au point d'un starter.
Dates	Since March 2023
Name of employer	UCB
Location	Douala, Cameroon
Activity sector	Brewery
Position occupied	Consultant/Researcher
Activities and responsibilities	- New product development - Research
Dates	Since January 2023
Name of employer	UNIPAC FARMING

Location	Yaoundé, Cameroon
Activity sector	Agri-food
Position occupied	Member
Activities and responsibilities	- New product development
Dates	Since January 2023
Name of employer	UNIPAC INVEST
Location	Yaoundé, Cameroon
Activity sector	Finance/Agri-food
Position occupied	Member
Activities and responsibilities	- Shareholder
Dates	Since March 2023
Name of employer	COOP-CA TRAALIT (the Littoral food processors' cooperative)
Location	Douala, Cameroon
Activity sector	Agri-food
Position occupied	Consultant/Researcher
Activities and responsibilities	- New product development - Research
Dates	Since November 2020
Name of employer	KINDAK
Location	Makak, Cameroon
Activity sector	Agri-food
Position occupied	Consultant/Researcher
Activities and responsibilities	- New product development - Research
Dates	Since November 2019
Name of employer	University of Ngaoundere/University Institute of Technology (UIT)
Location	Ngaoundéré, Cameroon
Activity sector	Higher education
Position occupied	Associate Professor/Researcher
Activities and responsibilities	- Lecturing - Research - Practicals - Students supervision
Dates	2021 until now
Name of employer	Journal of the American Society of Brewing Chemists
Location	USA
Activity sector	Scientific papers publication

Position occupied	Reviewer
Activities and responsibilities	- Review scientific papers
Dates	2021 until now
Name of employer	Heliyon
Location	Netherlands
Activity sector	Scientific papers publication
Position occupied	Reviewer
Activities and responsibilities	Review scientific papers
Dates	2020 until now
Name of employer	Journal of Food Processing and Preservation
Location	USA
Activity sector	Scientific papers publication
Position occupied	Reviewer
Activities and responsibilities	Review scientific papers
Dates	2019 until now
Name of employer	Journal of Food Stability
Location	Buea, Cameroon
Activity sector	Scientific papers publication
Position occupied	Associate Editor
Activities and responsibilities	Review scientific papers and journal paper editing
Dates	2019 until now
Name of employer	SCIREA Journal of Food
Location	New York, USA
Activity sector	Scientific papers publication
Position occupied	Editorial Board Member
Activities and responsibilities	Review scientific papers and journal paper editing
Dates	2012- 2019
Name of employer	University of Ngaoundere/University Institute of Technology (UIT)
Location	Ngaoundéré, Cameroon
Activity sector	Higher education
Position occupied	Senior Lecturer/Researcher

Activities and responsibilities	- Lecturing - Research - Practicals - Students supervision
Dates	2017 until now
Name of employer	American Society of Brewing Chemists
Location	USA
Activity sector	Scientific papers publication
Position occupied	Member
Activities and responsibilities	Review scientific papers
Dates	2017-2019
Name of employer	Ministry of Scientific Research and Innovation (MINRESI)
Location	Yaoundé, Cameroon
Activity sector	Research/Technology
Position occupied	Member of the National Technology Development Committee
Activities and responsibilities	- Biotechnology development policy in Cameroon - Project selection - Project supervision
Dates	2016-2017
Name of employer	GIZ Cameroon (Gesellschaft Für Internationale Zusammenarbeit)
Location	Yaoundé, Cameroon
Activity sector	- Environmental, climate and forest policy - Governance and decentralization - Rural development
Position occupied	Consultant
Activities and responsibilities	- Work meeting with the representatives GIZ, SNV, and the company SOCTRACAO. Definition of terms of reference and on-site visit of equipment - Evaluation of machine yields according to processing needs. - Analysis of the quality of materials related to hygienic requirements and sanitary and their availability on the market.
Dates	2015-2016
Name of employer	University of Johannesburg
Location	Johannesburg, South Africa
Activity sector	Higher education
Position occupied	Postdoctoral fellow at Biotechnology and Food technology department

Activities and responsibilities	- Lecturing - Research on brewing science - Practicals - Students supervision
Dates	2017 until now
Name of employer	African Journal of Science, Technology, Innovation and Development
Location	London, UK
Activity sector	Scientific papers publication
Position occupied	Reviewer
Activities and responsibilities	Review scientific papers
Dates	2017 until now
Name of employer	Journal of Food Science and Technology
Location	India
Activity sector	Scientific papers publication
Position occupied	Reviewer
Activities and responsibilities	Review scientific papers
Dates	2014- until now
Name of employer	Journal of Food and Bioprocess Technologies
Location	USA
Activity sector	Scientific papers publication
Position occupied	Reviewer
Activities and responsibilities	Review scientific papers
Dates	2014- until now
Name of employer	African Journal of Biotechnology
Location	Nigeria
Activity sector	Scientific papers publication
Position occupied	Editorial Board Member
Activities and responsibilities	Review scientific papers and paper editing
Dates	2013-2014
Name of employer	University of Johannesburg
Location	Johannesburg, South Africa
Activity sector	Higher education
Position occupied	Postdoctoral fellow at Biotechnology and Food technology department
Activities and responsibilities	- Lecturing - Research on brewing science

	- Practicals - Students supervision
Dates	2008-2012
Name of employer	University of Ngaoundere/University Institute of Technology (UIT)
Location	Ngaoundéré, Cameroon
Activity sector	Higher education
Position occupied	Assistant Lecturer
Activities and responsibilities	- Lecturing - Research - Practicals - Students supervision
Dates	2002-2008
Name of employer	University of Ngaoundere/ENSAI of Ngaoundere
Location	Ngaoundéré, Cameroon
Activity sector	Higher education
Position occupied	Research assistant
Activities and responsibilities	- Practicals in Agro-industrial process technology - Practicals in biodynamics - Practicals in Enzymology - Practicals in Mechanical and transfer unit operations
Dates	May – September 2001
Name of employer	CAM Assistance
Location	Douala, Cameroon
Activity sector	Food technology (Manufacture of machinery, development of new products)
Position occupied	Trainee (Internship End of Master of Engineering degree)
Activities and responsibilities	- Development of the manufacturing processes of juice and pineapple wine - Developing a taste panel - Development of the best process for juice and pineapple wine
Dates	June – August 2000
Name of employer	Cameroon Development Corporation (CDC)
Location	Tiko, Cameroon
Activity sector	Food Technology (Rubber, Tea, oil, etc...)
Position occupied	Trainee (Internship Master of Engineering, year 2)
Activities and responsibilities	- Investigations on the rubber quality issues - Development of a device to drain water from washing the coagulated latex to pretreatment zone
Dates	July – August 1999
Name of employer	SPC du Cameroon

Location	Bafoussam, Cameroon
Activity sector	Food Technology (Feed mills)
Position occupied	Trainee (Internship Master of Engineering, year 1)
Activities and responsibilities	- Contribution to the production of provender for poultry, pigs, rabbits, ... - Contribution to management of feed stocks and Maintenance

Dates	June – September 1998
Name of employer	Guinness Cameroon S.A.
Location	Douala, Cameroon
Activity sector	Food Technology (Brewery)
Position occupied	Trainee (Internship for end of degree, DUT)
Activities and responsibilities	- Bottle breakages analysis in Packaging service - Investigation on critical areas of thermal shock and breakage of bottles on the bottling line. - Bottle breakages and valuation costs

Dates	June – July 1997
Name of employer	Guinness Cameroon S.A.
Location	Douala, Cameroon
Activity sector	Food Technology (Brewery)
Position occupied	Trainee (Internship)
Activities and responsibilities	- Bottle breakages analysis in Packaging service - Investigation on critical areas of thermal shock and breakage of bottles on the bottling line. - Bottle breakages and valuation costs

Education and formation

Dates	2004 – 2012
Title of qualification awarded	Ph.D in Process engineering
Skills covered	General domain: Process Engineering and Chemical Engineering, Food technology, Mathematics, Statistics Specific Domain: Modeling, Process Optimization of Agricultural and Food Industries (Beverages)
Établissement Institution	National Advanced School of Agro-Industrial Sciences (ENSAI), Ngaoundere, Cameroon

Dates	2001 – 2003
Title of qualification awarded	Master of Science in Process Engineering (D.E.A - GP)
Skills covered	General domain: Process Engineering and Chemical Engineering, Food technology, Mathematics, Statistics

Établissement Institution	Specific Domain: Modeling, Process Optimization of Agricultural and Food Industries (Beverages)
	National Advanced School of Agro-Industrial Sciences (ENSAI), Ngaoundere, Cameroon
Dates	1998 – 2001
Title of qualification awarded	Master of Engineering
Skills covered	General domain: Process Engineering and Chemical Engineering, Food technology
Établissement Institution	National Advanced School of Agro-Industrial Sciences (ENSAI), Ngaoundere, Cameroon
Dates	1996 – 1998
Title of qualification awarded	University Institute of Technology Diploma (DUT)
Skills covered	General domain: Agro-Industrial Engineering
Établissement Institution	University Institute of Technology (UIT), Ngaoundere, Cameroon
Dates	1995 – 1996
Title of qualification awarded	Bachelor of Secondary Education (Series D)
Skills covered	General domain: Mathematics and Natural Sciences
Établissement Institution	Polyvalent Private Institute of Bonamoussadi (IPPB), Douala, Cameroon

Personal skills and competences

Language **French**

Self evaluation
English

Understand		Speak		Write
Listen	Read	Conversation	Orally	
Well	Well	Well enough	Well enough	Well

Social skills

- Good team skills, easy to adapt to multicultural environments
- Good communication skills

Organizational skills

- General Manager of the Ngaoundere Veterans Football Association (2018)
- Academic supervisor of the Bioengineering mention level 2 (2009-2011)

- Regular Interim position of Head of Department of Food Engineering and Quality Control (GACQ)
- President of the Association of Monitors and PhD students of the University of Ngaoundere **(2005)**
- Laboratory Technician at ENSAI of Ngaoundere **2004/2005 & 2005/2006**
- Member of the Junior Enterprise Club, Ngaoundere **(1998 – 2000)**
- Registrar of the Commission of projects in the Association of Ph.D Students of ENSAI, Ngaoundere **(2001-2004)**
- Auditor of the Agro - Industrial Club, UIT, Ngaoundere **(1996-1997)**

Computer skills

- Word, Excel, PowerPoint, Publisher, SigmaPlot, Statistica, Mathcad, Matlab, Minitab, Mathematica, Scientific Workplace, Statgraphics, SPSS, Systat, S Plus 2000, Derive 5, Adobe Photoshop, fortran, Origin, Sphinx, Design Expert, etc...

Artistic skills

- Practice drawing and poetry

Other skills

- Practice of Football, basketball, tennis, table tennis etc...

Driving Licence

- Driving licence B

Complement

- Special Distinction awarded for completion of all the lectures exams in a normal session by Prof. Maurice TCHUENTE Rector (Rector of the University of Ngaoundere) **(1998)**.
- Highest performance in Master of science results **(2003)**

List of lessons taught (ENSAI/IUT) in the University of Ngaoundere, National Polytechnic School of Douala (ENSPD) and ISAGO of Obala

- 1) Enzymology (IUT)
- 2) Process diagrams and technical drawing (IUT)
- 3) Industrial hygiene (IUT)
- 4) Fruits and vegetables (IUT)
- 5) Malting & Brewing (IUT/ENSAI)
- 6) Food process practical (beer, fruit juice, wine and spirit, etc...) (IUT)
- 7) Student professional project (IUT/ENSAI)
- 8) Advanced Brewing (IUT)
- 9) Wine and spirits (IUT)
- 10) Initiation to engineering equipment (IUT)
- 11) Industrial Enzymes and Applications (IUT)
- 12) Biostatistics and experimental design (ENSAI/IUT)
- 13) Processes of Food Industries II (Beverages) (ENSAI)
- 14) Mechanical and Transfer Unit Operations (ENSAI)
- 15) Experimental designs (ENSAI/IUT)
- 16) Agri-Food Processes 1 (ENSPD)

- 17) Introduction to process engineering (ISAGO)
- 18) Unit operations in food processing (BTS IUT)
- 19) Food formulation engineering (ENSAI)
- 20) Food industry processes (BTS IUT)

Academic direction of Ph.D students

List of Ph.D. completed

- 1) **Kadlezir Fiacre, 2024.** Boisson probiotique non-laitière: Modélisation et optimisation de l'extraction et de la fermentation du jus d'un cultivar de datte (*Phoenix dactilifera* L.) « Bournow » par la méthodologie des surfaces de réponses. Thèse de Doctorat Ph.D en Sciences de l'Ingénieur, Mention : Génie des Procédés. Ecole Doctorale Sciences, Techniques et Environnement. Faculté des Sciences Exactes et Appliquées. Université de Ndjamena, Tchad. (Directors: Pr. Mohagir, A.M. ; **Pr. Desobgo, Z.S.C.**)
- 2) **Makebe Calister Wingang. 2022.** Development of probiotic fermented beverage from soursop (*Annona muricata* Linn.) fruit. Ph.D in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)
- 3) **Wiyeh Claudette Bakisu Muala. 2022.** Probiotic beverage production from a mixture of baobab (*Adansonia digitata* L.) pulp and lemongrass (*Cymbopogon citratus* L.) extract using lactic acid bacteria. Ph.D in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)
- 4) **Ninga Kombele Aimé. 2021.** Liquéfaction d'une pulpe de goyave à chair blanche (*Psidium guajava* Linn) par les pectinases industrielles d'*Aspergillus niger* et clarification du jus par centrifugation. Thèse de Doctorat Ph.D en Génie des Procédés. Spécialité : Génie Alimentaire et Bioprocédés. Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)
- 5) **Nguemogne Annick Chancelle. 2020.** Purification de la dextrinase limite du cultivar de sorgho *Safrari* (*Sorghum bicolor* L. Moench). Thèse de Doctorat Ph.D en Génie des Procédés. Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)

List of Ph.D. students still in progress

- 1) **Acha Anna Affeck.** Modeling the production and clarification of a standardized « Nkang » beer. Ph.D in Process Engineering, National School of Agro-Industrial Science (ENSAI) (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)

- 2) **Assabjeu Djoufack Armel Césaire.** Study of the mechanism of enzymatic hydrolysis by fragmentation for the production of bioethanol from corn cobs. Ph.D in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)
- 3) **Brai Olivier.** Production of a probiotic sorghum beer. Ph.D in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)
- 4) **Tiemuncho Habass.** Construction and evaluation of a novel hybrid mash-tun for sorghum mashing. Ph.D in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)

Academic direction of Masters and Engineers Students

Masters and Engineers completed Masters completed

- 1) **Ngono awoumou, 2022.** Clarification of a *Dacryodes macrophylla* wine by *Corchorus olitorius*. Master of Science in Process Engineering. Specialty: Biomolecular and Molecular Food Engineering (GABM). National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: **Pr. Desobgo, Z.S.C.**; Dr. Agwanandé)
- 2) **Epée Willie Aurelien, 2022.** Etude de la potentialité de Khaya senegalensis dans l'amérisation des moûts et bières de sorgho. Master Recherche en Génie des Procédés, spécialité : Génie Alimentaire Biomoléculaire et Moléculaire (GABM). Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directeurs: **Pr. Desobgo, Z.S.C.**; Dr. Agwanandé)
- 3) **Amassoka Frédéric Jonathan, 2022.** Paramètres de fermentation de *Dacryodes macrophylla* et essai de carbonatation. Master Recherche en Génie des Procédés, spécialité : Génie Alimentaire Biomoléculaire et Moléculaire (GABM). Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directeurs: **Pr. Desobgo, Z.S.C.**; Dr. Agwanandé)
- 4) **Limegne Kameugne Daruis Gayus, 2020.** Potentialité d'un extrait de patate douce (*Ipomoea Batatas* Lam.) sur la saccharification de la maische de sorgho (*Sorghum bicolor*). Master Recherche en Génie des Procédés, spécialité : Génie Alimentaire et Bioprocédé. Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directeurs: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)
- 5) **Tiemuncho Habass Noulambeh, 2019.** Modeling the Operating Limits of a Mashing Process of Sorghum, Attainable Region Concept. Master of Science in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)

- 6) **Mekok Abizou Rostand, 2019.** Optimized seed roasting and ultrasound-assisted extraction of baobab oil (*Adansonia digitata* Linn). Master of Science in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Pr. Desobgo, Z.S.C.**)
- 7) **Kadlezir Fiacre, 2017.** Optimization of some physicochemical parameters during juice extraction of a date cultivar (*Phoenix dactylifera* L.). Master of Science in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Dr. Desobgo, Z.S.C.**)
- 8) **Wiyeh Claudette Bakisu Muala, 2016.** Influence of drying and mixture conditions on some physico-chemical characteristics of lemongrass (*Cymbopogon citratus*) blended with ginger (*Zingiber officinale*) tea. Master of Science in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Dr. Desobgo, Z.S.C.**)
- 9) **Makebe Calister Wingang, 2016.** Optimisation of the extraction process and investigation on must fermentation of ripe plantains. Master of Science in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Dr. Desobgo, Z.S.C.**)
- 10) **Tiyou Jules Padrik, 2014.** Application of hydrothermolysis and organosolv treatments for the solubilization of hemicellulosic fractions and lignin of sorghum stalks for the production of bioethanol. Master of Science in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Dr. Desobgo, Z.S.C.**)
- 11) **Nkoum Sem Ronald, 2014.** Optimising Sugar Release by the CSLF Technology and Dilute Acid Hydrolysis from Maize (*Zea mays* L.) Stalks as a Potential for Bioethanol Production. Master of Science in Process Engineering, National School of Agro-Industrial Science (ENSAI), Cameroon. (Directors: Pr Nso, E. J.; **Dr. Desobgo, Z.S.C.**)

Engineers completed

- 1) **Donfack Zambou Marthe Cathy, 2023.** Essais de production d'une bière à partir du sorgho non concassé : Application à une bière allégée en alcool. Diplôme d'Ingénieur de Conception en Industries Agricoles et Alimentaires. Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directeurs: **Pr. Desobgo, Z.S.C.** ; Dr. Nguemogné)
- 2) **Gnentedem Oumbe Romaric Brice, 2023.** Production d'un vin sans alcool à base d'ananas (*Ananas comosus* C) et d'oseille (*Hibiscus sabdariffa* L) sucré à la stevia (*Stévia rebaudiana* B). Diplôme d'Ingénieur de Conception en Industries Agricoles et Alimentaires.

Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directeurs: **Pr. Desobgo, Z.S.C.**; Dr. Agwanandé)

- 3) **Sabouang Ngouah Beaud Charles Fabrice, 2022.** Effet de l'aromatisation du rhum à partir des pommes d'anacarde sur les caractéristiques physico-chimiques d'un rhum arrangé. Diplôme d'Ingénieur de Conception des Industries Agricoles et Alimentaires. Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directeurs: **Pr. Desobgo, Z.S.C.**; Dr. Saidou)
- 4) **Tsafack Manedjou Andrelle Sherone, 2022.** Formulation d'un vin sans alcool à base de tamarin aromatisé à l'ananas. Diplôme d'Ingénieur de Conception des Industries Agricoles et Alimentaires. Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Cameroun. (Directeurs: **Pr. Desobgo, Z.S.C.**; Pr. Nkouam)
- 5) **Gnassiri Simon, 2021.** Formulation d'une bière à base sorgho rouge (*Sorghum bicolor*), de cannelle (*Cinnamomum zeylanicum*), de zeste de citron (*Citrus limon*) amérisée à l'écorce de caïcedrat (*Khaya senegalensis*). Diplôme d'Ingénieur de Conception. Ecole Nationale Supérieure Polytechnique de Douala (ENSPD), Cameroun. (Directeurs: Pr Mouangué Ruben; **Pr. Desobgo, Z.S.C.**)
- 6) **Fometeu Zamo Audrey Niquème, 2018.** Standardization of calcium and free amino nitrogen contents in the wort at the brewhouse. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (**Dr. Desobgo, Z.S.C.**, Dr Agwanande W.)
- 7) **Chano Kuitche Gaëlle, 2018.** Sulfur dioxide control in breweries. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (**Dr. Desobgo, Z.S.C.**, Dr Agwanande)
- 8) **Awah Noella, 2018.** Study of factors influencing saccharification during mashing. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Fombang Edith, **Dr. Desobgo, Z.S.C.**)
- 9) **Ayossa-Hanthe Lonam, 2018.** Ameliorating beer brightness: case of SABC Douala and Yaounde factories. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (**Dr. Desobgo, Z.S.C.**, Dr Agwanande W.)
- 10) **Minkoumou Augustin Caleb, 2018.** Brewer's yeast management for the improvement of the quality of the finished beer. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 11) **Kamche Josianna Clarence, 2018.** Formulation of tropical fruit compotes (pineapple, banana and papaya). Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (**Dr. Desobgo, Z.S.C.**, Dr Ngatchic J.)

- 12) **Tset III Steve F., 2017.** Improvement of stage times and reduction of extract losses during filtration in brewhouse 2. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 13) **Nsoh Cyril Fru, 2017.** Production of a malt drink from sorghum. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 14) **Tiemuncho Habass N., 2017.** Model-based design: A novel mash tun, mashing and fermentation process simulation software for food process applications. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 15) **Makamté Mukam F., 2017.** Analysis of the fermentation process at UBC: The case of King Beer. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 16) **Kembeu Kaleu Pavell, 2017.** Control of the duration of the stages and reduction of extract losses during the filtration of mash in brewhouse 1. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 17) **Metutu Kakoua Bessong V., 2017.** Evaluation of the impact of roller mills on the quality of the final product (flour). Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 18) **Fali Mbeh H., 2016.** Production of sorghum coffee-milk stouts and modelling of some physicochemical characteristics. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 19) **Takela Feudjo Moïse F., 2016.** Evaluation and reduction of water consumption during brewing. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Pr Nso E.J., **Dr. Desobgo, Z.S.C.**)
- 20) **Nana Njouyep Mervil, 2016.** Improvement and control of mash filtration. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (**Dr. Desobgo, Z.S.C.**, Amba)
- 21) **Nomo Nomo G., 2016.** Improvement and control of bitterness in isenbeck and high-density mother beers. Engineering degree, National School of Agro-Industrial Science (ENSAI), Cameroon. (Dr Fombang Edith, **Dr. Desobgo, Z.S.C.**)

Thesis & Reports

- 1) **Desobgo, Z.S.C., 2012.** Optimization of the action of hydrolases on some physico-chemical characteristics of the wort of two sorghum

cultivars. Ph.D Thesis in Process Engineering. ENSAI of the University of Ngaoundere.

- 2) **Desobgo, Z.S.C., 2003.** Study of the effect of some hydrolases on the filterability of unmalted sorghum-based worts (*Madjeru, Safrari, S.35*). Master of Science thesis in Process Engineering. ENSAI of the University of Ngaoundere.
- 3) **Desobgo, Z.S.C., 2001.** Production of juice and pineapple wine for a semi-automated unit. Master of Engineering thesis. ENSAI of the University of Ngaoundere.
- 4) **Desobgo, Z.S.C., 2000.** Investigation into rubber problems. Internship Report, Master of engineering year 2. ENSAI of the University of Ngaoundere.
- 5) **Desobgo, Z.S.C., 1999.** Contribution to the production of the Feed for poultry, pigs, rabbits. Internship report, Master of engineering year 1. ENSAI of the University of Ngaoundere
- 6) **Desobgo, Z.S.C., 1998.** Analysis bottles breakages of the packaging line No. 2 (1998). DUT end of thesis. UIT of the University of Ngaoundere.
- 7) **Desobgo, Z.S.C., 1997.** Monitoring of the packaging lines. Training report. UIT of the University of Ngaoundere.

Conferences

- 1) Man-Ikri B., **Desobgo Z.S.C., 2022.** Study of the impact of *Grewia mollis* on the clarification of *Mbayeri* sorghum cultivar worts. *Conference on Scientific and technological innovations: catalysts for growth and sustainable development. From 11 to 13 May 2022*, University Institute of Technology, University of Douala, Cameroon. N° SS 167
- 2) **Desobgo Z.S.C., 2021.** The wort boiling techniques and energy requirements: A Review. *The 1st International Conference on Local Resource Exploitation (LOREXP). April 20 – 23*. University Institute of Technology (UIT), University of Ngaoundere. REF: LOREXP_2021_A1070 Pages: 1218-1238
- 3) Nguemogne A. C., **Desobgo Z. S.C.*, Nso E. J., 2018.** A screening method for the expression of total amylase activity during malting of sorghum. *25th Annual Conference of the Cameroon Biosciences Society. November 28 – December 02*, University of Ngaoundere, Cameroon. CBS18_FAA_ORA 25.
- 4) Mbarga, M.J.A., **Desobgo, Z.S.C., Tatsadjieu, N.L., 2017.** Optimisation de quelques paramètres de fermentation de la sève de raphia (*Rafia farinifera*) par *Lactobacillus fermentum* et *Bifidobacterium bifidum*. *24th Annual Conference of the Cameroon Biosciences Society. November 28 – December 02*, University of Buea, Cameroon. CBS17-ORA-112. p 94.

- 5) **Desobgo, Z.S.C., 2013.** Introduction to experimental design in brewing science. University of Johannesburg Winter Beverage Symposium. 20 June 2013, Sedibeng Brewery, South Africa.
- 6) **Desobgo, Z.S.C., 2008.** Modeling and optimization of the effect of industrial enzymes on the filtrate yield of sorghum mash. 1st Scientific Days (JS) of the Process Engineering Network Applied to AgroIndustry (GP3A). September 11 and 12, 2008, Louvain-la-Neuve, Belgium. <http://www.gp3a.auf.org>
- 7) The polyphenol paradox in alcoholic beverages: a beer and wine paradox ?. 13th Chair J. DE CLERCK. 7-10 september 2008. Louvain-laNeuve, Belgium. <http://www.gp3a.auf.org>

Books Chapters

Associate Professor

- 1) **Desobgo, S.C.Z. 2024.** Utilization of Agricultural Waste for Water and Wastewater Treatment Processes. In: Arora, J., Joshi, A., Ray, R.C. (eds) Transforming Agriculture Residues for Sustainable Development. Waste as a Resource. Springer, Cham. https://doi.org/10.1007/978-3-031-61133-9_13
- 2) Mishra, S. S., Behera, S. S., Bari, M. L., Panda, S. K., & **Desobgo, Z. S. C. 2021.** Microbial bioprocessing of health promoting food supplements. In C. R. Ramesh (Ed.), Microbial Biotechnology in Food and Health (pp. 113–141). Elsevier.
- 3) **Desobgo, Z. S. C*. & Nso, E. J. 2021.** Winemaking: Control, Bioreactor and Modelling of Process. In Joshi, V.K. and Ramesh, C. Ray (Eds), *Wine Making: Basics and Applied Aspects*. 1st edition. (pp. 495-519). CRC Press, Taylor & Francis Group.

Senior Lecturer

- 4) **Desobgo, Z. S. C*. 2018.** Modernization of Fermenters for Large-Scale Production in the Food and Beverage Industry. In Panda, Sandeep Kumar, Halady, Prathap Kumar Shetty (Eds.), *Innovations in Technologies for Fermented Food and Beverage Industries*. (pp. 343). Springer International Publishing.
- 5) **Desobgo, Z. S. C.*, Mishra, S. S., Behera, S. K., & Panda, S. K. 2017.** Scaling-up and Modelling Applications of Solid-State Fermentation and Demonstration in Microbial Enzyme Production Related to Food Industries: An Overview. In R. C. Ray & C. M. Rosell (Eds.), *Microbial Enzyme Technology in Food Applications* (pp. 520). CRC Press, Taylor & Francis Group.

Publications

Associate Professor

- 1) Limegne D. G. K., **Desobgo Z. S.C.***, & Emmanuel Jong Nso. **2024.** Potential of β -Amylase from Sweet Potato (*Ipomoea batatas* Lam)

Extract on the Mashing of *Safrari* Sorghum. *Journal of Food Processing and Preservation*, 2024, 1-13.

- 2) Kadlezir, F., Mohagir, A. M., & **Desobgo Z. S.C.*** 2023. Extracting juice from dates (*Phoenix dactylifera* L.) using response surface methodology: Effect on pH, vitamin c, titratable acidity, free amino nitrogen (FAN) and polyphenols. *Applied Food Research*, 100375.
- 3) Brai, O., Ambindei, W. A., Ndasi, N. P., Wingang, M. C., Muala, W. C. B., **Desobgo, Z. S. C.**, & Nso, E. J. 2023. Production and Characterization of a Probiotic Sorghum Beverage Fermented with Lactic Acid Bacteria (*Lactobacillus fermentum* and *Bifidobacterium bifidum*) and *Bil-bil*. *American Journal of Food Science and Technology*, 11(3), 86–95. <https://doi.org/10.12691/ajfst-11-3-2>
- 4) Kadlezir, F., Mohagir, A.M. **Desobgo Z. S.C.***, 2023. Application of response surface methodology in date (*Phoenix dactylifera* L.) juice extraction: Effect of process parameters on Brix, color and sugar/acid ratio. *Journal of Food. Stability*, 6(2), 66-83.
- 5) Nguemogne A. C., **Desobgo Z. S.C.***, Nso E. J. 2023. Partial purification and characterization of limit dextrinase from *Safrari* sorghum malt. *Journal of Food. Stability*, 6(1), 34-50.
- 6) Man-Ikri B., **Desobgo, Z.S.C.**, 2022. Grewia mollis bark powder impact on the clarification of *Mbayeri* sorghum wort. *Applied Food Research*, 3(2023) 100243.
- 7) Ninga, K.A., **Desobgo, Z.S.C.**, Nso, E.J., Kayem, J., 2022. White-flesh guava juice clarification by a fixed-angle conical rotor centrifuge laboratory and characterization of continuous disk stack centrifuges. *Heliyon*, 8(2022) e11606
- 8) Hassana, B., **Desobgo, Z.S.C.**, Ngassoum, M., Barka, A., 2022. Development of enriched biochar from local materials by mixture design approach. *International Journal of Innovative Science and Research Technology*, 7(8), 60-71
- 9) **Desobgo, Z.S.C.**, 2021. The wort boiling techniques and energy requirements: A Review. Conférence Internationale LOREXP-2021 : « Chaines de Valeurs et Transformations Intégrales des Ressources Locales », Ngaoundéré, Cameroun, 20 au 23 Avril 2021.
- 10) Ninga, K.A., **Desobgo, Z.S.C.**, Nso, E.J., De, S., 2021. Pectinase hydrolysis of guava pulp: effect on the physicochemical characteristics of its juice. *Heliyon*, 7(10). e08141.
- 11) Acha Anna Afek, **Desobgo, Z.S.C.**, Nso E.J., 2021. A Field Survey to Assess the Consumption of Nkang for Standardization and Valorization in the North-West Region of Cameroon. *Green and Sustainable Chemistry*, 11, 107-123
- 12) Muala, W.C.B., **Desobgo, Z.S.C.**, Nso, E.J., 2021. Optimization of extraction conditions of phenolic compounds from *Cymbopogon*

citratatus and evaluation of phenolics and aroma profiles of extract. *Heliyon*, 7(4). e06744.

- 13) Mbarga, M.J.A., **Desobgo, Z.S.C.**, Tatsadjieu, L.N., Kavhiza, N., Kalisa, L., **2021**. Antagonistic effects of raffia sap with probiotics against pathogenic microorganisms. *Foods Raw Mater*, 9(1). 24–31.
- 14) Makebe, C.W., **Desobgo, Z.S.C.**, Ambindei, W.A., Billu A., Nso, E.J., Nisha, P., **2020**. Optimization of pectinase-assisted extraction of *Annona muricata* L. juice and the effect of liquefaction on its pectin structure. *Journal of the science of food and agriculture*, 100(15). 5487-5497.
- 15) Assabjeu, A.C., Noubissié, E., **Desobgo, Z.S.C.**, Ali, A., **2020**. Optimization of the enzymatic hydrolysis of cellulose of triplochiton scleroxylon sawdust in view of the production of bioethanol. *Science African*, 8: e00438.
- 16) Nguemogne A. C., **Desobgo Z. S.C.***, Nso E. J. **2020**. Optimization of the limit dextrinase extraction of the Cameroonian sorghum variety *Safrari*. *Journal of Food. Stability*, 3 (2). 9-26.

Senior Lecturer

- 17) Mbarga, M.J.A., **Desobgo, Z.S.C.**, Ngouné, T.L., Meisam, Z., Engeribo, A., Maryam, B., **2019**. Producing probiotic beverage based on raffia sap fermented by *Lactobacillus fermentum* and *Bifidobacterium bifidum*. *Research on Crops*, 20(3), 629-634
- 18) Taira, A., **Desobgo, Z.S.C.***, Nso, E.J., **2019**. Use of aqueous two-phase system for partial purification and characterization of α -amylase from *Burnatia enneandra* Micheli. *Journal of Food Stability*, 2(2), 26-41
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- 21) Ninga, K.A., Sengupta, S., Jain, A., **Desobgo, Z.S.C.**, Nso, E.J., De, S., **2018**. Kinetics of enzymatic hydrolysis of pectinaceous matter in guava juice. *Journal of Food Engineering*, 221, 158-166.
- 22) Nguemogne A. C., **Desobgo Z. S.C.***, Nso E. J., **2018**. A Screening approach for the expression of total amylase activity during malting of *Safrari* sorghum cultivar. *Research Journal of Science and Technology*, 10(4), 244-252

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- 25) **Desobgo, Z.S.C.***, Stafford, R.A. & Metcalfe, D.J.A., **2017**. Modeling of dimethyl sulfide stripping behavior when applying delayed onset of boiling during wort boiling. *Journal of the American Society of Brewing Chemists*, 75(3), 269-275
- 26) Nguemogne A. C., **Desobgo Z. S.C.***, Nso E. J. **2017**. Comparative study of limit dextrinase potential of three sorghum cultivars (*Safrari*, *Madjeru* and *S.35*). *Journal of the American Society of Brewing Chemists*, 75(3), 255-261
- 27) Adebo, O. A., Njobeh, P. B., Mulaba-Bafubiandi, A. F., Adebiyi, J. A., **Desobgo, Z. S. C.**, Kayitesi, E., **2017**. Optimization of fermentation conditions for ting production using response surface methodology. *Journal Food Processing and Preservation*, e13381. <https://doi.org/10.1111/jfpp.13381>
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- 31) Taira, A., **Desobgo, Z.S.C.***, Nso, E.J., **2015**. Partial purification of α - amylase from *Burnatia enneandra* Micheli. Part 1: Extraction using statistical model. *International Journal of Research in Biotechnology and Biochemistry*, 5 (1), 13-19
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- enneandra* and *Cadaba farinosa*. *Journal of Brewing and Distilling*, 4 (2), 46-50
- 34) NGouadjo, T.L., Youmssi, A., **Desobgo, Z.S.C.***, & Kayem, G.J., **2013**. Optimization of the extraction of roselle (*Hibiscus sabdariffa* L.) dried calyxes' juice. *International Journal of Innovation and Applied Studies*, 2 (4), 499-510.
- 35) **Desobgo, Z.S.C.***, & Nso, E.J., **2013**. Optimization of the impact of Hitempase 2XL, Bioglucanase TX and Brewers Protease on the turbidity of *Madjeru* sorghum cultivar wort. *International Journal of Applied Research and Studies*, 2 (1), 1-10.
- 36) **Desobgo, Z.S.C.***, Naponni, Y.F. & Nso, E.J., **2013**. Characterization of *Safrari* sorghum worts and beers hopped with *Vernonia amygdalina* and *Nauclea diderrichii*. *International Journal of Innovation and Applied Studies*, 2 (1), 83-91.

Assistant Lecturer

- 37) **Desobgo, Z.S.C.***, Nso, E.J. & Tenin, D., **2011**. The response surface methodology as a reliable tool for evaluating the need of commercial mashing enzymes for alleviating the levels of reducing sugars of worts of malted sorghum: Case of the *Safrari* cultivar. *Journal of Brewing and Distilling*, 2, 5-15.
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- 39) **Desobgo, Z.S.C.***, Nso, E.J. & Tenin, D., **2011**. Modeling the action of technical mashing enzymes on extracts and free-amino nitrogen yields of the *Madjeru* sorghum cultivar. *Journal of Brewing and Distilling*, 2, 29-44.
- 40) **Desobgo, Z.S.C.***, Nso, E.J. & Tenin, D., **2011**. Optimisation of the Action of Commercial Mashing Enzymes on Wort Extracts and Free Amino Nitrogen of the *Safrari* Sorghum Cultivar. *Technical Quarterly Master Brewers Association of the Americas*, 48, 77-86.
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Publications in Press



Pr. DESOBGO ZANGUE Steve Carly

I.4) Photocopie de l'acte de naissance

DEPARTEMENT

DIVISION

du Wouri

REPUBLIQUE UNIE DU CAMEROUN

Paix—Travail—Patrie

UNITED REPUBLIC OF CAMEROON

Peace—Work—Fatherland

RECETTES

MINISTRE DES FINANCES
DIRECTION GENERALE DES IMPOTS

FCFA 0001500

TIMBRE FISCAL-FISCAL STAMP
CMR21032

ARRONDISSEMENT

SUBDIVISION

de Douala

CENTRE D'ETAT CIVIL
CIVIL STATUS REGISTRATION CENTRE

de - Of *Guido et Alima Rod*

Acte de Naissance N° *9/77*
BIRTH CERTIFICATE

Nom de l'enfant *Georgio Franque Steve Linly*
Name of the child

Le - On the *Sept Janvier mil neuf cent soixante dix sept*
Est né à *Douala Cameroun*

Was born at
Nom de l'enfant *Georgio Franque Steve Linly*
Name of the child

De sexe - Sex *masculin*
De - Of *Georgio Gabriel*

Né à - Born at *Boulevard Tchang*
In - On the *13 Mai 1977*

Domicilié à *Douala*

Profession - Occupation *Tenue de*

Et de - And of *Guipi Tindjo Tindjo*

Né à - Born at
Le - On the *13 Mai 1977*

Domicilié à *Douala*
Resident at

Profession - Occupation *Ministre*

Dressé le *Sept Janvier mil neuf cent soixante dix sept*
Drawn up on the *Sept*

Sur la déclaration de *N° 2° de Mue H.*
In accordance with the declaration of
Tiliacos, sage femme

Lesquels ont certifié la sincérité de la présente déclaration.
Who attested to the truth of this declaration

Par Nous *Ekeurika Ekeurika, Officier et C. R. S.*
By Us *Officier du Centre Spécial de Guido*

Le Déclarant,
The declarant,
Signature de l'Officier de l'Etat-Civil
Signature of Registrar.

TIMBRE COMMUNAL
REPUBLIQUE DU CAMEROUN

500
FRANCS

NP0429785

REPUBLIQUE DU CAMEROUN
COMMUNAL STAMP

JOUE LE MAIN
1° 1^{er} 1^{er} Delegation

2^{ème} Adjoint

2024

2024

2024

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2024

II) PHOTOCOPIES DES DIPLÔMES

II.1) Baccalauréat série D

Ministère de l'Éducation Nationale
Office du Baccalauréat
du Cameroun

République du Cameroun
Paix Travail Patrie

Baccalauréat de l'Enseignement Secondaire

N° 03730

Vu les textes en vigueur portant organisation des Baccalauréats de l'Enseignement Secondaire

Vu le Procès-verbal des délibérations du jury, session de Juin 1996 Jury N° 069

Le diplôme du Baccalauréat de l'Enseignement Secondaire Général

série/spécialité BAD MATHÉMATIQUES ET SCIENCES DE LA NATURE

est conféré à M. DESOBGO ZANGUE STEVE CARLY

né(e) le 02/01/1977

à DOUALA

Matricule 509807

PASSABLE

Mention

Signature du titulaire

Pour en jouir avec les droits et prérogatives qui y sont attachés

Fait à Yaoundé le 23 Novembre 2001



Baccalauréat du Cameroun

Le Ministère de l'Éducation Nationale

Le Ministre de l'Enseignement Supérieur
Chancelier des ordres académiques

Alain

Joseph OWONA

Desobgo Zangue Steve Carly

Alain

II.2) Diplôme Universitaire de Technologie

RÉPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR
UNIVERSITÉ DE NGAOUNDÉRE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE



REPUBLIC OF CAMEROON
Peace - Work - Fatherland
MINISTRY OF HIGHER EDUCATION
UNIVERSITY OF NGAOUNDERE
UNIVERSITY INSTITUTE OF TECHNOLOGY

Diplôme Universitaire de Technologie "D.U.T." Diploma

N° 98/NT/008/10

Vu le décret n° 93/028 du 19 janvier 1993 portant organisation administrative et académique de l'Université de Ngaoundéré

Mindful of decree n° 93/028 of 19 January 1993 to organise the University of Ngaoundéré
Mindful of Ministerial Order n° 006/MINE-SUP/F-120 of 28 January 1993 to fix the domains of training and conditions of admission into the University of Ngaoundéré Institute of Technology

Vu les procès verbaux des délibérations du jury, session de
Mindful of the decisions of the board of examiners, session of
Octobre 1998

Le Diplôme Universitaire de Technologie The "D.U.T." Diploma

Spécialité
Major
Génie Agro-Industriel

Est délivré à
Is conferred on

Monsieur Desoboko Zomgue Steve-Carly

Né(e) le
Born on

02 janvier 1977

à Douala

N° Matricule
Registration N°
96A08210

Pour en jouir avec les droits et prérogatives qui y sont rattachés
With all the rights and privileges appertaining to the said degree.

Fait à Ngaoundéré, le
Done in Ngaoundere, on

17 NOV 2000

LE RECTEUR

Yves Tchoua

LE MINISTRE DE L'ENSEIGNEMENT SUPÉRIEUR,
CHANCELLIER DES ORDRES ACADEMIQUES

Alphonse Ndjongo
The Minister of Higher Education, Chancellor of Academic Orders

The holder

II.3) Diplôme d'Ingénieur

RÉPUBLIQUE DU CAMEROUN
Par - Travail - Paix

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR
UNIVERSITÉ DE NGAOUNDÉRE
ÉCOLE NATIONALE SUPÉRIEURE DES
SCIENCES AGRO-INDUSTRIELLES



REPUBLIC OF CAMEROON
Peace - Work - Fairness

MINISTRY OF HIGHER EDUCATION
UNIVERSITY OF NGAOUNDERE
NATIONAL ADVANCED SCHOOL OF
AGRO-INDUSTRIAL SCIENCES

Diplôme d'Ingénieur
Master of Engineering Degree

N° 01/ING/028/EN

Vu le décret n° 92/074 du 13 avril 1992 portant transformation des Centres Universitaires de Bua et de Ngaoundéré en Universités
Ministère de l'Éducation n° 92/074 of 13 April 1992 to transform Bua and Ngaoundere University Centres into Universities
Vu le décret n° 93/028 du 19 janvier 1993 portant organisation administrative et académique de l'Université de Ngaoundéré
Ministère de l'Éducation n° 93/028 of 19 January 1993 to organise the University of Ngaoundere
Vu le décret n° 95/061 du 03 avril 1995 portant organisation de l'École Nationale Supérieure des Sciences Agro-Industrielles de l'Université de Ngaoundéré
Ministère de l'Éducation n° 95/061 of 3 April 1995 to organise the National Advanced School of Agro-Industrial Sciences of the University of Ngaoundere

Vu les procès - verbaux des délibérations du jury, session de
Mindful of the decision of the board of examiners, session of

Octobre 2001

Le Diplôme d'Ingénieur
The Master of Engineering Degree

Spécialité
Specialty
Industries agricoles et Alimentaires

Spécialisation
Specialization

Desobogo Zangue Steve - Carly

Est délivré à
Is conferred on
Né(e) le 02 - 01 - 1977
Born on
à Douala - Cameroun
at Douala - Cameroun N° Matricule 98A005 EN
Registration N°

Pour en jouir avec les droits et prérogatives qui y sont rattachés
With all the rights and privileges appertaining to the said degree

19 MARS 2004

Fait à Yaoundé, le
Done in Yaounde, on

LE RECTEUR

[Signature]

Dr. Desobogo Zangue Steve - Carly

MAURICE TCHUENTE
Ministère de l'Enseignement Supérieur, Université et Académiques
Minister of Higher Education, University and Academic Affairs

LE MINISTRE DE L'ENSEIGNEMENT SUPÉRIEUR,
CHANCELLIER DES ORDRES ACADÉMIQUES

[Signature]

Hollog

II.4) Certificat de réussite du Diplôme d'Etudes Approfondies en Génie des Procédés



UNIVERSITE DE NGAOUNDERE
THE UNIVERSITY OF NGAOUNDERE

ECOLE NATIONALE SUPERIEURE DES
SCIENCES AGRO-INDUSTRIELLES
THE NATIONAL SCHOOL OF AGRO-INDUSTRIAL
SCIENCES

B.P.: 455 Ngaoundéré - Cameroun
Tél/Fax : (237) 225-27-51
E-mail : ensai_stages@yahoo.fr
Site Internet : http://ensai-nt.minesup.gov.cm



DIPLOME D'ETUDES APPROFONDIES EN GENIE DES PROCÉDES
CERTIFICAT DE REUSSITE / SUCCESS CERTIFICATE N° 003/003 /UN/D. ENSAI/DAACRS

Les soussignés

Vu le procès verbal du jury en date du **19 juillet 2003**

attestent que :

M/Mlle **DESOBGO ZANGUE Steve Carly**

Matricule n° **98A005EN**

Né(e) le **02 janvier 1977**

à

Douala

Titulaire du **Diplôme des Ingénieurs des Industries Agricoles et Alimentaires**

Ayant suivi les enseignements de **DEA de la filière de Génie des Procédés (GP)** pendant l'année académique **2001-2002** a validé la totalité des Modules / Unités de Valeurs avec les performances suivantes :

Code Module/UV	Intitulé des Modules / Unités de Valeurs	Note / 20	Mention	Année
MIGP6011	Mathématiques - Informatique pour le Génie des Procédés	18,0	TB	2002
PTGP6012	Phénomènes de Transport	15,0	B	2002
OU6014GP	Opérations unitaires de séparation et de Conservation	15,50	B	2002
TEGP6015	Techniques et Procédés nouveaux pour les IAA	13,50	B	2002
GA6013GP	Propriétés Phys., Physico-chimiques et Technol. des aliments	13,46	AB	2002
TA6113GP	Techniques Analytiques avancées	11,45	AB	2002
GBGP6312	Génie Biochimique et Biotechnologique	16,00	B	2002
MRGP6016	Méthodologie de la Recherche	15,60	B	2002
	Moyenne écrit	14,81	B	
IRGP6017	Stage d'Initiation à la recherche	15,50	B	2003
STGP6018	Mémoire de Recherche	14,00	B	2003
	Moyenne recherche :	15,50		
	MOYENNE GENERALE :	15,15		
MENTION : Bien				

En foi de quoi ce certificat lui est délivré pour servir et valoir ce que de droit./

Ngaoundéré le : **27 DEC. 2004**

Le Chef de Division des Affaires Académiques
de la Coopération, de la Recherche et de la Scolarité

Le Directeur,

Dr. Kamga Richard



II.5) Rapport de soutenance de Thèse de Doctorat PhD et attestation



RAPPORT DE SOUTENANCE DE THESE DE DOCTORAT/Ph.D

Spécialité : Génie des Procédés

VIVA VOCE REPORT OF THE Ph.D Thesis

Delof

Desobgo Zangue Steve Carly

Matricule : 98D005EN

Date: 14/09/2012

Sujet de Thèse/Title of Thesis

**Modélisation de l'action des hydrolases sur quelques caractéristiques
physico-chimiques des mouls de deux cultivars de sorgho**

Pr. Nso Emmanuel Jong

Jury / Board of Examiners:

- Président: Mbofung Carl Moses, Professeur, Université de Ngaoundéré
Rapporteurs : Ndjounekeu Robert, Professeur, Université de Ngaoundéré
Essia Ngang Jean Justin, Maître de Conférences, Université de Yaoundé 1
Examineurs : Fon Abi Charles, Maître de Conférences, Université de Yaoundé 1
Co-Directeurs : Kayem Joseph, Maître de Conférences, Université de Ngaoundéré
Nso Emmanuel, Maître de Conférences, Université de Ngaoundéré
Invité : Zo'obo, Directeur Process Qualité, Brasseries du Cameroun, Yaoundé

EVALUATION

Quelle mention accordez-vous par rapport à : Score the Viva voce on the following basis :	Mention / Merit			
	Excellent Excellent	Très Bien Very Good	Bien Good	Assez Bien Fairly Good
La clarté de la présentation / Clarity of the presentation	X			
La pertinence de la recherche menée par le candidat Relevance of research work carried out	X			
Défense de l'originalité de la thèse/ Defence of the originality of the thesis		X		
Techniques et méthodes de recherche Research methods and techniques		X		
Avancée dans la résolution du problème posé Contribution to the attainment of the general objectives of the thesis work	X			
Clarification des points d'ambiguïté dans la thèse Clarification of points of ambiguity		X		
Contribution à la Connaissance Scientifique Contribution to knowledge	X			
Possibilité de publication Potential of publishing results obtained	X			

Autres commentaires et Mention du Diplôme/ Other comments and Merit of Degree: Travail très intéressant à fort potentiel de valorisation industrielle et une opportunité pour la filière des céréales locales. La poursuite des travaux est à encourager. Le volume des résultats obtenus est important et a donné lieu à des publications scientifiques de très bon niveau, signe d'une reconnaissance internationale de la qualité de cette thèse. De ce qui précède, le jury reçoit la thèse de Monsieur Desobgo Zangue Steve Carly et lui décerne le diplôme de Docteur/PhD avec la mention très honorable.

Signatures :

Président(e)/President *Mbofung Carl Moses*

Membres 1 *ESSIA NGANG J. Justin*

2 *Ndjounekeu Robert*

3 *Fon Abi Charles*

4 *Fon Abi Charles*

5 *Kayem Joseph*

6 *Nso Emmanuel*

7

REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie

MINISTRE DE L'ENSEIGNEMENT SUPERIEUR

REPUBLIC OF CAMEROON
Peace – Work – Fatherland

MINISTRY OF HIGHER EDUCATION



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THE NATIONAL SCHOOL OF AGRO-INDUSTRIAL SCIENCES

B.P. : 455 Ngaoundéré – Cameroun Tél: (237) 77783840 Fax: (237) 22 15-81 89
E-mail : email_ngaoundere@yahoos.com Site Internet : <http://www.ensai-uni-ndg.com>



ATTESTATION

12012/087
N° UN/D. ENSAI/DAACRS/SSS

Le Directeur de l'Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI) de l'Université de Ngaoundéré, soussigné,
The Director of the National School of Agro-Industrial Sciences of the University of Ngaoundere, undersigned,

ATTESTE QUE
HEREBY CERTIFY THAT

M./Mme/Mlle **DESOBGO ZANGUE STEVE CARLY** Né(e) **02 Janvier 1977** à **DOUALA**

Matricule n° **98A005EN** de sexe **Masculin** *Born on the* **14 Septembre 2012** une thèse de
Reg. N° **Male** *sex* **has successfully defended on the 14 of September 2012** his thesis for the award of the

Doctorat/Ph.D
Doctor of Philosophy Degree Option: **Génie des Procédés**
in **Process Engineering**

Le travail de recherche a porté sur: **Modélisation de l'action des hydrolases sur quelques caractéristiques physico-chimiques des mouls de**
deux cultivars de sorgho.

The title of the research work is

Devant un jury dûment constitué par décision no **2012/479/UN/R/VRE-PDTC/DAAC/DEPE** du **04 Septembre 2012** composé de:
Before the jury constituted by decision no 2012/479/UN/R/VRE-PDTC/DAAC/DEPE of 04th September 2012 and made up of the following

Président : **Pr. MBOFUNG Carl Moses** Membres : **Pr. NDJOUENKEU Robert**

Rapporteurs : **Pr. NSO Emmanuel JONG**
Secrétaires: **Pr. KAYEM Joseph**

Pr. ESSIA NGANG Jean Justin
Pr. FON ABI Charles
Dr. ZO'OBO Jean Pierre

Ngaoundéré, le

Le Directeur
The Director



Pr. Nso Emmanuel Jong

NB : Il n'est délivré qu'une seule attestation. Le titulaire pourra en faire autant de copies certifiées conformes qu'il verra. Le Diplôme sera délivré ultérieurement.
Only one testimonial is issued. It is in the interest of the owner to make as many certified true copies as he/she may desire. The Certificate will be issued later.

III) ACTES ADMINISTRATIFS

III.1) Décision de recrutement et contrat de travail



Décision N°/UN/R/VR-EPDTIC/SG/DAAC/DEPE/SSPE

Portant recrutement de Monsieur DESOBGO ZANGUE Steve Carly en qualité d'Assistant au Département de Génie Alimentaire et Contrôle Qualité de l'Institut Universitaire de Technologie de l'Université de Ngaoundéré.

**LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE,
RAPPORTEUR DU CONSEIL D'ADMINISTRATION**

- Vu la Constitution ;
Vu la Loi n°2007/005 du 26 décembre 2007 portant Loi de Finances de la République du Cameroun pour l'exercice 2008 ;
Vu l'Ordonnance n°62/OF/4 du 07 février 1962 portant Régime Financier du Cameroun et les textes modificatifs subséquents ;
Vu le décret n° 2005/142 du 29 avril 2005 portant organisation du Ministère de l'Enseignement Supérieur ;
Vu le décret n° 92/74 du 13 avril 1992 transformant les Centres Universitaires de Ngaoundéré et Buéa en Universités ;
Vu le décret n°93/026 du 19 janvier 1993 portant création des Universités ;
Vu le décret n° 93/027 du 19 janvier 1993 portant dispositions communes applicables aux Universités, modifié et complété Par le décret n° 2005/342 du 10 septembre 2005 ;
Vu le décret n° 93/035 du 19 janvier 1993 portant statut spécial des personnels de l'Enseignement Supérieur, modifié et complété par le décret n° 2000/048 du 15 mars 2000 ;
Vu le décret n°93/028 du 19 janvier 1993 portant organisation administrative et académique de l'Université de Ngaoundéré ;
Vu le décret n° 93/028 du 19 janvier 1993 modifiant la rémunération des Fonctionnaires et Agents de l'Etat ;
Vu l'arrêté n° 2000/050 du 15 mars 2000 fixant les modalités de rémunération du personnel Assistant des Universités ;
Vu le décret n° 2000/209 du 27 juillet 2000 fixant la valeur du point d'indice des Fonctionnaires de l'Etat ;
Vu le décret n°2000/212 du 27 juillet 2000 modifiant certaines dispositions du décret n°91/324 du 09 juillet 1991 fixant les conditions d'attribution des logements administratifs ;
Vu le décret n° 2008/099 du 07 mars 2008 portant revalorisation de la rémunération mensuelle de base des personnels civils et militaires ;
Vu le décret n° 2008/100 du 07 mars 2008 portant revalorisation du taux de l'indemnité de non logement servie aux personnels civils et militaires ;
Vu le décret n° 2003/049 du 16 septembre 2003 portant nomination des Recteurs dans les Universités d'Etat ;
Vu les résolutions de la Commission Consultative de Recrutement des Assistants de l'Université de Ngaoundéré en sa séance du 23 janvier 2008 ;
Vu la décision du Conseil de l'Université de Ngaoundéré lors de sa vingt-deuxième session en date du 24 janvier 2008 ;
Vu l'avis favorable émis par le Conseil d'Administration de l'Université de Ngaoundéré lors de sa vingt unième session en date du 25 janvier 2008 ;
Vu la lettre n° 08/01866/L/MINESUP/SG/DDES/SCE/CEA1/ne du 31 mars 2008 portant autorisation de mise en service des Assistants et des ATER recrutés lors de la CCR du 23 janvier 2008 ;
Vu les prévisions budgétaires de l'exercice 2008.

DECIDE :

Article 1er : Monsieur DESOBGO ZANGUE Steve Carly titulaire d'un Diplôme d'Etudes Approfondies (DEA) en Génie des Procédés est pour compter du 16 avril 2008, date effective de sa prise de service, recruté en qualité d'Assistant, indice 1E/465, au Département de Génie Alimentaire et Contrôle Qualité de l'Institut Universitaire de Technologie de l'Université de Ngaoundéré.

Article2: L'Intéressé sera pris en charge par le budget de l'Etat Chap. 18 Art 102 Par 000 (18-102-000).

Article 3: La présente décision sera enregistrée et communiquée partout où besoin sera.

Ampliatiions

- MINESUP
- MINFI-SOLDE-TRESOR
- MINFOPRA/DPE/SDPFE/SAPE
- RECTEUR-UN
- VR-EPDTIC / VR-CIE
- SG UN / DAAC / DEPE / SSPE
- DAAF-UN / AC -UN
- CHEF D'ETABLISSEMENT



LE RECTEUR

Ngaoundéré, le

09 JUL. 2008

REPUBLIQUE DU CAMEROUN
PAIX-TRAVAIL-PATRIE

REPUBLIC OF CAMEROON
PEACE-WORK-FATHERLAND

UNIVERSITE DE NGAOUNDERE

THE UNIVERSITY OF NGAOUNDERE



CONTRAT DE TRAVAIL - CONTRACT OF ENGAGEMENT
2008/031
N°...../UN/R/VR-EPDTIC/SG/DAAC/DEPE/SSPE

Entre les Soussignés :

L'Université de Ngaoundéré, représentée par le Recteur
d'une part,

et Monsieur **DESOBGO ZANGUE Steve Carly**, de Sexe **masculin**, né le **02 Janvier 1977** à **Douala - Cameroun**, Arrondissement de **Douala 1**, Département du **Wouri**, Province du **Littoral**.

Fils de **SOBGO Gabriel**,
et de **GUEPI TEADJO Emilienne**,

de nationalité **camerounaise**, dont la résidence est à **Ngaoundéré**.

Ci-après dénommé Contractant
d'autre part,

conformément :

- aux dispositions prévues par la loi n° 92/007 du 14 août 1992 portant code du travail et à ses décrets et arrêtés d'application ;
- au décret n°92/74 du 13 avril 1992 transformant les centres universitaires de Ngaoundéré et Buéa en Universités ;
- aux textes régissant les universités et les institutions universitaires camerounaises, et notamment :
 - le décret n° 93/028 du 19 janvier 1993 portant organisation administrative et académique de l'Université de Ngaoundéré ;
 - le décret n° 93/027 du 19 janvier 1993 portant dispositions communes applicables aux universités ;
 - le décret n° 93/035 du 19 janvier 1993 portant statut spécial des personnels de l'Enseignement Supérieur ;
 - et le décret n° 93/021 du 19 janvier 1993 portant dispositions communes applicables aux Instituts Universitaires de Technologie ;
 - le décret n° 76/472 du 18 octobre 1976 portant certaines dispositions applicables aux



- personnels du cadre de l'Enseignement Supérieur ;
- le décret n° 78/484 du 09 novembre 1978 fixant les dispositions communes applicables aux agents de l'Etat relevant du code de travail ;
 - le décret réglementant le régime des déplacements au Cameroun ;
 - l'arrêté n° 2000/050 du 15 mars 2000 fixant les modalités de rémunération du personnel Assistant des Universités ;
 - le décret n° 2005/344 du 10 septembre 2005 portant nomination des Recteurs dans les Universités d'Etat ;
 - les résolutions de la Commission Consultative de Recrutement des Assistants (CCRA) de l'Université de Ngaoundéré en sa séance du 23 janvier 2008 ;
 - la décision du Conseil de l'Université de Ngaoundéré en sa vingt-deuxième session du 24 janvier 2008 ;
 - l'avis favorable émis par le Conseil d'Administration de l'Université de Ngaoundéré lors de sa vingt-unième session en date du 25 janvier 2008 ;
 - la lettre n° 08/01866/L/MINESUP/SG/DDES/SCE/CEA1/ne du 31 mars 2008 portant autorisation de mise en service des Assistants et ATER de l'Université de Ngaoundéré ;
 - le décret n° 2000/209 du 27 juillet 2000 fixant la valeur du point d'indice des Fonctionnaires de l'Etat ;
 - le décret n°2000/212 du 27 juillet 2000 modifiant certaines dispositions du décret n°91/324 du 09 juillet 1991 fixant les conditions d'attribution des logements administratifs ;
 - le décret n° 2008/099 du 07 mars 2008 portant revalorisation de la rémunération mensuelle de base des personnels civils et militaires ;
 - le décret n° 2008/100 du 07 mars 2008 portant revalorisation de l'indemnité de non logement servie aux personnels civils et militaires ;
 - vu le décret n° 2002/041 du 04 février 2002 fixant les montants et les modalités de paiement des primes allouées à certains personnels du Corps de l'Enseignement Supérieur ;
 - vu les prévisions budgétaires de l'Etat au Chap. 18 Art. 102 par 000 (18-102-000),

Il a été arrêté et convenu d'un commun accord ce qui suit :

Article 1 : Clauses générales

Quels que soient les titres donnés au Contractant et l'emploi occupé par lui, le présent contrat ne lui confère ni la qualité de fonctionnaire, ni le droit d'être nommé dans les cadres réguliers et permanents de l'Enseignement Supérieur, sauf dans les conditions fixées par les textes statutaires en vigueur.

Article 2 : Emploi à tenir

Le Contractant, titulaire d'un **DIPLÔME D'ETUDES APPROFONDIES (DEA)**, est appelé à remplir les fonctions d'**ASSISTANT, indice 1^E/465** au **Département de Génie alimentaire et Contrôle Qualité de l'Institut Universitaire de Technologie de l'Université de Ngaoundéré**.

Toutefois, conformément au décret n°93/027 du 19 janvier 1993, le Ministre de l'Enseignement Supérieur peut, pour nécessités de service, affecter le Contractant dans une autre Institution Universitaire camerounaise.

Article 3 : Rémunération

Le Contractant percevra une rémunération mensuelle calculée sur la base de l'indice **1E/465**. Outre la rémunération ci-dessus, le Contractant percevra mensuellement :

- une prime de technicité d'un montant de **TRENTE MILLE (30 000) Francs CFA** en vertu du décret n°2002/041 du 04 février 2002 fixant les montants et les modalités de paiement des primes allouées à certains personnels du Corps de l'Enseignement Supérieur ;
- une prime de l'Enseignement Supérieur d'un montant de **QUATRE VINGT DIX MILLE (90 000) Francs CFA** en vertu du décret n°2002/041 du 04 février 2002 susvisé ;
- une allocation mensuelle de logement non soumise à impôt dont le taux est égal à 20 % du salaire de base indiciaire ou catégoriel en vertu du décret n° 2008/100 du 07 mars 2008.
- il pourrait prétendre annuellement à une prime de recherche, conformément à la réglementation en vigueur.

Article 4 : Durée du Contrat

La durée du présent contrat est de deux (02) ans renouvelable deux (02) fois.

Toutefois les Assistants non titulaires d'une thèse de doctorat au moment de leur recrutement peuvent, sur décision des Recteurs, bénéficier d'une période supplémentaire de deux (02) ans, pour soutenir leur thèse de doctorat.

Article 5 : Congés

La période de service effectif ouvrant droit aux congés est de douze (12) mois. Toutefois, le Contractant bénéficiera annuellement de ses congés pendant les périodes d'interruption des classes, conformément à la réglementation en vigueur.

Article 6 : Voyages-Transport

A l'occasion de ses congés, le Contractant de nationalité camerounaise, a droit pour lui-même et sa famille au transport Aller et Retour gratuit conformément aux dispositions du décret n°91/134 du 22 février 1991 réglementant la prise en charge sur le budget de l'Etat des frais de déplacement des fonctionnaires et agents civils de l'Etat du lieu de service à leur localité d'origine entendue comme lieu de résidence habituelle justifiée par une attestation délivrée par le Préfet ;

a) au cas où le Contractant désirerait prendre ses congés ailleurs qu'à sa résidence habituelle, l'Université de Ngaoundéré n'aura à supporter que les frais exposés par l'intéressé sans que ceux-ci puissent excéder les frais qu'aurait nécessité son voyage Aller et Retour et éventuellement, celui de sa famille de son lieu de service à sa résidence habituelle.

b) le Contractant de nationalité étrangère bénéficie du transport Aller et Retour gratuit pour lui-même et sa famille à l'intérieur du Cameroun dans une ville de son choix.

c) la Contractante de nationalité étrangère épouse d'un Camerounais bénéficie du transport Aller et Retour gratuit à l'intérieur du pays dans les conditions de l'alinéa 1 de l'article 6 du présent contrat, s'il est prouvé qu'elle n'a pas bénéficié des congés au titre d'épouse dans l'Administration de son mari.

Les voyages et transports sont effectués par l'une des voies normales de transport selon la réglementation en vigueur.

Par famille, il faut entendre la conjointe légitime non divorcée, ni séparée et les enfants mineurs à charge.

Le Contractant ne peut toutefois prétendre au transport gratuit de sa conjointe, qu'au cas où celle-ci n'est pas salariée de l'Etat camerounais.

Article 7 : Déplacement temporaire

En cas de déplacement temporaire pour raison de service et lorsqu'il n'est ni logé, ni nourri par l'Université de Ngaoundéré, le Contractant bénéficiera des frais de mission conformément à la réglementation en vigueur.

Article 8 :

a) Appointement

Conformément au décret n° 2000/048 du 15 mars 2000 modifiant et complétant certaines dispositions du décret n° 93/035 du 19 janvier 1993 portant statut spécial des personnels de l'Enseignement Supérieur, la charge horaire du Contractant est de 200 heures de travaux dirigés, d'exercices ou de travaux pratiques par an. Au cas où le Contractant effectuerait des heures complémentaires au-delà de sa charge d'heures réglementaires ci-dessus mentionnées, celles-ci seront rémunérées conformément aux textes en vigueur.

b) Imputation

Les émoluments divers perçus par le Contractant sont pris en charge par le budget de l'Etat EXERCICE 2008 CHAPITRE 18 ARTICLE 102 PARAGRAPHE 000

Article 9 : Soins médicaux

Le Contractant a droit au remboursement sur les frais d'hospitalisation pour toutes les maladies ou accidents survenus du fait ou à l'occasion du travail conformément à la loi n°77/11 du 13 juillet 1997 portant réparation et prévention des accidents de travail et des maladies professionnelles. Il pourra bénéficier pour lui, sa conjointe et ses enfants mineurs à charge, des mêmes avantages que les fonctionnaires de classification correspondante en ce qui concerne les soins pour maladies et accidents survenus en dehors du travail jusqu'à l'instauration d'un régime de sécurité sociale.

Dans ce cas, l'Université de Ngaoundéré est tenue de lui verser une indemnité conformément aux dispositions du décret n° 78/484 du 09 novembre 1978.

Article 10 : Résiliation

Le contrat est résilié :

1/- de plein droit et sans préavis :

a) si, après acceptation et signature du contrat, le Contractant ne rejoint pas son poste sur première réquisition de l'Université de Ngaoundéré. Dans ce cas il sera tenu au remboursement de toutes les sommes perçues ainsi que des frais engagés pour ses bagages et transports et éventuellement ceux de sa famille.

En cas de refus, il y sera contraint par toutes les voies de droit.

b) pour faute lourde, conformément à la réglementation en vigueur.

2) Avec préavis (article 34 de la loi n° 92/007 du 14 août 1992 portant code du travail).

En cas de résiliation du contrat de travail : la durée du préavis réciproque est fixée, sauf pour faute lourde, conformément aux textes en vigueur.

Indemnité de licenciement : en cas de licenciement, si le Contractant a au moins deux (02) années d'ancienneté, il bénéficiera, sauf en cas de faute lourde, d'une indemnité de licenciement distincte du préavis et calculée conformément à l'article 14 du décret n° 78/484 du 09 novembre 1978.

Article 11 : Obligation du secret professionnel

Le Contractant s'engage à consacrer tout son temps et toute son activité, dans la limite de la réglementation en vigueur aux fonctions qui lui seraient confiées, à se conformer à toutes les instructions du présent contrat et à ne fournir aucune information de nature confidentielle dont il aurait connaissance à l'occasion de son travail.

CLAUSES PARTICULIÈRES

NEANT

ARTICLE 12 :

Le Contractant déclare formellement être libre de tout engagement antérieur.

Il déclare en outre avoir pris connaissance des clauses et conditions du présent contrat et en accepter sans réserve toutes les dispositions.

ARTICLE 13 :

Le présent contrat qui prend effet pour compter du **16 Avril 2008**, date effective de sa prise de service, est exempt de tous droits de timbre et d'enregistrement./-

Ngaoundéré, le **09 JUL. 2008**

LU ET APPROUVÉ

LU ET APPROUVÉ
[Signature]

LE CONTRACTANT

LE RECTEUR,

[Signature]
La Recteur
[Signature]

AMPLIATIONS

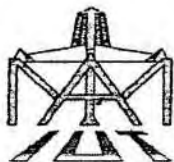
- MINESUP
- MINFI-SOLDE-TRESOR
- MINFOPRA/DPE/SDPFSE/SAPE
- RECTEUR-UN
- VR-EPDTIC-VR-CIE
- SG- UN/DAAC/DEPE/SSPE
- DAAF -UN/ AC -UN
- CHEF D'ETABLISSEMENT
- INTERESSÉ- CHRONO - ARCHIVES

III.2) Certificat de prise de service

REPUBLIQUE DU CAMEROUN

Paix – Travail – Patrie

UNIVERSITE DE NGAOUNDERE



Le Directeur

REPUBLIC OF CAMEROON

Peace - Work – Fatherland

THE UNIVERSITY OF NGAOUNDERE

**INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
DE NGAOUNDERE**

B.P. 455 NGAOUNDERE – Tél. 77 11 22 20 & 99 85 13 82

Ngaoundéré, le 16 AVR. 2008

2008/011 N° /UN/D.IUT

CERTIFICAT DE PRISE DE SERVICE

Le Directeur de l'Institut Universitaire de Technologie (IUT) de l'Université de Ngaoundéré soussigné, certifie que **Monsieur DESOBGO ZANGUE Steve Carly** né le 02 Janvier 1977 à Douala, actuellement en cours de recrutement, a effectivement pris service à l'Institut Universitaire de Technologie le 16 Avril 2008 en qualité d'Assistant au Département de Génie Alimentaire et Contrôle Qualité.

En foi de quoi ce certificat est délivré à l'intéressé pour servir et valoir ce que de droit.

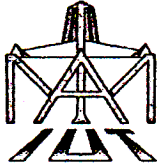
Ampliation:

-Chrono/Archives.

LE DIRECTEUR,

Dr.-Ing. Ali Ahmed

III.3) Attestation de présence effective



Le Directeur

Nº

UN/IUT/D/zlb

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
THE UNIVERSITY INSTITUTE OF TECHNOLOGY
NGAOUNDERE

B.P. 455 NGAOUNDERE - Tél. 00 (237) 677 11 22 20 / 242 25 40 23

Fax : 00 (237) 242 25 40 01

Site Web: www.iut.univ-ndere.cm

06 AUG 2024

Ngaoundéré, le

A T T E S T A T I O N D E P R E S E N C E E F F E C T I V E

Le Directeur de l'Institut Universitaire de Technologie (IUT) de
l'Université de Ngaoundéré soussigné **Pr. MOHAMMADOU Bouda Adji**,

Atteste que **Pr. DESOBGO ZANGUE Steve Carly**, Maître de Conférences, Matricule **652 117-M**, exerce la profession d'enseignant chercheur dans ledit Institut depuis le 16 Avril 2008, date de sa prise de service. Il est effectivement présent à son poste.

En foi de quoi la présente attestation est établie pour servir et valoir ce que de droit.

Ampliation

- Chrono/archives

Cette attestation est valable pour une période de 30 jours



P. Mohammadou P. 54 ha M.

III.4) Notes de service

REPUBLIQUE DU CAMEROUN

Paix - Travail - Patrie

UNIVERSITE DE NGAOUNDERE



REPUBLIC OF CAMEROON

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NGAOUNDERE

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Fax : 00 (237) 222 25 40 01 / 222 25 40 23

Site Web: www.iut.univ-ndere.cm

N° 118-2020
UN/D/DA

Ngaoundéré, le 11 OCT 2020

NOTE DE SERVICE

Les enseignants dont les noms suivent sont à compter de la date de signature de la présente note de service, nommés Responsables Techniques des Laboratoires d'enseignement à l'Institut Universitaire de Technologie pour le compte de l'année académique 2020/2021.

Il s'agit de:

Départements	Laboratoires	Responsables
	Laboratoire Spécialisé PCUE	Pr MOHAMMADOU BOUBA Adjì
Génie Alimentaire et Contrôle Qualité	Biochimie et Chimie Alimentaire	Pr TCHIEGANG Clergé
	Lait et produits laitiers	Pr JIOKAP NONO Yvette
	Microbiologie	Pr TATSADJIEU Léopold
	Analyse sensorielle	Pr MEZAJOUK K. Laurette
	Biologie Moléculaire	Pr MOHAMMADOU BOUBA Adjì
	Biologie Clinique	Dr AGUME Aurélie
	Brasseries	Pr DESOBGO Steve
	Technologie Alimentaire	Dr SAÏDOU Clément
Département de Génie Chimique	Chimie Analytique	Dr NOUBISSIE Éric
	Génie Chimique	Pr ALI Ahmed
	Chimie Organique	M. NGUEMTUE Thierry
	Technologie Environnementale	Dr HASSANA Boukar
Génie Electrique	Electronique et Signaux	Dr BOUSSAIBO André
	Electrotechnique	Dr Fabrice TSEGAING
	Asservissement et Régulation	Pr EDOUN Marcel
	Impression Typon	Dr BOUSSAIBO André
	Energies Renouvelables	Pr EDOUN Marcel
	Maintenance des équipements Biomédicaux	Dr YOUSOUFA Mohamadou
Génie Energétique	Froid et Climatisation	Dr BIKAI Jacques

Génie Mécanique	Salle CAO / DAO	Dr FTATSI Guy
	Prototypage	Pr NTENGA Richard
	Atelier Fabrication Mécanique	M. SAIDJO
	Matériaux, Essai et Métrologie	Dr FTATSI Guy
	Maintenance	M. EVENGA Didier
	Atelier GCD	M. DJOUATSA Aubin
	Bureau d'Etudes GCD	M. ENEME Gaétan

Ampliations :

- DA/IUT
- DFI/IUT
- Chefs de départements
- Intéressés
- Délégués de classe
- Archives



P. Mohammadou Bouda Adj

REPUBLIQUE DU CAMEROUN

Paix - Travail - Patrie

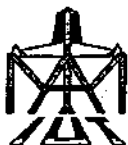
UNIVERSITE DE NGAOUNDERE



REPUBLIC OF CAMEROON

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INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
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Fax : 00 (237) 222 25 40 01 / 222 25 40 23

Site Web/ Web Site: www.iut.univ-ndere.cm

Le Directeur/ The Director

N°...../UN/IUT/D/DA

Ngaoundéré, le... 05 MARS 2024.....

NOTE DE SERVICE

NOTE DE SERVICE N° 160/2024 /UN/D.IUT/DA DU 05 MARS 2024

Désignant les personnels de l'Institut Universitaire de Technologie (IUT) de l'Université de Ngaoundéré comme membre du Comité d'Ethique de Lutte contre le Plagiat pour un mandat de deux (02) ans renouvelable un (01) fois.

N°	NOMS ET PRENOMS	Fonction	Grade	Qualité
1	MOHAMMADOU Bouba Adji	Directeur	Pr	Supervision Générale
2	EDOUN Marcel	Directeur Adjoint	Pr	Coordination
3	TATSADJIEU Léopold	Chef Division des Stages	Pr	Rapporteur
4	TCHIEGANG Clergé	Responsable Laboratoire LBP	Pr	Membre
5	Ali Ahmed	Responsable Laboratoire	Pr	Membre
6	EKOBENA Fouda Henri	Responsable Laboratoire LASE	Pr	Membre
7	NTENGA Richard	Chef Département Génie Mécanique	Pr	Membre
8	YAOUDAM ELISABETH	Coordonateur de la cellule de lutte contre la corruption et consommation des stupéfiants	MC	Membre
9	DESOBGO Steve	Responsable des activités de recherche	MC	Membre
10	YENKE Blaise Omer	Chef Département Génie Informatique	MC	Membre
11	SAIDOU Clément	Chef de Département Génie Alimentaire et Contrôle Qualité	MC	Membre
12	MEZAJOUG KENFACK Laurette	Chef de Département Enseignements Scientifiques de Base	MC	Membre
13	BOUSSAIBO André	Chef Département Génie Electrique	CC	Membre
14	BIKAI Jacques	Chef Département Génie Energétique	CC	Membre
15	HASSANA Boukar	Chef Département, Génie Chimique	CC	Membre

Copies :

- Recteur UN
- VRCIE/SG/UN
- Intéressés
- Affichage/Archives.



Le Directeur,

M. Mohammadou Bouba Adji

REPUBLIQUE DU CAMEROUN

Paix – Travail – Patrie

UNIVERSITE DE NGAOUNDERE



Le Directeur

N° IUT./D



REPUBLIC OF CAMEROON

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Site Web/ Web Site: www.iut.univ-ndere.cm

Ngaoundéré le 06 Mai 2024

COMMUNIQUE A TOUS LES ENSEIGNANTS DE L'IUT

Le Directeur de l'Institut Universitaire de Technologie (IUT) invite tous les Enseignants chercheurs de son établissement à participer à un séminaire sur « l'utilisation de l'outil de gestion des références bibliographiques Mendely Reference Manager » qu'organise le Laboratoire des Bioprocédés le Lundi 13 Mai 2024 de 10H à 12H dans la salle des Actes de l'IUT.

Il s'agit d'un levier essentiel pour améliorer la productivité, la qualité et la visibilité de la recherche au sein de notre institution en gagnant en temps et en efficacité dans la préparation des articles scientifiques, des mémoires et des thèses.

N.B. : Se munir d'un ordinateur avec connexion.

La présence de tous est vivement souhaitée.

Coordinateur : Monsieur le Professeur Mohammadou, Directeur de l'IUT

Présentateur : **Monsieur le Professeur Desobgo, Enseignant Chercheur au LBP**

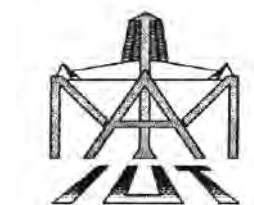
Modérateur : Monsieur le Professeur Tchiégang, Responsable du Laboratoire LBP



Le Directeur de l'IUT

Le Directeur

P. Mohammadou Boubou Adj



Le Chef de Département

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
Département de Génie Alimentaire et Contrôle Qualité
BP 455 - Ngaoundéré/ Tél: 699 52 37 27 / 679 32 75 96

Ngaoundéré, le 08 JANVIER 2020

NOTE D'INTERIM

Pendant l'absence du Chef de Département de Génie Alimentaire et Contrôle Qualité de l'IUT de Ngaoundéré du 09 au 13 Janvier 2020, l'intérim sera assuré par **Pr DESOBGO ZANGUE Stève Carly**, Enseignant au Département GACQ.

L'intéressé est tenu de remettre un rapport portant sur sa gestion académique et des affaires courantes du Département au Chef de Département dès son retour.

Le Chef de Département


Tatsadjieu Ngoune Léopold
Professeur



Ampliatiions

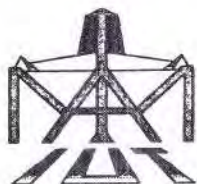
- Directeur de l'IUT
- Directeur Adjoint de l'IUT
- Chef de Division de la Formation Initiale
- Chefs de Division des Stages, de la Formation Continue et des Relations avec les Milieux Professionnels

REPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

REPUBLIC OF CAMEROON
Peace - Work - Fatherland

UNIVERSITE DE NGAOUNDERE

THE UNIVERSITY OF NGAOUNDERE



**INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
DE NGAOUNDERE**

DÉPARTEMENT GÉNIE ALIMENTAIRE ET CONTRÔLE QUALITÉ
BP 455 - Ngaoundéré/ Tél: 677 57 04 36

Le Chef de Département

Ngaoundéré le, 22 JAN 2021

N° 002/2021/IUT./DGACQ/sdo

NOTE DE SERVICE

Pendant l'absence du Chef de Département de Génie Alimentaire et Contrôle Qualité de l'IUT de Ngaoundéré du 23 au 30 janvier 2021, l'intérim sera assuré par le Pr. DESOBGO ZANGUE Steve, Enseignant dudit Département. L'intéressé est tenu de remettre un rapport portant sur sa gestion académique et des affaires courantes au Chef de Département dès son retour.

Le Chef de Département

Ampliations :

- Directeur /IUT
- Directeur-Adjoint /IUT
- Chef Division Formation Initiale
- Chef Division Stage
- RP/ IAB2 et 3, GEN2 et 3 et ABB2 et 3
- Affichage
- Archives/chrono/

Dr. Saidou Elémén
Chargé de Cours

REPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

REPUBLIC OF CAMEROON
Peace - Work - Fatherland

UNIVERSITE DE NGAOUNDERE

THE UNIVERSITY OF NGAOUNDERE



INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
DE NGAOUNDERE

DÉPARTEMENT GÉNIE ALIMENTAIRE ET CONTRÔLE QUALITÉ
BP 455 - Ngaoundéré/ Tél: 677 57 04 36

Le Chef de Département

Ngaoundéré le, 19 OCT 2023

N° 037/2023/IUT./DGACQ/sdo

NOTE DE SERVICE

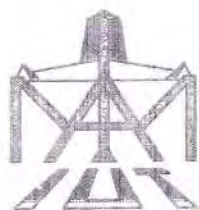
Pendant l'absence du Chef de Département de Génie Alimentaire et Contrôle Qualité de l'IUT de Ngaoundéré en mission du 20 au 27 octobre 2023, l'intérim sera assuré par le Pr. DESOBGO ZANGUE Steve Carly, Enseignant au Département. L'intéressé est prié de remettre un rapport portant sur la gestion académique et des affaires courantes au Chef de Département dès son retour.

Ampliations :

- Directeur /IUT
- Directeur-Adjoint /IUT
- Chef Division Formation Initiale
- Chef Division Stage
- RP/ IAB2 et 3, GEN2 et 3, ABB2 et 3 et BTS1 et 2
- Affichage
- Archives/chrono/

Le Chef de Département





Le Directeur

**INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
THE UNIVERSITY INSTITUTE OF TECHNOLOGY
NGAOUNDERE**

B.P. 455 NGAOUNDERE - Tél. 00 (237) 677 80 31 37 / 242 25 40 23

Fax : 00 (237) 242 25 40 01

Site Web: www.iut.univ-ndere.cm

Ngaoundéré le, 18 mai 2024

N° 9612024 UN/D/DA

NOTE DE SERVICE

Les Enseignants dont les noms suivent sont à compter de la date de signature de la présente note de service, chargés d'élaborer les contenus des unités d'enseignements du programme de formation en Master Universitaire de Technologie (MUT). L'accent sera mis sur les parcours et les spécialités retenus du Département de Génie Alimentaire et Contrôle Qualité (GACQ) de l'Institut Universitaire de Technologie (IUT) de l'Université de Ngaoundéré.

Supervision des commissions		
	Nom et prénoms	Responsabilité
/	Pr SAÏDOU Clément	Chef de Département GACQ, Superviseur des commissions
	Commission N°1 : mention Agroalimentaire ➤ Parcours 1 : Technologie Alimentaire ➤ Parcours 2 : Nutrition et diététique	
1	Pr. TCHIEGANG Clergé	Chef de commission
2	Pr. ALI Ahmed	Membre
3	Pr. DESOBGO ZANGUE Steve	Membre
4	Pr. JIOKAP NONO Yvette	Membre
5	Pr. MEZAJOUG Laurette B.	Membre
6	Dr. AGUME NTSO Aurélie	Membre
7	Dr. GHOMDIM NZALI Horliane	Membre
8	Dr. MAHAMA Abdoulaye	Membre
9	Dr. SOKAMTE Alphonse	Membre
	Commission N°2 : mention Agronomie et Environnement ➤ Parcours 1 : Gestion des déchets et Techniques d'Irrigations ➤ Parcours 2 : Alimentation et Nutrition Animale	
1	Pr. MOHAMMADOU Bouba Adjé	Chef de commission
2	Pr. NOUBISSIE Eric	Membre
3	Dr. HASSANA Boukar	Membre

4	Dr. FOGANG Bienvenu	Membre
5	Dr. NSOE MENGUE Nestor	Membre
6	Dr. NGOUFACK Cyrille	Membre
7	Dr. ESSOUNG Rosette Flaure	Membre
8	Dr. DONGMO Léonie	Membre
9	Dr. BENESSOUBO Kada Danielle	Membre
10	M. NGUEMTUE Thierry	Membre

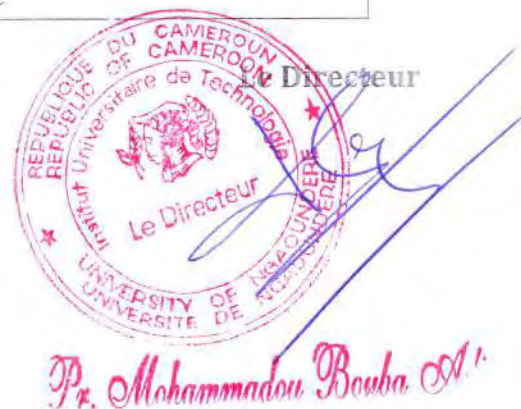
Commission N°3 : mention Biologie Moléculaire et Bio-Informatique

1	Pr. TATSADJIEU Léopold	Chef de commission
2	Pr. DJOULDE Darman Roger	Membre
3	Pr. MOHAMMADOU Bouba Adjil	Membre
4	Pr. YENKE Omer Blaise	Membre
5	Pr. NGANOU Nadège	Membre
6	Pr. NDAM NJOYA Harouna	Membre
7	Dr. EKOLLO MBANGE Aristid	Membre
8	Dr. NODEM SOHANANG Steve	Membre

Ampliatiions :

- Directeur-Adjoint /IUT
- Chef Division Formation Initiale
- Chef de Département GACQ
- Copies aux concernés

Le Directeur



Pr. Mohammadou Bouba A.



**INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
DE NGAOUNDERE**

DÉPARTEMENT GÉNIE ALIMENTAIRE ET CONTROLE QUALITÉ
BP 455 - Ngaoundéré/ Tél: 677 57 04 36

Le Chef de Département

Ngaoundéré le, 18 mai 2024

N° 024 / 2024 IUT / DGACQ /

Convocation

Les Enseignants dont les noms suivent sont conviés à prendre part à une séance de travail sur l'élaboration de programme de formation en Master Universitaire de Technologie (MUT). L'accent sera mis sur les parcours et les spécialités retenus du Département de Génie Alimentaire et Contrôle Qualité (GACQ) de l'Institut Universitaire de Technologie (IUT) de l'Université de Ngaoundéré. Cette réunion se tiendra le **mercredi 22 mai 2024 à 9 heures précises** à la salle des actes.

Commission N°1 : mention Agroalimentaire		
➤ Parcours 1 : Technologie Alimentaire		
➤ Parcours 2 : Nutrition et diététique		
	Nom et prénoms	Rôles
1	Pr. TCHIEGANG Clergé	Chef de commission
2	Pr. ALI Ahmed	Membre
3	Pr. DESOBGO ZANGUE Steve	Membre
4	Pr. JIOKAP NONO Yvette	Membre
5	Pr. MEZAJOUG Laurette B.	Membre
6	Dr. AGUME NTSO Aurélie	Membre
7	Dr. GHOMDIM NZALI Horliane	Membre
8	Dr. MAHAMA Abdoulaye	Membre
9	Dr. SOKAMTE Alphonse	Membre
Commission N°2 : mention Agronomie et Environnement		
➤ Parcours 1 : Gestion des déchets et Techniques d'Irrigations		
➤ Parcours 2 : Alimentation et Nutrition Animale		
1	Pr. MOHAMMADOU Bouba Adji	Chef de commission

2	Pr. NOUBISSIE Eric	Membre
3	Dr. HASSANA Boukar	Membre
4	Dr. FOGANG Bienvenu	Membre
5	Dr. NSOE MENGUE Nestor	Membre
6	Dr. NGOUFACK Cyrille	Membre
7	Dr. ESSOUNG Rosette Flaure	Membre
8	Dr. DONGMO Léonie	Membre
9	Dr. BENESSOUBO Kada Danielle	Membre
10	M. NGUMTUE Thierry	Membre

Commission N°3 : mention Biologie Moléculaire et Bio-Informatique

1	Pr. TATSADJIEU Léopold	Chef de commission
2	Pr. DJOULDE Darman Roger	Membre
3	Pr. MOHAMMADOU Bouba Adjji	Membre
4	Pr. NGANOU Nadège	Membre
5	Dr. EKOLLO MBANGE Aristid	Membre
6	Dr. NODEM SOHANANG Steve	Membre

Ampliatiions :

- Directeur /IUT
- Directeur-Adjoint /IUT
- Chef Division Formation Initiale
- Copies aux concernés

Le Chef de Département



Pr. Saïdou Clément
Food Technology

III.5) Actes d'avancement



13 SEP 2022

2022/685

Contrôle Financier Spécialisé auprès de
l'Université de Ngaoundéré

DECISION N°

JUN/RVR-EPDTIC/SG/DAAC/DEPE/SSPE

Portant avancement d'échelon de Monsieur DESOBGO ZANGUE
Steve Carly, Maître de Conférences.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE,

- Vu la Constitution;
- Vu la loi n°005 du 16 avril 2001 portant orientation de l'enseignement supérieur;
- Vu la loi n°2017/010 du 12 juillet 2017, portant statut général des établissements publics;
- Vu la loi 2018/011 du 11 juillet 2018, portant code de transparence et de bonne gouvernance dans la gestion des finances publiques au Cameroun;
- Vu la loi n°2021/025 du 16 décembre 2021, portant loi des Finances de la République du Cameroun pour l'exercice 2022;
- Vu le décret n°77/41 du 03 février 1977, fixant les attributions et l'organisation des contrôles financiers;
- Vu le décret n°92/74 du 13 avril 1992, transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités;
- Vu le décret n°93/028 du 29 janvier 1993, portant Organisation Administrative et Académique de l'Université de Ngaoundéré;
- Vu le décret n°2000/048 du 15 mars 2000, modifiant et complétant certaines dispositions du décret 93/035 du 19 janvier 1993 portant statut spécial des personnels de l'enseignement supérieur;
- Vu le décret n°2000/049 du 15 mars 2000, fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n°2005/342 du 10 septembre 2005, modifiant et complétant certaines le décret n°93/027 du 19 janvier 1993 portant dispositions communes aux Universités;
- Vu le décret n°2005/383 du 17 octobre 2005, fixant les règles financières applicables aux Universités;
- Vu le décret n°2008/099 du 07 mars 2008, revalorisant la rémunération mensuelle de base des personnels civils et militaires;
- Vu le décret n° 2017/318 du 27 juin 2017 portant nomination d'un "Vice-Chancellor" et des Recteurs dans certaines Universités d'Etat;
- Vu l'arrêté n° 005/MINFI du 19 septembre 2018, portant nomination du contrôleur financier à l'Université de Ngaoundéré;
- Vu la Circulaire n° 00000456/C/MINFI du 30 décembre 2021, portant Instructions relatives à l'Exécution des lois de Finances, au Suivi et au Contrôle de l'Exécution du Budget de l'Etat, des Etablissements Publics Administratifs, des Collectivités Territoriales Décentralisées et des autres Entités Publiques pour l'exercice 2022;
- Vu les résolutions du Conseil d'Université de l'Université de Ngaoundéré en sa session du 13 juin 2022;
- Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré en sa session du 14 juin 2022;
- Vu la résolution portant approbation du budget de l'Université de Ngaoundéré pour l'exercice 2022,

DECIDE :

Article 1^{er}: Monsieur DESOBGO ZANGUE Steve Carly (Mle 652117-M), Maître de Conférences de 2ème classe 5ème échelon (indice 1050), depuis le 16 avril 2020, est à compter du 16 avril 2022, avancé(e) au grade de Maître de Conférences, 6ème échelon de la 2ème classe (indice 1115).

Article 2 : L'intéressé (e) percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice 1115 correspondant

à son grade et pourra prétendre aux avantages accordées aux enseignants de même grade.

Article 3 : La présente décision sera enregistrée et communiquée partout où besoin sera./-

AMPLIATIONS

- MINESUP;
- MINFI/SOLDE/TRESOR;
- MINFOPRA/DRE/SDP/DE/SPE;
- RECTEUR;
- VR-EPDTIC;
- SG/DAAF/DAAC/DEPE/SSPE;
- CF/UN;
- AC/UN;
- INTERESSE(E);
- ARCHIVES/CHRONO.



27 SEPT 2022

Ngaoundéré, le

P.F. Uphie CHINJE



20/01094

DECISION N°-----UN/R/VR-EPDTIC /SG/DAAC/DEPE/SSPE
Portant avancement d'échelon de Monsieur DESOBGO ZANGUE Stève Carly,
Maître de Conférences.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE,

- Vu la Constitution;
- Vu La loi n°005 du 16 avril 2001, portant orientation de l'enseignement supérieur ;
- Vu la loi n°2018/012 du 11 juillet 2018, portant régime financier de l'Etat;
- Vu la loi n°2017/010 du 12 juillet 2017, portant statut général des établissements publics;
- Vu la loi n°2019/023 du 24 décembre 2019, portant loi de Finances de la République du Cameroun pour l'exercice 2020;
- Vu La loi n°2018/011 du 11 juillet 2018, portant code transparence et de bonne gouvernance dans la gestion des finances publiques au Cameroun;
- Vu le décret n°77/41 du 03 février 1977, fixant les attributions et l'organisation des contrôles financiers ;
- Vu le décret n° 92/74 du 13 avril 1992, transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités;
- Vu le décret n° 93/028 du 19 janvier 1993, portant Organisation administrative et académique de l'Université de Ngaoundéré;
- Vu Le décret n° 2000/048 du 15 mars 2000 modifiant et complétant certaines dispositions du décret n° 93 /035 du 19 janvier 1993, portant Statut Spécial des Personnels de l'Enseignement Supérieur;
- Vu le décret n° 2000/049 du 15 mars 2000, fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n° 2005/342 du 10 septembre 2005 modifiant et complétant le décret n° 93/027 du 19 janvier 1993, portant dispositions communes aux Universités;
- Vu le décret n° 2005/383 du 17 octobre 2005, fixant le régime financier applicable aux universités ;
- Vu le décret n° 2008/099 du 07 mars 2008, revalorisant la rémunération mensuelle de base des personnels civils et militaires ;
- Vu le décret n°2017/318 du 27 juin 2017, portant nomination d'un "Vice-Chancellor" et des Recteurs dans certaines Universités d'Etat;
- Vu L'arrêté n°005/MINFI du 19 septembre 2018, portant nomination du contrôleur financier à l'Université de Ngaoundéré ;
- Vu la circulaire n° 00008349/C/MINFI du 30 décembre 2019 Portant Instructions relatives à l'Exécution des lois de finances, au suivi et au Contrôle de l'Exécution du Budget de l'Etat, des Etablissements Publics Administratifs, des Collectivités Territoriales Décentralisées et des autres Organismes Subventionnés, pour l'Exercice 2020;
- Vu les résolutions du Conseil d'Université de l'Université de Ngaoundéré en sa session du 20 janvier 2020 ;
- Vu les résolutions du Conseils d'Administration de l'Université de Ngaoundéré en sa session du 21 janvier 2020.

DÉCIDE:

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly (Mle 652117-M), Maître de Conférences de 2^{ème} classe, 4^{ème} échelon (indice 1005) depuis le 30 novembre 2019 (ancienneté conservée 01 an, 07 mois 14 jours), est à compter du 16 avril 2020, avancé(e) au grade de Maître de Conférences de 2^{ème} classe, 5^{ème} échelon (indice 1050).

Article 2 : L'intéressé(e) percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice 1050 correspondant à son grade et pourra prétendre aux avantages accordés aux enseignants du même grade.

Article 3 : La présente décision sera enregistrée et publiée partout où besoin sera./-

AMPLIATIONS

- MINESUP ;
- MINFI/SOLDE/TRESOR ;
- MINFOPRA/DPE/SDPFSE/SAPE ;
- RECTEUR ;
- VR-EPDTIC ;
- SG/ DAAF/ DAAC/DEPE/SSPE ;
- CHEF D'ÉTABLISSEMENT ;
- CF/UN ;
- AC/UN ;
- INTERESSÉ ;
- ARCHIVES/CHRONO.



Ngaoundéré, le

30 NOV 2020

Handwritten signature in red ink.

REPUBLIQUE DU CAMEROUN

Paix - Travail - Patrie

MINISTÈRE DE L'ENSEIGNEMENT
SUPÉRIEUR

SECRÉTARIAT GÉNÉRAL

DIRECTION DU DÉVELOPPEMENT DE
L'ENSEIGNEMENT SUPÉRIEUR

MINISTÈRE DES FINANCES
DIRECTION GÉNÉRALE
DU BUDGET

REPUBLIQUE OF CAMEROON

Peace - Work - Fatherland

MINISTRY OF HIGHER EDUCATION

SECRETARIAT GENERAL

DEPARTMENT OF HIGHER EDUCATION
DEVELOPMENT

Contrôle Financier Central auprès de
MINESUP

ARRÊTÉ N° 20-0019/MINESUP/SG/DDES/CEPE/CFA2

Portant promotion de Monsieur DESOBGO ZANGUE Stève Carly au grade de
Maître de Conférences.

LE MINISTRE D'ETAT, MINISTRE DE L'ENSEIGNEMENT SUPERIEUR,

- Vu la Constitution;
- Vu la loi n°005 du 16 Avril 2001 portant orientation de l'Enseignement Supérieur;
- Vu la loi n° 2018/012 du 11 Juillet 2018 portant régime financier de l'Etat;
- Vu la loi n° 2019/023 du 24 décembre 2019 portant loi des Finances de la République du Cameroun pour l'exercice 2020;
- Vu le décret n° 41/77 du 03 février 1977 fixant les attributions et organisant les Contrôles Financiers ;
- Vu le décret n°2005/383 du 17 octobre 2005 fixant les règles financières applicables aux Universités;
- Vu le décret n°93/035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n° 2000/048 du 15 mars 2000;
- Vu le décret n° 75/459 du 26 juin 1975 déterminant le régime de rémunération des personnels civils et militaires, modifié et complété par le décret n° 79/64 du 03 mars 1979;
- Vu le décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n° 2008/099 du 07 mars 2008 portant revalorisation de la rémunération mensuelle de base des personnels civils et militaires;
- Vu le décret n° 2008/100 du 07 mars 2008 portant revalorisation du taux de l'indemnité de non logement servie aux personnels civils et militaires;
- Vu le décret n° 2011/408 du 09 décembre 2011 portant organisation du Gouvernement, modifié et complété par le décret n° 2018/190 du 02 mars 2018;
- Vu le décret n° 2019/002 du 04 Janvier 2019 portant réaménagement du Gouvernement;
- Vu le décret n° 2012/433 du 01 Octobre 2012 portant Organisation du Ministère de l'Enseignement Supérieur;
- Vu l'arrêté n°253 du 31 octobre 1994 portant Organisation et Fonctionnement du Comité Consultatif des Institutions Universitaires;
- Vu la Circulaire N° 00008349/C/MINFI du 30 décembre 2019 portant instructions relatives à l'exécution des lois de finances, au suivi et au contrôle de l'exécution du Budget de l'Etat et des Autres Entités Publiques pour l'exercice 2020;
- Vu l'arrêté n°20-00019/MINESUP/SP-CCIU du 07 janvier 2020 portant inscription sur la liste d'aptitude aux grades de Chargé de Cours, Maître de Conférences et Professeur dans les Institutions universitaires (session du 28 au 30 novembre 2019, en régularisation).
- Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré en sa session du 21 janvier 2020,

ARRETE :

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly (Mle 652117-M), né le 02 janvier 1977 à Douala, Chargé de Cours de 2ème classe 4ème échelon (indice 940) depuis le 16 avril 2018 est, à compter du 30 novembre 2019, date retenue par le Conseil d'Administration de l'Université, promu (e) au grade de Maître de Conférences de 2ème classe, 4ème échelon (indice 1005) de l'Enseignement Supérieur.

Article 2 : L'intéressé (e) percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice de son grade de Maître de Conférences et pourra prétendre à tous les avantages accordés aux enseignants du même grade.

Article 3 : La dépense totale résultant des dispositions de l'article 1er ci-dessus, est imputée sur le budget de l'Etat (Exercice 2020, Chapitre 18, Article 102, Paragraphe 000)

Article 4 : Le présent arrêté sera enregistré et communiqué partout où besoin sera./-

MINISTÈRE DES FINANCES
DU BUDGET

28 JUIL. 2020

VISA
BUDGETAIRE

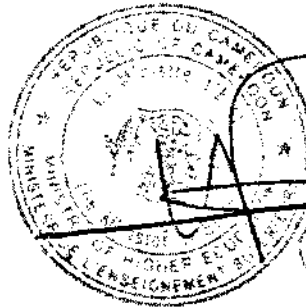
AMPLIATIONS

- SG/PM
- MINESUP/CAB/GS/IGA
- SG/MINESUP
- MINFOPRA
- MINFI
- RECTEUR/UN
- DDES/CEPE
- DAG/SIGIPES/SDPSP
- INTERESSE/DOSSIER
- CHRONO/ARCHIVES./-

Contrôle Financier Contrôl. auprès du
MINESUP

Yaoundé, le

25 AOÛT 2020



Jacques Fomonge



2019 / 163

DECISION N° _____ /UNR/VR-EPDTIC/SG/DAAC/DEPE/SSPE

Portant avancement d'échelon de Monsieur DESOBGO ZANGUE
Steve Carly, Chargé de Cours.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE,

- Vu la Constitution;
- Vu la loi n°005 du 16 avril 2001 portant orientation de l'enseignement supérieur;
- Vu la loi n°2007/006 du 26 décembre 2007, portant régime financier de l'Etat;
- Vu la loi n°2017/010 du 12 juillet 2017, portant statut général des établissements publics;
- Vu la loi n°2017/021 du 20 décembre 2017 portant loi de Finances de la République du Cameroun pour l'exercice 2018;
- Vu le décret n°77/41 du 03 février 1977, fixant les attributions et l'organisation des contrôles financiers;
- Vu le décret n°92/74 du 13 avril 1992 transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités;
- Vu le décret n°93/028 du 29 janvier 1993 portant Organisation Administrative et Académique de l'Université de Ngaoundéré;
- Vu le décret n°2000/048 du 15 mars 2000, modifiant et complétant certaines dispositions du décret 93/035 du 19 janvier 1993 portant statut spécial des personnels de l'enseignement supérieur;
- Vu le décret n°2000/049 du 15 mars 2000, fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n°2005/342 du 10 septembre 2005, modifiant et complétant certaines le décret n°93/027 du 19 janvier 1993 portant dispositions communes aux universitaires;
- Vu le décret n°2005/383 du 17 octobre 2005 fixant le régime financier applicable aux Universités;
- Vu le décret n°2008/099 du 07 mars 2008, revalorisant la rémunération mensuelle de base des personnels civils et militaires;
- Vu le décret n° 2017/318 du 27 juin 2017 portant nomination d'un "Vice-Chancellor" et des Recteurs dans certaines Universités d'Etat;
- Vu la décision n° 000721/MINFI du 11 novembre 2016 portant nomination du contrôleur financier à l'Université de Ngaoundéré;
- Vu la Circulaire n° 001/C/MINFI du 02 janvier 2018 portant Instructions relatives à l'Exécution des lois de Finances, au Suivi et au Contrôle de l'Exécution du Budget de l'Etat, des Etablissements Publics Administratifs, des Collectivités Territoriales Décentralisées et des autres Organismes Subventionnés, pour l'Exercice 2018;
- Vu la résolution n°05/CA/10/07/2018, entérinant les résolutions et recommandations de la 38ème session du Conseil d'Université de l'Université de Ngaoundéré;
- Vu la résolution n°06/CA/10/01/2018, portant approbation du budget de l'Université de Ngaoundéré, pour l'exercice 2018,

DECIDE :

Article 1^{er} : Monsieur DESOBGO ZANGUE Steve Carly (Mle 652117-M), Chargé de Cours de 2ème classe 3ème échelon (indice 870), depuis le 16 avril 2016, est à compter du 16 avril 2018, avancé(e) au grade de Chargé de Cours, 4ème échelon de la 2ème classe (indice 940).

Article 2 : L'intéressé (e) percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice 940 correspondant à son grade et pourra prétendre aux avantages accordées aux enseignants de même grade.

Article 3 : La présente décision sera enregistrée et communiquée partout où besoin sera./-

AMPLIATIONS

- MINESUP/MINFOPRA/MINFI
- CAB/R
- VREPTIC/VRRC/VRI
- SG/DAAC/DAAF
- Etablissements
- DEPE/SSPE
- CFS/AC
- Intéressé(e)
- Chrono

Ngaoundéré, le

06 MARS 2019



15 2017/210
DECISION N° 2017/210 /UN/R/VR/EPDTIC/SG/DAAC/DEPE/SSPE
Portant avancement d'échelon indiciaire de Monsieur DESOBGO ZANGUE Stève Carly,
Chargé de Cours à l'Institut Universitaire de Technologie de l'Université de Ngaoundéré.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE,

- Vu la Constitution;
- Vu La loi n° 99/016 du 22 décembre 1999, portant statut général des établissements publics et des entreprises du secteur public et parapublic;
- Vu la loi n° 005 du 16 avril 2005 portant orientation de l'enseignement supérieur;
- Vu la loi n° 2007/006 du 26 décembre 2007, portant régime financier de l'Etat ;
- Vu la loi n° 2016/018 du 14 décembre 2016 portant loi de finances de la République du Cameroun pour l'exercice 2017;
- Vu le décret n° 77/41 du 03 février 1977 fixant les attributions et l'organisation des contrôles financiers ;
- Vu le décret n° 92/74 du 13 avril 1992, transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités;
- Vu le décret n° 93/028 du 19 janvier 1993, portant Organisation administrative et académique de l'Université de Ngaoundéré ;
- Vu Le décret n° 2000/048 du 15 mars 2000 modifiant et complétant certaines dispositions du décret n° 93 /035 du 19 janvier 1993, portant Statut Spécial des Personnels de l'Enseignement Supérieur;
- Vu le décret n° 2000/049 du 15 mars 2000, fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n° 2003/250 du 16 septembre 2003 portant nomination du Recteur de l'Université de Ngaoundéré;
- Vu le décret n° 2005/342 du 10 septembre 2005 modifiant et complétant le décret n° 93/027 du 19 janvier 1993, portant dispositions communes aux Universités;
- Vu le décret n° 2005/383 du 17 octobre 2005, fixant le régime financier applicable aux universités ;
- Vu le décret n° 2008/099 du 07 mars 2008, revalorisant la rémunération mensuelle de base des personnels civils et militaires ;
- Vu le décret n° 2008/100 du 07 mars 2008, revalorisant le taux d'indemnité de non logement servie aux personnels civils et militaires ;
- Vu la décision n° 000721 MINFI du 11 novembre 2016, portant nomination du contrôleur financier à l'Université de Ngaoundéré ;
- Vu la circulaire n° 001/C/MINFI du 28 décembre 2016 Portant Instructions relatives à l'Exécution des lois de finances, au suivi et au Contrôle de l'Exécution du Budget de l'Etat, des Etablissements Publics Administratifs, des Collectivités Territoriales Décentralisées et des autres Organismes Subventionnés, pour l'Exercice 2017;
- Vu la résolution n° 05/CA/12/08/2016 du Conseil d'Administration de l'Université de Ngaoundéré, en sa session du 12 août 2016, entérinant les résolutions prises lors de la 35^{ème} session du conseil d'Université (bonifications et avancements d'échelon indiciaires des enseignants) ;
- Vu La résolution n° 11/CA/08/02/2017, portant approbation du budget de l'Université de Ngaoundéré, pour l'exercice 2017.

DÉCIDE:

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly, né le 02 janvier 1977 à Dschang, Matricule 652 117-M, Chargé de Cours 2^{ème} classe, 2^{ème} échelon (indice 785) depuis le 16 avril 2014, est à compter du 16 avril 2016, avancé au grade de Chargé de Cours, 2^{ème} classe, 3^{ème} échelon (indice 870).

Article 2 : L'intéressé percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice 870 correspondant à son grade et pourra prétendre aux avantages accordés aux Enseignants du même grade.

Imputation : Budget de l'Etat, Exercice 2017 Chap.18, Art.102, Para. 000.

Article 3 : La présente décision sera enregistrée et publiée partout où besoin sera.

19 JUIN 2017

Fait à Ngaoundéré, le
LE RECTEUR

AMPLIATIONS

- MINESUP ;
- MINFI/SOLDE/TRÉSOR ;
- MINFOPRA/DPE/SDPFSE/SAPE ;
- RECTEUR ;
- VR-EPDTIC ;
- SG/DAAP/DAAC/DEPE/SSPE ;
- CHEF D'ÉTABLISSEMENT ;
- CF/UN ;
- AC/UN ;
- INTERESSÉ ;
- ARCHIVES/CHRONO.



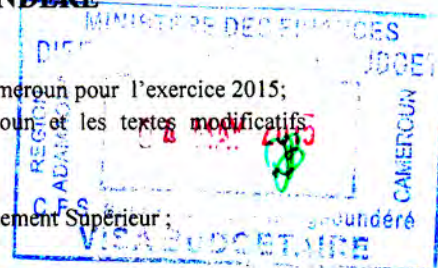
Amuam Zollo Paul Herve



2015/184

DECISION N°-----/UN/R/VR-EPDTIC/SG/DAAC/DEPE/SSPE
Portant avancement d'échelon indiciaire de Monsieur DESOBGO ZANGUE Stève Carly,
Chargé de Cours l'Institut Universitaire de Technologie de l'Université de Ngaoundéré.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE



- Vu la Constitution;
- Vu la loi n°2014/026 du 23 décembre 2014 portant loi de finances de la République du Cameroun pour l'exercice 2015;
- Vu l'ordonnance n°62/OF/4 du 07 février 1962 portant Régime Financier du Cameroun et les textes modificatifs subséquents ;
- Vu le décret n°2011/410 du 09 décembre 2011 portant formation du gouvernement ;
- Vu le décret n°2011/408 du 09 décembre 2011 portant Organisation du Ministère de l'Enseignement Supérieur ;
- Vu le décret n°93/026 du 19 janvier 1993 portant création d'Universités ;
- Vu le décret n° 93/027 du 19 janvier 1993 portant dispositions communes aux Universités, modifié et complété par le décret n° 2005/342 du 10 septembre 2005 ;
- Vu le décret n° 92/74 du 13 avril 1992 transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités;
- Vu le décret n°93/028 du 19 janvier 1993 portant Organisation administrative et académique de l'Université de Ngaoundéré ;
- Vu le décret n° 2005/383 du 17 octobre 2005 fixant le régime financier applicable aux universités ;
- Vu le décret n°2005/344 du 10 septembre 2005 portant nomination des Recteurs des Universités ;
- Vu le décret n°93 /035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n°2000/048 du 15 mars 2000 ;
- Vu le décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n°2008/099 du 07 mars 2008 revalorisant la rémunération mensuelle de base des personnels civils et militaires ;
- Vu le décret n°2008/100 du 07 mars 2008 revalorisant le taux d'indemnité de non logement servie aux personnels civils et militaires ;
- Vu la circulaire N° 00000683/C/MINFI du 31 décembre 2014 Portant Instructions relatives à l'Exécution des lois de finances, au suivi et au Contrôle de l'exécution du Budget de l'Etat, des Etablissements Publics Administratifs, des Collectivités Territoriales Décentralisées et des autres Organismes Subventionnés, pour l'Exercice 2015;
- Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré, en sa session du 06 mars 2015 ;
- Vu les prévisions budgétaires de l'exercice 2015.

DÉCIDE:

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly, Matricule 652 117-M, Chargé de Cours 2C/1E/715 depuis le 30/11/2012, est à compter du 16/04/2014, avancé au grade de Chargé de Cours 2C/2E/785.

Article 2 : L'Intéressé percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice 785 correspondant à son grade et pourra prétendre aux avantages accordés aux Enseignants du même grade.

Imputation : Budget de l'Etat, Exercice 2014 Chap.18, Art.102, Para. 000.

Article 3 : La présente décision sera enregistrée et publiée partout où besoin sera./-

Fait à Ngaoundéré, le 12 MAI 2015

LE RECTEUR

AMPLIATIONS

- MINESUP
- MINFI/SOLDE/TRÉSOR
- MINFOPRA/DPE/SDPFSE/SAPE
- VR-EPDTIC/ VR-CIE
- SG/ DAAF/ DAAC/DEPE/SSPE
- CHEF D'ÉTABLISSEMENT/CF
- INTERESSÉ



Stève Carly

SECRÉTARIAT GÉNÉRAL
DIRECTION DU DÉVELOPPEMENT DE
L'ENSEIGNEMENT SUPÉRIEUR
CELLULE DE L'ÉVALUATION ET DE LA
PROMOTION DE L'ENSEIGNANT
CHARGÉ D'ÉTUDES ASSISTANT N°1



ARRETE N° 14/0910 /MINESUP/SG/DDES/CEPE/CEA1

Portant promotion de Monsieur DESOBGO ZANGUE Stève Carly, au grade de Chargé de Cours.

LE MINISTRE DE L'ENSEIGNEMENT SUPERIEUR,



- Vu la Constitution;
- Vu la loi n°005 du 16 Avril 2001 portant orientation de l'Enseignement Supérieur;
- Vu la loi n° 2007/006 du 26 décembre 2007 portant régime financier de l'Etat;
- Vu la loi n° 2013/017 du 16 décembre 2013 portant loi des Finances de la République du Cameroun pour l'exercice 2014;
- Vu le décret n° 41/77 du 03 février 1977 fixant les attributions et organisant les Contrôles Financiers ;
- Vu le décret n°2005/383 du 17 octobre 2005 fixant les règles financières applicables aux Universités;
- Vu le décret n°93/035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n° 2000/048 du 15 mars 2000;
- Vu le décret n° 75/459 du 26 juin 1975 déterminant le régime de rémunération des personnels civils et militaires, modifié et complété par le décret n° 79/64 du 03 mars 1979;
- Vu le décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n° 2008/099 du 07 mars 2008 portant revalorisation de la rémunération mensuelle de base des personnels civils et militaires;
- Vu le décret n° 2008/100 du 07 mars 2008 portant revalorisation du taux de l'indemnité de non logement servie aux personnels civils et militaires;
- Vu le décret n° 2011/408 du 09 décembre 2011 portant organisation du Gouvernement;
- Vu le décret n° 2011/410 du 09 décembre 2011 portant formation du Gouvernement;
- Vu le décret n° 2012/433 du 01 Octobre 2012 portant Organisation du Ministère de l'Enseignement Supérieur;
- Vu l'arrêté n°253 du 31 octobre 1994 portant Organisation et Fonctionnement du Comité Consultatif des Institutions Universitaires;
- Vu la Circulaire N° 0001/C/MINFI du 06 janvier 2014 portant instructions relatives à l'exécution des lois de finances, au suivi et au contrôle de l'exécution du Budget de l'Etat, des établissements publics administratifs, des collectivités territoriales décentralisées et des autres Organismes subventionnés pour l'exercice 2014;
- Vu l'arrêté n° 12/0675/MINESUP/SP-CCIU du 17 décembre 2012 portant inscription sur la liste d'aptitude aux grades de Chargé de Cours, Maître de Conférences et Professeur dans les Institutions Universitaires (session du 27 au 30 novembre 2012);
- Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré en sa session du 19 mars 2013,

ARRETE :

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly (Mle 652117-M), né le 02 janvier 1977 à Douala, Assistant d'Université de 2ème classe 2ème échelon (indice 665) depuis le 14 septembre 2012 est, à compter du 30 novembre 2012, date retenue par le Conseil d'Administration de l'Université, promu (e) au grade de Chargé de Cours de 2ème classe, 1er échelon (indice 715) de l'Enseignement Supérieur.

Article 2 : L'intéressé (e) percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice de son grade de Chargé de Cours et pourra prétendre à tous les avantages accordés aux enseignants du même grade.

Article 3 : La dépense totale résultant des dispositions de l'article 1^{er} d-dessus, est imputée sur le budget de l'Etat (Exercice 2014, Chapitre 18, Article 102, Paragraphe 000)

Article 4 : Le présent arrêté sera enregistré et communiqué partout où besoin sera./-

Direction Générale du Budget

05 DEC 2014

AMPLIATIONS

- SG/PM
- MINESUP/CAB/IGS/IGA
- SG/MINESUP
- MINFI
- MINFOPRA
- Recteur/UN
- DDES/CEPE
- DAG/SIGIPES/SDPSP
- INTERESSE/DOSSIER
- CHRONO/ARCHIVES./-

Yaoundé, le



11 DEC 2014



Jacques Fame Ndonga



2013/232
DECISION N° /UN/R/VR/EPDTIC/SG/DAAC/DEPE/SSPE

Portant bonification d'échelon indiciaire de Monsieur DESOBGO ZANGUE Steve Carly, Assistant à l'Institut Universitaire de Technologie de l'Université de Ngaoundéré.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE

- Vu la Constitution;
- Vu la loi n°2012/014 du 21 décembre 2012 portant loi de finances de la République du Cameroun pour l'exercice 2013;
- Vu l'ordonnance n°62/OF/4 du 07 février 1962 portant Régime Financier du Cameroun et ses textes subséquents;
- Vu le décret n°2011/410 du 09 décembre 2011 portant formation du gouvernement;
- Vu le décret n°2011/408 du 09 décembre 2011 portant Organisation du Ministère de l'Enseignement Supérieur;
- Vu le décret n°93/026 du 19 janvier 1993 portant création d'Universités;
- Vu le décret n° 93/027 du 19 janvier 1993 portant dispositions communes aux Universités, modifié et complété par le décret n° 2005/342 du 10 septembre 2005;
- Vu le décret n° 92/74 du 13 avril 1992 transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités;
- Vu le décret n°93/028 du 19 janvier 1993 portant Organisation administrative et académique de l'Université de Ngaoundéré;
- Vu le décret n° 2005/383 du 17 octobre 2005 fixant le régime financier applicable aux universités;
- Vu le décret n°2005/344 du 10 septembre 2005 portant nomination des Recteurs des Universités;
- Vu le décret n°93 /035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n°2000/048 du 15 mars 2000;
- Vu le décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n°2008/099 du 07 mars 2008 revalorisant la rémunération mensuelle de base des personnels civils et militaires;
- Vu le décret n°2008/100 du 07 mars 2008 revalorisant le taux d'indemnité de non logement servie aux personnels civils et militaires;
- Vu la circulaire N°13/001/C/MINFI du 08 janvier 2013 portant Instructions relatives à l'Exécution, au suivi et au Contrôle de l'exécution du Budget de l'Etat, des Etablissements Publics Administratifs, des Collectivités Territoriales Décentralisées et des autres Organismes subventionnés pour l'Exercice 2013;
- Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré, en sa session du 19 mars 2013;
- Vu les prévisions budgétaires de l'exercice 2013.



DÉCIDE:

Article 1^{er} : Monsieur DESOBGO ZANGUE Steve Carly, Matricule 652 117-M, Assistant 3^E/605 depuis le 16/04/2012, est à compter du 14/09/2012, date de soutenance de sa Thèse, avancé au grade d'Assistant 2E/665.

Article 2 : L'Intéressé percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice 665 correspondant à son grade et pourra prétendre aux avantages accordés aux Enseignants du même grade.

Imputation : Budget de l'Etat, Exercice 2013 Chap.18, Art.102, Para. 000.

Article 3 : La présente décision sera enregistrée et publiée partout où besoin sera./-

Fait à Ngaoundéré, le

01 JUL 2013

AMPLIATIONS

- MINESUP
- MINFI/SOLDE/TRÉSOR
- MINFOPRA/DPE/SDPFSE/SAPE
- VR-EPDTIC/ VR-CIE
- SG/DAAC/DAAC/DEPE/SSPE
- CHEF D'ÉTABLISSEMENT/CF
- INTERRESSE



R. Amvam Zollo Paul Kono

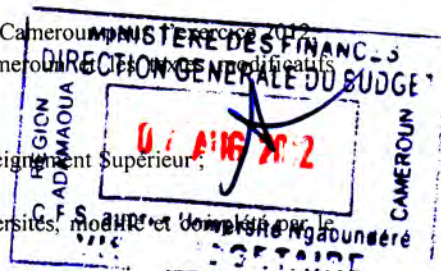


12012/426

DECISION N°----- /UN/R/VR-EPDTIC /SG/DAAC/DEPE/SSPE
Portant avancement d'échelon indiciaire de Monsieur DESOBGO ZANGUE Steve Carly,
Assistant à l'Institut Universitaire de Technologie de l'Université de Ngaoundéré.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE

- Vu la Constitution;
- Vu la Loi n°2011/020 du 14 décembre 2011 portant loi de finances de la République du Cameroun;
- Vu l'Ordonnance n°62/OF/4 du 07 février 1962 portant Régime Financier du Cameroun et ses modifications subséquents;
- Vu le Décret n°2011/410 du 09 décembre 2011 portant formation du gouvernement;
- Vu le Décret n°2011/408 du 09 décembre 2011 portant Organisation du Ministère de l'Enseignement Supérieur;
- Vu le Décret n°93/026 du 19 janvier 1993 portant création d'Universités;
- Vu le Décret n° 93/027 du 19 janvier 1993 portant dispositions communes aux Universités, modifié par le décret n° 2005/342 du 10 septembre 2005;
- Vu le Décret n° 92/74 du 13 avril 1992 transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités;
- Vu le Décret n°93/028 du 19 janvier 1993 portant Organisation administrative et académique de l'Université de Ngaoundéré;
- Vu le Décret n° 2005/383 du 17 octobre 2005 fixant le régime financier applicable aux universités;
- Vu le Décret n°2005/344 du 10 septembre 2005 portant nomination des Recteurs des Universités;
- Vu le Décret n°93 /035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n°2000/048 du 15 mars 2000;
- Vu le Décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le Décret n°2008/099 du 07 mars 2008 revalorisant la rémunération mensuelle de base des personnels civils et militaires;
- Vu le Décret n°2008/100 du 07 mars 2008 revalorisant le taux d'indemnité de non logement servie aux personnels civils et militaires;
- Vu la Circulaire N°0001/MINFI du 10 janvier 2012 portant Instructions relatives à l'Exécution, au suivi et au Contrôle de l'exécution du Budget de l'Etat, des Etablissements Publics Administratifs, des Collectivités Territoriales Décentralisées et des autres Organismes subventionnés pour l'Exercice 2012;
- Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré, en sa session du 03 février 2012;
- Vu les prévisions budgétaires de l'exercice 2012.



DÉCIDE:

Article 1^{er} : Monsieur **DESOBGO ZANGUE Steve Carly**, Matricule **652 117-M**, Assistant **2^E/530** depuis le 16/04/2010, est à compter du 16/04/2012, avancé au grade d'Assistant **3E/605**.

Article 2 : L'Intéressé percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice **605** correspondant à son grade et pourra prétendre aux avantages accordés aux Enseignants du même grade.

Imputation : Budget de l'Etat, Exercice 2012 Chap.18, Art.102, Para. 000.

Article 3 : La présente décision sera enregistrée et publiée partout où besoin sera. /-

Fait à Ngaoundéré, le

14 AOUT 2012

LE RECTEUR

AMPLIATIONS

- MINESUP
- MINFI/SOLDE/TRÉSOR
- MINFOPRA/DPE/SDPFSE/SAPE
- VR-EPDTIC/ VR-CIE
- SG/ DAAF/ DAAC/DEPE/SSPE
- CHEF D'ÉTABLISSEMENT/CF
- INTERESSE



Amvam Zollo Paul Henri



2010/687

DECISION N° _____ /UN/R/VE-EPDTIC/SC/DAAC/DEPE/SSPE
Portant avancement d'échelon indiciaire de Monsieur DESOBGO ZANGUE Stève Carly,
Assistant à l'Institut Universitaire de Technologie de l'Université de Ngaoundéré.

LE RECTEUR DE L'UNIVERSITE DE NGAOUNDERE

- Vu la Constitution ;
Vu la Loi n°2009/018 du 15 décembre 2009 portant loi de finances de la République du Cameroun pour l'exercice 2010 ;
Vu l'Ordonnance n°62/OF/4 du 07 février 1962 portant Régime Financier du Cameroun et les textes modificatifs subséquents ;
Vu le Décret n°2004/320 du 08 décembre 2004 portant organisation du gouvernement ;
Vu le Décret n°2004/322 du 08 décembre 2004 portant formation du gouvernement, modifié et complété par le décret n° 2007/269 du 07 septembre 2007 et le décret n° 2009/223 du 30 juin 2009 portant réaménagement du gouvernement ;
Vu le Décret n°2005/142 du 29 avril 2005 portant Organisation du Ministère de l'Enseignement Supérieur ;
Vu le Décret n°93/026 du 19 janvier 1993 portant création d'Universités ;
Vu le Décret n° 93/027 du 19 janvier 1993 portant dispositions communes aux Universités, modifié et complété par le décret n° 2005/342 du 10 septembre 2005 ;
Vu le Décret n° 92/74 du 13 avril 1992 transformant les Centres Universitaires de Buéa et Ngaoundéré en Universités ;
Vu le Décret n° 93/028 du 19 janvier 1993 portant Organisation administrative et académique de l'Université de Ngaoundéré ;
Vu le Décret n° 2005/383 du 17 octobre 2005 fixant le régime financier applicable aux universités ;
Vu le Décret n°2005/344 du 10 septembre 2005 portant nomination des Recteurs des Universités ;
Vu le Décret n°93 /035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n°2000/048 du 15 mars 2000 ;
Vu le Décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur ;
Vu le Décret n°2008/099 du 07 mars 2008 revalorisant la rémunération mensuelle de base des personnels civils et militaires ;
Vu le Décret n°2008/100 du 07 mars 2008 revalorisant le taux de l'indemnité de non logement servie aux personnels civils et militaires ;
Vu la Circulaire N°10/001/MINFI du 08 janvier 2010 portant Instructions relatives à l'Exécution et au Contrôle de l'exécution du Budget de l'Etat et des Organismes subventionnés pour l'Exercice 2010 ;
Vu les Résolutions du Conseil d'Administration de l'Université de Ngaoundéré, en sa session du 10 juillet 2010 ;
Vu les prévisions budgétaires de l'exercice 2010.

DÉCIDE :

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly, Matricule 652 117-M, Assistant 1^E/465 depuis le 16/04/2008 est à compter du 16/04/2010, avancé au grade d'Assistant 2^E/530.

Article 2 : L'Intéressé percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice 530 correspondant à son grade et pourra prétendre aux avantages accordés aux Enseignants du même grade.

Imputation : Budget de l'Etat, Exercice 2010 Chap.18, Art.102, Para. 000.

Article 3 : La présente décision sera enregistrée et publiée partout où besoin sera./-

Fait à Ngaoundéré, le

09 DEC. 2010

LE RECTEUR

AMPLIATIONS

- MINESUP
- MINFI/SOLDE/TRÉSOR
- MINFOPRA/DPE/SDPFSE/SAPE
- VR-EPDTIC/ VR-CIE
- SG/ DAAF/ DAAC/DEPE/SSPE
- DIRECTEUR- IUT/ CF
- INTÉRESSÉ.



Amvam Follo Paul Kenna

III.6) Actes de promotion aux grades de Chargé de Cours et Maître de Conférences

SECRÉTARIAT GÉNÉRAL
DIRECTION DU DÉVELOPPEMENT DE
L'ENSEIGNEMENT SUPÉRIEUR
CELLULE DE L'EVALUATION ET DE LA
PROMOTION DE L'ENSEIGNANT
CHARGÉ D'ETUDES ASSISTANT N°1



ARRETE N° 14/0910 /MINESUP/SG/DDES/CEPE/CEA1

Portant promotion de Monsieur DESOBGO ZANGUE Stève Carly, au grade de Chargé de Cours.

LE MINISTRE DE L'ENSEIGNEMENT SUPERIEUR,



- Vu la Constitution;
Vu la loi n°005 du 16 Avril 2001 portant orientation de l'Enseignement Supérieur;
Vu la loi n° 2007/006 du 26 décembre 2007 portant régime financier de l'Etat;
Vu la loi n° 2013/017 du 16 décembre 2013 portant loi des Finances de la République du Cameroun pour l'exercice 2014;
Vu le décret n° 41/77 du 03 février 1977 fixant les attributions et organisant les Contrôles Financiers ;
Vu le décret n°2005/383 du 17 octobre 2005 fixant les règles financières applicables aux Universités;
Vu le décret n°93/035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n° 2000/048 du 15 mars 2000;
Vu le décret n° 75/459 du 26 juin 1975 déterminant le régime de rémunération des personnels civils et militaires, modifié et complété par le décret n° 79/64 du 03 mars 1979;
Vu le décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
Vu le décret n° 2008/099 du 07 mars 2008 portant revalorisation de la rémunération mensuelle de base des personnels civils et militaires;
Vu le décret n° 2008/100 du 07 mars 2008 portant revalorisation du taux de l'indemnité de non logement servie aux personnels civils et militaires;
Vu le décret n° 2011/408 du 09 décembre 2011 portant organisation du Gouvernement;
Vu le décret n° 2011/410 du 09 décembre 2011 portant formation du Gouvernement;
Vu le décret n° 2012/433 du 01 Octobre 2012 portant Organisation du Ministère de l'Enseignement Supérieur;
Vu l'arrêté n°253 du 31 octobre 1994 portant Organisation et Fonctionnement du Comité Consultatif des Institutions Universitaires;
Vu la Circulaire N° 0001/C/MINFI du 06 janvier 2014 portant instructions relatives à l'exécution des lois de finances, au suivi et au contrôle de l'exécution du Budget de l'Etat, des établissements publics administratifs, des collectivités territoriales décentralisées et des autres Organismes subventionnés pour l'exercice 2014;
Vu l'arrêté n° 12/0675/MINESUP/SP-CCIU du 17 décembre 2012 portant inscription sur la liste d'aptitude aux grades de Chargé de Cours, Maître de Conférences et Professeur dans les Institutions Universitaires (session du 27 au 30 novembre 2012);
Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré en sa session du 19 mars 2013,

ARRETE :

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly (Mle 652117-M), né le 02 janvier 1977 à Douala, Assistant d'Université de 2ème classe 2ème échelon (indice 665) depuis le 14 septembre 2012 est, à compter du 30 novembre 2012, date retenue par le Conseil d'Administration de l'Université, promu (e) au grade de Chargé de Cours de 2ème classe, 1er échelon (indice 715) de l'Enseignement Supérieur.

Article 2 : L'intéressé (e) percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice de son grade de Chargé de Cours et pourra prétendre à tous les avantages accordés aux enseignants du même grade.

Article 3 : La dépense totale résultant des dispositions de l'article 1^{er} d-dessus, est imputée sur le budget de l'Etat (Exercice 2014, Chapitre 18, Article 102, Paragraphe 000)

Article 4 : Le présent arrêté sera enregistré et communiqué partout où besoin sera./-

Direction Générale du Budget

05 DEC 2014

AMPLIATIONS

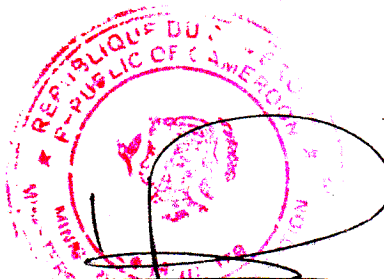
- SG/PM
- MINESUP/CAB/IGS/IGA
- SG/MINESUP
- MINFI
- MINFOPRA
- Recteur/UN
- DDES/CEPE
- DAG/SIGIPES/SDPSP
- INTERESSE/DOSSIER
- CHRONO/ARCHIVES./-

Direction Générale du Budget

VISA BUDGETAIRE

Yaoundé, le

11 DEC 2014



Jacques Fame Ndonga

REPUBLIQUE DU CAMEROUN

Paix - Travail - Patrie

MINISTÈRE DE L'ENSEIGNEMENT
SUPÉRIEUR

SECRÉTARIAT GÉNÉRAL

DIRECTION DU DÉVELOPPEMENT DE
L'ENSEIGNEMENT SUPÉRIEUR

MINISTÈRE DES FINANCES
DIRECTION GÉNÉRALE
DU BUDGET

REPUBLIQUE OF CAMEROON

Peace - Work - Fatherland

MINISTRY OF HIGHER EDUCATION

SECRETARIAT GENERAL

DEPARTMENT OF HIGHER EDUCATION
DEVELOPMENT

Contrôle Financier Central auprès de
MINESUP

ARRÊTÉ N° 20-0019/MINESUP/SG/DDES/CEPE/CFAZ

Portant promotion de Monsieur DESOBGO ZANGUE Stève Carly au grade de
Maître de Conférences.

LE MINISTRE D'ETAT, MINISTRE DE L'ENSEIGNEMENT SUPERIEUR,

- Vu la Constitution;
- Vu la loi n°005 du 16 Avril 2001 portant orientation de l'Enseignement Supérieur;
- Vu la loi n° 2018/012 du 11 Juillet 2018 portant régime financier de l'Etat;
- Vu la loi n° 2019/023 du 24 décembre 2019 portant loi des Finances de la République du Cameroun pour l'exercice 2020;
- Vu le décret n° 41/77 du 03 février 1977 fixant les attributions et organisant les Contrôles Financiers ;
- Vu le décret n°2005/383 du 17 octobre 2005 fixant les règles financières applicables aux Universités;
- Vu le décret n°93/035 du 19 janvier 1993 portant Statut Spécial des Personnels de l'Enseignement Supérieur, modifié et complété par le décret n° 2000/048 du 15 mars 2000;
- Vu le décret n° 75/459 du 26 juin 1975 déterminant le régime de rémunération des personnels civils et militaires, modifié et complété par le décret n° 79/64 du 03 mars 1979;
- Vu le décret n°2000/049 du 15 mars 2000 fixant l'échelonnement indiciaire du corps de l'Enseignement Supérieur;
- Vu le décret n° 2008/099 du 07 mars 2008 portant revalorisation de la rémunération mensuelle de base des personnels civils et militaires;
- Vu le décret n° 2008/100 du 07 mars 2008 portant revalorisation du taux de l'indemnité de non logement servie aux personnels civils et militaires;
- Vu le décret n° 2011/408 du 09 décembre 2011 portant organisation du Gouvernement, modifié et complété par le décret n° 2018/190 du 02 mars 2018;
- Vu le décret n° 2019/002 du 04 Janvier 2019 portant réaménagement du Gouvernement;
- Vu le décret n° 2012/433 du 01 Octobre 2012 portant Organisation du Ministère de l'Enseignement Supérieur;
- Vu l'arrêté n°253 du 31 octobre 1994 portant Organisation et Fonctionnement du Comité Consultatif des Institutions Universitaires;
- Vu la Circulaire N° 00008349/C/MINFI du 30 décembre 2019 portant instructions relatives à l'exécution des lois de finances, au suivi et au contrôle de l'exécution du Budget de l'Etat et des Autres Entités Publiques pour l'exercice 2020;
- Vu l'arrêté n°20-00019/MINESUP/SP-CCIU du 07 janvier 2020 portant inscription sur la liste d'aptitude aux grades de Chargé de Cours, Maître de Conférences et Professeur dans les Institutions universitaires (session du 28 au 30 novembre 2019, en régularisation).
- Vu les résolutions du Conseil d'Administration de l'Université de Ngaoundéré en sa session du 21 janvier 2020,

ARRETE :

Article 1^{er} : Monsieur DESOBGO ZANGUE Stève Carly (Mle 652117-M), né le 02 janvier 1977 à Douala, Chargé de Cours de 2ème classe 4ème échelon (indice 940) depuis le 16 avril 2018 est, à compter du 30 novembre 2019, date retenue par le Conseil d'Administration de l'Université, promu (e) au grade de Maître de Conférences de 2ème classe, 4ème échelon (indice 1005) de l'Enseignement Supérieur.

Article 2 : L'intéressé (e) percevra à ce titre une rémunération mensuelle calculée sur la base de l'indice de son grade de Maître de Conférences et pourra prétendre à tous les avantages accordés aux enseignants du même grade.

Article 3 : La dépense totale résultant des dispositions de l'article 1er ci-dessus, est imputée sur le budget de l'Etat (Exercice 2020, Chapitre 18, Article 102, Paragraphe 000)

Article 4 : Le présent arrêté sera enregistré et communiqué partout où besoin sera./-

MINISTÈRE DES FINANCES
DU BUDGET

28 JUIL. 2020

VISA
BUDGETAIRE

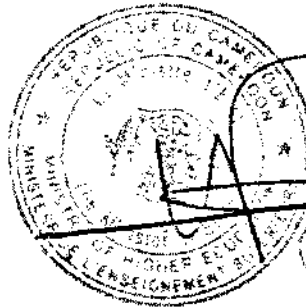
AMPLIATIONS

- SG/PM
- MINESUP/CAB/GS/IGA
- SG/MINESUP
- MINFOPRA
- MINFI
- RECTEUR/UN
- DDES/CEPE
- DAG/SIGIPES/SDPSP
- INTERESSE/DOSSIER
- CHRONO/ARCHIVES./-

Contrôle Financier Contrôl. auprès du
MINESUP

Yaoundé, le

25 AOÛT 2020



Jacques Fomengue

III.7) Listes d'aptitudes aux grades de Chargé de Cours et Maître de Conférences

EN 2/0675
ARRETE N° /MINESUP/SP-CCIU DU 07 DEC 2012
portant inscription sur la liste d'aptitude aux grades de Chargé de Cours, Maître de
Conférences et Professeur dans les Institutions Universitaires (session du 27 au 30 novembre
2012).

- Vu la Constitution ;
- Vu la loi n°005 du 16 avril 2001 portant orientation de l'Enseignement Supérieur ;
- Vu le décret n°2011/408 du 09 décembre 2011 portant organisation du Gouvernement ;
- Vu le décret n° 2011/410 du 09 décembre 2011 portant nomination des membres du
Gouvernement ;
- Vu le décret n°2012/433 du 1^{er} octobre 2012 portant organisation du Ministère de
l'Enseignement Supérieur ;
- Vu le décret n°93/026 du 19 janvier 1993 portant création d'Universités ;
- Vu le décret n°93/035 du 19 janvier 1993 portant dispositions communes aux
Universités, modifié et complété par le décret n°2005/342 du 10 septembre 2005 ;
- Vu le décret n°93/035 du 19 janvier 1993 portant Statut Spécial des personnels de
l'Enseignement Supérieur, modifié et complété par le décret n°2000/048 du 15 mars
2000 ;
- Vu l'arrêté n°253 du 31 octobre 1994 portant organisation et fonctionnement du Comité
Consultatif des Institutions Universitaires ;
- Vu les arrêtés fixant les critères de recrutement et de promotion dans divers grades de
l'Enseignement Supérieur répartis dans le cadre des Commissions Scientifiques
Spécialisées ;
- Vu les délibérations du Comité Consultatif des Institutions Universitaires en sa session
des 27, 28, 29 novembre et 03 décembre 2012,

ARRETE :

Article 1^{er} : Sont, pour compter des dates arrêtées par le Comité Consultatif des Institutions
Universitaires (CCIU), inscrits sur la liste d'aptitude au grade de **Chargé de Cours**, par ordre
alphabétique et par spécialité, les Assistants dont les noms suivent :

I- COMMISSION SCIENTIFIQUE SPECIALISEE DE DROIT, SCIENCES ECONOMIQUES ET SCIENCES POLITIQUES

A- Droit Privé et Sciences Criminelles

- | | |
|--------------------------------|--------------------------|
| 1. KAMWE MOUAFFO Marie-Colette | Université de Ngaoundéré |
| 2. NGO MBEM Stéphanie Rhodes | Université de Douala |

B- Droit Public

- | | |
|-----------------------|-------------------------|
| 1. MOUBITANG Emmanuel | Université de Yaoundé 2 |
|-----------------------|-------------------------|

2. BETI ETOA Christophe

Université de Yaoundé 2

C- Etudes Internationales

1. ELONO ESSONO Armand

Université de Yaoundé 2

D- Sciences Economiques

1. EPO Boniface NGAH

Université de Yaoundé 2

2. ESSAMA Pantaléon

Université de Yaoundé 2

3. MBANG Marthe Olga, épouse WERIWOH

Université de Yaoundé 2

4. MINKOUA NZHIE Jules René

Université de Yaoundé 2

5. MOHAMMADOU NOUROU

Université de Ngaoundéré

6. SAIDOU BABA OUMAR

Université de Buéa

7. TCHIEUZING AWOUTCHA Romuald

Université de Douala

8. ZOGO EKASSI Alphonse Marie Richard

Université de Yaoundé 2

E- Sciences et Techniques de Gestion

1. AYOU BENE Marius

Université de Douala

2. BIKOAH Robert

Université de Yaoundé 2

3. DJOUM KOUOMOU Serge

Université de Yaoundé 2

4. GOUANLONG KAMGANG Nadège Ingrid

Université de Ngaoundéré

5. NDANGWA, né le 10 mars 1968

Université de Ngaoundéré

6. ONOMO Michel Bertrand Cyrille

Université de Douala

7. SONE MBASSI Alain Noël

Université de Yaoundé 2 

II- COMMISSION SCIENTIFIQUE SPECIALISEE DE LETTRES ET SCIENCES HUMAINES

A- Sociologie et Anthropologie

1. ABOUNA Paul

Université de Yaoundé 1

2. ELLA ELLA Samuel Beni

Université de Yaoundé 1

3. FALNA TAUBIC

Université de Ngaoundéré

4. LEKA ESSOMBA Dieudonné Armand

Université de Yaoundé 1

B- Psychologie

1. GALY MOHAMADOU, né vers 1967

Université de Maroua

C- Littérature Négro-Africaine

1. AMOUGOU NDI Dagobert Stéphane

Université de Yaoundé 1

2. FOTIO JOUSSE Noël Ledoux

Université de Dschang

3. MAKANI François Guillaume

Université de Yaoundé 1

4. MOKWE Edouard

Université de Buéa

5. NGOH TOH Kelvin

Université de Buéa 

D- Langue et Littérature Françaises

- | | |
|---|-------------------------|
| 1. ABOUGA Yvette Marie Edmé | Université de Yaoundé 1 |
| 2. AMBASSA Marie Thérèse, épouse BETOKO | Université de Yaoundé 1 |
| 3. ATENKE ETOA Sosthène Marie Xavier | Université de Maroua |
| 4. AWOUNDJA Catherine Marie Ida, épouse NSATA | Université de Yaoundé 1 |
| 5. BONONO Chantal | Université de Yaoundé 1 |
| 6. EKORONG à MOUGNOL Alain Fleury | Université de Douala |
| 7. EVOUNG FOUDA Jean Bernard | Université de Yaoundé 1 |
| 8. NJOH KOME Ferdinand | Université de Douala |
| 9. SOL Mari Désiré | Université de Yaoundé 1 |

E- Linguistique et Phonétique

- | | |
|-------------------------|-------------------------|
| 1. BALGA Jean Paul | Université de Maroua |
| 2. FOSSI Pierre Achille | Université de Yaoundé 1 |
| 3. MAIRAMA Rosalie | Université de Maroua |

F- Langue et Littérature Anglaises et Nord-Américaines

- | | |
|-----------------------------|-------------------------|
| 1. EPOGE Napoléon KANG | Université de Yaoundé 1 |
| 2. NKWENTIAMA Carlous MULUH | Université de Maroua |

G- Sciences et Techniques de l'Information et de la Communication

- | | |
|--------------------------|-------------------------|
| 1. BILLE Olivier Anicet | Université de Yaoundé 2 |
| 2. ESSONO Thomas | Université de Yaoundé 2 |
| 3. NGUEGAN Jean François | Université de Yaoundé 2 |

H- Sciences Historiques et Archéologiques

- | | |
|----------------------|----------------------|
| 1. ADAM MAHAMAT | Université de Maroua |
| 2. BOUBA HAMAN | Université de Maroua |
| 3. NFI Joseph LON | Université de Buéa |
| 4. WASSOUNI François | Université de Maroua |

I- Géographie

- | | |
|-----------------------|-------------------------|
| 1. NGANA WARA Didier | Université de Yaoundé 2 |
| 2. WATANG ZIEBA Félix | Université de Maroua |

III- COMMISSION SCIENTIFIQUE SPECIALISEE DES MATHEMATIQUES

A- Mathématiques

- | | |
|--------------------------------|--------------------------|
| 1. AKONGNWI Clement MFORMBELE | Université de Bamenda |
| 2. DONGHO Joseph | Université de Maroua |
| 3. KIANPI Maurice | Université de Yaoundé 1 |
| 4. MBANG Joseph | Université de Yaoundé 1 |
| 5. TCHOUGONG NGONGANG Rodrigue | Université de Ngaoundéré |
| 6. TCHOUNDJA Edgar Landry | Université de Yaoundé 1 |
| 7. TSANOU Berge | Université de Dschang |

B- Mathématiques appliquées

- | | |
|-------------------|---|
| 1. TSOPZE Norbert | Université de Yaoundé 1  |
|-------------------|---|

IV- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES PHYSIQUES

A- Physique Nucléaire et Corpusculaire et Théories Physiques

- | | |
|---------------------------|-------------------------|
| 1. NANA Bonaventure | Université de Bamenda |
| 2. TABI Conrad Bertrand | Université de Yaoundé 1 |
| 3. TCHANGNWA NYA Fridolin | Université de Maroua |

B- Physique Atomique et Moléculaire et Physique du Solide

- | | |
|---------------------------------------|-------------------------|
| 1. HONA Jacques | Université de Yaoundé 1 |
| 2. NGUETCHO TCHAKOUTIO Aurélien Serge | Université de Maroua |
| 3. NKAM TCHOUOBIAP Serge | Université de Buéa |

C- Chimie Générale

- | | |
|--------------------------|-------------------------|
| 1. EBELLE Thierry | Université de Douala |
| 2. EMADAK Alphonse | Université de Yaoundé 1 |
| 3. MBIANGUE Yves Alain | Université de Maroua |
| 4. TCHEUMI Hervé Leclerc | Université de Maroua |

D- Chimie Minérale, Organique et Analytique

- | | |
|-------------------------------------|--|
| 1. NGONO BIKOBO Dominique | Université de Yaoundé 1 |
| 2. TABOPDA KUIATE Turibio | Université de Yaoundé 1 |
| 3. TATSIMO NDENDOUNG Simplicie Joël | Université de Maroua  |

V- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES DE LA TERRE ET DE LA VIE

A- Sciences de la Terre

- | | |
|---------------------------------------|-----------------------|
| 1. BASSEKA Charles Antoine | Université de Dschang |
| 2. BAYIGA Elie Constantin | Université de Douala |
| 3. MBOUDOU Germain Marie Monespérance | Université de Buéa |
| 4. TETSOPGANG Samuel | Université de Bamenda |

B- Biochimie

- | | |
|-------------------------------|-----------------------|
| 1. APINJOH Tobias OBEJUM | Université de Buéa |
| 2. SOFEU FEUGAING David Denis | Université de Buéa |
| 3. SIMO Gustave | Université de Dschang |

C- Biologie

- | | |
|--------------------------|-------------------------|
| 1. ALENE Désirée Chantal | Université de Yaoundé 1 |
| 2. KOSMA Philippe | Université de Maroua |
| 3. FOKAM Zephyrin | Université de Bamenda |

D- Physiologie

- | | |
|---------------------|-------------------------|
| 1. MEZUI Christophe | Université de Yaoundé 1 |
|---------------------|-------------------------|

VI- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES DE L'INGENIEUR

A- Electronique, Electrotechnique et Automatique

- | | |
|------------------------------|-------------------------|
| 1. KOM Guillaume Honoré | Université de Dschang |
| 2. LELE Chrislin Martial | Université de Yaoundé 1 |
| 3. Michael EKONDE SONE AJANG | Université de Buéa |

B - Génie Civil

- | | |
|--|--------------------------|
| 1. SIRI Lawrencia KONJE, épouse NUKENINE | Université de Ngaoundéré |
|--|--------------------------|

C - Chimie Appliquée et Génie Chimique

- | | |
|--------------------------------------|--------------------------|
| 1. NGUEMTCHOUIN MBOUGA Marie Coletti | Université de Ngaoundéré |
|--------------------------------------|--------------------------|

D - Biologie et Biochimie Appliquées

- | | |
|-------------------------------|--------------------------|
| 1. DESOBGO ZANGUE Stève Carly | Université de Ngaoundéré |
| 2. GOUDOUM Augustin | Université de Maroua |

E- Technologie et Génie Forestier

- | | |
|-------------------------------|----------------------|
| 1. TCHATCHOUA Dorothy TCHAPDA | Université de Maroua |
|-------------------------------|----------------------|

**VII- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES PHARMACEUTIQUES
ET MEDICALES**

A. Microbiologie, Immunologie, Hématologie, Hygiène et Hydrologie

- | | |
|----------------------------|-------------------------|
| 1. DISSONGO Jean II | Université de Douala |
| 2. KOUOTOU Emmanuel Armand | Université de Yaoundé I |
| 3. NGABA Guy Pascal | Université de Douala |
| 4. OWONA MANGA Léon Jules | Université de Douala |

B. Biophysique, Biochimie, Mathématiques, Statistiques et informatique Médicale

- | | |
|--------------------------|--------------------------|
| 1. NEOSSE GUENA Mathurin | Université de Ngaoundéré |
|--------------------------|--------------------------|

C. Disciplines Mixtes (Anatomie-pathologie, Hématologie et maladies du sang, Cancérologie)

- | | |
|-----------------------------------|-------------------------|
| 1. CHOUKEM Siméon Pierre | Université de Bouéa |
| 2. ESSOLA Basile | Université de Douala |
| 3. ESSOMBA MANY Achille | Université de Douala |
| 4. HANDY EONE Daniel | Université de Yaoundé I |
| 5. LEMOGOUM Daniel | Université de Douala |
| 6. MBATCHOU NGAHANE Bertrand Hugo | Université de Douala |
| 7. MOBY MPAH Edouard Hervé | Université de Douala |
| 8. NGATCHOU DJOMO William | Université de Douala |
| 9. PALLE John NGUNDE | Université de Bouéa |
| 10. WELEDJI Elroy Patrick HENSHEW | Université de Bouéa |

D. Sciences Cliniques

- | | |
|-----------------------------|-------------------------|
| 1. MAPOURE NJANKOUO YACOUBA | Université de Yaoundé I |
| 2. MVE KOH Valère Salomon | Université de Douala |
| 3. NGUEFACK Félicitée | Université de Yaoundé I |

Article 2 : Sont, pour compter des dates arrêtées par le Comité Consultatif des Institutions Universitaires (CCIU), inscrits sur la liste d'aptitude au grade de **Maître de Conférences**, par ordre alphabétique et par spécialité, les Chargés de Cours dont les noms suivent :

I- COMMISSION SCIENTIFIQUE SPECIALISEE DE DROIT, SCIENCES ECONOMIQUES
ET SCIENCES POLITIQUES

A- Droit Privé et Sciences Criminelles

- | | |
|---------------------------|-------------------------|
| 1. DIFFO TCHUNKAM Justine | Université de Yaoundé 2 |
| 2. TABE TABE Simon | Université de Dschang |

B- Science Politique

- | | |
|---------------------------|-------------------------|
| 1. GABSA NYONGBET Wilfred | Université de Yaoundé 2 |
|---------------------------|-------------------------|

C- Etudes Internationales

- | | |
|--|-------------------------|
| 1. NYAMNDING MESSANGA Charlemagne Pascal | Université de Yaoundé 2 |
|--|-------------------------|

D- Sciences Economiques

- | | |
|-----------------------|-------------------------|
| 1. NGO NONGA Fidoline | Université de Yaoundé 2 |
| 2. NJOM MOM Aloysius | Université de Bamenda |

E- Sciences et Techniques de Gestion

- | | |
|----------------------|-------------------------|
| 1. KONO ABE Jean Max | Université de Yaoundé 2 |
|----------------------|-------------------------|

II- COMMISSION SCIENTIFIQUE SPECIALISEE DE LETTRES ET SCIENCES HUMAINES

A- Psychologie

- | | |
|-------------------------|-------------------------|
| 1. EBALE MONEZE Chandel | Université de Yaoundé 1 |
| 2. MAYI Marc Bruno | Université de Yaoundé 1 |

B- Philosophie

- | | |
|-------------------|-------------------------|
| 1. KENMOGNE Emile | Université de Yaoundé 1 |
|-------------------|-------------------------|

C- Littérature Négro-Africaine

- | | |
|------------------------------------|----------------------|
| 1. AMABIAMINA W'AMAYINA Alda Flora | Université de Maroua |
|------------------------------------|----------------------|

D- Langue et Littérature Françaises

- | | |
|---------------------------------------|-------------------------|
| 1. BISSA ENAMA Marie Thérèse Patricia | Université de Yaoundé 1 |
| 2. NJIKE Emmanuel | Université de Bamenda |

E- Linguistique et Phonétique

- | | |
|--|-------------------------|
| 1. ASSOUMOU Jules | Université de Douala |
| 2. ATINDOGBE Gratien Gualbert | Université de Buéa |
| 3. BOUM Marie Anne, épouse NDONGO SEMENGUE | Université de Yaoundé 1 |

- | | |
|---|--------------------------|
| 4. NSEME Clédor | Université de Yaoundé 1 |
| 5. TATOU TSOBOZE Léonie Gisèle, épouse METANGMO | Université de Ngaoundéré |

F- Langue et Littérature Anglaises et Nord-Américaines

- | | |
|-------------------------|-------------------------|
| 1. TEKE Charles NGIEWIH | Université de Yaoundé 1 |
|-------------------------|-------------------------|

G- Sciences Historiques et Archéologiques

- | | |
|----------------|-------------------------|
| 1. KOUFAN Jean | Université de Yaoundé 1 |
|----------------|-------------------------|

H- Géographie

- | | |
|------------------------|---|
| 1. BENINGUISSE Gervais | Université de Yaoundé 2 |
| 2. MOUGOUE Benoît | Université de Yaoundé 1 |
| 3. TCHINDJANG Mesmin | Université de Yaoundé 1  |

III- COMMISSION SCIENTIFIQUE SPECIALISEE DES MATHEMATIQUES

A- Mathématiques appliquées

- | | |
|------------------|--|
| 1. NDOUNDAM René | Université de Yaoundé 1  |
|------------------|--|

IV- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES PHYSIQUES

A- Physique Nucléaire et Corpusculaire et Théories physiques

- | | |
|----------------------------|--------------------------|
| 1. KAMTA Martin | Université de Ngaoundéré |
| 2. KENFACK JIOTSA Aurélien | Université de Yaoundé 1 |
| 3. ZEKENG Serge Sylvain | Université de Yaoundé 1 |

B- Astronomie, physique Spatiale et Géophysique

- | | |
|-----------------------------|-------------------------|
| 1. NDOUGSA MBARGA Théophile | Université de Yaoundé 1 |
|-----------------------------|-------------------------|

C- Chimie Générale

- | | |
|----------------|---|
| 1. NGOUNE Jean | Université de Dschang  |
|----------------|---|

V- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES DE LA TERRE ET DE LA VIE

A- Sciences de la Terre

- | | |
|--------------------------------------|----------------------|
| 1. ETAME Jacques | Université de Douala |
| 2. NIDA Marie Joseph, épouse NTAMACK | Université de Douala |

B- Biologie

1. DIBONG Siegfried Didier
2. Lucy MANDE AYAMBA, épouse NDIP
3. NJAN NLOGA Alexandre Michel
4. NOUBISSIE TCHILAGAM Jean Baptiste
5. NWAGA Dieudonné

Université de Douala
Université de Buéa
Université de Ngaoundéré
Université de Ngaoundéré
Université de Yaoundé 1 

VI- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES DE L'INGENIEUR

A- Energétique et Thermodynamique

1. KEMAJOU Alexis

Université de Douala

B- Génie Civil

1. FOKWA Didier
2. NGAPGUE François

Université de Douala
Université de Dschang

C- Zootechnie

1. Julius AWAH NDUKUM

Université de Dschang 

VII- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES PHARMACEUTIQUES ET MEDICALES

A- Pharmacognosie et Pharmacotechnie

1. ASONGALEM Emmanuel ACHA
2. MPONDO MPONDO Emmanuel

Université de Buéa
Université de Douala

B- Microbiologie, Immunologie, Hématologie, Hygiène et Hydrologie

1. OKOMO ASSOUMOU Marie Claire

Université de Yaoundé 1

C- Disciplines Mixtes (Anatomie-Pathologie, Hématologie et Maladies du Sang, Cancérologie)

1. EYENGA Victor Claude

Université de Yaoundé 1

D- Sciences Cliniques

1. ONGOLO ONGOLO Pierre, (agréé CAMES, pour compter du 15 novembre 2012)

Université de Yaoundé 1

Article 3 : Sont, pour compter des dates arrêtées par le Comité Consultatif des Institutions Universitaires (CCIU), inscrits sur la liste d'aptitude au grade de **Professeur**, par ordre alphabétique et par spécialité, les Maîtres de Conférences dont les noms suivent :

I- COMMISSION SCIENTIFIQUE SPECIALISEE DE DROIT, SCIENCES ECONOMIQUES
ET SCIENCES POLITIQUES

A- Droit Privé et Sciences Criminelles

1. MEVOUNGOU NSANA Roger Université de Yaoundé 2

B- Droit Public

2. DONFACK SOKENG Léopold Université de Douala

C- Science Politique

1. MOUCHE Ibrahim Université de Yaoundé 2
2. ONANA Janvier Université de Douala

D- Sciences Economiques

1. BAYE MENJO Francis Université de Yaoundé 2 

II- COMMISSION SCIENTIFIQUE SPECIALISEE DE LETTRES ET SCIENCES HUMAINES

A- Sciences de l'Education

1. MVESSO André Université de Buéa

B - Art et Esthétique

1. MINKO ABOLO Marthe, épouse ATANGANA Université de Dschang

C- Langue et Littérature Françaises

1. MBALA ZE Barnabé Université de Yaoundé 1

D- Linguistique et Phonétique

1. KOUEGA Jean Paul Université de Yaoundé 1

E- Géographie

1. ELONG Joseph Gabriel Université de Yaoundé 1

IV- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES PHYSIQUES

A- Chimie Minérale, Organique et Analytique

1. TAPONDJOU AZEFACK Léon Université de Dschang 

V- COMMISSION SCIENTIFIQUE SPECIALISEE DES SCIENCES
DE LA TERRE ET DE LA VIE

A- Biologie

1. NJIOKOU Flobert

Université de Yaoundé 1

**LE MINISTRE DE L'ENSEIGNEMENT SUPERIEUR,
PRESIDENT DU COMITE CONSULTATIF DES INSTITUTIONS UNIVERSITAIRES**




Jacques FAME NDONGO



Arrêté n° 20-00019 /MINESUP/SP-CCIU DU 07 JAN 2020

Portant inscription sur la liste d'aptitude aux grades de **Chargé de Cours, Maître de Conférences et Professeur** dans les Institutions universitaires (session du 28 au 30 novembre 2019, en régularisation).

**LE MINISTRE D'ETAT,
MINISTRE DE L'ENSEIGNEMENT SUPERIEUR,**

- Vu la Constitution ;
Vu la loi n°005 du 16 avril 2001 portant orientation de l'Enseignement Supérieur ;
Vu le décret n°2011/408 du 09 décembre 2011 portant organisation du Gouvernement ;
Vu le décret n° 2019/002 du 04 janvier 2019 portant réaménagement du Gouvernement ;
Vu le décret n°2012/433 du 1er octobre 2012 portant organisation du Ministère de l'Enseignement Supérieur ;
Vu le décret n°93/026 du 19 janvier 1993 portant création d'Universités ;
Vu le décret n°93/027 du 19 janvier 1993 portant disposition communes aux Universités ;
Vu le décret n°93/035 du 19 janvier 1993 portant Statut Spécial des personnels de l'Enseignement Supérieur, modifié et complété par le décret n°2000/048 du 15 mars 2000 ;
Vu l'arrêté n°253 du 31 octobre 1994 portant organisation et fonctionnement du Comité Consultatif des Institutions Universitaires ;
Vu les arrêtés du 16 novembre 2010 fixant les critères de recrutement et de promotion dans divers grades de l'enseignement supérieur répartis dans le cadre des Commissions Scientifiques Spécialisées ;
Vu les délibérations du Comité Consultatif des Institutions Universitaires en sa session des 18, 19 et 20 décembre 2019 ;

ARRETE:

Article 1er : Sont, pour compter des dates arrêtées par le Comité Consultatif des Institutions Universitaires (CCIU), inscrits sur la liste d'aptitude au grade de **Chargé de Cours**, par ordre alphabétique et par spécialité, les Assistants dont les noms suivent:

I - COMMISSION SCIENTIFIQUE SPECIALISÉE DE DROIT, SCIENCES ÉCONOMIQUES ET SCIENCES POLITIQUES

A - Droit privé et sciences criminelles

- | | |
|---------------------------------|--------------------------|
| 1. ADNA EBUDE ENANG | Université de Yaoundé II |
| 2. MOUAFO TAMBO BLAISE DESIRE | Université de Yaoundé II |
| 3. MOUTIL FIRMIN GHISLAIN | Université de Douala |
| 4. NAH ANTHONY TETINWE | Université de Dschang |
| 5. OBI EPSE EYONG MARTINA AGBOR | Université de Douala |
| 6. STANLEY TAMBE TAMBE EBOT | University of Buea |
| 7. TCHABET KAMBO PATRICK ALDO | Université de Yaoundé II |
| 8. THOMAS OJONG | University of Buea |
| 9. TSOPBEING MARCEL WILLIAMS | Université de Yaoundé II |
| 10. YANI BENEDICTE | Université de Maroua |

B - Droit public

- | | |
|---------------------------|-----------------------|
| 1. DJUKWO NGUETEU DOROTHY | Université de Douala |
| 2. WANDJI KEMAJOU AXEL | Université de Dschang |

C - Science politique

1. ATANGANA EMMANUEL ALAIN
2. KIPOH BRIGITTE EPSE ABEL ZE
3. KOUNA BENGALA SERGE AUGUSTIN

Université de Yaoundé II
Université de Douala
Université de Douala

D - Sciences économiques

1. ABDOUL KARIM
2. ELOMO ZOGO THERESE
3. FOUDA FOE EUGENIE
4. GACHILI NDI GBAMBIE LADIFATOU EPSE POUMEYOU
5. IBRAHIM, né le 28 octobre 1986 à MAROUA
6. KOS A MOUGNOL ALICE
7. MVOGO GREGORY PAULIN
8. NKOUMOU NGOA GASTON BRICE
9. NYA PATRICK DANIEL
10. SONG JACQUES SIMON

Université de Maroua
Université de Yaoundé II
Université de Douala
Université de Yaoundé II
Université de Maroua
Université de Dschang
Université de Douala
Université de Dschang
Université de Douala
Université de Dschang

E - Sciences et Techniques de Gestion

1. ARABO YAYA
2. ATANGANA GUY CHRISTIAN BASILE
3. AYANKENG GODLOVE NKEMKIAFU
4. EWODO MEKA ROLAND
5. HIKOUATCHA KENFACK PRINCE DUBOIS
6. MBALLA MVONDO SUZANNE ANGELE EPSE ZAMA
7. MBARGA AXEL DIEUDONNE
8. MBASSI JEAN CLAUDE
9. MEDANG MEDANG JEAN YVES
10. MONDA LEA LARISSA
11. NEGOU ERNEST
12. ONOMO ONOMO MODESTE GHISLAIN
13. OUMAROU TADO
14. SAIDOU VICTOR
15. TAGNE AURELIEN BERTAND
16. TCHATCHOUA THIERRY
17. TCHINGNABE DANIEL
18. TEMGOUA EMILE

Université de Maroua
Université de Douala
Université de Yaoundé II
Institut Supérieur de technologie
appliquée et de Gestion
Université de Dschang
Université de Douala
Université de Douala
Université de Douala
Université de Yaoundé II
Université de Maroua
University of Buea
Institut National de la Jeunesse et
des Sports
Institut National de la Jeunesse et
des Sports
Institut National de la Jeunesse et
des Sports
Université de Douala
Université de Maroua
Université de Maroua
Université de Douala

II - COMMISSION SCIENTIFIQUE SPECIALISÉE DES LETTRES ET SCIENCES HUMAINES

A - Sociologie et Anthropologie

1. DAMAIGUE DANIEL
2. EFUET SIMON AKEM
3. HAROUNA, né le 04 mai 1979 à OUDDA
4. MBIDA NANA FRANK MICHAEL

Université de Douala
University of Buea
Université de Douala
Institut National de la Jeunesse et
des Sports

B - Sciences de l'Education

1. ETTA MERCY AKI
2. NJIOMO JOSEPH

University of Buea
Université de Maroua

C - Psychologie

1. DONG THIERRY
2. DZUETSO MOUAFO ACHILLE VICKY

Université de Dschang
Université de Maroua

D - Philosophie

1. DEUGA TCHEUGOU WILLIAM

Université de Maroua

E - Art et Esthétique

1. AFANE BELINGA RUTH COLETTE
2. ANELE OBIE MICHAEL
3. TIMMA OLIVIER

Université de Dschang
University of Bamenda
Université de Dschang

F - Littérature négro-africaine

1. JANET NGUNCHEKE NDULA
2. NGEH ERNESTILIA DZEKEM

Université de Yaoundé I
University of Bamenda

G - Langue et littérature françaises

1. KAMSU SOUOPTETCHA AMOS
2. SE NGUE DANIEL
3. TAKAM OMER

Université de Maroua
Université de Maroua
University of Buea

H - Linguistique et Phonétique

1. AMOUGOU MARTIAL PATRICE
2. ESSENGUE PIERRE
3. NAMA DIDIER
4. OUSMANOU, né le 20 juillet 1978 à YAGOUA
5. TABAH EMMANUEL NDZI

Institut National de la Jeunesse et
des Sports
University of Buea
Université de Maroua
Université de Yaoundé I
University of Buea

I - Langue et Littérature anglaises et Nord-américaines

1. AMABO OLIVIA BI-SOH
2. DAGASSO ETIENNE
3. MARY LOUISA LUM

Université de Dschang
Université de Maroua
Université de Douala

J - Langue et Littérature germaniques

1. LABA MAURICE

Université de Douala

K - Langue et Littérature espagnoles

1. FONE THOMAS
2. SIME SIME HORTENCE

Université de Douala
Université de Dschang

L - Sciences et Techniques de la Communication et de l'Information

1. CHAMOGNE KAMOLE CHANTAL EPSE MOUKOKO

Université de Douala

M - Sciences historiques et archéologiques

1. ETOA NDEDE ARLETTE
2. HABIT BIENVENU DIEUNEDORT
3. MEDJO PROTAIS PAMPHILE PATRICE
4. NTEDE EDONGO JEAN PHILIPPE
5. YANO YANO JEAN PIERRE

Université de Douala
Institut National de la Jeunesse et
des Sports
Université de Dschang
Université de Douala
Institut National de la Jeunesse et
des Sports

N - Géographie, Etudes démographiques

1. AGBORTOKO MANYIGBE AYUK NKEM
2. LEKANE TSOBGOU DIEUDONNE
3. NSEGBE ANTOINE DE PADOUE

University of Buea
Université de Dschang
Université de Dschang

III - COMMISSION SCIENTIFIQUE SPECIALISÉE DES MATHÉMATIQUES

A - Mathématiques appliquées

1. DANGBE EZEKIEL
2. DJORWE TEMOA
3. EBELE SERGE ALAIN
4. GUIEM RICHARD
5. MOULLA DONATIEN KOULLA

Université de Ngaoundéré
Université de Maroua
Université de Yaoundé I
Université de Maroua
Université de Maroua

6. NFOR VIVIAN NDFUTU
7. TIOGNING KUETI LAURAINÉ
8. WOUNDJIAGUE APOLLINAIRE

University of Bamenda
 Université de Yaoundé I
 Université de Maroua

IV - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES PHYSIQUES

A - Astronomie, Physique spatiale et Géophysique

1. MONO JEAN AIME

Université de Douala

B - Chimie générale

1. BECKLEY VICTORINE NAMONDO
2. DEUTCHOUA DJITILEU ARLETTE DANELLE

University of Buea
 Université de Douala

C - Chimie minérale, organique et analytique

1. NOUDOU SOLITAIRE BODRIX
2. TADJONG TCHO ALAIN

University of Bamenda
 University of Buea

V - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES DE LA TERRE ET DE LA VIE

A - Sciences de la Terre

1. BINELI AMBOMO ETIENNE
2. BOUBA LUCAS
3. EBONJI SETH CELESTIN RODRIGUE
4. ENGOME REGINA WORANY EPSE NGOMBA
5. FOWE KWETCHE PAUL GUSTAVE
6. LEWA SARA
7. MABEL NECHIA WANTIM
8. MAMA ANSELME CREPIN
9. NGO ELOGAN NTEM JEANNETTE

Université de Maroua
 Université de Maroua
 Université de Douala
 University of Buea
 Université de Douala
 Université de Maroua
 University of Buea
 Université de Douala
 Université de Yaoundé I

B - Biochimie

1. AYISEH RENE BILINGWE
2. MARCEL MOYEH NYUYLAM
3. TALOM TANGUE BENJAMIN

University of Buea
 University of Buea
 Université de Ngaoundéré

C - Biologie

1. AYUK ELIZABETH OROCK EPOUSE EBOTAGBO
2. BASGA EMMANUEL
3. ETEME ENAMA SERGE
4. GANGUE TIBURCE
5. KENGNE OLIVIER CLOVIS
6. MUTLEN MELVIN
7. NNANGA MEBENGA RUTH LAURE
8. NWAMO ROLAND DIDIER
9. NYAMSI TCHATCHO NECTAIRE LIE
10. TAIMANGA, né le 11 mai 1980 à BESSOUM

University of Buea
 Université de Maroua
 Université de Yaoundé I
 University of Bamenda
 Université de Maroua
 Université de Douala
 Université de Yaoundé I
 Université de Douala
 Université de Douala
 Université de Douala

D - Physiologie

1. EBAL MINYE EDMOND
2. GUESSOGO WILIAM RICHARD
3. HAMADOU ANDRE
4. MBOUH SAMUEL
5. PALE SIMON

Institut National de la Jeunesse et des Sports
 Institut National de la Jeunesse et des Sports
 Institut National de la Jeunesse et des Sports
 Institut National de la Jeunesse et des Sports
 University of Buea

VI - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES DE L'INGENIEUR

A - Thermodynamique et Energétique

1. ABDOURAMANI DADJE	Université de Ngaoundéré
2. BOMBA VALENTIN N°4	Université de Maroua
3. FOTSING METEGAM ISABELLE FLORA	Université de Dschang
4. FOTSING TALLA CYRILLE	Université de Maroua
5. KODJI DELI	Université de Maroua
6. MEDJO NOUADJE BRIGITTE ASTRID	Université de Dschang
B - Génie civil	
1. BAHÉL BENJAMIN	Université de Douala
2. MOUNDOM AMADOU	Université de Dschang
3. NGWEM BAYIHA BLAISE	Université de Douala
C - Génie mécanique	
1. FTATSI MBETMI GUY-DE-PATIENCE	Université de Ngaoundéré
D - Chimie appliquée et Génie chimique	
1. HASSANA BOUKAR	Université de Ngaoundéré
2. YANNE ETIENNE	Université de Maroua
E - Biologie et Biochimie appliquées	
1. NGUIKWIE KWANGA SYLVIE EPSE MEKONDANE	Université de Ngaoundéré
2. VOUNDI OLUGU STEVE HENRI	Université de Douala
F - Génie rural	
1. FITA DASSOU ELISABETH EPOUSE MOKSIA	Université de Maroua
G - Phytotechnie	
1. YAKOUBA OUMAROU	Université de Maroua
H - Zootechnie	
1. TCHOWAN GUY MERLIN	University of Buea
I - Technologie et Génie forestier	
1. ROY LYONGA MBUA	University of Buea

VII - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES PHARMACEUTIQUES ET MÉDICALES

A - Pharmacognosie et Pharmacotechnie	
1. MBOLE JEANNE MAURICETTE EPSE MVONDO MENDIM	Université de Yaoundé I
B - Microbiologie, Immunologie, Hématologie, Hygiène et Hydrologie	
1. LEM EDITH ABONGWA ÉPSE LUZAH	University of Bamenda
C - Microbiologie	
1. GAKE BOUBA	Université de Ngaoundéré
D - Sciences cliniques	
1. BEKOLO NGA WINNIE TATIANA	Université de Douala
2. BINYOM PIERRE RENE	Institut Supérieur de Technologie Médicale
3. DIVINE ENORU EYONG ETA	University of Buea
4. ETOA NDZIE MARTINE CLAUDE EPSE ETOGA	Université de Douala
5. MBANGO NGOH EDISARI NOEL DESIREE EPSE EKOUTA	Université de Douala
6. METOGO MBENGONO JUNETTE ARLETTE EPSE NJOKI	Université de Douala
7. NAIZA NGOWO MONONO EPSE MONOKO	University of Buea
8. TEUWAFEU DENIS GEORGES	University of Buea

Article 2 : Sont, pour compter des dates arrêtées par le Comité Consultatif des Institutions Universitaires (CCIU), inscrits sur la liste d'aptitude au grade de **Maître de Conférences**, par ordre alphabétique et par spécialité, les Chargés de Cours dont les noms suivent:

I - COMMISSION SCIENTIFIQUE SPECIALISÉE DE DROIT, SCIENCES ÉCONOMIQUES ET SCIENCES POLITIQUES

A - Droit privé et sciences criminelles

- | | |
|---|--------------------------|
| 1. BIBOUM BIKAY FRANCOIS | Université de Douala |
| 2. EYANGO DJOMBI ANDRE DESMONDS | Université de Douala |
| 3. KAMWE MOUAFFO MARIE COLETTE | Université de Ngaoundéré |
| 4. KOUAM SIMEON PATRICE, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Ngaoundéré |
| 5. LOWE GNINTEDEM PATRICK JUVET, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Dschang |
| 6. MBIFI RICHARD | University of Bamenda |
| 7. SUNKAM KAMDEM ACHILLE, agrégé CAMES, pour compter du 13 novembre 2019 | University of Buea |
| 8. TJOUEN ALEX FRANCOIS, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Yaoundé II |
| 9. VOUDWE BAKREO, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Douala |
| 10. YANPELDA VIRGINIE | Université de Douala |

B - Droit public

- | | |
|---|--------------------------|
| 1. AKONO OMGBA SEDENA, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Yaoundé II |
| 2. BEGNI BAGAGNA, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Douala |
| 3. EBANG MVE URBAIN NOEL | Université de Yaoundé II |
| 4. OWONA MFEGUE KOURRA FELICITE | Université de Yaoundé II |

C - Science politique

- | | |
|-------------------------------|--------------------------|
| 1. ATANGANA MVOGO FLORENT GUY | Université de Ngaoundéré |
| 2. KIVEN JAMES KEWIR | University of Buea |
| 3. MEDOU NGOA FRED JEREMIE | Université de Douala |

D - Etudes internationales

- | | |
|----------------------------------|--------------------------|
| 1. NGOUYAMSA MEFIRE MARCEL BRUCE | Université de Ngaoundéré |
|----------------------------------|--------------------------|

E - Sciences économiques

- | | |
|--|--------------------------|
| 1. BIDIASSE HONORE, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Douala |
| 2. GANDJON FANKEM GISLAIN STEPHANE, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Yaoundé II |
| 3. KAMDEM CYRILLE BERGALY, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Yaoundé II |
| 4. NLOM JEAN HUGUES, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Yaoundé II |
| 5. ONGO NKOA BRUNO EMMANUEL, agrégé CAMES, pour compter du 13 novembre 2019 | University of Buea |
| 6. SAIDOU BABA OUMAR | University of Bamenda |
| 7. TEKAM OUMBE HONORE | Université de Dschang |
| 8. VUKENKENG ANDREW WUJUNG | University of Bamenda |

F - Sciences et Techniques de Gestion

- | | |
|--|--------------------------|
| 1. BELLO, né le 02 septembre 1971 à YAGOUA | Université de Douala |
| 2. DJEUDJA ROVIER | Université de Yaoundé II |
| 3. GOUALONG KAMGANG NADEGE INGRID, agrégée CAMES, pour compter du 13 novembre 2019 | Université de Ngaoundéré |
| 4. MATH MAZRA | Université de Ngaoundéré |
| 5. MFOUAPON GEORGES KRIYOSS, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Ngaoundéré |
| 6. NJOMO LOUIS MOSAKE | Université de Douala |
| 7. OMENGUELE RENE GUY | University of Bamenda |
| 8. SONE MBASSI ALAIN NOEL, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Yaoundé II |
| 9. TAKOUDJOU NIMPA ALAIN, agrégé CAMES, pour compter du 13 novembre 2019 | Université de Dschang |

II - COMMISSION SCIENTIFIQUE SPECIALISÉE DES LETTRES ET SCIENCES HUMAINES

A - Sociologie et Anthropologie

- | | |
|--|-------------------------|
| 1. BIOS NELEM CHRISTIAN | Université de Yaoundé I |
| 2. MELI MELI VIVIEN | Université de Dschang |
| 3. NGADJIFNA, né le 28 septembre 1975 à YAGOUA | Université de Douala |

B - Sciences de l'Education

- | | |
|---------------------------------|-------------------------|
| 1. DJEUMENI TCHAMABE MARCELLINE | Université de Yaoundé I |
|---------------------------------|-------------------------|

C - Psychologie

- | | |
|--------------------------|-----------------------|
| 1. FOMBA EMMANUEL MBEBEB | University of Bamenda |
|--------------------------|-----------------------|

D - Philosophie

- | | |
|------------------------------|-----------------------|
| 1. MBIH JEROME TOSAM | University of Bamenda |
| 2. VALENTINE BANFEGHA NGALIM | University of Bamenda |

E - Art et Esthétique

- | | |
|----------------------------------|-------------------------|
| 1. NGUFOR EMELDA AMBO EPSE SAMBA | Université de Yaoundé I |
|----------------------------------|-------------------------|

F - Littérature négro-africaine

- | | |
|----------------------------------|-------------------------|
| 1. ANGO MEDJO MARTIN PAUL | Université de Yaoundé I |
| 2. YAOUDAM ELISABETH EPSE SAIDOU | Université de Maroua |

G - Linguistique et Phonétique

- | | |
|------------------------------------|-------------------------|
| 1. MEUTEM KAMTCHUENG LOZZI MARTIAL | Université de Maroua |
| 2. NGUE UM EMMANUEL | Université de Yaoundé I |

H - Langue et Littérature anglaises et Nord-américaines

- | | |
|-----------------------|----------------------|
| 1. NGALA DONATUS BEFI | Université de Douala |
|-----------------------|----------------------|

I - Langue et Littérature germaniques

- | | |
|---------------------------------------|-------------------------|
| 1. MBONGUE JOSEPH | Université de Yaoundé I |
| 2. MIGUQUE JEAN BERTRAND | Université de Yaoundé I |
| 3. NSANGOU MARYSE EPSE NJIKAM MOULIOM | Université de Yaoundé I |

J - Langue et Littérature espagnoles

- | | |
|---------------------------|----------------------|
| 1. ESSISSIMA MICHEL YVES | Université de Maroua |
| 2. HATOLONG BOHO ZACHARIE | Université de Maroua |
| 3. NGOUABA NYA JEAN PAUL | Université de Douala |

K - Sciences et Techniques de la Communication et de l'Information

- | | |
|-----------------------------------|--------------------------|
| 1. DJIMELI TAFOPi ALEXANDRE | Université de Dschang |
| 2. MPESSA MOUANGUE MARIE MARCELLE | Université de Yaoundé II |

L - Sciences historiques et archéologiques

- | | |
|-------------------------------------|-------------------------|
| 1. ANJOH FRII-MANYI ROSE | University of Buea |
| 2. BATENGUENE ASSIL RAPHAEL | Université de Douala |
| 3. ENOH RICHARD AGBOR AYUKNDANG | University of Buea |
| 4. LINDA ANKIAMBOM LAWYER EPSE YANG | Université de Yaoundé I |
| 5. MAHAMAT ABBA OUSMAN | Université de Maroua |
| 6. MEYOLO JOEL NARCISSE | Université de Yaoundé I |
| 7. MOUSSA II | Université de Yaoundé I |
| 8. NZOGUE JEAN BAPTISTE | Université de Douala |
| 9. SAMBO ARMEL | Université de Maroua |

M - Géographie, Etudes démographiques

- | | |
|-----------------------|-------------------------|
| 1. ELENO MANKA'A FUBE | Université de Yaoundé I |
|-----------------------|-------------------------|

III - COMMISSION SCIENTIFIQUE SPECIALISÉE DES MATHÉMATIQUES

A - Mathématiques

1. MBA ALPHONSE, pour compter du 30 novembre 2018
2. MBEHOU MOHAMED

Université de Yaoundé I
Université de Yaoundé I

B - Mathématiques appliquées

1. AYISSI ETEME ADOLPHE

Université de Ngaoundéré

IV - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES PHYSIQUES

A - Physique nucléaire et Corpusculaire et Théories physiques

1. EMA'A EMA'A JEAN MARIE
2. FAUTSO KUIATE GAETAN

Université de Ngaoundéré
University of Bamenda

B - Physique atomique et moléculaire et Physique du Solide

1. LOUDODOP FOTSO PATRICK HERVE

Université de Dschang

C - Astronomie, Physique spatiale et Géophysique

1. MEYING ARSENE

Université de Ngaoundéré

D - Chimie générale

1. BIKELE MAMA DESIRE
2. NDI NSAMI JULIUS
3. NJANJA TCHEUNKEU EVANGELINE EPSE BETNGA

Université de Douala
Université de Yaoundé I
Université de Dschang

E - Chimie minérale, organique et analytique

1. AWOUAFACK MAURICE DUCRET
2. FOTSO WABO GHISLAIN
3. NOTE LOUGBOT OLIVIER PLACIDE

Université de Dschang
Université de Yaoundé I
Université de Yaoundé I

V - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES DE LA TERRE ET DE LA VIE

A - Sciences de la Terre

1. MVONDO OWONO FRANCOIS
2. NGUEUTCHOUA GABRIEL

Université de Douala
Université de Yaoundé I

B - Biochimie

1. AZANTSA KINGUE GABIN BORIS
2. MBAVENG TSAFACK ARMELLE

Université de Yaoundé I
Université de Dschang

C - Biologie

1. ASANGA PATRICIA BI EPSE FAI
2. DJONWANGWE DENIS
3. MISSOUP ALAIN DIDIER
4. NANA PAULIN
5. TONJOCK ROSEMARY ÉPSE KINGE

University of Bamenda
Université de Maroua
Université de Douala
Université de Dschang
University of Bamenda

D - Physiologie

1. BILANDA DANIELLE CLAUDE EPOUSE DZEUFIE
2. DAVID EMERY TSALA
3. DJIOGUE SEFIRIN
4. TONFACK LIBERT BRICE

Université de Yaoundé I
Université de Maroua
Université de Yaoundé I
Université de Yaoundé I

VI - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES DE L'INGENIEUR

A - Electronique, Electrotechnique et Automatique

1. BOUKAR OUSMAN
2. DZONDE NAOUSSI SERGE RAOUL
3. KOMBE TIMOTHEE
4. MBOUPDA PONE JUSTIN ROGER
5. NDJIYA NGASOP

Université de Ngaoundéré
Université de Douala
Université de Douala
Université de Dschang
Université de Ngaoundéré

B - Génie mécanique

1. BETENE EBANDA FABIEN

Université de Douala

2. MTOPI FOTSO BLAISE EUGENE	Université de Dschang
C - Chimie appliquée et Génie chimique	
1. HENRIETTE ZANGUE ADJIA	Université de Ngaoundéré
2. KOFA GUILLAUME PATRICE	Université de Ngaoundéré
3. NCHARE MOMINOU	Université de Ngaoundéré
D - Biologie et Biochimie appliquées	
1. DESOBGO ZANGUE STEVE CARLY	Université de Ngaoundéré
2. DJANTOU DJANTOU ELIE BAUDELAIRE	Université de Ngaoundéré
3. KAKTCHAM PIERRE MARIE	Université de Dschang
4. LENG MARLYSE SOLANGE EPSE NDOUNTENG	Université de Douala
E - Pédologie	
1. CHRISTOPHER NGOSONG	University of Buea
F - Economie et Education rurale	
1. SOTAMENOU JOEL	Université de Yaoundé II
G - Technologie et Génie forestier	
1. SIMON NGOMBA LONGONJE	University of Buea

VII - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES PHARMACEUTIQUES ET MÉDICALES

A - Pharmacognosie et Pharmacotechnie	
1. TEMBE FOKUNANG ESTELLA	Université de Yaoundé I
B - Microbiologie, Immunologie, Hématologie, Hygiène et Hydrologie	
1. ESSOMBA NOEL EMMANUEL	Université de Douala
C - Biophysique, Biochimie, Mathématiques, Statistiques et Informatique médicale	
1. AROWOSHEG ADUNI UFUAN EPSE ACHIDI	University of Buea
2. NEOSSI GUENA MATHURIN	Université de Ngaoundéré
D - Disciplines mixtes (anatomie-pathologie, hématologie et maladies du sang, cancérologie)	
1. ATEUDJIEU JEROME	Université de Dschang
2. DONGHO TSAKEU EVELINE EPSE NGOUADJEU	Université de Douala
E - Sciences cliniques	
1. KEDY MANGAMBA DANIELE CHRISTIANE EPSE KOUM	Université de Douala
2. MAFFOSSOG MARIE PATRICE EPSE HALLE EKANE	Université de Douala
3. MVE KOH VALERE SALOMON	Université de Yaoundé I
4. ONGOTSOYI ANGELE HERMINE EPSE PONDY	Université de Yaoundé I
5. PALLE JOHN NGUNDE	University of Buea
6. PENDA CALIXTE IDA	Université de Douala
7. VERLA VINCENT SIYSI	University of Buea

Article 3 : Sont, pour compter des dates arrêtées par le Comité Consultatif des Institutions Universitaires (CCIU), inscrits sur la liste d'aptitude au grade de **Professeur**, par ordre alphabétique et par spécialité, les Maîtres de Conférences dont les noms suivent:

I - COMMISSION SCIENTIFIQUE SPECIALISÉE DE DROIT, SCIENCES ÉCONOMIQUES ET SCIENCES POLITIQUES

A - Droit privé et sciences criminelles	
1. NGOUE WILLY JAMES	Université de Douala
2. NGUIHE KANTE PASCAL	Université de Dschang
3. SAMGENA DAIGA GALEGA	Université de Yaoundé II
4. SIMON TABE TABE	Université de Dschang
B - Droit public	
1. KAM YOGO EMMANUEL DIEUDONNE	Université de Douala
C - Etudes internationales	

1. MOUKOKO MBONJO PIERRE

Université de Yaoundé II

D - Sciences économiques

1. ABESSOLO YVES ANDRE

Université de Yaoundé II

2. NGOUHOUE IBRAHIM

Université de Dschang

E - Sciences et Techniques de Gestion

1. HALIDOU MAMOUDOU

Université de Maroua

2. MAYEGLE FRANCOIS XAVIER

Université de Ngaoundéré

3. NGOK EVINA JEAN FRANCOIS

Université de Ngaoundéré

II - COMMISSION SCIENTIFIQUE SPECIALISÉE DES LETTRES ET SCIENCES HUMAINES

A - Sciences de l'Education

1. EINSTEIN MOSES EGBE ANYI

University of Bamenda

B - Psychologie

1. EBALE MONEZE CHANDEL

Université de Yaoundé I

2. MAYI MARC BRUNO

Université de Yaoundé I

C - Philosophie

1. MALOLO DISSAKE EMMANUEL

Université de Douala

D - Langue et littérature françaises

1. BANDOLO CHRISTINE ROSALIE EPSE ONGUENE ESSONO

Université de Yaoundé I

2. MBASSI ATEBA RAYMOND

Université de Maroua

3. MBASSI BERNARD

Université de Yaoundé I

4. NOUMSSI GERARD MARIE

Université de Yaoundé I

E - Linguistique et Phonétique

1. EMMANUEL NFORBI

Université de Dschang

F - Géographie, Etudes démographiques

1. YEMMAFOUO ARISTIDE

Université de Dschang

III - COMMISSION SCIENTIFIQUE SPECIALISÉE DES MATHÉMATIQUES

A - Mathématiques

1. LELE CELESTIN

Université de Dschang

2. NNANG HUBERT MADELEINE

Université de Yaoundé I

3. TIEUDJO DANIEL

Université de Ngaoundéré

B - Mathématiques appliquées

1. BOWONG TSAKOU SAMUEL

Université de Douala

IV - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES PHYSIQUES

A - Physique nucléaire et Corpusculaire et Théories physiques

1. EFFA JOSEPH YVES

Université de Ngaoundéré

2. EKOBEA FOU DA HENRI PAUL

Université de Ngaoundéré

B - Physique atomique et moléculaire et Physique du Solide

1. DIKANDE ALAIN MOISE

University of Buea

2. NANA ENGO SERGE GUY

Université de Yaoundé I

3. TCHOFFO MARTIN

Université de Dschang

C - Astronomie, Physique spatiale et Géophysique

1. LENOUE ANDRE

Université de Douala

2. NOUAYOU ROBERT

Université de Yaoundé I

D - Chimie générale

1. NGOUNE JEAN

Université de Dschang

V - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES DE LA TERRE ET DE LA VIE

A - Sciences de la Terre

1. NGOS 3 SIMON

Université de Yaoundé I

B - Biochimie

1. JUDE DAIGA BIGOGA
2. TCHOUMBOUNANG FRANCOIS

Université de Yaoundé I

Université de Douala

C - Biologie

1. AJEAGAH GIDEON AGHAINDUM
2. FONKOU THEOPHILE
3. IBRAHIMA ADAMOU
4. NOUBISSIE TCHIAGAM JEAN BAPTISTE
5. ZEBAZE TOGOUET SERGE HUBERT

Université de Yaoundé I

Université de Dschang

Université de Ngaoundéré

Université de Ngaoundéré

Université de Yaoundé I

D - Physiologie

1. MEGUENI CLAUTILDE EPSE TCHIEGANG
2. NIEMENAK NICOLAS
3. SOKENG DONGMO SELESTIN

Université de Ngaoundéré

Université de Yaoundé I

Université de Ngaoundéré

VI - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES DE L'INGENIEUR**A - Electronique, Electrotechnique et Automatique**

1. ELE PIERRE

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B - Génie mécanique

1. ATANGANA ATEBA

Université de Douala

C - Chimie appliquée et Génie chimique

1. NGOMO HORACE MANGA

Université de Yaoundé I

D - Biologie et Biochimie appliquées

1. KANSCI GERMAIN
2. NSO EMMANUEL JONG

Université de Yaoundé I

Université de Ngaoundéré

VII - COMMISSION SCIENTIFIQUE SPECIALISÉE DES SCIENCES PHARMACEUTIQUES ET MÉDICALES**A - Toxicologie, Physiologie, Pharmacodynamique Biophysique et Biochimie Pharmaceutiques**

1. SANDO ZACHARIE

Université de Yaoundé I

B - Microbiologie

1. KAMGA FOUAMNO HENRI LUCIEN

University of Bamenda

C - Sciences cliniques

1. BI SUH MARY EPSE ATANGA
2. ESSI MICHELINE MARIE JOSE
3. KAMGA GUEGNE HORTENSE YVETTE EPSE GOMSU FOTSING

University of Bamenda

Université de Yaoundé I

Université de Yaoundé I

**LE MINISTRE D'ETAT,
MINISTRE DE L'ENSEIGNEMENT SUPERIEUR,
PRESIDENT DU COMITE CONSULTATIF DES INSTITUTIONS UNIVERSITAIRES**



JACQUES FAME NDONGO

IV) ACTIVITES ACADEMIQUES

IV.1) Notes de présentation

IV.1.1) Contribution à l'enseignement

Depuis mon recrutement au Département de Génie Alimentaire et Contrôle Qualité de l'Institut Universitaire de Technologie (IUT) en 2008, j'ai embrassé ma vocation d'enseignant avec un enthousiasme indéfectible. Chaque jour, je me suis plongé corps et âme dans la transmission du savoir, guidé par une passion ardente pour former les futures générations.

Lorsque j'ai franchi l'étape décisive de ma promotion au grade de Maître de Conférences en novembre 2019, cet accomplissement a ravivé mon engouement et renforcé ma détermination à exceller dans mon rôle. Cette reconnaissance de mes compétences et de mon dévouement a été comme un vent frais, attisant la flamme de ma motivation et me poussant à repousser sans cesse les limites de l'excellence académique.

Avec une motivation renouvelée, j'ai continué à dispenser mes enseignements dans diverses disciplines liées aux sciences de l'ingénieur, telles que l'enzymologie, la malterie/brasserie, le dessin technique et les schémas de procédés, les biostatistiques et l'ingénierie des équipements. Chaque cours, chaque travail dirigé, chaque travaux pratique était une occasion unique de transmettre non seulement des connaissances, mais aussi une passion contagieuse pour ces domaines fascinants.

Mon expertise étant largement reconnue, mes services ont été sollicités au-delà des murs de l'IUT. À l'ENSAI de l'Université de Ngaoundéré et à l'Institut supérieur agricole (ISAGO) d'Obala, j'ai eu l'honneur de partager mes connaissances avec des étudiants avides d'apprendre, contribuant ainsi à la formation d'une nouvelle génération de professionnels compétents.

Mais mon rôle ne s'est pas limité à l'enseignement. J'ai activement participé à la restructuration des programmes d'études conformément au système LMD, apportant ma pierre à l'édifice de l'excellence académique. De plus, j'ai encadré avec dévouement des dizaines d'étudiants de tous niveaux, des DUT aux doctorats, les guidant dans leur cheminement intellectuel et leur offrant un soutien indéfectible. À ce jour, j'ai la fierté d'avoir accompagné 5 doctorants jusqu'à la soutenance de leur thèse de doctorat, dont 4 Camerounais de l'ENSAI de Ngaoundéré et 1 Tchadien de l'Université de N'Djamena. De plus, 6 étudiants de Master ont également soutenu leurs travaux sous ma supervision depuis mon accession au grade de Maître de Conférences, sans compter les nombreux étudiants ingénieurs que j'ai eu l'honneur d'encadrer.

Chaque jour, je puise mon inspiration dans la réussite de mes étudiants, dans leurs regards brillants de compréhension et dans leur soif insatiable de connaissances. C'est cette étincelle qui alimente mon engouement intact pour l'enseignement, une flamme qui ne cessera jamais de

brûler en moi. Voir ces jeunes esprits s'épanouir et atteindre leurs objectifs académiques est la plus grande récompense que je puisse espérer dans ma carrière d'enseignant passionné.

En tant que passionné de l'enseignement supérieur, j'ai toujours eu à cœur de promouvoir l'intégrité académique et de favoriser l'excellence dans les programmes d'études. C'est pourquoi mon implication au sein du comité de lutte contre le plagiat est une véritable source de motivation et de fierté.

Avec mes collègues, nous travaillons d'arrache-pied pour sensibiliser les étudiants aux enjeux éthiques du plagiat et leur inculquer les bonnes pratiques de citation et de référencement. Nos efforts permettront de renforcer la rigueur académique et de préserver la crédibilité de notre institution.

En repensant à ce parcours, je suis fier d'avoir contribué à l'excellence de notre institution et à la formation de futurs professionnels compétents et éthiques. C'est avec passion et dévouement que je continuerai à œuvrer pour l'enseignement supérieur de qualité.

IV.1.2) Contribution à la recherche, au développement de la science et de la culture

IV.1.2.a) Articles scientifiques

Dans le paysage dynamique de l'agro-industrie camerounaise, où les défis de l'insuffisance alimentaire et de la crise potentielle se profilent à l'horizon, la recherche scientifique revêt une importance cruciale pour catalyser le développement socio-économique. En tant que membre actif et collaborateur engagé au sein de plusieurs laboratoires de renom, dont le Laboratoire de Génie et Technologie Alimentaire (LAGETA) de l'ENSAI de l'Université de Ngaoundéré, le Laboratoire de BioProcédés (LBP) de l'IUT de l'Université de Ngaoundéré, et le laboratoire de Biophysique, Biochimie Alimentaire et Nutrition (LABBAN), ainsi que des institutions étrangères prestigieuses telles que l'Université de Johannesburg en Afrique du Sud, Nous nous sommes investi dans une quête scientifique visant à optimiser les techniques d'extraction et de traitement des boissons.

Le contexte camerounais, riche en ressources agroalimentaires diversifiées, offre un potentiel immense pour stimuler la croissance économique à travers l'exploitation et la valorisation de ces précieuses matières premières. Cependant, les défis démographiques croissants et les pressions économiques soulèvent des préoccupations quant à la sécurité alimentaire et à la pérennité de l'industrie agroalimentaire. Face à cette réalité, il devient impératif de mobiliser les sciences de l'ingénieur au service de l'agro-industrie nationale, en vue de développer des solutions innovantes pour valoriser les productions agricoles et réduire les pertes post-récolte.

Nos recherches s'inscrivent dans cette perspective globale, visant à exploiter le potentiel des sciences de l'ingénieur pour comprendre les phénomènes biochimiques et améliorer la qualité des produits agroalimentaires. Sous le thème central de **Avancées technologiques dans la production des boissons**, mes travaux se déclinent en trois sous-thèmes distincts (*Sous-thème 1 : Extraction et clarification enzymatique des jus de fruits ; Sous-thème 2 : Optimisation de l'extraction enzymatique et de la clarification du moût pour les boissons à base de sorgho ; Sous-thème 3 : Boissons probiotiques et fonctionnelles*), chacun représentant une facette essentielle de l'industrie agroalimentaire. Ainsi, le fil conducteur commun est l'utilisation des outils des sciences de l'ingénieur, tels que la planification expérimentale, la modélisation et l'optimisation des procédés, pour améliorer les techniques d'extraction, de traitement et de valorisation des boissons issues de diverses sources (fruits, céréales, fermentations traditionnelles). Cette approche vise à optimiser les rendements, la qualité et les propriétés fonctionnelles des boissons, tout en valorisant les ressources locales et le patrimoine culturel du Cameroun et du monde en général.

VI-2.1) Sous-thème 1 : Extraction et clarification enzymatique des jus de fruits

À travers une série d'études approfondies, nous avons cherché à améliorer les méthodes d'extraction des jus de fruits tout en explorant les effets de différents processus sur la qualité et les propriétés des produits finis.

L'un des domaines clés de notre recherche a été l'optimisation de l'extraction assistée par la pectinase du jus d'*Annona muricata* L. et l'effet de la liquéfaction sur sa structure de pectine. Cette étude a permis de déterminer les conditions optimales pour extraire le jus de corossol en utilisant des enzymes pectinolytiques, tout en évaluant l'impact de la liquéfaction sur la structure et les propriétés de la pectine, ce qui ouvre la voie à des améliorations significatives dans le processus d'extraction des jus de fruits.

Une autre recherche importante a porté sur l'hydrolyse de la pulpe de goyave par la pectinase et son effet sur les caractéristiques physico-chimiques de son jus. En analysant les changements induits par l'hydrolyse enzymatique sur la pulpe de goyave et la qualité de son jus, nous avons contribué à une meilleure compréhension des mécanismes impliqués dans le processus de clarification des jus de fruits, offrant ainsi des perspectives pour l'optimisation des procédés industriels.

Parallèlement à ces études, nous avons exploré les possibilités de clarification du jus de goyave à chair blanche en utilisant des centrifugeuses à rotor conique fixe et des centrifugeuses à disque empilé continu en laboratoire. En comparant les performances de différents équipements de clarification, nous avons identifié des méthodes efficaces pour améliorer la clarté et la qualité des jus de fruits, contribuant ainsi à l'optimisation des processus de fabrication à l'échelle industrielle.

Dans une perspective plus méthodologique, nous avons appliqué la méthodologie de surface de réponse dans l'extraction du jus de datte pour évaluer l'effet des paramètres de processus sur les caractéristiques du jus, notamment le Brix, la couleur et le rapport sucre/acide. Cette approche statistique nous a permis de déterminer les conditions optimales pour extraire le jus de datte tout en maintenant ses propriétés sensorielles et nutritionnelles, ouvrant ainsi la voie à une production plus efficace et standardisée de cette boisson populaire.

Enfin, nous avons étudié l'extraction du jus de datte en utilisant la méthodologie de surface de réponse pour évaluer son effet sur le pH, la vitamine C, l'acidité titrable, l'azote amino libre (FAN) et les polyphénols. En analysant l'impact de différents paramètres de processus sur les

propriétés physico-chimiques et nutritionnelles du jus de datte, nous avons contribué à une meilleure compréhension de ses qualités nutritionnelles et de son potentiel pour la santé.

Le fil conducteur entre ces articles réside dans ma volonté d'optimiser les techniques d'extraction des jus de fruits en utilisant des méthodes enzymatiques et statistiques avancées, tout en explorant les effets de différents processus sur la qualité, la clarté et les propriétés nutritionnelles des produits finis. En combinant une approche multidisciplinaire avec des méthodologies innovantes, nous avons cherché à améliorer la compréhension scientifique et la pratique industrielle dans le domaine de la production de boissons à base de fruits, en adéquation avec l'objectif global d'appliquer les sciences de l'ingénieur à l'agroalimentaire.

1. **Makebe, C. W., Desobgo, Z. S. C., Ambindei, W. A., Billu, A., Nso, E. J., & Nisha*, P. (2020). Optimization of pectinase-assisted extraction of *Annona muricata* L. juice and the effect of liquefaction on its pectin structure. *Journal of the Science of Food and Agriculture*, 100(15), 5487-5497.**

Cet article présente une étude visant à optimiser l'extraction assistée par pectinase du jus d'*Annona muricata* L. (corossol) et à étudier l'effet de la liquéfaction sur sa structure pectinique. Le corossol est un fruit tropical sous-utilisé qui présente des avantages nutritionnels et thérapeutiques importants, mais il est confronté à d'énormes pertes post-récolte en raison de sa grande périssabilité. Le problème abordé dans cette recherche est la difficulté de transformer la pulpe de corossol en jus en raison de sa nature pectinée, qui empêche la diffusion des solutés pendant l'extraction.

Nous avons utilisé la méthodologie de la surface de réponse (RSM) en utilisant un plan de Doehlert pour optimiser les conditions d'extraction, à savoir le temps d'incubation, la concentration enzymatique et la température d'incubation. Les réponses évaluées sont le rendement en jus, le pH, la limpidité, le total des solides solubles (TSS) et l'acidité titrable. Les conditions optimales obtenues sont un temps d'incubation de 172 minutes, une concentration enzymatique de 0,04 % (p/p) et une température d'incubation de 42,9 °C, ce qui donne un rendement de 75,2 %, un pH de 3,75, une limpidité de 87,06 %, un extrait sec total de 7,35°Brix et une acidité titrable équivalente à l'acide malique de 0,46 %. La microscopie électronique à balayage (MEB) a révélé que la pectinase hydrolysait la pectine du corossol, provoquant des ruptures et des rides à la surface, facilitant la libération des solutés et améliorant l'extraction du jus. La spectroscopie infrarouge à transformée de Fourier (FTIR) a confirmé la rupture de la structure de la pectine du corossol au cours de la liquéfaction, comme le montre l'intensité plus

élevée des bandes correspondant à l'étirement des liaisons hydroxyle et glycosidique dans la pectine hydrolysée du corossol.

En conclusion, l'étude a fourni des conditions optimisées pour l'extraction assistée par pectinase du jus de corossol, qui pourrait être une méthode prometteuse pour la valorisation de ce fruit sous-utilisé. L'application du traitement enzymatique a entraîné la décomposition de la structure de la pectine, comme l'ont montré les analyses SEM et FTIR, ce qui a permis d'améliorer le rendement et la qualité de l'extraction du jus.

2. Ninga, K. A., Desobgo, Z. S. C. *, De, S., & Nso, E. J. (2021). *Pectinase hydrolysis of guava pulp: Effect on the physicochemical characteristics of its juice. Heliyon, 7(10), 1-11.*

L'article se concentre sur l'étude de l'effet du traitement enzymatique de la purée de goyave sur les caractéristiques physicochimiques de son jus. La goyave est un fruit très périssable, et sa transformation en jus peut prolonger sa durée de conservation. Cependant, la présence de pectine dans la goyave rend le jus très visqueux, ce qui entraîne un faible rendement de récupération du jus lors de la clarification. Le traitement enzymatique avec des pectinases peut décomposer les molécules de pectine, réduisant ainsi la viscosité et améliorant la clarification.

Nous avons traité la purée de goyave avec différentes concentrations (0,033 %, 0,055 %, 0,078 % et 0,1 % p/p) de pectinase d'*Aspergillus niger* à 43 °C pendant des durées de traitement variables (3 à 90 minutes). Ils ont analysé les paramètres physicochimiques du jus non traité et du jus traité, notamment la viscosité, le pH, la conductivité électrique, les solides solubles totaux (SST), la couleur, la teneur en protéines et en polyphénols, la teneur en acide galacturonique et la capacité antioxydante. La microscopie électronique à balayage à émission de champ (FESEM) a été utilisée pour visualiser les changements morphologiques dans les échantillons de jus.

Les résultats ont montré que le traitement enzymatique réduisait significativement la viscosité du jus de 91% dans les 3 premières minutes, accompagné d'une augmentation de la teneur en acide galacturonique. Le pH, la teneur en protéines et en polyphénols ont diminué, tandis que la conductivité, la couleur et le TSS ont augmenté. La capacité antioxydante n'a pas été affectée. Les images FESEM ont révélé une diminution de la taille des particules, une amélioration de l'homogénéité et une augmentation de la proportion de la phase continue avec l'augmentation de la concentration enzymatique et du temps de traitement.

En conclusion, le traitement enzymatique de la purée de goyave a permis de réduire efficacement la viscosité et d'améliorer les caractéristiques physicochimiques du jus, ce qui le rend apte à la clarification et au traitement ultérieur. L'étude fournit des indications précieuses sur l'optimisation des paramètres de traitement enzymatique pour une production efficace de jus de goyave.

3. *Ninga, K. A., Desobgo, S. C. Z. *, Nso, E. J., & Kayem, J. (2022). White-flesh guava juice clarification by a fixed-angle conical rotor centrifuge laboratory and characterization of continuous disk stack centrifuges. Heliyon, 8(2022), 1-12.*

L'étude visait à étudier l'impact des paramètres de centrifugation sur les caractéristiques physicochimiques du jus de goyave clarifié à chair blanche et à déterminer les caractéristiques opérationnelles des centrifugeuses continues à assiettes sur la base des performances d'une centrifugeuse de laboratoire.

L'étude a montré que l'augmentation de la force g pendant la centrifugation entraînait une diminution de la taille moyenne des particules des échantillons centrifugés et de leurs gammes de tailles. Ces résultats sont cohérents avec ceux de recherches antérieures. La centrifugation a permis d'obtenir des rendements d'extraction du jus de goyave allant de 89,72 % à 97,07 %. Le rendement de l'extraction du jus a été constamment élevé, avec une variation minimale entre les différentes forces g et durées de centrifugation. L'efficacité de clarification de la centrifugeuse a diminué avec la capacité du séparateur pour toutes les accélérations centrifuges étudiées. Une augmentation de la force g a conduit à une augmentation de l'efficacité de la clarification en raison de l'augmentation correspondante de la surface de sédimentation.

L'étude a conclu que les paramètres de centrifugation, en particulier la force g et la durée de centrifugation, influencent de manière significative les caractéristiques physicochimiques du jus de goyave. Il a été observé que des forces g plus élevées amélioraient l'efficacité de la clarification et réduisaient la taille des particules dans les échantillons de jus clarifiés. La recherche a fourni des informations précieuses sur les aspects opérationnels des centrifugeuses en continu en se basant sur les performances d'une centrifugeuse de laboratoire, offrant des applications potentielles dans les environnements industriels pour les processus de clarification des jus.

4. *Kadlezir, F, Mohagir, A. M., & Desobgo, S. C. Z. * (2023). Application of response surface methodology in date (Phoenix dactylifera L.) juice extraction: Effect of*

process parameters on Brix, color and sugar/acid ratio. Journal of Food Stability, 4(1), 1-18.

La demande des consommateurs pour des aliments fonctionnels a suscité l'intérêt pour la transformation de fruits moins connus comme les dattes du désert, qui sont davantage connues pour leurs propriétés médicinales que pour leur valeur culinaire. Le cultivar de datte « *Bournow* » du Tchad est très productif et bien adapté à la région saharienne, mais il présente des caractéristiques physiques médiocres qui le rendent invendable pour la consommation directe. L'étude visait à développer des modèles mathématiques pour prédire le profil physico-chimique de l'extrait de jus de datte « *Bournow* », et optimiser l'extraction des sucres, de la couleur, et du rapport sucre/acide par la méthodologie de la surface de réponse. Les effets de la température, de la durée, du rapport eau/pulpe et du volume de l'enzyme pectinase sur le Brix, la couleur et le rapport sucre/acide ont été étudiés à l'aide d'un plan d'expérience composite centré. L'objectif de ce travail était de valoriser cette variété de datte en produisant un jus adapté à l'industrie des boissons.

La caractérisation physique a confirmé la mauvaise qualité des dattes de *Bournow*, justifiant le besoin de valorisation. Des modèles polynomiaux du second degré avec interactions ont été obtenus pour relier les facteurs aux réponses. La température, le temps, le rapport et le volume d'enzymes ont tous eu des effets significatifs singuliers, quadratiques et interactifs sur l'augmentation ou la diminution du Brix, de la couleur et du rapport sucre/acide. Les conditions optimales pour maximiser toutes les réponses étaient les suivantes : 95°C, 10 min, rapport eau/pulpe 2:1, 0 mL de pectinase, produisant 21,89 °Brix, rapport sucre/acide 13,99, et 197,49 unités de couleur ASBC. D'autres analyses physicochimiques ont montré que le jus était riche en vitamine C, en composés phénoliques, etc.

L'extraction a mis en évidence la richesse en nutriments du jus de dattes *Bournow*. L'optimisation a permis de maximiser sa valeur Brix, sa couleur et son goût sucré équilibré, ce qui suggère une bonne qualité qui pourrait être encore améliorée pour être utilisée dans d'autres industries telles que les boissons fermentées. Cela valorise une variété de dattes de qualité médiocre.

5. *Kadlezir, F., Mohagir, A. M., & Desobgo, S. C. Z.* (2024). Extracting juice from dates (Phoenix dactylifera L.) using response surface methodology: Effect on pH, vitamin C, titratable acidity, free amino nitrogen (FAN) and polyphenols. Applied Food Research, 4(1), 100375.*

Les dattes (*Phoenix dactylifera* L.) sont une culture fruitière de grande valeur nutritionnelle et d'importance économique mondiale. Elles sont riches en nutriments tels que les vitamines, les minéraux, les fibres et les antioxydants tels que les polyphénols. Cependant, le potentiel de certains cultivars de dattes sous-utilisés, comme le « *Bournow* », en tant qu'aliments fonctionnels et nutraceutiques reste inexploré. Cette étude visait à optimiser l'extraction de composés bioactifs tels que le pH, la vitamine C, l'acidité titrable, l'azote aminé libre (AAL) et les polyphénols du cultivar de datte « *Bournow* » afin de produire un jus riche en nutriments adapté à l'industrie des boissons.

La méthodologie de la surface de réponse a été employée en utilisant un plan composite centré à 4 facteurs pour étudier les effets de la température, de la durée, du rapport eau/pulpe et du volume d'enzymes sur l'extraction des composés ciblés. Des modèles polynomiaux du second degré avec interactions ont été développés et validés pour chaque réponse. L'optimisation multi réponse a ensuite été utilisée pour maximiser la vitamine C, les polyphénols et les FAN, tout en maintenant le pH dans une fourchette acceptable.

Les paramètres d'extraction ont influencé de manière significative les réponses, avec des contributions de facteurs singuliers, d'effets quadratiques et d'interactions. La température a augmenté le pH, la vitamine C, les polyphénols et le FAN, tandis que le temps a augmenté le pH et les polyphénols, mais a diminué la vitamine C et le FAN. Le rapport eau/pulpe a réduit la vitamine C, les polyphénols et les FAN par dilution. Le volume d'enzymes a eu des effets mitigés. Les conditions optimales étaient 95°C, 10 min, rapport eau/pulpe 2:1, et 0,5 mL de pectinase, donnant un pH de 4,13, 116,5 mg/L de vitamine C, 6,25 g GA/100 g de polyphénols, et 587,88 mg/L de FAN, avec une désirabilité composite de 0,856.

L'étude a optimisé avec succès l'extraction d'un jus de dattes riche en nutriments à partir du cultivar « *Bournow* » en utilisant la méthodologie de la surface de réponse. Le jus optimisé est un produit prometteur pour l'industrie des boissons en raison de ses propriétés physicochimiques favorables et de ses composés bioactifs. Il est recommandé de poursuivre les recherches sur les attributs sensoriels, la durée de conservation et les applications dans d'autres produits alimentaires.

En somme, mes recherches dans le domaine de l'extraction et de la clarification enzymatique des jus de fruits ont contribué de manière significative à l'avancement des connaissances scientifiques et à l'amélioration des pratiques industrielles dans le secteur agroalimentaire. En combinant une approche multidisciplinaire avec des méthodologies innovantes, nous avons

cherché à développer des solutions durables pour répondre aux défis croissants de la production alimentaire et à promouvoir le développement socio-économique dans ma communauté et au-delà.

V-2.2) Sous-thème 2 : Optimisation de l'extraction enzymatique et de la clarification du moût pour les boissons à base de sorgho

Mon parcours dans le domaine de la recherche et du développement scientifique a été également marqué par une contribution significative à l'optimisation des processus de production de boissons à base de sorgho, ainsi qu'à la valorisation des ressources locales et durables. À travers une série d'articles de recherche, nous avons démontré un engagement sans faille envers l'avancement de la science, tout en mettant en lumière l'importance de la culture et de l'agriculture dans ce contexte.

L'article intitulé "Optimisation de l'Extraction de la Dextrinase Limite de la Variété de Sorgho Camerounais *Safrari*" représente l'un des premiers jalons de votre parcours scientifique. Dans cet article, nous avons exploré les techniques d'extraction de la dextrinase limite, une enzyme cruciale dans le processus de clarification du moût de sorgho. Notre recherche a permis de mettre en évidence l'importance de cette enzyme dans l'amélioration de la qualité des boissons à base de sorgho, ouvrant ainsi la voie à des méthodes plus efficaces et économiques de production.

Notre engagement envers la recherche se manifeste également à travers l'article "Purification partielle et caractérisation de la dextrinase limite du malt de sorgho *Safrari*". En caractérisant cette enzyme essentielle dans le processus de clarification du moût de sorgho, nous avons contribué à une meilleure compréhension des mécanismes enzymatiques impliqués, ouvrant ainsi la voie à des innovations dans la production de boissons à base de sorgho.

Dans une étude ultérieure, intitulée "*Grewia mollis* bark powder impact on the clarification of *Mbayeri* sorgho wort", nous avons élargi votre champ d'investigation en explorant l'impact de la poudre d'écorce de *Grewia mollis* sur la clarification du moût de sorgho *Mbayeri*. Cette recherche témoigne de votre volonté d'explorer des solutions novatrices et durables pour améliorer les processus de clarification, tout en mettant en valeur le potentiel des ressources locales dans l'industrie agroalimentaire.

Enfin, notre recherche s'est étendue à l'exploration de nouvelles sources enzymatiques pour améliorer les processus de brassage du sorgho, comme en témoigne l'article "Potential of Beta-Amylase from Sweet Potato (*Ipomoea batatas* Lam) Extract on the Mashing of *Safrari* Sorgho".

Cette étude met en évidence votre volonté d'explorer de nouvelles voies pour optimiser les processus de production, tout en soulignant le potentiel prometteur des ressources locales dans ce domaine.

À travers ces articles, un fil conducteur scientifique émerge, mettant en évidence notre engagement à explorer de nouvelles voies pour optimiser les processus de production de boissons à base de sorgho. Notre recherche se concentre sur l'identification et l'optimisation des enzymes impliquées dans la clarification et le brassage du sorgho, avec un accent particulier sur l'utilisation de ressources locales et durables. En contribuant à une meilleure compréhension des mécanismes enzymatiques impliqués dans ces processus, nous ouvrons la voie à des innovations significatives dans l'industrie agroalimentaire, tout en valorisant le patrimoine culturel et agricole du Cameroun.

1) Nguemogne, A. C., **Desobgo, Z. S. C.***, & Nso, J. E. (2020). *Optimisation de l'Extraction de la Dextrinase Limite de la Variété de Sorgho Camerounais Safrari. Journal of Food Stability*, 3(2), 9-26.

Les enzymes peuvent être extraites de sources végétales pour diverses applications. L'optimisation des conditions d'extraction est importante pour obtenir une activité enzymatique maximale. L'enzyme dextrinase limite des malts de céréales a des applications utiles, notamment dans l'industrie brassicole pour réduire la turbidité du moût. Cependant, la plupart des études antérieures sur l'extraction de la dextrinase limite ont utilisé des approches classiques d'optimisation d'un facteur à la fois plutôt que des méthodes de conception expérimentale statistique plus efficaces. Le but de ce travail était d'optimiser l'extraction de la dextrinase limite de la variété de malt de sorgho *Safrari* du Cameroun en utilisant la méthodologie de la surface de réponse (MSR). Les effets de la température, du pH, du rapport malt/tampon et du temps d'extraction sur l'activité enzymatique ont été évalués à l'aide d'un plan d'expérience de Doehlert à 4 facteurs.

Le modèle quadratique généré reliant l'activité limite de la dextrinase aux facteurs a été validé statistiquement. L'analyse de la variance a montré que la température, le rapport malt/tampon, le temps, les effets quadratiques des quatre facteurs et l'interaction pH-temps influençaient de manière significative l'activité enzymatique pendant l'extraction. Tous les effets significatifs, à l'exception de l'interaction pH-temps, ont contribué négativement à l'activité enzymatique. Les conditions optimales d'extraction déterminées étaient 23°C, pH 5,0, rapport malt/buffer 5/32, et 10 h de temps, prédisant une activité enzymatique théorique maximale de 140 mU/mL. La

vérification expérimentale a donné 76% de cette valeur à 106 mU/mL. Des tests supplémentaires ont montré que les antioxydants, l'EDTA, le calcium et l'albumine de sérum bovin n'ont pas amélioré les rendements d'extraction par rapport au contrôle.

La MSR a permis d'optimiser rapidement l'extraction de la dextrinase limite du malt de sorgho *Safrari*. Les conditions optimales étaient comparables à celles rapportées pour d'autres céréales. La température douce et la dilution modérée requises sont avantageuses pour les applications brassicoles potentielles utilisant cette variété locale de sorgho comme source d'enzymes, sans nécessiter d'additifs d'extraction. L'extraction optimisée pourrait constituer un moyen rentable d'obtenir une dextrinase limite pour réduire la turbidité du moût lors de l'utilisation de sorgho et d'autres céréales comme adjuvants de brasserie.

2) Nguemogne, A. C., Desobgo, Z. S. C.*, & Nso, J. E. (2023). Purification partielle et caractérisation de la dextrinase limite du malt de sorgho Safrari. Journal of Food Stability, 6(1), 34-50.

L'article porte sur la purification partielle et la caractérisation de l'enzyme dextrinase limite dans le malt de Sorgho *Safrari*. L'étude vise à optimiser le processus de purification de cette enzyme en vue d'applications industrielles potentielles. Les chercheurs ont procédé à diverses étapes de purification, y compris la dialyse et la précipitation au sulfate d'ammonium, pour isoler l'enzyme dextrinase de l'extrait de malt. Les problèmes rencontrés concernaient l'évaluation de l'activité et de la spécificité de l'enzyme à différents stades de la purification.

Les résultats ont révélé des activités et des spécificités enzymatiques variables après chaque étape de purification, ce qui indique l'efficacité du processus de purification. En outre, l'étude a évalué les propriétés enzymatiques de la dextrinase, telles que le pH et la température optimaux pour l'activité et la stabilité. Il a été constaté que l'enzyme présentait une thermorésistance et une activité optimale à un pH compris entre 5,0 et 5,5. L'amélioration de l'efficacité de la filtration et de la teneur en sucre dans les brassins de sorgho traités avec l'enzyme dextrinase partiellement purifiée a constitué une découverte importante. Cela suggère le rôle de l'enzyme dans la liquéfaction des brassins et l'amélioration de leur filtrabilité. L'étude a mis en évidence l'importance de l'optimisation des conditions de synthèse enzymatique au cours de la transformation du malt de céréales afin d'obtenir des quantités d'enzymes utiles à l'industrie.

En conclusion, la recherche a permis de purifier partiellement l'enzyme dextrinase limite du malt de Sorgho *Safrari* par une série d'étapes de purification. Les activités et propriétés spécifiques de l'enzyme ont été évaluées, indiquant son utilité industrielle potentielle pour

améliorer l'efficacité de la filtration et la teneur en sucre des brassins de sorgho. L'étude souligne l'importance de l'optimisation des techniques de purification des enzymes pour les applications industrielles et donne un aperçu des caractéristiques spécifiques de l'enzyme dextrinase pour la recherche future et l'utilisation industrielle.

3) Man-Ikri, Bertin, & Desobgo, Z. S. C.*. (2023). *Grewia mollis* bark powder impact on the clarification of Mbayeri sorgho wort. *Applied Food Research*, 3(1), 100243.

L'article examine l'utilisation de *Grewia mollis* pour la clarification du moût de sorgho d'un point de vue physicochimique. L'étude visait à examiner l'impact de paramètres tels que le rapport moût/*Grewia*, le temps d'agitation et la vitesse sur le processus de clarification. En utilisant la modélisation statistique et mathématique, les caractéristiques physicochimiques des moûts traités ont été analysées pour évaluer l'efficacité de la *Grewia mollis* dans la clarification du moût de sorgho. La problématique abordée par l'étude était la turbidité élevée du moût de sorgho de Mbayeri, nécessitant une clarification pour améliorer la qualité.

L'expérimentation a permis de découvrir que l'optimisation du rapport moût/*Grewia*, du temps d'agitation et de la vitesse conduisait à une réduction significative de la turbidité, de la couleur et des polyphénols, tout en augmentant la teneur en protéines. Les résultats ont indiqué une réduction de la turbidité d'environ 84% dans des conditions optimales, soulignant l'efficacité de la *Grewia mollis* dans le traitement du moût de sorgho opaque sans impact sur la lixiviation des nutriments. L'étude a également mis en évidence le rôle significatif de *Grewia mollis* dans le traitement des problèmes de turbidité et le potentiel d'utilisation de coagulants naturels peu coûteux pour la clarification du moût. En conclusion, l'étude a confirmé l'impact positif de la poudre d'écorce de *Grewia mollis* sur la clarification du moût de sorgho de Mbayeri, démontrant son potentiel en tant que coagulant naturel pour l'amélioration des caractéristiques physicochimiques du moût. Les paramètres optimisés se sont avérés efficaces pour réduire la turbidité tout en conservant les propriétés essentielles requises pour une fermentation réussie.

Dans l'ensemble, la recherche a souligné la faisabilité et les avantages de l'utilisation de *Grewia mollis* dans les processus de clarification du moût, offrant une voie prometteuse pour améliorer la qualité des boissons à base de sorgho.

4) Limegne, D. G. K., Desobgo, Z. S. C.*, & Nso, E. J. (2024). *Potential of β -Amylase from Sweet Potato (*Ipomoea batatas* Lam) Extract on the Mashing of Safrari Sorgho. *Journal of Food Processing and Preservation*, 2024.*

L'article explore l'utilisation de l'extrait enzymatique de patate douce pour améliorer le processus de saccharification dans le moût de sorgho. L'étude vise à remédier à la carence en β -amylases dans le malt de sorgho, qui affecte le processus de saccharification et se traduit par un faible extrait fermentescible dans le moût. En utilisant la patate douce comme source de β -amylases, les chercheurs cherchent à améliorer l'efficacité de la saccharification lors de la production de bière de sorgho.

L'étude a utilisé des plans de Box-Behnken pour déterminer les conditions optimales d'extraction des enzymes brutes et de saccharification du moût de sorgho *Safrari*. Les principaux facteurs pris en compte étaient le rapport masse-volume, le temps d'extraction et la température.

Les résultats ont démontré que dans les conditions optimales d'un rapport masse-volume de 0,1, d'un temps d'extraction de 210 minutes et d'une température de 60°C, l'activité enzymatique était maximisée, les activités enzymatiques théoriques et expérimentales étant proches. En outre, les paramètres physicochimiques du moût, y compris la turbidité, le pH, le brix, les sucres réducteurs et l'acidité titrable, se situaient dans des fourchettes spécifiques, démontrant l'efficacité de l'extrait d'*Ipomoea batatas* dans la saccharification du moût de sorgho.

En conclusion, les résultats de la recherche indiquent que l'extrait enzymatique de patate douce contient des β -amylases qui peuvent être utilisées efficacement dans le processus d'empâtage du sorgho *Safrari* malté. L'optimisation du processus d'extraction et l'amélioration subséquente de l'efficacité de la saccharification suggèrent une approche prometteuse pour surmonter les défis associés aux β -amylases déficientes dans le malt de sorgho. En utilisant des sources naturelles d'enzymes comme la patate douce, il est possible d'améliorer la qualité et la fermentescibilité du moût lors de la production de bière de sorgho, offrant ainsi une solution durable et efficace pour améliorer le processus de brassage.

En résumé, mes recherches dans le domaine de l'extraction et de la caractérisation des enzymes végétales et des composés bioactifs ont contribué de manière significative à l'avancement des connaissances scientifiques et à l'innovation dans l'industrie agroalimentaire et de la santé au Cameroun. En combinant une approche multidisciplinaire avec des méthodologies de pointe, nous avons cherché à développer des solutions durables pour répondre aux défis croissants de la production alimentaire et à promouvoir le développement socio-économique dans ma communauté et au-delà.

VI-2.3) Sous-thème 3 : Boissons probiotiques et fonctionnelles

À travers une série d'articles de recherche, nous avons exploré divers aspects de la production de boissons bénéfiques pour la santé, tout en valorisant les ressources locales et traditionnelles.

L'article intitulé "Antagonistic effects of raffia sap with probiotics against pathogenic microorganisms" témoigne de notre intérêt pour les interactions entre les ingrédients traditionnels et les probiotiques dans la lutte contre les micro-organismes pathogènes. Notre recherche met en évidence les propriétés bénéfiques de la sève de raphia en tant qu'agent antimicrobien naturel, ouvrant ainsi la voie à de nouvelles approches pour améliorer la sécurité alimentaire.

En parallèle, notre travail de terrain dans l'article "A field survey to assess the consumption of Nkang for standardization and valorization in the North-West region of Cameroon" reflète notre engagement envers la préservation et la valorisation des traditions culturelles locales. En évaluant la consommation de Nkang dans la région du Nord-Ouest du Cameroun, nous contribuons à la préservation de ce patrimoine culinaire des bières et à sa reconnaissance dans le cadre de la recherche scientifique.

Dans une autre étude, "Production and Characterization of a Probiotic Sorghum Beverage Fermented with Lactic Acid Bacteria (*Lactobacillus fermentum* and *Bifidobacterium bifidum*) and *Bil-bil*", nous avons exploré le potentiel des bactéries lactiques et du bil-bil dans la fermentation du sorgho pour produire une boisson probiotique. Cette recherche illustre notre volonté d'exploiter les propriétés bénéfiques des micro-organismes pour développer des produits alimentaires fonctionnels, tout en mettant en valeur les ingrédients locaux.

Enfin, dans "Optimization of extraction conditions of phenolic compounds from *Cymbopogon citratus* and evaluation of phenolics and aroma profiles of extract", nous explorons les propriétés antioxydantes et aromatiques des composés phénoliques extraits de la citronnelle. Cette recherche met en évidence notre intérêt pour les composés bioactifs présents dans les plantes locales, ouvrant la voie à de nouvelles applications dans l'industrie alimentaire.

Un fil conducteur scientifique émerge de ces articles, mettant en évidence votre engagement à explorer les interactions entre les micro-organismes, les ingrédients traditionnels et les composés bioactifs pour développer des produits alimentaires bénéfiques pour la santé. Notre recherche s'inscrit dans une démarche holistique qui intègre les aspects scientifiques, culturels et traditionnels, contribuant ainsi à la promotion de la santé et à la préservation du patrimoine culturel local.

1. *Arsene, M. M. J.**, *Desobgo, Z. S. C.*, *Ngoune, T. L.*, *Nyasha, K.*, & *Louis, K.* (2021). *Antagonistic effects of raffia sap with probiotics against pathogenic microorganisms. Foods and Raw materials*, 9(1), 24-31.

L'article présente une étude sur les effets antagonistes de la sève de raphia fermentée avec des probiotiques (*Lactobacillus fermentum* et *Bifidobacterium bifidum*) contre des micro-organismes pathogènes tels que *Escherichia coli*, *Listeria monocytogenes*, *Salmonella sp.* et *Bacillus cereus*.

Le contexte est que les probiotiques sont connus pour leurs propriétés bénéfiques, et de nombreuses études ont été menées pour explorer leurs avantages. Nous avons voulu évaluer les propriétés fonctionnelles de la sève de raphia, une boisson camerounaise, fermentée avec des probiotiques en étudiant son activité antagoniste contre les bactéries pathogènes. Ceci est important car les micro-organismes pathogènes deviennent résistants aux antibiotiques, et les probiotiques pourraient être une nouvelle alternative pour trouver de nouveaux composés ou organismes antimicrobiens.

L'étude a utilisé un plan expérimental Box-Behnken avec quatre facteurs (taux d'ensemencement de *L. fermentum* et *B. bifidum*, température et temps d'incubation) pour générer des modèles mathématiques. La méthode de diffusion sur disque a été utilisée pour évaluer l'effet antagoniste en mesurant les zones d'inhibition des bactéries pathogènes.

Les résultats ont montré que les zones d'inhibition étaient comprises entre 0 et 23 mm pour les différentes bactéries pathogènes. L'analyse des données a révélé que *L. fermentum* était efficace contre *B. cereus*, tandis que *B. bifidum* était efficace contre *Salmonella sp.*, *E. coli* et *B. cereus*. Le temps d'incubation a augmenté de manière significative toutes les zones d'inhibition. L'optimisation des modèles a révélé des zones d'inhibition maximales pour des taux d'ensemencement de *L. fermentum* et de *B. bifidum* de 2 % et 10 %, respectivement, une durée d'incubation de 48 heures et une température de 37 °C.

En conclusion, l'étude a démontré que la sève de raphia fermentée par *L. fermentum* et *B. bifidum* avait un effet antagoniste contre les bactéries pathogènes telles que *E. coli*, *L. monocytogenes*, *Salmonella sp.* et *B. cereus*. D'autres études ont été recommandées pour déterminer le mécanisme d'action et confirmer les effets bénéfiques sur des modèles animaux.

2. *Afek, A. A.*, *Desobgo, Z. S. C.*, & *Jong, N. E.* (2021). *A field survey to assess the consumption of nkang for standardization and valorization in the North-West region of Cameroon. Green and Sustainable Chemistry*, 11, 107-123.

L'article présente une enquête de terrain menée dans la région du Nord-Ouest du Cameroun pour évaluer la consommation de la bière de maïs, en particulier la Nkang, en vue de sa normalisation et de sa valorisation, potentielles. Le contexte est que les bières africaines traditionnelles comme la Nkang sont souvent opaques, moins attrayantes que les bières occidentales, et souffrent d'une mauvaise qualité hygiénique, d'une faible teneur en alcool, de variations organoleptiques et d'une conservation insatisfaisante.

Le problème est que la Nkang, malgré son importance culturelle et son appréciation par les consommateurs, se raréfie sur les marchés et que la petite quantité disponible est de mauvaise qualité, ce qui indique un risque d'extinction. L'étude visait à examiner les modes de consommation, les préférences et la pertinence culturelle du Nkang afin d'éclairer sa normalisation potentielle et l'amélioration de sa qualité.

Les résultats obtenus à partir des entretiens et des questionnaires ont révélé qu'il existe trois types de bière de maïs dans la région : Kwacha, Sha-ah et Nkang. La Nkang s'est avérée être la plus préférée des consommateurs en raison de son goût, de sa faible teneur en alcool, de sa clarté, de sa faible viscosité, de sa couleur attrayante et de son impact culturel. Cependant, il n'était pas souvent présent sur les marchés et la quantité disponible était de mauvaise qualité. Les consommateurs ont exprimé leur volonté d'acheter du Nkang clarifié et amélioré à un prix plus élevé et leur désir de préserver la tradition culturelle entourant sa consommation.

La conclusion tirée de l'enquête est que le Nkang est une bière de maïs culturellement importante et appréciée dans la région, mais que son processus de production doit être normalisé et sa qualité améliorée afin d'augmenter sa durée de conservation et ses possibilités de commercialisation. L'étude recommande de poursuivre les travaux visant à améliorer la qualité de la Nkang et les méthodes de conservation afin de la rendre durable et d'empêcher son extinction potentielle, car les consommateurs apprécient sa pertinence culturelle et sont prêts à soutenir sa production et sa consommation.

3. **Olivier, B., Ambindei, W. A., Ngwasiri, P. N., Wingang, M. C., Desobgo, Z. S. C., & Emmanuel Jong, N. (2023). Production and Characterization of a Probiotic Sorghum Beverage Fermented with Lactic Acid Bacteria (*Lactobacillus Fermentum* and *Bifidobacterium Bifidum*) and Bil-Bil. American Journal of Food Science and Technology, 11(3), 86-95.**

L'article présente une recherche sur la production et la caractérisation d'une boisson probiotique à base de sorgho fermenté avec des bactéries lactiques (*Lactobacillus fermentum* et *Bifidobacterium bifidum*) et de *bil-bil* (une bière traditionnelle à base de sorgho).

La demande de produits enrichis en probiotiques a augmenté en raison de leurs effets bénéfiques potentiels sur la santé. Toutefois, le marché des probiotiques est encore limité, essentiellement aux produits laitiers. L'introduction de boissons probiotiques provenant de sources non laitières, telles que les céréales, pourrait remédier à cette limitation et répondre à diverses préférences alimentaires.

L'étude visait à déterminer les conditions optimales de fermentation aérobie pour produire une boisson probiotique à base de sorgho en utilisant *L. fermentum*, *B. bifidum* et *bil-bil* comme ferments. Les objectifs spécifiques étaient d'optimiser la température de fermentation, la durée et les proportions de chaque composant fermentaire.

Les analyses microbiologiques ont montré que le *bil-bil* contenait des bactéries lactiques, ce qui en fait une source potentielle de probiotiques. Les grains de sorgho présentaient les caractéristiques requises pour la production de malt et de bière. Grâce à un plan de mélange D-optimal, nous avons optimisé les paramètres physicochimiques et microbiologiques de la boisson. Les conditions optimales étaient un taux d'inoculation de 10 % de *L. fermentum*, 7 % de *B. bifidum* et 83 % de *bil-bil*, avec une température de fermentation de 39,5 °C et une durée de 2 jours.

La boisson obtenue avait une acidité titrable de 3,15 mEqg ac.mal/mL, un pH de 3,05, une teneur en vitamine C de 74,28 mg/L, une teneur en polyphénols de 0,46 mg/mL, une teneur en sucres réducteurs de 0,86 mg/mL, des solides solubles totaux de 4,88°Brix, une charge probiotique de $25,11 \times 10^6$ CFU/mL, une turbidité de 409,38 EBC et une viscosité de 5,18 mPa.s.

L'étude a démontré la production réussie d'une boisson probiotique à base de sorgho par fermentation mixte, la fermentation en l'alcool étant réalisée par les levures présentes dans le *bil-bil* et la fermentation en l'acide lactique par *L. fermentum*, *B. bifidum* et les bactéries lactiques du *bil-bil*. La boisson obtenue respecte la concentration en probiotiques recommandée pour les effets bénéfiques potentiels sur la santé.

Muala, W. C. B., Desobgo, Z. S. C.*, & Jong, N. E. (2021). Optimization of extraction conditions of phenolic compounds from *Cymbopogon citratus* and evaluation of phenolics and aroma profiles of extract. *Heliyon*, 7(2021), 1-10.

L'article présente une recherche visant à optimiser l'extraction des composés phénoliques des feuilles de *Cymbopogon citratus* (citronnelle) à l'aide d'une méthode de décoction. Des auteurs ont souligné la demande croissante d'antioxydants naturels et de composés aromatiques dans diverses industries, telles que l'alimentation (boissons) et les cosmétiques, en raison de la préférence croissante des consommateurs pour les additifs naturels.

L'étude a utilisé un plan composite central (CCD) pour étudier les effets de trois variables indépendantes : le rapport solide/liquide, la température et le temps d'extraction, sur la teneur totale en polyphénols (TPC) et l'activité antioxydante (DPPH) de l'extrait de citronnelle. Le processus d'optimisation a été réalisé à l'aide de la méthodologie de la surface de réponse (RSM).

Les résultats ont montré que le rapport solide/liquide était le facteur le plus significatif affectant la TPC et l'activité DPPH. Des rapports plus élevés ont conduit à une augmentation des rendements d'extraction jusqu'à un point critique, après quoi un déclin a été observé en raison de la congestion des molécules qui entrave le transfert de masse. La température a eu un effet positif jusqu'à un certain niveau, au-delà duquel une dégradation des composés phénoliques s'est produite. Un temps d'extraction prolongé a d'abord augmenté le TPC mais a finalement conduit à une diminution du TPC et de l'activité DPPH en raison de la nature thermolabile des composés phénoliques. Les conditions optimales déterminées étaient un rapport solide/liquide de 5g/100mL, une température de 93,8°C et un temps d'extraction de 11,3 minutes. Dans ces conditions, les valeurs expérimentales obtenues étaient de $71,98 \pm 0,33$ mg GAE/100mL pour le TPC et de $80,63 \pm 0,49$ mg TE/100mL pour le DPPH, ce qui était en bon accord avec les valeurs prédites. Une analyse plus poussée de l'extrait optimisé a révélé que les acides caféique et syringique étaient les composés phénoliques prédominants, tandis que le citral et le géraniol étaient les principaux composés volatils contribuant au profil aromatique.

Les études ont permis de conclure que l'extrait de citronnelle obtenu dans des conditions optimisées pourrait être une source potentielle d'antioxydants naturels et de composés aromatiques pour diverses applications industrielles, en particulier dans l'industrie des boissons.

Dans l'ensemble, Notre contribution à la recherche, au développement de la science et de la culture repose sur une approche multidisciplinaire et collaborative, mobilisant les connaissances et l'expertise de plusieurs laboratoires et équipes de recherche de renom, tant sur le plan national qu'international. En combinant les sciences de l'ingénieur avec une compréhension approfondie des phénomènes biochimiques, nous nous efforçons de proposer

des solutions innovantes pour relever les défis complexes auxquels est confrontée l'industrie agroalimentaire au Cameroun. En définitive, Notre objectif est de contribuer à la création de produits de haute qualité, tout en valorisant les richesses du terroir camerounais/africain et en favorisant le développement durable de l'agro-industrie.

IV.1.2.b) Chapitre de livre

Desobgo, Z. S. C*. & Nso, E. J. 2021. Winemaking: Control, Bioreactor and Modelling of Process. In Joshi, V.K. and Ramesh, C. Ray (Eds), Wine Making: Basics and Applied Aspects. 1st edition. (pp. 495-519). CRC Press, Taylor & Francis Group.

Le document fournit un aperçu général de la fabrication du vin, de la surveillance, de la sécurité et du contrôle de la qualité, mettant en évidence les activités liées à chaque opération unitaire, aux caractéristiques et utilisations des bioréacteurs, et enfin, aux approches innovantes visant à optimiser l'efficacité du processus. L'agro-industrie est enracinée dans les systèmes de production, d'où les nombreuses procédures actuelles faisant l'objet d'études approfondies sur les méthodes visant à développer de meilleurs systèmes pour la sécurité et la qualité. La bioconversion est essentielle dans la production de vins de qualité, avec un accent sur les processus de fermentation. Le document explore les étapes d'extraction et de préparation du jus, mettant en lumière la nécessité d'une manipulation soignée pour éviter les goûts astringents et garantir la qualité. Il examine également les processus de fermentation et de fermentation malolactique, mettant l'accent sur l'impact de la température et de l'activité microbienne sur la qualité du vin. Le document discute en outre de la typologie et des utilisations des bioréacteurs, ainsi que des approches innovantes pour optimiser les opérations de la cave. Dans l'ensemble, le document met en valeur les processus complexes impliqués dans la fabrication du vin et l'importance de surveiller, de garantir la sécurité et de contrôler la qualité pour produire des vins exceptionnels répondant à la demande des consommateurs en matière de produits de meilleure qualité.

Les principaux objectifs intéressants dans le domaine de la vinification incluent l'optimisation des opérations de la cave pour réduire les coûts, accroître la vitesse et améliorer l'utilisation des ressources. Des avancées technologiques telles que les actions enzymatiques, l'utilisation de levures sélectionnées, la modification des démarreurs microbiens et l'immobilisation sont d'une importance capitale pour améliorer la qualité des vins et produire des vins aux caractéristiques variées. L'informatisation est également un aspect essentiel pour optimiser les processus de la cave en améliorant l'efficacité de la production, de la main-d'œuvre, des matériaux, de l'eau et de l'énergie. L'objectif est de mettre en œuvre des procédures et des technologies qui permettent de produire du vin de qualité à moindre coût, face à des marges bénéficiaires plus serrées et à une demande croissante de vins de qualité supérieure. La maturation du vin, le contrôle de la fermentation et l'application de méthodes d'ajustement sont également des objectifs clés pour produire des vins de qualité avec des caractéristiques sensorielles et microbiologiques stables. La recherche de vins excellents, répondant aux normes de qualité les plus élevées, tout en

maintenant une approche durable et respectueuse de l'environnement, est une priorité pour les producteurs de vin du monde entier.

IV.1.2.c) Doctorats Ph.D soutenus

Thèse de Doctorat PhD de Dr. Nguemogne Annick Chancelle

(ENSAI, Université de Ngaoundéré, Cameroun)

Directeurs : Pr Desobgo ZSC ; Pr Nso EJ

Thème : *Purification de la dextrinase limite du cultivar de sorgho Safrari (Sorghum bicolor L. moench)*

L'étude porte sur la purification et la caractérisation de la dextrinase limite du cultivar de sorgho « *Safrari* », une enzyme cruciale pour l'hydrolyse complète de l'amidon pendant le maltage et le brassage. Les objectifs étaient de maximiser la précipitation de la dextrinase limite à l'aide de sulfate d'ammonium suivi d'une dialyse, de caractériser le pH optimal de l'enzyme, la température, les paramètres cinétiques et les effets de divers additifs, et d'évaluer son impact sur la filtrabilité et la teneur en sucre des moûts de sorgho. Les hypothèses suggéraient que cette méthode de purification permettrait d'obtenir une enzyme partiellement purifiée comparable à celles d'autres céréales et qu'elle améliorerait la filtrabilité du moût. La méthodologie a consisté à optimiser le maltage du sorgho, à affiner la précipitation au sulfate d'ammonium pour obtenir une activité enzymatique spécifique maximale, à utiliser deux méthodes de dosage pour mesurer l'activité et à déterminer le pH optimal, la température, les paramètres cinétiques et les effets des additifs. L'étude a également évalué l'impact de l'enzyme sur la filtrabilité du moût et la teneur en sucre. Les résultats ont montré une purification de 6,5 fois et un rendement de 75 % à une saturation de 20 % en sulfate d'ammonium, l'enzyme présentant une activité optimale à un pH de 5,0 à 5,5 et à une température de 50 à 60°C. Les paramètres cinétiques étaient un K_m de 2,4 mg/mL et un V_{max} de 0,03 mg-1.mL-1.sec-1. Les agents réducteurs, le chlorure de calcium et l'albumine de sérum bovin ont renforcé l'activité enzymatique, et l'ajout de la dextrinase limite purifiée a amélioré la filtrabilité du moût. La méthode de purification a permis d'isoler avec succès la dextrinase limite, en éliminant l'interférence d'autres enzymes. Son pH et sa température optimaux la destinent à des applications industrielles, notamment à la brasserie. La forte affinité et l'efficacité catalytique de l'enzyme vis-à-vis de l'amidon, ainsi que l'amélioration par les agents réducteurs et le calcium, s'alignent sur le fait qu'il s'agit d'une enzyme sulfhydryle. L'amélioration de la filtrabilité du moût souligne son potentiel dans le domaine de la brasserie. Cette étude fournit des informations précieuses sur la purification et la caractérisation de l'enzyme, soulignant son application dans les processus de conversion de l'amidon. Bien que la purification ait été partielle, d'autres études pourraient permettre d'obtenir

une purification complète et une caractérisation plus détaillée, les travaux futurs portant sur ses performances dans les processus de brassage réels.

Thèse de Doctorat PhD de Dr. Ninga Kombélé
(ENSAI, Université de Ngaoundéré, Cameroun)

Directeurs : Pr Desobgo ZSC ; Pr Nso EJ

Thème : *Liquéfaction d'une pulpe de goyave à chair blanche (*Psidium guajava* Linn) par les pectinases industrielles d'*Aspergillus niger* et clarification du jus par centrifugation*

L'étude porte sur la dépectinisation de purée de goyave (*Psidium guajava*) à chair blanche à l'aide de pectinases commerciales provenant d'*Aspergillus niger* et sur la clarification ultérieure du jus de goyave dépectinisé par centrifugation. Les objectifs principaux comprennent la détermination du mécanisme de dépectinisation enzymatique, dont on suppose qu'il ne suit pas la cinétique classique de Michaelis-Menten ; l'évaluation de l'impact de la concentration de pectinase et du temps de contact enzyme-purée sur les caractéristiques physicochimiques du jus dépectinisé ; et l'évaluation de l'effet de la durée et de la vitesse de centrifugation sur le jus clarifié afin de prédire les performances d'une centrifugeuse à assiettes en continu basée sur une centrifugeuse à rotor conique à l'échelle du laboratoire. La méthodologie a consisté à modéliser la cinétique de la dégradation de la pectine commerciale et de la dépectinisation de la pectine de goyave à l'aide de l'équation de Hill, à analyser l'impact de la concentration enzymatique et du temps de contact sur les propriétés physicochimiques du jus, et à utiliser une centrifugeuse à rotor conique de laboratoire pour clarifier le jus à différentes vitesses et à différents moments. Les propriétés physicochimiques, la distribution de la taille des particules et l'efficacité de la clarification ont été évaluées, et les performances de la centrifugeuse de laboratoire ont été utilisées pour estimer les caractéristiques de fonctionnement d'une centrifugeuse à disques en continu. Les résultats indiquent que la dégradation de la pectine commerciale suit le modèle de Michaelis-Menten, tandis que la dépectinisation de la pectine de goyave présente une tendance sigmoïdale, le coefficient de Hill diminuant avec l'augmentation de la concentration enzymatique. Le traitement enzymatique a augmenté de manière significative la teneur en acide galacturonique et en solides solubles totaux et a modifié la couleur du jus, mais a eu des effets négligeables sur la capacité antioxydante. La centrifugation à différentes vitesses a permis d'obtenir des propriétés physicochimiques similaires pour le jus clarifié, la vitesse optimale étant de 1343 g. Les limites de séparation des particules et les seuils de coupure estimés ont été calculés à partir des résultats de la centrifugation. Les limites de séparation des particules et les tailles de coupure estimées pour les centrifugeuses ont également été déterminées. La cinétique sigmoïdale de la dépectinisation de la pectine de goyave a été attribuée aux mécanismes de

processivité et à l'inhibition substrat/produit des isoformes de pectinase, la diminution du coefficient de Hill indiquant des changements dans l'affinité enzyme-substrat. Les propriétés similaires du jus clarifié à des vitesses plus élevées suggèrent que la vitesse de 1343 g est optimale pour la centrifugeuse de laboratoire, tandis que l'augmentation de la durée de centrifugation conduit à un jus clarifié avec des particules plus fines. La méthodologie a permis de mieux comprendre la cinétique de dépectinisation enzymatique, les conditions optimales du processus et les effets des paramètres de centrifugation sur les propriétés du jus. Elle a également permis de développer une approche prédictive pour les opérations de centrifugation en continu de la pile de disques, basée sur les performances à l'échelle du laboratoire. Les contributions comprennent des aperçus de la cinétique non-Michaelis-Menten de la dépectinisation de la pectine de goyave, des conditions optimisées pour la dépectinisation de la purée de goyave, des effets démontrés de la centrifugation sur les propriétés du jus, et une méthodologie développée pour l'augmentation des opérations de centrifugation. L'étude est bien structurée, progresse logiquement au fil des chapitres et aboutit à des résultats significatifs applicables aux processus industriels. Les limites de l'étude sont l'accent mis sur un seul cultivar de goyave et la dépendance à l'égard des données de laboratoire pour les prévisions de centrifugation, ce qui suggère des recherches futures pour explorer différents cultivars et valider les performances de la centrifugeuse à empilement continu de disques à l'échelle industrielle.

Thèse de Doctorat PhD de Dr. Wiyeh Claudette Bakisu Muala

(ENSAI, Université de Ngaoundéré, Cameroun)

Directeurs : Pr Desobgo ZSC ; Pr Nso EJ

Thème : *Probiotic Beverage Production from a Mixture of Baobab (*Adansonia digitata* L.) Pulp and Lemongrass (*Cymbopogon citratus* L.) Extract using Lactic Acid Bacteria*

L'étude porte sur la création d'une boisson probiotique à partir d'un mélange de pulpe de baobab (*Adansonia digitata* L.) et d'extrait de citronnelle (*Cymbopogon citratus* L.) à l'aide de bactéries lactiques (LAB). La pulpe de baobab, riche en nutriments mais à forte teneur en pectine, et la citronnelle, source d'azote contribuant à l'arôme de la boisson, ont été combinées à cette fin. L'objectif principal était de produire une boisson fermentée probiotique à l'aide de LAB provenant de la pulpe de baobab. Les objectifs spécifiques comprenaient l'optimisation des conditions d'hydrolyse de la pectine de la pulpe de baobab, la détermination des meilleures conditions de décoction pour l'extraction des composants bioactifs de la citronnelle et l'identification des paramètres cinétiques de la fermentation lactique du jus de baobab et de citronnelle. La méthodologie comprenait l'hydrolyse de la pectine de baobab avec différentes concentrations de pectinase, l'optimisation de l'extrait de citronnelle à l'aide d'un plan composite central pour la teneur en polyphénols et l'activité antioxydante, ainsi que l'isolement et l'évaluation des attributs probiotiques du LAB (*Lactobacillus fermentum*) à partir de la pulpe de baobab. Les essais de fermentation ont modélisé la cinétique de croissance, l'utilisation du substrat et la formation du produit. Les résultats ont montré une hydrolyse optimale de la pectine avec 0,2% de pectinase pendant 120 minutes, augmentant la teneur en sucre réducteur de 11,84 g/L à 25,23 g/L, et des conditions optimales d'extraction de la citronnelle produisant une teneur élevée en polyphénols et une activité antioxydante. Le jus de baobab et de citronnelle 50/50 fermenté avec *L. fermentum* a atteint une biomasse maximale de 13,91 log CFU/mL. La discussion a mis en évidence l'amélioration du profil nutritionnel du jus et l'efficacité de la fermentation par *L. fermentum*, en particulier dans le mélange 50/50. L'étude contribue au domaine en démontrant le potentiel des LAB autochtones pour la production de boissons probiotiques à partir de ressources sous-utilisées comme le baobab et la citronnelle, avec une hydrolyse optimisée, des conditions d'extraction et une cinétique de fermentation détaillée qui fournissent des informations précieuses sur le développement. La thèse est bien structurée, organisée de manière logique, et les résultats font progresser de manière significative la compréhension du potentiel de ces ressources végétales dans les boissons fonctionnelles.

Toutefois, l'étude manque de données sur l'évaluation sensorielle et la stabilité de la durée de conservation, ce qui suggère que les recherches futures devraient aborder ces aspects et explorer les avantages de la boisson pour la santé par le biais d'études in vivo.

Thèse de Doctorat PhD de Dr. Calister Makebe, Wingang

(ENSAI, Université de Ngaoundéré, Cameroun)

Directeurs : Pr Desobgo ZSC ; Pr Nso EJ

Thème : *Development of probiotic fermented beverage from soursop (Annona muricata Linn.) fruit*

L'étude vise à développer une boisson fermentée probiotique à partir du corossol (*Annona muricata* Linn.), un fruit tropical hautement périssable aux propriétés nutritionnelles et fonctionnelles significatives, afin de réduire les pertes post-récolte par la transformation en d'autres produits. Les principaux objectifs étaient d'optimiser les conditions d'extraction assistée par pectinase du jus de corossol et de modéliser sa fermentation à l'aide de *Lactobacillus casei*, *Lactobacillus acidophilus* et leur consortium. En utilisant le plan de Doehlert, l'étude a optimisé l'extraction assistée par pectinase en examinant les effets du temps d'incubation, de la concentration enzymatique et de la température. Le jus extrait a été fermenté en mode discontinu avec les souches probiotiques sélectionnées, et la cinétique de fermentation a été modélisée à l'aide des modèles de Monod, Luedeking-Piret et logistique. Les conditions optimales d'extraction étaient 172 minutes d'incubation, 0,04% de concentration enzymatique, et 42,9°C, produisant un taux d'extraction de 75,2%, avec un pH de 3,74, 87,06% de clarté, 7,35°Brix TSS, et 0,44% d'acidité titrable. Le consortium de *Lactobacillus acidophilus* et *Lactobacillus casei* a atteint la croissance cellulaire la plus élevée, soit 11,5 log CFU/mL après 36 heures de fermentation. Le traitement à la pectinase a permis de liquéfier efficacement la pulpe de corossol, et la cinétique de fermentation a été décrite avec précision par les modèles utilisés, le consortium probiotique affichant la meilleure croissance et la meilleure production de métabolites. Cette étude donne des indications précieuses sur l'optimisation de l'extraction du jus de corossol et le développement d'une boisson fermentée probiotique à partir de ce fruit tropical sous-utilisé. L'article est bien structuré, progressant logiquement du contexte à la méthodologie, aux résultats, à la discussion et aux conclusions. La recherche a optimisé avec succès les conditions d'extraction et développé une boisson probiotique à partir du corossol, démontrant le potentiel du fruit pour la valeur ajoutée et la diversification des produits alimentaires. Cependant, l'étude n'a pas abordé les attributs sensoriels, la durée de conservation ou l'acceptabilité de la boisson par le consommateur, ce qui suggère que les recherches futures devraient se concentrer sur ces aspects pour mieux évaluer sa viabilité commerciale.

Thèse de Doctorat PhD de Dr. Katlezir Fiacre

(Université de Ndjamena, Tchad)

Directeurs : **Pr Desobgo ZSC** ; Pr Mohagir Ahmed Mohammed

Thème : *Modélisation et optimisation de l'extraction et de fermentation de jus de datte (*Phoenix dactylifera* L.) « Bournow » par la méthodologie des surfaces de réponses*

L'étude vise à modéliser et à optimiser les processus d'extraction et de fermentation du jus de datte (*Phoenix dactylifera* L.) « Bournow » en utilisant la méthodologie de la surface de réponse (RSM). Les objectifs étaient de caractériser les propriétés physicochimiques et morphologiques de la variété de dattes Bournow, de déterminer les conditions optimales d'extraction des composés majeurs et mineurs, et d'optimiser les conditions de fermentation du jus avec des souches probiotiques. L'hypothèse suggère que les dattes Bournow ont des caractéristiques morphologiques médiocres, mais que des conditions d'extraction optimales pourraient produire des éléments physicochimiques souhaitables et maximiser les composés mineurs. Un plan composite central (CCD) à quatre facteurs a été utilisé pour étudier les effets des paramètres d'extraction (température, durée, rapport eau/pulpe et volume d'enzymes) sur les composés majeurs et mineurs, tandis que le processus de fermentation a été analysé à l'aide d'un plan de Box-Behnken pour étudier les effets de la durée, de la dose de ferment et du Brix initial. Les résultats ont révélé que les dattes Bournow présentaient des caractéristiques de qualité médiocres. Les conditions d'extraction optimales (95°C, 10 minutes, rapport eau/pulpe de 2:1, volume d'enzyme de 0,5 ml) ont produit des composés majeurs avec un Brix de 21,89°B, un rapport sucre/acide de 13,99, et une couleur de 197,49ASBC. Pour les composés mineurs, les mêmes conditions d'extraction ont donné des acides aminés libres à 587,88 mg/L, des polyphénols totaux à 6,25 g GAE/100 g MS, de la vitamine C à 116,5 mg/L et un pH de 4,13. La fermentation optimisée a modifié de manière significative les propriétés physicochimiques du jus, démontrant une fermentation efficace par les souches probiotiques. Cette étude donne des indications précieuses sur l'utilisation de la variété de dattes Bournow pour la production de boissons fonctionnelles, en utilisant la méthodologie de la surface de réponse pour améliorer les propriétés nutritionnelles et fonctionnelles du jus. L'article bien structuré présente logiquement le contexte, les objectifs, la méthodologie, les résultats, la discussion et les conclusions, le tout agrémenté de sous-titres et d'aides visuelles. Les résultats soulignent le potentiel des variétés de dattes sous-utilisées pour le développement de boissons fonctionnelles et la diversification des produits. Les recherches futures pourraient valider les effets

probiotiques, évaluer le profil aromatique de la boisson et augmenter le processus de production.

IV.1.3) Contribution à la gouvernance de l'université et de l'enseignement supérieur

La gouvernance universitaire est un processus complexe qui nécessite l'implication de tous les acteurs clés. En tant qu'enseignant, pierre angulaire de l'institution, je considère qu'il est de mon devoir de contribuer activement à cette gouvernance afin de garantir l'excellence de notre établissement.

Avant tout, mon rôle premier est d'offrir aux étudiants un enseignement et un encadrement de qualité supérieure. C'est en formant des apprenants compétents et engagés que nous bâtissons les fondations d'une société prospère. Mais mon implication ne s'arrête pas aux murs de la salle de classe. J'effectue régulièrement des missions de prospection et d'encadrement de stagiaires, partageant mon expertise avec la prochaine génération de professionnels.

Au fil des années, j'ai eu l'honneur d'assurer l'intérim de Chef de département à plusieurs reprises, notamment pour le Génie Alimentaire et Contrôle Qualité. Ces responsabilités m'ont permis de contribuer directement à la gestion et à l'organisation de nos programmes, veillant à ce que nos actions soient toujours guidées par l'excellence académique.

Cependant, la gouvernance ne se limite pas à la gestion quotidienne. Elle implique également une vision à long terme et une planification stratégique rigoureuse. C'est pourquoi je me suis pleinement impliqué dans la refonte de nos programmes pour les arrimer au système Licence-Master-Doctorat (LMD). Cette réforme ambitieuse vise à aligner notre offre de formation sur les normes internationales, garantissant ainsi la compétitivité de nos diplômés sur le marché du travail. Mais mon engagement ne s'est pas limité à cette noble cause. J'ai également l'honneur de faire partie de l'équipe chargée de concevoir les programmes de master universitaire de technologie (MUT) en technologie alimentaire et en nutrition et diététique. C'est un véritable défi, mais aussi une opportunité unique de façonner l'avenir de ces disciplines cruciales. Avec mes collègues experts, nous avons analysé les besoins du marché du travail, les dernières tendances scientifiques et les meilleures pratiques pédagogiques. Ensemble, nous sommes entrain de concevoir des programmes d'études innovants, équilibrés et adaptés aux réalités contemporaines. Les programmes que nous allons élaborer offriront aux étudiants une formation de pointe, alliant théorie et pratique, et leur permettant de développer les compétences requises pour exceller dans leurs domaines respectifs. Qu'il s'agisse de maîtriser les procédés de transformation alimentaire ou de promouvoir une alimentation saine et équilibrée, nos diplômés seront armés pour relever les défis de demain.

Ma contribution à la gouvernance universitaire va au-delà de simples tâches administratives. C'est un engagement profond envers l'excellence académique, la formation de qualité et le progrès constant de notre institution. En collaborant étroitement avec mes collègues, en

partageant nos visions et en unissant nos efforts, nous façonnons l'avenir de l'enseignement supérieur, pierre par pierre.

Car au final, la véritable gouvernance universitaire réside dans notre capacité à transcender les silos et à travailler ensemble, dans un esprit d'ouverture et de synergies constructives, pour offrir aux générations futures un environnement d'apprentissage et de recherche à la hauteur de leurs aspirations.

IV.1.4) Contribution au développement de la nation

En tant qu'académicien passionné, je considère qu'il est de mon devoir de contribuer activement au développement de notre nation. L'enseignement supérieur et la recherche ne doivent pas être des tours d'ivoire déconnectées des réalités du terrain. Au contraire, elles doivent être des moteurs de progrès, des catalyseurs de changement et des sources d'inspiration pour l'ensemble de la société.

C'est dans cet esprit que j'ai noué des partenariats fructueux avec des acteurs clés du développement économique national. Ma collaboration avec l'Agence de Promotion des Petites et Moyennes Entreprises (APME) m'a permis de mettre mon expertise au service des entrepreneurs camerounais, leur offrant un accompagnement sur mesure pour développer leurs activités.

De même, mon travail avec l'Agence des Normes et de la Qualité (ANOR) a été une expérience enrichissante. En partageant mes connaissances en matière de contrôle qualité et de normalisation, j'ai pu aider de nombreuses entreprises à améliorer leurs processus de production et à garantir la conformité de leurs produits aux normes en vigueur.

Depuis 2023, j'ai eu l'honneur de siéger au sein du prestigieux comité scientifique du projet de développement et de modernisation des procédés de transformation, de conservation et de conditionnement agroalimentaire (PDEMOPTRACCA). En tant qu'expert reconnu dans le domaine, mon rôle a été d'apporter mon expertise et ma vision éclairée pour évaluer, sélectionner et accompagner les projets les plus prometteurs. Avec rigueur et discernement, j'ai examiné de nombreuses propositions novatrices visant à révolutionner les méthodes de transformation, de conservation et de conditionnement des produits alimentaires. Mon objectif était d'identifier les solutions les plus durables, efficaces et respectueuses de l'environnement, capables de répondre aux défis actuels et futurs de l'industrie agroalimentaire. Grâce à une collaboration étroite avec les autres membres du comité, ces projets ont pu aller jusqu'à leur maturation et leur financement, contribuant ainsi à l'émergence de nouvelles technologies et pratiques de pointe dans ce secteur stratégique.

J'ai également l'insigne privilège de faire partie du comité scientifique du prestigieux "Salon International des Industries et Techniques Agro-pastorales du Septentrion" (SIAGROS). En cette qualité, je contribue activement à la sélection des innovations les plus prometteuses dans les domaines de l'agriculture, de l'élevage et des technologies associées. Mes conseils avisés guident les exposants vers l'excellence, garantissant ainsi aux visiteurs une immersion de qualité dans les dernières avancées du secteur. C'est avec passion et dévouement que j'œuvre au rayonnement de cet événement incontournable.

Mais mon engagement ne s'arrête pas là. Je suis fier d'accompagner plusieurs entrepreneurs du "Made in Cameroon" dans le secteur des boissons, entre autres l'Union Camerounaise des Brasseries (UCB), qu'il s'agisse de bières, de vins, de jus ou de spiritueux. Ensemble, nous travaillons à développer de nouveaux produits innovants, répondant aux goûts et aux attentes des consommateurs locaux.

Cette démarche s'inscrit pleinement dans la perspective de l'import-substitution, un enjeu crucial pour notre pays. En valorisant nos ressources nationales et en stimulant la production locale, nous renforçons notre indépendance économique et créons de nouvelles opportunités d'emploi pour notre jeunesse.

Ma contribution ne se limite pas à un simple transfert de connaissances techniques. Je m'efforce d'insuffler une véritable culture entrepreneuriale auprès de ces acteurs économiques, les encourageant à innover, à prendre des risques calculés et à repousser constamment les limites de leur créativité.

Car c'est en libérant le potentiel de nos entrepreneurs, en les aidant à transformer leurs idées en réalités tangibles, que nous bâtissons un avenir prospère pour notre nation. Chaque nouvelle entreprise florissante, chaque emploi créé, chaque produit "Made in Cameroon" qui conquiert les marchés, est une victoire collective qui nous rapproche un peu plus de notre objectif de développement durable.

Bien sûr, ce chemin n'est pas sans embûches. Mais c'est précisément dans ces moments de défis que mon rôle prend tout son sens. En tant que guide, mentor et facilitateur, je m'efforce de lever les obstacles, de combler les lacunes et d'ouvrir de nouvelles perspectives pour ces entrepreneurs courageux.

Car au final, contribuer au développement de notre nation, c'est bien plus qu'une simple mission académique. C'est un engagement profond envers notre avenir commun, une détermination inébranlable à bâtir un Cameroun fort, prospère et fier de ses réalisations.

IV.2) Liste des enseignements dispensés

REPUBLIQUE DE CAMEROUN

Paix - Travail - Patrie

MINISTRE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE



REPUBLIC OF CAMEROON

Peace - Work - Fatherland

MINISTRY OF HIGHER EDUCATION
THE UNIVERSITY OF NGAOUNDERE



INSTITUT UNIVERSITAIRE DE TECHNOLOGIE

DEPARTEMENT DE GENIE ALIMENTAIRE ET CONTRÔLE QUALITE

B.P. 455 NGAOUNDERE Tel. (237) 699 52 37 27 / 6 79 32 75 96

Le Chef de Département

Ngaoundéré, le 10 JUIN 2024

Unités d'enseignement dispensées par Pr DESOBGO ZANGUE Steve Carly à l'IUT de
l'Université de Ngaoundéré

Unité d'enseignement	Mention/Parcours	Année académique
Enzymologie	GBIO 1	2008-2020
Dessin technique et Schémas des Procédés	GBIO 1	Depuis 2016
Opérations unitaires des procédés alimentaires	BTS IAL	2024
Process de l'industrie alimentaire	BTS IAL	Depuis 2022
Projet de production alimentaire	BTS IAL	Depuis 2022
Malterie – Brasserie	IAB 2	Depuis 2009
Sucrierie	IAB 2	2024
Projet tutoré	IAB 2	Depuis 2009
Malterie - Brasserie	IAB 3	Depuis 2009
Projet professionnel et personnel	IAB 3	Depuis 2009
Biostatistiques	IAB 3	Depuis 2011
Ingénierie des équipements	IAB 3	Depuis 2016
Enzymes industrielles et applications	IAB 3	2009-2020
Vins et spiritueux	IAB 3	2009-2017, 2024

Le Chef de Département



Pr. Saïdou Clement
Food Technology

REPUBLIQUE DU CAMEROUN

Paix – Travail – Patrie

UNIVERSITE DE NGAOUNDERE

ECOLE NATIONALE SUPERIEURE
DES SCIENCES INDUSTRIELLES

Département de Génie des Procédés et
d'Ingénierie

B.P. 454 Ngaoundéré

Tél. (237) 222 25 40 22

Courriel: ensai@univ-ndere.cm



REPUBLIC OF CAMEROON

Peace – Work – Fatherland

THE UNIVERSITY OF NGAOUNDERE

NATIONAL SCHOOL OF AGRO-
INDUSTRIAL SCIENCES

Department of Process Engineering

P.O. Box: 454 Ngaoundéré

Tel. (237) 222 25 40 22

E-mail: ensai@univ-ndere.cm

Unités d'enseignement dispensées par Pr DESOBGO ZANGUE Steve
Carly au Département de Génie des Procédés et d'Ingénierie

Unité d'enseignement	Mention/Parcours	Année académique
Plans d'expériences	Master 2	Depuis 2016
Génie de la formulation	Master 2	Depuis 2021
Opérations unitaires de transfert	Master 1	2016-2021
Projet professionnel et personnel	IAA 2	Depuis 2016
Production et maturation des spiritueux	Master PGIF	Depuis 2023
Méthodes d'analyse et de modélisation des données	Master PGIF	Depuis 2023

Le Chef de Département



P. Koko Guillaume P.
Maitre de Conférences

10 JUIN 2024

IV.3) Activités d'encadrement des étudiants

IV.3.1) Thèses de Doctorat PhD

**DEPART EMENT DE GENIE DES PROCEDES ET INGENIERIE****DEPARTMENT OF PROCESS ENGINEERING****UNITE DE FORMATION DOCTORALE GENIE DES PROCEDES****PURIFICATION DE LA DEXTRINASE LIMITE DU CULTIVAR DE SORGHO****SAFRARI (*Sorghum bicolor* L. Moench)****THESE**Présentée en vue de l'obtention du diplôme de **Doctorat/Ph.D**

Parcours/Spécialité : Génie des Procédés

17⁰ JUIN 2024

Par :

NGUEMOGNE Annick Chancelle*Master es Sciences et Technologie**(Option : Génie Alimentaire et Bioprocédés)***Matricule : 09S181EN****Jury :**

NDJOUENKEU Robert	Professeur	Université de Ngaoundéré	Président
MBOUGUENG Pierre Désiré	Maitre de Conférences	Université de Ngaoundéré	Examineur
DJOULDE DARMAN Roger	Maitre de Conférences	Université de Maroua	Rapporteur
FOMBANG Edith	Maitre de Conférences	Université de Ngaoundéré	Rapporteur
JIOKAP NONO Yvette	Maitre de Conférences	Université de Ngaoundéré	Rapporteur
NSO Emmanuel JONG	Professeur	Université de Ngaoundéré	Directeur
DESOBGO ZANGUE Steve C.	Maitre de Conférences	Université de Ngaoundéré	Co-Directeur

Année académique 2019/2020



Ecole Nationale des Sciences Agro-
Industrielles (ENSAI)

National School of Agro-Industrial Sciences
(ENSAI)

RAPPORT DE SOUTENANCE DE THESE DE DOCTORAT/Ph.D

GENIE DES PROCÉDES (GP)

VIVA VOCE REPORT OF THE Ph.D Thesis

Delof

NGUEMOGNE Annick Chancelle

Matricule : 09S181EN

Date : 18/05/2020

Sujet de Thèse/Title of Thesis

Purification de la dextrinase limite du sorgho Safrari (Sorghum bicolor L. Moench)

Jury / Board of Examiners:

Président: M. NDJOUENKEU Robert, Professeur, Université de Ngaoundéré
Rapporteurs: M. DJOULDE DARMAN Roger, Maître de Conférences, Université de Maroua
Mme FOMBANG Edith, Maître de Conférences, Université de Ngaoundéré
Mme JIOKAP NONO Yvette, Maître de Conférences, Université de Ngaoundéré
Membres: M. MBOUGUENG Pierre Désiré, Maître de Conférences, Université de Ngaoundéré
Directeurs: M. NSO Emmanuel JONG, Professeur, Université de Ngaoundéré
M. DESOBGO ZANGUE Steve C., Maître de Conférences, Université de Ngaoundéré

EVALUATION

Quelle mention accordez-vous par rapport à : Score the Viva voce on the following basis :	Mention / Merit			
	Excellent Excellent	Très Bien Very Good	Bien Good	Assez Bien Fairly Good
La clarté de la présentation / Clarity of the presentation	X			
La pertinence de la recherche menée par le candidat Relevance of research work carried out	X			
Défense de l'originalité de la thèse/ Defence of the originality of the thesis		X		
Techniques et méthodes de recherche Research methods and techniques		X		
Avancée dans la résolution du problème posé Contribution to the attainment of the general objectives of the thesis work	X			
Clarification des points d'ambiguïté dans la thèse Clarification of points of ambiguity		X		
Contribution à la Connaissance Scientifique Contribution to knowledge	X			
Possibilité de publication Potential of publishing results obtained	X			

Autres commentaires et Mention du Diplôme/ Other comments and Merit of Degree:

Mademoiselle NGUEMOGNE a présenté en une quarantaine de minutes, avec dynamisme et clarté, un travail scientifique de haute facture portant sur l'optimisation des conditions de production, d'extraction et de purification de la dextrinase limite du sorgho safrari. Les résultats présentés mettent en évidence les potentialités de la dextrinase limite du safrari dans l'amélioration de la filtrabilité des moûts. La méthodologie utilisée, la qualité et la densité des résultats, associées à leur applicabilité, confirment la pertinence de la recherche menée. La clarté de la présentation et la qualité des réponses apportées lors du débat témoignent des bonnes aptitudes scientifiques de la candidate. Compte tenu de ce qui précède, le jury à l'unanimité, accepte la thèse et décerne à Mademoiselle NGUEMOGNE Annick Chancelle le Doctorat/PhD en Sciences de l'Ingénieur, Mention Génie des Procédés, avec la MENTION TRES HONORABLE.

Signatures :

Président ... NDJOUENKEU Robert

Membres 1. MBOUGUENG Pierre Désiré

2. DJOULDE DARMAN Roger

3. FOMBANG Edith

4. JIOKAP NONO Yvette

5. NSO Emmanuel JONG

6. DESOBGO ZANGUE Steve C.

UNIVERSITE DE NGAOUNDERE

*Ecole Nationale Supérieure des
Sciences Agro-Industrielles*



THE UNIVERSITY OF NGAOUNDERE

*National School of Agro-Industrial
Sciences*

DEPARTEMENT DE GENIE DES PROCEDES ET D'INGENIERIE

DEPARTMENT OF PROCESS ENGINEERING

UNITE DE FORMATION DOCTORALE GENIE DES PROCEDES

**Liquéfaction d'une pulpe de goyave à chair blanche (*Psidium guajava*
Linn) par les pectinases industrielles d'*Aspergillus niger* et
clarification du jus par centrifugation**

THESE

Présentée en vue de l'obtention du diplôme de **DOCTORAT/Ph.D. en Sciences de l'Ingénieur**

Mention : Génie des Procédés

Spécialité : Génie Alimentaire et Bioprocédés

Par

NINGA KOMBELE Aimé

Master en Sciences et Technologie en Génie des Procédés

Matricule : 08I032EN

Jury:

ESSIA NGANG Jean Justin	Professeur	Université de Yaoundé I	Président – Rapporteur
TAVEA Frédéric Marie	Maître de Conférences	Université de Douala	Rapporteur
TCHATUENG Jean Bosco	Maître de Conférences	Université de Ngaoundéré	Rapporteur
KAYEM Joseph	Maître de Conférences	Université de Ngaoundéré	Examineur
NSO Emmanuel JONG	Professeur	Université de Ngaoundéré	Directeur
DESOBGO ZANGUE Steve C.	Maître de Conférences	Université de Ngaoundéré	Directeur

Année académique 2020/2021



RAPPORT DE SOUTENANCE DE THESE DE DOCTORAT/Ph.D

JUN 2024

Spécialité : Génie des Procédés (GP)
VIVA VOCE REPORT OF THE Ph.D Thesis

De/of
NINGA KOMBELE Aimé

Matricule : 081032EN

Date: 27/09/2021

Sujet de Thèse/Title of Thesis

Liquéfaction d'une pulpe de goyave à chair blanche (*Psidium guajava* Linn) par les pectinases industrielles d'*Aspergillus niger* et clarification du jus par centrifugation

Jury / Board of Examiners:

Président/Rapporteur : ESSIA NGANG Jean Justin, Professeur, Université de Yaoundé I
Rapporteurs : TCHATCHUENG Jean Bosco, Maître de Conférences, Université de Ngaoundéré
Examineur : KAYEM Joseph, Maître de Conférences, Université de Ngaoundéré
Directeurs : NSO Emmanuel JONG, Professeur, Université de Ngaoundéré
DESOBGO ZANGUE Steve Carly, Maître de Conférences, Université de Ngaoundéré

EVALUATION

Quelle mention accordez-vous par rapport à : Score the Viva voce on the following basis :	Mention / Merit			
	Excellent Excellent	Très Bien Very Good	Bien Good	Assez Bien Fairly Good
La clarté de la présentation / Clarity of the presentation		X		
La pertinence de la recherche menée par le candidat Relevance of research work carried out	X			
Défense de l'originalité de la thèse/ Defence of the originality of the thesis	X			
Techniques et méthodes de recherche Research methods and techniques	X			
Avancée dans la résolution du problème posé Contribution to the attainment of the general objectives of the thesis work		X		
Clarification des points d'ambiguïté dans la thèse Clarification of points of ambiguity	X			
Contribution à la Connaissance Scientifique Contribution to knowledge	X			
Possibilité de publication Potential of publishing results obtained	X			

Autres commentaires et Mention du Diplôme/ Other comments and Merit of Degree:

Monsieur NINGA KOMBELE Aimé a fait un exposé clair, précis et très pédagogique de ses travaux sur la liquéfaction d'une pulpe de goyave à chair blanche (*Psidium guajava* Linn) par les pectinases industrielles d'*Aspergillus niger* et clarification du jus par centrifugation. Il a établi le mécanisme de dépectinisation enzymatique sur la base du modèle de Hill montrant la différence avec le modèle classique de Michaelis Menten. Une mise en évidence des particules résiduelles par microscopie électronique après traitement enzymatique lui a permis de montrer la nécessité de clarifier son jus par centrifugation, opération unitaire dont il a déterminé les paramètres optimaux pour son application à grande échelle.

Au regard de tout ce qui précède, le jury reçoit le mémoire de thèse de M. NINGA KOMBELE Aimé et lui attribue le titre de Docteur / Ph.D en Sciences de l'Ingénieur, mention Génie des Procédés, parcours Génie Alimentaire et Bioprocédés avec la Mention Très Honorable

Signatures :

Président ... ESSIA NGANG Jean Justin

Membres 1. TCHATCHUENG Jean Bosco

2. KAYEM Joseph

3. NSO Emmanuel JONG

4. DESOBGO ZANGUE Steve Carly

UNIVERSITE DE NGAOUNDERE
*Ecole Nationale Supérieure des
Sciences Agro-Industrielles*



THE UNIVERSITY OF NGAOUNDERE
*National School of Agro-Industrial
Sciences*

DEPARTEMENT DE GENIE DES PROCEDES ET D'INGENIERIE

DEPARTMENT OF PROCESS ENGINEERING

UNITE DE FORMATION DOCTORALE GENIE DES PROCEDES

11th 0 JUN 2024

**Probiotic Beverage Production from a Mixture of *Baobab (Adansonia digitata* L.) Pulp and Lemongrass (*Cymbopogon citratus* L.) Extract
using Lactic Acid Bacteria**

A THESIS

Submitted in partial fulfilment for the award of a **DOCTORATE/Ph.D. Degree in Engineering Sciences**

Option: Process Engineering

Specialty: Food Engineering and Bioprocessing

BY:

WIYEH Claudette BAKISU MUALA

Master of Science and Technology in Process Engineering

Registration number: 11I092EN

Jury:

ESSIA NGANG Jean Justin	Professor	University of Yaounde I	President/ Rapporteur
TATSADJIEU NGOUNE L.	Professor	University of Ngaoundere	Rapporteur
NGAH Esther	Associate Professor	University of Ngaoundere	Examiner
MBOUGUENG Pierre D.	Associate Professor	University of Ngaoundere	Examiner
NSO Emmanuel JONG	Professor	University of Ngaoundere	Supervisor
DESOBGO ZANGUE Steve C.	Associate Professor	University of Ngaoundere	Supervisor

Academic year 2021/2022



Ecole Nationale des Sciences Agro-
Industrielles (ENSAI)

National School of Agro-Industrial Sciences
(ENSAI)

RAPPORT DE SOUTENANCE DE THESE DE DOCTORAT/Ph.D

GENIE DES PROCÉDES (GP)

VIVA VOCE REPORT OF THE Ph.D Thesis

Del/of

WIYEH Claudette BAKISU MUALA

Matricule : 111092EN

Date: 13/01/2022

Sujet de Thèse/Title of Thesis

Probiotic Beverage Production from a mixture of Baobab (*Adansonia digitata* L.) Pulp and Lemongrass (*Cymbopogon citratus* L.) Extract using Lactic Acid Bacteria.

Jury / Board of Examiners:

Président/Rapporteur : M. ESSIA NGANG Jean Justin, Professeur, Université de Yaoundé
Rapporteur : M. TATSADJIEU NGOUNE Léopold, Professeur, Université de Ngaoundéré
Membres : Mme NGAH Esther, Maître de Conférences, Université de Ngaoundéré
M. MBOUGUENG Pierre Désiré, Maître de Conférences, Université de Ngaoundéré
Directeurs : M. NSO Emmanuel JONG, Professeur, Université de Ngaoundéré
M. DESOBGO ZANGUE Steve Carly, Maître de Conférences, Université de Ngaoundéré

EVALUATION

Quelle mention accordez-vous par rapport à : Score the Viva voce on the following basis :	Mention / Merit			
	Excellent Excellent	Très Bien Very Good	Bien Good	Assez Bien Fairly Good
La clarté de la présentation / Clarity of the presentation	X			
La pertinence de la recherche menée par le candidat Relevance of research work carried out	X			
Défense de l'originalité de la thèse/ Defence of the originality of the thesis		X		
Techniques et méthodes de recherche Research methods and techniques	X			
Avancée dans la résolution du problème posé Contribution to the attainment of the general objectives of the thesis work		X		
Clarification des points d'ambiguïté dans la thèse Clarification of points of ambiguity		X		
Contribution à la Connaissance Scientifique Contribution to knowledge	X			
Possibilité de publication Potential of publishing results obtained	X			

Autres commentaires et Mention du Diplôme/ Other comments and Merit of Degree:

Madame WIYEH Claudette BAKISU MUALA a fait un exposé clair, précis et très pédagogique de ses travaux de recherche. Elle a déterminé les conditions optimales d'hydrolyse enzymatique de la pectine de baobab et celles d'extraction des composés bioactifs de *C. citratus*. A partir de ces intrants, elle a su définir les conditions et le meilleur mélange permettant la survie de *Lactobacillus fermentum* isolé dans la pulpe de baobab pour la mise en place d'une boisson à base de probiotique. La qualité des résultats obtenus, les perspectives d'industrialisation, la pertinence des réponses aux questions et la qualité de sa publication dans une revue spécialisée justifient que le jury accepte la thèse de Mme WIYEH Claudette BAKISU MUALA et lui décerne à l'unanimité le titre de Docteur/Ph.D en Sciences de l'Ingénieur, Mention Génie des Procédés, Parcours Génie Alimentaire et Bioprocédés de l'Université de Ngaoundéré avec la Mention Très Honorable.

Signatures :

Président ... ESSIA NGANG Jean J.

Membres 1. TATSADJIEU NGOUNE L.

2. Mme NGAH Esther.....

3. MBOUGUENG Pierre D.

4. NSO Emmanuel JONG

5. DESOBGO ZANGUE S. C.



DEPARTEMENT DE GENIE DES PROCEDES ET D'INGENIERIE

DEPARTMENT OF PROCESS ENGINEERING

UNITE DE FORMATION DOCTORALE GENIE DES PROCEDES

**Development of probiotic fermented beverage from
soursop (*Annona muricata* Linn.) fruit**

A THESIS

Submitted in partial fulfilment for the award of a DOCTORATE/Ph.D. Degree in Engineering Sciences

Option: Process Engineering

Specialty: Food Engineering and Bioprocessing

BY

MAKEBE Calister WINGANG

Master of Sciences and Technology in Process Engineering

Registration number: 111068EN

Jury:

NDJOUENKEU Robert	Professor	University of Ngaoundere	President
TAVEA Frédéric Marie.	Associate Professor	University of Douala	Rapporteur
MBAWALA Augustin	Professor	University of Ngaoundere	Rapporteur
NDI KOUNGOU Sylvere	Associate Professor	University of Ngaoundere	Examiner
NSO Emmanuel JONG	Professor	University of Ngaoundere	Supervisor
DESOBGO ZANGUE Steve C.	Associate Professor	University of Ngaoundere	Supervisor

Academic year 2021/2022



Ecole Nationale des Sciences Agro-
Industrielles (ENSAI)

National School of Agro-Industrial Sciences
(ENSAI)

RAPPORT DE SOUTENANCE DE THESE DE DOCTORAT/Ph.D

GENIE DES PROCÉDES (GP)

VIVA VOCE REPORT OF THE PH.D THESIS

Del of

MAKEBE Calister WINGANG

Matricule : 111068EN

Date: 14/01/2022

Sujet de Thèse/Title of Thesis

Development of probiotic fermented beverage from soursop (*Annona muricata* Linn) fruit

Jury - Board of Examiners

Président : M. NDJOUENKEU Robert, Professeur, Université de Ngaoundéré
Rapporteurs : M. MBAWALA Augustin, Professeur, Université de Ngaoundéré
M. TAVEA Frédéric Marie, Maître de Conférences, Université de Douala
Membres : M. NDI KOUNGOU Sylvere, Maître de Conférences, Université de Ngaoundéré
Directeurs : M. NSO Emmanuel JONG, Professeur, Université de Ngaoundéré
M. DESOBGO ZANGUE Steve Carly, Maître de Conférences, Université de Ngaoundéré

EVALUATION

Quelle mention accordez-vous par rapport à : Score the Viva voce on the following basis :	Mention / Merit			
	Excellent Excellent	Très Bien Very Good	Bien Good	Assez Bien Fairly Good
La clarté de la présentation / Clarity of the presentation	X			
La pertinence de la recherche menée par le candidat Relevance of research work carried out	X			
Défense de l'originalité de la thèse/ Defence of the originality of the thesis		X		
Techniques et méthodes de recherche Research methods and techniques		X		
Avancée dans la résolution du problème posé Contribution to the attainment of the general objectives of the thesis work		X		
Clarification des points d'ambiguïté dans la thèse Clarification of points of ambiguity	X			
Contribution à la Connaissance Scientifique Contribution to knowledge	X			
Possibilité de publication Potential of publishing results obtained	X			

Autres commentaires et Mention du Diplôme/ Other comments and Merit of Degree:

Madame MAKEBE Calister WINGANG a fait en 39 minutes, un exposé clair, précis et pédagogique de ses travaux de recherche. Elle a déterminé les conditions optimales d'hydrolyse enzymatique de la pectine d'*Annona muricata* et a montré l'impact de cette liquéfaction sur la structure de cette pectine par la méthode FTIR et la Microscopie Electronique à Balayage. Une modélisation et une optimisation des conditions de fermentation du jus obtenu faite, en utilisant *L. acidophilus*, *L. casei* et un mélange des deux souches, a montré que les modèles cinétiques de Luedeking, Piret et Monod sont adaptés. Elle a par la suite fait une caractérisation des boissons probiotiques ainsi que le suivi de son stockage au réfrigérateur. Elle a démontré que le profil rhéologique des boissons fermentées est le même et indépendant de la souche et que, ces boissons peuvent garder leur propriétés probiotiques pendant 28 jours, malgré la baisse de quelques caractéristiques physicochimiques.

La qualité des résultats obtenus, leurs potentiels d'exploitation pour la production d'une boisson probiotique, la pertinence des réponses aux questions et la qualité de sa publication dans une revue spécialisée justifient que le jury accepte la thèse de Mme MAKEBE Calister WINGANG et lui décerne à l'unanimité le titre de Docteur/Ph.D en Sciences de l'Ingénieur, Mention Génie des Procédés, Parcours Génie Alimentaire et Bioprocédés de l'Université de Ngaoundéré avec la Mention Très Honorable.

Signatures :

Président ... NDJOUENKEU Robert

Membres 1. MBAWALA Augustin

2. TAVEA Frédéric Marie

3. NDI KOUNGOU Sylvere

4. NSO Emmanuel JONG

5. DESOBGO ZANGUE S. C.

UNIVERSITE DE NDJAMENA

ECOLE DOCTORALE
SCIENCES, TECHNIQUE ET
ENVIRONNEMENT



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والتقنيات والبيئة

N° attribué par le Secreteriat Scientifique de l'ED-STE (ou la bibliotheque centrale)

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THÈSE DE DOCTORAT

Mention : **Sciences de l'Ingénieur**

Spécialité : **Génie des Procédés**

Titre : Modélisation et optimisation de l'extraction et de fermentation de jus de datte
(*Phoenix dactylifera* L) « Bournow » par la méthodologie des surfaces de réponses.

Thèse de Doctorat

Présentée par M. **KADLEZIR Fiacre**

Sous la direction de M. AHMED MOHAMMED Mohagir, Maitre de Conférences/

M. DESOBGO ZANGUE Steve Carly, Maitre de Conférences

Composition du jury :

M. MAHMOUT YAYA, Professeur titulaire, Université de N'Djamena (Tchad), President

M. GUILLAUME KOFA, Maitre de Conférences, Université N'Gaoundere (Cameroun), Rapporteur

M. NDI KOUNGOU Sylvère, Maitre de Conférences, Université N'Gaoundere (Cameroun), Rapporteur

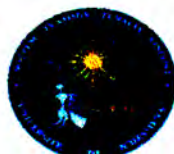
M. ABDELSALAM TIDJANI, Professeur titulaire, Université de N'Djamena (Tchad), Rapporteur

M. HIMEDA MAKHLOUF, Maitre de Conférences, Université de N'Djamena (Tchad), Examinateur

M. AHMED MOHAMMED Mohagir, Maitre de Conférences, Université de N'Djamena (Tchad), Directeur

M. DESOBGO ZANGUE Steve Carly, Maitre de Conférences, Université N'Gaoundere (Cameroun), Co Directeur

Année univeritaire : 2022



Rapport de Soutenance de thèse

Titre :

Modélisation et optimisation de l'extraction et de la fermentation de jus de datte (*Phoenix dactylifera* L.) « Bournow » par la méthodologie des surfaces réponses.

Jury

Président : MAHMOUT YAYA, Professeur Titulaire, Université de N'Djaména (Tchad)
Rapporteurs : KOFA Guillaume Patrice, Maître de Conférences, Université de Ngaoundéré (Cameroun)
NDI KOUNGOU Sylvere, Maître de Conférences, Université de Ngaoundéré (Cameroun)
ABDELSALAM TIDJANI, Professeur Titulaire, Université de N'Djaména (Tchad)
Examineur : HIMEDA MAKHLOUF, Maître de Conférences, Université de N'Djaména (Tchad)
Directeur : AHMED MOHAMED MOHAGIR, P Maître de Conférences, Université de N'Djaména (Tchad)
Co Directeur : DESOBOGO ZANGUE Steve Carly, Maître de Conférences, Université de Ngaoundéré (Cameroun)

Évaluation

Quelle mention accordez-vous par rapport à	Mention			
	Excellent	Très bien	Bien	Assez Bien
La clarté de la présentation	X			
La pertinence de la recherche menée par le candidat	X			
Défense de l'originalité de la thèse	X			
Techniques et méthodes de recherche	X			
Avancée dans la résolution du problème posé	X			
Clarification des points d'ambiguïté dans la thèse	X			
Contribution à la connaissance scientifique	X			
Possibilités de publication	X			

Autres commentaires et Mention du Diplôme

Le candidat a fait une présentation scientifique très pédagogique et magistrale. La recherche est pertinente et permet d'apporter une solution à un problème de santé publique et de développement : production des boissons probiotiques non laitières à base de dattes. Cette étude basée sur la modélisation et l'optimisation par la méthodologie des surfaces de réponse a permis de montrer que pour les conditions suivantes (temps de fermentation 72h, dose de ferment 0,015 IU, Brix 18,12 °B) le jus de datte obtenu possède les caractéristiques de boisson probiotique de qualité acceptable (acidité titrable 2,72 g/l, rapport sucre/acidité 6,12, couleur 60,87 ASBC, pH 4,11 et Brix 16,34 °B). Les perspectives sont nombreuses et très prometteuses. De ce qui précède le jury reçoit la thèse de Monsieur KADLEZIR FIACRE, et lui décerne le Diplôme de Docteur de l'Université de N'Djaména avec la mention très honorable avec félicitations du jury.

Signatures

Président :	MAHMOUT YAYA	
Rapporteurs :	KOFA Guillaume Patrice	
	NDI KOUNGOU Sylvere	
	ABDELSALAM TIDJANI	
Examineur :	HIMEDA MAKHLOUF	
Directeur :	AHMED MOHAMED MOHAGIR	
Co-Directeur	DESOBOGO ZANGUE Steve Carly	

REPUBLICQUE DU TCHAD

PRESIDENCE DE TRANSITION

PRIMATURE

MINISTRE D'ETAT A
L'ENSEIGNEMENT SUPERIEUR,
A LA RECHERCHE SCIENTIFIQUE ET
A L'INNOVATION

SECRETARIAT D'ETAT A
L'ENSEIGNEMENT SUPERIEUR
A LA RECHERCHE SCIENTIFIQUE ET
A L'INNOVATION

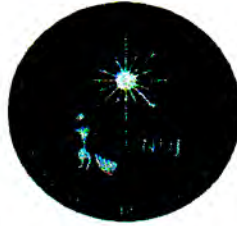
SECRETARIAT GENERAL DU
MINISTRE

UNIVERSITE DE N'DJAMENA

SECRETARIAT GENERAL

ECOLE DOCTORALE DES SCIENCES,
TECHNIQUES ET ENVIRONNEMENT

Unité - Travail - Progrès
وحدة - عمل - تقدم



جمهورية تشاد

رئاسة الانتقالية

رئاسة الوزراء

وزارة دولة للتعليم العالي والبحث العلمي والابتكار

امانة الدولة في التعليم العالي والبحث العلمي والابتكار

الامانة العامة للوزارة

جامعة لنجمينا

الامانة العامة

عمادة الدراسات العليا
في العلوم التقنية والبيئة

PROCES-VERBAL DE SOUTENANCE DE THESE

Nom : KADLEZIR Prénom : Fiacre

N° Carte d'Identité Nationale (CIN avec NNI) : R27208278

N° de matricule : 0188012012328422290620

Date et lieu de naissance : 30 août 1988

Date de première inscription en doctorat : 2019-2020

Directeur de thèse : AHMED MOHAMED Mohagir

Co-directeur(s) : DESOBGO ZANGUE Steve Carly

Ecole Doctorale : Science Technique Environnement

Formation Doctorale : Physique - Science de l'Ingénieur

Laboratoire d'accueil : Laboratoire Génie et Technologie alimentaire

Thèse intitulée : Boisson probiotique non-laitière

Soutenue le : (date et heure) 18 Janvier à 16h00

Au siège de l'établissement (Faculté) _____ à N'Djaména pour
l'obtention du Doctorat.

Devant le jury :

Civilité, Nom, Prénom	Qualité	Titre	Etablissement de rattachement	Visio-conférence	signature
Pr MAHMOUD YAYA	Président	Prof	Université de N'Djaména	☐ Oui ☒ Non	
Pr KOFA Guillaume	Rapporteur	MC	Université de N'Djaména	☐ Oui ☒ Non	
Himeda Makhlouf	Examinateur	MC	Université de N'Djaména	☐ Oui ☒ Non	
Pr Aboloulou Tidjane	Rapporteur	PT	Univ. N'Djaména	☐ Oui ☒ Non	
Abdi Karim Sylvain	Rapporteur	MC	Université de N'Djaména	☐ Oui ☒ Non	
M. DESOBGO ZANGUE Steve Carly	Co-Directeur	MC	UNT de N'Djaména	☒ Oui ☐ Non	
Ahmed Mohamed Mahagr	Directeur	MC	Université N'Djaména	☒ Oui ☐ Non	

Après avoir entendu la présentation, les éclaircissements et les arguments du candidat, les membres se sont retirés pour délibérer.

I. Après délibération, les membres du jury acceptent unanimement la thèse.

a. La thèse est acceptée.

i. Pour sa performance lors de la soutenance, l'étudiant se voit accorder la mention :

☒ Excellent ☐ très bien ☐ bien ☐ passable.

ii. Par ailleurs, à l'égard de la qualité et de la valeur de la thèse, le jury considère cette dernière :

☒ Excellente ☐ très bien ☐ bien ☐ passable.

iii. Ainsi, la thèse a été acceptée avec la mention :

☐ Honorable ☐ très honorable ☒ Très honorable avec les félicitations du jury

iv. La thèse a été acceptée sous réserve d'apporter les corrections préconisées par le jury, avec la mention :

☐ Honorable ☐ très honorable ☐ Très honorable avec les félicitations du jury

II. A la suite de la soutenance, les membres du jury n'acceptent pas unanimement la thèse.

a. La thèse a été jugée irrecevable.

Fait à N'Djaména, le 18/01/2024

IV.3.2) Mémoires de Master recherche

REPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

UNIVERSITE DE NGAOUNDERE

ECOLE NATIONALE SUPERIEURE DES SCIENCES
AGRO- INDUSTRIELLES



REPUBLIC OF CAMEROON
Peace - Work - Fatherland

UNIVERSITY OF NGAOUNDERE

NATIONAL SCHOOL OF AGRO-INDUSTRIAL
SCIENCES

DEPARTEMENT DE GENIE DES PROCEDES INGENIERIE
UNITE DE FORMATION DOCTORALE DE GENIE DES PROCEDES OPTION GENIE
ALIMENTAIRE BIOCHIMIQUE ET BIOMOLECULAIRE

MEMOIRE DE FIN D'ETUDE

En vue de l'obtention du Diplôme de MASTER recherche en génie des procédés

**PARAMETRES DE FERMENTATION DE DACRYODES
MACROPHYLLA ET ESSAI DE CARBONATATION**

Rédigé et soutenu par :

AMASSOKA Frédéric Jonathan

Licence technologique en IAB

Matricule : 20S249EN

Encadreurs académiques :

Dr AGWANANDE wilson

(Chargé de cours, université de
Ngaoundéré)

Pr DESOBGO steve

(Maître de conférences, université de
Ngaoundéré)

Année Académique 2021/2022





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Division des Affaires Académiques, de la Coopération,
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NATIONAL SCHOOL OF AGRO-INDUSTRIAL SCIENCES

B. P. 455 – Ngaoundéré, CAMEROON / FAX: +237 22-15-81-89

Tel : +237 22 16 65 00

CYCLE DES FORMATIONS MASTER

Rapport de soutenance de mémoire de Master recherche

OPTION (SAN, GP, CIE, IEAI) :.....GP.....

Nom du candidat : AMASSOKA Frederic Jonathan Matricule : 20S249EN.....

Date de naissance : 02/06/94 Lieu de naissance : Mbandjock.....

Titre du mémoire : Paramètres de fermentation de *Dacryodes Macrophylla* et essai de carbonatation

Encadreur : Dr AGWANANDE, Pr DESOBGO

Appréciation du jury

A) MEMOIRE :

Rédaction et Présentation (note/20)

Fond – Valeur Scientifique et Technique (note/30)

Total mémoire (note/50)

B) SOUTENANCE :

i) NOTE DE FORME :

Clarté de la présentation (note/10)

Qualité de la prestation (note/5)

Personnalité du candidat (note/5)

ii) NOTE DE FOND :

Maîtrise scientifique et technique du sujet (note/15)

Présentation, et discussion des résultats (note/15)

Total soutenance (note/50)

I- Total (mémoire + soutenance) note/100

II- Mention **TB**

REMARQUES DU JURY :

*Sujet d'intérêt économique
faire des corrections demandées.*

Décision du jury :

ADMIS/REFUSE

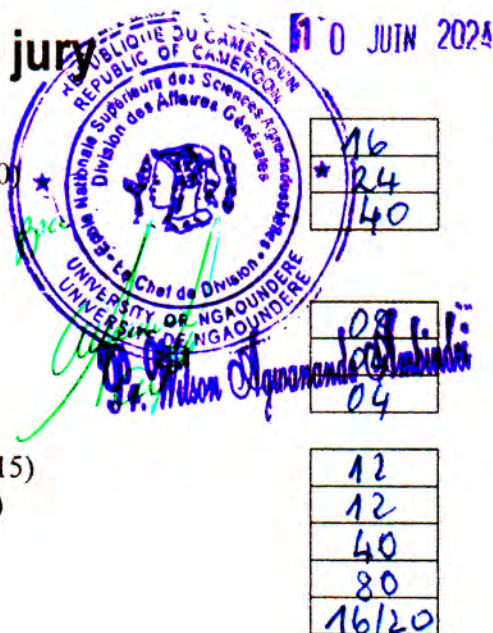
Président : Pr NSO

LE JURY

Examineur Pr MBOUENG

Rapporteur Dr AGWANANDE, Pr DESOBGO

Date de la soutenance...31. 05. 2023...



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REPUBLIQUE DU CAMEROUN

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NATIONAL SCHOOL OF AGRO-
INDUSTRIAL SCIENCES

Département de Génie des procédés et Ingénierie

*Mémoire de Fin d'Etudes En vue de l'obtention du Diplôme de
MASTEUR en génie des procédés*

Mention : génie des procédés et ingénierie

Spécialité : Génie Alimentaire Biochimique et Biomoléculaire

POTENTIALITE DE *Khayr senegalensis* Juss DANS
L'AMERISATION D'UN MOUT POUR LA PRODUCTION D'UNE
BIERE DE SORGHO

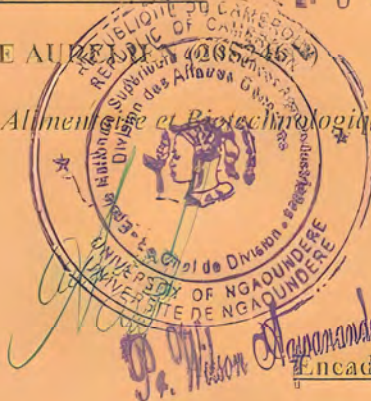
Rédigé et soutenu par

10 JUIN 2024

EPEE WILLIE AURELIEN

(Licencié ès Industrie Alimentaire et Biotechnologique)

af/c



Encadreur académique :

Dr. AGWANANDE Wilson

(Charge de cours, ENSAI)

Encadreur académique :

Pr. DESOBGO Steve Carly

(Maitre de conférences, IUT)

Année Académique 2021/2022



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Tel : +237 22 16 65 00

CYCLE DES FORMATIONS MASTER

Rapport de soutenance de mémoire de Master recherche

OPTION (SAN, GP, CIE, IEAI) :GP.....

Nom du candidat : EPPE Willie Aurelien Matricule : 20S246EN.....

Date de naissance : 03/09/97 Lieu de naissance : Ngaoundere.....

Titre du mémoire : Potentialité de *Khaya senegalsis* Juss dans l'amérisation d'un mout pour la production d'une bière de sorgho

Encadreurs : Dr AGWANANDE, Pr DESOBGO

Appréciation du jury

F) MEMOIRE :

Rédaction et Présentation (note/20)

Fond – Valeur Scientifique et Technique (note/30)

Total mémoire (note/50)

B) SOUTENANCE :

i) NOTE DE FORME :

Clarté de la présentation (note/10)

Qualité de la prestation (note/5)

Personnalité du candidat (note/5)

ii) NOTE DE FOND :

Maîtrise scientifique et technique du sujet (note/15)

Présentation, et discussion des résultats (note/15)

Total soutenance (note/50)

XI- Total (mémoire + soutenance) note/100

XII- Mention

REMARQUES DU JURY :

TBF Très bonne soutenance sur une
thématique made in Cameroon
Corrections à faire

Décision du jury : ADMIS/REFUSE

LE JURY

Président : Pr NSO

Examineur Dr MAKA

Rapporteurs Dr AGWANANDE, Pr DESOBGO

Date de la soutenance...31. 05. 2023.....

REPUBLIQUE DU CAMEROUN
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Site web: www.ensai.univ-ngoundere.com



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Email: ensai@univ-ngoundere.cm ensai@univ-ngoundere.cm

Website: www.ensai.univ-ngoundere.com

END OF STUDY DISSERTATION IN VIEW OF OBTAINING A MASTER'S DEGREE IN PROCESS ENGINEERING

ETAT ACHIEVE A L'ISSUE DE BACHELORUS MAI ROBERT ET LA VINE
L'ANNEE 2022-2023

Internship carried out in 2022 at the Food Engineering and Technology Laboratory (FAGETA)

Specialty: biochemical and molecular food engineering (GABM)

10 JUN 2023

Written by:

NGONO AWOUMOU

(Bachelor in Food Engineering)

Master 2 in Process Engineering

Matricule: 20824805

Supervisors:

Prof. DESOBGO ZANGUE

Associate professor at ENSAI University of
Ngaoundere

Prof. KOFA GUILLAUME

Associate professor at IUT University of
Ngaoundere





**ECOLE NATIONALE SUPERIEURE DES
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B. P. 455 – Ngaoundéré, CAMEROON / FAX: +237 22-15-81-89

Tel : +237 22 16 65 00

CYCLE DES FORMATIONS MASTER

Rapport de soutenance de mémoire de Master recherche

OPTION (SAN, GP, CIE, IEAI) :.....GP.....

Nom du candidat : NGONO AWOUMOU Esther Lois Matricule : 20S248EN.....

Date de naissance : 05/03/98 Lieu de naissance : Mbalmayo.....

Titre du mémoire : Clarification d'un vin de *Dacryodes Macrophylla* par *Conchurus Olitoris*

Encadreurs : Pr DESOBGO, Pr KOFA

Appréciation du jury

C) MEMOIRE :

Rédaction et Présentation (note/20)

Fond – Valeur Scientifique et Technique (note/30)

Total mémoire (note/50)

B) SOUTENANCE :

i) NOTE DE FORME :

Clarté de la présentation (note/10)

Qualité de la prestation (note/5)

Personnalité du candidat (note/5)

ii) NOTE DE FOND :

Maîtrise scientifique et technique du sujet (note/25)

Présentation, et discussion des résultats (note/25)

Total soutenance (note/50)

V- **Total (mémoire + soutenance) note/100**

VI- **Mention**

REMARQUES DU JURY :

*Très bonne soutenance, tout le résultat prouvé et fondé
à la mise sur pied d'agents floraux pour les herbivores
Décision du jury : ADMIS/REFUSE*

Décision du jury :

ADMIS/REFUSE

LE JURY

Président : Pr MBOUGUENG

Examineur Dr NSOE

Rapporteurs Pr DESOBGO, Pr KOFA

Date de la soutenance : 31. 15. 2023.....

16
24
40

8
4
4

12
12
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80
100

REPUBLIQUE DU CAMEROUN

Paix – Travail – Patrie

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SUPERIEUR

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SAI

DÉPARTEMENT DE GENIE DES PROCÉDES ET D'INGENIERIE

CLARIFICATION DE LA BIÈRE DE SORGHO À L'AIDE DE LA GOMME DE NKUI (*Triumfetta cordifolia.*)

Mémoire présenté en vue de l'obtention du Diplôme de Master recherche en Génie des Procédés

Option : Génie Alimentaire et Bioprocédé

Par :

BASSA Zacharie Carime

Licence en Industries Alimentaires et Biotechnologies

18S151EN

Encadreur :

DESOBGO ZANGUE Steve Carly

Maître de Conférences

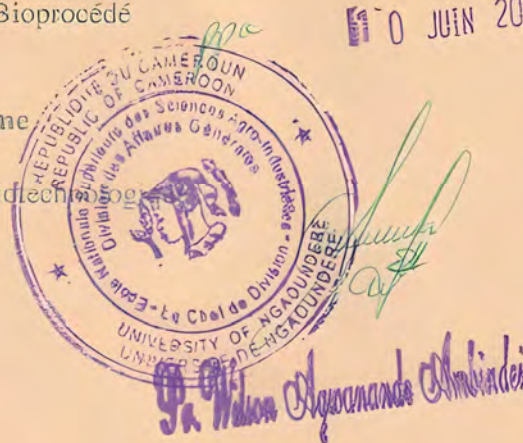
IUT, Université de Ngaoundéré

Pr NSO Emmanuel Jong

Professeur

ENSAI, Université de Ngaoundéré

Année académique 2019 – 2020





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Tel : +237 22 16 65 00

CYCLE DES FORMATIONS MASTER

Rapport de soutenance de mémoire de Master recherche

OPTION : Génie des Procédés / Spécialité : GAB

Nom du candidat : BASSA Zacharie Carime Matricule : 18S151EN
Date de naissance : 08/04/1995 Lieu de naissance : NLOBISSON II
Titre du mémoire : Clarification de la bière de sorgho à l'aide de la gomme nkui
Encadreur : Pr NSO Emmanuel / Pr DESOBGO Steve

Appréciation du jury 17 JUIN 2024

A) MEMOIRE :

Rédaction et Présentation (note/20)
Fond – Valeur Scientifique et Technique (note/20)
Total mémoire (note/50)

B) SOUTENANCE :

i) NOTE DE FORME :

Clarté de la présentation (note/10)
Qualité de la prestation (note/5)
Personnalité du candidat (note/5)

ii) NOTE DE FOND :

Maîtrise scientifique et technique du sujet (note/15)
Présentation, et discussion des résultats (note/15)
Total soutenance (note/50)

I- Total (mémoire + soutenance) note/100

II- Mention

TRES BIEN

REMARQUES DU JURY :

Sujet intéressant avec des résultats clairs et exploitables

Décision du jury :

ADMIS/REFUSE

LE JURY

Président : Pr KOFA Guillaume

Examineur : Dr AMBA Victoria

Rapporteur : Pr DESOBGO Steve

Date de la soutenance : 23/11/2024

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MASTER RECHERCHE

UNITE DE FORMATION DOCTORALE – GENIE DES PROCEDES ET INGENIERIE

Potentialité d'un extrait de patate douce (*Ipomoea Batatas* Lam.) sur la saccharification de la maische de sorgho (*Sorghum Bicolor* L.)

Mémoire présenté en vue de l'obtention du Diplôme de Master en Science et Technologie

Spécialité : Génie Alimentaire et Bioprocédés

Rédigé et soutenu par :

LIMEGNE KAMEUGNE Daruis Gayus

Matricule : 18S183EN

Licence en Biochimie

Sous la supervision de :

17⁰ JUIN 2001



Pr. Nelson Ngoumou Ndjongo

Pr DESOBGO ZANGUE Steve Carly

Maître de Conférences

IUT. Université de Ngaoundéré

Pr NSO Emmanuel Jong

Professeur

ENSAI. Université de Ngaoundéré

Année académique 2019 – 2020



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Tel : +237 22 16 65 00

CYCLE DES FORMATIONS MASTER
Rapport de soutenance de mémoire de Master recherche

OPTION : Génie des Procédés / Spécialité : GAB

Nom du candidat : LIMEGNE KAMEUGNE Daruis Gayus Matricule : 185183EN

Date de naissance : 18/04/1997 Lieu de naissance : BALENG

Titre du mémoire : Potentialité d'un extrait de patate douce (Ipoema Batatas Lam.) sur la saccharification de la maïs de sorgho (Sorghum Bicolor L.)

Encadreur : Pr NSO Emmanuel / Pr DESOBGO ZANGUE Steve

Appréciation du jury

A) MEMOIRE :

Rédaction et Présentation (note/20)

Fond – Valeur Scientifique et Technique

Total mémoire (note/50)

B) SOUTENANCE :

i) NOTE DE FORME :

Clarté de la présentation (note/10)

Qualité de la prestation (note/5)

Personnalité du candidat (note/5)

ii) NOTE DE FOND :

Maîtrise scientifique et technique du sujet (note/15)

Présentation, et discussion des résultats (note/5)

Total soutenance (note/50)

I- Total (mémoire + soutenance) note/100

II- Mention TRES BIEN

REMARQUES DU JURY :

Sujet très intéressant sur la valorisation des déchets agro-industriels.

Décision du jury :

ADMIS/REFUSE

LE JURY

Président : Pr MBOUGUENG Pierre

Examineur : Dr Wilson AGWANANDE

Rapporteur : Pr DESOBGO Steve

Date de la soutenance : 23/11/2021

IV.3.3) Mémoires de fin d'études Ingénieur

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MEMOIRE DE FIN D'ETUDES

*En vue de l'obtention du diplôme d'ingénieur de conception des industries
agricoles et alimentaires*

**Formulation d'un vin à base des pommes
d'*Anacardium occidentale* et des racines
de *Beta vulgaris***

Stage effectué du 03 Août au 13 Novembre 2020 au Laboratoire de Génie et
Technologie Alimentaire (LAGETA) de l'ENSAI de Ngaoundéré

KAHOU SONKENG Péguy Servain

Matricule : 17I021EN

Licence ès Biochimie

Encadreurs :

Pr DESOBGO ZANGUE Steve

Maitre de conférences, IUT

Dr NGATCHIC Thérèse Josiane

Chargé de cours, ENSAI

ANNEE ACADEMIQUE : 2019/2020



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FICHE DE SYNTHESE DES NOTES²³ DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

ETUDIANT	
NOMS :	KAHOU SONKENG PEGUY SERVAIN
FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	FABRICATION D'UN VIN A BASE DES ANACARDE (ANARDIUM OCCIDENTAL L)

NOTE DE FOND	Maîtrise technique du sujet	18/20
	Présentation des résultats	09/10
NOTE DE FORME	Clarté de la présentation	09/10
	Qualité de la prestation orale	09/10
	Personnalité du candidat	09/10
TOTAL		54/60
MOYENNE		18/20

OBSERVATIONS :

I- JURY

Tres bonne Soutenance, travail d'un intérêt économique important
TBF

PRESIDENT	RAPPORTEUR
NOM : PR NSO	NOM : PR DESOBGO
SIGNATURE :	SIGNATURE :

AUTRES MEMBRES	
NOM	DR AGWAGNANDE <i>Raymond</i>
SIGNATURE	

DATE :



10 JUIN 2024

²³ Moyenne des notes attribuées par chaque membre du jury.

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Mémoire de fin d'études

En vue de l'obtention du diplôme d'Ingénieur des Industries Agricoles et Alimentaires (IAA)

Sur le thème :

**FORMULATION D'UNE COMPOTE A BASE DE POMME D'ANACARDE
(*Anacardium Occidentale L.*), DE PAPAYE (*Carica papaya L.*), DE
POUDRE DE BAOBAB (*Adansonia digitata L.*).**

Stage effectué du 03 Aout au 16 novembre 2020 au laboratoire de génie et technologie

Alimentaire (LAGETA)



Rédigé et soutenu par :

BISSABAN YENYEMB Pierre-Eric

Licence ès Biochimie

Matricule : 17I008EN



Dr. Wilson Ngananda Ambinda

Encadreur académique

Pr. DESOBGO Carly
Maître de conférences
IUT

Encadreur académique

Dr. NGATCHIC Josiane
Chargé de cours
ENSAI

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FICHE DE SYNTHESE DES NOTES⁹ DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

ETUDIANT	
NOMS :	BISSABAN YENYEMB PIERRE ERIC
FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	FORMULATION D'UNE COMPOTE A BASE DE POMME D'ANACARDE, DE PAPAYE ET DE POUDRE DE BAOBAB

NOTE DE FOND	Maîtrise technique du sujet	18 /20
	Présentation des résultats	8 /10
NOTE DE FORME	Clarté de la présentation	9 /10
	Qualité de la prestation orale	9 /10
	Personnalité du candidat	9 /10
TOTAL		54 /60
MOYENNE		18 /20

OBSERVATIONS :

I- JURY

*Sujet intéressant et innovant avec des perspectives d'applications industrielles
Très bien avec félicitations*

PRESIDENT	RAPPORTEUR
NOM : PR MEZAJOUG	NOM : DR NGATCHIC
SIGNATURE : <i>[Signature]</i>	SIGNATURE : <i>[Signature]</i>

AUTRES MEMBRES	
NOM	DR SAIDOU
SIGNATURE	<i>[Signature]</i>

DATE : 15/12/2020



10 JUIN 2020

⁹ Moyenne des notes attribuées par chaque membre du jury.

[Signature]
187

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**Mémoire de stage fin d'études en vue de l'obtention du
diplôme d'Ingénieur des Industries Agricoles et Alimentaires**

**Thème : Production d'un vin blanc à base de
carica papaya (papaye solo 8)**

*Stage effectué du 03 Août au 13 Novembre 2020 au Laboratoire de Génie et
Technologie Alimentaire (LAGETA) de l'ENSAI de Ngaoundéré*

Rédigé et soutenu par

YAKAM FUT Leslie Karen

DUT en Industrie Agroalimentaire et Biotechnologique

Matricule : 1710481EN



Dr. Wilson Agwanande Ambindei

Pr. DESOBGO ZANGUE Steve Carly

Maître de Conférences

IUT / UN

Dr. AGWANANDE Wilson AMBINDEI

Chargé de Cours

ENSAI / UN

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FICHE DE SYNTHÈSE DES NOTES⁵² DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

ETUDIANT	
NOMS :	YAKAM FUT LESLIE KAREN
FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	CONCEPTION D'UN VIN A BASE DE CARICA PAPYA (PAPAYE SOLO 8)

NOTE DE FOND	Maitrise technique du sujet	18/20
	Présentation des résultats	09/10
NOTE DE FORME	Clarté de la présentation	09/10
	Qualité de la prestation orale	09/10
	Personnalité du candidat	09/10
TOTAL		54/60
MOYENNE		18/20

OBSERVATIONS :

I- JURY

Très bonne soutenance sur une thématique de grande importance économique. Faire les corrections demandées. Mention : T B - F

PRESIDENT	RAPPORTEUR
NOM : PR NSO	NOM : DR AGWANANDE
SIGNATURE :	SIGNATURE :

AUTRES MEMBRES	
NOM	MME MOUTO
SIGNATURE :	

DATE :

18/12/2020



10 JUIN 2024

⁵² Moyenne des notes attribuées par chaque membre du jury.

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MEMOIRE DE FIN D'ETUDES
EN VUE DE L'OBTENTION DU DIPLOME D'INGENIEUR DE CONCEPTION DES
INDUSTRIES AGRICOLES ET ALIMENTAIRES

THEME : Formulation d'un vin sans alcool à base de
pommes d'*Anacardium occidentale* L.

*Stage effectué au Laboratoire de génie et technologie alimentaire (LAGETA) de
l'ENSAI de l'Université de Ngaoundéré*

Du 01 Juin au 30 septembre 2021

Rédigé et Soutenu Par :

EPOH BEUGANG Rouben

Licence ès Science, option Biochimie

Matricule : 181014EN

Encadreurs académique :

Pr. DESOBGO ZANGUE Steve Carly
Maitre de Conférences
IUT/UN

Dr. Wilson AGWANANDE AMBINDEI
Chargé de Cours
ENSAI/UN

Année académique 2020/2021



19 06 2021

Dr. Wilson Agwanande Ambindei

FICHE DE SYNTHESE DES NOTES¹⁷
DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

ETUDIANT	
NOMS :	EPON BEUGANG ROMEO
FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	CONCEPTION D'UN VIN SANS ALCOOL A BASE DE POMMES D'ANACADIUM OCCIDENTALE

NOTE DE FOND	Maitrise technique du sujet	16/20
	Présentation des résultats	08/10
NOTE DE FORME	Clarté de la présentation	08/10
	Qualité de la prestation orale	08/10
	Personnalité du candidat	08/10
TOTAL		48/60
MOYENNE		16/20

OBSERVATIONS :

I- JURY

PRESIDENT	RAPPORTEUR
NOM : PR NSO	NOM : PR DESOBGO
SIGNATURE :	SIGNATURE :

AUTRES MEMBRES	
NOM	DR NGATCHIC
SIGNATURE	

DATE :

19-11-2022



¹⁷ Moyenne des notes attribuées par chaque membre du jury.

Production d'un vinaigre à base de pommes d'anacarde (*Anacardium occidentale L.*)

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40 27/679 828 800
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web site : www.ensai.univ-ndere.cm

Mémoire de fin d'études

En vue de l'obtention du diplôme d'Ingénieur de Conception
en Industries Agricole et Alimentaire

Production d'un vinaigre à base des pommes d'anacarde (*Anacardium occidentale L.*)

Stage effectué du 06 juin au 31 septembre 2022 au laboratoire de Génie et Technologie
Alimentaire (LAGETA) de l'ENSAI de Ngaoundéré

OBOUNOU OYONO Merlin Jordan

Matricule : 19IO38EN

Licence en biochimie

Encadreurs académiques :

Pr DESOBGO

Maitre de Conférences, IUT

Pr NGATCHIC
Maitre de Conférences, ENSAI

Année académique 2021-2022

RÉDIGÉ PAR OBOUNOU OYONO MERLIN JORDAN

FICHE DE SYNTHÈSE DES NOTES¹
DE SOUTENANCE DE MÉMOIRE DE FIN D'ÉTUDES

ÉTUDIANT	
NOMS :	OBOUNOU OYONO MERLIN JORDAN
FILIÈRE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MÉMOIRE :	PRODUCTION DU VINAIGRE À PARTIR DES POMMES D'ANACARDE

NOTE DE FOND	Maîtrise technique du sujet	11/20
	Présentation des résultats	8,5/10
NOTE DE FORME	Clarté de la présentation	8,5/10
	Qualité de la présentation orale	8,5/10
	Personnalité du candidat	8,5/10
TOTAL		51,00
MOYENNE		47,50

OBSERVATIONS :

I- JURY

Mention Très bien
Travail de grande intérêt économique : "Made in Cameroon"
C'est sûr, c'est bon
Cameroon 1/1

PRESIDENT	RAPPORTEUR
NOM : PR NSO	NOM : PR DESOBGO
SIGNATURE :	SIGNATURE

AUTRES MEMBRES	
NOM	DR AGWAWANDE
SIGNATURE	
DATE :	



FICHE DE SYNTHÈSE DES NOTES⁵¹
DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

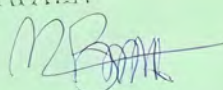
ETUDIANT	
NOM : TSAFACK MANEDJOU ANDREILLE SHERONE	
FILIERE : INDUSTRIES AGRICOLES ET ALIMENTAIRES	
SUJET DU MEMOIRE : FORMULATION D'UN VIN SANS ALCOOL A BASE DE TAMARIN DOUCE INDICA ANANAS COMOSUS	

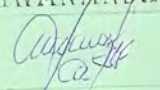
NOTE DE FOND	Maîtrise technique du sujet	18/20
	Présentation des résultats	9/10
NOTE DE FORME	Clarté de la présentation	9/10
	Qualité de la présentation orale	9/10
	Personnalité du candidat	9/10
TOTAL		54/60
MOYENNE		18/20

OBSERVATIONS

LE JURY

Mention TBF
Excellente présentation et contribution à la
valorisation des ressources locales en vinification

PRESIDENT		RAPPORTEUR	
NOM : PR MBAWALA		NOM : PR DESOBGO	R. NKOUAM
SIGNATURE		SIGNATURE	

AUTRES MEMBRES	
NOM	DR AGVANANDE
SIGNATURE	

DATE : 03/11/2022



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E-mail : ensai@univ-ndere.cm/ ensai_stage@yahoo.fr

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Mémoire de fin d'Etudes

Pour l'obtention du Diplôme d'Ingénieur de Conception des Industries Agricoles et Alimentaires

EFFET DE L'AROMATISATION DU RHUM A PARTIR DES POMMES D'ANACARDE SUR LES CARACTERISTIQUES PHYSICO-CHIMIQUES D'UN RHUM ARANGE

Stage effectué du 06 Juin au 30 septembre 2022 au Laboratoire

(LAGETA) à l'ENSAI

Rédigé et Soutenu par :

SABOUANG NGOUAH Beaud Charles Fabrice

Licence ès Option Biochimie

Matricule 191040EN



10 JUIN 2024

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Encadreurs industriels

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Directeur Général FBE

Année Académique 2021/2022

Encadreurs académiques :

Pr DESOBGO ZANGUE Stève Carly

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Dr SAIDOU Clément

Chargé de cours, IUT

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FICHE DE SYNTHÈSE DES NOTES⁴⁶
DE SOUTÈNANCE DE MÉMOIRE DE FIN D'ÉTUDES

ÉTUDIANT	
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FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MÉMOIRE :	PRODUCTION D'UNE EAU DE VIE A PARTIR DES POMMES D'ANACARDE

NOTE DE FOND	Maîtrise technique du sujet	17	20
	Présentation des résultats	8,5	10
NOTE DE FORME	Clarté de la présentation	8,5	10
	Qualité de la prestation orale	8,5	10
	Personnalité du candidat	5/8,5	10
TOTAL			60
MOYENNE		17	20

OBSERVATIONS

1- JURY

Mention très Bien
Sujet de grand intérêt économique avec une exploitation totale de la matière locale pour les produits made in Cameroon

PRESIDENT	RAPPORTEUR
NOM : PRÉSIDENT : PR NSO	NOM : DR SAIDOU / Pr. DESORGO
SIGNATURE	SIGNATURE

AUTRES MEMBRES	
NOM	M. BIYANZI
SIGNATURE	

DATE :

10 JUIN 2021



Dr. Willem Agnesando Ambindé

⁴⁶ Moyenne des notes attribuées par chaque membre du jury

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MEMOIRE DE FIN D'ETUDE

EN VUE DE L'OBTENTION DU DIPLOME D'INGENIEUR DE CONCEPTION EN INDUSTRIES
AGRICOLLES ET ALIMENTAIRES

THEME :

**ESSAIS DE PRODUCTION D'UNE BIÈRE À PARTIR DU
SORGHO NON CONCASSÉ : APPLICATION À UNE
BIÈRE ALLÉGÉE EN ALCOOL**

Stage effectué au sein de l'École Nationale Supérieure des Sciences Agro-Industrielles
(ENSAI) au laboratoire de Génie et Technologie Alimentaire

Juin 2023



Rédigé et soutenu par

DONFACK ZAMBOU Marthe

Matricule : 201020EN

Ingénieur en Science

Encadreur Académique :

Dr NGUEMOGNE Annick C.

(Assistant- ENSAI)



10 JUIN 2023

Encadreur Académique :

Pr DESOBGO ZANGUE Steve Carly

(Maître de Conférences- IUT)

Année académique 2022/2023

FICHE DE SYNTHÈSE DES NOTES¹⁹
DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

ETUDIANT	
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FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	OPTIMISATION DE LA PRODUCTION DE LA BIÈRE A PARTIR DU SORGHO NON-CONCASSE

NOTE DE FOND	Maîtrise technique du sujet	17/20
	Présentation des résultats	8,5/10
NOTE DE FORME	Clarté de la présentation	8,5/10
	Qualité de la prestation orale	8,5/10
	Personnalité du candidat	8,5/10
TOTAL		51/60
MOYENNE		17/20

OBSERVATIONS :

Mention: TB

Très bonne soutenance pour un sujet innovant. Candidate doit apprendre à défendre son travail.

I- JURY

PRESIDENT	RAPPORTEUR
NOM : PR NSO	NOM : DR NGUEMOGNE
SIGNATURE :	SIGNATURE :

AUTRES MEMBRES	
NOM	DR AGWANANDE
SIGNATURE	

DATE :

25/07/2023

¹⁹ Moyenne des notes attribuées par chaque membre du jury.

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MEMOIRE DE FIN D'ETUDES

EN VUE DE L'OBTENTION DU DIPLOME D'INGENIEUR DE CONCEPTION DES
INDUSTRIES AGRICOLES ET ALIMENTAIRES

*PRODUCTION D'UN VIN SANS ALCOOL À BASE
D'ANANAS (*Ananas comosus* C) ET D'OSEILLE (*Hibiscus
sabdariffa* L) SUCRÉ À LA STEVIA (*Stévia rebaudiana* B)*

Stage effectué au sein du laboratoire de Génie et Technologie
l'ENSAI

Du 15 Mars au 30 Juin 2023



Rédigé et soutenu par :

GNENTEDEM OUMBE ROMARIC BRIC

Matricule : 201024EN

Licence ès Biochimie

Encadreur :

Dr Wilson AGWANANDE A.

Chargé de Cours

ENSAI / Université de Ngaoundéré

Encadreur :

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Maitres de Conférences

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Année académique 2022/2023

FICHE DE SYNTHESE DES NOTES²³
DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

ETUDIANT	
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FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	PRODUCTION D'UN VIN MOELLEUX SANS ALCOOL A BASE D'ANANAS ET SUCRE A LA STEVIA

NOTE DE FOND	Maîtrise technique du sujet	18/20
	Présentation des résultats	09/10
NOTE DE FORME	Clarté de la présentation	09/10
	Qualité de la prestation orale	09/10
	Personnalité du candidat	09/10
TOTAL		54/60
MOYENNE		18/20

OBSERVATIONS :

TBF

Très bonne travail avec un intérêt
économique. Prendre attache avec le jury pour
faire les corrections.

I- JURY

PRESIDENT	RAPPORTEUR
NOM : PR NSO	NOM : DR AGWANANDE
SIGNATURE :	SIGNATURE :

AUTRES MEMBRES	
NOM	DR BEKA
SIGNATURE	

DATE : 25/07/2023

²³ Moyenne des notes attribuées par chaque membre du jury.

P. Wilson Agwanande Ambinda

FICHE DE SYNTHÈSE DES NOTES⁷
DE SOUTENANCE DE MEMOIRE DE FIN D'ETUDES

ETUDIANT	
NOMS :	BANGBANG BONGTANG LAURENT VALDESE
FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	PRODUCTION D'UN WHISKY A BASE DE MAÏS ET AROMATISE AU FEVES DE CACAO

NOTE DE FOND	Maîtrise technique du sujet	17/20
	Présentation des résultats	8/10
NOTE DE FORME	Clarté de la présentation	8/10
	Qualité de la prestation orale	7/10
	Personnalité du candidat	7/10
TOTAL		59/60
MOYENNE		17/20

OBSERVATIONS :

TRES BIEN Sujet très intéressant cours des
présentés qui peuvent s'appliquer à l'agro-industrie

I- JURY

PRESIDENT	RAPPORTEUR
NOM : PR MBOUGUENG	NOM : PR DESOBGO
SIGNATURE :	SIGNATURE :

AUTRES MEMBRES			
NOM	DR AGWANANDE		
SIGNATURE			

DATE : 19/11/2021

⁷ Moyenne des notes attribuées par chaque membre du jury.



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FILIERE :	INDUSTRIES AGRICOLES ET ALIMENTAIRES
SUJET DU MEMOIRE :	FORMULATION D'UNE BIERE A PARTIR DE MATIERE AMYLACEE (SORGHO-BANANE-MIEL) HOUBLON

NOTE DE FOND	Maîtrise technique du sujet	8,5	17	/20
	Présentation des résultats	8,5	17	/10
NOTE DE FORME	Clarté de la présentation	8,5	17	/10
	Qualité de la prestation orale	8,5	17	/10
	Personnalité du candidat	5	10	/10
TOTAL			17	/20
MOYENNE				

OBSERVATIONS :

I- JURY

PRESIDENT	RAPPORTEUR
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SIGNATURE :	SIGNATURE :

AUTRES MEMBRES	
NOM	DR AMBA
SIGNATURE	

DATE : 14 12 2020



⁶ Moyenne des notes attribuées par chaque membre du jury.



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SUJET DU MEMOIRE :	CONCEPTION D'UNE EAU-DE-VIE A BASE DE FRUITS LOCAUX

NOTE DE FOND	Maîtrise technique du sujet	15/20
	Présentation des résultats	8/10
NOTE DE FORME	Clarté de la présentation	8/10
	Qualité de la prestation orale	7/10
	Personnalité du candidat	7/10
TOTAL		45/60
MOYENNE		15/20

OBSERVATIONS: Very interesting work with great originality. Good presentation with good points but some lapses in some technical aspects.

I- JURY

PRESIDENT	RAPPORTEUR
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DATE : 18/12/2020



²⁵ Moyenne des notes attribuées par chaque membre du jury.

V) PUBLICATIONS AU GRADE DE MAITRE DE CONFERENCES

V.1) Potential of β -Amylase from
Sweet Potato (*Ipomoea batatas* Lam)
Extract on the Mashing of *Safrari*
Sorghum

Journal of Food Processing and Preservation

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Research Article

Potential of β -Amylase from Sweet Potato (*Ipomoea batatas* Lam) Extract on the Mashing of *Safrari* Sorghum

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The aim of this study was to investigate the use of sweet potato as a local source of enzymatic extract for the saccharification of sorghum mash. Box-Behnken designs were employed to determine the optimal conditions for extracting crude enzymes and saccharifying *Safrari* sorghum mash. The optimal conditions for maximizing enzymatic activity were found to be a mass-to-volume ratio of 0.1, an extraction time of 210 min, and a temperature of 60°C. The theoretical and experimental enzymatic activities under these conditions were 23.83 U/mg and 23.49 U/mg, respectively. The extraction of enzymes under these optimal conditions resulted in wort with physicochemical parameters within the following ranges: turbidity (0.79 to 4.52 NTU), pH (5.40 to 8.85), brix (14.80 to 17.50°B), reducing sugars (0.17 to 0.2114 mg/mL), and titratable acidity (3.54 to 5.24 g/L). These findings demonstrate that the extract from *Ipomoea batatas* contains enzymes that can be effectively used in the mashing process of malted *Safrari* sorghum.

1. Introduction

In tropical regions like Africa, the production of lager beers often involves using a high proportion of cereals other than barley malt [1]. Among these cereals, sorghum is the primary one used as a substitute for barley in beer production [2]. Sorghum is rich in starch, with a content of approximately 69.5% [3], and has an amylopectin/amylose ratio of 75/25 [4], making it a valuable source of carbohydrates for efficient mashing and saccharification. However, brewing with sorghum presents challenges due to the complex mashing process. Sorghum malt has a low level of β -amylases, which are responsible for breaking down starch into maltose. The deficiency of β -amylases in sorghum malt affects the saccharification of the mash [5], resulting in a low level of fermentable extract in the wort. Insufficient hydrolysis of starch also leads to difficulties during wort filtration and

cloudiness of the beer [2, 3]. To overcome these challenges, researchers have explored various sources of enzymes to enrich sorghum mash. For example, Desobgo et al. [6] used commercial enzymes to increase the level of fermentable sugars in the wort when brewing the *Safrari* cultivar. However, Lyumugabe et al. [5] suggested that the β -amylase deficiency in sorghum malt can be addressed without using commercial enzymes. Other researchers have substituted a portion of sorghum malt with cereals, roots, and tubers to improve saccharification. Etim and EtokAkpan [7] found that substituting 20% of sorghum malt with sweet potato flour increased the level of β -amylases in sorghum mash and promoted saccharification. This is attributed to the presence of both β -amylases and β -glucanases in sweet potatoes [7, 8]. Studies have also explored the combination of sweet potato and sorghum for mash saccharification [9]. However, there is still a lack of optimization of enzymatic activity in these studies.

Therefore, this study is aimed at investigating the potential of an enzymatic extract from *Ipomoea batatas* (sweet potato) to improve the saccharification of sorghum mash. By utilizing sweet potato as a local source of β -amylases, this research seeks to facilitate the saccharification process during the production of sorghum beer.

2. Material and Methods

2.1. Material. Marimar sweet potatoes were bought in the Dang area of Ngaoundere, a city in Cameroon. The specific variety of sorghum (*Sorghum bicolor* L. Moench) used in the study is *Safrari*, sourced from the Institute of Agronomic Research for Development (IRAD) in Maroua, Cameroon.

For the statistical analysis, Minitab 2022 software was employed, while OriginLab 2022 was utilized to create the graphs.

2.2. Methods

2.2.1. Raw Material Treatment. The sweet potato was washed using tap water prior to being peeled and cut into strips. These strips were then dried at a temperature of 40°C for a duration of 72 hours. The dried strips were subsequently crushed and passed through a 500 μ m sieve to obtain sweet potato powder. The powder was stored in kraft paper at a temperature of 25°C [10].

After obtaining the sorghum cultivar (*Safrari*), it was sifted to remove any impurities. To ensure its ability to germinate and produce malt suitable for brewing, the grains obtained through this method underwent a series of viability tests.

2.2.2. Characterization of Raw Material. The pH of *Ipomoea batatas* Lam powder was measured using the method described by Tamsen et al. [11]. After the samples were mineralized following the Kjeldahl method [12], the total nitrogen content was calculated using the colorimetric technique of Devani et al. [13]. The reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNSA) method, with a slight modification to the approach described by Krivorotova and Sereikaite [14]. The dry matter and ash content of the sample were determined using the AFNOR [15] method. The germination capacity, germination energy, thousand kernel weight, and moisture content of *Safrari* sorghum were determined according to the ASBC standard method [16].

2.2.3. Extraction of Amylases from Sweet Potato Powder and Activity Determination. After weighing the sweet potato, 100 mL of distilled water was added to it. The mixture was then homogenized and left to stand at a temperature of 25°C. Following this, the mixture was centrifuged at 4000 rpm for a duration of 20 minutes at a temperature of 4°C. Once the centrifugation process had separated the different phases, the less dense phase, known as the supernatant, was collected and stored at a temperature of 4°C. It is important to note that a slightly modified technique, as described by Rajagopal et al. [17] and Ramakrishnan and Rathnasamy [18], was employed for this purpose. To deter-

mine amylase activity, the methodology outlined by Raul et al. [19] was utilized.

The alpha amylase activity in the extract was quantified using the DNS method, as described in reference [20]. To summarize, the experimental procedure involved combining a reaction mixture consisting of 1% soluble starch, 20 mM phosphate buffer with a pH of 7, and fermented extract. This mixture was then incubated at a temperature of 37°C for 20 min. Following the incubation, 3,5-dinitrosalicylic acid (DNS) was added to the mixture. The amount of reducing sugar released during the assay was determined by measuring the intensity of color development at a wavelength of 540 nm using a UV-VIS spectrophotometer. One unit (1 U) of amylase activity is defined as the amount of enzyme that releases one micromole of maltose within a minute, under standardized assay conditions.

2.2.4. Modeling the Extraction of β -Amylase from Sweet Potatoes. The study employed response surface methodology (RSM) to investigate the impact of three variables, namely, the ratio of sample mass to solvent mass (g/mL), extraction time (min), and extraction temperature (°C), on α -amylase activity. RSM involves establishing regression equations that describe the relationships between the components (factors) of a product and its characteristics (responses), thereby reducing the number of experimental trials while maintaining the desired level of precision. Additionally, RSM helps identify the interaction effects among multiple variables [21]. In this study, a second-order polynomial model and the Box-Behnken RSM design were employed to assess the influence of these process variables on the desired outcome.

$$y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum_{i < j} \beta_{ij} x_i x_j, \quad (1)$$

where y is the measured response; x is the factor; β_0 is the constant; k is the number of factors; β_j is the coefficient of linear effects; β_{jj} is the coefficient of the quadratic effects; β_{ij} is the interaction coefficients.

Table 1 provides an overview of the range of factors that can impact enzyme activity. Equations (2) and (3) establish the relationship between coded values and their corresponding real values.

$$x_j = \frac{U_j - U_j^0}{\Delta U_j}, \quad (2)$$

with

$$U_j^0 = \frac{U_j^{\max} + U_j^{\min}}{2}, \quad (3)$$

where x_j is the value of the coded variable; U_j is the value of the actual variable; U_j^0 is the value of the real variable at the center of the domain; ΔU_j is the variation step; $U_j^{\min}$ is the

TABLE 1: Domain of the factors for enzyme activity.

Factors	Units	Low level (-1)	Center (0)	High level (+1)
Ratio (X_1)	m/v	0.050	0.075	0.100
Time (X_2)	min	30	120	210
Temperature (X_3)	°C	10	35	60

value of the real variable at the lower bound; $U_j^{\max}$ is the value of the real variable at the upper bound of the domain.

2.2.5. Validation of the Mathematical Model. The model underwent validation using four different methods: calculating the absolute average deviation (AAD), determining the accuracy factors (A_f) and bias factor (B_f) using Excel 2021, and calculating the coefficient of determination (R^2) and adjusted coefficient of determination (adjusted R^2) using Minitab 2022 software. These calculations were performed according to the formulas mentioned by Desobgo et al. [22]. The validation parameters for the models are listed in Table 2.

2.2.6. Optimization of Model Parameters. The Minitab 2022 optimization tool was utilized to maximize the enzymatic activity of the crude extract of *Ipomoea batatas* Lam.

2.2.7. Experimental Sorghum Malting Procedure. For three times, one kilogram of sorghum grain was washed with three liters of distilled water. The purpose of this process was to remove dust and other unnecessary substances. Additionally, the grains were soaked in three liters of distilled water for 48 h at room temperature (25°C), with the water being changed three times every 12 h. Throughout the four-day germination period, the grains were watered every six hours in a Heraeus D-63450 oven (Kendro Laboratory Product, Hanau, Germany) set at 25°C. The malt was then dried in a CKA 2000 AUF dryer for four days at 40°C. Prior to storage, the subsequent rootlets were separated from the malted sorghum.

2.2.8. Scanning Electron Microscopy. Malt from *Safrari* was immersed in liquid nitrogen at a temperature of -196°C. The frozen samples were then sliced through the germ using a sharp blade and attached to metal stubs. The samples, coated with a layer of gold, were examined using a Zeiss Evo LS15 scanning electron microscope (SEM) from Carl Zeiss in Oberkochen, Germany, operating at an acceleration voltage of 20 kV.

2.2.9. Mashing of Safrari Malt with Addition of Sweet Potato Enzyme Extract. A 600 mL beaker was filled with 250 mL of distilled water. Then, 50 g of *Safrari* (malted) flour (sieve passage, 1 mm) was added to the beaker and continuously homogenized until the mixture became uniform. The mixture was incubated at 45°C in a water bath (Mettler brand) for one hour, with intermittent stirring every five minutes. After the incubation, the mixture was decanted, and 100 mL of the liquid above the sediment was separated and set aside. The sorghum starch was gelatinized by boiling the corn for 40 min, stirring every 5 min, and then cooled to a temperature between 55°C and 65°C. The *Ipomoea batatas* Lam enzyme extracts were added to the separated liquid in a

TABLE 2: Model validation parameters.

Validation parameters	Standard values	Acceptable values
R^2	1	$\geq 92\%$
Adjusted R^2	1	$\geq 80\%$
AAD	0	[0-0.3]
Bias factor (Bf)	1	[0.75-1.25]
Accuracy factor (Af)	1	[0.75-1.25]

ratio ranging from 0 to 0.1 (v/v), based on the Box-Behnken matrix. After adding this mixture back into the mash, stirring continued for 30 to 90 min at a temperature range of 55 to 65°C, in 5-minute intervals. Once the mash had cooled to 25°C, it was filtered for 1 h 30 min using Whatman No. 4 paper (GE Healthcare Whatman, Fisher Scientific, France).

2.2.10. Modeling. The criteria for selection were determined based on the existing literature. Therefore, the following factors are taken into consideration during the saccharification process of sorghum mash: the ratio of the volume of crude enzymatic extract to the volume of mash (v/v), the duration of saccharification (min), and the saccharification temperature (°C). A Box-Behnken design with three components and fifteen trials was used for modeling. Each trial was conducted twice, and the ranges for the factors are listed in Table 3. These models are presented in quadratic form, and the same validity criteria used for the previous extraction were applied.

2.2.11. Characterization of Sorghum Wort. According to the ASBC standards, measurements were taken for turbidity, pH, extract (°B), reducing sugar, and titratable acidity [16].

2.2.12. Statistical Analysis. Statistical analysis was carried out using ANOVA, and only factors with a probability P of less than 0.05 were considered to have a significant impact.

3. Results and Discussion

3.1. Characteristics of Raw Material

3.1.1. Sweet Potato Powder (*Ipomoea batatas* Lam). Table 4 provides important information about the quality and characteristics of the sweet potato flour being analyzed. The pH value of 5.85 falls within the range established by Tortoe et al. [23], which suggests that the flour is within an acceptable acidity level for storage and consumption. The moisture content of the sweet potato flour is measured at $5.49 \pm 0.07\%$, indicating that it has a relatively low moisture content. This is a positive characteristic for storage,

TABLE 3: Domain of factors for *Safrari* malt mashing.

Factors	Units	Low level (-1)	Center (0)	High level (+1)
Ratio (X_1)	(v/v)	0	0.05	0.1
Time (X_2)	min	30	60	90
Temperature (X_3)	°C	55	60	65

TABLE 4: General chemical properties of *Ipomoea batatas* powder.

Features	Value
pH	5.85 ± 00
Moisture content (% DM)	5.49 ± 0.07
Ash content (% DM)	0.32 ± 0.01
Protein content (% DM)	2.51 ± 0.1
Reducing sugar content (% DM)	1.57 ± 0.04

as lower moisture levels can help prevent microbial growth and extend the shelf life of the product. It is worth noting that this moisture content is lower than the range reported by Tortoe et al. [23], who found moisture content between 7 and 10%. The difference in moisture content could be attributed to variations in the sweet potato cultivar used, processing methods, or storage conditions. The ash content of the sweet potato powder is measured at $0.32 \pm 0.01\%$. This indicates a relatively low mineral concentration in the flour. However, it is important to note that this value is lower than the ash content reported by Nogueira et al. [24], which was $1.64 \pm 0.06\%$. The lower ash content in the current study could be attributed to the authors' utilization of the sweet potato harvest season and drying treatment to make the flour. The protein concentration of the sweet potato flour is measured at $2.51 \pm 0.1\%$. This value is smaller than the protein concentration reported by Nogueira et al. [24], which was $2.91 \pm 0.04\%$. The difference in protein concentration suggests that both studies used different sweet potato cultivars or processing methods that did significantly affect the protein content of the flour.

The sugar level in the sweet potato flour is measured at $1.57 \pm 0.04\%$. This is significantly lower than the sugar level reported by Nogueira et al. [24], which was $6.23 \pm 0.56\%$. The decrease in sugar level could be attributed to the specific sweet potato cultivar used in the current study, as different cultivars can have varying sugar content.

3.1.2. Unmalted *Safrari* Sorghum Grain. The results presented in Table 5 indicate that the moisture content of the *Safrari* cultivar sample is 8.34%, which falls within the recommended range for grain preservation (less than or equal to 13%). This information is supported by previous studies conducted by Briggs [25], Briggs et al. [26], [27], and Hough et al. [28]. Furthermore, the germinative capacity of the *Safrari* cultivar sample is 98%, indicating that a high percentage of the grains have the potential to germinate. The germinative energy, measured at 4 mL and 8 mL, is 96% and 94%, respectively, suggesting that the grains have a strong ability to initiate germination. These values are comparable to those reported by Deso-

TABLE 5: Physicochemical characteristics of unmalted *Safrari*.

Features	<i>Safrari</i>
Moisture content (%)	8.33
Germination capacity (%)	98.00
Germination energy (4 mL) (%)	96.00
Germination energy (8 mL) (%)	94.00
1000 grains' weight (g)	50.65

bgo et al. [29], further validating the potential of the *Safrari* cultivar for malting and brewing purposes. Additionally, the weight of one thousand kernels for the *Safrari* cultivar sample is determined to be 50.65 g. This value provides an indication of the size and density of the kernels, which is an important factor in the malting and brewing process.

3.1.3. Scanning Electron Microscopy of Unmalted and Malted *Safrari*. The results of the study showed that there was a network of starch-free cell walls in the endosperm along the endosperm-scutellum interface on day four of malting, as observed through scanning electron microscopy (SEM) of the grain's proximal sections (Figure 1(a)). This suggests that some changes occurred in the endosperm during the malting process. When comparing malted *Safrari* to unmalted *Safrari*, it was found that the malted variety exhibited starch degradation specifically at the endosperm-scutellum interface (Figure 1(b)). This indicates that the modification process began in this region and then progressed throughout the floury endosperm. The enzymatic hydrolysis of starch granules, protein bodies, and the protein matrix is a key mechanism underlying the endosperm transformation observed in malted grains. This process involves the action of enzymes that break down starch into smaller molecules, such as sugars, as well as the degradation of proteins. As a result of these enzymatic activities, air gaps are generated within the endosperm, leading to a decrease in hardness and density.

3.2. Modeling and Optimizing Beta-Amylase Activity Contained in Sweet Potato Powder Extract. The enzyme activity model can be deduced by utilizing the extraction matrix provided in Table 6. This model incorporates individual factors, interactions, and quadratic effects.

$$Y_{EA}(\text{U/mg}) = 11.16 + 9.624x_1 + 1.044x_2 + 0.128x_3 + 0.69x_1^2 - 0.87x_2^2 + 0.59x_3^2 + 0.3x_1x_2 + 0.83x_1x_3 + 0.34x_2x_3, \quad (4)$$

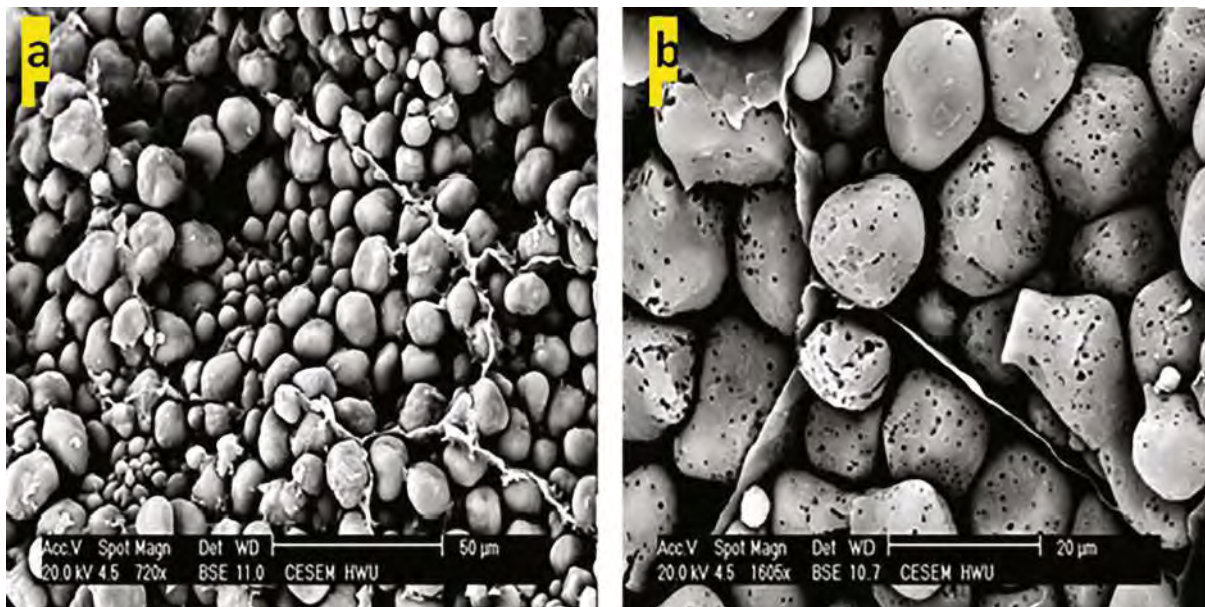


FIGURE 1: SEM for Safrari sorghum: (a) unmalted; (b) malted.

TABLE 6: Box-Behnken coded and transformed experimental values for *Ipomoea batatas* powder β -amylase extraction matrix with response.

Run	Coded values			Real values			Response
	x_1	x_2	x_3	Ratio (v/v) (X_1)	Time (min) (X_2)	Temperature ($^{\circ}$ C) (X_3)	β -Amylase activity (U/mg) (Y)
1	-1	-1	0	0.05	30	35	2.49
2	1	-1	0	0.10	30	35	20.43
3	-1	1	0	0.05	210	35	0.93
4	1	1	0	0.10	210	35	20.07
5	-1	0	-1	0.05	120	10	3.28
6	1	0	-1	0.10	120	10	21.57
7	-1	0	1	0.05	120	60	1.65
8	1	0	1	0.10	120	60	23.27
9	0	-1	-1	0.075	30	10	8.41
10	0	1	-1	0.075	210	10	12.87
11	0	-1	1	0.075	30	60	8.21
12	0	1	1	0.075	210	60	14.02
13	0	0	0	0.075	120	35	12.03
14	0	0	0	0.075	120	35	11.66
15	0	0	0	0.075	120	35	9.79

where Y_{EA} is the enzyme activity; x_1 is the sweet potato powder/water ratio; x_2 is the extraction time; x_3 is the extraction temperature.

The utilization of this model considers the validation of the model. Table 7 showcases the criteria for model validity.

According to the validation requirements of Table 7, it is confirmed that the model is valid. Therefore, it is suitable to conduct an exploratory analysis of the model's components. In this analysis, the impact of a factor will only be taken into consideration if its probability is less than 0.05. Table 8 presents the factors that are relevant to this condition and will be investigated.

3.2.1. Effect of the Ratio (x_1) on the Enzyme Activity. The results presented in Figure 2 show a significant increase in enzyme activity as the ratio of sweet potato mass to solvent volume increased from 0.05 to 0.1 during the extraction process. The enzyme activity increased from 2.22 U/mg to 21.47 U/mg, and this difference was found to be statistically significant ($p \leq 0.001$, Table 8). This increase in enzyme activity can be attributed to the addition of sweet potato powder during the extraction. The sweet potato powder acts as a source of enzymes, leading to a higher concentration of enzymes in the extract. As a result, the extract becomes more active and concentrated, leading to the observed increase in

TABLE 7: Validation of the model parameters.

Model	R ² (>92%)	R ² adjusted (>80%)	AAD [0-0.3]	Af [0.75-1.25]	Bf [0.75-1.25]
Y _{EA}	97.12%	91.94%	0.243	1.208	0.988

TABLE 8: Estimation of regression coefficients for enzyme activity of *Ipomoea batatas* powder extract.

Term	Coeff	Coef ErT	T value	p value
Constant	11.16	1.23	9.10	≤0.001
x ₁	9.624	0.751	12.82	≤0.001
x ₂	1.044	0.751	1.39	0.223
x ₃	0.128	0.751	0.17	0.872
x ₁ * x ₁	0.69	1.11	0.63	0.558
x ₂ * x ₂	-0.87	1.11	-0.79	0.466
x ₃ * x ₃	0.59	1.11	0.53	0.616
x ₁ * x ₂	0.30	1.06	0.28	0.789
x ₁ * x ₃	0.83	1.06	0.78	0.469
x ₂ * x ₃	0.34	1.06	0.32	0.763

enzyme activity. This finding is consistent with a study conducted by Sun et al. [30], who also observed a similar increase in enzyme activity with the addition of sweet potato powder. This suggests that the mechanism behind this observation is likely to be the same in both studies.

3.2.2. Optimization of Beta-Amylase Extraction from Sweet Potato Powder. The text discusses the optimization of enzymatic activity by identifying the values of certain parameters. Table 9 presents the results, showing that a m/v ratio of 0.10, extraction period of 210 min, and extraction temperature of 60°C provide the optimal compromise among the various parameters. The predicted enzyme activity under these conditions is 23.83 U/mg, while the observed enzyme activity is 23.49 U/mg. This observed value is higher than that achieved by Hesam et al. [31], which was 16.37 U/mg. The underlying mechanism behind these results can be explained by the influence of environmental conditions, harvest period, and nutrient content in the soil on the protein content and beta-amylase activity. It is known that the total protein content in a sample can affect the enzymatic activity, and factors such as environmental conditions and nutrient availability can impact the protein synthesis in plants. Therefore, variations in these factors can lead to differences in enzyme activity [10].

3.3. Modeling the Impact of *Ipomoea batatas* Lam. Crude Extract on Safrari Wort. This study employed a Box-Behnken design, consisting of three components and three levels, to investigate the impact of process variables on turbidity, pH, brix, reducing sugars, and titratable acidity during the mashing of sorghum malt. Each batch involved 15 tests with three central points, following a statistically designed approach. The results are presented in Table 10.

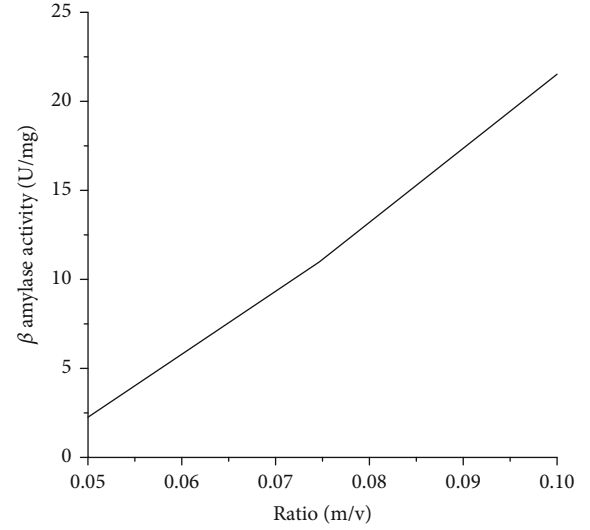


FIGURE 2: Evolution of the enzyme activity as a function of the ratio. Extraction time and temperature are fixed, respectively, at 120 min and 35°C.

Box-Behnken models establish relationships between individual factors, interactions, and quadratic effects with the response variables. These models comprise the following:

$$Y_{RS}(\text{mg/ml}) = 0.1714 - 0.0051x_1 + 0.0046x_2 - 0.007x_3 \\ + 0.0166x_1^2 + 0.0143x_2^2 + 0.0019x_3^2 \\ + 0.0049x_1x_2 + 0.0115x_1x_3 + 0.0044x_2x_3,$$

$$Y_{TA}(\text{g/L}) = 4.315 - 0.287x_1 + 0.046x_2 + 0.158x_3 \\ - 0.158x_1^2 - 0.16x_2^2 + 0.164x_3^2 + 0.125x_1x_2 \\ - 0.367x_1x_3 + 0.047x_2x_3,$$

$$Y_{pH} = 6.07 + 0.021x_1 + 0.723x_2 - 0.169x_3 \\ - 0.056x_1^2 + 0.031x_2^2 + 1.624x_3^2 - 0.145x_1x_2 \\ - 0.162x_1x_3 + 0.2x_2x_3,$$

$$Y_T(\text{NTU}) = 1.455 - 0.098x_1 + 0.39x_2 - 0.249x_3 \\ - 0.503x_1^2 + 0.816x_2^2 + 0.786x_3^2 \\ - 0.157x_1x_2 + 0.75x_1x_3 - 1.051x_2x_3,$$

$$Y_B(^{\circ}\text{B}) = 16.55 - 0.606x_1 + 0.215x_2 - 0.031x_3 \\ - 0.331x_1^2 - 0.194x_2^2 + 0.694x_3^2 \\ + 0.175x_1x_2 + 0.037x_1x_3 - 0.05x_2x_3, \quad (5)$$

TABLE 9: Optimal conditions for maximum β -amylase activity.

m/v ratio (g/ml)	Time (min)	Temperature ($^{\circ}$ C)	Theoretical enzyme activity (U/mg)	Experimental enzyme activity (U/mg)	Desirability
0.1	210	60	23.83	23.49	1

TABLE 10: Experimental matrix for Box-Behnken with responses.

Run	Coded values			Real values			Responses				
	x_1	x_2	x_3	Ratio (x_1)	Time (x_2)	Temperature (x_3)	Turbidity (NTU)	pH	Soluble solids ($^{\circ}$ B)	Reducing sugars (mg/mL)	Titrateable acidity (g/L)
1	0	-1	0	0.05	30	60	1.1400	5.40	16.60	0.205801	4.37025
2	0.1	-1	0	0.10	30	60	1.4400	5.75	14.80	0.189150	3.53774
3	0	1	0	0.05	90	60	2.4100	6.63	16.90	0.205801	4.20561
4	0.1	1	0	0.10	90	60	2.0800	6.40	15.80	0.208755	3.87324
5	0	0	-1	0.05	60	55	3.0650	7.75	17.50	0.211441	4.03631
6	0.1	0	-1	0.10	60	55	1.1900	8.10	16.45	0.174916	4.20561
7	0	0	1	0.05	60	65	0.7870	7.50	17.30	0.182167	5.17099
8	0.1	0	1	0.10	60	65	1.9100	7.20	16.40	0.191567	3.87167
9	0.05	-1	-1	0.075	30	55	1.8100	7	16.90	0.198550	4.19942
10	0.05	1	-1	0.075	90	55	4.5200	8.55	17.20	0.198550	4.20561
11	0.05	-1	1	0.075	30	65	3.6955	6.50	17.00	0.167933	4.33769
12	0.05	1	1	0.075	90	65	2.2000	8.85	17.10	0.185659	4.53336
13	0.05	0	0	0.075	60	60	1.4340	6.03	16.65	0.170082	4.37025
14	0.05	0	0	0.075	60	60	1.4500	6.08	16.50	0.171693	4.37025
15	0.05	0	0	0.075	60	60	1.4800	6.10	16.50	0.172560	4.20561

where Y_{RS} is the reducing sugars; Y_{TA} is the titrateable acidity; Y_{pH} is the pH; Y_T is the turbidity; Y_B is the brix; x_1 is the ratio; x_2 is the time; x_3 is the temperature.

The effectiveness of these models, all of which are interactive second-degree models, depends on the accuracy of a few input variables. Based on Table 11, all models are reliable and appropriate for conducting a thorough analysis of the components. Table 12's ANOVA only includes variables with a probability less than 0.05, indicating that these are the only variables that are relevant to this particular condition.

3.3.1. Impact of Singular and Quadratic Factors on the Different Responses

(1) *Impact of Ratio (x_1 and x_1^2)*. The results presented in Figure 3 show several changes in the parameters measured. The titrateable acidity decreased from 4.44 g/L to 3.87 g/L, indicating a decrease in the overall acidity of the solution. This can be attributed to the transformation of potassium and sodium found in sweet potatoes into an alkaline solution when added during mashing. The presence of OH⁻ ions in the solution can neutralize the acidity, resulting in a reduction in titrateable acidity [32].

The brix, which is a measure of the sugar content, decreased from 16.82 $^{\circ}$ B to 15.61 $^{\circ}$ B (Figure 3). This decrease can be explained by the participation of sugars in Maillard reactions. Maillard reactions occur between reducing sugars and amino groups, leading to the formation of browning compounds [33]. In this case, the rate of sugar involvement

TABLE 11: Validation criteria of the different models from wort attributes.

Settings	R^2	R^2_{adj}	AADM	Bf	Af
Y_{RS}	0.9556	0.8757	0.014	1,001	1.014
Y_{TA}	0.9823	0.9503	0.009	1,000	1,009
Y_{pH}	0.9590	0.8852	0.025	1,000	1.025
Y_B	0.9617	0.8929	0.006	1,000	1.006
Y_{Tu}	0.9806	0.9457	0.050	1.007	1,050

in nonenzymatic browning is faster than the rate of starch hydrolysis into sugars. As a result, the overall sugar content decreases, leading to a decrease in brix [34].

The turbidity of the solution decreased from 1.05 NTU to 0.85 NTU (Figure 3). This decrease can be attributed to the presence of β -amylase in the sweet potato extract. β -Amylase is an enzyme that hydrolyzes starch into maltose. The hydrolysis of starch results in the production of linear and branched dextrins. These dextrins contribute to the turbidity of the solution. However, β -amylase also removes the nonreducible dextrin terminations, resulting in the production of maltose and a decrease in turbidity [35, 36].

The study conducted by Desobgo et al. [29] investigated the engagement of reducing sugars in Maillard reactions and its impact on the decrease in reducing sugar content. The authors proposed that the initial explanation for the decrease in reducing sugar content is the involvement of reducing

TABLE 12: ANOVA for the significance of the factors used during mashing of *Safrari* malt.

Term	Reducing sugars	Titrateable acidity	p value	pH	Brix	Turbidity
Constant	≤ 0.001	≤ 0.001		≤ 0.001	≤ 0.001	≤ 0.001
x_1 -ratio (v/v)	0.044	≤ 0.001		0.872	0.001	0.299
x_2 -time (min)	0.058	0.162		0.002	0.040	0.006
x_3 -temperature ($^{\circ}\text{C}$)	0.014	0.003		0.235	0.701	0.032
x_1^2	0.002	0.013		0.772	0.033	0.010
x_2^2	0.004	0.012		0.872	0.148	0.001
x_3^2	0.521	0.011		≤ 0.001	0.002	0.001
x_1x_2	0.129	0.027		0.450	0.169	0.244
x_1x_3	0.008	≤ 0.001		0.401	0.744	0.001
x_2x_3	0.161	0.292		0.310	0.665	≤ 0.001

A factor has a significant impact on the response if its probability is $p < 0.05$ (data in bold).

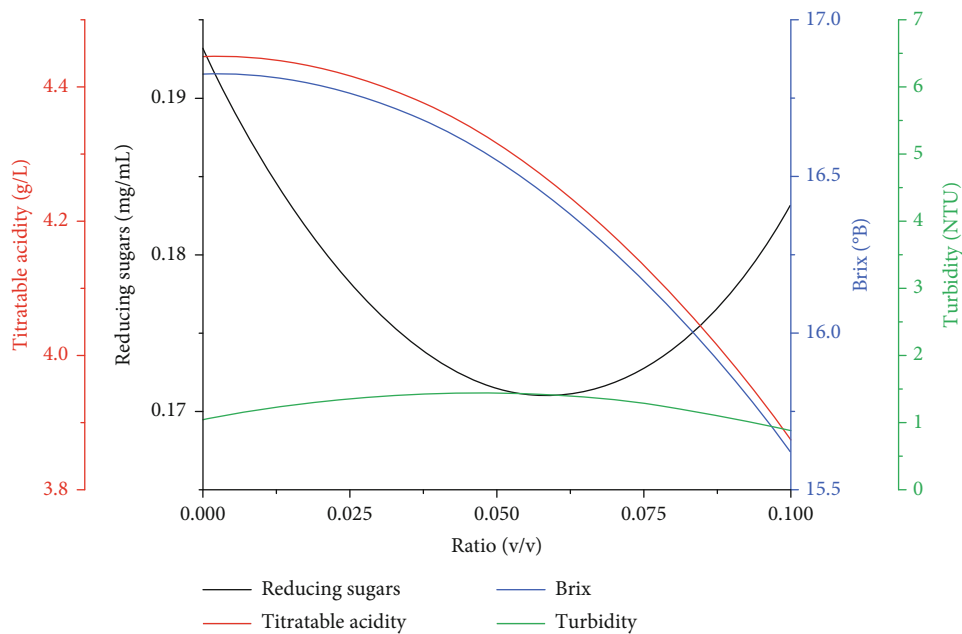


FIGURE 3: Evolution of titratable acidity, reducing sugars, brix, and turbidity as a function of ratio. Mashing time and temperature are fixed, respectively, at 60 min and 60°C .

sugars in Maillard reactions (Figure 3). Maillard reactions are complex chemical reactions that occur between reducing sugars and amino acids, leading to the formation of various compounds responsible for the browning and flavor development in food products. The reducing sugars, such as glucose and fructose, are known to participate in these reactions as reactive components [37–39]. According to Chevalier et al. [40], reducing sugars exhibit surprising reactivity during Maillard reactions. The variable rate of the reaction implies that the concentration of reducing sugars can either increase or decrease depending on the conditions and the presence of other reactants. In the context of the study, it was observed that as the ratio increased, the concentration of reducing sugars also increased. This observation can be explained by

the variable rate of Maillard reactions. When the ratio is low, the rate of starch hydrolysis may surpass the rate of Maillard reactions, leading to an increase in reducing sugar content. However, as the ratio increases, the rate of Maillard reactions becomes more dominant, resulting in a decrease in reducing sugar content. The underlying mechanism of this phenomenon can be elucidated by considering the competition between starch hydrolysis and Maillard reactions. When the ratio of reducing sugars to starch is low, the breakdown of starch into reducing sugars occurs at a higher rate than the formation of Maillard reaction products. As a result, the concentration of reducing sugars increases. However, as the ratio increases, the rate of Maillard reactions becomes more significant, leading to a decrease in reducing sugar content.

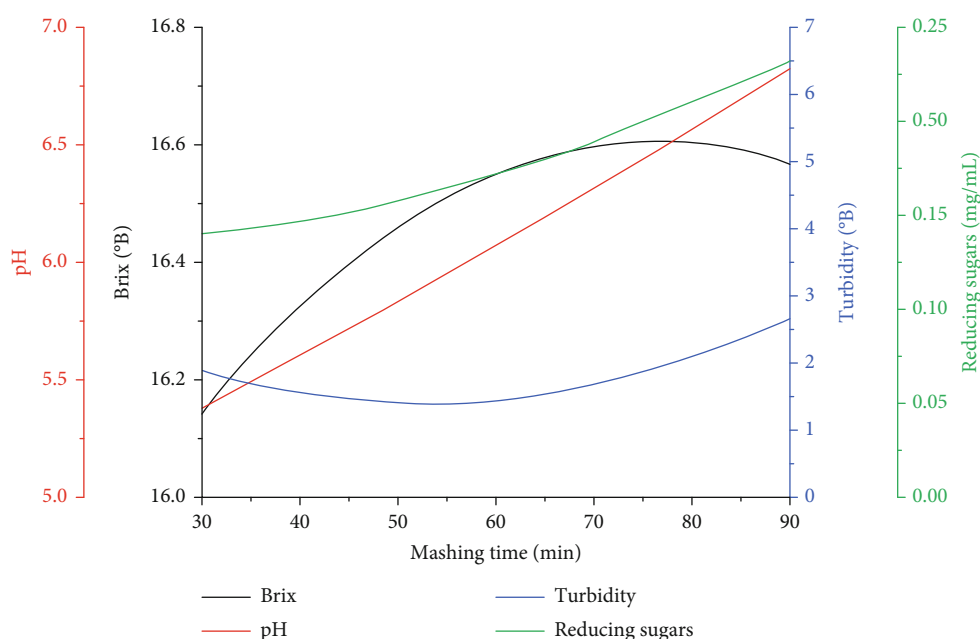


FIGURE 4: Evolution of pH, brix, turbidity, and reducing sugars as a function of mashing time. Ratio and mashing temperature are fixed, respectively, at 0.05 and 60°C.

(2) *Impact of Mashing Time (x_2 and x_2^2)*. In this study, Figure 4 provides a graphical representation of the changes observed in various parameters as the mashing time is increased from 30 to 90 min. The turbidity of the mash, measured in nephelometric turbidity units (NTU), is seen to increase from 1.88 NTU to 2.66 NTU as the mashing time is prolonged. Similarly, the brix level, which indicates the sugar content of the mash, is observed to climb from 16.14°B to 15.59°B. The pH of the mash also shows an increase from 5.37 to 6.82, and the concentration of reducing sugars rises from 0.14 mg/mL to 0.23 mg/mL. These findings can be explained by the enzymatic hydrolysis of starch in the mash.

As the mashing time increases, the amylolytic enzymes have more time to act on the starch molecules, breaking them down into simpler sugars such as maltose and glucose. This enzymatic reaction becomes more efficient over time, leading to an increase in the concentration of reducing sugars in the medium. This is consistent with the peak activity of these enzymes during this particular time interval, as reported by Nso et al. [41]. The incomplete hydrolysis of starch, resulting in the release of insoluble residues (dextrin), and the interaction between polyphenols/proteins and protein/protein leading to cloudiness formation are two potential factors contributing to the increase in wort turbidity. During the hot mashing stage, high molecular weight proteins tend to lose water, coagulate, and form a stubborn material known as hot trub [42, 43]. It is worth mentioning that the addition of raw sweet potato extract, up to a concentration of 0.1%, may also contribute to the cloudiness of the mixture. The presence of sodium and potassium ions in the enzymatic extract of sweet potato can explain the increase in

pH observed during the mashing process. These ions, by creating alkaline solutions in the medium, help neutralize a growing number of organic acids. Basilio et al. [44] also noted a similar pH elevation when cooking orange sweet potatoes.

(3) *Impact of Mashing Temperature (x_3 and x_3^2)*. As depicted in Figure 5, an increase in mashing temperature resulted in a decrease in brix levels from 17.27°B to 17.21°B, a decrease in pH from 7.86 to 7.52, and a decrease in reducing sugars from 0.18 mg/mL to 0.16 mg/mL.

In this study, the increase in mashing temperature resulted in an increase in titratable acidity from 4.32 g/L to 4.64 g/L. Additionally, at a temperature of 60.8°C, the turbidity decreased from 2.49 NTU to 1.43 NTU before rising again to 1.99 NTU at 65°C (Figure 5). These changes in temperature can trigger Maillard reactions, where sugars combine with proteins, leading to a significant decrease in brix [34]. The appropriate temperature conditions allow for the release of specific *Safrari* malt components and extract acids, which are responsible for the decrease in pH. The decrease in pH is caused by the release of H^+ ions from the ionized latter alone. The decrease in reducing sugars may be a result of ongoing Maillard reactions, which require the presence of free amino acids, reducing sugars, pH, and temperature [34]. The increase in titratable acidity may be attributed to the components of malted *Safrari* and the organic acids present in the enzymatic extract, which are released into the medium and contribute to the increase in acidity [45]. The decrease in turbidity may be caused by the involvement of proteins in the medium in Maillard processes. However, the increase in turbidity in the wort, which is promoted by the formation

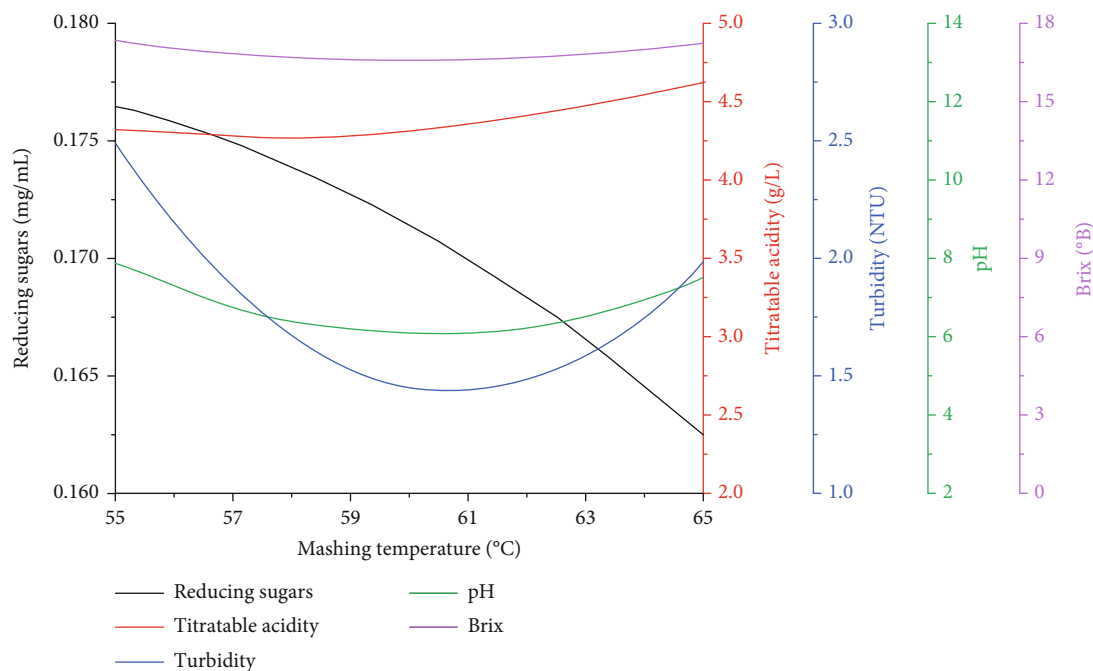


FIGURE 5: Evolution of reducing sugars, titratable acidity, turbidity pH, and brix, as a function of mashing temperature. Ratio and mashing time are fixed, respectively, at 0.05 and 60 min.

of a polyphenol-protein bond and partial precipitation of the complex, is not definitively explained by the significant release of polyphenols, insoluble proteins, and enzyme inactivation [46].

3.3.2. Impact of Interactions on the Different Responses

(1) *Impact of Interaction Ratio/Mashing Time (x_1x_2)*. The study found that the interaction between x_1 and x_2 has a significant impact on the titratable acidity ($p = 0.027$; Table 12). Specifically, decreasing the ratio of x_1 and prolonging the saccharification time of x_2 lead to an increase in titratable acidity (Figure 6). This increase in titratable acidity can be attributed to the slower dissolution of K^+ and Na^+ ions in the extract as the ratio decreases over time. As a result, the production of bases in the medium is limited, ultimately leading to the rise in titratable acidity.

(2) *Impact of Interaction Ratio/Mashing Temperature (x_1x_3)*. The interaction between the parameters x_1 and x_3 significantly increases the concentration of reducing sugars ($p = 0.008$; Table 12) and the turbidity ($p = 0.001$; Table 12), while significantly lowering the titratable acidity ($p \leq 0.001$; Table 12).

Reducing sugars increase as the ratio (x_1) and saccharification temperature (x_3) decrease. The decrease in ions that may affect the mash pH, caused by the smaller sweet potato extract, could help explain the rise in reducing sugars in the wort. Sweet potato β -amylase continues to function and assist in hydrolysis at its optimal pH. For a mashing time of 60 minutes, the optimal temperature for sweet potato β -amylase is between 55 and 59°C (Figure 7). This finding

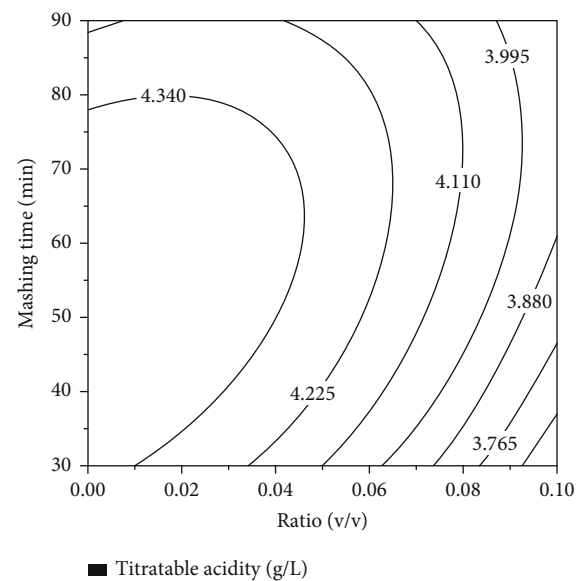


FIGURE 6: Contour plot showing the combined effect of ratio and mashing time on titratable acidity of wort.

aligns with previous studies that reported the maximum β -amylase activity of sweet potatoes at the optimal temperature [31, 47, 48].

When both the ratio (x_1) and saccharification temperature (x_3) are decreased, turbidity increases. This may be due to being within the optimal temperature range for extracting polyphenols from sorghum ($<60^\circ\text{C}$) [49], which could explain the increase in turbidity. The combination of protein in the extract and malt also contributes to turbidity.

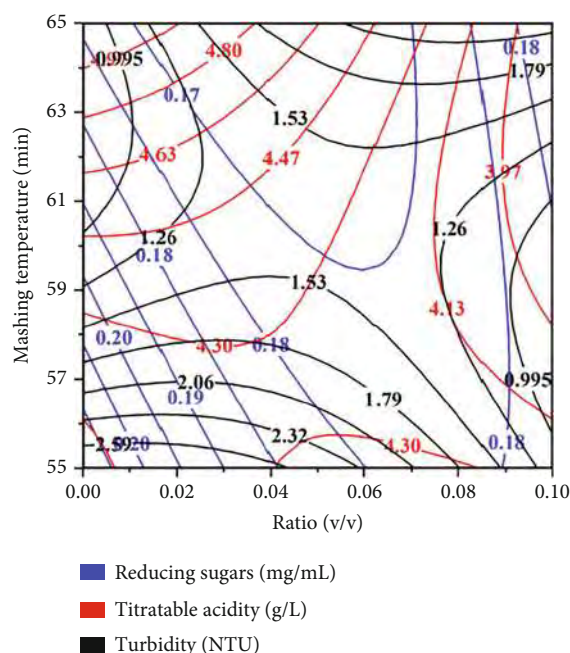


FIGURE 7: Contour plots showing the combined effect of ratio and mashing temperature on reducing sugars, titratable acidity, and turbidity of wort.

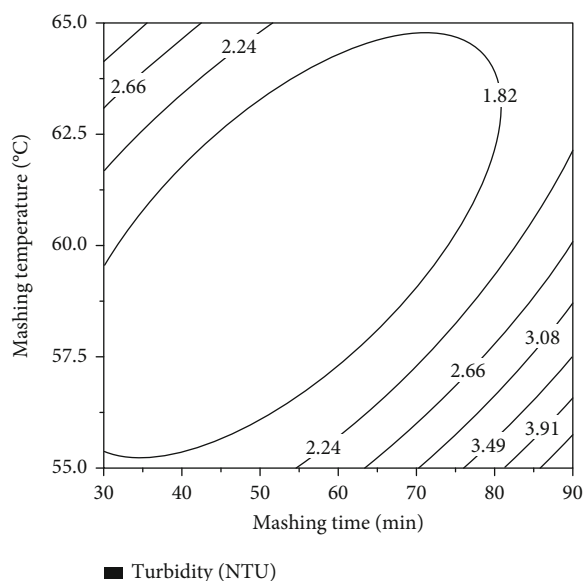


FIGURE 8: Contour plot showing the combined effect of mashing time and mashing temperature on the turbidity of wort.

On the other hand, when both the ratio (x_1) and saccharification temperature (x_3) are increased, turbidity also increases. Increasing the temperature can deactivate β -amylase [50–52] and disrupt the pH balance due to the presence of K^+ and Na^+ ions, potentially reducing the starch-hydrolyzing capacity of this enzyme as well as *Safrari* malt enzymes. These factors may lead to a significant amount of insoluble residue and a hazy wort.

When the ratio (x_1) is increased and the saccharification temperature (x_3) is decreased, the titratable acidity decreases. This decrease can be explained by the fact that as the ratio increases, more alkali ions dissolve and are neutralized by organic acids to form salts. Consequently, the titratable acidity declines.

(3) *Impact of Interaction Mashing Time/Mashing Temperature (x_2x_3)*. The interaction between mashing time (x_2) and temperature (x_3) has a significant impact on turbidity, as indicated by a $p \leq 0.001$ in Table 12. Turbidity increases when mashing time is decreased and temperature is increased. This can be attributed to inadequate starch hydrolysis when the mashing time is shortened, leading to an increase in turbidity [53]. Additionally, as the temperature increases, the activity of β -amylase decreases gradually, resulting in poor hydrolysis and increased turbidity. Figure 8 illustrates that increasing mashing time and temperature leads to a decrease in turbidity. The formation of trub, a mixture of protein and other solids, can also contribute to turbidity. During mashing, proteins are released from the malt and can interact with polyphenols, forming complexes that can precipitate and contribute to trub formation and reduce turbidity.

4. Conclusion

This research is aimed at exploring the potential use of sweet potatoes as a source of β -amylase in the mashing process of malted *Safrari* sorghum. Through the development of mathematical models, optimal conditions were determined to achieve the highest β -amylase activity in the crude aqueous extract of *Ipomoea batatas* Lam. By incorporating this extract into the mashing of malted *Safrari*, the impact of *Ipomoea batatas* Lam's β -amylase on key wort characteristics such as brix, pH, titratable acidity, turbidity, and reducing sugar was assessed. It was found that each situation requires a specific combination of β -amylase utilization, mashing time, and temperature to achieve the desired wort characteristic.

Data Availability

Data are available within the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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V.2) Production and Characterization of
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Fermented with Lactic Acid Bacteria (
Lactobacillus fermentum and
Bifidobacterium bifidum) and *Bil-bil*

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Production and Characterization of a Probiotic Sorghum Beverage Fermented with Lactic Acid Bacteria (*Lactobacillus fermentum* and *Bifidobacterium bifidum*) and Bil-bil

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Abstract The main objective of this study was to determine optimum conditions for aerobic fermentation for the production of a probiotic sorghum beverage using as ferment *Lactobacillus fermentum*, *Bifidobacterium bifidum* and bil-bil (a sorghum based traditional beer). Microbiological analyses on bil-bil showed 5×10^4 CFU/mL total mesophilic flora and 3.5×10^3 CFU/mL lactic acid bacteria. Physicochemical characterization on sorghum grains gave a water content, thousand corn weight, germinative energy and germinative capacity of $7.02 \pm 0.16\%$, $19.2 \pm 0.1g$, $99.82 \pm 0.11\%$, 100% respectively. Optimization of physicochemical and microbiological parameters of the beverage through a D-optimal plan after maximizing reducing sugars, polyphenols, vitamin C, total soluble sugars, antioxidant activity, probiotic load and minimizing titratable acidity, pH, turbidity and viscosity, resulted in an inoculation rate of 10 % *L. fermentum* and *B. bifidum* and 80 % bil-bil, a fermentation temperature of 37°C and fermentation time of 3 days. These operating conditions resulted in a beverage with a titratable acidity of 3.15 mEqg ac.mal/mL, pH of 3.05, vitamin C content of 74.28 mg/L, polyphenol content of 0.46 mg/mL, reducing sugar content of 0.86 mg/mL, TSS of 4.88°Brix, a probiotic load of 25.11×10^6 CFU/mL, turbidity of 409.38 EBC, and a viscosity of 5.18 mPa.s. Mix fermentation could be exploited in the production of a probiotic sorghum beer.

Keywords: Fermentation, Lactic Acid Bacteria, Sorghum, Beverage, Probiotic, beer, bil-bil

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1. Introduction

The artisanal production of sorghum beer (locally called bil-bil in the northern regions of Cameroon) is of a remarkable socio-economic pertinence as it is widely used in traditional ceremonies and is an important source of income for producers [1]. The relative success of bil-bil among consumers could be due to the therapeutic virtues attributed to it and the diet improvement of millions of people [2] partly due to the presence of lactic acid bacteria. The interest needed for the recognition of probiotic microorganisms as important health agents was renewed by the characterization of specific probiotic cultures and by the scientific demonstration of their positive influence on health [3]. The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization

(WHO) joint Working Group defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [4,5,6].

Over the past decade there has been a steady increase in demand for probiotic-enriched products where consumption increase of up to 150% has been recorded [7]. The probiotic market currently accounts for 10% of the functional food market [8]. Although dairy products are currently the main suppliers of probiotics [9,10], more and more non-dairy products containing probiotics are being developed from cereals, chocolate bars, cookies, soft bars and probiotic-enriched juices [11,12,13]. There is a growing emphasis on healthy and beneficial eating and a positive public perception of the positive effects of "good bacteria".

Despite its increasing diversification, the probiotic market still appears to be too limited to ensure sufficient

consumption of probiotics necessary to achieve the beneficial effects they provide [14,15]. Moreover, it remains mainly limited to dairy products, which makes it not easily accessible to low consumers of this range of products [10]. Indeed, habits, food tastes and behaviors, such as the increasingly important emergence of vegetarianism and veganism, keep consumers away from dairy probiotic products [16]. Moreover, a good percentage of the world's population is limited in their access to dairy probiotics due to lactose intolerance [17].

Whole grain cereals and cereal components offer a route of having probiotic vehicles with a dual advantage of providing beneficial bioactive constituents and fibers [18,19,20]. Such constituents comprise soluble fiber, non-digestible carbohydrates and phytochemicals such as antioxidants (phenolic compounds, vitamin C, carotenoids), phytoestrogens and phytic acids [18,19].

Despite being the principal source of dietary nutrients, cereal grains are deficient in some basic food constituents such as amino acids [6,21]. Fermentation may improve the nutritional value, sensory attributes and functional qualities of cereals [21,22], and their products such as beer.

In order to maximize consumption and diversify the sources of probiotics, and to give an added value to beer, the concept of developing a probiotic sorghum beer-like beverage fits. The main objective of this study is to determine the optimal aerobic fermentation conditions for the production of a probiotic sorghum beverage using *Lactobacillus fermentum*, *Bifidobacterium bifidum* and bil-bil. Specifically, the fermentation temperature, duration and the proportion of each component in the ferment – mix has to be determined.

2. Material and Methods

2.1. Material

The plant material used for the production of the beer was a sorghum cultivar, S.35, which was purchased from the Institute of Agricultural Research for Development (IRAD) Maroua in April 2020.

One of the ferment, a traditional beer (bil-bil), produced from a random mixture of two sorghum cultivars: dark red ndjigari and pale red mbayéri was obtained from local vendor in the Ngaoundere locality. The lactic ferments used, whose probiotic potential were prior determined, were *Lactobacillus fermentum* and *Bifidobacterium bifidum*, obtained from the Food Microbiology Laboratory of ENSAI, University of Ngaoundere.

2.2. Methods

2.2.1. Microbiological characterization of traditional beer

- **Revival and multiplication of strains**

The probiotic strains, *Lactobacillus fermentum* and *Bifidobacterium bifidum* were initially lyophilized and therefore necessitates revival before it could be applied. Into 10 mL of maximum recovery dilution saline (DS; 0.85% NaCl and 0.1% peptone in distilled water) was added 1 g of lyophilizate of each strain and the resulting suspension well agitated for 10 min. The solution was

then transferred to 1 L of previously prepared and sterilized MRS (De Man, Rogosa and Sharpe) broth. After incubation for 48 h at 42 °C, the MRS broth containing the multiplicates was centrifuged at 6500 g for 15 min at 4 °C. The supernatant was then removed, the pellet washed in DS without being re-suspended and then re-centrifuged as before. The supernatant was discarded and the pellet finally re-suspended in 10 mL of DS, and the volume later made up to 250 mL with DS. The probiotic concentration of this solution was obtained by serial dilutions of factor 10 in tubes containing 9 mL of SD. The dilutions were spread on MRS Petri dishes and incubated for 24 h at 42 °C and the colonies counted.

- **Determination of the cell concentration of multiplicates**

Serial dilutions of factor 10 were carried out in tubes containing 9 mL of normal saline solution. Dilutions were inoculated by the spreading method on MRS plated Petri dishes and incubated at 42 °C for 24 h before colony counts were conducted.

2.2.2. Physicochemical characterization of sorghum grains

In order to determine if the sorghum grains cultivar S.35 could be malted, several preliminary analyses were carried out: water content (about 5 g of crushed grains were dried at 105 °C for 24 hrs. The difference in mass before and after drying was expressed as the percentage water content per the massing before drying), germinative capacity (hydrogen peroxide method), germinative energy (4 mL and 8 mL test) and the weight of a thousand grains (to predict density). All tests were carried out according to the European Brewing Convention (EBC) methods [23].

2.2.3. Malting

Sorghum grains were sorted to be cleared of bad grains and foreign matter and then washed with distilled water. The grains were then steeped in distilled water at ambient temperature (22-25 °C) for 48 hrs. The steep liquor was changed after every 18 hours with 30 minutes of air-rest before re-steeping in a fresh liquor. At the end of steeping, the grains were put in a germination chamber at an initial temperature of 25 °C to allow for germination. Germination is a traditional and well-known technique to ameliorate the nutrient composition of grains. Furthermore, according to Gunenc *et al.* [24], germination has continuously been applied in softening grain structure and reducing anti-nutritional factors. Germination lasted for 48 hours upon development of shoots and rootlets. The germinated grains were kilned in a ventilated oven at a temperature of 45 °C for two days in order to arrest germination, conserve enzymes and stop further degradation of the grains' starchy endosperm [25].

2.2.4. Mashing

Decoction mashing was adopted for sweet wort production. The malted sorghum grains were ground to a coarse flour using a Polymix PX-MFC 90D grinder. Using a PAKWA DAHONGYING brand electronic scale, 4 kg of coarse flour were weighed and added to 25 L of distilled water (at 45 °C) contained in the brewing tank (BRAUMEISTER). The pump was turned on to agitate

the media to avoid floc formation and the vessel was held at 45°C (optimal temperature for protein hydrolyses) for one hour. Upon resting to allow for decantation, part of the supernatant (21 L) was removed. The rest, containing that starch granules, was brought to boiling while stirring intermittently at regular intervals, in a separate vessel to allow for starch gelatinization. After gelatinization, the resulting paste and supernatant were returned to the Braumeister and the temperature raised to 65 °C, marking the start of saccharification (amylase activity). After one and a half hour, the temperature was raised to 72°C and agitation continued at this temperature for 30 min. The spent grains were then filtered off and the resulting wort was boiled for one hour. Cooling immediately followed to prepare for fermentation.

2.2.5. Fermentation

A 5-factor D-optimal mixing plan (three components of the mixture and two process factors) was used for the fermentation of cultivar S.35. The five factors were the quantity of *L. fermentum*, the quantity of *B. bifidum*, the amount of bil-bil, the fermentation temperature and fermentation time. For the seeding rates of *L. fermentum* and *B. bifidum*, the choice of levels (1-10%) took account of exploratory studies. For the bil-bil rate, the interval (80-98 %) was chosen, taking into account the fundamental stress after setting the rate of *L. fermentum* and *B. bifidum*. The interval (37-42°C) was considered for temperature based on the optimal growth temperature of the two lactic ferments. Exploratory studies showed a considerable contamination after three days of fermentation, thus, 1-3 days was considered. Fermentation was carried out in an anaerobic condition. The fermentation operation was repeated trice and each fermentation sample analyzed.

2.2.6. Physicochemical analyses

- **Probiotic load**

The probiotic loading was determined by the seeding dilution method.

- **Total Soluble Solids (Brix)**

Total soluble solid was measured with the help of an optical refractometer (Hanna Instruments HI-96801). Distilled water was used to calibrate the instrument before reading of samples.

- **pH**

The pH of samples were measured directly using a CONSORT C830 pH meter.

- **Titrateable acidity**

The AFNOR [26] method was used to determine titrateable acidity using 0.1 N NaOH with phenolphthalein as an indicator.

In a conical flask, 5 mL of each beverage sample was introduced, and three drops of phenolphthalein was added. The sample was titrated with a 0.1 N NaOH solution till a persistent pink coloration was attained. The volume of NaOH consumed, was noted in mL.

The total titrateable acidity was calculated thus:

$$\text{Titrateable Acidity} = \frac{75 \cdot V \cdot N}{T} \quad \text{Eq 1}$$

V = volume of NaOH used,

N = concentration of NaOH T = sample volume

- **Antiradical activity**

Antiradical activity at DPPH (2,2 diphenyl-1-picrylhydrazine) was evaluated using the method described by Muanda et al. [27].

- **Turbidity**

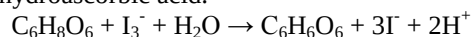
Beverage sample was inserted in an HACH 2100N turbidimeter and the turbidity read directly.

- **Viscosity**

It was evaluated using a NDJ-5S rotary viscometer.

- **Vitamin C content**

The iodine titration method was employed in the determination of vitamin C as described by Helmenstine [28]. In the form of triiodide, iodine oxidizes vitamin C to form dehydroascorbic acid.



After complete oxidation of vitamin C, excess iodine and triiodide will react with starch to form a blue-black complex, marking the endpoint of the titration.

A 1 % starch solution was prepared by adding 0.5 g of soluble starch in 50 mL of distilled water at 90 °C. The solution was well agitated to enable complete dissolution.

Iodine solution was prepared by dissolving 5 g of KI and 0.268 g of KIO₃ in 200 mL of distilled water. 30 mL of 3 M sulfuric acid was then added. The volume was then completed to 500 mL with distilled water.

A standard solution of vitamin C was prepared by dissolving 0.25 g of vitamin C in 100 mL of distilled water. After complete dissolution, distilled water was used to make up 250 mL.

The vitamin C content of the standard solution was determined using 25 mL in a 250 mL conical flask and 5 drops of 1 % starch solution added to it. The resulting solution was titrated with iodine until the endpoint and the volume noted. A similar procedure was carried out for the probiotic beer samples.

The vitamin C content was calculated as the ratio of the volume of iodine used for the standard solution compared to beer samples.

- **Polyphenol Content**

Phenolic compounds were extracted using 70% ethanol, then determined by the Folin-Ciocalteu reagent method [29].

Beer samples were diluted at a ratio of 1:5 with distilled water and placed in a volumetric flask. To prepare 5000 mg/L mother solution, 50 mg of gallic acid was added in a 10 mL volumetric flask and dissolved in 2 mL of methanol, and distilled water was used to complete the volume. From the mother solution, 1 mL was added to a 50 mL volumetric flask and the volume completed with distilled water. A standard was prepared with 1, 2, 3, 8, 12, and 24 mg/L and distilled water was used as blank. The calibration standard curve was achieved by adding 3, 6, 12, 24, 36, and 72 µL of 100 ppm gallic acid to a final volume of 208 µL with distilled water. For the beer samples, 24 µL were mixed with 184 µL distilled water in a thermos-microtiter 96-well plate (TM Roskilde), adding 12 µL of Folin-Ciocalteu reagent and 30 µL of sodium carbonate (200 g/L). The mixtures were incubated in the dark for 1 hr at ambient temperature. After the incubation period, 50

μL of distilled water was added, and absorbance was read at 765 nm.

The results were expressed as milligram equivalent of gallic acid in 100 g of dry product from equation 2 obtained from the gallic acid standard curve.

$$\text{Optical density} = aQ + b \quad \dots\dots\dots \text{Eq 2}$$

Q: the amount of phenolic compounds; a, b constants to be determined.

• **Reducing Sugars**

The method described by Alejandro *et al.* [30] for the determination of reducing sugar was modified and used. In a 50 mL flask, 0.5 g of DNS was weighed and dissolved in 10 mL of 10 % NaOH. Then 15 g of Na and K double tartrate was dissolved in 25 mL of distilled water. The two solutions were mixed and made up to 50 mL with distilled water.

A standard solution, S_1 , of maltose with a concentration of 2 mg/mL was prepared by mixing 0.1 g of maltose in 50 mL of distilled water. Standard solutions S_2 , S_3 , S_4 , and S_5 , of concentrations 0.25, 0.5, 1, and 1.5 mg/mL, were prepared by serial dilution of solution S_1 . Using standard solutions S_1 , S_2 , S_3 , S_4 , and S_5 of maltose, the calibration range was prepared, and the test of the samples was carried out as indicated. The quantity of reducing sugars in each test sample was determined by referring to the regression equation calibration curve as in equation 2.

2.2.7. Modeling

The factors chosen were those which would have a significant influence on the microbiological and physicochemical characteristics of the beverage. These include the seeding rate of *L. fermentum* (A), the seeding rate of *B. bifidum* (B), the seeding rate of bil-bil (C), the fermentation temperature (D) and the fermentation time (E). The D-optimal mixing plan was used to define the different tests.

The mathematical models obtained took into account the coded variables. These were polynomial mathematical models of the quadratic type taking into account the elements of the first degree (A, B, C, D, and E), the second degree (A^2 , B^2 , C^2 , D^2 and E^2) and interactions (AB, AC, AD, AE, BC, BD, BE, CD, CE and DE). These factors were considered statistically significant if the probability (p) was ≤ 0.05 .

The responses that were measured were: pH, Brix, probiotic load, reducing sugar content, Vitamin C content, total polyphenols, antioxidant activity, turbidity, viscosity and titratable acidity.

2.2.8. Optimization

Digital optimization was carried out with the software Design-Expert 11. To optimize a response or all of the responses, it was a question of setting the optimal conditions for each response and then using the software for practical modalities. The latter made it possible to obtain the theoretical optimum (maximum or minimum) of all the responses.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical characteristics of sorghum grains

Prior to malting, sorghum grains were tested for their malting potential and aptitude for beer production. The physico-chemical characteristics of the grains are presented in Table 1.

Table 1. Physico-chemical characteristics of S.35 sorghum grains

Characteristics	Experimental values	Reference value
Water content (%)	7.02 ± 0.16	≤ 13 [31]
Thousand corn weight (g)	19.2 ± 0.1	7 – 61 [31]
Germinative energy (4 ml) (%)	99 ± 0.0	60 – 100 [23]
Germinative energy (8 ml) (%)	97 ± 0.0	40 – 100 [23]
Germinative capacity (%)	100	92–100 [23]

The water content of the sorghum S35 cultivar was 7.02 ± 0.16 %, a value that is less than the recommended 13 % [31] for long term storage of cereal grains. This implies that the grains could be stored for a long period of time. More so, appropriate steeping duration will be required to increase the water content to facilitate germination during malting.

The thousand corn weight is an indication on grain size. For cereals destined for beer making, this parameter gives an estimate of the yield of wort density if starch is totally hydrolyzed [31]. The thousand corn weight also had a value within the recommended range.

The germinative energy gives the percentage of grains which can be expected to germinate fully if the sample is malted normally at the time of the test. The values obtained were 99 ± 0.0 and 97 ± 0.0 % for the 4 and 8 mL tests respectively, giving a water sensitivity value of 2 %. The germinative capacity gives a measure of living grains in the sample. A value of 100 % was obtained, implying that the sample was entire viable.

According to Analytica-EBC [23] and Briggs *et al.* [31], the sorghum S35 grains met with malting expectations owing to the analyzed characteristics.

3.2. Physico-chemical characteristics of sorghum wort and bil-bil

After malting of sorghum grains and subsequent mashing of the malt, wort to be fermented was obtained. The wort, and one of the ferment, the traditional beer bil-bil, were characterized. The results of the physico-chemical analyses are shown in Table 2.

The reducing sugar content and brix are indications of wort to undergo fermentation as they indicate the nutrient source (fermentable sugar) to be converted by specific microorganisms during the fermentation process [31]. The relative high values of these parameters makes the wort suitable as a good fermentation medium.

The pH of the wort was found to be 6.12 ± 0.55 , a pH value suitable for the growth of *B. bifidum*, whose optimal pH range is 6.5 – 7.0, and does not grow below pH 4.5 – 5.0 [32, 33]. *L. fermentum*, on the other hand, is known to tolerate pH values down to 4.5 [34].

Vitamin C content was lower in the sorghum wort compared to that of the bil-bil. This could be due to the fact that the bil-bil has undergone fermentation and is therefore richer in vitamin C. Similarly, polyphenols were lower in the sorghum wort compared to that in the bil-bil. On the other hand, the titratable acidity, reducing sugar content, total soluble sugars and pH were relatively lower in bil-bil, due to fermentation metabolism [35].

Table 2. Physico-chemical characteristics of sorghum wort and bil-bil

Parameters	Sorghum wort	Bil-bil
Titratable acidity (mEqg Ac.gal/mL)	5.84 ± 0.36	3.72 ± 0.13
Reducing sugar content (mg/mL)	15.12 ± 0.18	0.02 ± 0.11
Polyphenol content (mg/mL)	0.2 ± 0.01	0.3 ± 0.02
Brix (°Brix)	11.43 ± 0.20	5.90 ± 1.10
pH	6.12 ± 0.55	3.26 ± 0.16
Viscosity (mPa.s)	3.50 ± 0.50	8.50 ± 0.50
Turbidity (EBC)	98.57 ± 1.04	612.68 ± 0.10
Vitamin C (mg/L)	7.61 ± 0.08	16.82 ± 0.24

3.3. Microbiological Characteristics of bil-bil

Microbiological analyses showed that bil-bil contained 5×10^4 CFU/mL of total mesophilic bacteria and 3.5×10^3 CFU/mL of lactic acid bacteria. These values were relatively low, enabling the bil-bil to be a non-pathogenic source. Worthy of note is the fact that bil-bil is a consortium of different microorganism, among which are lactic acid bacteria which are of probiotic interest [36].

Though bil-bil contains probiotics, it is far from being considered a probiotic drink because of its relatively low concentration of probiotic bacteria compared to the limits (10^6 to 10^9 CFU/mL) stipulated by WHO. If used as a starter culture, upon cell multiplication during fermentation, the eventual product obtain could have the recommended dose of probiotic concentration.

Yeast cells were identified but the concentration was not determined since the point of interest was on probiotic strains.

3.4. Modelling and Evolution of Parameters during Fermentation

3.4.1. Probiotic Load Evolution

The mathematical model obtained following the evolution of probiotic load took into consideration coded and real variables, as presented in equation 2:

$$Y_{CP} (A,B,C,D,E) = +44.43A + 16.96B + 0.9765C - 38.39AB - 39.77AC + 2.77AD + 13.60AE - 10.03BC - 15.22BD + 14.32BE + 0.0470CD + 0.4498CE + 20.75ABD - 51.17ABE - 7.79ACD - 23.06ACE + 21.19BCD - 25.72BCE$$

Eq 3

With Y_{CP} : Probiotic load

- **Influence of single factors on probiotic load**

Figure 1a presents the evolution of the probiotic load as a function of the inoculation rates of *L. fermentum* (A) and bil-bil (C). Taken individually, the two ferments contribute significantly in increasing the probiotic load, as shown by their positive coefficients in the model equation. A similar tendency was obtained with *L. fermentum* (A) and *B. bifidum* (B) as shown in Figure 1. Effectively, the probiotic load increased from 2 to 23×10^6 CFU/mL. This is due to microbial multiplication during the fermentation process [37].

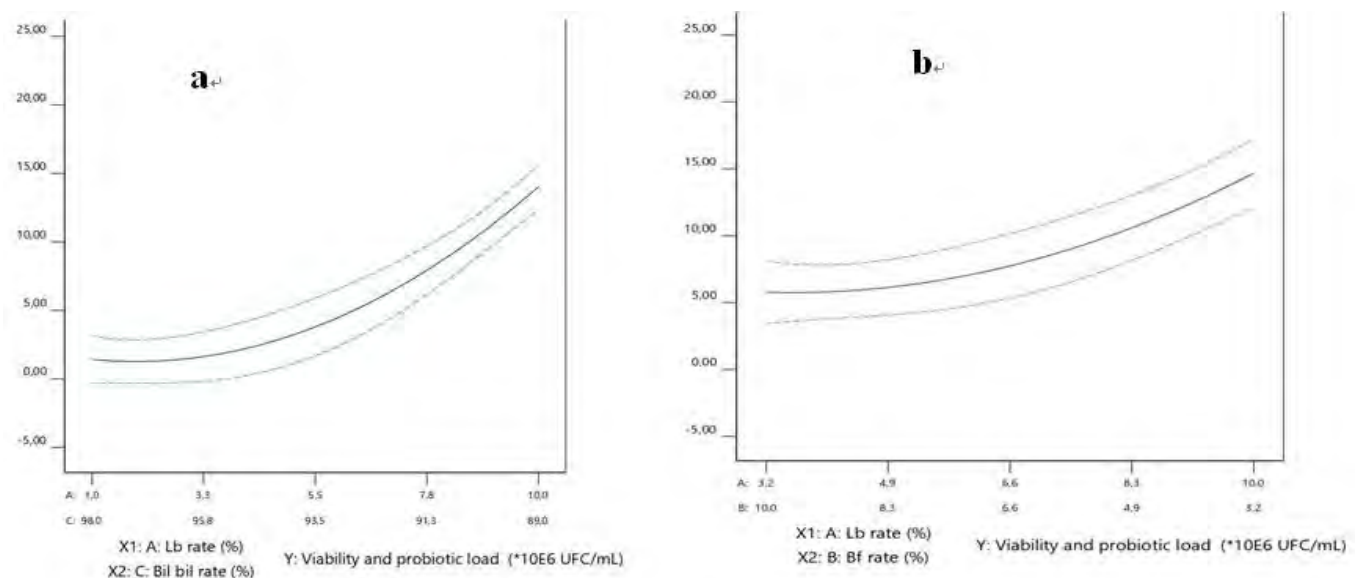


Figure 1. Evolution of probiotic load as a function of (a) *L. fermentum* and bil bil and (b) *L. fermentum* and *B. bifidum* seeding rates

- **Influence of ferment seeding rate interactions on probiotic load**

Evolution of the probiotic load as a function of the seeding rates of *L. fermentum* (A), *B. bifidum* (B) and bil-bil (C) is as shown in Figure 2. The three ferments, taken

individually, contribute significantly in increasing the probiotic load as the concentration of the ferment increases. This is shown by the coefficients of the model which are positive for all three factors. Indeed, the probiotic load increases from 2 to 23x10⁶ CFU/mL. This could be due to the multiplication of microorganisms in the medium as a function of fermentation time [37].

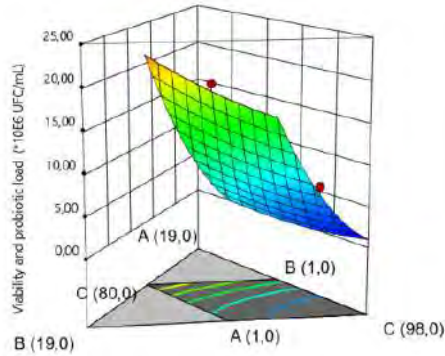


Figure 2. Evolution of probiotic load as a function of seeding rates of *L. fermentum*, *B. bifidum* and bil-bil

3.4.2. Monitoring Titratable Acidity

The mathematical model obtained for titratable acidity is shown in equation 4:

$$Y(A,B,C,D,E) = +3.64 \times A + 3.634 \times B + 3.59 \times C + 0.0268 \times A \times D - 0.3694 \times A \times E + 0.0477 \times B \times D - 0.2705 \times B \times E + 0.0050 \times C \times D - 0.5421 \times C \times E - 0.0410 \times A \times D \times E + 0.0036 \times C \times D \times E + 0.0042 \times C \times D \times E + 0.2918 \times A \times D^2 - 0.4541 \times A \times E^2 + 0.1511 \times A \times C \times D - 0.3485 \times B \times E^2 - 0.2590 \times C \times D^2 + 0.6876 \times C \times E^2$$

Eq 4

Evolution of titratable acidity of the beers as a function of fermentation time (E) and the seeding rates of *L. fermentum* (A) and *B. bifidum* (B) is shown in Figure 3a, while Figure 3b shows the evolution of the titratable acidity of the beers as a function of the fermentation time (E), *L. fermentum* seeding rate (A) and bil-bil rate (C).

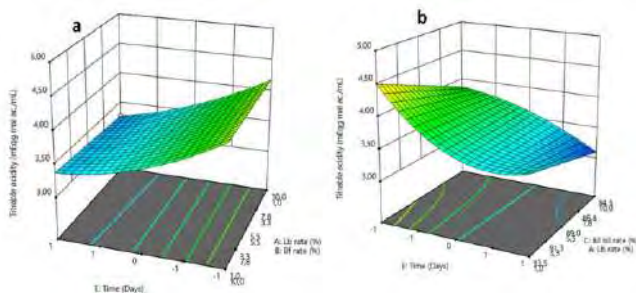


Figure 3.: Evolution of titratable acidity of beers as a function of (a) fermentation time, seeding rates of *L. fermentum* and *B. bifidum* and (b) fermentation time, seeding rate of *L. fermentum* and bil-bil

In each case, all three factors combined contribute significantly in increasing the titratable acidity. Indeed, the acidity decreased from 4.3 to 3.4 mEqg of malic acid/mL for fermentation times ranging from 1 to 3 days and for the seeding rates of *L. fermentum* and *B. bifidum* varying from 1 to 10%. During aerobic fermentation, organic acids are released over time by the microorganisms present, thus acidifying the medium [38]. This is done either by genetic mutation of the microorganisms or by co-culture (bacteria-yeast).

3.4.3. Monitoring Brix

The sugar saturation model is depicted by equation 5:

$$Y(A,B,C,D,E) = +3.80 \times A + 7.04 \times B + 4.60 \times C + 1.37 \times A \times B + 6.06 \times A \times C - 0.3232 \times A \times D - 0.8585 \times A \times E - 0.2682 \times B \times C + 0.2493 \times B \times D - 1.42 \times B \times E + 0.1761 \times C \times D - 1.23 \times C \times E + 0.0987 \times A \times B \times D + 0.0998 \times A \times B \times E + 0.4151 \times A \times C \times D + 0.2924 \times A \times C \times E + 0.3237 \times A \times D \times E - 0.7112 \times B \times C \times D + 1.30 \times B \times C \times E - 0.5855 \times B \times D \times E + 0.1292 \times C \times D \times E - 0.3027 \times A \times D^2 + 1.85 \times A \times E^2 + 1.20 \times B \times D^2 - 2.05 \times B \times E^2 + 0.4459 \times C \times D^2 + 0.3363 \times C \times E^2 + 0.5623 \times A \times B \times D \times E - 0.3180 \times A \times C \times D \times E + 1.25 \times B \times C \times D \times E - 1.32 \times A \times B \times D^2 + 0.8051 \times A \times B \times E^2 - 0.3697 \times A \times C \times D^2 - 5.25 \times A \times C \times E^2 - 3.39 \times B \times C \times D^2 + 2.32 \times B \times C \times E^2$$

Eq 5

Figure 4 shows the evolution of the Brix of beers as a function of the *L. fermentum* seeding rate (A) and the bil-bil (C). The Brix increases when the *L. fermentum* seeding rate increases in the beer and a decrease in the bil-bil rate. Furthermore, the Brix value increases from 4.6 to 5.72 °Brix for *L. fermentum* seeding rates ranging from 1 to 10% and for bil-bil seeding rates ranging from 98 to 89%. This could be due to the fact that bil-bil contains a consortium of microorganisms capable of transforming the sugars contained in the wort.

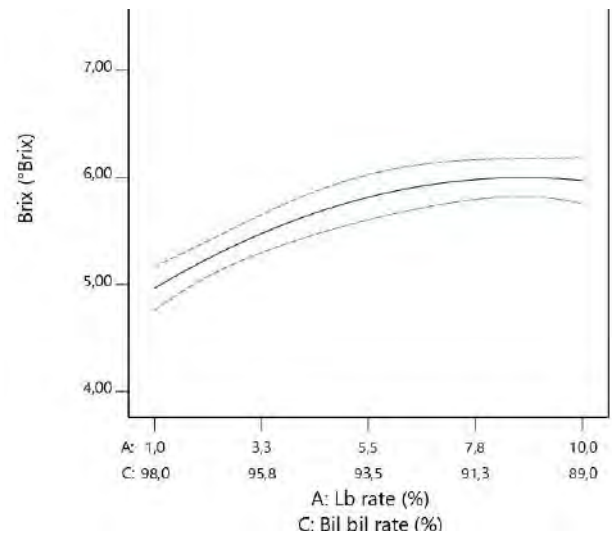


Figure 4. Evolution of the Brix of beers per the seeding rate of *L. fermentum* and bil-bil

Figure 5a shows Brix evolution in beers as a function of the *B. bifidum* seeding rate (B) and the fermentation time (E). The *B. bifidum* seeding rate alone does not significantly influence the Brix. However, combined with the fermentation time, they have a significant impact on Brix. They contribute significantly to lowering the Brix. For any value of *B. bifidum* rate and for a long fermentation time (E = 3 days), the Brix was the lowest possible (4.5 °Brix). This could be explained by the multiplicative metabolism of *B. bifidum* over time, although it was in small quantities compared to bil-bil.

The Brix evolution of the beers as a function of the bil-bil seeding rate (C) and the fermentation time (E) is shown in Figure 5b.

The interaction of these two factors significantly influences the Brix. They contribute significantly in lowering the Brix. For a high value of bil-bil (C = 98%) and for a long fermentation time (E = 3 days), the Brix is the lowest possible (4.5 °Brix). This could be as a result of the fermentative metabolism of the microorganisms

contained in bil-bil. Indeed, these microorganisms need nutrients (mainly sugars) to ensure their survival over time. It is the phenomenon of glycolysis that is involved.

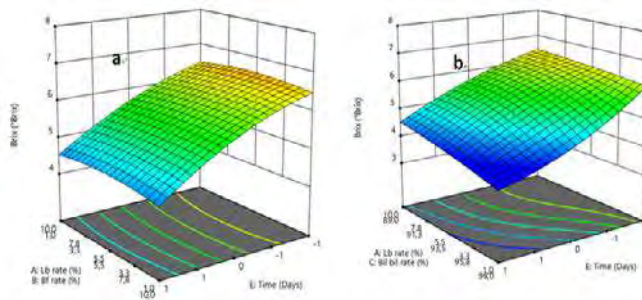


Figure 5. Evolution of the Brix of beers according to (a) seeding rate of *B. bifidum* and fermentation time, and (b) seeding rate of bil-bil and fermentation time.

3.4.4. pH monitoring

Equation 6 represents the mathematical model for pH

$$Y(A,B,C,D,E) = 3.17 \times A + 3.30 \times B + 3.11 \times C - 0.5817 \times A \times B - 0.2470 \times A \times C - 0.2112 \times A \times D + 0.3646 \times A \times E - 0.4436 \times B \times C + 0.2694 \times B \times D + 0.2287 \times B \times E + 0.0715 \times C \times D - 0.0831 \times C \times E + 0.3071 \times A \times B \times D - 1.75 \times A \times B \times E + 0.2017 \times A \times C \times D - 1.02 \times A \times C \times E + 1.03 \times A \times D \times E - 0.9669 \times B \times C \times D - 0.6183 \times B \times C \times E - 0.6137 \times B \times D \times E - 0.0706 \times C \times D \times E - 0.5078 \times A \times D^2 + 0.0053 \times A \times E^2 + 1.02 \times B \times D^2 - 1.77 \times B \times E^2 - 0.1636 \times C \times D^2 + 0.1028 \times C \times E^2 - 1.22 \times A \times B \times D \times E - 1.68 \times A \times C \times D \times E + 1.53 \times B \times C \times D \times E - 0.5873 \times A \times B \times D^2 + 3.53 \times A \times B \times E^2 + 1.52 \times A \times C \times D^2 - 0.2343 \times A \times C \times E^2 - 1.28 \times B \times C \times D^2 + 3.04 \times B \times C \times E^2 \quad \text{Eq 6}$$

The evolution of the pH of the beers as a function of the bil-bil seeding rate (C), the *L. fermentum* seeding rate (A) and the fermentation time (E) is shown in Figure 6.

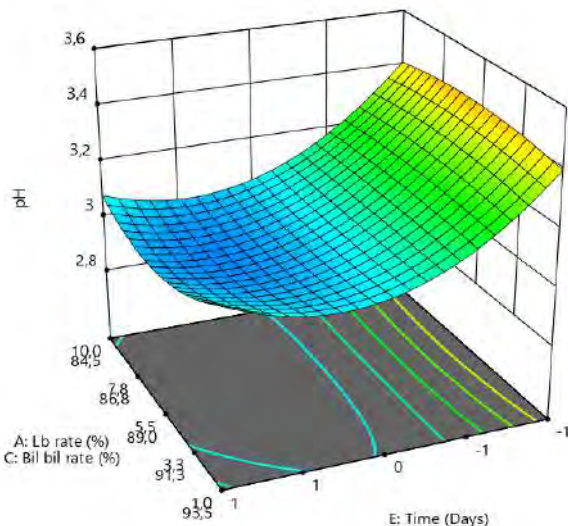


Figure 6. Evolution of pH of beers as a function of the bil-bil and *L. fermentum* seeding rates and the fermentation time.

The interaction of these factors significantly decreases the pH. This decrease in pH could be due to the fact that the microorganisms consume the sugars present in the medium to produce ethanol. The latter is then oxidized into organic acids by lactic acid bacteria and yeasts, releasing organic agents. The lactic acids and organic agents released contribute to the formation of free H^+ ions in the medium, resulting in acidification of the medium [39,40].

3.4.5. Monitoring antioxidant activity

The mathematical model for antioxidant activity is depicted in equation 7:

$$Y(A,B,C,D,E) = +53.85 + 2.52 \times D + 20.35 \times E \quad \text{Eq 7}$$

The evolution of the antioxidant activity of beers as a function of fermentation time is shown in Figure 7.

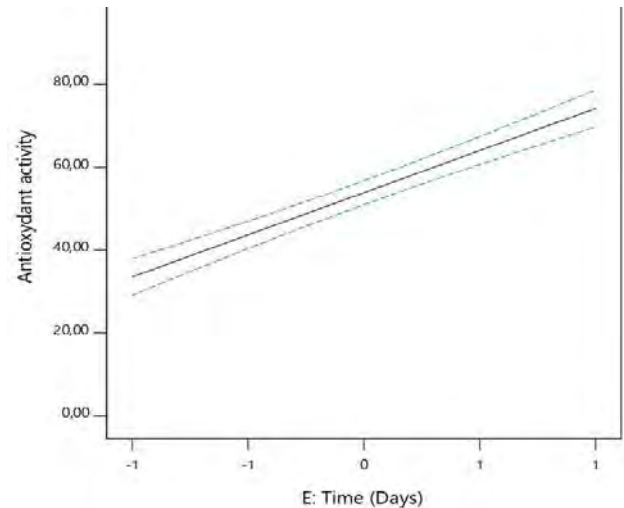


Figure 7. Evolution of the antioxidant activity of beers as a function of fermentation time

Time contributes significantly to the increase in antioxidant activity. This increase could be due to the secretion of free radical scavenging compounds such as vitamin C and phenolic compounds from the medium with time, which over time act as primary antioxidants. The action of these antioxidants is thought to be due to their ability to donate hydrogen atoms or electrons derived mainly from the hydroxyl ring of flavonoids [41].

3.4.6. Monitoring reducing sugars

Equation 8 shows the mathematical model for reducing sugars evolution during fermentation.

$$Y(A,B,C,D,E) = +1.37 \times A + 1.26 \times B + 0.0.8334 \times C + 0.2214 \times A \times D - 0.7687 \times A \times E - 0.0212 \times A \times D - 0.6016 \times B \times E + 0.0628 \times C \times D - 0.5547 \times C \times E \quad \text{Eq 8}$$

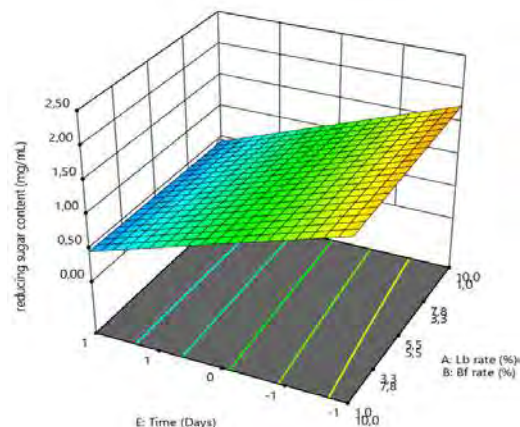


Figure 8. Evolution of reducing sugars as a function of fermentation time and the seeding rate of bil-bil

Figure 8 shows the evolution of reducing sugars as a function of fermentation time and bil-bil seeding rate.

Reducing sugars decreases significantly with increase in fermentation time combined with the bil-bil seeding rate. Indeed, the values range from 1.5 mg/mL to 0.0 mg/mL for fermentation time values ranging from 1 to 3 days and for ferment rate values varying between 1 and 10%. The bil-bil used is a consortium of microorganisms including yeasts. The yeast will consume the sugar present in the medium, producing alcohol. The higher the bil-bil seeding rate for a fix volume of wort, the greater the yeast concentration and the more reducing sugar is being consumed.

3.4.7. Monitoring phenolic content

Evolution of the phenolic content of beers during fermentation is shown in the mathematical model of equation 9:

$$Y(A,B,C,D,E) = +0.2860 \times A + 0.2676 \times B + 0.3261 \times C - 0.0160 \times A \times D + +0.2038 \times A \times E + 0.0080 \times C \times D \times E + 0.0362 \times A \times D^2 - 0.0861 \times A \times E^2 + 0.2172 \times B \times D^2 - 0.0598 \times B \times E^2 + 0.0152 \times C \times D^2 + 0.0973 \times C \times E^2 \quad \text{Eq 9}$$

The evolution of phenolic content as a function of fermentation time and bil-bil seeding rate is shown in Figure 9. The phenolic compound content increases significantly with increase in fermentation time combined with the bil-bil seeding rate. Indeed, it goes from 0.00 mg/mL to a value of 5.59 mg/mL for fermentation time values ranging from 1 to 3 days and for bil-bil values varying between 84.5 and 93.5%. This growth could be due to the release of polyphenols during fermentation. Indeed, microbial metabolism is characterised by the production of antioxidant compounds including polyphenols [42].

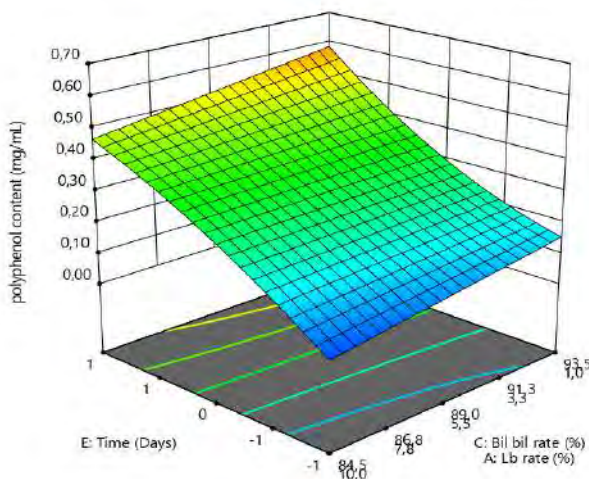


Figure 9. Evolution of the polyphenols of beers according to the rate of sowing of bil-bil, the rate of seeding of *L. fermentum* and the fermentation time.

3.4.8. Monitoring Vitamin C content

The mathematical model of vitamin C evolution is depicted in equation 10.

$$Y(A,B,C,D,E) = +50.84 \times A + 36.81 \times B + 42.14 \times C - 2.54 \times A \times D + 26.45 \times A \times E - 2.28 \times B \times D + 16.39 \times B \times E + 3.84 \times C \times D + 20.01 \times C \times E - 6.57 \times A \times D \times E + 1.65 \times B \times D \times E + 1.13 \times C \times D \times E - 22.34 \times A \times D^2 -$$

$$26.90 \times A \times E^2 - 2.48 \times B \times D^2 + 8.89 \times B \times E^2 + 5.93 \times C \times D^2 + 6.47 \times C \times E^2 \quad \text{Eq 10}$$

Figure 10 shows the evolution of vitamin C as a function of the fermentation time, the seeding rates of bil-bil and *L. fermentum*.

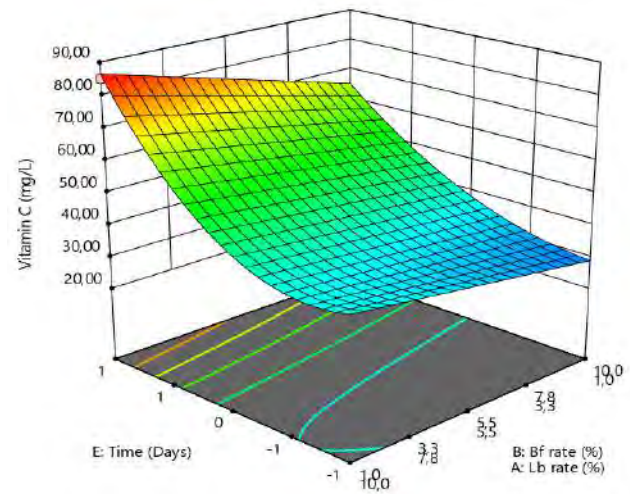


Figure 10. Evolution of vitamin C content as a function of fermentation time, the seeding rates of *B. bifidum* and *L. fermentum*.

The vitamin C content increases significantly with fermentation time combined with the *B. bifidum* rate. In fact, it goes from 26 mg/L to a value of 83 mg/L for values of fermentation time ranging from 1 to 3 days and for values of bil-bil rate varying between 1 and 10% (possibly 1 to 10% *L. fermentum*). This growth could be due to the release of antioxidants during fermentation. Indeed, microbial metabolism is characterized by the production of antioxidant compounds, including vitamin C [42].

3.4.9. Monitoring turbidity

Turbidity evolution was modelled mathematically as shown in equation 11.

$$Y(A,B,C,D,E) = +3184.04 \times A - 4616.04 \times B + 865.56 \times C + 4486.02 \times A \times B - 5968.64 \times A \times C + 9636.49 \times B \times C - 5555.16 \times A \times B \times C - 9848.94 \times A \times B(A-B) - 3943.58 \times A \times C(A-C) + 6762.36 \times B \times C(B-C) \quad \text{Eq 11}$$

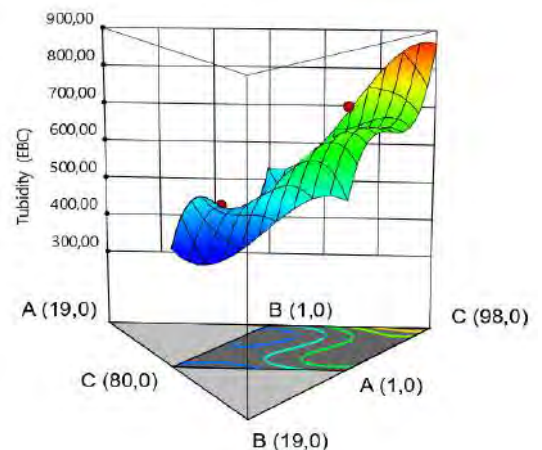


Figure 11. Evolution of the turbidity of beers as a function of the seeding rate of the ferments

Figure 11 shows the evolution of the turbidity of beers as a function of the different ferments. It can be seen that all factors significantly influence turbidity, even when combined. Furthermore, the turbidity value increases between 300 and 800 EBC for A and B values ranging from 1 to 10% and for bil-bil seeding rates varying between 84.5 and 93.5%. This could be due to the effect of microbial metabolism whereby the microorganisms present release biopolymers into the medium thereby increasing turbidity [43].

3.4.10. Monitoring viscosity

The mathematical model for viscosity is shown in equation 12:

$$Y(A,B,C,D,E) = +44.57 \times A - 15.84 \times B + 10.24 \times C - 36.96 \times A \times B - 81.41 \times A \times C + 37.60 \times B \times C + 40.42 \times A \times B \times C - 75.14 \times A \times B \times (A-B) - 55.98 \times A \times C \times (A-C) + 24.06 \times B \times C \times (B-C)$$

Eq 12

Figure 12 shows the evolution of beer viscosity as a function of temperature, *B. bifidum* seeding rate (B) and bil-bil rate (C). It was found that the viscosity decreased significantly when these factors were combined. Furthermore, the viscosity value decreases from 8.8 to 5.00 mPa.s for seeding rate values of *L. fermentum* and *B. bifidum* ranging from 1 to 10%. This could be due to the effect of microbial metabolism whereby the microorganisms present release biopolymers into the medium which make it more viscous.

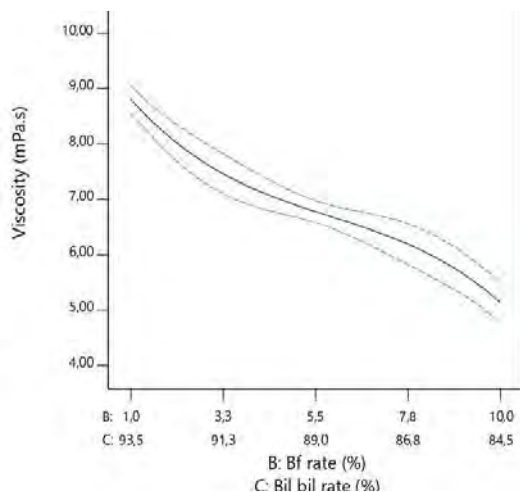


Figure 12. Evolution of viscosity of beers as a function of *B. bifidum* and bil-bil seeding rates

3.5. Fermentation Optimization

In order to find ideal and optimal physicochemical and microbiological characteristics for consumption, an optimization was carried out using the Design Expert 11 software. The aim was to find an optimum for each response. The conditions obtained were: Inoculation rate of *L. fermentum* (A) = 10%; Inoculation rate of *B. bifidum* (B) = 7%; Inoculation rate of bil-bil (C) = 83%; Fermentation temperature (D) = 39.5 °C; Fermentation time (E) = 2 days. The optima responses obtained are presented in Table 4.

To perceive a positive health effect from consumption, a least concentration of probiotic microbes is essential. The recommended quantity is between $10^6 - 10^{11}$

CFU/day [44]. With a probiotic load of 17.9×10^6 CFU/mL, the beer produced under optimised conditions could be considered as a source of probiotics.

Table 4. Physico-chemical characteristics of beer produced under optimized conditions.

Characteristics	Optimum values
Titrateable acidity (mEqg Mal.Ac./mL)	3.63
pH	3.06
Brix (°Brix)	5.74
Probiotic load ($\times 10^6$ CFU/mL)	17.90
Reducing sugars content (mg/mL)	0.95
Phenolic compound content (mg/mL)	0.28
Vitamin C content (mg/L)	44.61
Percentage of inhibition	51.80
Turbidity (mPa.s)	446.98
Viscosity (EBC)	5.43

Conclusion

The outcome of this study showed that mixed fermentation can be successfully carried out on sorghum wort to produce a probiotic beer. Alcohol fermentation was achieved by yeast present in bil-bil, while *L. fermentum*, *B. bifidum* and lactic acid bacteria found in bil-bil were responsible, not only for lactic acid fermentation, but also for the probiotic load.

Conflict of Interest

The authors declare no conflict of interest.

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V.3) Extracting juice from dates (*Phoenix dactylifera* L.) using response surface methodology: Effect on pH, vitamin C, titratable acidity, free amino nitrogen (FAN) and polyphenols

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Extracting juice from dates (*Phoenix dactylifera* L.) using response surface methodology: Effect on pH, vitamin c, titratable acidity, free amino nitrogen (FAN) and polyphenols

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ABSTRACT

The aim of this text is to investigate the effects of temperature, time, volume/mass ratio, and enzyme volume on various properties of date juice extracted from the "Bournow" cultivar, and to optimize the extraction process to maximize the levels of free amino acids, total polyphenols, vitamin C, and pH. The extraction technique was used to obtain date juice for use in the beverage industry from the "Bournow" cultivar. The effects of temperature, time, volume/mass ratio, and enzyme volume on free amino acid, total polyphenols, vitamin C, and pH levels were then investigated using a design centered on four parameters. All multivariate polynomial models of second degree with interactions were discovered and validated. To optimize responses, multiresponse optimization was utilized. The center composite design (CCD) determined the following response ranges: 4.21 to 5.62 pH; 45 to 126.66 mg/L vitamin C; 2.7 to 6.87 g GA/100 g total polyphenols; and 429.94 to 615.55 mg/L free amino acids. The selected factors had varying effects on the pH, vitamin C, total polyphenols, and free amino acid responses, with simple, quadratic, and interaction contributions leading to significant increases or decreases. Multi-response optimization, the objective of which was to maximize all responses besides pH in order to produce an abundant juice, resulted in the following compromise: 95 °C temperature; 10 min duration; 2:1 water/pulp ratio; and 0.5 ml of pectinase. The optimal values simulated yielded the following respective maxima: pH is 4.13; vitamin C is 116.5 mg/L; total polyphenols are 6.25 g GA/100 g; and free amino acids are 587.88 mg/L. This study successfully optimized the extraction technique for obtaining date juice from the "Bournow" cultivar. The results provide valuable insights for the beverage industry. Future prospects include further research on the sensory properties and shelf life of date juice, as well as exploring its potential applications in other food and beverage products.

1. Introduction

Dates (*Phoenix dactylifera* L.) are a globally significant fruit crop due to their nutritional value and economic advantages. Dates are used in high-value products such as candies, syrups, colas, beverages, and chocolates (Younas et al., 2020). They are the most cost-effective source of nutrients for combating food insecurity and rising food demand, especially in developing nations (Ghnimi et al., 2017). Date seeds are nutritious, but the food industry underutilizes them as agricultural refuse. They offer tremendous potential for the development of high-value natural health products. Dates are sacred fruits in all three major religions, but particularly Islam, and must be offered during

Ramadan, when the breaking of the fast is both religious and caloric (Alghamdi et al., 2018). Dates alleviate a variety of health issues and provide nutritional and pharmacological advantages. Dates provide fast energy from their simple sugars and provide health benefits from their fiber content (Al-Shahib & Marshall, 2002). According to Al-Farsi et al. (2007), the sugar content of dates varies from 35 % to 88 % based on maturation. Fresh or dried, dates are primarily composed of mono-saccharides and disaccharides (glucose, fructose, and sucrose), the amounts of which are used to identify them (Al-Hilphy et al., 2023). Dates are an excellent source of vitamins and offer numerous health advantages. Dates are potassium and magnesium abundant. Smaller amounts of calcium, zinc, copper, and selenium are present (Al Hilfi

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et al., 2019). Minerals are required for the development of bones, dentition, soft tissues, hemoglobin, muscles, and nerve cells (Vayalil, 2012). Underutilized date (Bournow), rich in nutrition and potential for high-value health products, are neglected by the food industry as agricultural waste. This research text highlights the gap in investigating their potential as functional food and nutraceuticals, despite the current focus on plant-based foods and optimal nutrition.

This paper's objective is to identify the optimal conditions for extracting secondary compounds (pH, titratable acidity, vitamin C, total polyphenols, and free amino acids) from date cultivar juice. Consequently, the optimal combination of extraction of minor compounds permits the production of a juice with adequate physicochemical characteristics in minor elements and a close to 1 desirability.

2. Materials and methods

2.1. Biological material

Date (*Phoenix dactylifera* L.) sampling was carried out on the N'Djamena market in collaboration with the Chadian Institute of Agricultural Research for Development (ITRAD). The "Bournow" variety (Fig. 1) was the most suitable because of its availability, good keeping qualities, appreciation by producers and high productivity. This variety accounts for 70 % of production in the Bornou district (Allarangaye et al., 2011).

In this study, the pectinase A, rapidase, was purchased from DSM (Food & Beverage, Netherlands).

2.2. Establishment of mathematical models

Factors affecting the extraction of constituents from dates were used. These factors are: Extraction temperature (x1), extraction time (x2), water/pulp ratio (x3) and pectinase volume (x4). The same 4-factors Centered Composite Design (CCD) were used to run the manipulations. After extraction, the responses considered were pH, free amino nitrogen (FAN) content, total polyphenols and titratable acidity.

For manipulation purposes, the coded variables were transformed into real variables (Wr matrix). The effects of real variables, which are

not necessarily expressed in the same units, can be compared using this transformation technique (Desobgo et al., 2010; Mathieu & Phan-tan-luu, 1997):

$$x_j = \frac{U_j - U_j^0}{\Delta U_j} \quad (1)$$

Where: x_j : value of coded variable j ; U_j = value of natural variable j ; U_j^0 = value of natural variable j at the center of the domain; ΔU_j is called the variation "step".

And;

$$U_j^0 = \frac{U_j^{\max} + U_j^{\min}}{2} \quad (2)$$

$U_j^{\max}$: maximum value of natural variable j ; $U_j^{\min}$: minimum value of natural variable j .

The model equation was defined and the coefficients of the model equation were predicted after the design of the experiment (the CCD in our case) had been chosen. In response surface method, the model used was generally a full quadratic equation or a diminutive form of this equation. The second-degree model can be written as follows (Mathieu & Phan-tan-luu, 1997):

$$y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum \sum_{i < j} \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

With β_0 : the constant, ε : the error and β_j , β_{jj} and β_{ij} were the coefficients of the model.

The following equation provides the matrix expression of the model:

$$y = W\beta + \varepsilon \quad (4)$$

It has been translated as follows:

$$\begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{bmatrix} = \begin{bmatrix} 1 & x_{11} & x_{12} & \cdots & x_{1k} \\ 1 & x_{21} & x_{22} & \cdots & x_{2k} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 1 & x_{n1} & x_{n2} & \cdots & x_{nk} \end{bmatrix} \times \begin{bmatrix} \beta_0 \\ \beta_1 \\ \vdots \\ \beta_k \end{bmatrix} + \begin{bmatrix} \varepsilon_0 \\ \varepsilon_1 \\ \vdots \\ \varepsilon_k \end{bmatrix} \quad (5)$$

The method of least squares was used to solve the system of equations given above. In this case, the Least Squares Method (LSM) is a multiple regression technique. Here's a summary:



Fig. 1. Date cultivar in its raw state (a,b); date cultivar crushed to different particle sizes (c,d).

Table 1
Experimental matrix and chemical evidence of Bournow date juice.

Run	x1	x2	x3	x4	pH	Vitamin C (mg/L)	Polyphenols (g GA/100 g)	Free Amino Nitrogen (mg/L)
1	0	0	0	0	4.46	78.333	3.72	550.310
2	−1	−1	1	1	4.37	45.000	2.70	440.712
3	1	1	1	1	5.62	88.333	6.72	484.564
4	1	1	−1	−1	4.82	70.000	6.06	605.210
5	−1	−1	−1	−1	4.95	126.667	5.66	524.222
6	0	0	1.607	0	4.63	63.333	3.98	472.649
7	0	0	−1.607	0	4.43	86.667	5.57	617.552
8	1	−1	−1	−1	4.37	116.667	5.45	606.022
9	−1	1	−1	1	4.40	100.000	3.50	503.586
10	0	−1.607	0	0	4.51	71.667	2.81	532.105
11	1.607	0	0	0	4.79	121.667	5.46	566.274
12	1	1	1	−1	5.08	113.333	5.84	497.512
13	0	1.607	0	0	4.66	73.333	4.57	522.800
14	1	1	−1	1	4.64	105.000	5.98	586.278
15	0	0	0	1.607	4.54	70.000	4.11	496.755
16	−1	1	1	−1	4.35	75.000	5.21	435.508
17	0	0	0	0	4.61	66.667	4.57	540.000
18	−1.607	0	0	0	4.44	81.667	3.69	456.660
19	1	−1	1	−1	4.78	103.333	3.52	499.324
20	−1	−1	−1	1	4.37	110.000	4.49	513.354
21	−1	1	−1	−1	4.55	95.000	5.10	515.134
22	0	0	0	0	4.69	63.333	4.06	545.000
23	0	0	0	−1.607	4.61	83.333	5.86	515.891
24	−1	1	1	1	4.39	58.333	3.90	429.944
25	0	0	0	0	4.62	63.333	3.50	540.000
26	1	−1	1	1	4.64	80.000	3.44	487.056
27	−1	−1	1	−1	4.43	95.000	3.84	445.596
28	1	−1	−1	1	4.21	120.000	6.87	587.770

Table 2
Validation criteria for different models based on juice attributes.

Paramètres	R ²	R ² _{adj}	AAD	B _f	A _f
Y _{pH}	0.9352	0.8654	0.029	1.006	1.029
Y _{vitC}	0.9221	0.8381	0.142	0.876	1.170
Y _{Pol}	0.9378	0.8708	0.116	0.925	1.132
Y _{aal}	0.9990	0.9980	0.045	1.034	1.045

It has been assumed that the random errors in the MMC, are distributed in the same way as the mean zeros and common unknown variances, and that they are independent of each other. The value obtained and the fitted value (y) for the second observation is represented by:

$$\varepsilon_i = y_i - y$$

(6)

The residual (error) ε is the evaluation of the corresponding residual (error). This residual takes two aspects into account. The first is a lack of fit, which reflects the potential mismatch between the polynomial model and the real model. The second is experimental errors, which are linked to the random nature of the response.

Evaluations of β_j were chosen on the basis that they should minimize the sum of squared residuals, also known as the sum of squared errors and has been noted SSE.

$$SSE = \sum_{i=1}^n \varepsilon_i^2 = \sum (y_i - y)^2$$

(7)

Residuals were evaluated using the following equation:

$$\varepsilon = y - W\beta$$

(8)

And SSE used the following expression:

Table 3
ANOVA for the significance of factors used in the extraction of certain constituents from Bournow date juice (*Phoenix dactylifera* L.).

Terms	pH df	MS	P	Vitamin C df	MS	P	Polyphenols df	MS	P	Free amino nitrogen df	MS	P
Const	/	/	0.000	/	/	0.000	/	/	0.000	/	/	0.000
x ₁	1	0.4007	0.000	1	1149.04	0.002	1	7.17653	0.000	1	24,618.0	0.000
x ₂	1	0.1835	0.001	1	374.15	0.046	1	3.97158	0.000	1	177.4	0.000
x ₃	1	0.1319	0.004	1	2339.00	0.000	1	5.20427	0.000	1	43,021.0	0.000
x ₄	1	0.0304	0.112	1	569.21	0.018	1	1.64039	0.009	1	750.3	0.000
x ₁ ²	1	0.0079	0.398	1	2812.99	0.000	1	0.92178	0.038	1	2175.1	0.000
x ₂ ²	1	0.0022	0.654	1	138.94	0.202	1	0.08501	0.496	1	577.4	0.000
x ₃ ²	1	0.0009	0.768	1	234.79	0.104	1	1.54493	0.011	1	0.9	0.703
x ₄ ²	1	0.0010	0.753	1	312.58	0.065	1	2.37146	0.003	1	2906.6	0.000
x ₁ x ₂	1	0.4192	0.000	1	1.56	0.889	1	1.15562	0.023	1	68.5	0.004
x ₁ x ₃	1	0.4935	0.000	1	1083.51	0.002	1	0.18922	0.315	1	788.0	0.000
x ₁ x ₄	1	0.0410	0.069	1	291.84	0.073	1	3.38560	0.001	1	54.5	0.009
x ₂ x ₃	1	0.0315	0.106	1	826.56	0.006	1	6.25000	0.000	1	1.0	0.682
x ₂ x ₄	1	0.0885	0.012	1	451.56	0.031	1	0.08122	0.506	1	0.5	0.780
x ₃ x ₄	1	0.1314	0.004	1	1254.34	0.001	1	0.00303	0.897	1	35.8	0.026
Error	13	0.0104	/	13	76.98	/	13	0.17337	/	13	5.7	/
Lack of fit	10	0.0108	0.510	10	84.86	0.369	10	0.16026	0.688	10	0.2	1.000
Pure error	3	0.0093	/	3	50.69	/	3	0.21709	/	3	24.2	/

Const: constant; df: degree of freedom; MS: mean square; P: probability.

$$SSE = \varepsilon^T \varepsilon = (y - W\beta)^T (y - W\beta) \quad (9)$$

Dividing the SSE as a function of β , a vector of partial derivatives was found as follows:

$$\frac{\partial}{\partial \beta} (SSE) = -2W^T (y - W\beta) \quad (10)$$

By setting this derivative equal to 0, it was obtained:

$$y = W\beta \quad (11)$$

It was possible to solve this system of equations directly to obtain the β coefficients:

$$W^T W \beta = W^T y \quad (12)$$

After that, the formal solution of these equations was given by:

$$\beta = (W^T W)^{-1} W^T y = CM^T y \quad (13)$$

With:

$$C = (W^T W)^{-1} \quad (14)$$

With C, the square matrix.

The model equation took its final form thanks to the values of the coefficients. The Minitab 21 program was used to perform matrix operations to evaluate the β vector. OriginLab 2022 was used to plot the graphs. It is important to note that the experimenter, who is aware of the stakes and risks of the study, determines the final model.

2.3. Validation of mathematical models

The coefficient of determination R^2 represented the goodness of fit of the second-degree equations. The models were validated using two techniques. The first strategy was the Absolute Average Deviation analysis (AAD) (Baş & Boyaci, 2007), while the second strategy used the accuracy factor and the polarized factor.

Method 1: The aim of the statistical analysis was to give the model's representativeness scientific legitimacy. The model equation was used to easily calculate the predicted response after obtaining the regression coefficients. Since system behavior is generally unknown, it was necessary to check whether the models corresponded correctly to the experimental data. Several methods are used to determine whether the model is adequate. Residual analysis measuring the residuals, the sum of the prediction errors of the residuals and the lack-of-fit test are some of these methods. The coefficient of determination (R^2) has generally been used to explain the predictive potential of the model as a whole. It should be noted that the coefficient of determination (R^2) is not the only measure of model accuracy. It is a measure of the amount of reduced response variability that has been achieved using the model's repressor variables. However, a high R^2 value does not mean that the regression model is good. Regardless of whether the additional variable is statistically significant or not, adding an additional variable to the model will always increase R^2 . Consequently, models with large R^2 values may provide poor predictions for new observations or evaluations of the mean response. If we compare experimental results with model results, we should obtain a straight line with a 45° angle passing through the origin. However, it is possible to obtain such a line using the formula $[y=ax+b]$. Absolute average deviation analysis (AAD), a direct method for describing deviations, was used to eliminate these types of errors.

The following equations were used to calculate the coefficient of determination R^2 and AAD:

$$R^2 = \frac{\sum_{i=1}^n (y_{i,cal} - \bar{y})^2}{\sum_{i=1}^n (y_{i,exp} - \bar{y})^2} \quad (15)$$

$$AAD = \frac{\left[\sum_{i=1}^n \left(\frac{|y_{i,exp} - y_{i,cal}|}{y_{i,exp}} \right) \right]}{n} \quad (16)$$

Where n is the number of experiments performed, $\bar{y}$ is the mean of the experimental responses and $y_{i,exp}$ and $y_{i,cal}$ are the experimental and calculated responses respectively.

To check the accuracy of the model, the combined evaluation of R^2 and AAD values should be more effective. The AAD between predicted and observed data should be as small as possible and R^2 should be close to 1 (Baş & Boyaci, 2007). The model equation defines the true behavior of the system and can be used for interpolation in the experimental domain, depending on the acceptable values of R^2 and AAD. It is important to consider the issue of extrapolation outside the area where the initial observations were made. It is quite possible that a model that works well with the initial data will no longer work with the outside data.

Method 2: Observed and theoretical values were compared to assess model validation. Equations for the polarized factor, Bf, and the polarized accuracy factor, Afl, were provided (Ross, 1996):

$$B_f = 10 \frac{\frac{1}{n} \sum_{i=1}^n \log \left(\frac{y_{i,cal}}{y_{i,exp}} \right)}{\left| \log \left(\frac{y_{i,cal}}{y_{i,exp}} \right) \right|} \quad (17)$$

$$A_{fl} = 10 \frac{\frac{1}{n} \sum_{i=1}^n \left| \log \left(\frac{y_{i,cal}}{y_{i,exp}} \right) \right|}{\left| \log \left(\frac{y_{i,cal}}{y_{i,exp}} \right) \right|} \quad (18)$$

$y_{i,theo}$, response obtained using the model; $y_{i,exp}$, response obtained by experiment; n, number of trials.

In the perfect predictive model, $A_{fl} = B_f = 1$. The acceptable predictive model is defined as $0.75 < B_f$ or $A_{fl} < 1.25$ (Dalgaard & Jørgensen, 1998).

2.4. Optimization

Optimization was carried out using a multi-response approach that included maximization of pH, vitamin C, polyphenols and titratable acidity as specifications. Minitab 21.3.1 was used to find the best combination meeting all specifications. Response optimization refers to a set of variable parameters that work together to optimize a single response or a set of responses. This method is useful for determining the effect of several variables on a response. Minitab assigns an individual desirability to each response and determines it according to the importance attributed to it. These values were added together to determine the overall desirability of the multiple-response system. When the composite desirability reached its maximum, an optimal solution was found. Individual and composite desirability were used to determine how well a combination of variables met the objectives of the response. Individual desirability measures the extent to which parameters optimize a single response, while composite desirability measures the extent to which parameters optimize a group of responses. The desirability scale runs from 0 to 1. A value of 1 would be ideal, while a value of 0 would indicate that one or more responses are outside the acceptable range. The weighted geometric mean of the individual desirability of the different responses is the composite desirability. Minitab determined the optimal parameters for the input variables by maximizing the composite desirability.

2.5. Date juice extraction

Solid-liquid extraction was carried out here. Extractions were carried out in accordance with the parameters of the four-factor centered composite design (CCD). The date juice extraction process was carried out according to Kadlezir et al. (2023).

Dates were sorted, washed and pitted. They were then crushed to increase the exchange surface and facilitate juice extraction. Extraction

was performed by immersing beakers containing crushed dates and water in a water bath, using a centered composite design with the variables temperature, time, water/pulp ratio, and pectinase (enzyme) volume ranging from 25 °C to 95 °C, 10 min to 120 min, 2 to 5, and 0 mL to 0.5 mL, respectively. This yielded 28 assays, each of which was filtered through a filter cloth, then pasteurized for 15 s at 72 ± 2 °C (Burapalit, 2019). Pasteurized juices were stored in the refrigerator at 4 °C.

2.6. Determination of free amino acid content in juices

The ninhydrin technique was used to determine the concentration of free amino acids in extracts by colorimetry (EBC-Analysis-Committee, 1998).

To obtain a 1/100 dilution, 99 ml distilled water was mixed with 1 ml extract. The sample was diluted and separated into three test tubes. Each test tube received 1 ml of color reagent (100 g/L Na_2HPO_4 , 60 g/L KH_2PO_4 , 5 g/L ninhydrin and 3 g/L fructose). Tubes were immersed in boiling water for 16 min. They were then cooled in a water bath to 20–25 °C. Each received 5 ml dilution solution (2 g KIO_3 , 1 L H_2O /Ethanol 96 % (600:400, v/v)). A Jenway 6405 UV/Visible spectrophotometer was used to measure absorbance at 570 nm (Jenway Ltd Felsted, Dunmow, Essex CM6 3LB, UK). The results obtained were compared with those of the control and the standard. To create the blank, 2 ml of distilled water was used in place of the diluted extract. The standard was 2 ml glycine (10.72 mg/L) instead of the diluted extract. The following relationship was used to determine the proportion of free amino acids:

$$FAN(\text{mg} / \text{L}) = \frac{2 \times A_1}{A_2} \times d \quad (19)$$

FAN: free amino nitrogen (mg/L); A1: absorbance of test solution at 570 nm; A2: mean absorbance of standard solution; d: dilution factor.

2.7. pH measurement of date juice

The pH meter electrode (Jual HANNA HI9813–6 Portable pH/ EC/ TDS Meter Harga Murah) was dipped into the beaker containing 20 ml of

sample measured with a graduated pipette, followed by 5 ml of the metaphosphoric acid-acetic acid solution (v/v) and 10 ml of distilled water. A second empty Erlenmeyer flask was filled with standard ascorbic acid solution (250 mg/L). Vitamin C (Vit C) was titrated with dichlorophenolindophenol (DCPIP) solution (8.61×10^{-3} mol/L) for 30 s until a pink tint persisted. The procedure was repeated three times. The following formula was used to calculate vitamin C content.

$$\text{VitC}(\text{mg} / \text{L}) = \frac{[\text{DCPIP}] \times V \times M}{V_0} \quad (20)$$

M: molar mass of vitamin C (176 g/mol); V: volume of DCPIP (ml); V₀: volume of sample (mL)

2.9. Determination of total phenolic content in date juice

Polyphenols in date juice were measured using the Folin-Ciocalteu reagent (Matloob & Balakita, 2016), which produces a blue phosphotungstic-phosphomolybdenum complex. Two milliliters distilled water and 1.0 mL Folin-Ciocalteu reagent (diluted 1:10) were added to 100 µL sample extract. After allowing the mixture to stand for 5 min, 0.75 mL Na_2CO_3 solution (60 g/L) was added. After 90 min, absorbance was measured at 765 nm using a UV-visible spectrophotometer (Jenway Ltd Felsted, Dunmow, Essex CM6 3LB, UK) against water as a blank. For three replicates, total phenol concentration was expressed as g gallic acid equivalent (GAE) per 100 g fresh sample.

3. Results and discussion

3.1. Modeling pH, vitamin C, free amino acids and polyphenols

The influence of process parameters (temperature, time, enzyme ratio and volume) on the extraction of some responses (pH, vitamin C, free amino acids and polyphenols) from date juice was determined. The results are shown in Table 1.

CCD models relate singular factors, interactions and quadratic effects to response variables. These models consisted of:

$$Y_{\text{pH}} = 4.5771 + 0.0856x_1 + 0.058x_2 + 0.0491x_3 - 0.0236x_4 + 0.0095x_1^2 + 0.005x_2^2 - 0.0033x_3^2 + 0.0035x_4^2 + 0.06268x_1x_2 + 0.06801x_1x_3 + 0.0196x_1x_4 + 0.01718x_2x_3 + 0.0288x_2x_4 + 0.03509x_3x_4 \quad (21)$$

$$Y_{\text{vitC}} = 66.36 + 4.59x_1 - 2.62x_2 - 6.54x_3 - 3.23x_4 + 5.623x_1^2 + 1.25x_2^2 + 1.625x_3^2 + 1.874x_4^2 + 0.121x_1x_2 + 3.187x_1x_3 + 1.654x_1x_4 + 2.783x_2x_3 + 2.057x_2x_4 - 3.429x_3x_4 \quad (22)$$

$$Y_{\text{Pol}} = 3.935 + 0.3624x_1 + 0.2696x_2 - 0.3086x_3 - 0.1732x_4 + 0.1018x_1^2 - 0.0309x_2^2 + 0.1318x_3^2 + 0.1633x_4^2 + 0.1041x_1x_2 - 0.0421x_1x_3 + 0.1781x_1x_4 + 0.242x_2x_3 - 0.0276x_2x_4 - 0.0053x_3x_4 \quad (23)$$

$$Y_{\text{aal}} = 544.08 + 21.223x_1 - 1.801x_2 - 28.055x_3 - 3.705x_4 - 4.945x_1^2 - 2.548x_2^2 + 0.099x_3^2 - 5.716x_4^2 + 0.801x_1x_2 - 2.718x_1x_3 - 0.715x_1x_4 - 0.097x_2x_3 - 0.066x_2x_4 + 0.579x_3x_4 \quad (24)$$

sample at 25 °C; the pH value was read. The operation was repeated three times.

2.8. Determination of vitamin C content in date juice

An Erlenmeyer flask was filled with a volume V' equal to 5 ml of

With, Y_{pH}: pH; Y_{vitC}: Vitamin C; Y_{Pol}: Polyphenols; Y_{aal}: Free amino acids; x₁: Temperature; x₂: Time; x₃: Water/pulp ratio; x₄: Pectinase volume.

These second-order models are useful if some of the input variables

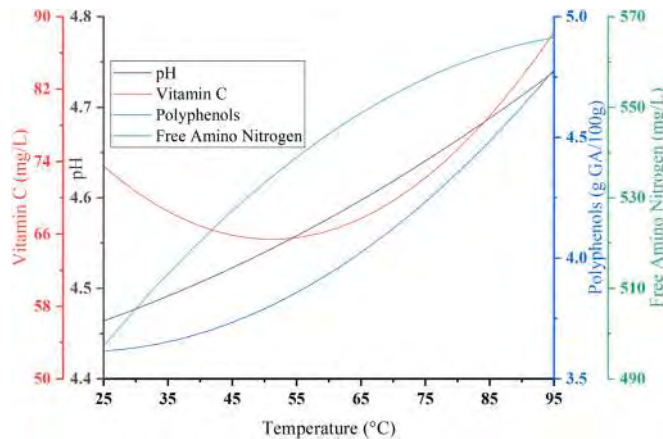


Fig. 2. Changes in vitamin C, pH, polyphenols and free amino acids as a function of temperature (time, water/pulp ratio and pectinase volume fixed at 65 min, 3.5 and 0.25 mL respectively).

are precise. Table 2 shows that all the models are valid and allow a thorough evaluation of the components.

The ANOVA in Table 3 only takes into account variables with a probability of less than 0.05. These are therefore the only relevant elements. These are therefore the only relevant elements.

3.1.1. Impact of singular factors on responses

3.1.1.1. Impact of temperature. The factor corresponding to extraction temperature, as a singular factor (extraction time (x2), water/pulp ratio (x3), and enzyme volume (x4) being fixed at their central values of 65 min; 3.5 and 0.25 mL respectively), has a significant impact on vitamin C, pH, polyphenols and free amino acids (Table 3).

All these responses increase with increasing temperature. Indeed, pH, vitamin C, polyphenols and free amino acids increase significantly (Table 3) from 4.46; 73.5 mg/L; 3.61 g GA/100 g and 497.2 mg/L respectively at 25 °C, to values of 4.73; 88.26 mg/L; 4.78 g GA/100 g and 565.42 mg/L at 95 °C (Fig. 2).

In the case of vitamin C and the free amino acids present in dates (Ashraf & Hamidi-Esfahani, 2011), the temperature would weaken the pulp, allowing greater release of these two components into the juice. In fact, for vitamin C, the extraction kinetics would be superior to those of denaturation.

For pH, this could be explained by the fact that potassium, one of the most abundant mineral compounds in dates (Ibrahim et al., 2001; Mohamed, 2000), could increase the pH. Secondly, high-temperature extraction would rapidly deactivate pectinase, resulting in an extract with a higher pH. Because of the loss of volatile acids and carbon dioxide due to increased temperature, the acidity of the juice would decrease, resulting in a higher pH.

The higher temperature would enable polyphenols to be extracted more efficiently. In this case, the rate of polyphenol extraction would be higher than the rate of degradation. This may be explained by the presence of hydrolyzable tannins, which are thermodegradable. In reality, hydrolyzable tannins were degraded at high temperature (100 °C), resulting in an increase in non-tannin content. Al-Farsi et al. (2005) reported an increase in the total phenolic content of sun-dried dates due to temperature-induced tannin degradation during the drying process. In addition, Jeong et al. (2004) reported a significantly higher concentration of polyphenols in heated citrus peels than in unheated peels.

3.1.1.2. Impact of extraction time. The factor corresponding to extraction time, as a singular factor (extraction temperature (x1), water/pulp ratio (x3), and enzyme volume (x4) being fixed at their central values of 60 °C; 3.5 and 0.25 mL respectively), has a significant impact on vitamin

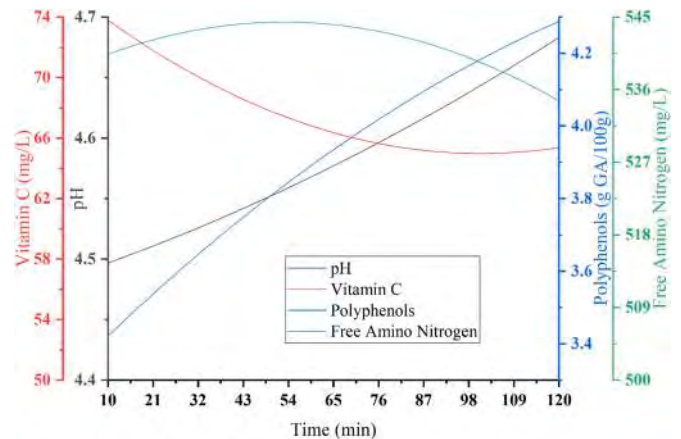


Fig. 3. Changes in vitamin C, pH, polyphenols and free amino acids as a function of time (temperature, water/pulp ratio and pectinase volume fixed at 60 °C, 3.5 and 0.25 mL respectively).

C, pH, polyphenols and free amino acids (Table 3). pH and polyphenol content increase significantly (Table 3) with increasing extraction time. On the other hand, vitamin C and free amino acids decrease significantly (Table 3) with increasing extraction time (Fig. 3).

Indeed, for pH and polyphenols, we start from 4.49 to 3.42 g GA/100 g respectively for a time of 10 min, to increase to 4.68 and 4.28 g GA/100 g respectively for a time of 120 min Fig. 3. In addition to the above-mentioned reasons concerning temperature (here it is fixed at 60 °C), a longer extraction time would allow greater accumulation in the juice, justifying the increase. It's important to note that different types of polyphenols have different effects. According to Kaack and Austed (1998), anthocyanidins are protected from oxidative destruction by vitamin C, which acts as an inhibitor. This protection is provided by vitamin C. According to Aka et al. (2013), the protective effect described is most likely due to the decrease in the oxidized form of the polyphenol caused by ascorbic acid. This form of the polyphenol is then oxidized, as has been discovered for chlorogenic acid and epicatechin.

The decrease in vitamin C and free amino acids can be seen in Fig. 3. For vitamin C and free amino acids, we start from 73.79 mg/100 g and 540.39 mg/L for a time of 10 min, and decrease to 65.37 mg/100 g and 534.61 mg/L respectively, for a time of 120 min Fig. 3.

The main route of vitamin C degradation in aqueous liquid systems involves the oxidation of ascorbic acid to dehydroascorbic acid, which rapidly degrades to 2,3-diketogulonic acid (Washko et al., 1992). The hydrolysis of dehydroascorbic acid results in the loss of the vitamin property of the molecule. Ascorbic acid degradation increases with increasing water activity or moisture content (Lee & Labuza, 1975). The reaction of ascorbic acid with its oxidized form, dehydroascorbic acid, and subsequent hydrolysis to 2,3-diketogulonic acid occur simultaneously in water in the absence of oxidizing or reducing compounds (Serpén & Gökmen, 2007).

The carbonyl groups of proteins, peptides and amino acids condense with the carbonyl groups of sugars to trigger Maillard reactions, forming Schiff bases that can be rearranged into Amadori or Heyns products (Hellwig & Henle, 2014). These macromolecules are decomposed or modified to generate reactive dicarbonyl species, which can react readily with other nucleophiles such as amines, guanidines and thiols. By reacting with free amino acids to produce imines, these intermediates can undergo Strecker degradation, leading to the formation of Strecker aldehydes (Lund & Ray, 2017). This would justify the decrease in free amino acid content over time.

3.1.1.3. Impact of the water/pulp ratio. The factor that corresponds to the water/pulp ratio, as a singular factor (with extraction temperature (x1), extraction time (x2), and enzyme volume (x4) fixed at their central

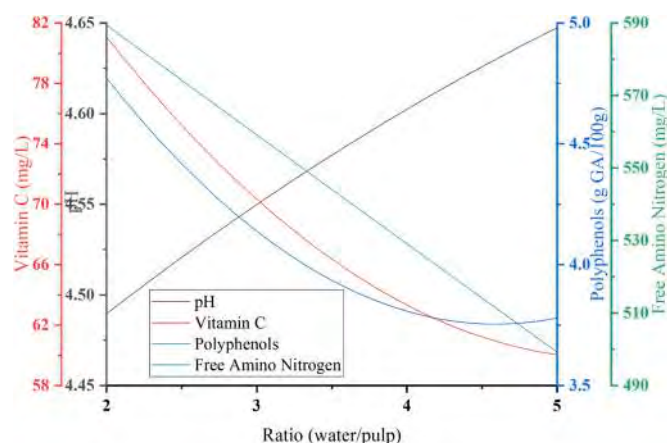


Fig. 4. Changes in vitamin C, pH, polyphenols and free amino acids as a function of water/pulp ratio (temperature, time and pectinase volume fixed at 60 °C, 65 min and 0.25 mL respectively).

values of 60 °C; 65 min; and 0.25 mL respectively), has a significant impact on vitamin C, pH, polyphenols and free amino acids (Table 3).

Vitamin C, polyphenols and free amino acids decreased with increasing water/pulp ratio, while pH increased with increasing water/pulp ratio (Fig. 4).

In fact, vitamin C, polyphenols and free amino acid contents go from 81.06 mg/100 g; 4.77 g GA/100 g and 589.42 mg/L respectively for a water/pulp ratio of 2 (Fig. 4), to 60.04 mg/100 g; 3.77 g GA/100 g and 499.25 mg/L respectively for a water/pulp ratio of 5 (Fig. 4). On the other hand, pH rose from 4.48 for a water/pulp ratio of 2 to 4.64 for a water/pulp ratio of 5. All these observations can be explained simply by the dilution effect, which reduces the concentration of vitamin C, polyphenols and free amino acids, while increasing the pH.

3.1.1.4. Impact of enzyme volume. The factor corresponding to enzyme volume, as a singular factor (extraction temperature (x1), extraction time (x2), and water/pulp ratio (x3) being fixed at their central values of 60 °C; 65 min; and 3.5 respectively), has a significant impact on vitamin C, pH, polyphenols and free amino acids (Table 3).

Vitamin C and polyphenol contents undergo a significant decrease, followed by a non-significant increase with increasing enzyme volume (Fig. 5), while free amino acid content undergoes a non-significant increase on the one hand, followed by a significant decrease with increasing enzyme volume (Fig. 5).

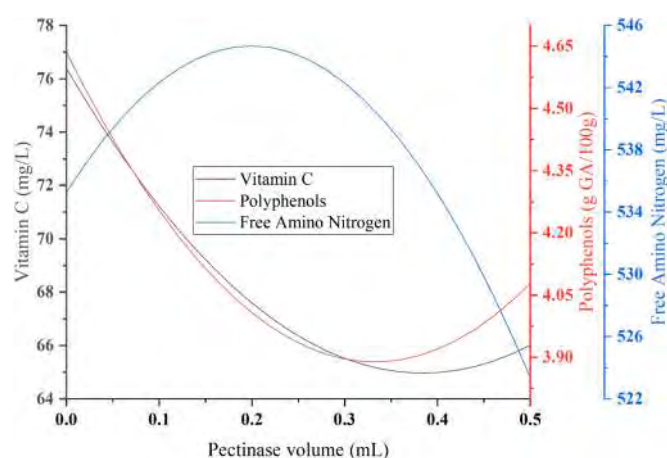


Fig. 5. Changes in vitamin C, polyphenols and free amino acids as a function of pectinase volume (temperature, time and water/pulp ratio set at 60 °C, 65 min and 3.5 respectively).

In fact, for vitamin C, we go from 76.39 mg/100 g without the addition of pectinase, followed by a decrease to a minimum value of 64.96 mg/100 g at a pectinase volume of 0.38 mL and then a non-significant increase to 66 mg/100 g at a pectinase volume of 0.5 mL (Fig. 5). The polyphenol content ranges from 4.63 g GA/100 g with no pectinase added, to a minimum value of 3.89 g GA/100 g with 0.33 mL pectinase added, followed by a non-significant increase to 4.07 g GA/100 g with 0.5 mL pectinase added (Fig. 5). The free amino acid content starts at 535.27 mg/L without the addition of pectinase, followed by a non-significant increase to 544.68 mg/L with the addition of 0.2 mL pectinase, and then a significant decrease to 523.36 mg/L with the addition of 0.5 mL pectinase (Fig. 5).

The reason for the drop in polyphenols and amino acids with increasing pectinase volume is hydrolysis of the date matrix, releasing these two constituents, which are involved in the disorder formation process. The most common cause of cloudiness in beverages is the interaction between proteins and polyphenols. Proline is present in proteins that bind polyphenols, and the more proline present, the greater the disorder-forming activity. Proline-rich proteins with high binding affinity to polyphenols via hydrogen bonding and hydrophobic interactions have been shown to be responsible for haze formation (Schulte et al., 2016). At least two sites of haze-causing polyphenols can bind to proteins, enabling them to crosslink proteins and produce insoluble, light-scattering particles. At least initially, the interaction between protein and polyphenol is non-covalent and reversible. The ratio of haze-active polyphenols (HA) to HA protein affects both haze particle size and haze intensity (Schulte et al., 2016).

3.1.2. Impact of interactions on responses

The x1 × x2 interaction (temperature/extraction time) contributes to a significant increase in pH, polyphenols and free amino acids (Table 3). Indeed, for pH, polyphenols and free amino acids, this increase is observed with a simultaneous increase in extraction temperature and time (Fig. 6).

Increased temperature and extraction time contribute to pulp embrittlement and greater release of minerals such as potassium and calcium (Ibrahim et al., 2001; Mohamed, 2000), which in aqueous solution form bases that raise the pH.

The increase in polyphenols with a simultaneous increase in temperature and extraction time can be explained by the fact that polyphenol extraction is better at higher temperatures (Jeong et al., 2004), and with longer extraction times there is an accumulation of these polyphenols in the date juice, resulting in an increase.

The increase in free amino acid content with increasing temperature and extraction time is explained by the fact that these compounds are extracted much more quickly, so that the extraction kinetics are greater than those for the formation of Maillard reaction compounds (Hellwig &

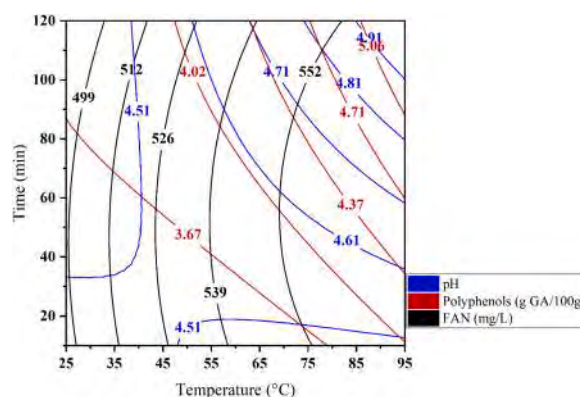


Fig. 6. Changes in pH, polyphenols and free amino acids as a function of temperature/time interaction. The pulp/water ratio and pectinase volume are fixed at 3.5 and 0.25 mL respectively.

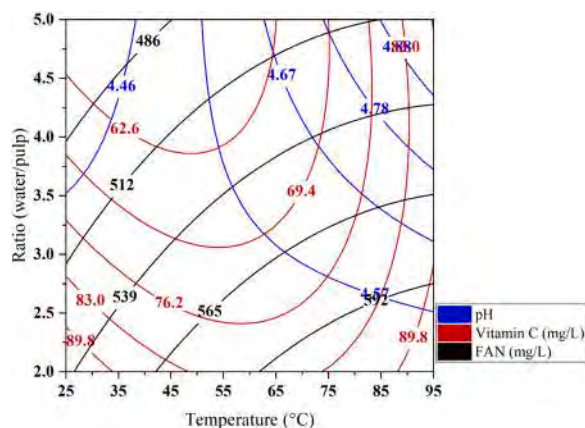


Fig. 7. Changes in pH, vitamin C and free amino acids as a function of temperature/water/pulp ratio interaction. The time and volume of pectinase are fixed at 65 min and 0.25 mL respectively.

Henle, 2014) and haze.

The $x1 \times 3$ (temperature/pulp water ratio) interaction contributes to a significant increase in pH and vitamin C (Table 3), while it contributes to a significant decrease in free amino acid content (Table 3).

The increase in the pH is achieved through a simultaneous increase in temperature and water/pulp ratio (Fig. 7). The water/pulp ratio, which favors dilution of the juice, contributes to raising its pH, while the increase in temperature contributes to better extraction of minerals, such as potassium, which forms bases in solution.

The increase in vitamin C is observed by reducing the extraction temperature simultaneously with an increase in the pulp water ratio (Fig. 7). This would lead to an accumulation of extracted vitamin C, and with the temperature reduced, vitamin C would not be destroyed by the latter.

The drop in free amino acids is observed when the temperature is reduced simultaneously with an increase in the water/pulp ratio (Fig. 7). This decrease is explained more by the dilution effect than by an increase in the ratio.

The $x1 \times 4$ interaction contributes to a significant increase in polyphenols and a simultaneous significant decrease in free amino acids (Table 3).

In fact, the increase in polyphenols is obtained via a simultaneous increase in temperature and pectinase volume (Fig. 8). This increase is explained by the fact that the extraction temperature weakens the pulp and, combined with the volume of pectinase, hydrolyzes the pectins, thus enabling greater extraction of polyphenols.

The decrease in free amino acid content can be observed by lowering

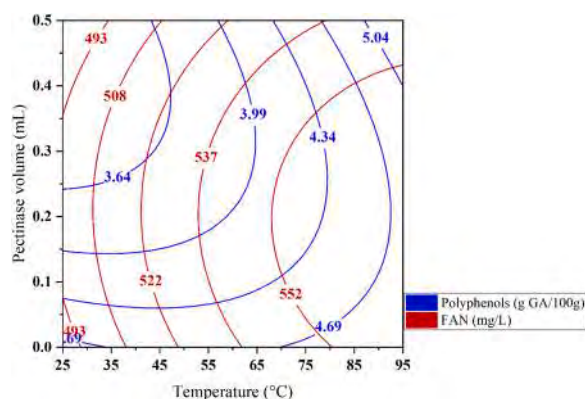


Fig. 8. Changes in polyphenols and free amino acids as a function of pectinase temperature/volume interaction. The pulp/water ratio and time are set at 3.5 and 65 min respectively.

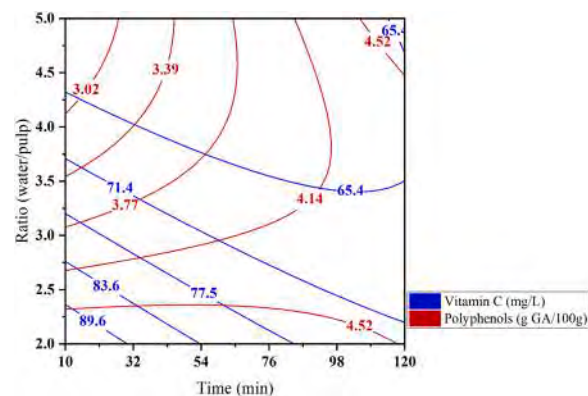


Fig. 9. Evolution of vitamin C and polyphenols as a function of time/water/pulp ratio interaction. Temperature and pectinase volume set at 60 °C and 0.25 mL respectively.

the extraction temperature simultaneously with an increase in pectinase volume (Fig. 8). In fact, this effect can be explained by the fact that not only would the lower temperature not allow better extraction, it would also not allow hydrolysis of the pulp by pectinase.

The $x2 \times 3$ interaction contributes to a significant increase in vitamin C and polyphenols (Table 3). The increase in vitamin C content is observed via a simultaneous decrease in time and water/pulp ratio (Fig. 9). This could be explained by the fact that, on the one hand, a decrease in the water/pulp ratio leads to an increase in concentration, and a reduction in time contributes to a decrease in the effect of temperature on vitamin C degradation, and thus to an increase in vitamin C content.

The increase in polyphenol content is observed with an increase in extraction time simultaneously with a decrease in the water/pulp ratio (Fig. 9). Indeed, increasing the extraction time contributes to an accumulation of polyphenols in the juice, and concomitantly a decrease in the water/pulp ratio reduces the effect of dilution and, in turn, creates an increase in concentration.

The $x2 \times 4$ interaction contributes to a significant increase in pH and vitamin C content (Table 3). This increase in pH is achieved by increasing extraction time and decreasing pectinase volume (Fig. 10). The increase in pH would be due simultaneously to an accumulation of ions responsible for the basic character (such as potassium, sodium and others), while the reduction in pectinase volume would not favor hydrolysis of the pulp and would therefore reduce extraction of the organic acids present in the date.

The increase in vitamin C can be observed via a simultaneous reduction in extraction time and pectinase volume. In fact, the thermal

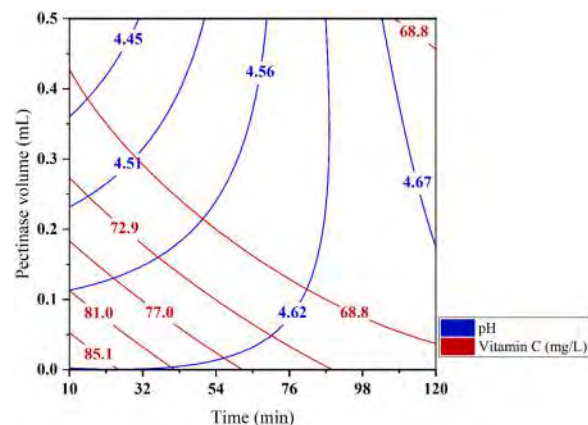


Fig. 10. Evolution of pH and vitamin C as a function of pectinase time/volume interaction. Temperature and water/pulp ratio set at 60 °C and 3.5 respectively.

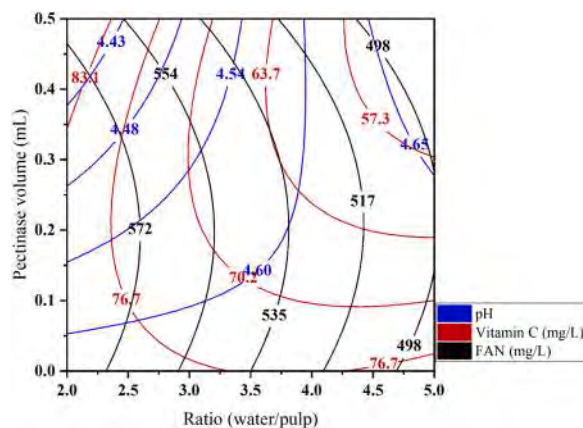


Fig. 11. Changes in pH, vitamin C and free amino acids as a function of the water/pulp ratio/pectinase volume interaction. Temperature and time set at 60 °C and 65 min respectively.

degradation of vitamin C is slowed down as the time of heat application is reduced. At the same time, according to studies, the stability of vitamin C depends largely on the temperatures applied and the presence of oxygen, and its degradation is largely due to oxidation reactions occurring during the adiabatic heating phase (Oey et al., 2008). The applied temperature inactivates endogenous enzymes, notably ascorbic acid oxidase, which can cause vitamin C degradation (Leong & Oey, 2012; Munyaka et al., 2010).

The x3×4 interaction contributes to an increase in pH, free amino acid content and a decrease in vitamin C content (Table 3).

The increase in pH is achieved by increasing the water/pulp ratio simultaneously with a decrease in pectinase volume. Indeed, the increase in the water/pulp ratio would contribute to this pH increase via the dilution effect, and the simultaneous decrease in pectinase volume would contribute to the reduction of the hydrolytic effect of this enzyme, which could have enabled the release of organic acids (Fig. 11).

The increase in free amino acid content would occur via a reduction in the water/pulp ratio and simultaneously with the increase in pectinase volume (Fig. 11). In fact, reducing the water/pulp ratio would bring about a concentration effect, while at the same time increasing the pectinase volume would contribute to better pulp hydrolysis and, in turn, an increase in free amino acid content.

The decrease in vitamin C content can be seen in the simultaneous increase in the water/pulp ratio and enzyme volume (Fig. 11). Although the increase in pectinase volume would contribute to an increase in vitamin C content through hydrolysis of the pulp, the gradient of the water/pulp ratio would be greater, and consequently lead to a decrease in the vitamin C content of the juice.

3.2. Optimization of minor constituents (pH, vitamin C, polyphenols and FAN)

Multi-response optimization was used to maximize the minor physicochemical properties of date juice. To this end, vitamin C, polyphenols and free amino acids (FAN) were all taken into account. pH, on the other hand, was left unchanged. The final compromise for Minitab 21.3 optimization was as follows: extraction at 95 °C for 10 min, water/pulp ratio 2 and pectinase volume 0.5 mL. Using this combination, free amino acids of 587.88 mg/L, polyphenols of 6.25 g GA/100 g, vitamins C of 116.5 mg/100 g and a pH of 4.13 were obtained. Individual desirabilities for free amino acids, polyphenols and vitamin C were 0.841, 0.852 and 0.875 respectively. Composite desirability was 0.856. In the study, composite desirability (0.856) was close to 1, suggesting that the parameters appeared to have a positive impact on all responses. However, the individual desirability data showed that the parameters were more effective at maximizing vitamin C (0.875) than polyphenols

(0.852) and FANs (0.841).

4. Conclusion

In this study, the response surface methodology was used to analyze the characteristics of extracted date juices. The juice extraction method affected the pH, vitamin C, free amino acids, and polyphenols in the juice. The extraction process was optimized to maximize desired responses and improve the formulation. The results showed that the extraction method effectively extracted both major and minor compounds from the date fruit. This suggests that the parameters used in the study were successful in achieving favorable outcomes. The study had a high composite desirability value of 0.8560, indicating that the response surface methodology could be a suitable approach for date juice extraction when optimal conditions are established. The findings of this study have broader implications, as the production of date juice could contribute to the development of functional food products with added health benefits. Date juice has the potential to serve as a prebiotic for fermentation using probiotics, highlighting its nutritional value.

CRediT authorship contribution statement

Fiacre Kadlezir: Conceptualization, Data curation, Investigation, Writing – original draft. **Ahmed Mohammed Mohagir:** Validation, Visualization, Writing – review & editing. **Steve Carly Zangué Desobgo:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

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V.4) Application of response surface methodology in date (*Phoenix dactylifera* L.) juice extraction: Effect of process parameters on Brix, color and sugar/acid ratio

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**ORIGINAL ARTICLE****Application of response surface methodology in date (*Phoenix dactylifera* L.) juice extraction: Effect of process parameters on Brix, color and sugar/acid ratio**¹Kadlezir Fiacre / ²Mohagir Ahmed Mohammed / *³Desobgo Zangué Steve Carly/**Authors' Affiliation**¹University of Ndjamena,
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A technique called "extraction" was used to get juice from the "*Bournow*" date cultivar so that it could be used in the beverage industry. To reach this goal, dates were first studied to find out what their physical traits were. Then, the four-factor centered composite design was used to find out how temperature, time, volume to mass ratio, and enzyme volume affected Brix, color, and sugar/acid ratio. All second-degree multivariate polynomial models with interactions were obtained and validated. To maximize the responses, a multi-response optimization was done. The physical characterization permitted to get values below the criteria for classification. This enabled to say that the *Bournow* dates have bad qualities, which was why they needed to be valued. From center composite design the following responses ranges were obtained: Brix, between 13.2 and 25.9 °B; color, between 125.5 and 206 ASBC; sugar/acid ratio, between 6.24 and 46.90. It was observed that the selected factors have different effects on the Brix, color, and sugar/acid ratio responses, increasing or decreasing them in a significant way in single, quadratic, and interaction contributions. The multi-response optimization, whose goal was to get the maximum of all responses in order to make nutrient-rich juice, came up with the following compromise: temperature, 95 °C; time, 10 min; ratio water/pulp, 2:1, and pectinase volume, 0 mL. The simulated optimum values resulted in the following respective maximums: Brix, 21.89 °B; sugar/acid ratio, 13.99 and color, 197.49 ASBC.

Keywords: *Phoenix dactylifera* L., Juice, Extraction, Optimization**1. Introduction**

Consumer demand for functional foods has resulted in the processing of lesser-known fruits such as desert dates, which are known for their medicinal properties rather than their culinary value. These products, such as *Phoenix dactylifera* L., contain antioxidants, vitamins, and other nutritive and functional compounds (Chirinos *et al.*, 2013). It is important for human and animal nutrition because of its productivity, nutritional quality of its fruits, and ability to adapt to Saharan regions. Chad is Africa's eighth-largest producer, with an annual output of about

21,134.59 tons (FAOSTAT, 2022). The cultivar most widely used in almost all production areas in Sahara is "*Bournow*" due to its high productivity, ease of storage, the quality of its fruits for marketing (dry date), and climate adaptation. Date palm products and byproducts are widely used from the Sahara to the Sahel (Mahmoud *et al.*, 2022). The date can be used as a raw material in the development of many products including liquid sugar, date pastes, juices, syrups, soft drinks, confectionery, alcohol, vinegar (Estanove, 1990). It is primarily the dry dates, soft dates, and palms that are used in human or animal food, either locally or through traders throughout



almost the entire Chad (Mahmoud *et al.*, 2022). Date juice could be an excellent substitute. That juice is a beverage with a unique flavor and a lot of nutritional benefits (Mahmoud *et al.*, 2022). Heat treatment causes several biological, physical, and chemical changes in foods, resulting in sensory, textural, and nutritional changes. Heating has been shown to improve food safety by destroying or inhibiting microorganisms and inactivating anti-nutritional factors. It is also involved in the formation of desired compounds such as flavor compounds, antioxidants, and coloring agents. Furthermore, heating improves food digestibility and nutrient bioavailability (Echegaray *et al.*, 2021). Chemical constituents of food products must be affected by processing methods. Understanding the effect of processing on functional components is critical for preserving or improving the original activity of dates during date juice preparation (Burapalit, 2019).

The food and beverage industry still views popular fruits as the only raw materials for juice production; however, the discovery of previously unknown nutritional facts about lesser-known fruits (Amadou & Le, 2017) such as the *Bournow* date presents an alternative source of juice raw material. The study's overarching goal is to contribute to the valorization of the date sector by producing an adequate juice. This will entail specifically developing mathematical models that will allow the prediction of the profile of some physicochemical characteristics of the "Bournow" date extract as well as optimizing the extraction of sugars, color, and the sugar/acid ratio.

2. Materials and Methods

2.1 Biological material

Date sampling was conducted at the N'Djamena market in collaboration with Chadian Institute of Agricultural Research for Development (ITRAD)

The variety "*Bournow*" was the best indicated because of its availability, good conservation, producer appreciation, and high productivity. This variety accounts for 70 % of the Bornou district's production (Allarangaye *et al.*, 2011).

2.2 Physical characterization of date fruits

The physical characterization of date fruits was performed according to Sawaya *et al.* (1983). The color of the date fruits was assessed visually; the measurements of the entire fruit and its core (length and width) were taken with a caliper; and the mass (pulp and core) was determined using an analytical balance.

2.3 Determination of the water content of the whole date

The mass lost during drying under typical conditions determines the water content of dates (EBC-Analysis-Committee, 1998). It was calculated using 3 grams of date powder. To achieve a constant mass, the sample was crushed, spread in a porcelain capsule, and dried in a 103 ± 2 °C for 24 h.

$$W(\%) = \frac{\text{loss in weight}}{\text{initial weight of sample}} \times 100 \quad (1)$$

loss in weight = *initial weight of sample* – *final weight of sample (after drying)*

With, W (%): water content

2.4 Response Surface Methodology (RSM)

A mathematical model is an equation or set of equations that best describes the reality it depicts. This model will then be used to describe any analogous phenomenon as a whole, as well as to make predictions (interpolation, extrapolation). First, a phenomenon must be observed, followed by the construction of a model that reproduces it as precisely as possible, the identification of the model's limits, and finally, its validation. This mathematical modeling method corresponds well

to the scientific method steps used in the research program, namely observation, analysis, hypothesis, and validation (Gervais, 2007).

Temperature, extraction time, ratio (water/pulp), and pectinase volume were all variables. A centered composite design (CCD) was used to select the experiments values.

$N = N_f + N + N_0$, or $24 + 24 + 4 = 28$ trials, is the total number of tests performed.

The value of alpha for CCD was calculated to satisfy the condition of near orthogonality. The proposed model is a second-degree dual-interaction model. Each technique is carried out in triplicate. Minitab 21.3.1 was used to create the experimental design. For the laboratory experiments, the coded variables were converted into real variables (Desobgo *et al.*, 2010; Mathieu & Phan-tan-luu, 1997), resulting in the experimental design shown in Table 1. The transformation operations listed below were used:

$$U_j = U_j^0 + x_j \Delta U_j \quad (2)$$

$$U_j^0 = \frac{U_j^{\min} + U_j^{\max}}{2} \quad (3)$$

With: x_j , value of the coded variable j ; U_j , value of the real variable j ; U_j^0 , value of the real variable j at the center of the domain; ΔU_j , increment; $U_j^{\max}$, value of the real variable at the upper bound of the domain; $U_j^{\min}$, value of the real variable at the lower bound of the domain.

The a priori postulated mathematical model is of the second-degree polynomial type with interactions. It is expressed as follows

$$y = a_0 + \sum a_i x_i + \sum a_{ij} x_i x_j + \sum a_{ii} x_i^2 + \varepsilon \quad (4)$$

Where: y , the response; x_i and x_j , the independent variables; a_0 , constant; a_i , a_{ij} and a_{ii} , model coefficients and ε , error or residual.

2.5 Validation and optimization

To express the fit of second-degree equations, the determination coefficient R^2 was used. This coefficient of determination was insufficient for model validation on its own (Desobgo, 2012). The absolute average deviation (AAD) (Baş & Boyaci, 2007) was required to validate a model, as was the use of the bias factor and the accuracy factor (Ross, 1996). As a result, the model validation criterion was calculated using the formulas:

$$AAD = \frac{\left[\sum_{i=1}^n \left(\frac{|Y_{i,\text{exp}} - Y_{i,\text{theo}}|}{Y_{i,\text{exp}}} \right) \right]}{n} \quad (5)$$

$$B_f = 10^{\frac{1}{n} \sum_{i=1}^n \log \left(\frac{Y_{i,\text{theo}}}{Y_{i,\text{exp}}} \right)} \quad (6)$$

$$A_f = 10^{\frac{1}{n} \sum_{i=1}^n \left| \log \left(\frac{Y_{i,\text{theo}}}{Y_{i,\text{exp}}} \right) \right|} \quad (7)$$

With: AAD, absolute average deviation; B_f , bias factor; A_f , accuracy factor; $Y_{i,\text{theo}}$, response obtained using the model; $Y_{i,\text{exp}}$, response obtained via experiment; n , number of trials.

The calculated values must fall within the following ranges: AAD, 0-0.3; B_f , 0.75-1.25, and A_f , 0.75-1.25 (Dalgard & Jørgensen, 1998).

The optimization was carried out using a multi-response approach that included maximizing the brix, color and sugar/acid ratio as specifications. The Minitab 21.3.1 software was used to find the best combination respecting all the specifications. Response optimization referred to a set of variable parameters that worked together to optimize a single response or a set of responses. This was useful for determining the effect of

multiple variables on a response. Minitab assigned an individual desirability to each response and determined it based on the importance assigned to it. These values were added together to determine the overall desirability of the multi-response system. When the composite desirability reached its maximum, an optimal solution emerged. Individual and composite desirability determined how well a combination of variables met the response objectives. Individual desirability measured how well the parameters optimized a single response, whereas composite desirability measured how well the parameters optimized a group of responses. Desirability is scaled from 0 to 1. A value of 1 would be ideal, while a value of 0 would indicate that one or more responses were out of the acceptable range. The weighted geometric mean of individual desirability for various responses would be composite desirability. Minitab determined the optimal parameters for the input variables by maximizing the composite desirability.

2.6 Juice extraction

A solid-liquid extraction was done here. Extractions were carried out in accordance with the parameters of the four-factor centered composite design (CCD). The extraction process of the date juice was carried out as shown in Figure 1.

The dates were sorted, washed, and pitted. They were then crushed to increase the exchange surface and facilitate juice extraction. This extraction was carried out by immersing beakers containing crushed dates and water in a water bath, using a centered composite design with the variables temperature, time, water/pulp ratio, and pectinase (enzyme) volume ranging from 25°C to 45°C, 10 min to 60 min, 2 to 5, and 0 mL to 0.5 mL, respectively. This resulted in 28 trials (Table

1), each of which was filtered through a filter cloth and then pasteurized for 15 seconds at $72 \pm 2^\circ\text{C}$ (Burapalit, 2019). The pasteurized juices were kept in the fridge at 4°C .

2.7 Physicochemical analysis of date extracts

Every analysis was performed in triplicate at each level, according to the experimental design. Brix, color, and sugar/acid ratio were all measured.

2.7.1 Determination of the Brix degree of date extracts

After calibrating the refractometer (Digital Refractometer HI96801) with distilled water (as a zero), a few drops of the sample were dispersed on the refractometer's prism, and the amount of soluble dry residue was recorded from the instrument's digital display. After each assay, the prism plate is cleaned with distilled water and a soft cloth. The operation is repeated three times for each sample.

2.7.2 Measurement of titratable acidity of date extracts

The titratable acidity was determined according to (Martínez *et al.*, 2012) as follows. About 10 mL of sample was introduced in a 250 mL beaker followed by 50 mL of distilled water. Three drops of phenolphthalein at 1% concentration were added while stirring. When titrating, a solution of 0.1 N sodium hydroxide (NaOH) was used until a pink color was maintained for 10 seconds. The procedure was performed in triplicate. The formula to determine titratable acidity was given by:

$$A(\%) = \frac{0.0067V_1}{V_0} \times 100 \quad (8)$$

With: V_0 : volume of sample taken for titration (mL); V_1 : volume of 0.1 N sodium hydroxide solution used (mL); 0.0067: acidity conversion factor in malic acid equivalent.

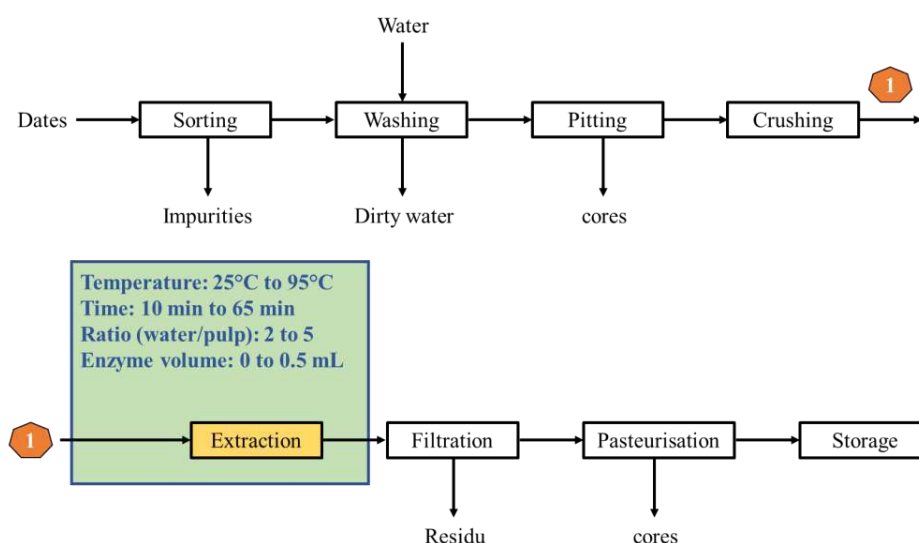


Figure 1: Schematic of date juice extraction process

2.7.3 Determination of the juice color

After collecting the juice sample, an absorbance reading was taken at 430 nm using a Jenway 6405 UV/Visible (Jenway Ltd Felstd, Dunmow, Essex CM6 3LB, UK) spectrophotometer so that the level of color intensity could be determined (ASBC, 2009).

$$\text{Color (ASBC)} = 12.7 \times \text{Abs (430 nm)} \times F \quad (9)$$

With: Abs, absorbance; F, dilution factor; ASBC, American Society of Brewing Chemists color units.

2.7.4 Determination of the juice color

Sugar/acid ratio (taste) was determined using TSS and TA values, as previously stated (Solomakhin & Blanke, 2010).

2.7.5 Determination of the free amino acid content of extracts

The ninhydrin technique was used to determine the concentration of free amino acids in extracts using colorimetry (EBC-Analysis-Committee, 1998).

To make a 1/100 dilution, 99 ml of distilled water was mixed with 1 ml of extract. The sample was diluted and separated into three test tubes. Each test tube received 1ml of color reagent (100g/L Na_2HPO_4 , 60g/L KH_2PO_4 , 5g/L ninhydrin, and 3g/L fructose). The tubes were immersed in boiling water for 16 minutes. They were then chilled in a water bath to 20-25 °C. Each received 5ml of dilution solution (2g of KIO_3 , 1L of a mixture of H_2O /Ethanol 96% (600:400, v/v)). A Jenway 6405 UV/Visible spectrophotometer was used to measure absorbance at 570 nm (Jenway Ltd Felsted, Dunmow, Essex CM6 3LB, UK). The obtained results were compared to the control and standard results. To create the blank, 2ml of distilled water was used instead of the diluted extract. The standard was 2 ml of glycine (10.72 mg/L) instead of the diluted extract. The following relationship determined the proportion of free amino acids:

$$\text{FAN (mg / L)} = \frac{2 \times A_1}{A_2} \times d \quad (10)$$

FAN: free amino nitrogen (mg/L); A_1 : Absorbance of the test solution at 570 nm; A_2 : Average absorbance of the standard solution; d: dilution factor

Table 1: Matrix of coded and real variables in the centered composite design

Trials	x1	x2	x3	x4	Temperature	Time	Ratio	Enzyme volume
					(°C)	(min)	(water/pulp)	(ml)
1	0	0	0	0	60.0	65	3.5	0.25
2	-1	-1	1	1	38.2	31	4.4	0.41
3	1	1	1	1	81.8	99	4.4	0.41
4	1	1	-1	-1	81.8	99	2.6	0.09
5	-1	-1	-1	-1	38.2	31	2.6	0.09
6	0	0	1.607	0	60.0	65	5.0	0.25
7	0	0	-1.607	0	60.0	65	2.0	0.25
8	1	-1	-1	-1	81.8	31	2.6	0.09
9	-1	1	-1	1	38.2	99	2.6	0.41
10	0	-1.607	0	0	60.0	10	3.5	0.25
11	1.607	0	0	0	95.0	65	3.5	0.25
12	1	1	1	-1	81.8	99	4.4	0.09
13	0	1.607	0	0	60.0	120	3.5	0.25
14	1	1	-1	1	81.8	99	2.6	0.41
15	0	0	0	1.607	60.0	65	3.5	0.50
16	-1	1	1	-1	38.2	99	4.4	0.09
17	0	0	0	0	60.0	65	3.5	0.25
18	-1.607	0	0	0	25.0	65	3.5	0.25
19	1	-1	1	-1	81.8	31	4.4	0.09
20	-1	-1	-1	1	38.2	31	2.6	0.41
21	-1	1	-1	-1	38.2	99	2.6	0.09
22	0	0	0	0	60.0	65	3.5	0.25
23	0	0	0	-1.607	60.0	65	3.5	0.00
24	-1	1	1	1	38.2	99	4.4	0.41
25	0	0	0	0	60.0	65	3.5	0.25
26	1	-1	1	1	81.8	31	4.4	0.41
27	-1	-1	1	-1	38.2	31	4.4	0.09
28	1	-1	-1	1	81.8	31	2.6	0.41

2.7.6 pH measurement of date juice

The electrode of the pH meter (Jual HANNA HI9813-6 Portable pH/ EC/ TDS Meter Harga Murah) was dipped into the beaker containing 20 mL of sample at 25° C; the pH value was read. The operation was repeated three times.

2.7.7 Determination of the Vitamin C content of date juice

An Erlenmeyer flask was filled with a volume V equal to 5 mL of sample measured with a graduated pipette, followed by 5 mL of the metaphosphoric acid-acetic acid solution (v/v) ($\text{HPO}_4\text{-CH}_3\text{ COOH}$) and 10 mL of distilled water. A second empty Erlenmeyer flask was filled with a standard ascorbic acid solution (250 mg/L). Vitamin C (Vit C) was titrated with dichlorophenolindophenol (DCPIP) solution (8.61×10^{-3} mol/L) for 30 seconds until a pink hue persists. The procedure was repeated thrice. The following formula was used to calculate the vitamin C content.

$$\text{Vit C (mg/L)} = \frac{[\text{DCPIP}] \times V \times M}{V_0} \quad (11)$$

M: molar mass of vitamin C (176g/mol); V: volume of DCPIP (mL); V_0 : volume of sample (mL)

2.7.8 Determination of total phenolic content in date juice

The polyphenols in date juice were measured using the Folin-Ciocalteu reagent (Matloob & Balakita, 2016), which produces a blue phosphotungstic-phosphomolybdenum complex. 2 mL of distilled water and 1.0 mL of Folin-Ciocalteu reagent (diluted at 1/10) were added to 100 μL of sample extract. After allowing the mixture to stand for 5 min, 0.75 mL of Na_2CO_3 solution (60 g/L) was added. After 90 min, absorption was measured at 765 nm using an UV-visible spectrophotometer (Jenway Ltd Felstd,

Dunmow, Essex CM6 3LB, UK) against water as a blank. For three replicates, the total phenol concentration was expressed as g of gallic acid equivalent (GAE) per 100 g of fresh sample.

3. Results and Discussion

3.1 Morphological characterization of date

The morphological characteristics of the *Bournow* date were measured as well as its water content. The results are presented in Table 2.

Bournow dates have a water content of 12% (Table 2). This characteristic leads to a relative stability of their quality over a relatively long period of time. This value is consistent with those discovered in the literature which ranged from 12% to 45% (Acourene *et al.*, 2001). Given the low moisture content of this date, it is classified as having a dry consistency according to Djoudi, (2013). In terms of date length, the cultivar *Bournow* produced shorter dates, which measured 1.78 cm (Table 2). This value was lesser than the maximum reported for other date cultivars, which ranged from 2.59 to 6 cm (Acourene *et al.*, 2001; Djoudi, 2013). In Table 2, it can be seen that the cultivar *Bournow* has a diameter of 0.72 cm. This value differed from those found in the 58 cultivars studied by Acourene *et al.* (2001) in the Ziban region which diameter was between 1.43 cm and 2.40 cm. This could be attributed to varietal differences. According to the literature classification (Mohammed *et al.*, 1983), the fruit and pulp masses of 5.56 g and 4.69 g are low because they are less than 6 g and 5 g, respectively (Table 2). Definitely, *Bournow* dates have a poor character globally for all measured characteristics. This would justify their use in Chad as animal feed. It could also be considered for valorization in beverages.

Table 2: Water content and morphological characterization of *Bournow* dates.

Characteristics	Values obtained	Limit	Character	decision	Reference
Water content (%)	12	12 % - 24 %	Medium	Reasonable	
Diameter (cm)	0.72	< 1.5	Very little	Bad character	
Length (cm)	1.78	< 3.5	Short	Bad character	(Mohammed et al., 1983)
Mass of the date (g)	5.56	< 6	Low	Bad character	
Pulp weight (g)	4.69	< 5	Low	Bad character	

3.2 Physicochemical characterization of the date juice

Although focused on the responses that are the subject of this work (Brix, color and sugar/acid ratio), other physicochemical characteristics were determined to have a global view on the

properties of date juice. These data are exhibited in Table 3. From there it was seen that the pH ranged from 4.21 ± 0.02 to 5.62 ± 0.01 , vitamin C from 45 ± 3 to 126 ± 6 mg/100 g, titratable acidity from 0.37 ± 0.04 to 2.13 ± 0.03 g/L, free amino nitrogen 430 ± 8 to 617 ± 11 mg/L and polyphenols from 270 ± 6 to 687 ± 8 mg GAE/100 g DM. When compared with literature, it was noticed that, the value obtained were for some characteristics (pH and titratable acidity) within the range obtained by Abbès *et al.* (2011) after applying pectinase/cellulase to obtain juice from date. Indeed, the values obtained by Abbès *et al.* (2011) were for pH and titratable acidity, respectively 3.12 to 4.87, 0.18 to 1.29 g/L. The result obtained demonstrates the impact of chosen process variables.

Table 3: Other physicochemical characteristics of *Bournow* date juice

	value	
	min	max
pH	4.21 ± 0.02	5.62 ± 0.01
Vit C (mg/100g)	45 ± 3	126 ± 6
Titratable acidity (g/L)	0.37 ± 0.04	2.13 ± 0.03
Free amino nitrogen (mg/L)	430 ± 8	617 ± 11
Polyphenols (mg GAE/100g DM)	270 ± 6	687 ± 8

Table 4: Summary of physicochemical analysis of *Bournow* date
(*Phoenix dactylifera* L.) juice

x1	x2	x3	x4	Brix (°B)	Color (ASBC)	Sugar/acid ratio
0	0	0	0	18	148	12.0624
-1	-1	1	1	15.5	172.5	10.9313
1	1	1	1	14.4	141.75	18.5757
1	1	-1	-1	21.2	180.5	46.9027
-1	-1	-1	-1	20.9	198	18.3041
0	0	1.607	0	13.2	103.25	12.5756
0	0	-1.607	0	25.9	168	34.078
1	-1	-1	-1	21.9	201	57.4171
-1	1	-1	1	21.5	153.5	21.278
0	-1.607	0	0	16.8	171.75	11.6193
1.607	0	0	0	17.3	145.25	35.2342
1	1	1	-1	13.4	148.25	36.2084
0	1.607	0	0	18	167.5	17.8241
1	1	-1	1	20.5	145.75	16.2319
0	0	0	1.607	17.1	179.25	11.0824
-1	1	1	-1	13.6	155	16.6667
0	0	0	0	17.1	143.75	9.6042
-1.607	0	0	0	17.3	166.5	13.9247
1	-1	1	-1	13.8	125.5	19.1332
-1	-1	-1	1	21.4	198	17.9681
-1	1	-1	-1	20.8	206	27.8075
0	0	0	0	16.7	134	11.016
0	0	0	-1.607	15.6	178.25	10.5774
-1	1	1	1	14.5	175.75	16.3848
0	0	0	0	16.3	150	8.8889
1	-1	1	1	13.9	167.5	16.1053
-1	-1	1	-1	13.3	158.75	6.2441
1	-1	-1	1	22.3	186.5	30.883

Table 5: Validation criteria of the different models from juice attributes

Settings	R ²	R ² _{adj}	AAD	Bf	Af
Y _{Bx}	98.34	96.55	0.061	0.994	1.064
Y _{Col}	93.37	86.24	0.088	0.934	1.096
Y _{S/A}	98.56	97.00	0.062	0.982	1.066

3.3 Modeling and optimization of physicochemical parameters of date juice

The influence of operating parameters (temperature, time, ratio, and enzyme volume) on the extraction of date juice was determined. The findings are provided in Table 4.

CCD models linked singular factors, interactions, and quadratic effects to response variables. These models consisted of:

$$Y_{Bx} = 17.117 - 0.0014x_1 - 0.0286x_2 - 2.3075x_3 + 0.2208x_4 + 0.0055x_1^2 + 0.0180x_2^2 + 0.3454x_3^2 - 0.1294x_4^2 - 0.0411x_1x_2 - 0.0670x_1x_3 - 0.0895x_1x_4 + 0.0460x_2x_3 - 0.0282x_2x_4 + 0.0783x_3x_4 \quad (12)$$

$$Y_{S/A} = 10.11 + 0.186x_1 - 0.12x_2 - 1.46x_3 + 0.131x_4 + 0.042x_1^2 + 0.046x_2^2 + 0.248x_3^2 - 0.076x_4^2 - 0.006x_1x_2 - 0.086x_1x_3 - 0.053x_1x_4 + 0.056x_2x_3 - 0.018x_2x_4 + 0.047x_3x_4 \quad (13)$$

$$Y_{Col} = 143.66 - 4.55x_1 - 3.18x_2 - 9.65x_3 - 0.89x_4 + 1.889x_1^2 + 3.951x_2^2 - 1.148x_3^2 + 5.319x_4^2 - 0.659x_1x_2 - 0.902x_1x_3 + 0.103x_1x_4 + 2.281x_2x_3 - 2.765x_2x_4 + 4.157x_3x_4 \quad (14)$$

With, Y_{Bx}: Brix; Y_{S/A}: Sugar/acid ratio; Y_{col}: Color; x₁: Temperature; x₂: Time; x₃: Ratio water/pulp; x₄: Pectinase volume.

These interactive second-degree models are beneficial if a few input variables are precised. Table 5 shows that all models are valid and can thoroughly evaluate components. Table 5 ANOVA only considers variables with probability <0.05. Thus, they are the only relevant elements.

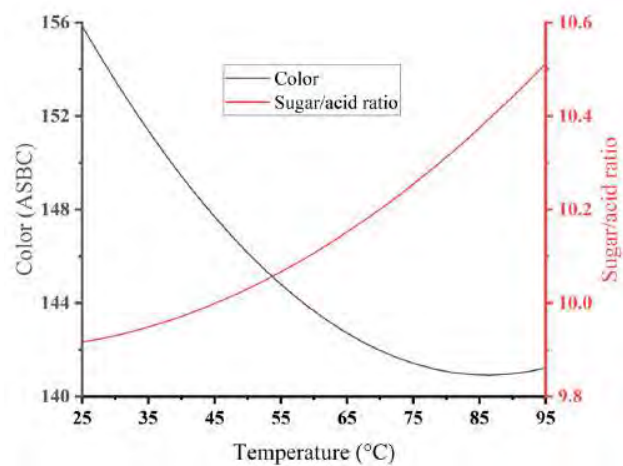


Figure 2: Evolution of color and sugar/acid ratio, as a function of temperature (time, water/pulp ratio, and pectinase volume fixed respectively at 65 min, 3.5, and 0.25 mL)

Table 6: ANOVA for the significance of the factors used during extraction of some constituents from *Bournow* date (*Phoenix dactylifera* L.) juice

Terms	Brix	Color	Sugar/acid ratio
	Probabilities (P)		
Constant	0.000	0.000	0.000
x_1 -Temperature (°C)	0.988	0.002	0.003
x_2 -Time (min)	0.744	0.020	0.037
x_3 -Ratio (water/pulp)	0.000	0.000	0.000
x_4 -Enzyme volume (mL)	0.023	0.475	0.024
x_1^2	0.935	0.067	0.319
x_2^2	0.792	0.001	0.277
x_3^2	0.100	0.246	0.000
x_4^2	0.076	0.000	0.081
x_1x_2	0.514	0.458	0.865
x_1x_3	0.295	0.314	0.036
x_1x_4	0.168	0.907	0.176
x_2x_3	0.467	0.020	0.152
x_2x_4	0.653	0.007	0.629
x_3x_4	0.224	0.000	0.224

3.3.1 Impact of singular factors on responses

3.3.1.1 Impact of temperature and time

The sugar/acid ratio increased significantly ($P=0.000$, Table 6) from 9.92 to 10.51 as the temperature increased from 25°C to 95°C (Figure 2). Although temperature has no effect on Brix in

this scenario, it does cause cell weakening and thus more mineral extraction. Because the juice has alkaline ions in solution, they would engage in acid-base reactions, lowering the titratable acidity by that means. This resulted in an increase in the ratio. The sugar/acid ratio range (7.82-15.4)

indicated a healthy balance of sweetness and acidity, which was important for must quality and flavor. The findings were consistent with the literature, which placed this ratio at 10.78 for the production of good juice (Benidir *et al.*, 2020).

For temperatures ranging from 25 to 95 °C, the color of date juice decreased significantly ($P=0.000$, Table 6) from 155 to 141 ASBC. Date pigments include chlorophyll, carotene, and anthocyanins, which provide green, yellow, and red colors, respectively (Ashraf & Hamidi-Esfahani, 2011). Date syrups' color intensity may be affected by pigments such as carotenoids, flavonoids, tannin derivatives, and polyphenols (Benamara *et al.*, 1999). Lutein is the most abundant carotenoid pigment in dates, followed by β -carotene (Boudries *et al.*, 2007). Carotenoids were susceptible to destruction during processing and storage due to exposure to high temperatures, light, or pro-oxidant chemicals due to their highly unsaturated nature. As a result, carotenoid loss during food processing was documented and quantified in various articles (Aman *et al.*, 2005; Hiranvarachat *et al.*, 2008). The two major carotenoid changes that occurred during processing was isomerization and oxidation. Carotenoids occurred naturally in all-trans form. Heating, on the other hand, caused isomerization of all-trans-carotene to cis forms (Achir *et al.*, 2010).

For a duration of 10 to 120 min, the sugar/acid ratio decreased significantly ($P=0.037$, Table 6) from 10.42 to 10.03 (Figure 3). The consequence of the Maillard reactions was a reduction in the sugar/acid ratio, which is the opposite of what was shown for the temperature. In essence, the reducing sugar like glucose first condensed with a substance that had a free amino group (most commonly the E-amino group of lysine, but also the α -amino groups of terminal amino acids in

proteins) to produce N-substituted glycosilamine, which then rearranged to produce the Amadori rearrangement product (Martins *et al.*, 2000). The residual amount lowers when the sugars were involved in that process, which caused the ratio to fall. This Maillard reactions induced also the reduction of amino nitrogen with time.

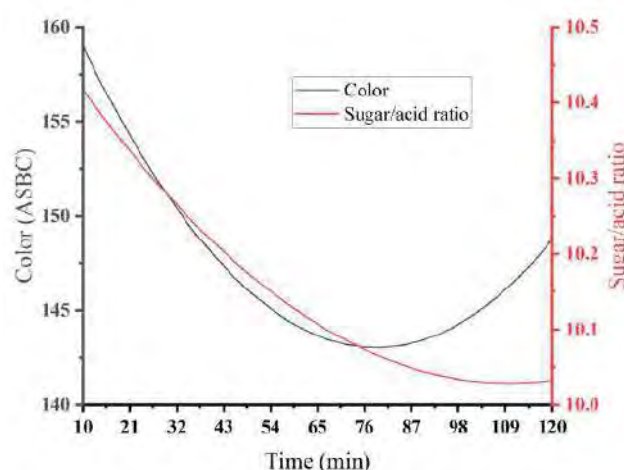


Figure 3: Evolution of color and sugar/acid ratio, as a function of time (temperature, water/pulp ratio, and pectinase volume fixed respectively at 65 min, 3.5, and 0.25 mL)

At 10 min, the color was 158.97 ASBC, and at 79 min, it was 143.02 ASBC, therefore it drops significantly ($P=0.020$, Table 6). Following that, there is a significant increase ($P=0.001$, Table 5) till 148.75 ASBC at 120 min (Figure 3). During the extraction, this phenomenon of date juice discolouration as a function of time and temperature was noticed (Benahmed Djilali & Adiba, 2012). Several pigments, including carotenoids, anthocyanins, flavones, flavonoles, lycopene, carotenes, flavoxanthin, and lutein, were found in dates (Echegaray *et al.*, 2021). The alteration of color and pigment during food products' heat processing is controlled by a variety

of factors. These include browning and process conditions that are enzymatic and non-enzymatic. Numerous studies on the qualitative characteristics of fruit-based pigments, particularly on carotenoid loss after heat processing, have been published in the literature (Ahmed & Ramaswamy, 2005). Indeed, compounds such as anthocyanins are sensitive to heat treatment (Weber & Larsen, 2017) and, the time of application of heat treatment would therefore contribute to a reduction of anthocyanins and therefore color. The color enhancement in the second stage could be due to Maillard reactions that increased with time. Thermally treated food can cause a chain reaction of reactions known as the Maillard reactions or nonenzymatic browning. These reactions are crucial in the development of flavor and color in heated food. Under certain conditions, the reaction happens between carbonyl groups of reducing sugars and free amino groups of amino acids, peptides, or proteins. The final stage of the Maillard reaction, where condensation of carbonyls and amines generates brown-colored high molecular weight molecules known as melanoidins, has been related to color development (Starowicz & Zieliński, 2019).

3.3.1.2 Impact of ratio water/pulp

Brix declined significantly ($P=0.000$, Table 6) from 21.72 °B to 14.30 °B as the water/pulp ratio increased from 2 to 5 (Figure 4). This could be explained by the diluting effect that an increase in water would have on sugar extraction. This was consistent with the literature, which reported the similar pattern for plantain juice extraction (Bentahar *et al.*, 2014; Makebe *et al.*, 2017). Like the brix, the decrease in all the other responses with the increase of water/pulp ratio (Figure 4) could be explained by the dilution brought with the increase of water.

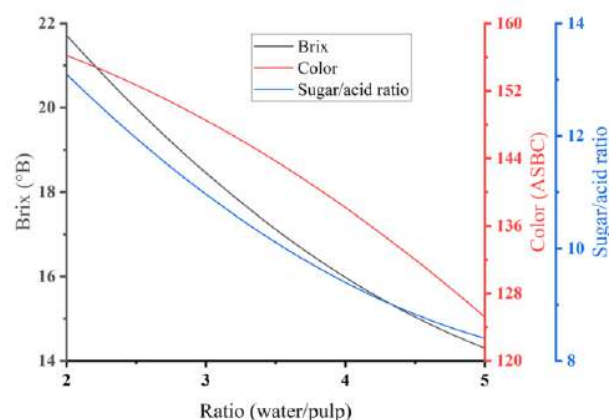


Figure 4: Evolution of brix, color and sugar/acid ratio, as a function of ratio water/pulp (temperature, time, and pectinase volume fixed respectively at 60°C, 65 min, and 0.25 mL)

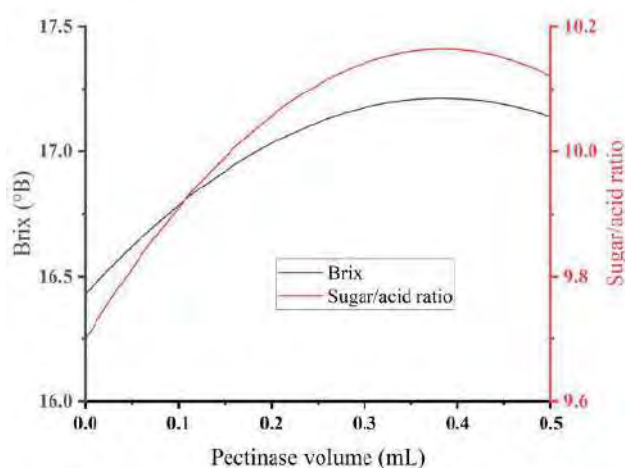


Figure 5: Evolution of brix and sugar/acid ratio, as a function of pectinase volume (temperature, time, and ratio water/pulp fixed respectively at 60°C, 65 min, and 3.5)

3.3.1.3 Impact of pectinase volume

Brix began at 16.42 °B before increasing significantly ($P=0.023$, Table 6) to 17.14 °B, with the addition of 0.5 mL pectinase. Abbès *et al.* (2011) made the same observation for the production of date syrups. This was related to the

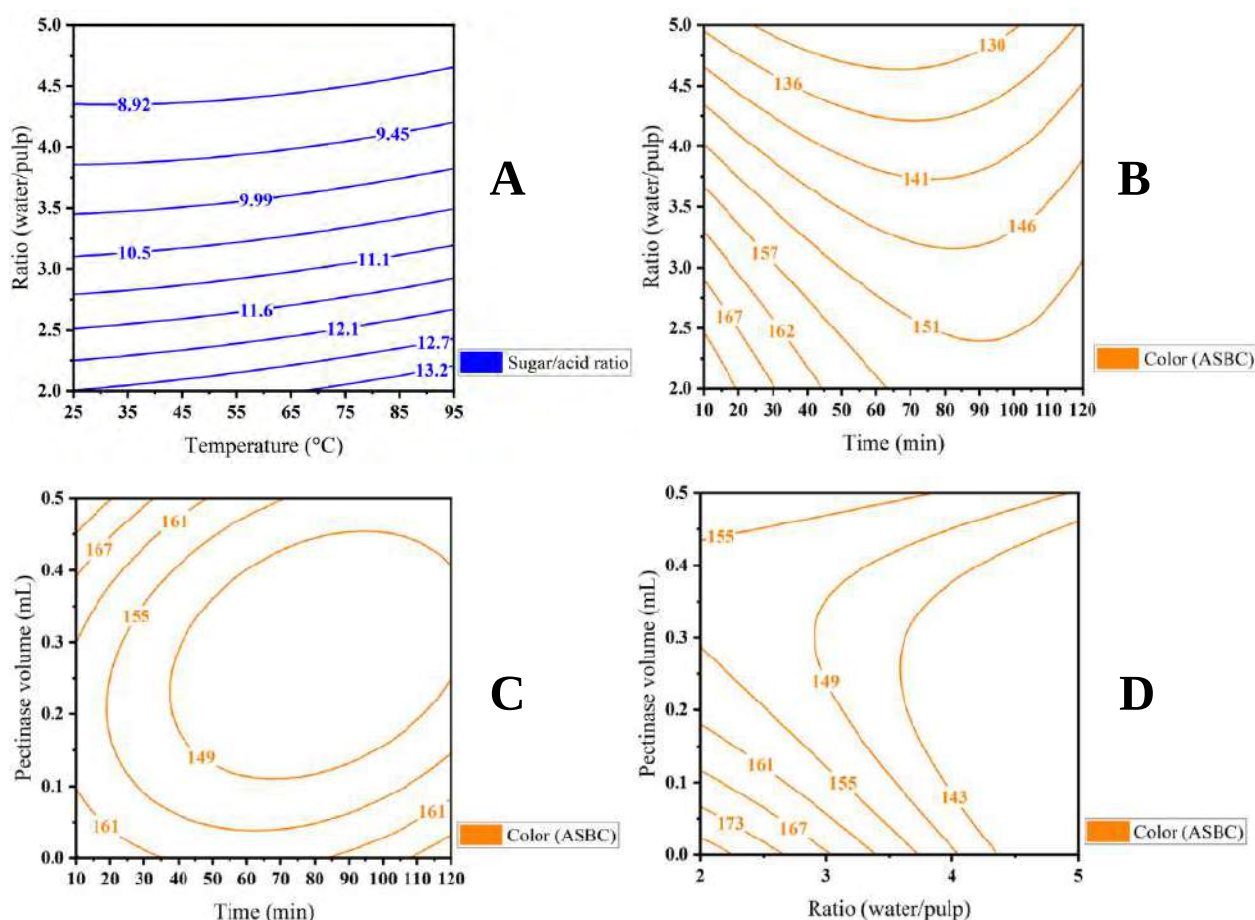


Figure 6: Evolution of sugar/acid ratio and color as a function of temperature/ratio (A), time/ratio (B), time/pectinase volume (C) and ratio/pectinase volume (D). All the other factors for each curve are fixed at the central value of the domain

breakdown of pectin into galacturonic acid units and sugars in the plant cell wall matrix and middle lamella (Abbès *et al.*, 2011; Demir *et al.*, 2001; Landbo *et al.*, 2007).

The volume of pectinase that was used, resulted in a significant increase ($P=0.024$, Table 6) in the sugar/acid ratio. It ranged from 9.7 when there was no pectinase present to 10.12 when there was 0.5 mL of pectinase present in volume (Figure 5). This rise can be explained by the fact that, as was observed earlier, the sugar release kinetics seemed faster than the titratable acid release kinetics,

which resulted in an overall increase in the sugar/acid ratio.

3.2.2 Impact of interactions on responses

The interaction between x_1 and x_3 (temperature/water/pulp ratio) greatly reduced ($P=0.036$, Table 6) the sugar/acid ratio. In fact, this effect was accentuated simultaneously by a fall in temperature and a rise in the water/pulp ratio (Figure 6 A). This might be explained by the fact that the dilution effect dropped the brix quicker than the titratable acidity, which decreased the sugar/acid ratio. Paul *et al.* (2018), who evaluated

the effect of dilution on banana fermentation, made similar observation. The simultaneous drop in temperature and sugar/acid ratio demonstrates that the date's structure plays a crucial role in fixing sugar and restricting its release.

The x2x3 interaction (time, water/pulp ratio) significantly contributed to the color increase ($P=0.020$, Table 6). This was seen when the extraction time increased while the water/pulp ratio decreased (Figure 6 B). Indeed, a longer extraction time would help to extract the elements that contributed to the color, such as pigments and polyphenols, while a decrease in the water/pulp ratio would concentrate the medium, resulting in an increase of the date juice's color. Furthermore, a longer extraction time would permit an increase in color due to Maillard reactions since the temperature was fixed at 60 °C. Melanoidins are compounds formed during the late stages of the Maillard reaction from reducing sugars and proteins or amino acids. In food, they have been identified as anionic and colored compounds (Echavarría *et al.*, 2012).

The x2x4 interaction (time, pectinase volume) significantly contributed to color reduction ($P=0.007$, Table 6). This occurred when the extraction time increased while the pectinase volume reduced (Figure 6 C). With time, the pigments responsible for the color degrade (Benahmed Djilali & Adiba, 2012), and the decrease in the volume of pectinase contributes to the non-destruction of the pulp structure, reducing the extraction of the compounds responsible for the color.

The x3x4 interaction (time, pectinase volume) significantly contributed to the color increase ($P=0.000$, Table 6). This was seen when the volume of pectinase increased while the water/pulp ratio decreased (Figure 6 D). Indeed, lowering the ratio helps to reduce the dilution

effect, while increasing the pectinase volume helps to weaken the pulp through pectin hydrolysis (Srivastava & Tyagi, 2013), resulting in the release of the compounds responsible for the color.

3.2.3 Optimization

To achieve the best physicochemical properties of the date juice, multi-response optimization was used. Brix, color, and sugar/acid ratio were all maximized for this purpose. At the end of this Minitab 21.3 optimization, the compromise was as follows: extraction temperature 95 °C, time 10 min, water/pulp ratio 2 and pectinase volume 0 mL. This combination produced a Brix of 21.89 °B, a sugar/acid ratio of 13.99, and a color of 197.49 ASBC. The individual desirability for the brix, color and ratio sugar/acid were respectively 0.683, 0.917 and 0.768. While the composite desirability was 0.784. The composite desirability (0.7840) in the study was close to 1, indicating that the parameters appeared to produce favorable results for all responses as a whole. Individual desirability, on the other hand, indicated that the parameters were more effective in maximizing color (0.91718) than for sugar/acid ratio (0.76893), and finally for brix (0.68336).

4. Conclusion

The goal of this work was to contribute to the valorization of the date *Bournow* variety in the field of drinks. Its poor physical character, which renders it unmarketable and consumable as is, confirmed the need for valorization. The response surface methodology was used to extract the juice, which allowed us to see that the selected factors, which are temperature, time, water/pulp ratio, and pectinase volume, have different effects on the responses Brix, color, and sugar/acid ratio, either increasing or decreasing them significantly. This extraction allowed us to highlight the nutrient richness of date juice, as observed by other

authors on other varieties of dates. The optimization, which consisted of maximizing all of the responses (Brix, color, and sugar/acid ratio), resulted in the quadruplet: temperature 95 °C, time 10 min, water/pulp ratio 2, and pectinase volume 0 mL. The resulting Brix was 21.89 °B, the sugar/acid ratio was 13.99, and the color was 197.49 ASBC. The nutrient richness of the juice produced under optimal conditions suggests that the juice's quality could be improved, and the Bournow date could be used in other industries such as fermented beverages.

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Conflict of interest

The authors declare that there are not conflicts of interest.

Ethics

This Study does not involve Human or Animal Testing.

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V.5) Purification Partielle et
Caractérisation de la Dextrinase
Limite du Malt de Sorgho *Safrari*

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**ORIGINAL**

Purification partielle et caractérisation de la dextrinase limite du malt de sorgho *Safrari*

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Abstract

Limit dextrinase from *Safrari* sorghum malt was purified by maximizing its ammonium sulfate precipitation followed by dialysis. This purification led to a partially purified extract whose yield was 75% for a purification factor of 6.5. The characteristics of the purified limit dextrinase are as follows: optimum pH for activity and stability of 5.0-5.5; optimum temperature for activity and stability of 50 – 60°C. The kinetic parameters were 0.03 mg.mL⁻¹.sec⁻¹ and 2.4 mg.mL⁻¹ for V_{max} and K_m respectively. Limit dextrinase is a sulfhydryl enzyme and its activity is enhanced in the presence of ascorbic acid; but also, in the presence of bovine serum albumin or calcium chloride. The filtration of sorghum mash is improved in the presence of purified exogenous limit dextrinase.

Keywords: *Safrari*; Limit dextrinase; Natural pullulan; Megazyme kit; Purification; Enzyme activity.

Résumé

La dextrinase limite du malt du sorgho *Safrari* a été purifiée en maximisant sa précipitation au sulfate d'ammonium suivie d'une dialyse. Cette purification a conduit à un extrait partiellement purifié dont le rendement est de 75% pour un facteur de purification de 6,5. Les caractéristiques de la dextrinase limite ainsi purifiée sont les suivantes : pH optimal pour l'activité et la stabilité de 5,0-5,5 ; température optimale pour l'activité et la stabilité de 50 - 60°C. Les paramètres cinétiques étaient de 0,03 mg.mL⁻¹.sec⁻¹ et 2,4 mg.mL⁻¹ pour V_{max} et K_m respectivement. La dextrinase de limite est une enzyme sulfhydryle et son activité est renforcée en présence d'acide ascorbique; mais aussi, en présence de sérum albumine bovine ou de chlorure de calcium. La filtration des moûts de sorgho est améliorée en présence de dextrinase limite exogène purifiée.

Mots clés : *Safrari* ; dextrinase limite ; pullulan naturel ; kit Megazyme ; purification ; activité enzymatique.

1. Introduction

Les enzymes, dans les conditions d'exploitation industrielle (température, pression, agitation, pH, présence d'oxygène etc.), sont sujets à des

dénaturations (Kotzia *et al.*, 2012; Lewis & Bamforth, 2006). L'utilisation des micro-organismes comme source d'enzyme satisfait bien



à cette exigence, vu leur permanente disponibilité (Andrés Illanes, Altamirano, & Wilson, 2008). Les enzymes d'origine végétales y sont également exploitées (Illanes *et al.*, 2008). L'avantage de pouvoir optimiser les conditions de synthèse enzymatique chez les céréales pendant le procédé de maltage, pourrait permettre d'aboutir à des quantités d'enzyme exploitables à l'échelle industrielle. Toutefois, la présence d'autres composés dans l'extrait enzymatique brut pourrait constituer une gêne pour certaines applications, d'où le recours à la purification. La purification des enzymes amylolytiques endogènes au malt de sorgho a concerné pour la plupart des travaux l' α -et la β -amylase (El Nour & Yagoub, 2010), mais pour ce qui est de la dextrinase limite, seuls les travaux de Hardie *et al.* (1976) ont porté sur de telles investigations en Afrique du Sud. Ceux-ci ont eu en effet à purifier la dextrinase limite de la variété de sorgho *Kaffircorn*. Toutefois, les techniques de purification des enzymes sont connues et pour ce qui est du cas particulier de la dextrinase limite à partir des céréales, ces techniques vont des méthodes de précipitation simple (au sulfate d'ammonium) aux techniques chromatographiques sophistiquées. Celles-ci tournent essentiellement autour de deux schémas principaux: la précipitation au sulfate d'ammonium suivie de l'électrophorèse continue puis de la gel-filtration (Whitaker, Voragen, & Wong, 2002) d'une part, et d'autre part la précipitation au sulfate d'ammonium suivie des techniques chromatographiques diverses (Whitaker *et al.*, 2002). Or, les enzymes utilisées à l'échelle industrielle sont généralement purifiées par des techniques de faible résolution à l'instar de la précipitation au sulfate d'ammonium, ce qui n'aboutit pas nécessairement à des extraits totalement purifiés (Khattak, Ul-Islam, Ullah, Khan, & Park, 2015). Le but de ce travail est donc de purifier la dextrinase limite du malt de sorgho

Safrari extraite dans des conditions optimales, par précipitation au sulfate d'ammonium et dialyse.

2. Matériel et méthodes

2.1 Matériel

2.1.1. Matériel biologique

Le matériel biologique était constitué du sorgho *Safrari* obtenu à l'Institut de Recherche Agronomique pour le Développement (IRAD) de Maroua/Cameroun.

2.1.2 Logiciels

Le logiciel Sigmaplot version 14.5 (Systat Software, Inc., 501 Canal Blvd, Suite E, Point Richmond, CA 94804-2028, USA) a servi aux tracés des courbes.

2.2 Méthodes

2.2.1 Procédé d'extraction de la dextrinase limite

Le broyat de malt vert (100 g) a été pesé et mélangé à 640 mL de tampon acétate (100 mM, pH 5,0), incubé à 23 °C au bain-marie (HH-S, Chine). L'ensemble a été homogénéisé et laissé au repos à 23 °C pour extraction pendant 10 h avec agitation lente pendant cinq secondes toutes les heures. Après extraction, l'ensemble a été centrifugé (Heraeus Biofuge Primo R, Allemagne) à 6000 g pendant 30 min à 4 °C ; puis le surnageant a constitué l'extrait enzymatique brut. L'utilisation du kit enzymatique K-PullG6 a exigé la présence de dithiothréitol (10 mM de DTT) pendant l'extraction.

2.2.2. Purification de la dextrinase limite

La purification de la dextrinase limite est décrite à la figure 1.

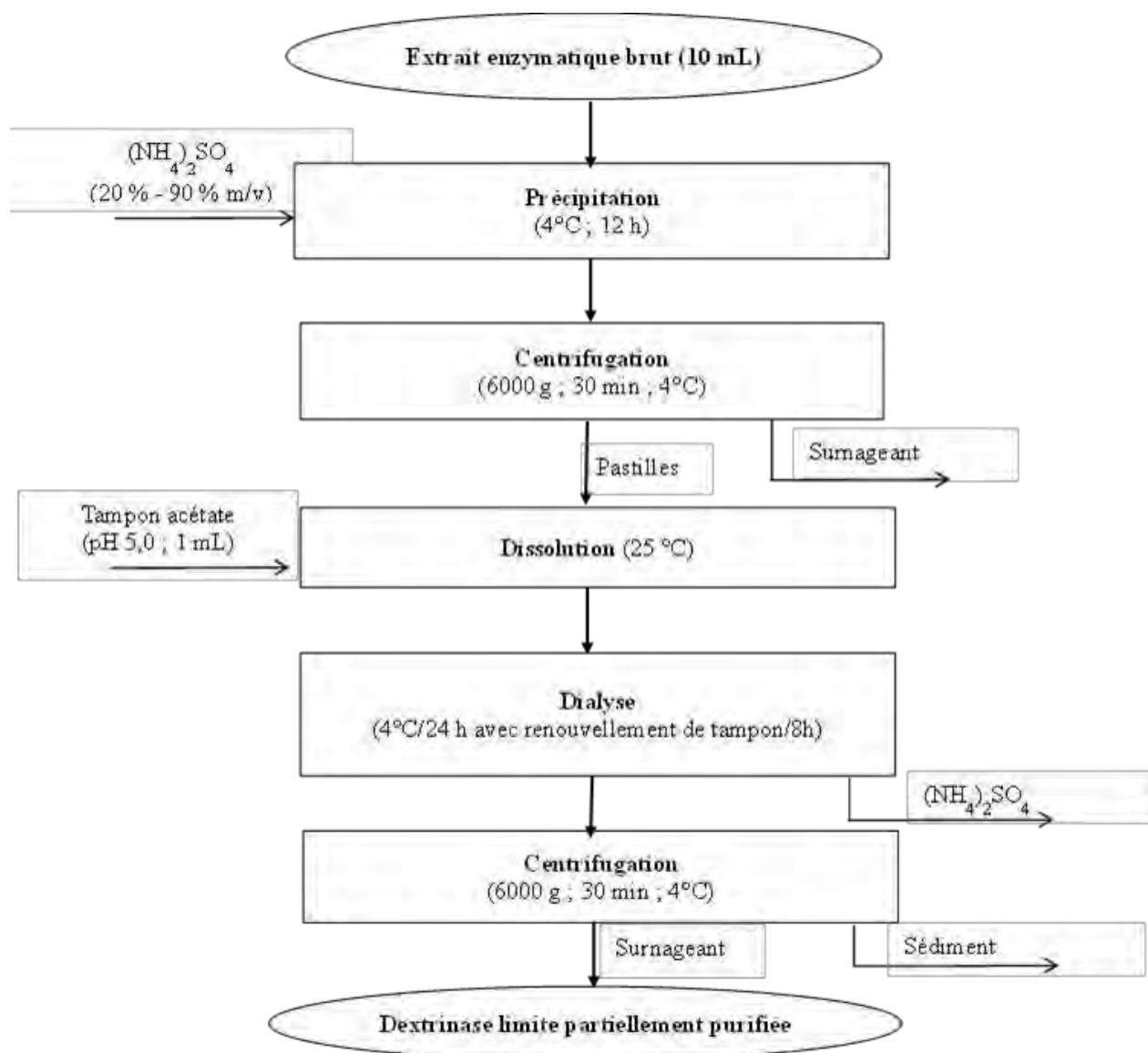


Figure 1 : Procédé de purification partielle de la dextrinase limite

2.2.2.1 Précipitation au sulfate d'ammonium

La maximisation de la précipitation de la dextrinase limite au sulfate d'ammonium a été faite selon la technique proposée par Barros *et al.* (2001) avec modification, en variant les pourcentages de saturation de l'extrait enzymatique brut de 20 % à 90 % m/v.

Le sulfate d'ammonium (AR, Chine) a été ajouté lentement dans l'extrait enzymatique incubé à 4 °C dans un incubateur (SHP biochemical incubator), en agitant pour éviter de fortes concentrations zonales qui pourraient causer la précipitation des protéines non désirées.

2.2.2.2 Dialyse à l'équilibre

La dialyse a été effectuée selon [Barros *et al.* \(2001\)](#) avec modification. En effet, l'échantillon à dialyser (60 mL) a été introduit dans le sac de dialyse (seuil de coupure de 11311 daltons et de 33 mm de largeur ; Sigma Aldrich) ; qui a été placé dans un récipient contenant une grande quantité (1 L) de solution tampon acétate de sodium (100 mM, pH 5,0). La séparation a été menée pendant 24 h à 4 °C dans l'incubateur, avec changement du tampon toutes les 8 heures.

2.2.3 Détermination de l'activité de la dextrinase limite

La détermination de l'activité de la dextrinase limite a été faite par deux méthodes : celle au pullulan naturel et celle au kit enzymatique de Megazyme ([Megazyme, 2016](#)).

2.2.3.1 Détermination de l'activité de la dextrinase limite par la méthode au pullulan naturel

L'activité de la dextrinase limite a été déterminée par dosage des sucres réducteurs ([Fischer & Stein, 1961](#)).

Une solution (0,25 mL) à 0,5 % de pullulan (Sigma Aldrich, USA) a été ajoutée à 0,25 mL d'extrait enzymatique contenant 100 mM de tampon acétate de sodium pH 4,0 (0,75 mL). L'ensemble a été incubé à 37°C au bain-marie (Memmert, Allemagne et HH-S, Chine) pendant 30 min. La réaction a été stoppée avec 0,25 mL de réactif au DNS (2,5 g d'acide dinitrosalicylique +75 g de tartrate double sodium potassium +50 mL de NaOH 2 N). Le mélange a été porté dans un bain bouillant pendant cinq minutes. L'ensemble a été ensuite refroidi dans un bain de glace et 4 mL d'eau distillée y ont été ajoutées. L'intensité de la coloration a été déterminée par spectrophotométrie contre un blanc ne contenant

pas le pullulan. La concentration en sucres réducteurs, exprimée en mg de maltose par mL a été déterminée à l'aide d'une courbe d'étalonnage :

$$DO = aQ + b \quad (1)$$

Avec, DO : Densité Optique et Q : quantité de sucres réducteurs (mg/mL) ; a, b : Constantes à déterminer.

L'activité enzymatique est la quantité d'enzyme qui libère une μmol de maltose équivalent du pullulan en 1 min à 37 °C, à pH 4,0 et s'exprime en U/mL. Elle a été calculée comme suit :

$$A_{\text{Enz}} (\text{U} / \text{mL}) = \frac{[\text{Sucres}]}{t} \quad (2)$$

A_{Enz} : activité enzymatique ; [Sucres] : Concentration en sucre ($\mu\text{mol/mL}$) ; t : temps (min)

2.2.3.2 Détermination de l'activité de la dextrinase limite par la méthode au kit Megazyme

Le substrat K-PullG6 et l'extrait de dextrinase limite ont été pré-incubés séparément à 40°C pendant 5 min. Par la suite, à chaque tube contenant le substrat K-PullG6 (0,10 mL), 0,1 mL d'extrait de dextrinase limite a été ajouté. L'ensemble a été incubé à 40 °C pendant 30 min. Après, 1,5 mL de tampon Tris (2 % m/v) pH 9,0 a été ajouté à chaque tube et agité vigoureusement. Les absorbances des solutions ont été lues à 590 nm.

Une unité enzymatique est définie comme la quantité d'enzyme (présence d'un excès d' α -glucosidase et de β -glucosidase thermostables) nécessaire pour libérer une micromole de 4-nitrophenol du substrat 4,6-*O*-benzylidene-4-nitrophenyl-6³- α -D-maltotriosyl-maltotriose

(BPNPG₃G₃) en une minute dans les conditions définies par le test.

L'activité A_{DL} de la dextrinase limite (en U/g de malt) est :

$$A_{DL} = \frac{DA_{400}}{t} \cdot \frac{V_t}{V_e} \cdot \frac{1}{e_{mM}} \cdot \frac{V_{ext}}{M_m} \cdot D \quad (3)$$

Avec :

ΔA_{400} (absorbance de la réaction - absorbance du blanc), t (temps d'incubation), V_t (Volume total dans le tube à essai), V_e (Volume de la prise d'essai), ϵ_{mM} du *p*-nitrophenol (à 400 nm) dans 2 % de tampon Tris, M_m (masse de malt), V_{ext} (volume d'extraction), D (facteur de dilution de l'extrait enzymatique de départ).

2.2.4 Dosage des protéines solubles

La détermination des teneurs en protéines solubles a été faite avec la méthode de (Lowry, Rosenbrough, Farr, & Randall, 1951).

A 1 mL de chaque solution stock d'une part et d'échantillon à doser d'autre part, contenus dans des tubes à essai, 1 mL de solution alcaline de cuivre a été ajouté, les tubes ont été agités et laissés au repos pendant 10 min. Ensuite, 0,5 mL de réactif de Folin-Ciocalteu (DC Panreac Quimica, Espagne) a été ajouté à chaque tube, l'ensemble a été agité et incubé à l'obscurité pendant 30 min. Après incubation, les contenus des tubes ont été à nouveau homogénéisés avant la lecture des absorbances à 660 nm au spectrophotomètre.

2.2.5 Le tableau de purification enzymatique

Le tableau de purification sert à montrer le rôle et l'efficacité de chaque étape dans le processus de purification (GE-Healthcare, 2010). Plusieurs termes le constituent :

Pour construire un tel tableau, on dose à chaque étape de purification la quantité de protéines totales et l'activité de l'enzyme en question. Les valeurs du volume (mL), de l'activité enzymatique totale (U/mL) et de la concentration protéique (mg/mL) sont mesurées expérimentalement et sont utilisées pour le calcul des autres termes du tableau. Les calculs ont été effectués comme suit :

$$A_{EnzT} (U) = A_{Enz} \cdot V_{Enz} \quad (4)$$

Avec : A_{EnzT} : Activité enzymatique totale (U); A_{Enz} : Activité enzymatique (U/mL); V_{Enz} : volume de l'extrait enzymatique

$$A_{EnzS} (U / mg) = \frac{A_{EnzT}}{Q_p} \quad (5)$$

Avec : A_{EnzS} : Activité enzymatique spécifique (U/mg); Q_p : masse de protéines (mg)

$$Rdt (\%) = \frac{A_{EnzT}^i}{A_{EnzT}} \cdot 100 \quad (6)$$

Rdt : Rendement (%); A_{EnzT}^i : Activité enzymatique totale à l'étape i de purification (U); A_{EnzT} : Activité enzymatique totale (U)

$$F_p = \frac{A_{EnzS}^i}{A_{EnzS}} \quad (7)$$

Avec : F_p : Facteur de purification; A_{EnzS}^i : Activité enzymatique spécifique à l'étape i de purification (U/mg)

2.2.6 Détermination du pH optimum d'activité et de stabilité

Les solutions enzymatiques ont été incubées à des pH variant de 3,0 à 6,5 en utilisant le tampon phosphate à 100 mM. Les tests d'activité au kit et au pullulan ont été conduits comme décrit précédemment. Pour déterminer le pH de stabilité de la dextrinase limite, les extraits enzymatiques (0,1 mL) ajoutés de 1 mL de

tampon acétate au pH correspondant ont été incubés sur une durée de 90 minutes à 40 °C. Après quoi le substrat du kit (0,1 mL) y a été ajouté pour le test d'activité.

2.2.7 Détermination de la température optimale d'activité et de stabilité

Les tests d'activité au pullulan et au kit ont été menés en incubant les milieux réactionnels aux températures d'étude (20 °C à 70 °C). Le tampon acétate (100 mM pH 5,0) a été utilisé.

La stabilité thermique de la dextrinase limite a été évaluée en incubant l'extrait enzymatique (0,1 mL) aux températures de 50 à 65 °C pendant 90 min, barèmes température/temps généralement employés pour le brassage des moûts en brasserie (Dewar, Orovan, & Taylor, 1997). Les 90 min d'incubation achevées, les extraits ont été refroidis à 40 °C pour le test d'activité avec la méthode du kit Megazyme K-PullG6.

2.2.8 Détermination des propriétés catalytiques K_m et V_{max}

Les paramètres cinétiques K_m et V_{max} ont été déterminés par linéarisation de l'équation de Michaelis-Menten en utilisant la représentation graphique de Lineweaver-Burk et selon le protocole décrit par (Dicko, 2006). L'iode (I_2) interagit avec l'amidon pour donner une coloration bleue qui absorbe à une longueur d'onde de 580 nm.

Les amylases sont des enzymes obéissant à la loi cinétique de Michaelis-Menten donnée par l'équation :

$$V_i = \frac{V_{max} [S]}{[S] + K_m} \quad (8)$$

Avec : V_i : vitesse initiale de la réaction enzymatique ; V_{max} : Vitesse maximale de l'enzyme ; K_m : constante de Michaelis.

La linéarisation permet d'obtenir l'équation en double inverse de Lineweaver-Burk suivante :

$$\frac{1}{V_i} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \quad (9)$$

2.2.9 Détermination de l'activité de la dextrinase limite en présence de quelques effecteurs d'activité enzymatique (EDTA, DTT, $CaCl_2$, SAB, acide ascorbique)

L'activité enzymatique a été évaluée par ajout de solutions tampons (0,75 mL pour le test au pullulan et 1 mL pour le test au kit) contenant chacune l'additif à évaluer (DTT à 1 mM, ou acide ascorbique à 5 mM, ou $CaCl_2$ à 5 mM, ou EDTA à 1 mM, ou SAB à 10 mg/mL) dans des tubes à essai contenant l'extrait enzymatique (0,1 mL pour le test au kit et 0,25 mL pour le test au pullulan), avant l'ajout du substrat (K-PullG6 ou pullulan selon le test) dans le milieu. Les tests au pullulan et au kit ont été conduits tels que précédemment décrits.

2.2.10. Application de la dextrinase limite à des brassins de sorgho

L'extrait enzymatique purifié a été appliqué comme source exogène de dextrinase limite à différentes concentrations à des brassins de sorgho. La filtrabilité de ces brassins et les teneurs en sucres réducteurs des moûts qui en sont issus ont été déterminées.

2.2.10.1 Détermination de l'effet de la dextrinase limite au brassage

Le sorgho *Safari* a été malté dans les conditions optimales définies par Nguemogne *et al.* (2020). Le malt obtenu a été touraillé à 50 °C pendant 24 h, broyé et tamisé ($\phi \leq 1$ mm). Quant au brassage, le procédé a été celui décrit par Desobgo *et al.* (2011) (brassage par décoction) et est présenté à la figure 2.

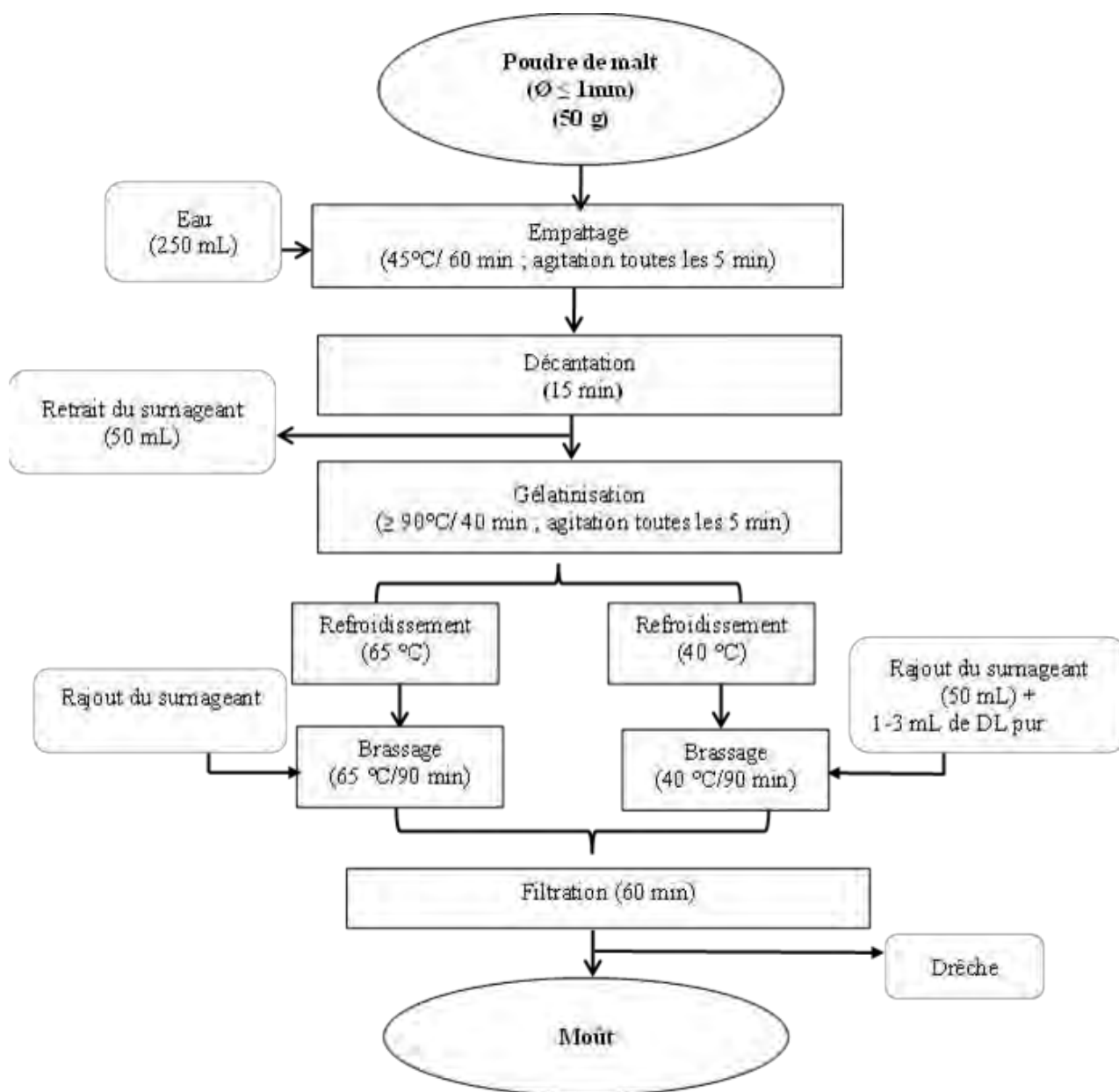


Figure 2 : Procédé de purification partielle de la dextrinase limite

Quatre brassins ont été ainsi préparés : le premier (brassin témoin), a été à l'étape de refroidissement, refroidi à 65 °C sans ajout de la dextrinase limite purifiée ; les second, troisième et quatrième brassins (brassins tests) ont été refroidis à 40 °C (température optimale de stabilité de la dextrinase limite) avant ajout des différents

surnageants mélangés avec 1 mL, 2,5 mL et 3 mL de dextrinase limite purifiée, respectivement.

2.2.10.2 Détermination de la teneur en sucres réducteurs des moûts

La teneur en sucres réducteurs a été déterminée comme précédemment indiqué.

2.2.10.3 Test de filtrabilité des brassins

La filtration des brassins a été faite avec du papier filtre Whatman N° 1. Cette filtrabilité a été déterminée en relevant les volumes cumulatifs de filtrats toutes les cinq minutes sur une durée de 1 h. Les courbes donnant les volumes cumulatifs de filtrat en fonction du temps ont par la suite été tracées et ont servi à observer les vitesses de filtration desdits brassins.

3. Résultats et discussion

3.1 Précipitation de la dextrinase limite au sulfate d'ammonium

Le maximum d'activité en dextrinase limite a été obtenu à 20 % m/v de saturation au sulfate d'ammonium (Tableau 1). Cette valeur se rapproche de 30 % utilisée par Yellowlees, (1980) avec la dextrinase limite des pois non germés, mais reste inférieure aux valeurs obtenues par Dunn *et al.* (1973) et Hardie *et al.* (1976) qui étaient de 30 - 50 % avec le sorgho malté.

Tableau 1 : Activités enzymatiques des précipités au sulfate d'ammonium

% m/v de saturation en sulfate d'ammonium	Activité totale (U/mL)	[Protéines] (mg/mL)	Activité spécifique (mU/mg)
0 - 20	0,002	0,082	23,5
0 - 30	0,001	1,418	0,5
0 - 40	0,002	2,282	0,8
0 - 50	0,000	nd	nd
0 - 60	0,000	nd	nd
0 - 70	0,003	1,079	2,5
0 - 80	0,002	1,864	1,0
0 - 90	0,005	2,686	1,9

nd : non déterminé car activité enzymatique totale non détectée.

Les différences observées avec Yellowlees (1980), Dunn *et al.* (1973) et Hardie *et al.* (1976) pourraient s'expliquer par le fait que la précipitation varie d'une protéine à une autre; et aussi, en fonction de la source, une même protéine peut avoir des tailles et des solubilités variables (Labrou, 2014). Par ailleurs, la concentration de 20 % de saturation en sulfate d'ammonium pourrait permettre de postuler que la dextrinase limite du sorgho *Safrari* aurait une masse moléculaire beaucoup plus élevée que celle des précédents auteurs, étant donné que plus la masse moléculaire d'une protéine est élevée, moins de sulfate d'ammonium sera utilisé pour sa précipitation (Fiechter, 1983).

3.2 Purification de la dextrinase limite

Pour le test au kit (Tableau 2), l'activité enzymatique totale augmente après la première étape de purification (précipitation au SAM), puis diminue après la dialyse ; tandis que l'effet inverse est observé pour ce qui est du test au pullulan naturel. Ces observations se répercutent sur les rendements de purification. Quel que soit le test d'activité appliqué, l'activité enzymatique spécifique augmente après chaque étape de purification. Quel que soit le test d'activité appliqué, le facteur de purification augmente après chaque étape de purification. Cette activité totale évolue en dents de scie.

Pour ce qui est de l'activité enzymatique spécifique (Tableau 2), les conditions idéales d'augmentation de celle-ci après chaque étape de purification sont observées. Ce qui atteste de l'effectivité de la purification. Toutefois, l'activité enzymatique spécifique finale (après dialyse) avec le test au pullulan est d'environ 0,5 fois supérieure à celle obtenue avec le kit enzymatique.

Tableau 2 : Purification de la dextrinase limite

	Activité totale (mU)		Protéines (mg)		Activité spécifique (mU/mg)		Rendement (%)		Facteur de purification	
	Kit	Pullulan	Kit	Pullulan	Kit	Pullulan	Kit	Pullulan	Kit	Pullulan
Extrait brut	20,9	97,6	3314,285	3303,714	4	18	100,00	100,00	1,0	1,0
20% SAM	41,5	7,1	154,285	3,157	16	77	198,80	0,50	4,0	4,2
Dialyse	15,7	9,5	35,526	2,742	26	134	75,24	0,73	6,5	7,4

SAM : sulfate d'ammonium

La hausse d'activité dans les cas où cela est observée pour le cas du kit megazyme, serait due aux pertes de matières impliquant des inhibiteurs qui gêneraient l'activation de l'enzyme à purifier ; pertes d'inhibiteurs qui seraient considérables par comparaison à une éventuelle perte d'enzyme. Ces observations se reflètent sur les rendements de purification. La même tendance a été obtenue

par [Emenike, Chibuzo, & Sabinus \(2015\)](#) avec l' α -amylase des grains germés de l'arbre à pain.

La plus grande activité observée pour le pullulan par rapport au kit megazyme (Tableau 2) peut s'expliquer par le fait de la haute spécificité du test au kit pour des extraits enzymatiques purifiés ou non, contrairement au test au pullulan qui n'est spécifique que pour des extraits purs en dextrinase limite. N'ayant pas utilisé des techniques de purification de haute résolution telles les techniques chromatographiques, l'on serait donc en présence d'un extrait en dextrinase limite partiellement purifié, d'où la surestimation de l'activité observée avec le test au pullulan naturel. En plus, la haute spécificité du test au kit enzymatique se reflète même dans la valeur du rendement final, qui est d'environ 100 fois supérieur à celui du test au pullulan.

3.3 pH optimum d'activité et de stabilité de la dextrinase limite

On peut observer sur la figure 3, deux pics d'activité : à pH 3,5 et à pH 5,5, le maximum se situant à 3,5. La présence de ces 02 pics suggérerait que l'extrait enzymatique présenterait une activité liée à la possible présence d'autres enzymes amylolytiques. On constate une meilleure activité enzymatique située dans la plage 5,0-5,5 pour ce qui est du Kit. Etant donné que le substrat utilisé est spécifique à cette enzyme, une conclusion peut donc être tirée faisant état de ce que la plage de pH d'activité de 5,0 - 5,5 est celle correspondante à la dextrinase limite.

La variation du pH de 3,5 à 5,5 révèle que la dextrinase limite est plus active et stable à pH 5,5. Seulement 5 % d'activité sont perdues par rapport au contrôle (Tableau 3). En deçà du pH 5,0 (correspondant au contrôle), la chute d'activité est rapide et atteint son minimum au pH 3,5 (Figure 4).

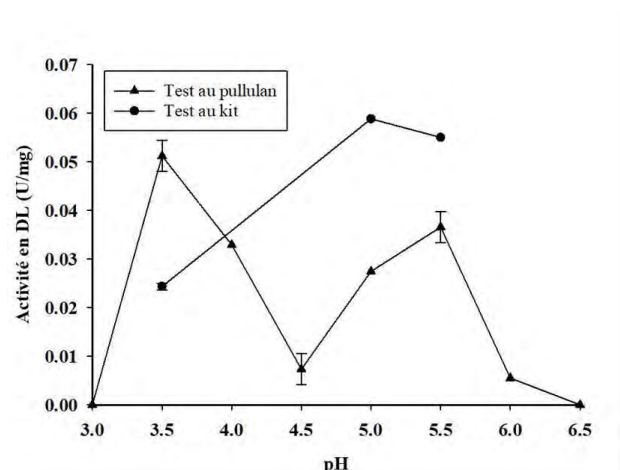


Figure 3 : Activité de la dextrinase limite (DL) en fonction du pH (test au pullulan et au Kit)

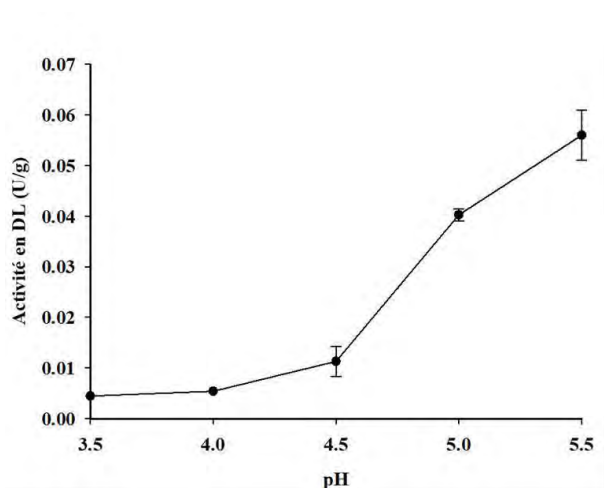


Figure 4 : Stabilité de la dextrinase limite en fonction du pH (40 °C/90 min)

A l'observation de la figure 3, le pic à pH 3,5 serait celui de l' α -glucosidase dont le pH optimum d'activité dans les malts de sorgho d'après Taylor & Dewar (1994) et Agu & Palmer (1997) est de 3,75. La plage de pH optimum de 5,0 - 5,5 obtenue avec la dextrinase limite du malt de sorgho *Salfrari* dénote de la préférence de cette enzyme pour des pH légèrement acides. Ce

comportement est similaire à celui des amylases céréalières en général dont la plage de pH optimale d'activité est de 4,0 - 5,5 (Osman, Coverdale, Cole, Hamilton, & de Jersey, 2002). La chute d'activité (Figure 4) peut s'expliquer soit par la formation de formes ioniques inadaptées de l'enzyme ou du substrat, soit par l'inactivation de l'enzyme ou la combinaison de ces phénomènes (Andrés Illanes *et al.*, 2008; Kotzia *et al.*, 2012; Mohan, Long, & Mutneja, 2013; Singh, Saini, & Kennedy, 2010).

Tableau 3: Activités relatives de la dextrinase limite en fonction du pH

pH	Activité relative en dextrinase limite (%)
Contrôle	100
5,5	95
5,0	68,5
4,5	19,2
4,0	9,2
3,5	7,7

43

3.4 Température optimale d'activité et de stabilité de la dextrinase limite

D'après le test au pullulan (figure 5), l'augmentation d'activité devient très rapide au-delà de 30 °C pour atteindre le pic d'activité ($\approx 0,12$ U/mg) à 40 °C. Au-delà de 40 °C, l'on observe une chute abrupte de 40 à 45 °C (0,02 U/mg), puis faible de 45 à 50 °C (0,005 U/mg). L'on peut donc faire le constat qu'à 35 et 45 °C on a une perte d'activité respectivement de 55 et 80 % par rapport à 40 °C.

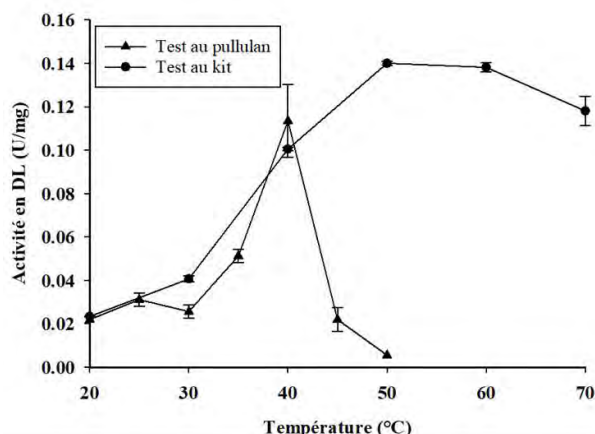


Figure 5 : Activité de la dextrinase limite en fonction de la température (test au pullulan et au kit megazyme)

D'après cette figure 5, l'activité de la dextrinase limite (test au kit megazyme) croît avec l'augmentation de la température pour atteindre le maximum ($\approx 0,14$ U/mL) entre 50 et 60 °C ; puis décroît légèrement au-delà de 60 °C pour atteindre environ 0,12 U/mL à 70 °C.

D'après la figure 6, la dextrinase limite est plus stable aux températures ≤ 55 °C. Toutefois, l'exposition de celle-ci pendant 90 min entraîne près de 80 % de perte d'activité entre 50 et 55 °C par rapport au contrôle (Tableau 4). Ce résultat est similaire à celui de [Stenholm & Home \(1999\)](#) qui ont obtenu une perte d'activité en dextrinase limite de 70 % après 1 h d'incubation à 55 °C. Au-delà de 55 °C, la perte d'activité continue jusqu'à atteindre les 95 % à 65 °C.

Les vitesses des réactions catalysées par les enzymes croissent avec l'augmentation de la température jusqu'à l'atteinte d'un maximum dit température optimale ([Andrés Illanes *et al.*, 2008](#); [Kotzia *et al.*, 2012](#); [Lewis & Bamforth, 2006](#); [Mohan *et al.*, 2013](#)). Au-delà de cette

température, la vitesse chute généralement drastiquement.

Tableau 4: Activités relatives de la dextrinase limite en fonction de la température

Barème température /temps	Activité relative en dextrinase limite (%)
Contrôle (50°C/30 min)	100
50°C/90 min	20
55°C/90 min	16,5
60°C/90 min	8,6
65°C/90 min	5

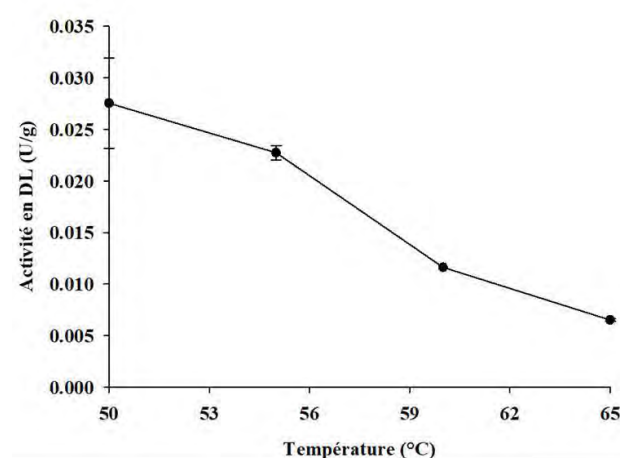


Figure 6: Stabilité de la dextrinase limite en fonction de la température

Du fait que l'augmentation de l'énergie des molécules due à l'élévation de température, il y aurait une rupture des liaisons qui maintiendrait les enzymes dans leur forme optimale d'activité et progressivement, l'enzyme se dénaturerait ([Andrés Illanes, 2008](#); [Kotzia *et al.*, 2012](#); [Mohan *et al.*, 2013](#)).

La température optimale de 40 °C obtenue ici a également été similaire à celle obtenue par des auteurs ayant travaillé avec le riz (Furegon, Peruffo, & Curioni, 1997).

Les résultats obtenus pour le test au kit (figure 5) sont similaires à ceux de Stenholm & Home (1999) qui ont obtenus l'optimum de 50 °C avec l'orge, et à ceux de Yamasaki *et al.* (2008), qui ont obtenus la valeur optimale de 55 °C avec le riz. Par ailleurs, près de 85 % d'activité est préservée à 70 °C. La dextrinase limite du malt de sorgho *Safiri* serait donc une enzyme thermorésistante.

D'après la figure 6 et en comparaison au résultat de Stenholm & Home (1999) où la perte d'activité était complète après 20 min d'incubation à 60 °C avec l'orge, il pourrait s'en déduire que la dextrinase limite du malt de sorgho *Safiri* est plus thermostable. La stabilité de la dextrinase limite à sa température optimale d'activité (50 °C/30 min, Tableau 4) est comparable à celle de la pullulanase 65 - 70 °C/30 min (Nakamura, Sashihara, Nagayama, & Horikoshi, 1989). La dextrinase limite du malt de sorgho *Safiri* peut être utilisée comme enzyme industrielle au même titre que les pullulanases.

3.5 Propriétés catalytiques K_m et V_{max}

La constante de Michaelis (K_m) et la vitesse maximale (V_{max}) ont été calculées à l'aide de la représentation graphique de Lineveaver-Burk (Figure 7). Ces valeurs sont de 0,2128 mg.mL⁻¹ et de 0,0033 mg.mL⁻¹.sec⁻¹ respectivement pour K_m et V_{max} .

La valeur de K_m obtenue à la figure 7 est inférieure de celle du riz (Yamasaki *et al.*, 2008), et à celle obtenue par Singh *et al.* (2010) avec la pullulanase de *Aureobasidium pullulans*. Ceci laisserait penser que la dextrinase limite de *Safiri* aurait plus d'affinité pour l'amidon que la pullulanase microbienne. Par ailleurs, le pouvoir

catalytique de la pullulanase serait similaire à celle de la dextrinase limite du sorgho *Safiri* sur l'amidon. En effet, on a pour V_{max} les valeurs de 0,21 et 0,20 U.min⁻¹ respectivement pour la pullulanase (Singh *et al.*, 2010) et pour la dextrinase limite de *Safiri*.

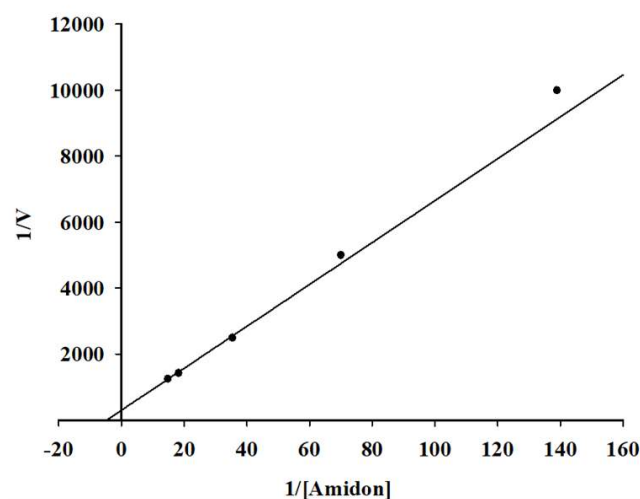


Figure 7: Représentation de Lineveaver-Burk ($1/V = f (1/[Amidon])$) pour la détermination de K_m et V_{max}

45

3.6 Effets de l'EDTA, le DTT, le CaCl₂, le SAB et l'acide ascorbique sur l'activation de la dextrinase limite

3.6.1 Cas du test au pullulan naturel

L'activité de la dextrinase limite est améliorée uniquement en présence d'agents réducteurs (Figure 8). Ceci de l'ordre de 80 % avec le DTT et de 400 % avec l'acide ascorbique. Tandis qu'elle baisse avec le SAB et le CaCl₂ respectivement de l'ordre de 46 % et 65 %, pour s'annuler presque à 92 % avec l'EDTA.

Le comportement de l'enzyme pendant l'extraction serait différent de celui au cours de la purification (Figure 8). En effet, pendant l'extraction, il y avait plutôt inhibition en présence

de ces composés pour le même test d'activité (Nguemogne *et al.*, 2020). Ceci voudrait signifier que quoique la dextrinase limite n'aie pas besoin d'agents réducteurs pour son extraction (d'après le test au pullulan), ceux-ci sont plutôt indispensables pour l'activation de l'enzyme purifiée, la préférence étant pour l'acide ascorbique. L'accroissement d'activité de la dextrinase limite purifiée en présence d'agents réducteurs à l'instar du DTT a également été observé par Yamasaki *et al.* (2008). L'inhibition en présence d'EDTA ou de CaCl_2 (Figure 8) voudrait signifier que la dextrinase limite aurait besoin pour son activation, de la présence d'ions métalliques qui ne seraient pas les ions calcium car étant inhibiteurs. Cette inhibition en présence de CaCl_2 a également été observée par Morinaga *et al.* (1997). La différence de pourcentage d'inhibition avec celui de Morinaga *et al.* (1997) serait due à la différence de concentration en CaCl_2 . L'inhibition en présence d'EDTA a également été observée par Hardie *et al.* (1976).

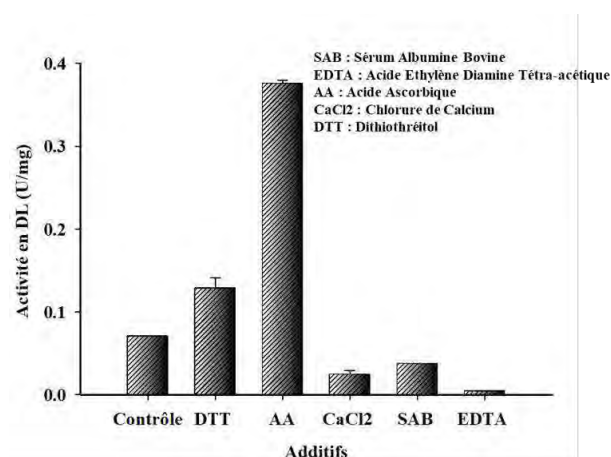


Figure 8: Effet de quelques effecteurs sur l'activité en dextrinase limite (test au pullulan)

3.6.2 Cas du test au kit Megazyme K-Pull G6

Avec le test au kit Megazyme, on observe un accroissement d'activité de la dextrinase limite

quel que soit l'effecteur (Figure 9). Toutefois, l'effet d'activation et/ou de stabilisation dû aux agents réducteurs se confirme. En effet, la méthode du kit Megazyme voudrait que la dextrinase limite soit extraite en présence de DTT. Par ailleurs, l'activité de l'enzyme extraite dans ces conditions et purifiée est d'avantage améliorée en présence d'acide ascorbique qui est également un agent réducteur. La dextrinase limite du malt de sorgho *Safrari* serait donc une enzyme sulfhydrile et demanderait un environnement réduit pour son extraction, son activation et même sa stabilisation.

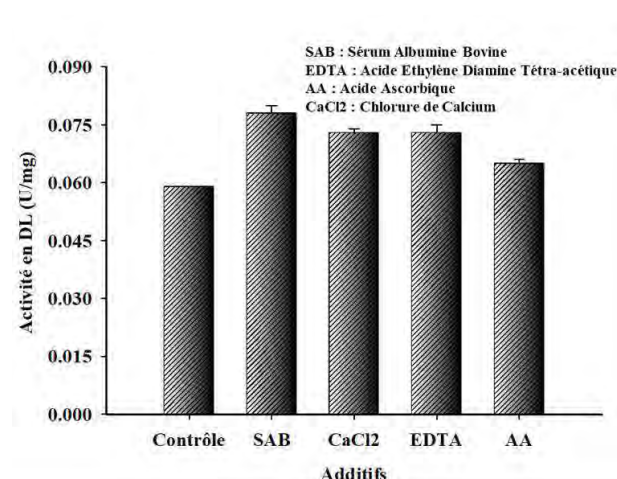


Figure 9: Effet de quelques effecteurs sur l'activité de la dextrinase limite (test au kit K-PullG6)

L'accroissement d'activité de la dextrinase limite observée en présence d'agents réducteurs à l'instar du DTT est en accord avec les travaux de Sissons (1991), MacGregor, Macri, Schroeder, & Bazin (1994), Kristensen *et al.* (1998). Notons que le pic d'activité enzymatique est obtenu en présence de SAB est similaire aux travaux de MacGregor *et al.* (1994) qui, ont montré que l'activité de la dextrinase limite se multiplie par trois en présence de SAB. En outre, l'activité de la dextrinase limite bien qu'améliorée, est la même en présence d'EDTA qu'en présence de

CaCl₂. Ceci voudrait signifier que la dextrinase limite et des inhibiteurs éventuels présents dans le milieu seraient des métalloprotéines, mais la concentration en EDTA ne serait pas assez pour complexer les ions calcium de manière à inhiber la dextrinase limite d'une part, et d'autre part, cette concentration serait assez pour complexer les ions métalliques nécessaires à l'action des inhibiteurs.

3.7 Impact de l'ajout de la dextrinase limite purifiée sur la filtrabilité et la teneur en sucres des brassins de sorgho

Le constat peut être fait de ce que la vitesse de filtration croît avec l'augmentation de la quantité d'extrait en dextrinase limite (Figure 10). Mais pour ce qui est de la teneur en sucres réducteurs les comportements entre le témoin et les moûts ajoutés de dextrinase limite sont différents (Tableau 5). Il résulte en effet une différence statistiquement significative de la teneur en sucres réducteurs entre le moût témoin et ceux ajoutés d'extrait de dextrinase limite (Tableau 5).

Tableau 5 : Test des étendues multiples pour sucres réducteurs par moût

Moût	Teneur en sucres réducteurs (g/L)
Témoin	3,90±0,03 ^a
1 mL de DL	4,18±0,03 ^b
2,5 mL de DL	4,34±0,30 ^b
3 mL de DL	4,33±0,32 ^b

La différence statistique observée au tableau 5 pourrait s'expliquer par le fait de la présence à la fois de la dextrinase limite et d'autres enzymes saccharifiantes endogènes au brassin témoin, ayant atteint leur maximum d'activité. Par ailleurs,

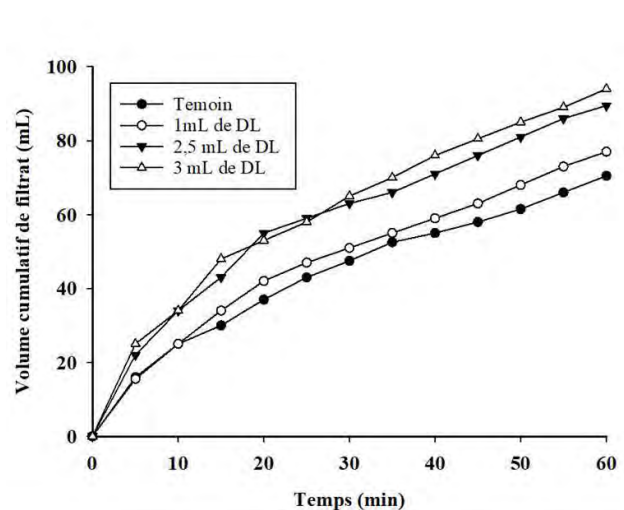


Figure 10: Volumes cumulatifs de filtrats des brassins en fonction du temps de filtration (DL mis pour dextrinase limite)

le substrat de la dextrinase limite dans ces conditions n'est plus le pullulan, mais plutôt les dextrines ramifiées dont l'hydrolyse donne les dextrines linéaires (Macgregor, 1996), d'où son action liquéfiant. L'absence de différence statistiquement significative en sucres réducteurs pour les moûts ajoutés de dextrinase limite partiellement purifiée témoigne de l'action majoritaire de cette enzyme (exogène) dans la fluidification desdits brassins. Comme conséquence directe, l'on a une meilleure filtrabilité de ces moûts par rapport au brassin témoin (Figure 10). Par ailleurs, les dextrines linéaires obtenues après activité de la dextrinase limite sont des substrats à l'action d'autres enzymes amylolytiques; enzymes dont l'action saccharifiante entraînerait nécessairement l'augmentation de la teneur en sucres réducteurs. Mais il n'en est rien pour les moûts ajoutés de dextrinase limite partiellement purifiée (Tableau 5) ; ce qui justifierait l'action majoritaire de cette enzyme dans la liquéfaction du milieu. Ceci met à nouveau à l'évidence que l'optimisation partant

du maltage jusqu'à la purification, en passant par l'extraction aurait permis d'obtenir un extrait enrichi en dextrinase limite et appauvri en d'autres enzymes amylolytiques.

4. Conclusion

L'objectif de ce travail était de purifier de manière partielle la dextrinase limite du malt de sorgho *Safrari*, purification faite par maximisation de la précipitation au sulfate d'ammonium suivie de la dialyse. La disparité observée dans les caractéristiques de la dextrinase limite en fonction du test d'activité appliqué a permis de dire que le procédé de purification appliqué a conduit à un extrait en dextrinase limite partiellement purifié pouvant renfermer des traces éventuelles d'autres enzymes amylolytiques. Comme corollaire à cela, la méthode de test d'activité de la dextrinase limite basée sur la mesure du pouvoir réducteur n'était pas adaptée en raison de la possible augmentation de ce pouvoir par l'action de l' α -glucosidase (éventuellement) sur le maltotriose (pour donner d'autres sucres réducteurs), produit de l'hydrolyse du pullulan par la dextrinase limite. Les caractéristiques spécifiques à la dextrinase limite retenues ont donc été celles obtenues avec le kit enzymatique K-PullG6. L'activité enzymatique a été rehaussée en présence d'agents réducteurs (dithiothréitol, acide ascorbique), de sérum albumine bovine et aussi de chlorure de calcium. Par ailleurs, l'application de l'extrait en dextrinase limite à des brassins de sorgho a permis d'améliorer leur filtrabilité, classifiant la dextrinase limite comme enzyme liquéfiant. Ce pouvoir liquéfiant a été également évalué sur l'amidon afin de déterminer les paramètres cinétiques de la dextrinase limite.

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Conflit d'intérêt

Les auteurs déclarent qu'ils n'ont pas de conflits d'intérêt.

Éthique

Cette étude n'est pas faite sur des hommes ou des animaux.

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V.6) *Grewia mollis* Bark Powder
Impact on the Clarification of *Mbayeri*
Sorghum Wort

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Grewia mollis bark powder impact on the clarification of Mbayeri sorghum wort

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ABSTRACT

A study was undertaken on the clarity of Mbayeri sorghum wort. *Grewia mollis* has not, to our knowledge, been used to clarify sorghum wort. Therefore, it was described alongside malted and brewed Mbayeri sorghum from a physicochemical standpoint. *Grewia mollis* was incorporated into the design using a Box-Behnken model with three parameters (wort/*Grewia mollis* powder ratio, stirring speed, and stirring time) to clarify the wort. On the acquired worts' physicochemical studies, statistical and mathematical modeling was employed. Statistical methods such as bias and accuracy factors, among others, were utilized to validate the developed models. According to the results of the physicochemical investigation, Mbayeri sorghum was suitable for brewing. The same holds true for the classification of *Grewia mollis*, which was shown to be appropriate for the clarifying method. During mathematical modeling, it was determined that the ratio of wort to *Grewia*, stirring speed, and stirring time had a substantial effect on the selected physicochemical criteria (responses). Multi-response optimization performed according to a specified specification, which includes minimizing pH and titratable acidity and maximizing Brix, color, turbidity, while decreasing sugar, soluble protein, and polyphenol content, yielded the following results: pH, 5.2; turbidity, 224 NTU; brix, 12.97 °P, colour, 46 ASBC; reducing sugars, 94.03 mg/mL; soluble protein, 502.42 mg/L; polyphenols, 54.21 mg/L; and titratable acidity, 1.2 g/L H₂T. The optimal blend of 0.25 g/L *Grewia mollis* powder, 45 rpm, and 60 minutes yielded this result. These characteristics suggest that *Grewia mollis* could be used as a brewing filter assist.

1. Introduction

In 2020, Cameroon produced approximately 1,215,377 t of sorghum out of a total of 3,733,377 t of cereal (FAOSTAT, 2022). It represented almost one-third of the nation's cereal output. Sorghum is the primary cereal ingested by the majority of people in northern Cameroon, who frequently endure recurrent famines (Desobgo et al., 2011; Desobgo and Nso, 2013). Bread, couscous, dumplings, fermented and unfermented porridges were typical sorghum-based cuisines in Africa and Cameroon. The production of this crop has quadrupled over the past two decades, from 420,000 t in 2000 to 1,217,377 t in 2020 (FAOSTAT, 2022). This increasing demand is attributable to Bili-increased Bili's output. It was the ideal grain for brewing traditional African brews and was regarded as Africa's grain of the 21st century (Taylor, 2003). Bili-Bili is a typical Cameroonian beer manufactured and drunk primarily in the country's northern region. In the competitive environment of multinational corporations, sorghum was the best substitute for malted barley when producing beer (Davana and Revanna, 2021). Unlike barley, however, the absence of straw in sorghum grain was until recently seen as a significant obstacle to the use of sorghum in the production of light and lager beers.

This indicates the difficulty conventional brewers have in clarifying this beer (Goode et al., 2002; Goode and Arendt, 2003; Nso et al., 2003). Due to its foggy look, Bili-Bili was sometimes referred to as an opaque beer. Typically, filtering agents such as Irish moss, isinglass, gelatin, and fish glue were added to beer to diminish its cloudiness. Their price may be a constraint. *Grewia mollis*, a naturally occurring coagulant/flocculant that has already been utilized for water purification, was a less expensive, more practical, and more accessible choice for traditional and industrial brewers (Ngounou et al., 2021). *Grewia mollis*, a common shrub or tree in the Sudano-Sahelian region, is also present in Cameroon and Nigeria. Some regional recipes call for the dried and pulverized inner bark of the stem as a thickener (Muazu et al., 2009). Particularly in the Adamawa region of Cameroon, the powder is used as a binder in the preparation of fried maize cakes. In Nigeria, it has been used to produce soups and Hausa-named native pastries called "Kosai" by crushing it and combining it with bean flour (Emeje et al., 2008). Mucilage, a naturally occurring polysaccharide, has been associated with the functional properties of *Grewia* powder (Nep and Conway, 2011). Since the eventual goal is to replace existing clarifying agents, it would be best to use the powder as-is initially, assuming it does not pose any issues

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during wort treatment. Production, quality, structure, and bioactive properties of plant polysaccharides can vary depending on the extraction method employed (Chen et al., 2018; Ghori et al., 2017). Determining the operating conditions for the use of *Grewia mollis* powders in clarifying and evaluating its effect on some physicochemical properties of *Mbayeri* sorghum wort was the purpose of this study.

2. Material and methods

2.1. Biological material

In January 2022, the *Grewia mollis* bark was acquired in the northern region of Cameroon, specifically in Guider's town, while the *Mbayeri* sorghum cultivar was purchased at the little market of the city of Ngaoundere.

2.2. *Grewia mollis* characterization

Utilizing a procedure developed by the Association of Official Analytical Chemists, the moisture, ash, fat, total soluble solids, and protein levels were determined (AOAC, 2006).

The total phenolic content was calculated using the Folin-Ciocalteu reagent (Meda et al., 2005). A methanol/water solution (1:1 v/v) was used to create samples, which were then filtered through a 0.45 µm disc filter at a concentration of 0.05 g/mL. In a ratio of 1:25:25, the filtrate was mixed with sodium carbonate solution (75 mg/mL) and Folin-Ciocalteu reagent solution (1:10 v/v). After one hour of incubation at room temperature in the dark, absorbance was measured using a spectrophotometer (Spectro UV-VIS Dual Beam UVS-2800, Labomed, Inc., USA) at a wavelength of 760 nm. Various amounts of gallic acid were utilized to produce a standard curve with detection and quantification limits of 6.38 and 19.34 mg/L, or almost 14.0 to 112.0 mg/L.

2.3. *Mbayeri* sorghum characterization

Before malting, the grain was subjected to a number of physicochemical studies, including those for moisture content, germination capability, germination energy, thousand-corn weight, and protein content. All of these analyses were conducted using the standard ASBC technique (ASBC, 2009).

2.4. Sorghum malting

One kilogram of the sorghum cultivar's grain was washed three times with three liters of distilled water to eliminate dust and contaminants. The grains were steeped in 3 L of distilled water at room temperature (25°C) for 48 hours with three 12-h water changes. Germination was conducted in Heraeus D-63450 (Kendro laboratory product, Hanau, Germany) for four days at a temperature of 25°C, with watering every six hours. The malt was subsequently dried for four days at 40°C with the CKA 2000 AUF dryer. The malted sorghum was then removed of its rootlets and stored.

2.5. *Mbayeri* sorghum brewing

The malted sorghum was milled to a 0.7 mm thickness. Five kilograms of flour were weighed and added to a Braumeister along with twenty liters of 45°C water. For 30 min during the proteolytic phase, the medium was agitated at this temperature to prevent floc formation. Next, the starch was gelatinized by draining the supernatant (15 L) and heating the remaining material at 95–98°C for 40 min on a gas plate. Following gelatinization and cooling to 60–65°C, the amylolytic stage commenced, and the supernatant and gelatinized starch were reintroduced into the Braumeister at 65°C. At this temperature, stirring lasted 1 h and thirty minutes. The following process was saccharification, which included raising the temperature to 72°C for one hour. The mash was finally mixed and chilled to 25°C.

Table 1
Range of different factors

Factors	Interval of factors
Mass/volume ratio	1/1000 – 1/10000
Stirring time (min)	5 – 60
Stirring speed (rpm)	0 – 200

2.6. Clarification methods

2.6.1. Hot test

The cloudy wort was added to five jars for the coagulation/flocculation experiments, which were then carried out in a 100°C water bath. The mixture was stirred using a spatula after the powdered *Grewia mollis* bark was added. The jars were removed from the water bath after 10 min, and turbidity measurements were taken every five minutes.

2.6.2. Cold test

In a subsequent phase, before adding the bark, a Jar test agitator was employed to conduct the cold test.

2.7. Wort characterisation

The extract, titratable acidity, pH, polyphenols, soluble proteins, color, and turbidity were all assessed using standard ASBC techniques (ASBC, 2009). This equation was utilized to determine the efficacy of turbidity elimination:

$$\%Turbidity = \frac{Turb_i - Turb_f}{Turb_i} \times 100 \quad (1)$$

Where $Turb_i$ and $Turb_f$ are the initial and final turbidity (EBC), respectively.

2.8. Experimental design

When constructing an experimental design (Table 1), the mass/volume ratio, stirring time, and stirring speed were evaluated in order to investigate the interaction between the various parameters (these factors were chosen based on the preliminary tests).

2.9. Choice of experimental responses

The experimental responses used were Brix, pH, reducing sugar concentration, soluble protein content, total polyphenol content, and titratable acidity. These seven reactions served as criteria for evaluating the effect of *Grewia mollis* on the quality of the worts. Table 2 displays the experiment matrix. Three factors comprised the experimental matrix of a Box Behnken response surface design: mass-to-volume ratio (x1), stirring time (x2), and stirring speed (x3).

2.10. Modeling

The criteria for selection were those that promoted flocculation/coagulation of suspended particles and aided in wort clarifying. Adjustments were made using a Box-Behnken experimental design with three parameters. The experimental design represented in the matrix was the result of the selected factors. Physical and biological relevance led to the selection of these components. In actuality, time was an essential part of clarity, since it permitted the largest extraction yields possible within a given time limit. The rate of agitation would maximize the polymer extraction from *Grewia mollis* bark. Using the mass of *Grewia mollis* powder, we would be able to establish the optimal quantity for enhancing the clarity of a volume of wort.

The subsequent mathematical models took into account the coded variables (Eq. 2). These mathematical models employed polynomials

Table 2
Experience matrix

Experiences Order of trials	Factors		Stirring speed (rpm)		Stirring time (min)	
	Mass/volume ratio (m/v)					
	X ₁ Coded variable	Real variable	X ₂ Coded variable	Real variable	X ₃ Coded variable	Real variable
1	0	1/5500	-1	0	-1	5
2	1	1/10000	0	100	1	60
3	1	1/10000	1	200	0	32.5
4	0	1/5500	1	200	-1	5
5	1	1/10000	0	100	-1	5
6	1	1/10000	-1	0	0	32.5
7	-1	1/1000	0	100	1	60
8	0	1/5500	0	100	0	32.5
9	0	1/5500	1	200	1	60
10	-1	1/1000	-1	0	0	32.5
11	0	1/5500	0	100	0	32.5
12	0	1/5500	0	200	0	32.5
13	0	1/5500	-1	0	1	60
14	-1	1/1000	0	100	-1	5
15	-1	1/1000	1	200	0	32.5

with several variables. The model's factors consisted of first-degree factors (x_1 , x_2 , and x_3), second-degree factors (x_1^2 , x_2^2 , and x_3^2), and interactions (x_1x_2 , x_1x_3 and x_2x_3). If the probability (p) was less than or greater than 0.05, these factors were deemed statistically significant.

$$y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum \sum_{i < j} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

With β_0 : the constant, ε : the error, and the β_j , β_{jj} and β_{ij} were the coefficients of the model and y: the response.

The model's validity was determined by comparing theoretical and observed values. Ross, (1996) provided the following equations for the polarisation factor, Bf, and the polarized accuracy factor, Afl:

$$B_f = 10^{\frac{1}{n} \sum_{i=1}^n \log \left(\frac{y_{i,cal}}{y_{i,exp}} \right)} \quad (3)$$

$$A_{f1} = 10^{\frac{1}{n} \sum_{i=1}^n \left| \log \left(\frac{y_{i,cal}}{y_{i,exp}} \right) \right|} \quad (4)$$

The perfect predictive model leads to: $Af_1 = B_f = 1$. The acceptable predictive model was: $0.75 < B_f$ or $Af_1 < 1.25$ (Dalgaard and Jørgensen, 1998). The software Minitab 21 was used to generate the models and the statistics, while OriginLab 2022 was used to plot the graphs.

2.11. Optimization

This optimization aimed to find a satisfactory compromise for each response. Consequently, the objective was to locate the combination that matched all of the criteria needs adequately. The objectives of this specification were to obtain a wort that could ferment rapidly, to have wort with standard hues, and to benefit from the antioxidant impact of the polyphenols while minimizing turbidity, so reducing the wort's opacity and thereby clarifying it. After establishing these conditions, the Minitab 21 software was utilized to identify the optimal triplet that satisfied them. Using the same software, the theoretical results for each of the four responses were determined. Utilizing the optimal theoretical combination, the wort was clarified. The powder of *Grewia mollis* and the wort were placed in the freezer for further analysis to ascertain the wort's characteristics. Prior to this clarification, the wort was brought to room temperature (25°C). The protein content, color, polyphenol content, and turbidity were evaluated physicochemically. In addition, the content of extract, titratable acidity, pH, and reducing sugar was assessed.

Table 3
Physicochemical characteristics of the bark of *Grewia mollis* and viability test of unmalted *Mbayeri* sorghum

Chemical composition	bark (<i>Grewia mollis</i>)	<i>Mbayeri</i> sorghum
Moisture (%)	9.8 ± 0.1	8.1 ± 0.0
Ash (%)	7.4 ± 0.1	/
Proteins (%)	12.6 ± 0.1	12.2 ± 0.6
Lipids (%)	1.9 ± 0.7	/
Total sugars (%)	35.3 ± 0.1	/
Total polyphenols (%)	17.2 ± 0.1	/
Germinative capacity (%)	/	98 ± 0.1
Germinative energy (4 mL) (%)	/	98.4 ± 1.1
Germinative energy (8 mL) (%)	/	97 ± 0.9
Thousand corn weight (g)	/	43.3 ± 0.1

3. Results and discussion

3.1. Proximate analysis of *Grewia mollis* and viability test of *Mbayeri* sorghum cultivar

The chemical composition of *Grewia mollis* powder, expressed as a percentage of dry matter, is shown in Table 3. Water, ash, protein, lipids, total sugars, and polyphenols were 9.8 ± 0.1%, 7.4 ± 0.13%, 12.6 ± 0.11%, 1.9 ± 0.7%, 35.3 ± 0.10%, and 17.8 ± 0.11%, respectively, according to this table. All of these data fell within the ranges specified in the literature for a variety of *Grewia* species: 6.30-8.71% for ash, 12.91-18.8 % for protein, 2.64-3.86% for lipids, and 28.6-40.1% for total sugars (Muhammad et al., 2021; Nep and Conway, 2011; Panyoo et al., 2014; Pradip, 2020). The total polyphenols content achieved was greater than what Zia-Ul-Haq et al., (2013) found, which was between 0.95 to 2.05%. This could be attributable to the species or the geographical location. The powdered bark had a low moisture level and was composed of sugars and other substances. High levels of total sugars in the sample of bark suggested the existence of polysaccharide gum. Due to the amount of sugars, Panyoo et al. (2014) were driven to extract the polysaccharide-rich *Grewia* gum. *Grewia mollis* gum included the neutral monosaccharide carbohydrates glucose, rhamnose, galactose, arabinose, and xylose (Nep and Conway, 2011; Panyoo et al., 2014). The protein content was ordinary whereas the ash amount was high. This was likewise noted by Panyoo et al., (2014).

The moisture level of unmalted *Mbayeri* sorghum, which was 8.12 ± 0.02%, was within the acceptable limit for grain preservation, as shown in Table 3. In fact, this figure should go below or equal 13% (Hough et al., 2012). Germinative capacity, germinative energy (4 mL),

Table 4
Summary of the physicochemical characteristics of the malted *Mbayeri* wort after mashing

Characteristics	Wort
Extract (°P)	12.9 ± 0.6
Turbidity (EBC)	356 ± 2
Turbidity (NTU)	1463 ± 1
Colour (ASBC)	49.1 ± 0.5
Reducing sugars (mg/mL)	108.2 ± 1.3
Soluble protein (mg/L)	548.5 ± 0.9
Polyphenols (mg/L)	61.5 ± 9.2
pH	4.4 ± 0.4
Titrate acidity (meq g/100 g DM H ₂ T)	1.5 ± 0.5

and germination energy (8 mL), with respective values of 98 ± 0.15 %, 98.41 ± 1.15 %, and 97 ± 0.96 %, were within the EBC-Analysis-Committee, (1998). Therefore, the grains were suitable for malting. The weight of 1000 grains, 43.36 ± 0.13 g, fell within the range determined by Desobgo et al. (2013). Protein content was 12.26 ± 0.57 %. According to these data, *Mbayeri* sorghum is suitable for malting and brewing.

3.2. Physicochemical characteristics of the wort after mashing

The observed extract in Table 4 was 12.9 ± 0.60 °P. Indeed, it was the most important factor to consider when determining the brewing capability of wort (Briggs et al., 2004). Because they are the principal source of energy for yeast metabolism, reducing sugars (108.2 ± 1.3 mg/mL) were required for alcoholic fermentation in brewing. Moreover, proteins are important to a successful fermentation process. Therefore, the protein level was crucial not only for the fermenting capacity of the yeast, but also for the flavor of the beer. The *Mbayeri* wort was appropriate for fermentation into sorghum beer, according to Table 4. It was essential to take note of the extremely high turbidity value and confirm the opacity of the sorghum wort as mentioned in the literature (Embashu et al., 2019; Kayode et al., 2011). Therefore, it was vital that the wort be clarified.

3.3. Wort clarification using *Grewia mollis*

3.3.1. During wort boiling

Molecular instability of polysaccharides, possibly by hydrolysis, at this temperature (100°C) would account for the observed increase in Plato, turbidity, color, reducing sugars, soluble proteins, and polyphenols with increasing concentrations of *Grewia mollis* or decreasing ratios (Table 5). In addition, *Grewia mollis* gums would experience a mass loss between 30 and 140°C. This may be attributable to the loss of structural and adsorbed water from biopolymers (Kittur et al., 2002; Vendruscolo et al., 2009) or to the desorption of structural water from polysaccharides. The results preclude the use of *Grewia mollis* during the boiling of wort. The decline in sorption capacity as temperature rises can be rationalized as follows: Athletic activities were exothermic. Consequently, an increase in temperature should diminish sorption. The solubility of the solute changes as the temperature changes. At elevated temperatures, the size of the sorbent decreases, resulting in a less porous sorbent with fewer bonding sites, which decreases the distribution ratio values (Alessandro, 2001).

3.3.2. After wort boiling

Table 6 contains the physicochemical analyses of *Mbayeri* wort purified with *Grewia mollis* bark powder. The response surface methodology was used to statistically model the following physicochemical characteristics: color (ASBC), soluble protein content (mg/L), polyphenol content (mg/L), and turbidity (EBC). As indicated in Table 7, the mathematical models obtained were all second-order with interactions and validated (Dalgaard and Jørgensen, 1998). Additionally, a factor was considered important if its probability was less than 0.05 (Table 8).

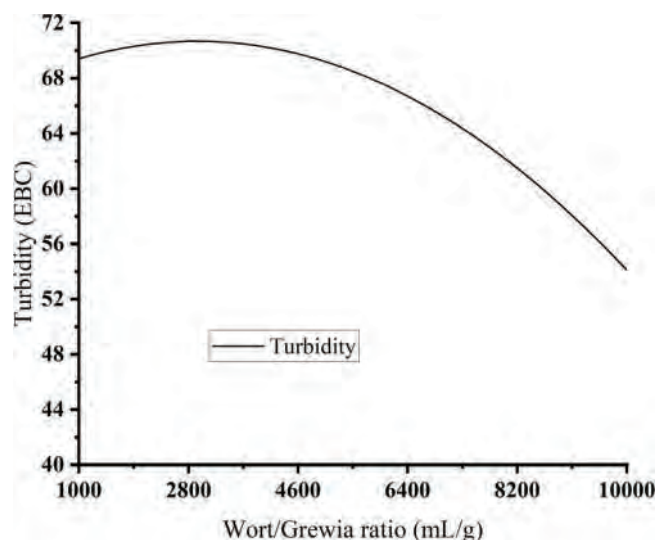


Fig. 1. Evolution of the turbidity as a function of wort/*Grewia* ratio (Stirring speed and stirring time fixed at 0 rpm and 5 min)

3.4. Effect of singular contribution

3.4.1. Effect of wort/*Grewia mollis* ratio (x_1)

The ratio of wort volume to *Grewia mollis* mass (x_1) has only a significant influence on wort turbidity (whose model equation is documented in Table 7) and the quadratic effect ($P = 0.031$, Table 8). Fig. 1 demonstrates that wort turbidity decreases with increasing dilution (with decreasing *Grewia mollis*). Delele et al. (2018) also made this observation when treating river water with *Moringa* seed powder. With a high concentration of *Moringa* grain powder, the turbidity of river water did rise. The wort's turbidity was a measurement of the suspended colloidal particles. The coagulation/flocculation phenomenon could not occur if the stirring speed was zero and the time was five minutes. The immediate result was that the solid particles of *Grewia mollis* powder stayed suspended in the wort, hence increasing the turbidity.

3.4.2. Effect of stirring speed (x_2)

Mbayeri sorghum worts' turbidity, color, polyphenols, and proteins are strongly affected by the stirring speed (x_2) (Table 8). Each model is described in Table 9. In fact, a decline in each of these physicochemical properties was found as stirring speed increased (Fig. 2).

Increasing the agitation speed would produce flocs from the *Grewia* powder and wort haze. *Grewia* biopolymer may favor adsorption and bridging effects, promote floc's tightly packed aggregate structure, and accelerate floc settling due to its large molecular mass (Li et al., 2006; Niu et al., 2013). The outcome was a reduction in turbidity.

When *Grewia* powder was added to the wort and the stirring speed was increased, the proteins would have generated charges similar to magnets, attracting mostly oppositely charged particles (Schwarz, 2001). The flocculation process happened when these proteins bound the charges and the wort particles collected into flocs. Due to the high molecular weight of *Grewia* gum, the flocs settled, reducing the amount of protein in the cleared wort.

Hot trub contained insoluble, denaturated proteins, complex polysaccharides, lipids, tannins, polyphenols, and various other minerals (Kühbeck et al., 2006). The rate of stirring facilitates contact between the haze and the flocculating ingredients in the *Grewia* powder. The decrease in polyphenols in cleared wort is justifiable given that they permit the settling of cloudiness.

Depending on the form and size of the molecules, phenolic chemicals affect the beer's color, flavor, froth, and chemical-physical stability. They are present in both the aleuronic layer and grain chaff. When

Table 5Physicochemical characteristics of malted *Mbayeri* wort after introduction of *Grewia mollis* during boiling

Wort/ <i>Grewia mollis</i> ratio (mL/g)	°Plato	Turbidity (EBC)	Colour (ASBC)	Reducing sugar (mg/L)	Soluble protein (mg/L)	Polyphenols (mg/mL)
500/1	14.5±0.6	1421±1	86.52±0.80	126.21±0.40	569.02±2.00	64.24±5.10
1000/1	14.1±0.2	954±0	77.11±0.30	114.04±0.70	551.15±0.80	61.72±3.70
5000/1	13.2±0.2	120±0	71.20±0.40	102.11±0.40	478.21±0.50	58.54±8.60
10000/1	12.2±0.3	98±1	58.14±0.70	103.26±0.40	428.43±0.60	51.24±1.20

Table 6Physicochemical analysis of malted *Mbayeri* wort after clarification using *Grewia mollis*

x1	x2	x3	Colour (ASBC)	Protein (mg/L)	Polyphenols (mg/mL)	Turbidity (EBC)
0	-1	-1	56.8	479.5	39.5	70.61
1	0	1	58.1	490.8	40.8	55.36
1	1	0	67.7	501.4	50.4	55.34
0	1	-1	52.6	485.3	41.23	47.85
1	0	-1	57.6	491.3	41.3	59.73
1	-1	0	59.9	491.6	42.6	49.97
-1	0	1	58.8	491.5	41.5	48.51
0	0	0	68.1	500.8	50.72	70.35
0	1	1	57.1	489.8	39.8	48.45
-1	-1	0	68.6	501.3	51.3	69.50
0	0	0	67.4	502	50.1	72.73
0	0	0	64.7	499.4	49.4	68.57
0	-1	1	52.5	485.2	35.2	47.96
-1	0	-1	58	490.7	40.7	56.09
-1	1	0	59.5	493.2	42.2	48.60

Table 7

Mathematical models and validation criteria

Equations	R ²	Af1	Bf
$Y_{Colour(ASBC)} = 66.733 - 0.2x_1 - 0.112x_2 + 0.188x_3 + 0.283x_1^2 - 3.092x_2^2 - 8.892x_3^2 + 4.225x_1x_2 - 0.075x_1x_3 + 2.2x_2x_3$ (5)	0.9832	1.014	1.002
$Y_{Turbidity(EBC)} = 70.55 - 0.29x_1 - 4.73x_2 - 4.25x_3 - 6.75x_1^2 - 7.95x_2^2 - 8.88x_3^2 + 6.57x_1x_2 + 0.8x_1x_3 + 5.81x_2x_3$ (6)	0.9287	1.040	1.004
$Y_{proteins} = 500.73 - 0.2x_1 + 1.512x_2 + 1.312x_3 + 1.13x_1^2 - 4.99x_2^2 - 10.79x_3^2 + 4.47x_1x_2 - 0.33x_1x_3 - 0.3x_2x_3$ (7)	0.9611	1.022	1.001
$Y_{polyphenols(mg/L)} = 50.073 - 0.075x_1 + 0.629x_2 - 0.679x_3 - 0.653x_1^2 - 2.795x_2^2 - 8.345x_3^2 + 4.225x_1x_2 - 0.325x_1x_3 + 0.717x_2x_3$ (8)	0.9654	1.021	1.002

Table 8

Significance of factors

	Colour	Turbidity	Protein	Polyphenols
x_1	0.653	0.859	0.812	0.899
x_2	0.799	0.028	0.116	0.315
x_3	0.673	0.040	0.160	0.283
x_1^2	0.665	0.031	0.378	0.467
x_2^2	0.004	0.017	0.008	0.020
x_3^2	0.000	0.011	0.000	0.000
x_1x_2	0.001	0.030	0.011	0.003
x_1x_3	0.904	0.728	0.784	0.700
x_2x_3	0.014	0.044	0.800	0.409

Grewia mollis powder, which aided in haze decantation by increasing stirring speed, decreased wort color due to the complexation of proteins that create haze by polyphenols.

3.4.3. Effect of stirring time (x_3)

The stirring time (x_3) has a major influence on the turbidity, color, polyphenols, and proteins in *Mbayeri* sorghum worts (Table 8). All models are displayed in Table 7. A decline in these physicochemical properties was noticed when stirring duration increased (Fig. 3).

Regarding turbidity reduction, the clarification process was effective. The absorption was generated by the diffusion of wort components onto the surface of the sorbent. The extremely porous nature of sorbents (Osemeahon et al., 2016) and the particle size (powder) provide a large surface area for the sorption of wort components on the binding sites. All other components associated with the cloudiness of the worts (proteins, polyphenols, and color) underwent the same process and were adsorbed on the *Grewia mollis* powder.

amino acids and fermentable carbohydrates react, pigmented molecules called melanoidin are produced. Together with polyphenols, these compounds determine the final hue of wort and beer (Hodzić et al., 2007).

Table 9Physicochemical characteristics of optimized malted *Mbayeri* wort

Characteristics	Theoretical values (From models)	Experimental values
Colour (ASBC)	46	48.5±1.5
Turbidity (EBC)	56	57.6±2.2
Soluble protein (mg/L)	502.42	511±4
Polyphenols (mg/L)	54.21	52.3±2.9
Reducing sugars (mg/mL)	/	98.7±1.8
Titrate acidity (g/L)	/	1.2±0.1
pH	/	5.3±0.3
Plato (°P)	/	13.2±0.7

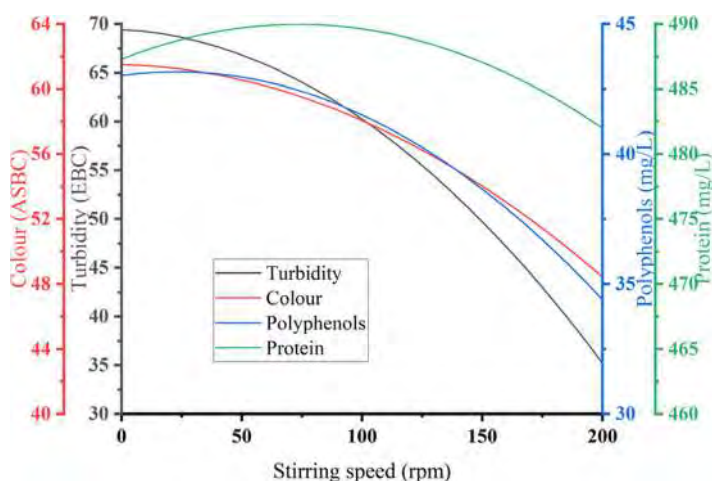


Fig. 2. Evolution of turbidity, colour, polyphenols and protein as a function of stirring speed (ratio wort/*Grewia* and stirring time fixed respectively at 1000 mL/g and 5 min)

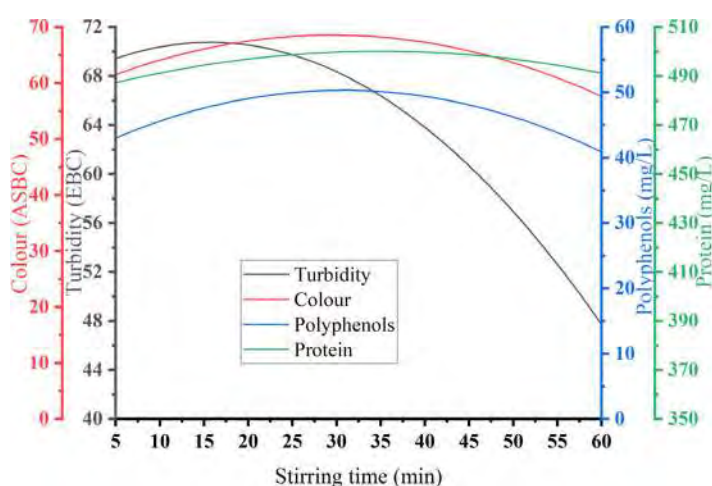


Fig. 3. Evolution of turbidity, colour, polyphenols and protein as a function of stirring time (ratio wort/*Grewia* and stirring speed fixed respectively at 1000 mL/g and 0 rpm)

It was required to allow sufficient time for the formation of particles large enough to be removed efficiently during the sedimentation process. In every clarifying process, including coagulation-flocculation activities, the time of macro flocs generation was a critical operating component (Wang et al., 2005).

3.5. Effect of interaction contribution

3.5.1. Effect of interaction wort/*Grewia mollis* ratio and stirring speed (x_1x_3)

The interaction $x_1 \times x_3$ has a substantial effect on the turbidity, hue, polyphenols, and proteins of *Mbayeri* sorghum worts (Table 8).

For polyphenols content (Fig. 4), an increase in wort was noted with an increase in *Grewia mollis* content (from 10,000 mL/g to 1,000 mL/g, which corresponds to 0.1 g/L to 1 g/L of *Grewia mollis* concentration) at stirring speeds ranging from 0 to 100 rpm. In addition, an increase in polyphenols and a decrease in *Grewia mollis* between 100 and 200 rpm. This could be attributed to the low agitation speed (0 to 100 rpm) during the first phase, which prevented the fixation of polyphenols on *Grewia mollis* powders. The increase in polyphenols would originate from the raw *Grewia mollis* powder, which includes polyphenols (Sambo et al., 2015) and would therefore bring some along with it. For the second phase, agitation speeds between 100 and 200 rpm would increase the exchange surface of the *Grewia mollis* powders, resulting in enhanced polyphenol adsorption and subsequent removal during decantation. Due to its high viscosity, *Grewia mollis* powder including gum will also blend more quickly. Consequently, the gum flocs were more stable. Perng and Bui, (2015) made this discovery while investigating the effect of agi-

tation speed on the ability of reactive dyeing wastewater to decolorize *Cassia fistula* gum.

The same pattern was noted for color (Fig. 4). In fact, polyphenols were principally responsible for the color of sorghum worts, which resulted from the Maillard reaction triggered by kilning and wort boiling. Therefore, the same explanations for polyphenols would be relevant.

The protein trend was comparable to the hue (Fig. 4). In fact, the polyphenol/protein complex contributed to the cloudiness of the wort (Briggs, 1998; Kühbeck et al., 2006). Additionally, the hue was due to the polyphenols (Hough et al., 2012). Therefore, the complex would have the same adsorption as the color.

With increased stirring speed and a decrease in the wort/*Grewia mollis* ratio, turbidity decreased (Fig. 4). The synergy between these two forces allowed for a decrease in turbidity. In fact, the agitation speed would aid in the expansion of the exchange surface. Simultaneously, the fall in *Grewia mollis* amount would reduce steric hindrance and allow the adsorption of wort chemicals at all active sites (Perng and Bui, 2015).

3.5.2. Effect of interaction stirring speed and stirring time (x_2x_3)

Fig. 5 demonstrates that the turbidity and color of the worts drop dramatically (Table 8) as agitation speed and time increase. This is due to the fact that quick mixing was used in the coagulation process to spread the coagulant throughout the cloudy solution (Saritha et al., 2017). It is believed that the agitation speed is responsible for this synergistic effect, since it helps to expose the majority or all of the active sites in the *Grewia mollis* powder, so making the turbidity easier to absorb. In contrast, the agitation time maximizes the absorption of the turbidity components, including the polyphenols, and consequently the color.

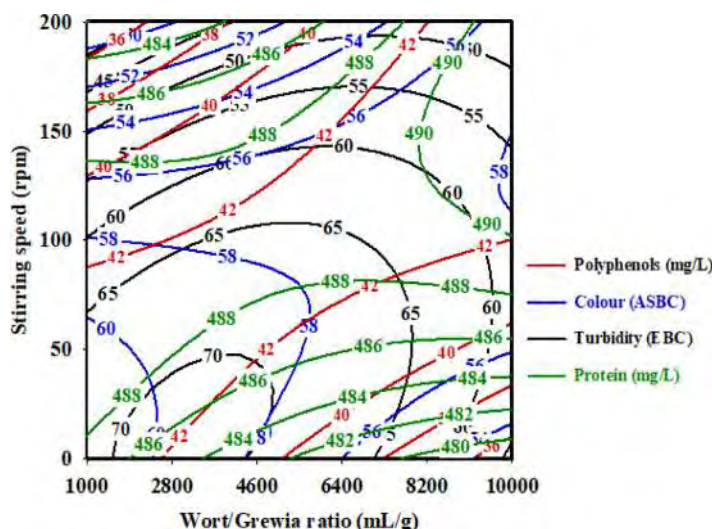


Fig. 4. Evolution of turbidity, colour, polyphenols and protein as a function of wort/Grewia ratio and stirring speed (stirring time fixed at 5 min)

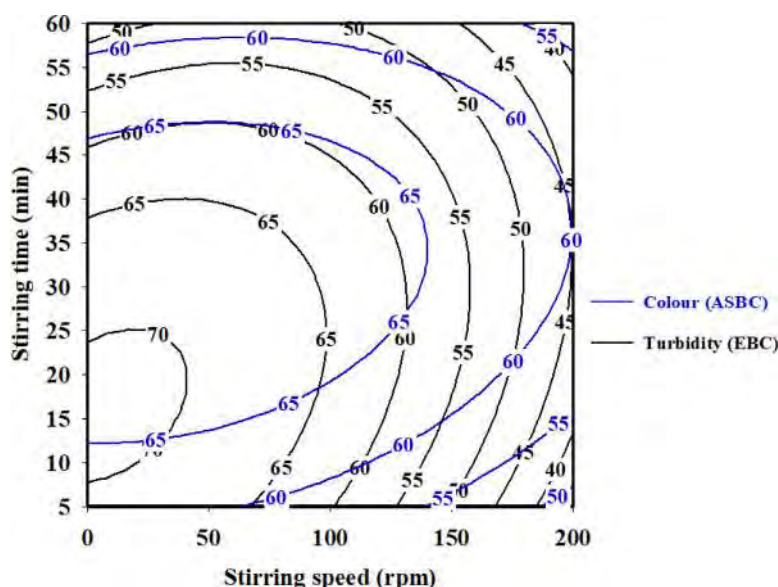


Fig. 5. Evolution of turbidity and colour as a function of stirring speed and stirring time (wort/Grewia ratio fixed at 1000 mL/g meaning 1 g/L)

According to other sources, frequent and gentle stirring promotes the formation and consolidation of flocs (Ndabigengesere et al., 1995).

3.6. Optimization

The following optimal trio was obtained via a multi-response optimization criterion in order to increase color, protein, and polyphenol content while limiting turbidity in order to produce an appetizing wort. The wort-to-*Grewia mollis* ratio was 4000 mL/g (0.25 g/L of *Grewia mollis*), and the stirring rate and duration were 45 rpm for 60 minutes. *Grewia* concentrations of 0.25 g/L were within the range reported in the literature when *Moringa oleifera* was used to clear various substrates (Villaseñor-Basulto et al., 2018). In addition, *Grewia*'s figure of 45 rpm fell within the range (40 rpm) determined by Arnoldsson et al. (2008) for *Moringa* water treatment using the jar test. Nevertheless, the optimal duration was longer than the 17-min estimate provided by the same authors. This may be due to the dissimilar nature of the suspended solids that should coagulate or flocculate. Table 9 displays the theoretical physicochemical attributes that came from this optimal theoretical value. After laboratory confirmation of these data, it was determined that the range of deviations from the theoretical results for turbidity, color, soluble proteins, and polyphenols were 2.85 %, 5.43 %, 1.70 %, and 3.55 %, respectively.

As a result, theoretically optimal conditions for clarifying *Mbayeri* malt wort could be validated.

A comparison of the properties of cleared wort and starting wort revealed that, under optimal conditions, only turbidity fell significantly by 84.27 percent (Tables 9 and 4). This result falls within the range of the relevant literature. Guar gum was used to cleanse water, and turbidity was reduced by 88.1% (Mukherjee et al., 2013), whereas *Grewia* gum eradicated chromium from 47% to 98% (Kofa et al., 2019). The other characteristics did not considerably change. As a result, *Grewia mollis* powder substantially enhanced the clarity of wort by eliminating cloudiness.

4. Conclusion

Grewia mollis, a shrub native to the Adamawa region of Cameroon, was used to clarify and reduce the opacity of sorghum wort. Characterization of this substance revealed the existence of components that may allow this clarity to be achieved with a high sugar concentration, indicating the polysaccharide nature of the gum. The opacity (356 EBC) and the need for clarification were validated by the physicochemical parameters of the generated mash. The viability characteristics of *Mbayeri* sorghum permitted malting of this crop. Mathematical modeling re-

vealed the major impact of clarification-related elements (wort/*Grewia* ratio, stirring time, and speed, among others) on the wort's particular physicochemical features (turbidity, among others). With the parameters tuned, a turbidity reduction of approximately 84% was possible. This demonstrated the efficacy of *Grewia mollis* in treating opaque sorghum wort due to the absence of other nutrient leaching. This study emphasized opacity concerns and the possibilities for clarifying wort with inexpensive natural coagulants.

Ethical Statement

The authors declare that the study does not involve animals and humans

Declaration of Competing Interest

The authors declare that they have no conflict of interest

Data Availability

No data was used for the research described in the article.

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V.7) White-Flesh Guava Juice
Clarification by a Fixed-Angle Conical
Rotor Centrifuge Laboratory and
Characterization of Continuous Disk
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Research article

White-flesh guava juice clarification by a fixed-angle conical rotor centrifuge laboratory and characterization of continuous disk stack centrifuges



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ABSTRACT

The two objectives of this paper were to determine the effect of centrifugation parameters on guava juice physicochemical characteristics and to identify operational characteristics of continuous disc-stack centrifuges based on the performance of a laboratory centrifuge. Effects of g-force (149 g–3731 g) and centrifugation time (10–40 min) on juice physicochemical characteristics (protein, pectin, galacturonic acid, dry matter, total soluble sugars contents; pH; electrical conductivity, clarity and particle size distribution) were assessed. Laboratory centrifuge performance was evaluated for 1343 g, 2388 g and 3731 g. At 1343 g, separation limits (x_{max}), feed flow-rates of disc-stack centrifuges and cut-off sizes (x_{50}) were determined for corresponding laboratory centrifuge operation times. Significant decrease in average particle size, protein and pectin contents was observed, contrary to juice clarity. Similar clarification efficiency was obtained for g-forces ≥ 1343 g. x_{max} were 2669 nm, 2200 nm and 1783 nm, with corresponding x_{50} for continuous centrifuges, 1887 nm, 1556 nm, 1261 nm, for 20 min, 30 min and 40 min, respectively.

1. Introduction

Guava (*Psidium guajava*) is a well-known tropical and sub-tropical fruit. Originated from USA, Columbia, Peru and Mexico, it is nowadays widely spread in different countries in Asia, Africa and South America (Ninga et al., 2021). Sometimes called “superfruit”, it is well appreciated for its high vitamin A and C content (more than 4 times that of orange) (Surajbhan et al., 2012; Akesson and Choonhahirun, 2013). Antioxidant capacity of its phenolic compound makes it suitable to trap free radicals in human body, therefore inhibiting formation and spreading of cancer cells (Flores et al., 2015). When stored at room temperature, guava fruit's shelf-life is 3–4 days, demonstrating its high perishability.

Due to its short shelf-life, guava can be processed into juice. Ninga et al. (2018) described kinetic models of hydrolysis of pectinaceous matter of guava pulp during depectinization. The effect of enzymatic treatment of guava pulp on the physicochemical parameters of the juice were described by Nso et al. (1998), Kaur et al. (2011), Le et al. (2012), Surajbhan et al. (2012), Akesson and Choonhahirun (2013), Nguyen et al. (2013) and Marcelin et al. (2017). FESEM images were collected

and analyzed, showing particles breakdown during depectinization of the pulp (Ninga et al., 2021).

After depectinization, some chemical interactions can take place in guava juice. Polyphenols and proteins can interact with haze or tannin formation. The former, because of their positive charge, will behave as a bridge and glue, thus implying the agglomeration of the latter (negatively charged). Tannin complexes could also be the result of polymerization of phenolic compounds into denser units (McLellan and Padilla-Zakour, 2005). There could also be some interactions between proteins and oligogalacturonates from pectins hydrolysis (Shomer et al., 1999; Ninga et al., 2021). These interactions can lead to particles formation coupled with stone cells, affecting juice stability during storage (Wu et al., 2005).

Depectinized guava juice should be clarified to slow down haze formation during storage. Clarification is necessary to settle down the macromolecular particles (protein, polyphenol, cell wall, pectin and derived products) in fruit juice, to increase its acidity and to minimize the sucrose inversion level for longer shelf life (Ghosh et al., 2018). It can be done using filter-aids, centrifugation and microfiltration through membrane. Although some filter-aids such as chitosan may be non-toxic, the use of filter-aids requires the determination of suitable concentrations

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and the combination of filtration to remove residues. Handling of these materials and the cost of waste disposal constitute a hurdle for the use of filter-aid (Sharma et al., 2015). Practical applications of membrane processes such as microfiltration face the major problem of reduction of the permeate flux due to irreversible fouling of membranes. Fouling occurs due to accumulation of pectin substances, tannins, proteins and fibers present in the juice on the membrane surface (known as concentration polarization) or blocking of the membrane pores (Rai and De, 2009; Sagu et al., 2014). Another unit operation for clarification of fruit juice is centrifugation during which particles will settle depending on their sedimentation velocity, thus forming a pellet at the sedimentation front and leaving clearer juice. Centrifugation describes less effect on the physicochemical and nutritional composition of the juice compared to microfiltration, since 4.6%–21.4% of polyphenols, and 19.9%–53.5% of proteins are removed during clarification compared to 28.6%–67.5% and 26.0–59.6% for polyphenol and proteins, respectively with microfiltration. Though producing more turbid juice due to less removal of suspended particles than microfiltration, centrifugation is proved to result in greater juice yield and is chemical free (Chhaya et al., 2013; Sagu et al., 2014; Biswas et al., 2016). Centrifugation of guava juice had been the subject of many research works. Kaur et al. (2009, 2011), Surajbhan et al. (2012), Marcelin et al. (2017) determined guava yield after centrifuging depectinized guava pulp at fixed rotation speed or g-force and centrifugation time. They also determined some physicochemical parameters of the juice without assessing the effect of processing conditions (g-force and time) on them. Moreover, clarification performance of different centrifuges used in those works was not assessed. Finally, the study of the dimensional and operational characteristics of a continuous centrifuge as a function of clarification performance of the guava juice of the laboratory centrifuge has not been done. This paper has two objectives: To determine the effect of g-force and centrifugation time on the physicochemical characteristics of clarified guava juice; and to identify operational characteristics of a continuous centrifuge from the clarification performance of a laboratory centrifuge.

2. Material and methods

2.1. Biological materials

Guava fruits (*Psidium guajava*) CV Lucknow 49, were purchased from local market in Kharagpur, West Bengal, India. Degree of maturity and ripeness were selection criteria.

Pectinase (Polygalacturonase) from *Aspergillus niger* (activity: 8000–12,000 U/g) was purchased from HiMedia laboratories Pvt. Ltd. (Mumbai, India).

2.2. Experimental set-up

The centrifuge used in this study was a refrigerated and programmable fixed-angle conical rotor laboratory centrifuge (Remi, R - 236M,

Table 1. Geometrical characteristics of the rotor R - 236 M.

Features	Minimum radius (mm)	Minimum study radius (mm)	Maximum radius (mm)	Average radius (mm)	Inclination angle to the vertical (°)
Values	≈30	≈54	133.5	≈82	20

Remi Elektrotechnik Ltd, West Bengal, India) (Figure 1A). It was equipped with a stainless-steel truncated conical bowl (rotor) model R - 236M (Figure 1B) with six tube accommodating holes. The tubes' inclination angle to the vertical axis was 20°. Geometric characteristics of the rotor are given in Table 1.

2.3. Methods

2.3.1. Preparation of white guava juice for clarification

The extraction of guava juice was done following the procedure described by Ninga et al. (2021). Preliminary steps consisted of washing and rinsing of matured ripened guava fruits with tap water, followed by removal of blemishes by trimming, manual deseeding by a 16 – mesh sieve, blending using a food processor and mixing of the puree with demineralized water at a ratio of 35% w/w. The juice obtained underwent enzymatic treatment at $45 \pm 2^\circ\text{C}$ under continuous stirring using a Remi Motor agitator, type RQ 122 (Elektrotechnik Ltd, Kolkata, India) at 1500 rpm. Operating conditions were: enzyme concentration = 0.078 weight of dried extract/weight of initial guava pulp; incubation time = 40 min. Enzyme preparation was inhibited by heat treatment of depectinized guava juice for 10 min at $95 \pm 2^\circ\text{C}$. The juice was cooled at room temperature, then filtered using a cheese cloth (200 μm mesh size). The filtrate was collected and kept in polyethylene terephthalate (PET) bottles, and stored in the freezer for further analysis.

2.3.2. Clarification of guava juice by centrifugation

Hundred grams of filtered guava juice were weighed at room temperature in 250 ml centrifuge tubes. Those tubes were introduced in the rotor of a laboratory centrifuge (Remi, R - 236M, Remi Elektronik Ltd, West Bengal, India). Centrifugation was done at $35 \pm 3^\circ\text{C}$. Centrifugation speeds used were 149 g (1000 rpm), 597 g (2000 rpm), 1343 g (3000 rpm), 2388 g (4000 rpm) and 3731 g (5000 rpm). Centrifugation times at constant speed were 10 min, 20 min, 30 min and 40 min. At the end of centrifugation, supernatant was collected in PET plastic bottles and kept in refrigerator for further analyses; whereas the pellets were discarded.

2.3.3. Determination of process parameters and physicochemical characteristics of guava juice

- Determination of the yield

The extraction yield of guava juice was determined using Eq. (1):



Figure 1. Image of the R - 236M rotor (A) and the laboratory fixed-angle conical rotor centrifuge (B).

$$\eta = \frac{m_s}{m_i} \times 100 \quad (1)$$

with η : the yield of guava juice (%); m_s and m_i weights of supernatant and initial guava juice (g), respectively.

- Particle size analysis of juice samples

The determination of the particle size of the supernatant was done at 25 °C using the Zetasizer based on the principle of dynamic light scattering (Malvern, Zetasizer nano, Malvern Instruments limited, UK). For this purpose, the dispersing medium used was demineralized water. For non-centrifuged guava juice, particle size distribution was obtained with the Mastersizer 2000 E (Version 5.20, Serial Number MAL1017204, Malvern Instruments Limited, United Kingdom). Average particle sizes were determined thanks to incorporated softwares. Particle size distribution curves of the centrifuged samples were fitted with Minitab 14 software using the log-normal model to determine the relationship between frequency (%) and particle size (nm) for each operating condition (g-force - centrifugation time) (Eq. (2)). An analysis of variance was performed to determine the significance levels of the parameters.

$$y = \frac{a}{x} \times e^{\left[-0.5 \left(\frac{\ln\left(\frac{x}{x_0}\right)}{b} \right)^2 \right]} \quad (2)$$

with x : particle size (nm); y : frequency (%). a and b are the model's constants, x_0 is the geometric average particle size.

For each operating condition, the parameters a , b and x_0 were estimated using Minitab 19.0 software and coefficients of determination were determined. The surface (%*nm) of each curve was determined by calculating the integral of log-normal models between two given particle sizes using Scientific Workplace version 5.5 software.

- Determination of Dry Matter Content

The determination of the dry matter content (DM) was done as follows. In a clean empty Petri dish (initial weight M_0), 5 g of guava juice was weighed and the total weight before drying (M_1) noted. The Petri dish was placed in an oven at 105 °C for 24 h. After drying, Petri dishes were removed and placed in a desiccator for cooling to room temperature. Final weight of the Petri dish (after drying) was determined (M_2). The dry matter content was determined using Eq. (3):

$$DM (\%) = \frac{M_2 - M_0}{M_1 - M_0} \times 100 \quad (3)$$

- Determination of other characteristics

Other physicochemical characteristics of the juice were determined. They represent quality attributes of juice and give more information on the nutritional value of the juice, its acidity and cloudiness. These characteristics were used to determine optimal conditions for centrifugation of guava juice and to determine the clarification performance of the laboratory centrifuge. These were: total soluble sugars (TSS), galacturonic acid, pectin, proteins contents; pH and electrical conductivity.

Protein content was determined using the method of Lowry; pectin content with the method using carbazole developed by McCready and McComb (1952) with some modifications as described by Ninga et al. (2021).

The clarity of guava juice samples was determined by measuring their transmittance at 660 nm using a UV-visible spectrophotometer (M/s Perkin Elmer, Connecticut, USA) against demineralized water as blank (Jain and De, 2016).

Total soluble sugars (TSS) were determined with a laboratory refractometer with digital display (Digital Lab Refractometer Salinity - 300034, Sper Scientific, Scottsdale, Arizona, United States of America) using demineralized water as blank (Ninga et al., 2021).

The galacturonic acid content was determined with the method using 2-cyanoacetamid with galacturonic acid as standard (Ninga et al., 2021).

pH and electrical conductivity were determined at room temperature with a pocket tester (Eutech Instruments Ltd, Singapore) by immersing electrodes in juice sample after calibration with demineralized water as described by Ninga et al. (2021).

For each characteristics, the ANOVA was done using Statgraphics Centurion software Version XV.II and 5 % significance level was utilized to check the difference between samples.

2.3.4. Determination of the dimensional and operational characteristics of continuous centrifuges

The objective was to determine the dimensional characteristics and feed flow-rate of a continuous centrifuge based on the performance of the laboratory centrifuge. Since the technological objective desired was centered on the supernatant (guava juice), centrifugal decanting was preferred over centrifugal dewatering (Towler and Sinnott, 2008; Koller, 2009). The bowl of the laboratory centrifuge used is a conical (truncated cone) type with a fixed angle (Figure 2A). The type of continuous centrifuge chosen in this study is the continuous disk stack-centrifuge. This choice was justified by the geometric similarity between the rotor of the laboratory centrifuge and a disc of the disk stack centrifuge (Figure 2B). This geometric similarity leads to a similarity in particle flow through the centrifuge tube and between two consecutive discs.

The determination of dimensional and operational characteristics of continuous disc stack-type centrifuges was done following the approach described by Maybury et al. (2000). They conducted a comparative study of the clarification performance of a laboratory centrifuge and a continuous disc stack centrifuge. The goal of this research was to develop a relationship between the clarification performance of laboratory centrifuge and that of continuous one. The study's purpose was to use the performance of a laboratory centrifuge to predict feed flow-rates of disc stack centrifuges.

- Determination of operating characteristics of the laboratory centrifuge

The determination of the clarification performance of the laboratory centrifuge required the determination of its sigma factor (Σ) or theoretical equivalent area using the equation described by Rayat et al. (2016). This represents the surface area of a gravity sedimentation tank capable of ensuring similar clarification performance to the centrifuge for the same suspension feed flow-rate (Svarovsky, 2000).

Considering the acceleration and deceleration times negligible compared to the operating time, the sigma factor of the conical rotor laboratory centrifuge was obtained from Eq. (4). This was generated by assuming the presence of a suspension consisting mainly of the cut-off size particle (x_{50}) with equal frequency in the supernatant and the pellet. For the calculation of this area, a laminar particle fall regime was assumed, thus neglecting mass transfer phenomena during centrifugation.

$$\Sigma = \frac{V_1 \times \omega^2}{6 \times g \times \ln\left(\frac{2R_2}{R_1+R_2}\right)} = \frac{V_1 \times (2\pi N)^2}{3600 \times 6 \times g \times \ln\left(\frac{2R_2}{R_1+R_2}\right)} \quad (4)$$

with Σ_1 : the sigma factor of the centrifuge (m^2); g : the acceleration of gravity ($m.s^{-2}$); ω : the angular velocity ($rad.s^{-1}$); N : the rotational speed (rpm); V_1 : the volume of the feed (m^3); R_1 and R_2 minimum and maximum radial distances of sedimentation from the rotor axis (m).

The sigma factor was determined for the speeds 1343 g, 2388 g and 3731 g.

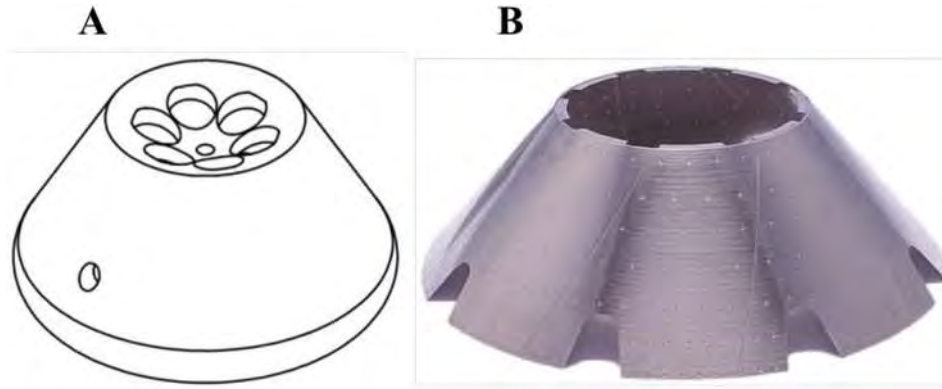


Figure 2. Three-dimensional representation of (A) the rotor of a laboratory fixed-angle conical rotor centrifuge and (B) a disc of a disc stack centrifuge (Alpha Laval).

- Determination of clarification performance of the laboratory centrifuge

This determination required that of the clarification efficiency (Eq. (5)) following the procedures described by Maybury et al. (2000) with some modifications. The optical density of each sample (feed and clarified samples) was determined at 660 nm using a UV-visible spectrophotometer (M/s Perkin Elmer, Connecticut, USA) against demineralized water as a blank. The centrifuged sample with the minimum optical density was used as the control.

$$CE (\%) = \left[1 - \left(\frac{OD_{\text{sample}} - OD_{\text{control}}}{OD_{\text{ali}} - OD_{\text{control}}} \right) \right] \times 100 \quad (5)$$

with: CE : the clarification efficiency (%); OD_{sample} , OD_{ali} and OD_{control} optical densities of the sample feed and control sample, respectively.

For centrifugation speeds 1343 g, 2388 g and 3731 g, the clarification efficiency was plotted against separator capacity ($V_1/t/\lambda_{\text{lab}}\Sigma_{\text{lab}}$). V_1 is the volume of the processed sample (in m^3); λ_{lab} is the correction factor to characterize the deviation of the particle flow regime from the ideal regime in the case of the laboratory centrifuge. Σ_{lab} its theoretical equivalent area (in m^2) corresponding to the centrifugation speed, and t the centrifugation time (in s). For laboratory centrifuges, λ_{lab} is most often equated to 1 (Maybury et al., 2000; Boychyn et al., 2004). The optical density of the feed sample was equated to 2 with respect to the detection limit of the UV-visible spectrophotometer.

- Determination of maximum particle size (x_{max})

The centrifugation speed used for this purpose is 1343 g. This choice was guided by the significant variations of the parameters observed at this speed compared to the lower speeds and the negligible variations compared to the higher speeds (2388 g and 3731 g).

For that purpose, a preliminary determination of probability or grade efficiency of each particle to settle during centrifugation if the feed was exclusively made of it was done. Particle's frequency in distribution curves of both guava feed sample and clarified juice (for 20 min, 30 min and 40 min) were used as well as guava yield for each centrifugation condition using Eq. (6).

$$G_t(x) = 1 - (1 - E_{Tt}) \times \frac{dF_t(x)}{dF(x)} \quad (6)$$

with $G_t(x)$: the grade efficiency corresponding to the particle of size x ; $dF_t(x)$ and $dF(x)$: frequency of the particle of size x in the centrifuged and raw guava juice, respectively; E_{Tt} : the yield of guava juice centrifuged at 1343 g for a time t in min.

For the determination of x_{max} , grade efficiency was plotted against particle size. The particle size with the greatest grade efficiency closer to 1 was considered as the limit of separation.

- Determination of dimensional and operational characteristics of a continuous centrifuge for guava juice clarification

In case of linear correlation between clarification efficiency and separator capacity, equal performance of laboratory and disc-stack centrifuges could be predicted (Eq. (7)), thus enabling the determination of the feed flow-rate latter using its sigma factor and the separation capacity of laboratory centrifuge (Eq. (8)) as described by Maybury et al. (2000) and Rayat et al. (2016).

$$\frac{V_1}{t \sum_{\text{lab}} \lambda_{\text{lab}}} = \frac{Q}{\sum_{\text{ds}} \lambda_{\text{ds}}} \quad (7)$$

$$Q = \frac{V_1}{t \sum_{\text{lab}} \lambda_{\text{lab}}} \sum_{\text{ds}} \lambda_{\text{ds}} \quad (8)$$

with Q , Σ_{ds} and λ_{ds} , the feed flow-rate ($\text{m}^3 \cdot \text{s}^{-1}$), the sigma factor (m^2), and the correction factor for a deviation of the flow regime from the ideal regime of the continuous disk stack centrifuge. λ for disk stack centrifuges is equal to 0.4 (Maybury et al., 2000; Boychyn et al., 2004).

Intrinsic characteristics of a disc stack centrifuges found in literature (Table 2) were used to determine their sigma factor (Eq. (9)) for an equivalent g-force at 1343 g assuming that their volume flow rate is maximum (qV_{max}).

$$\Sigma = \frac{2}{3} \times \frac{\omega^2}{g} \times N \times \frac{\pi \times (r_2^3 - r_1^3)}{\tan \theta} \times F_L \quad (9)$$

with Σ : the sigma factor of the centrifuge (m^2); θ : inclination angle of discs from the vertical ($^\circ$); g : the acceleration of gravity ($\text{m} \cdot \text{s}^{-2}$); ω : the angular velocity ($\text{rad} \cdot \text{s}^{-1}$); N : number of discs; F_L : the correction factor of caulks on discs; r_1 and r_2 inner and outer radii of the discs (m), respectively.

In case of available discs' specifications; F_L was determined using Eq. (10); otherwise; it was equated to 1.

$$F_L = 1 - \left(\frac{3Z_L B_L}{4\pi r_2} \right) \left(\frac{1 - \left(\frac{r_1}{r_2} \right)^2}{1 - \left(\frac{r_1}{r_2} \right)^3} \right) \quad (10)$$

with: Z_L the number of caulks on each disc; B_L : the height of a caulk (m).

Feed flow-rates of each centrifuge were determined at 1343 g for 20 min, 30 min and 40 min, respectively. The disk stack centrifuge with the greatest feed flow-rate of guava juice for a given operating time of the laboratory centrifuge was selected.

- Determination of the theoretical cut-off size (x_{50}) for the disc stack centrifuges

Table 2. Characteristics of commercial disc stack centrifuges identified in the literature.

Models	Manufacturers	$r_{\min}$ – $r_{\max}$ (mm)	N	Z	B (m)	θ (°)	Sources
Westfalia SAOOH	Westfalia Separator AG (Oelde, Germany)	21; 53	62	6	0.005	45	(Boychnyn et al., 2004)
GEA OTC 2-03-107	Westfalia Separator Systems GmbH	22; 44	36		0.0025	40	(Cambielli et al., 2006)
SC6-06-076	Westfalia Separator (Oelde, Germany)	31; 62	76		0.0005	40	(Chlup et al., 2008)
Westfalia CSA-1	Westfalia Separator GmbH (Oelde, Germany)	26; 55	45			38.5	(Espuny, 2016)
Frau CN2S	Frau (Italy)	15; 54.5	51			52	(Jukkola et al., 2019)
Culturefuge 100™	Alfa Laval AB (Lund, Sweden)	36; 84.5	82	8	0.004	40	(Shekhawat et al., 2018)

Particle's theoretical grade efficiency or probability was plotted against its size for 20 min, 30 min and 40 min at 1343 g using Eq. (11), corresponding to disk stack centrifuges (Svarovsky, 2000). The cut-off size (x_{50}) is the particle size of probability 0.5. This particle size is independent of the initial suspension.

$$G(x) = \left(\frac{x}{x_{\max}} \right)^2 \quad (11)$$

with: $G(x)$: the probability corresponding to particles of size x (nm); $x_{\max}$: the separation limits (nm).

3. Results and discussion

3.1. Effect of centrifugation parameters on the physicochemical characteristics of guava juice

3.1.1. Effect of centrifugation parameters on the extraction yield of guava juice

Centrifugation resulted in the guava juice at a yield between 89.72 ± 0.69 and $97.07 \pm 0.69\%$ w/w (Figure 3). The yield of juice extraction is almost 90% for all combinations of parameters applied. The juice yields obtained for 149 g are around 90% ($91 \pm 2\%$ w/w). For g -forces between 597 g and 3731 g, extraction yields are greater than 95%.

An increase in g -force does not significantly affect the yield of guava juice, regardless of centrifugation times. The maximum percentage of pellets in the initial juice is approximately $2.5 \pm 0.5\%$.

3.1.2. Effect of centrifugation parameters on the particle size distribution of clarified guava juice

The particle size distribution of the raw guava juice shows two peaks, the first around 2250 nm and the second around 90,000 nm (Figure 4). It appears that the particles with size between 22,440 nm and 251,785 nm are those with the highest proportion. They account for 72.77% of

particles present in the feed sample. The average particle size is 72,138 nm. Particles greater than that represent 45.45% of all particles. It appears that non-centrifuged guava juice contains a broad range of large particles and aggregates which could either be those which passed through the filter cloth after enzymatic treatment or those resulting from interactions between deactivated proteins and oligogalacturonates from pectins hydrolysis (Shomer et al., 1999; McLellan and Padilla-Zakour, 2005).

For centrifuged guava juice samples, the distribution is of Fischer (Figure 5). For each centrifugal force, the increase in centrifugation time is accompanied by a progressive narrowing of the curves around the mean size. The smallest particle diameter is 68 nm for all each operating condition. The particle size distribution curve for the 149 g samples shows a predominance of particles with diameters between 615 nm and 1718 nm (Figure 5A). The decrease in the frequency of particles of a given diameter as a function of time for a given g -force would reflect a decrease in the number of these particles during centrifugation. The reduction in the width of the particle size spectrum with increasing centrifugation time for a given acceleration is accompanied by an increase in the proportion of finer particles.

Solid lines on each curve represent the fitting log-normal model. Model constants for different operating conditions were determined and reported (Table 3), as well as values of the coefficient of determination and analysis of variances.

These coefficients show an acceptable fitting of the log-normal model for particle size distribution curves. The surface areas of curves were obtained by calculating the integer of each model between 68 and 3091 nm and plotted for each operating condition (Figure 6).

Centrifugation has a remarkable effect for centrifugal accelerations of 597 g and 1343 g on the profile of the particle size distribution curves, whereas a quasi-linear trend was observed for 149 g, 2388 g and 3731 g (Figure 6).

For a given g -force, an increase in the centrifugation time led to a decrease in the width of particle size range as displayed in the surface vs particle size curve (Figure 6), therefore leading to a decrease in the average particle size of centrifuged guava juice samples (from 332 nm to

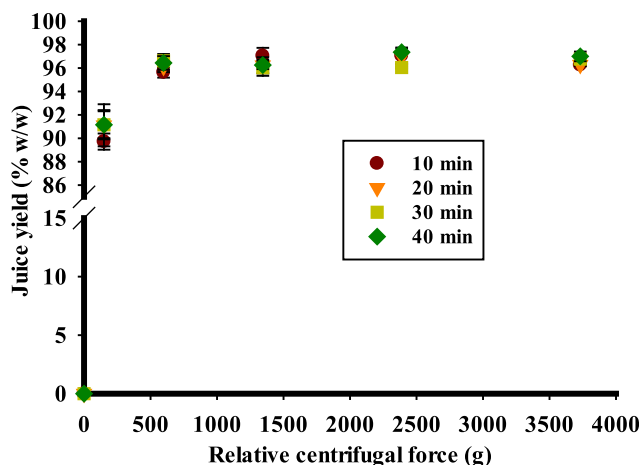


Figure 3. Yield of guava juice as a function of the relative centrifugal force applied.

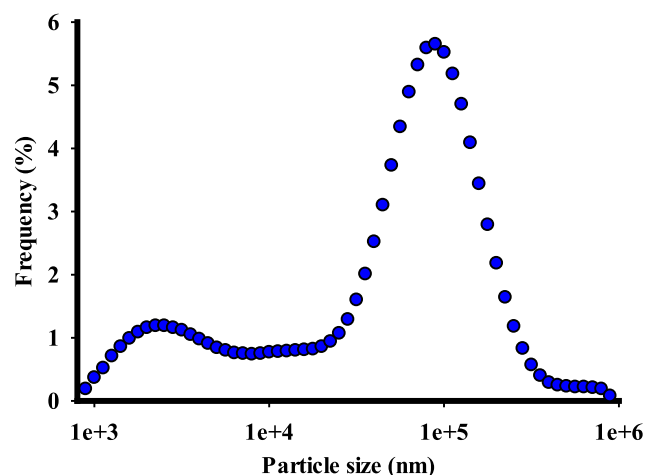


Figure 4. Particle size distribution of non-centrifuged guava juice.

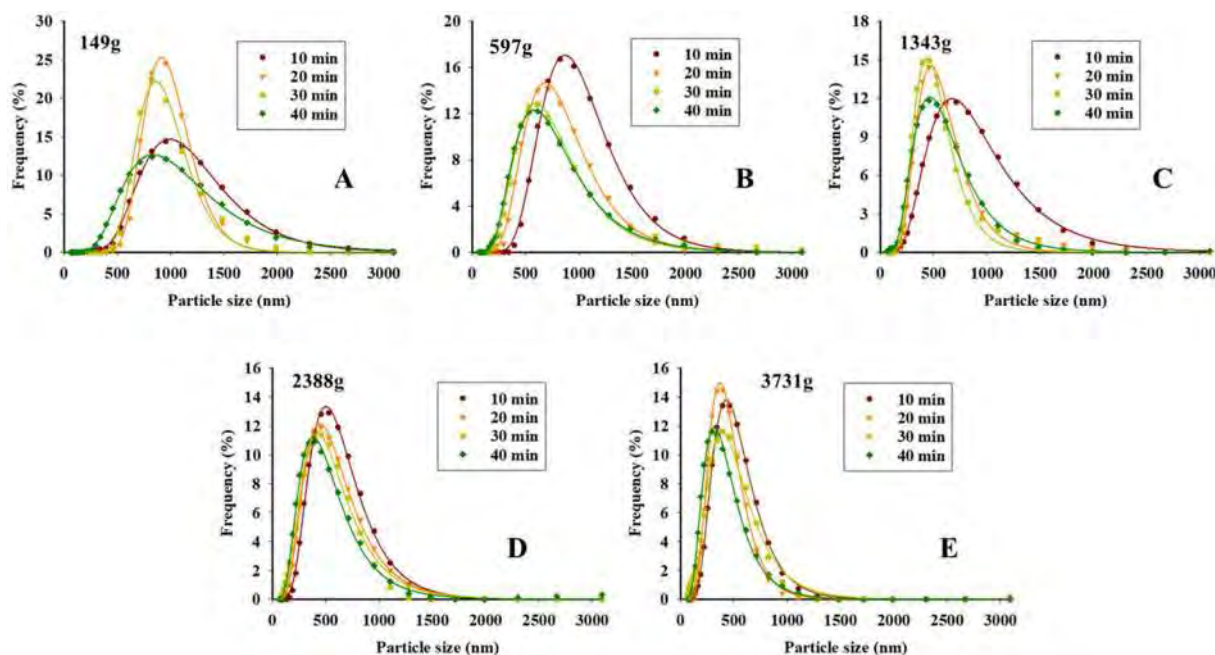


Figure 5. Particle size distribution of clarified guava juice. (A), (B), (C), (D) and (E) guava juices centrifuged at the indicated accelerations and centrifugation times.

1140 nm) (Figure 7). This could be explained by the fact during centrifugation, particles are submitted to centrifugal forces leading to their settling. According to Stokes' Law, the sedimentation velocity of a particle is a function of particle size, and the difference between the density of the particle and that of the fluid. The greater and denser the particle, the greater its sedimentation velocity. Particles would settle in the same magnitude of their sedimentation velocity, denser and larger particles settling first followed by lighter and smaller ones (Svarovsky, 2000). This led to a decrease in the average particle size observed in centrifuged sample, as the result of the narrowing of particle size distribution curves. The consequence is an increase in the clarity of guava juice as described further.

Increasing g-force while fixing the centrifugation time led to a decrease in the average particle size of centrifuged samples and their particle size ranges (Figures 6 and 7). This is in accordance to the result described by Chawafambira et al. (2015). The sedimentation velocity being a function of the centrifugal acceleration, increasing the g-force led to an increase in that velocity, thereby to the settling of particles in the same order of their velocity. Moreover, frictional forces acting on the particles and decreasing their sedimentation velocity would therefore be increasingly negligible compared to the centrifugal force (Robatel and Borel, 1989). An increase in g-force would lead to a progressive decrease in the effect of Brownian diffusion forces that act on fine particles, keeping them in suspension and opposing their sedimentation in the case

Table 3. Values of constants of the log-normal models, coefficients of determination and analysis of variances for each clarified guava juice sample.

Centrifugation parameters		Model Constants and Analysis of Variance Parameters					
g-force	Time (min)	a	b	x_0	R^2	Value of F	Probability (p)
149 g	10	15,631.2303	0.3732	1142.2940	0.9991	6884.3455	<0.0001
	20	23,822.1460	0.2298	966.6824	0.9984	3815.1160	<0.0001
	30	19,769.6168	0.2612	922.6080	0.9955	1324.8144	<0.0001
	40	11,801.2795	0.4658	1040.1947	0.9990	6295.6201	<0.0001
597 g	10	15,787.0226	0.3482	983.7673	0.9987	4668.9589	<0.0001
	20	10,913.1193	0.4006	806.1014	0.9980	2985.3766	<0.0001
	30	8533.0774	0.4463	731.5867	0.9976	2477.1187	<0.0001
	40	8068.4398	0.4746	733.1956	0.9996	16,730.3293	<0.0001
1343 g	10	9094.7421	0.4892	857.2055	0.9994	9681.9720	<0.0001
	20	7461.1000	0.3900	555.1124	0.9975	2348.0955	<0.0001
	30	7115.7952	0.3769	505.2511	0.9966	1734.6698	<0.0001
	40	6269.7107	0.4735	580.7774	0.9992	7287.9592	<0.0001
2388 g	10	7383.4772	0.4339	608.2819	0.9987	4756.9925	<0.0001
	20	6075.1861	0.4797	560.3254	0.9993	8461.0370	<0.0001
	30	5485.8938	0.4968	537.9501	0.9949	1156.6211	<0.0001
	40	4887.3994	0.5077	495.4160	0.9973	2195.9962	<0.0001
3731 g	10	6444.7337	0.4115	508.3661	0.9991	7024.2033	<0.0001
	20	5986.8620	0.3838	429.2792	0.9992	7350.1895	<0.0001
	30	5034.3938	0.4840	486.5226	0.9927	811.4094	<0.0001
	40	4235.7907	0.4733	397.8415	0.9973	2182.9271	<0.0001

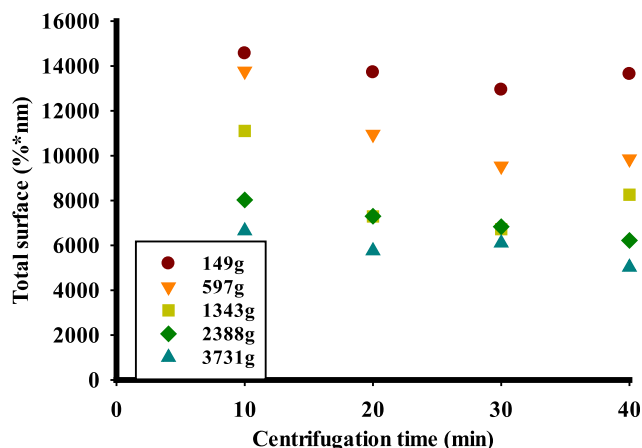


Figure 6. Particle size spectrum surface as a function of centrifugation parameters.

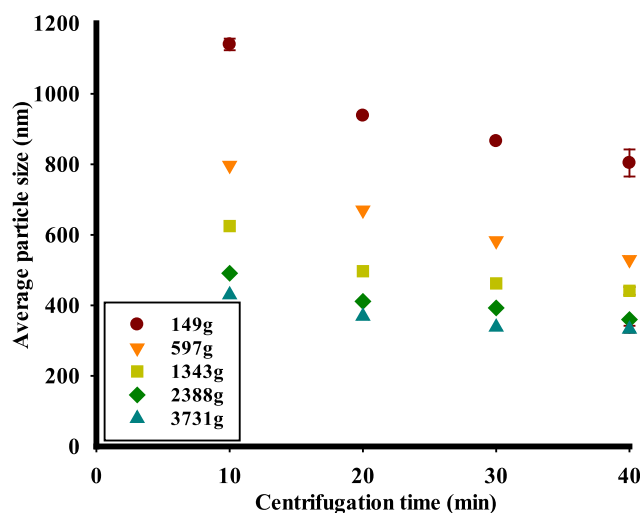


Figure 7. Average particle size of clarified juice as a function of centrifugation parameters.

of gravity sedimentation (Svarovsky, 2000). Resulting supernatants (juice) contained finer particles with decreasing maximum particle sizes as the g-force increased.

For g-force ranging from 1343 g to 3731 g, there was no significant effect of centrifugation on the average particle size for centrifugation times greater than 20 min (Figure 7). This is similar for the surface vs particle size curves. This could be explained by the fact that there could be no significant difference in the sedimentation velocity of particles for those clarification conditions.

3.1.3. Effect of centrifugation parameters on the clarity of guava juice

The clarity of the different guava juices ranged from 0 to $68.72 \pm 2.18\%$ Transmittance. That of the feed sample was not measurable by the spectrophotometer (Figure 8). The effect of time and centrifugal acceleration on clarity showed a trend opposite to that of average particle size. An increase in time or centrifugal acceleration is accompanied by an increase in clarity of banana juice as observed by Sagu et al. (2014). Ghosh et al. (2018) also highlighted an increase in clarity of the jamun juice with an increase in time and centrifugation speed. A maximum clarity of 83.60 %T was described by them with 60 min of centrifugation at 8000 rpm. Details of the rotor used by them being absent, g-forces corresponding to their rotation speeds could not be calculated, therefore a comparison could not be made between their work and this research work.

The increase in percentage transmittance during centrifugation could be due to the increase in scattered light intensity caused by the reduction in the particle size spectrum of the colloidal particles in guava juice. The clarity of guava juice would reflect the behavior of suspended particles and macromolecules of samples towards the incident light beam at 660 nm. Values of electrical conductivity revealed the presence of charges in the samples. When a light beam passes through the sample, it could be scattered by negatively charged particles of the juice due to the presence of electric field and a diffuse layer around them. Settling of particles based on their sedimentation velocity could result in an increase in the clarity. This results in the presence of smaller particles in greater proportion in the juice. The smallest hydrodynamic diameter measured in each sample is 68 nm, i.e. greater than 1/10 of measurement wavelength (660 nm) (Figure 5 B–E). The intensity of scattered light depends on the particle size. The larger the particle, the greater the intensity of the scattered light (Malvern Instruments, 2013). Settling of larger particles results in the presence of smaller one in clarified juice. That could result in an increase in the intensity of transmitted light coming out from the cuvette. The intensity of light scattered by particles depends on their size and number. The larger the particle, the greater the intensity of the scattered light (Malvern Instruments, 2013).

Guava juice samples corresponding to g-force 149 g and 597 g (10 min) contained large particles ($d > 660$ nm) that could cause complete scattering of light due to multiple light scattering, resulting in a 0 % Transmittance (Figure 8). For g-forces ranging from 1343 g to 3731 g, for $t > 10$ min, particles with diameter less than 660 nm accounted for 75% in each sample. Less light scattering could be observed in these samples, resulting in an increase in clarity with increasing time. The same phenomena could be observed for 597 g.

For 3731 g, a maximum clarity of 70 %Transmittance is obtained (Figure 8). Sagu et al. (2014) obtained similar results after centrifuging banana juice at 6000 g for 60 min, showing that more energy was consumed in their case. The increase in clarity between 30 and 40 min of centrifugation is not significant.

3.1.4. Effect of centrifugation parameters on the pectin content of guava juice

The pectin content of the non-centrifuged sample was 426.70 ± 34.54 mg/L. Centrifugation contributes to decrease the pectin content to a value between 238.69 and 19.23 mg/L (Figure 9). For each g-force, increasing the centrifugation time led to a decrease in pectin content of clarified juice. 85% of pectin content reduction was recorded for 1343 g, whereas 93 and 96% were depicted for 2388 g and 3731 g, respectively. That significant decrease was followed by an insignificant one, with slopes from 10 min to 40 min of 1.52%, 0.84% and 0.03% for 1343 g, 2388 g and 3731 g, respectively. For 149 g and 597 g, the decrease in

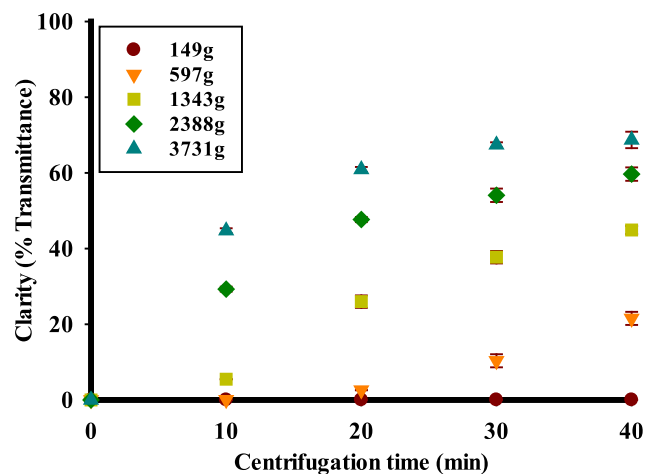


Figure 8. Clarity of guava juice as a function of centrifugation parameters.

pectin content is correlated with a decrease in the values of slopes between two consecutive time intervals.

The decrease in pectin content, just like that of average particle size, could mean that among the particles which settled during centrifugation could there be those made of pectins of pectinaceous matters. Oligogalacturonates resulting from pectins hydrolysis are negatively charged; they could therefore be subject to ionic interactions with positively charged compounds such as proteins, yielding in the formation of complex particles (Jaarsveld et al., 2005; Ninga et al., 2021). During centrifugation, these particles, depending on their size, sedimentation velocity and the clarification conditions, could settle forming the pellet. Particles in the supernatant could have a non-significant difference in affinity with ethanol solution used to precipitate pectins and pectinaceous matters during analysis. That could explain linear decreasing trends with small values of slopes observed between 10 and 40 min for 1343 g, 2388 g and 3731 g. This corroborated with the decrease in average particle size with respective slopes 10.74%, 7.65% and 5.65% for 1343 g, 2388 g and 3731 g (Figure 9). This could be supported by the progressive narrowing of the particle size distribution curves around 200 nm–1000 nm for all samples obtained using these centrifugal forces (Figure 5 C–E).

An increase in g-force was accompanied by a decrease in pectin content. This corroborates with results described by Sagu et al. (2014). When centrifuging depectinized banana juice, they observed a decrease in alcohol insoluble solids (AIS) (among which pectins) with an increase in acceleration.

3.1.5. Effect of centrifugation parameters on the protein content of guava juice

The protein content of non-centrifuged guava juice is 713.24 ± 23.87 mg/L. The protein content decreases over the clarification time for all centrifugal accelerations applied (Figure 10). For samples centrifuged at 1343 g, 2388 g, 3731 g, after 10 min of separation, more than 45% of protein content reduction could be observed. This was followed by insignificant decrease up to 40 min (slope 5.64% and 3.46%, respectively), as can be seen with error bars. In the case of centrifugal acceleration of 597 g, there is a decrease in protein content with a gradual decrease in the slope between two consecutive values of centrifugation time. This decrease is not considerable between 20 min and 40 min. For 149 g, the decrease in protein content after the first 10 min (21% of the value of the non-centrifuged guava juice) is followed by a moderate decrease (16.10% of the value at 10 min) between 10 and 40 min. Ghosh et al. (2018) described a decrease in protein content of jamun juice with an increase in time and centrifugation speed.

Protein content during centrifugation showed a similar trend to that of pectin content and particle size. Proteinaceous particles would be among those that settle during centrifugation. These particles could be

the results of the complexation of protein molecules with other molecules, such as oligogalacturonates, polyphenols and chelating anions present in the milieu (Siebert, 1999).

The decrease in protein content with increasing g-forces was also recorded by Sagu et al. (2014) in case of banana juice with protein content decreasing from 1060 mg/L to 610 mg/L with acceleration increased from 2000 g to 10,000 g. They also reported that centrifugation had less effect on the juice protein content compared to microfiltration. Biswas et al. (2016) described similar results with bottle gourd. Juice obtained with centrifugation could have greater nutritional values compared to that obtained with microfiltration. A comparative study needs to be done in the case of guava.

3.1.6. Effect of centrifugation parameters on other physicochemical characteristics of guava juice

- Effect of centrifugation parameters on the total soluble sugars (TSS) and galacturonic acid content

The total soluble sugar content of the initial sample is $2.3 \pm 0.0^\circ\text{Bx}$. Centrifugation results in an increase in the total soluble sugar content (Figure 11). The total soluble sugar content of centrifuged samples is greater than that of non-centrifuged one. However, the increase in time and centrifugal acceleration does not show a significant effect between different centrifuged samples. This corroborates with the results obtained by Ghosh et al. (2018) for jamun juice. They also obtained almost equal values of TSS both with centrifugation and microfiltration. Márquez-Montes et al. (2022) described an increase in TSS after cryocentrifugation of prickly pear juice. When clarifying guava juice with ultrafiltration using 100 kDa polyethersulfon membrane, Omar et al. (2020) observed a 7–17% decrease in TSS value compared to that of fresh juice. This was similar to the observations done by Ghosh et al. (2018) and Biswas et al. (2016) who obtained smaller values of TSS with microfiltration than that of centrifugation. This means that membrane processes could lead to a decrease in soluble sugars, therefore affecting the nutritional value of the juice. The biological and the microbial stabilities of the juice were not studied in the present work.

Centrifugation is not appropriate for separating soluble compounds from juice with a low molecular weight. This could also explain the insignificant effect of centrifugation parameters on the galacturonic acid content of guava juice (Figure 12).

- Effect of centrifugation parameters on dry matter content

The dry matter content of the initial sample is $2.85 \pm 0.12\%$ w/w. It appears that centrifugation contributes to a considerable decrease in the

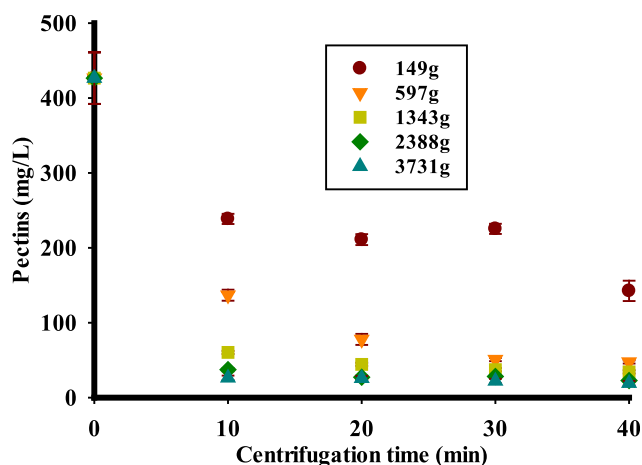


Figure 9. Pectin content of clarified guava juice as a function of centrifugation parameters.

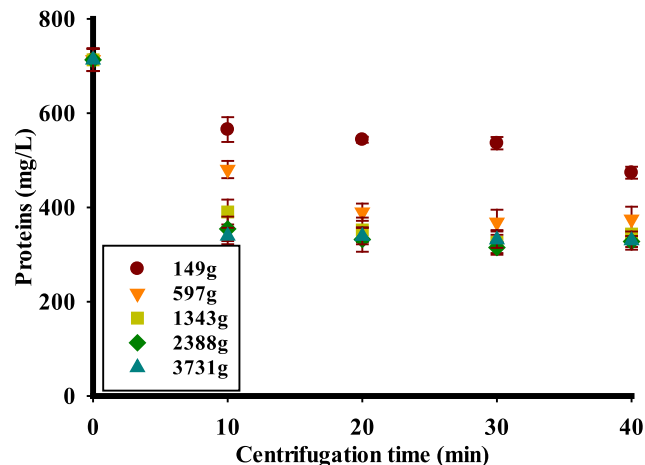


Figure 10. Protein content of clarified guava juice as a function of centrifugation parameters.

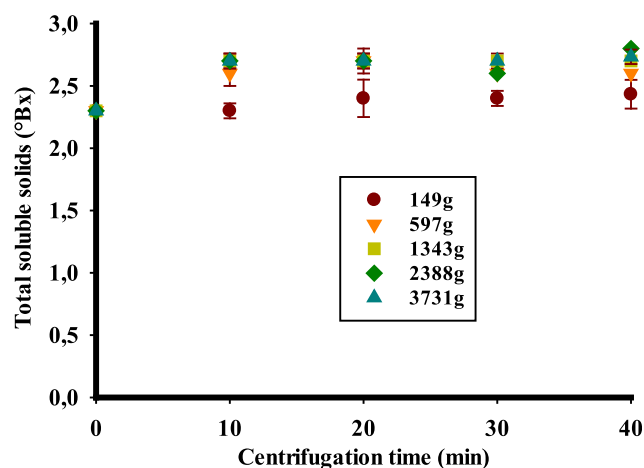


Figure 11. Total soluble sugar content of clarified juice as a function of centrifugation parameters.

dry matter content of guava juice compared to the non-centrifuged one (Figure 13). The significant decrease in dry matter content could be explained by particles sedimentation during centrifugation. Varying the centrifugation parameters does not significantly affect the dry matter content of the clarified samples (Figure 13).

- Effect of centrifugation parameters on pH value

The pH of the non-centrifuged guava juice is 3.71 ± 0.00 . The pH does not vary significantly with increasing centrifugation parameters (Figure 14). Monomers and oligomers of galacturonic acid could display buffering behavior leading to an insignificant change in pH (Ninga et al., 2021). Ghosh et al. (2018) described an increase in pH value with an increase in centrifugation time; however, a decrease in pH values with an increase in centrifugation speed was recorded. Similar results were obtained for prickly pear juice by Márquez-Montes et al. (2022). Clarification of guava juice with ultrafiltration did lead to significant change in pH value as observed by Omar et al. (2020).

- Effect of centrifugation parameters on electrical conductivity

Centrifugation contributes to a decrease in the electrical conductivity of the clarified samples (Figure 15).

The electrical conductivity of the non-centrifuged guava juice is 1828.33 ± 7.51 mS/cm. Greater proportion of charged particles are

present in non-centrifuged sample than in centrifuged ones with not-significant difference among them. Colloidal particles of the different samples would have electrical charges whose total does not lead to a considerable variation in conductivity regardless of the values of the clarification parameters. Conductivity of the samples is also the result of the charge of dissolved ions present in samples.

3.2. Determination of the dimensional and operational characteristics of a continuous disk stack centrifuge

3.2.1. Determination of the operating characteristics of the laboratory fixed-angle conical rotor centrifuge

Thanks to the geometrical characteristics given in Table 1, sigma factors of lab centrifuge rotor were calculated for 1343 g, 2388 g and 3731 g; these were 0.475 m^2 , 0.844 m^2 and 1.318 m^2 , respectively. An increase in the acceleration led to an increase in sigma factor, thus a greater sedimentation surface.

3.2.2. Determination of separation performance of the laboratory centrifuge

The clarification efficiency is an important factor in comparing the separation performance of the centrifuge for the different operating conditions. The performance of the centrifuge for different operating conditions is shown in Figure 16.

For all centrifugal accelerations, the clarification efficiency decreases with separator capacity (Figure 16). For the 1343 g acceleration, it decreases from 95% to 71% for separator capacities ranging from $8.77 \times 10^{-8} \text{ m.s}^{-1}$ to $3.51 \times 10^{-7} \text{ m.s}^{-1}$. For 2388 g and 3731 g, it decreases from 98.41% to 90.35% and from 100% to 95.15% for capacities ranging from $4.94 \times 10^{-8} \text{ m.s}^{-1}$ to $1.97 \times 10^{-7} \text{ m.s}^{-1}$ and from $3.16 \times 10^{-8} \text{ m.s}^{-1}$ to $1.26 \times 10^{-7} \text{ m.s}^{-1}$, respectively. Separator capacity is the ratio of feed flow-rate per unit area of sedimentation. Clarification efficiency reflects the centrifuge performance under operating conditions. It describes the behavior of a gravity sedimentation tank with a specific sedimentation area, if it was fed with a given flow-rate of guava juice. The decrease in clarification efficiency as a function of separator capacity could be explained by the increase in the amount of settled solids with the decrease in feed flow-rate. The suspension feed flow-rate being small coupled with reduced effect of leaching, its residence time in the tank would be long resulting in an increase in the amount of settled solids (Koller, 2009). Moreover, an increase in g-force led to an increase in the clarification efficiency because of an increase in the corresponding surface area for sedimentation.

The increase of the g-force leads to a progressive narrowing of the scatter (Figure 16). For each acceleration, linear regression fitting was done with R^2 values being 99.70%, 99.90% and 99.80%, for 1343 g,

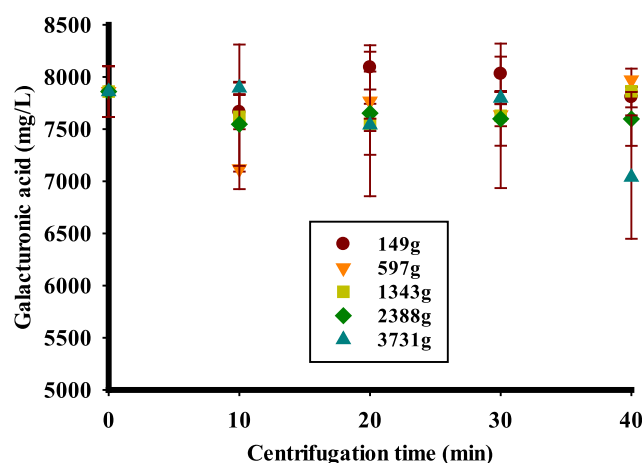


Figure 12. Galacturonic acid content of clarified juice as a function of centrifugation parameters.

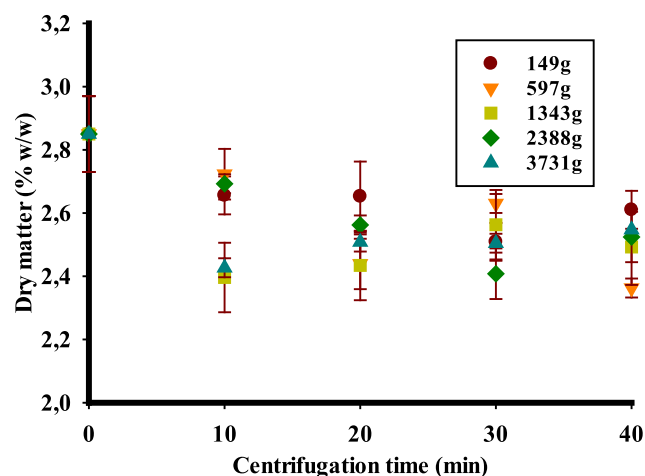


Figure 13. Dry matter content of clarified juice as a function of centrifugation parameters.

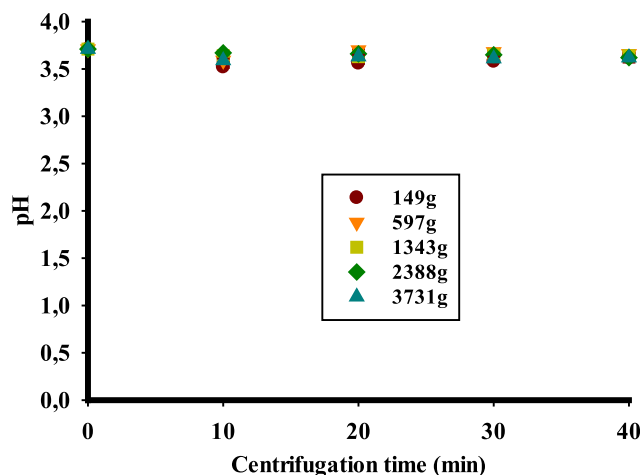


Figure 14. PH of clarified juice as a function of centrifugation parameters.

2388 g and 3731 g, respectively, with corresponding slopes $-9,20 \times 10^7$, $-5,39 \times 10^7$ and $-5,27 \times 10^7$. Decreasing absolute value of slopes with increasing g-forces could be related to the decrease in surfaces of particle size distribution curves (Figure 6). This is contrary to the results obtained by Boychyn et al. (2004), who explained greater absolute value of slope with narrowing of particle size distribution without showing the graph.

The linear correlation between clarification efficiency and separator capacity for each acceleration concluded that the performance of the laboratory centrifuge could be used to predict that of a continuous disc stack centrifuge with a given sigma factor (Maybury et al., 2000). The equation of regression line can be used to predict the feed flow-rate of that disc-stack centrifuge corresponding clarification efficiency.

Except the clarified juice sample at 1343 g for 10 min, a difference in clarification efficiency of less than 10% was obtained for all operating conditions bestowing a similar performance for 1343 g, 2388 g and 3731 g (Figure 16). This is in agreement with the results observed by Maybury et al. (2000). They reported a non-significant difference in clarification efficiency at 1312 g and 3870 g. Moreover, this non-significant difference was also observed for several physicochemical parameters obtained with these operating conditions. 1343 g was used as optimal g-force for further works.

3.2.3. Determination of maximum particle size (x_{max})

The grade efficiency varies from 0 to 1 for all centrifugation times (Figure 17). The particle size range varies from 68 nm to 3010 nm. Grade efficiency of particle less than 893 nm was assumed to be 0 due to limit of detection of the Mastersizer. Beyond 893 nm, grade efficiency increased with particle size for each centrifugation time. Depending on

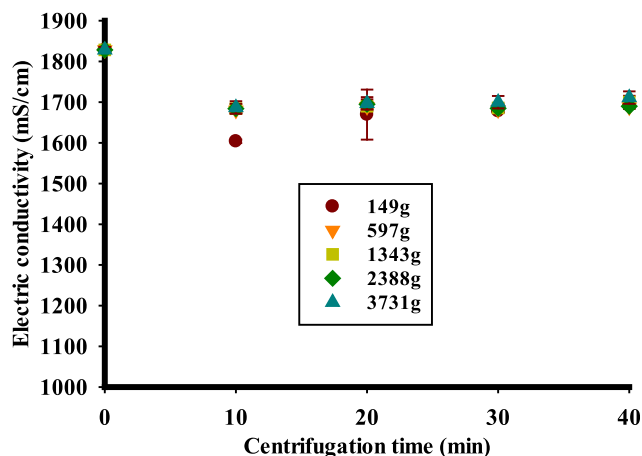


Figure 15. Electrical conductivity of clarified juice as a function of centrifugation parameters.

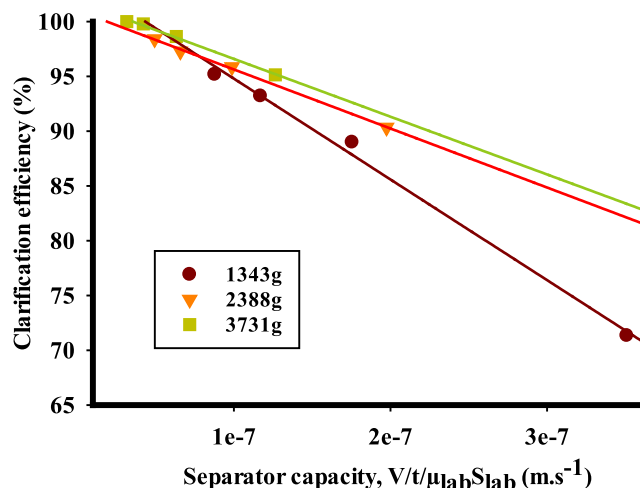


Figure 16. Performance of the laboratory centrifuge for 1343 g, 2388 g and 3731 g.

centrifugation conditions, there was a given particle size beyond which probability is equal to 1. It corresponds to the particle having the smallest probability to be detected in the supernatant if the feed was exclusively made of it. That is separation limit (x_{max}) and is 2669 nm, 2200 nm and 1783 nm, for 20 min, 30 min and 40 min respectively. It can be noticed that as separation time increased, limit of separation decreased. Particles bigger than the separation limit could easily be removed during centrifugation, since their probability is equal to 1 (Svarovsky, 2000).

For particle diameters between 68 nm and 1262 nm, the probability increases steeply with the particle size (Figure 17). For that range, an insignificant increase (less than 100 nm) in the particle size led to a significant increase in the probability (probability difference greater than 0.1). It gives information on the sensitivity of centrifugation. This can be noticed in the particle size distribution curves, since two particles of size difference less than 100 nm were having frequency difference greater than 1% (Figure 5 C-E). During centrifugation, their sedimentation velocity can be significantly different with a rapid settling of the greater one.

Above 1262 nm, the probabilities increase non-significantly towards 1 (Figure 17). Thus, two particles with a particle size difference of 500 nm have equal probabilities of being removed by centrifugation. In addition, a negligible difference in probabilities is observed with increasing centrifugation times. This corroborates with the frequencies obtained for each particle diameter (Figure 5 C-E).

3.2.4. Determination of feed flow-rates for disc stack centrifuges of known dimensions

Sigma factors used are those corresponding to 1343 g. Table 4 shows feed flow-rates for disc stack centrifuges for each run time of the laboratory centrifuge.

For a given continuous centrifuge, increasing the run time of the laboratory centrifuge results in a decrease in its feed flow-rate to ensure similar performance to that of the latter (Table 4). Similarly, increasing the sigma factor results in an increase in the feed flow-rate for a given run time of the laboratory centrifuge.

For a given run time of the laboratory centrifuge, the Culturefuge 100™ model has the highest feed flow-rate. With neither centrifuge prices nor technical specifications available, the main criterion for selection was feed flow-rate. However, the choice of a disk stack centrifuge model will depend on the operator depending on the volume of sample to be processed and the corresponding feed flow-rate.

3.2.5. Determination of theoretical cut-off size (x_{50})

For each centrifugation time at 1343 g, the increase in probability with increasing particle size for those smaller than x_{max} is observed (Figure 18). For those greater than x_{max} , the probability was reduced to 1.

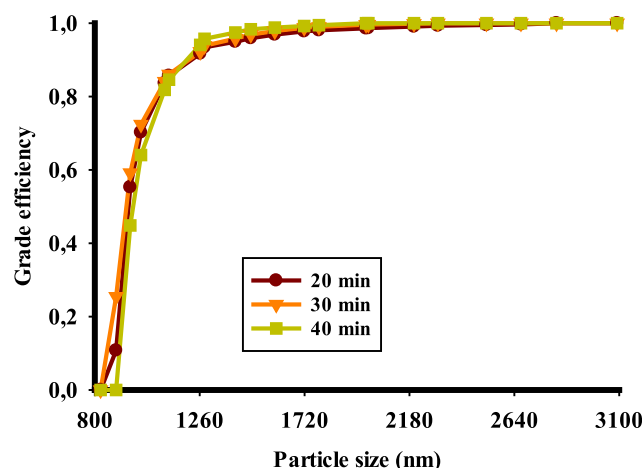


Figure 17. Variation in grade efficiency as a function of particle size for samples clarified at 1343 g.

Table 4. Estimated feed flow-rates for each commercial disk stack centrifuges.

Models	Σ Factor (m ²)	Operating time of the laboratory centrifuge (min)	Feed flow-rate of disc stack centrifuges (L/h)
Westfalia SAOOH	404.351	20	102.152
		30	68.101
		40	51.076
GEA OTC 2-03-107	204.469	20	51.655
		30	34.437
		40	25.828
SC6-06-076	857.071	20	216.523
		30	144.349
		40	108.262
Westfalia CSA-1	387.536	20	97.904
		30	65.269
		40	48.952
Frau CN2S	326.023	20	82.364
		30	54.909
		40	41.182
Culturefuge 100™	1666.380	20	420.980
		30	280.653
		40	210.490

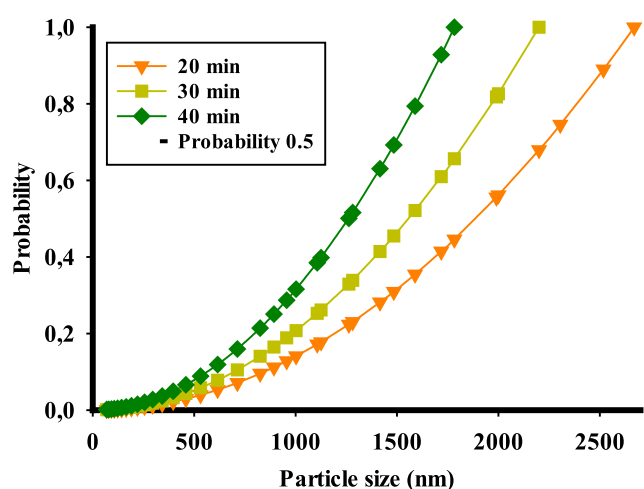


Figure 18. Probability as a function of particle size for continuous disc stack centrifuges.

This curve describes the probability for particles of a given particle size to be removed by centrifugation if the initial suspension consisted exclusively of them. This is the probability that could be obtained if the guava juice was clarified using a continuous disk stack centrifuge operating at 1343 g.

The cut-off size (x_{50}) corresponding to probability 0.5 was predicted. It varies for each treatment time; being 1887 nm, 1556 nm, 1261 nm, for 20 min, 30 min and 40 min, respectively. Increasing the centrifugation time would result in a decrease in the cut-off size. The decrease in surface area of particle size distribution curves with an increase in centrifugation time is accompanied by a decrease in the probability of particles of a specific size found in the supernatant after centrifugation.

4. Conclusion

In sum, the first objective of this paper was to determine the effect of centrifugal time and g-force on the physicochemical characteristics of clarified white-fleshed guava juice. The second objective was to identify the operational characteristics of a continuous centrifuge based on the performance of a fixed-angle conical rotor laboratory centrifuge. 97% w/w of clear guava juice could be recovered through centrifugation. Particle sizes of clear centrifuged juice samples ranged from 68 nm to 3100 nm compared to the feed sample with particle sizes comprised between 68 nm and 900,000 nm. 95.49% w/w of pectin and pectinaceous matters was removed during centrifugation with a decrease in dry matter content of 10.53% w/w. No significant change in physicochemical parameters and in the clarification efficiency of the fixed-angle conical rotor laboratory centrifuge used was observed for g-forces greater than 1343 g. With that acceleration, limits of separation or maximal particle sizes were determined and ranged from 1783 nm to 2669 nm for centrifugation times ranging from 20 to 40 min. Thanks to the clarification performance of the laboratory centrifuge, operational parameters of identified commercial disc stack centrifuges with given geometrical characteristics were determined. The predicted cut-off sizes ranged from 1261 nm to 1887 nm. Among these commercial centrifuges, Culturefuge 100™ model was the one with the highest predicted feed flow-rate.

Declarations

Author contribution statement

Kombele Aime Ninga: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Steve Carly Zangue Desobgo; Emmanuel Jong Nso: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Joseph Kayem: Analyzed and interpreted the data.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

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Additional information

No additional information is available for this paper.

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V.8) Pectinase Hydrolysis of Guava Pulp: Effect on the Physicochemical Characteristics of its Juice

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Pectinase hydrolysis of guava pulp: effect on the physicochemical characteristics of its juice



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ABSTRACT

The objective of this research is to assess the effect of enzymatic treatment of guava puree on the physicochemical parameters of the juice. Pectinases from *Aspergillus niger* were applied to the puree at 43 ± 3 °C under constant stirring. Enzyme concentrations used were: 0.033 % (w/w), 0.055% (w/w), 0.078 % (w/w) and 0.1 % (w/w). For each enzyme concentration, the treatment times were varied from 3 – 90 min. Physicochemical parameters of raw puree and enzymatically treated juice were determined. These were: viscosity, pH, electric conductivity, protein and polyphenol content, galacturonic acid content, color, TSS, and antioxidant capacity. Particle distribution, homogeneity of raw puree and juice samples dried extracts were assessed using a Field Emission Scanning Electron Microscopy (FESEM). A 91% viscosity decrease was recorded for each enzyme concentration after 3 min of enzyme reaction. That decrease was accompanied by an increase in galacturonic acid content with increasing depectinization factors. Enzyme treatment of guava puree led to a decrease in pH, protein and polyphenol contents and an increase in conductivity and color. Analysis of FESEM images of guava samples bestowed a decrease in particle size, a scattering of particles in the medium, an increase in continuous phase proportion and an improvement of sample homogeneity with increasing values of processing parameters, due to the breaking-down of bigger particles and the solubilization during depectinization.

1. Introduction

Native from Mexico, Peru, USA and Columbia, Guava known as *Psidium guajava*, belonging to family of *Myrtaceae*, is a tropical and sub-tropical fruit widely found in different countries throughout South America, Asia, Europe, and Africa (Ninga et al., 2018). Guava is well appreciated for antioxidant capacity of its phenolic compound and its vitamin A and C content. It contains high concentration of vitamin C (100–200 mg/100 g), more than fresh orange juice (60–80 mg/100 g) (Akesowan and Choonhahirun, 2013; Surajbhan et al., 2012). It is a highly perishable climacteric fruit with a shelf-life of 3–4 days when stored at room temperature. It can be consumed freshly or processed into different products: juice, puree, jam, jelly, concentrate, nectar (Wu et al., 2005).

Guava is rich in pectin, a complex polysaccharide made of galacturonic acid units linked by α – (1–4) galactosidic bonds. Two different chains can be found pectin structure, homogalacturonan, also known as

“smooth” region, and rhamnogalacturonan I, known as “hairy” region, branched to the former one (Combo). Due to its molecular weight, its degree of methoxylation (DM) and its hydroxyl groups, pectin exhibit a high water retention capacity yielding to a high viscosity of the puree. Moreover during guava juice clarification, pectin molecules cause membrane fouling, resulting in low juice recovery yield (Lee et al., 2006). To increase that yield, clarification process may require several preliminary extraction steps that are: hot, cold and enzymatic extraction. Compared to the first two preliminary steps, the enzymatic one is known to be that with an appreciable juice recovery yield (Sharma Harsh et al., 2016).

Pectinases, used during enzymatic treatment of guava puree, will break-down pectin molecules into smaller oligagalacturonans causing pectin-protein complexes to flocculate, so that the resultant juice has much lower pectin and viscosity. The resulting enzymatically treated can therefore be easily clarified through centrifugation or filtration. Attributes of the juice such as clarity, aroma and flavor increase after

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clarification (Sharma Harsh et al., 2014; Ninga et al., 2018). Research works published on enzymatic treatment of guava puree highlighted a reduction in viscosity, turbidity, and pH, an increase in the juice extraction efficiency, increased color attributes, titratable acidity, total soluble solids, and clarity. There was also an increase in the concentration of neutral sugar, uronic acids, and methanol (Akesowan and Choonhahirun, 2013; Kaur et al., 2011; Marcelin et al., 2017; Nguyen et al., 2013; Nso et al., 1998; Surajbhan et al., 2012; Thi Thuy Le et al., 2012). Guava juice contains proteins and polyphenols which are susceptible of haze formation due to interactions between them, leading to cloud and tannin appearance. The effect of enzymatic treatment of guava puree on these two parameters as well as the forementioned ones will help in assessing the storage stability of the juice. It is also essential to correlate changes in physicochemical parameters with morphological modifications of the particle due to macromolecular network break-down during the enzymatic depectinisation of guava juice. Hence, this paper aims to assess the impact of enzyme hydrolysis of guava puree on the physicochemical characteristics of its juice and visualize the morphological modifications that occur all along the enzymatic depectinisation.

2. Materials and methods

2.1. Biological material

Guava fruits (*Psidium guajava*), used for the experiments, were purchased from local market in Kharagpur, West Bengal, India. They were selected based on their degree of maturity and ripeness.

Pectinase used in the present study (isolated from *Aspergillus niger*, activity: 8000–12000 U/g, dry extract) was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Other chemicals are: bovine serum albumin; pectin; sodium potassium tartrate; gallic acid; D-galacturonic acid monohydrate; 2,2-Diphenyl-1-picrylhydrazyl (DPPH); copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$); Folin Ciocalteu's phenol reagent. All chemicals were of analytical grade.

2.2. Methods

2.2.1. Guava juice extraction

Guava juice was prepared following the procedure described by Ninga et al. (2018). Mature and ripened guava fruits were processed into guava puree following these steps: washing followed by rinsing using tap water, removal of blemishes by trimming, manual deseeding by a 16 – mesh sieve, blending using a food processor and mixing of the puree with demineralized water at a ratio of 35% w/w. The guava fruits of proper maturity and ripeness were washed using tap water, rinsed, trimmed to remove blemishes, cut in small pieces and deseeded manually by using a 16-mesh sieve. The puree was blended using a food processor without addition of water. The bulk solution was packaged in plastic bottles and stored in the freezer for further experiments.

2.2.2. Enzyme treatment of the pulp

The guava pulp was thawed before use, and 100 g of pulp was weighed in a 250 mL beaker, as described by Ninga et al. (2018). The beaker was put in a water bath at $45 \pm 2^\circ\text{C}$ temperature for 5 min under continuous stirring. Dried *A. niger* pectinase extract was added in each sample under continuous stirring using a Remi Motor agitator, type RQ 122 (supplied by Elektrotechnik Ltd, Kolkata, India) at 1500 rpm. The depectinization parameters were as followed: enzyme concentrations: 0.033%, 0.055%, 0.078% and 0.1% weight of dried extract/weight of initial guava pulp; incubation time: 3, 5, 8, 12, 15, 18, 20, 40, 60, 80 and 90 min. The depectinisation was stopped by a heat treatment at 95°C for 5 min. That required the transferring of the solution into a glass sampling bottle followed by heat treatment in boiling water bath. After cooling at the room temperature, the mixture was filtered by a cheese cloth (200 μm mesh size). The filtrate was collected and kept in polyethylene

terephthalate (PET) bottles, and stored in the freezer for further analysis. The experiments were performed in triplicate.

2.3. Analysis of physicochemical parameters of guava juice samples

For each sample of juice, some analyzes were executed to estimate the parameters which are: the viscosity, the reducing sugar content, the polyphenol content, the protein content, the antioxidant capacity. To these parameters are added the pH, the conductivity, total soluble solids, and the color.

2.3.1. Determination of viscosity

The juice viscosity was estimated at ambient temperature ($30 \pm 2^\circ\text{C}$) using capillary viscometer said Ostwald (Pisco, Calcutta, India) (Jain and De, 2016).

Knowing the dynamic viscosity of water at room temperature, the dynamic viscosity of the juice was determined from the equality of the flow time ratio and dynamic viscosity as shown in Eq. (1).

$$\mu_{jus}(\text{mPa.s}) = \frac{t_1}{t_0} \times \mu_{eau} \quad \text{Eq. 1}$$

with μ_{jus} μ_{eau} the dynamic viscosity of the juice and water in mPa.s, respectively.

To evaluate the effect of processing parameters on the viscosity of the juice, the viscosity value was plotted against time and the enzyme concentration.

2.3.2. Determination of pH, electric conductivity and total soluble sugars

The pH and conductivity were measured using a pocket tester (Eutech Instruments Ltd, Singapore) at room temperature ($30 \pm 2^\circ\text{C}$). Demineralized water (obtained from Millipore reverse osmosis device, Surepro pre-filtration System Merck Life Science Private Limited, Bangalore, India) was utilized as blank. The calibration of the apparatus required a preliminary step electrode washing with deionized water. Then, in a beaker containing deionized water, the electrodes were immersed. The electrical conductivity and pH were measured of the juice samples were measured by introducing the electrode in the sample. After this determination, electrodes were rinsed with deionized water.

The total soluble solids (TSS) were calculated by a laboratory refractometer with digital display (Digital Lab Refractometer Salinity - 300034, Sper Scientific, Scottsdale, Arizona, United States of America). Demineralized water was therefore used as blank. For this, a few droplets of water were put on the playback interface. The determination of the value of TSS was made by pressing the Read and the value of TSS was posted on the screen. The playback interface has been cleaned with kimwipe to remove any trace of water that may influence the reading. Some guava juice droplets were also filed on the playback interface and the value of TSS measured as in the case of deionized water.

2.3.3. Determination of color

The color of guava juice was established using the spectrophotometric method by reading the absorbance at 420nm using a UV-visible spectrophotometer (M/s Perkin Elmer, Connecticut, USA) (Jain and De, 2016). For this, the sample was diluted to one-tenth with deionized water in 25mL test tubes. The sample color value was obtained multiplying the absorbance value by 10. The deionized water was utilized as blank.

2.3.4. Determination of protein content

Sodium bicarbonate solution (Lowry Solution A) was realized by dissolving 1 g of Na_2CO_3 (Merck Specialties Private Limited, Mumbai, India) in 50 mL of a 0.1N NaOH solution. The Lowry B solution was obtained by dissolving in water (5 mL), a mixture of 25 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Merck Specialties Private Limited, Mumbai, India), and 50 mg of $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (Loba Chemie Pvt. Ltd., Mumbai, India). The two solutions were subsequently mixed gradually to prepare the solution of

Lowry. The commercial Folin-Ciocalteu reagent (Loba Chemie Pvt. Ltd., Mumbai, India) was diluted to half (1/2) using distilled water. The calibration range was achieved as follows. From a BSA solution (bovine serum albumin) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) standard of 2.5 g/L, a suitable concentration of the standard assay solution was prepared to achieve a range from 0 to 250 mcg of protein. Each tube was filled to 1 mL with distilled water. 5 mL of Lowry reagent was thereafter adjoined to each tube. After stirring, the mixture was permitted to rest for 10 min. The Folin-Ciocalteu solution (0.5 mL) was then introduced and the tube was homogenized, then permitted to stay in the dark for 30 min. Absorbance was obtained at 660 nm against the blank prepared by replacing 1 mL of the sample by deionized water (1 mL). The concentration of protein guava juice was obtained using the previous protocol and, replacing the BSA solution by 1 mL of the sample. The amount of protein in each test sample was calculated as in Eq. (2):

$$[\text{protein}] (\text{mg.l}^{-1}) = \frac{\text{Abs}}{\alpha} \quad \text{Eq. 2}$$

with: [Protein]: protein concentration; Abs: sample absorbance; α : calibration curve slope.

2.3.5. Determination of galacturonic acid

The determination of the concentration of galacturonic acid in the samples was obtained using the method cyanoacetamide (OIV, 2009) with some modifications. The supernatant (2 mL) from the centrifugation of the sample mixture of ethanol (99 % v/v) was diluted in a volumetric flask. This dilution (2 mL) was inserted in a test tube, and then augmented with 4 mL of borate buffer (100 mM; pH 9.0). (Loba Chemie Pvt Ltd., Mumbai, India). and 2 mL of cyanoacetamide solution (1% w/v) (Spectrochem Pvt. Ltd. Mumbai, India). The test tube was deposited in a water bath (Remi model RSB - 12, Remi Elektronik Ltd, Maharashtra, India) at 95 °C for 10 min. After heating, the test tubes were cooled to room temperature by putting them in a water bath (30 ± 2 °C). The optical density of samples was read at 273 nm. The concentration of galacturonic acid was calculated by considering the calibration curve using the galacturonic acid at concentrations of 0–250 µg/mL, with a dilution factor of 50 and using Eq. (3).

$$[\text{AcG}] (\text{mg.l}^{-1}) = \frac{\text{Abs}}{\alpha} \times 50 \quad \text{Eq. 3}$$

with: [AcG]: the galacturonic acid content; Abs: absorbance of the sample; α : the slope of the calibration curve.

2.3.6. Determination of the total polyphenol content

The protocol used for the estimation of the total polyphenol content is related to the literature (Sagu et al., 2014). The juice/blank/standard (0.5 mL) was introduced in 25 mL tubes and supplemented with 0.5 mL of Folin Ciocalteu reagent. The mixture was incubated for 5 min to facilitate the reaction with stirring. The anhydrous sodium carbonate (75 g/L, 10 mL) was introduced and mixed. The tube was then filled to 25 mL with distilled water. After mixing, the tubes were permitted to rest at room temperature (30 ± 2 °C) for 1 h. Absorbance was measured at 750 nm using UV spectrophotometer - Visible. Gallic acid (Sigma Aldrich, Slovakia) was utilized for the calibration curve. The results were formulated as mg gallic acid equivalents per 100 mL (See Equation 4).

$$[\text{polyphenols}] (\text{mgGAE} / 100\text{mL}) = \frac{\text{Abs}}{\alpha} \times 50 \quad \text{Eq. 4}$$

with: [polyphenols]: the phenolic compounds content; Abs: absorbance of the sample; α : the slope of the calibration curve.

2.3.7. Determination of the antioxidant capacity

The antioxidant capacity determination protocol (Brand-Williams et al., 1995) of every sample was utilized with some modifications. The

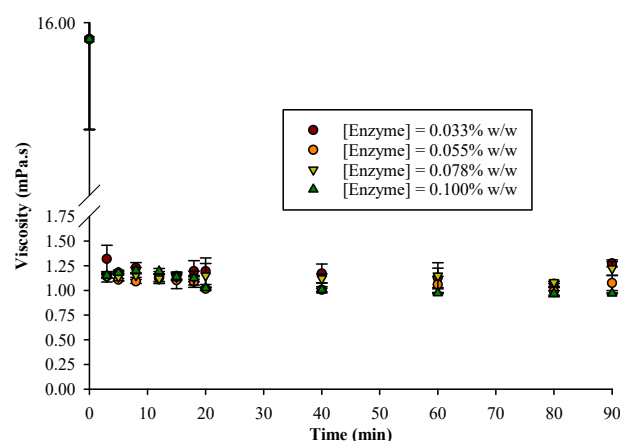


Figure 1. Evolution in the viscosity of guava juice with time and enzyme concentration.

reaction mixture was composed of 2.5 mL of supernatant from the centrifugation of the sample-ethanol (99 % v/v), 4.5 mL absolute alcohol, and 0.6 mL of DPPH solution (0, 5 mM in pure ethanol) (Sigma Aldrich, Slovakia). When the DPPH reacts with antioxidants which may form hydrogen, it is reduced. This modification of color (from dark purple to light yellow) was characterized by absorbance at 517nm after a reaction of 100 min. The blank was composed of 2.5 mL of supernatant and 5.1 mL of pure ethanol. The control solution was achieved using a mixture of 7 mL ethanol and 0.6 mL of DPPH solution. The antioxidant capacity was determined as in Eq. (5).

$$\text{AC}(\%) = 100 - \left[\frac{(\text{Abs}_{\text{éch}} - \text{Abs}_{\text{blanc}})}{\text{Abs}_{\text{contrôle}}} \times 100 \right] \quad \text{Eq. 5}$$

AC: Antioxidant capacity of the sample; $\text{Abs}_{\text{éch}}$: Absorbance of the sample; $\text{Abs}_{\text{blanc}}$: Absorbance white; $\text{Abs}_{\text{contrôle}}$: Absorbance of the control solution.

The ANOVA was done using the software Statgraphics Centurion Version XV.II and 5 % significance level was utilized to check the difference between samples.

2.3.8. Morphological study of the juice samples by Field Emission Scanning Electron Microscopy (FESEM)

The imaging technique made it possible to visualize the structure of the suspension by studying the morphology of the particles, the profile of the mixture, and the distribution of the granules and particles. The image analysis required the untreated or treated juice to be dehydrated. For this, juice samples were previously spread on microscope slides and dried under vacuum in a desiccator for 10 days. The images were produced by FESEM (JEOL, model JSM-7610F, Tokyo, Japan).

3. Results and discussion

3.1. Effect of enzymatic treatment parameters on viscosity

The reduction of viscosity with treatment time for each enzyme concentration used is observed in Figure 1. The viscosity of untreated juice is 15.90 ± 0.522 mPa.s. At the end of the third minute of treatment (after deactivating the enzyme in a boiling bath for 5 min), it decreased significantly to a viscosity of 1.32 ± 0.2 mPa.s for all enzyme concentrations; a decrease of more than 90% of the initial viscosity. This observation is similar to that made by the literature (Akesowan and Choonhahirun, 2013; Kaur et al., 2011; Nso et al., 1998; Surajbhan et al., 2012). This could be elucidated by the hydrolysis of pectin macromolecules by pectinases. Industrial pectinase preparation of *Aspergillus niger* is a conglomerate of several isoforms with different action mechanisms and

kinetic parameters (Benen et al., 2003). Hydrolysis of pectin macromolecules and subsequent accumulation of the reaction products would occur in the first min of the reaction (Combo et al., 2012). These scientists have demonstrated that at the fifth minute of the enzyme reaction, products of degree of polymerization (DP) 1 to 10 are already formed. These low degree of polymerization products compared to pectin macromolecule would exhibit lower hydraulic radius and water retention capacity than the substrate (pectin) from which they were derived. Therefore, the resulting mixture would get a better flowability, thus justifying the low viscosity values obtained from the treated samples. PGII isoform is a highly active enzyme that hydrolyzes pectin molecules in the first few min of reaction. Without substrate inhibition, this isoform would easily attack pectin or polygalacturonic acid macromolecules (its natural substrate would be pectin) and release oligomers (Benen et al., 1999). The rapid decrease in viscosity from the third minute of reaction may be due to the low affinity of PGII isoform for pectin or polygalacturonic acid molecules and its high activity.

It is also observed that after the sudden decrease in viscosity, another more moderate and non-significant decrease occurs between the 3rd and 80th min (1.32–1.27; 1.15 to 0.97 0.033% and 0.1% w/w of enzyme respectively) with the asymptotically tending viscosity value (0.9 mPa.s for the enzyme concentration of 0.1% m/m; 1 mPa.s for other concentrations). The phenomenon observed during this phase may be due to both the complementarity between isoforms and the phenomenon of processivity that would occur both during the treatment period. Some isoforms (PGI and PGD) would be sensitive to steric hindrance due to substrate size and therefore the molecular weight. As a result, these isoforms would prefer low molecular weight substrates from the hydrolysis of oligomers by other isoforms (Benen et al., 1999; Pařenicová et al., 2000). Besides, the phenomenon of processivity would be accompanied by a gradual decrease in the number of oligomers in the decreasing order of their molecular weight and an accumulation of lower molecular weight products. This phenomenon is described in the evolution profile of reaction products for all isoforms (Benen et al., 1999; Benen et al., 2003; Pařenicová et al., 1998, 2000). It was shown with the increase in enzyme processing time (from the 15th-minute reaction), there was a gradual decrease in the concentration of oligomers of polymerization degree between 4 and 10 with the formation of mono, di, and trimers (Combo et al., 2012; Nikolić and Mojovic, 2007). These secondary substrates and their derivatives would form the compounds encountered during the slow decrease in viscosity. The non-significant change in viscosity observed is because these compounds would describe a range of hydraulic radii that would not significantly affect the viscosity of the samples. Also, the hydrolysis of pectin molecules (a highly hydroxylated compound) leads to the release of oligomers of galacturonic acid weakly hydroxylated compared to pectin. Hydrolysis would be accompanied by a decrease in water retention capacity; therefore, the release of water molecules into the system would result in a decrease in viscosity (Akesowan and Choonhahirun, 2013).

After the non-significant decrease, the same figure shows a non-significant increase in viscosity after 90 min of enzyme treatment (about 10% of the viscosity value at 80 min). This could be due to the formation of particles after cooling the juice. These particles are reported to be the result of the coagulation phenomenon observed by the interaction between protein or polyphenol molecules with mono, di, and certain galacturonic acid oligomers (Shomer et al., 1999). The heat treatment of the samples to stop the enzyme reaction has the effect of denaturing protein molecules. The latter being positively charged to the natural pH of the juice (3.5 ± 0.2) could form ionic interactions with the negatively charged galacturonic monomers and oligomers. The result would be the creation of particles known as "cloud" (Rai et al., 2004; Shomer et al., 1999). The formation of these particles could be promoted by the high monomer content after 90 min of enzymatic treatment. The particles obtained would affect the viscosity of the medium due to their higher hydraulic radius than those of galacturonic acid monomers and protein molecules taken individually.

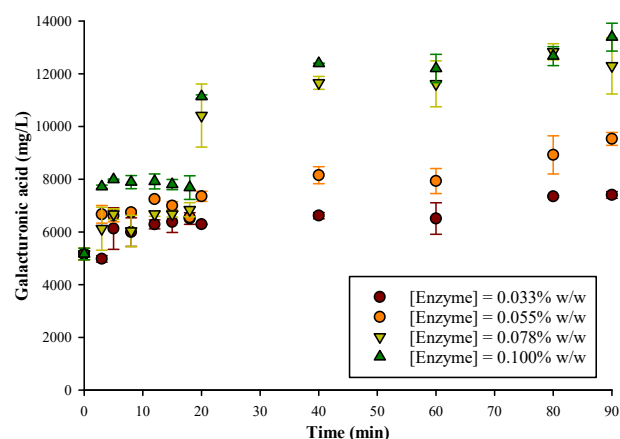


Figure 2. Evolution of the galacturonic acid content as a function of time for the different enzyme concentrations.

Finally, this figure shows that an increase in enzyme concentration goes hand in hand with a decrease in viscosity value (Akesowan and Choonhahirun, 2013; Kaur et al., 2011; Surajbhan et al., 2012). This could be because increased enzyme concentration would mean an increase in the number of catalysts. The rate of reaction is a function of enzyme concentration. The increase in enzyme concentration would be followed by an increase in the number of products formed (Nikolić and Mojovic, 2007).

3.2. Effect of enzymatic treatment parameters on the galacturonic acid content

The raw juice has a galacturonic acid content of 5161.510 ± 216.461 mg/L. For each enzyme concentration, increased treatment time was followed by a significant increase in galacturonic acid content (Figure 2). The rise in galacturonic acid content as a function of contact time may be due to the liberation of galacturonic acid following the reaction of pectin hydrolysis by pectinases. Galacturonic acid is the smallest galacturonate which can be released after the enzymatic treatment of guava puree by pectinases. In most of the mechanisms involved (simple attack on single chain, multiple attack on single chain or multiple chain of pectin or oligogalacturonates) during that treatment, that product is known to be the final product. In the single attack mechanism, an α (1–4) linkage of the pectin substrate is hydrolyzed by the enzyme with the liberation of two low molecular weight products. Processivity or multiple attack on a single chain involves the release of the lower molecular weight product after hydrolysis and the binding of the bigger product with the enzyme. That product will later shift to a new position on the enzyme for another hydrolysis reaction to take place. The release of low molecular weight oligogalacturonates would be followed by their hydrolysis by the various PG isoforms; the last product released regardless of the polymerization degree of the substrate is the galacturonic acid monomer (Benen et al., 2003).

For an enzyme concentration of 0.033% w/w, the galacturonic acid content progressively increases in a non-significant way over the contact time. This trend is obtained between 3 and 90 min for 0.055% w/w enzyme concentration. For 0.078% and 0.1% w/w enzyme concentrations, three distinct zones can be obtained: an area of non-significant variation in galacturonic acid content between 3 and 18 min and between 20 and 90 min and also, a significant increase between 0 and 3 min.

Findings on each PG isoform showed that the progression curve of products formed during the hydrolysis of pectin exhibited a rise in oligomeric levels followed by a decline in these contents justifying the

phenomena of processivity and/or simple attack. This decrease was accompanied by a rise in monomer content (Jacques A. E. Benen et al., 1999; Pařenicová et al., 1998, 2000). PG conglomerate of *A. niger* for polygalacturonic acid hydrolysis showed the appearance of galacturonic acid monomers and products of DP = 2 to DP = 10 at the end of the fifth minute of treatment (Combo et al., 2012). These researchers also showed that with an increase in treatment time, there was a progressive decrease (in descending order of polymerization) in the content of oligomers of molecular weight between 2 and 10. The release of galacturonic acid at the third minute of reaction may be due to the enzyme activity of the commercial preparation used in this study. That was between 8000 and 12000 U/g; it is, therefore, higher than that used (3160 U/ml) in the literature (Combo et al., 2012). This high value of enzyme activity would result in the acceleration of the above phenomena. The progressive increase in galacturonic acid content for enzyme concentrations of 0.033% and 0.055% m/m could be due to the progressive hydrolysis of the DP oligomers above 1 with the progressive release of galacturonic acid monomers. The same phenomenon could explain the variation in concentrations of 0.078 % and 0.1 % w/w above 20 min. Indeed, Combo et al. (2012) observed that after the 60th-minute reaction, the medium was saturated mainly with galacturonic acid monomers with very small amounts of dimer and trimer of galacturonic acid. They also showed an increase in the monomer/dimer ratio over the treatment time reflecting progressive hydrolysis of dimers to monomers. With the predominance of these two oligomers, PGD isoform is the one that would be most active since it is the only one to hydrolyze the galacturonic acid dimer. Hydrolysis would occur at a relatively low rate (0.67×10^{-3} μ kat/mg and 0.67×10^{-4} μ kat/mg for 50 and 500 μ M dimer, respectively) (Pařenicová et al., 2000). This dimer hydrolysis may explain the nonsignificant increase in galacturonic acid content observed with enzyme concentrations of 0.078 % and 0.1% w/w.

For enzyme concentrations of 0.078% and 0.1% w/w, a significant and abrupt increase was observed between 18 and 40 min of reaction. This increase could be the result of the processivity phenomenon involving the processes of hydrolysis of substrates, the release of low DP products, migration of high DP products along the enzyme molecule to the active site, and secondary hydrolysis. The simple attack mechanism for the subsequent release of products following hydrolysis could also occur during this period. Since the amount of enzyme was high compared with the other two concentrations, the accumulation of low-polymerization oligomers for the first 20 min of these two enzyme concentrations would be faster. This accumulation would be followed by hydrolysis of these oligomers into others of lower degrees of polymerization. These new products would undergo secondary hydrolysis with the progressive release of monomers. These sequenced hydrolysis reactions would occur between 18 and 40 min. It was highlighted the release of oligomers of DP between 3 and 8 from 15 min of reaction (Nikolić and Mojovic, 2007). The amount of these oligomers increased over time and after 120 min of treatment, the amount of DP oligomer between 6 and 8 showed a decrease. These phenomena would be observed in this study and more accelerated concerning the activity of the enzyme preparation used (8000–12000 U/g), i.e. at least 8 times that of the enzyme preparation used by these two researchers (1000 U/g).

For a given duration of treatment (Figure 2), increased enzyme concentration results in a significant increase in galacturonic acid content (Wang et al., 2007). These researchers have shown that increased pectinase concentration leads to more production of galacturonic acid for a given period of cotton fiber treatment. For a period of treatment of guava juice, increased enzyme concentration would lead to an increase in the amount of reducing ends as shown by (Nikolić and Mojovic, 2007) with apple pectin. These reductive ends are those of the galacturonic acid molecules and their oligomers. All released after the enzyme reaction. Increasing enzyme concentration and processing time would result in an acceleration of the reaction of enzyme hydrolysis of pectin, and thus of simple attack and processivity. Progressive spacing between the evolutionary curves of the number of galacturonic acids would reflect the fact

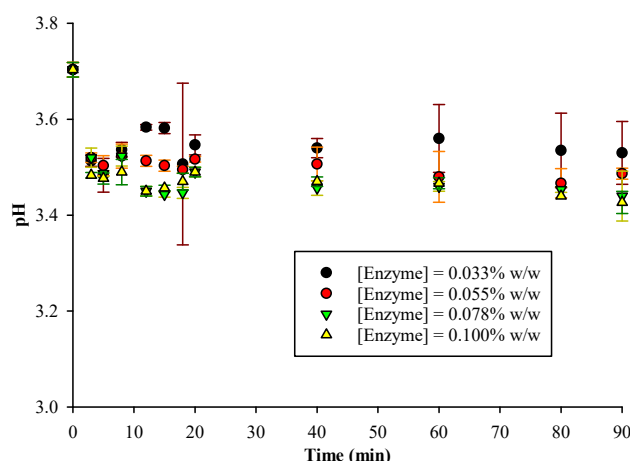


Figure 3. Guava juice pH evolution based on the enzymatic processing time.

that increased treatment time would result in the progressive release of the reaction products with an increase in enzyme concentration. A gradual disappearance of oligomers of a degree of polymerization between 2 and 10 coupled with an increase in the quantity of galacturonic acid monomers was observed. It is this acceleration of the reaction that may explain the fact that, as the treatment duration increases, the gap between the galacturonic acid content curves increases with increasing enzyme concentration. As a consequence of these different observations, the effect of inhibition by substrate molecules could be reduced with increasing enzyme concentration.

3.3. Effect of operating conditions on the parameters of juice

3.3.1. Effect of enzymatic treatment on the pH of guava juice

The initial pH was 3.70 ± 0.02 , and it was found to decrease significantly with treatment at the third minute of treatment (Figure 3). This decrease is accompanied by near-constant variation (plateau presence) between 3 and 90 min. It was shown that the enzyme treatment of guava juice resulted in a decrease in the pH value for all factor combinations (Ahmed et al., 2014; Combo et al., 2012).

Figure 3 also shows that, with increasing enzyme concentration, the pH value decreases over a given treatment period. This could be explained that with increased enzyme concentration there would be a more significant production of galacturonic acid monomers (Nikolić and Mojovic, 2007; Wang et al., 2007). The decrease in pH with increased enzyme concentration may also be due to the release of ascorbic acid molecules during pectin hydrolysis (Akesowan and Choonhahirun, 2013). The non-significant difference between enzyme concentrations of 0.078% and 0.1% m/m corroborates the observation for time-dependent variation of galacturonic acid content for both concentrations.

A decrease in pH during enzyme treatment could also be attributed to the release of carboxylic groups (acid groups) during pectin hydrolysis. Pectin is a complex macromolecule consisting of so-called "hairy" zones built around the "smooth" zones. The smooth areas that make up the skeleton of the pectin are a polymer of galacturonic acids bound by an α -(1–4) galactosidic bond. Pectin hydrolysis by pectinases is accompanied by the release of galacturonic acid oligomers. Mono (Figure 4a), di, tri, and oligomer (Figure 4b) all have a reductive end that may cause a decrease in pH. Besides, galacturonic acid has two carboxylic groups, including C1 and C6 carbon, as shown in Figure 4a. Hydrolysis of methoxyl groups by Pectin Esterase (PE) results in the formation of a carboxylic group in the C6 position as shown in Figure 4c (Garg et al., 2016). This demethoxylation reaction is essential for the activity of polygalacturonases which will hydrolyze the oligomers of galacturonic acid at the level of galactosidic

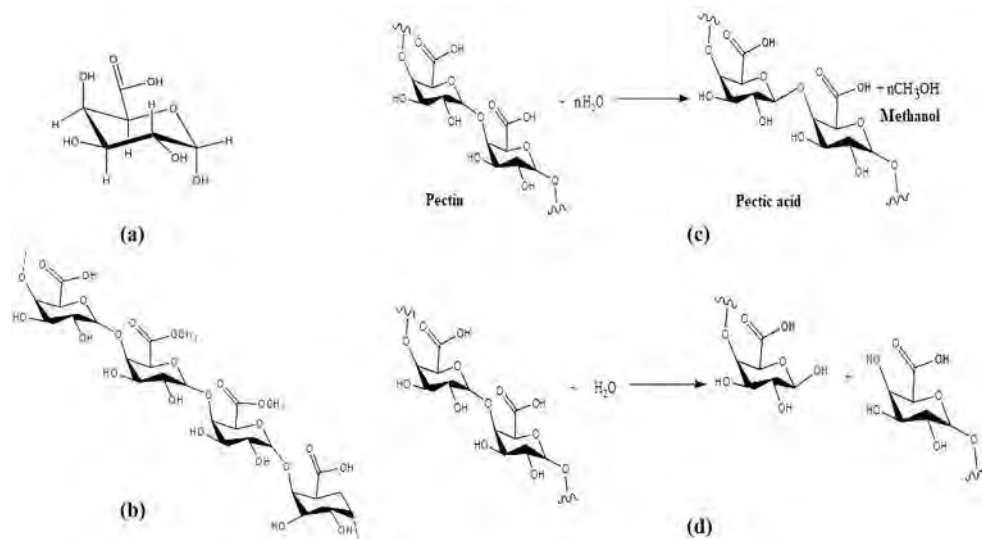


Figure 4. Identification of carboxylic groups in the reaction medium. (a) and (b) schematic representations of galacturonic acid and a segment of the pectin chain with certain methoxylated residues of galacturonic acid, respectively; (c) hydrolysis reaction of the methoxyl bond by Pectin esterase with the formation of a carboxylic group; (d) hydrolysis reaction of the galactoside bond by PG with the release of a reducing end at C1 carbon.

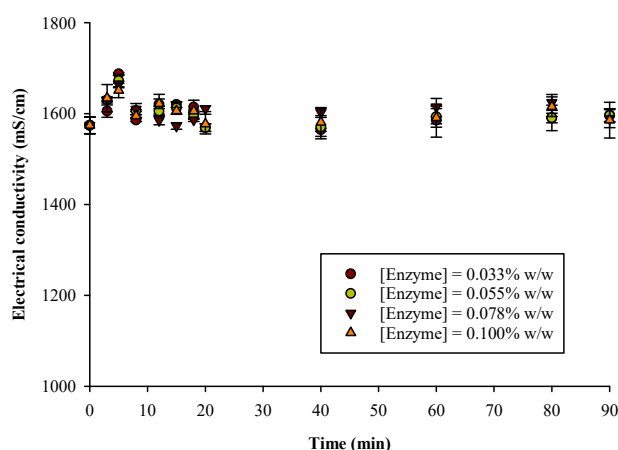


Figure 5. Evolution of electrical conductivity during the enzymatic treatment.

bonds with the release of a reducing end (Figure 4d). Some PG isoforms would prefer partially demethoxylated pectin substrates; methoxyl group describing an inhibitory effect on their activity due to its conformation (Benen et al., 2003).

The near-constant pH variation observed between 3 and 90 min for all enzyme concentrations could be explained by the buffer effect exerted by galacturonic acid molecules. Hydrolysis of pectin molecules by the synergistic action of pectinases would be accompanied by the release and accumulation of galacturonic acid molecules (Combo et al., 2012). This monomer is a molecule with two carboxylic groups and could, therefore, be considered a low organic acid (Figure 4a). In the reaction medium, this acid would coexist with its conjugate base.

Depending on the pH value, the acidic species could release an H^+ ion in the medium or the basic species could capture an H^+ ion from the reactive medium to maintain stable pH values. This buffer effect is optimally expressed when the pH value of the medium is in the order of $pK_a \pm 0.5$; however, the maximum pH limit for expression of the buffer effect is $pK_a \pm 1$ (Dennison, 2002; Stoll and Blanchard, 2009). Galacturonic acid pK_a is 3.47 (Holvik and Høiland, 1977). The buffer effect of galacturonic acid and the base species may be explained by the fact

that the pH values of the juice obtained after enzymatic treatment are all within the range of optimal expression of activity ($2.97 \leq pH \leq 3.97$).

3.3.2. Effect of enzyme treatment on the electrical conductivity of guava juice

The electrical conductivity of the initial juice is 1574.33 ± 18.48 mS/cm. It appears that for all enzyme concentrations, three trends are generally observed according to time intervals (Figure 5). The first-time range is between 0 and 5 min where the enzyme treatment causes a significant increase in the conductivity values of the juice compared to the untreated juice. The second is the one between 5 and 20 min where the treatment causes a non-significant decrease in the conductivity. The third range is from 20 to 90 min; in this, there is a non-significant increase in conductivity for all enzyme concentrations, except for 0.078% and 0.1% w/w where there is a decrease between 80 and 90 min of treatment.

The increase in electrical conductivity for the first five min could be due to the release of calcium ions (Ca^{2+}) during hydrolysis. Pectin is present in biological systems as a complex network with an “egg-box” structure. Pectin chains due to their negative charge can be chelated by calcium ions (Prasanna et al., 2007). During the hydrolysis of pectin, the appearance of the products of the reaction results from the destruction of these networks with the possible release of calcium ions in the reaction medium. This release would increase the conductivity value observed with the samples having undergone the enzymatic treatment.

The decrease of the conductivity between 5 and 20 min of treatment could be due to the chelation phenomena that may exist between certain cations and the anionic groups of the reaction products. These chelation phenomena could be the result of ionic interactions between these groups of opposite charge. Guava is a fruit that has a high mineral content; it contains inter alia several types of cations (K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} , Na^+ , Fe^{2+}). The magnesium content is the highest compared to other cations (Flores et al., 2015). These cations would contribute to the ionic strength. These different cations could be involved in interactions with a galacturonic acid monomer or oligomer molecules or protein molecules present in the medium. These interaction phenomena could be driven by the buffering effect of monomers, galacturonic acid oligomers, and proteins. At the pH of the juice samples, conjugated species of galacturonic acid could be dominant. This is negatively charged and could be associated with ionic interactions with cations. These interactions would have the effect of regulating the buffering effect by promoting the release

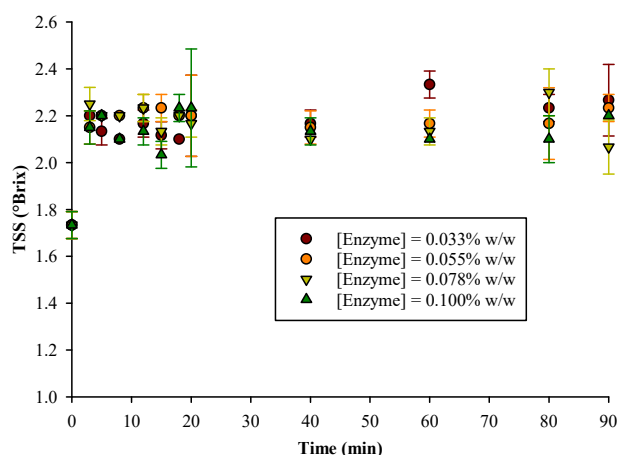


Figure 6. Evolution of TSS content of guava juice during the enzymatic treatment.

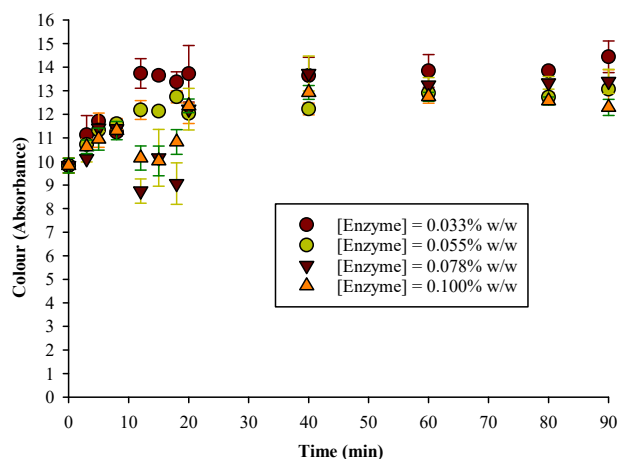


Figure 7. Changes in the color of the juice during the enzymatic treatment.

of H^+ ions in the reaction medium, as they would help to reduce the quantity of the negative species (Dennison, 2002).

The increase in conductivity between 20 and 90 min could be explained by the presence of other types of interaction involving galacturonic acid oligomers and proteins as mentioned above (Shomer et al., 1999). These could render some monomer or oligomer molecules unavailable for cation chelation reactions. Moreover, the decrease in pH that occurs in this time range and the predominance of the conjugated species of galacturonic acid due to the pH of the samples greater than the pKa of the galacturonic acid buffer, there would be an increase of conductivity as shown by (Dennison, 2002).

3.3.3. Effect of enzyme treatment on the value of TSS (total soluble sugar) of guava juice

The TSS content of the initial juice is 1.7 ± 0.1 . It appears from Figure 6 that the enzymatic treatment leads to a significant increase in total soluble sugar content from the third minute of reaction for all enzyme concentrations. Beyond 3 min and up to 90 min, the TSS content does not describe significant variation during treatment. This increase in TSS content compared to initial juice was also observed (Ahmed et al., 2014; Akesowan and Choonhahirun, 2013). It could be explained by the hydrolysis of pectin which releases galacturonic acid monomers that can be assimilated to glucose molecules and thereby lead to an increase in the

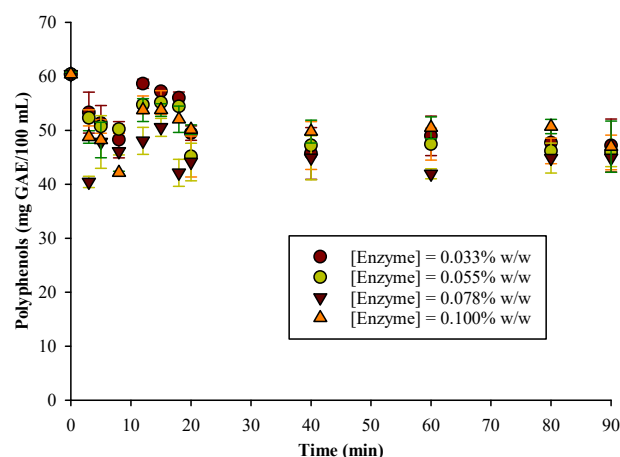


Figure 8. Evolution of juice polyphenols in the enzymatic treatment.

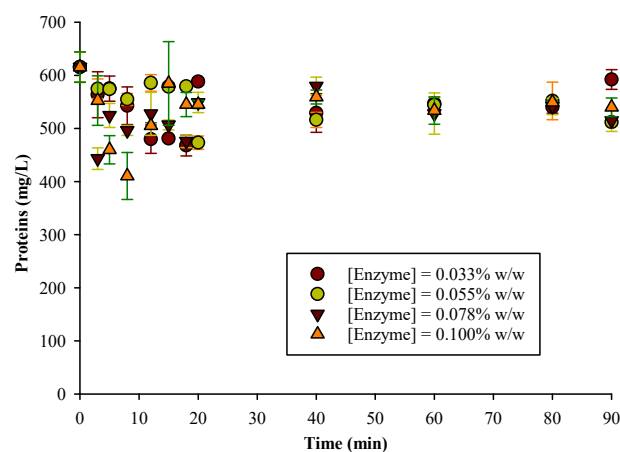


Figure 9. Evolution of the juice protein content during the enzyme treatment.

TSS value. It could also be due to the release of sugar due to the hydrolysis of guava starch by endogenous amylases. It was demonstrated the presence of α and β - amylases and starch during the ripening of guava (Jain et al., 2001). During enzymatic treatment of guava juice, these endogenous amylases hydrolyze the starch resulting in the release of glucose molecules that can lead to an increase in the value of TSS.

3.3.4. Effect of enzymatic treatment on color samples of juice

The initial juice color value is 9.8 ± 0.3 . Figure 7 shows that for each enzyme concentration, two-time ranges are distinct according to the observed trends in color: a range where there is an increase in color and a second where it does not record significant variation. Overall the increase in color occurs during the first 12 min. Beyond this time, the color is almost constant for all enzyme concentrations.

The increase in the color attribute during the treatment could be due to the enzymatic browning of the initial juice, resulting from the oxidation of the phenolic compounds by the polyphenol oxidases (PPO) present in the initial juice. PPOs are enzymes that catalyze the conversion of compounds with a phenolic ring to o-quinone. They possess in their structure a copper atom as a prosthetic group which is very indispensable for their activity.

The conversion of phenolic compounds to quinone would require the presence of oxygen and could occur in two specific steps (Taranto et al., 2017; Yoruk and Marshall, 2003). The first is the hydroxylation of the

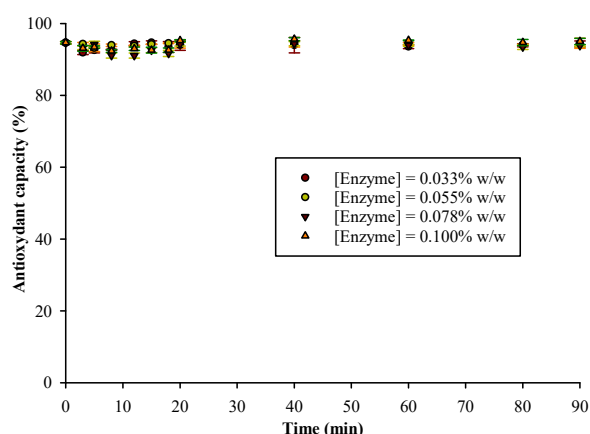


Figure 10. Evolution of the antioxidant capacity of guava juice during the enzymatic treatment.

phenolic nucleus in the ortho position of a hydroxyl group of a monophenolic compound. During this reaction, which has phenol and oxygen as substrates, one oxygen atom is introduced into the monophenolic compound while the other oxygen atom is reduced to water. This reaction is known as monophenolase activity or hydroxylase activity or cresolase activity. It is facilitated by the presence of ascorbic acid or *o*-diphenol compounds that act as electron donors. The product of this first reaction is an *o*-diphenolic compound. The second step is known as diphenolase activity or oxidase activity or catecholase activity. It is an oxidation reaction of the preceding *o*-diphenolic compound with the formation of an *o*-quinone compound which would be highly active. It would undergo non-enzymatic condensation reactions with amino acid molecules, proteins, and other phenolic compounds to give colored complex polymers known as melanin or to form polymers with protein residues such as -SH or -NH₂ groups. During this reaction where two *o*-diphenol compounds are oxidized to two *o*-quinone, the two oxygen atoms are reduced to a water molecule. The first reaction would necessarily involve the second, but the opposite would not always be true. PPOs, include three enzyme groups: tyrosinases, catechol oxidase, and laccases. The latter would be the only ones to catalyze the oxidation of a *p*-diphenolic compound. Tyrosinases possess both cresolase and catecholase activities. Catechol oxidases catalyze the conversion of *o*-diphenols to *o*-quinone (Taranto et al., 2017; Yoruk and Marshall, 2003). Before enzymatic treatment, the guava juice did not undergo

preliminary heat treatment to inactivate endogenous enzymes, therefore stopping enzymatic reactions occurring. As a result, the polyphenol oxidases present in the juice would still be active during enzymatic treatment that took place under aerobic conditions (the reactor was not closed). Stirring during the treatment would have allowed the mixing of the ambient oxygen in the reaction medium. This mixing would have increased the dissolved oxygen content in the juice, which would have increased the substrate content for the oxidation of polyphenols. In addition to this dissolution of oxygen, ascorbic acid present in the medium would also have played a role in browning by promoting monophenolase activity. To these aerobic conditions coupled with the presence of ascorbic acid could be added the temperature and the reaction time. The enzymatic treatment was done at a temperature of 43 ± 2 °C. This temperature is substantially equal to the optimum activity temperature of the guava polyphenol oxidase which is 48 °C (Razzaque et al., 2000).

Also, during processing, the color value increases for a given enzyme concentration. This could reflect the progressive oxidation of phenolic compounds with the appearance of melanins. The increase in enzyme concentration has no significant effect on the color of the samples of given treatment time. This could be because the industrial preparation used is mainly composed of pectinase. Increasing the pectinase concentration would not lead to a change in the polyphenol oxidase concentration already present in the juice.

3.3.5. Effect of enzymatic treatment on the levels of polyphenols and protein of guava juice

The content of phenolic compounds of the initial juice is 60.3 ± 0.4 mg GAE/100mL. Figure 8 shows a decrease in polyphenol content during enzymatic treatment for all enzyme concentrations compared to that of the initial juice. Between 20 and 90 min, the polyphenol content does not change significantly with time for all enzyme concentrations. The decrease in the polyphenol content in the treated samples was reported in the case of enzymatic treatment of banana juice (Sagu et al., 2014). The decrease in the polyphenol content could, among other things, be explained by their oxidation to *o*-quinone by the PPO. By this oxidation, the hydroxyl groups of the preliminary phenolic compounds would be oxidized to a quinone. This new grouping would have low reactivity with a mixture of phosphotungstic and phosphomolybdic acids; it would have little influence on the reduction of this mixture. This would lead to the formation of a less colored blue complex than that of the unoxidized phenolic compound (Blainski et al., 2013). The formation of this *o*-quinone would lead to condensation reactions and complexation with amino acids and proteins resulting in the formation of melanins. The decrease in the polyphenol content could also be due to the

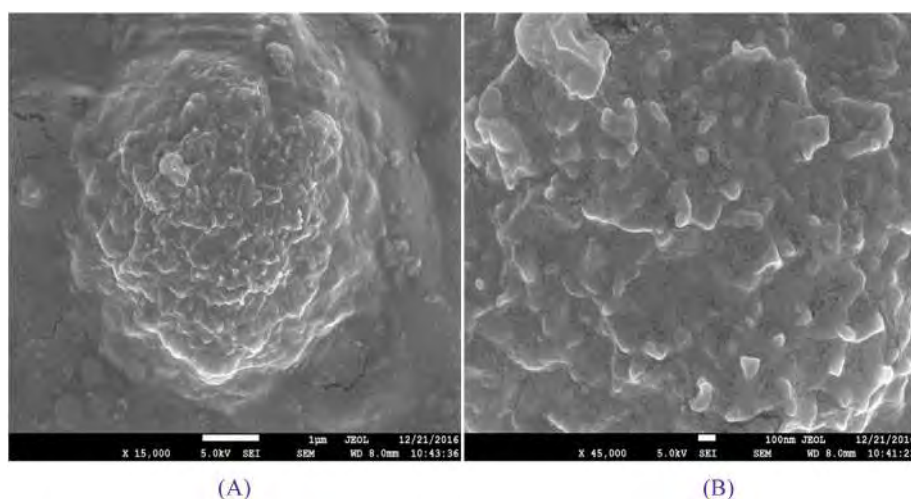


Figure 11. Images FESEM guava puree without any enzymatic treatment (A) and (B) Enlargement 15000 and 45000 respectively.

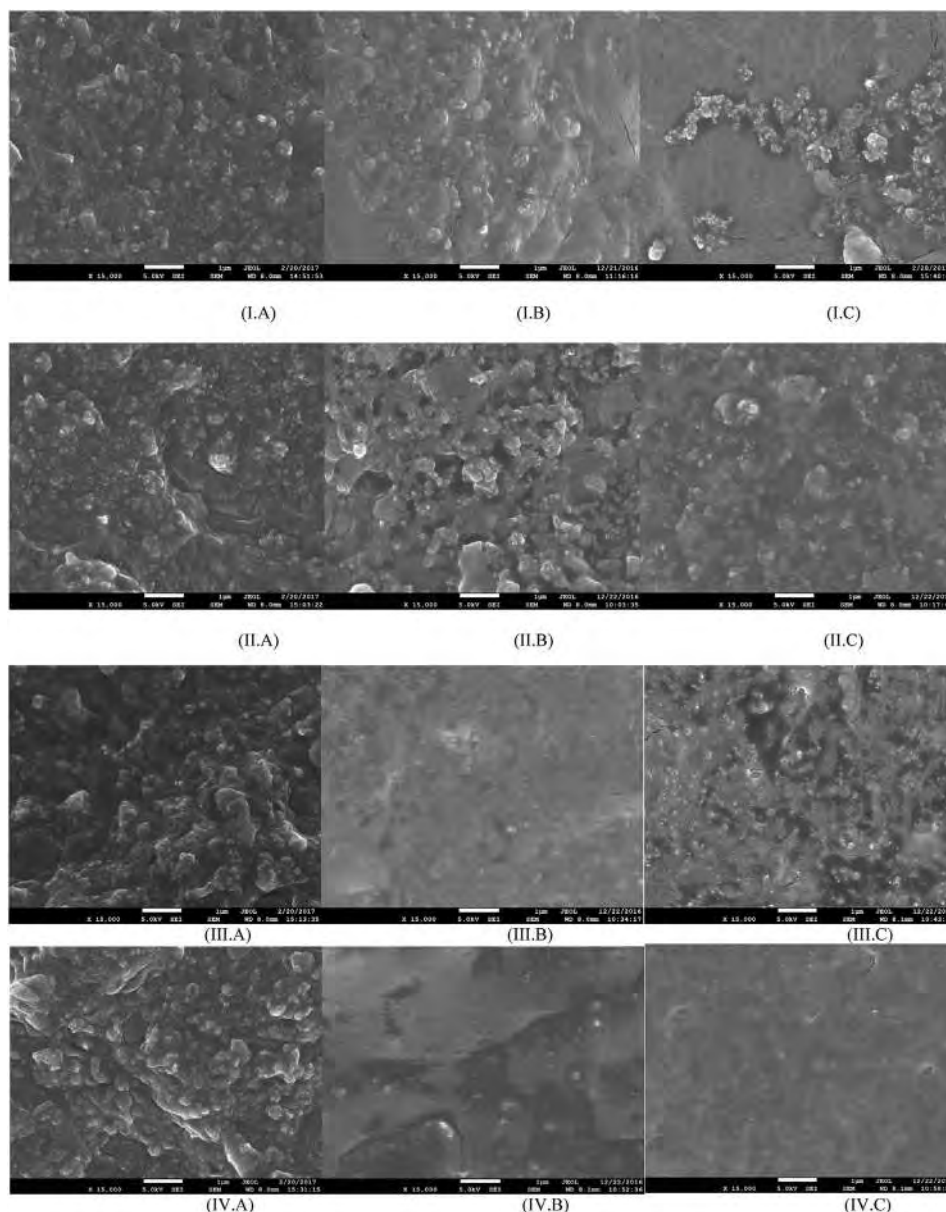


Figure 12. Images FESEM of guava juice samples. (I), (II), (III), and (IV) Images guava samples having undergone an enzymatic treatment with 0.033% w/w, 0.055% w/w, 0.078% w/w, and 0.1% w/w enzyme concentration, respectively. The indices A, B, and C represent respectively the time of enzyme treatment of 8, 20, and 90 min for a given concentration of enzyme.

non-reactivity of the polyphenols involved in the formation of complexes with other molecules such as proteins and other polyphenols. The non-reactivity of these polyphenols would reflect the non or slight reduction of the Folin Ciocalteu reagent because of the involvement of their hydroxyl groups in the formation of the complexes. Indeed, polyphenol molecules act as bridge and the "glue" to promote the precipitation of protein chains, resulting in the formation of trouble or tannin (McLellan and Padilla-Zakour, 2005). This phenomenon could be accelerated by the addition of enzymes during treatment, increasing protein content. Also, polyphenols could be involved in the formation of clouds due to their polymerization to form larger complexes (McLellan and Padilla-Zakour, 2005).

The protein content of the initial juice is 615.6 ± 28.5 mg/L. Figure 9 shows that the enzymatic treatment results in a decrease in the protein content of the samples compared to that of the raw juice for all enzyme concentrations. During processing, the protein content does not describe a monotonous trend with increasing time for all enzyme concentrations.

The decrease in the protein content of the treated samples compared to the initial one could be explained by the non-reactivity with the reagents (the Folin Ciocalteu Reagent and the Lowry solution) used for their detection and the complexes that these proteins form.

These complexation phenomena would lead to unavailability in the reaction medium of the protein molecules. This decrease could, among other things, be attributed to the oxidation of certain tyrosine residues of proteins by PPOs (polyphenol oxidases) (Taranto et al., 2017; Yoruk and Marshall, 2003). Indeed, this amino acid has in its structure an aromatic ring having a hydroxyl group in para position concerning the carbon 1 of the ring. This position of the hydroxyl group would make tyrosine an ideal substrate for the expression of monophenolase activity. Since the reduction of the Folin Ciocalteu reagent is, among other things, dependent on the presence of tyrosine, a group of tyrosine oxidized to o-quinone by the PPOs would, therefore, be unavailable for the blue color formation characteristic of the reduced Folin Ciocalteu reagent. The decrease in protein content could also be due to the formation of

melanins between o-quinine compounds derived from the oxidation of polyphenols and protein molecules. The formation of these melanins would involve -SH or -NH₂ residues. These SH residues are essential for the reduction of Folin Ciocalteu reagent; their involvement in melanin formation would reduce the intensity of the blue color (Noble and Bailey, 2009). The decrease in protein content could further be explained by the complexation phenomena involving protein molecules and galacturonic acid monomers (Shomer et al., 1999). These phenomena could be dominant with the reaction products (monomers and oligomers) than with pectin macromolecules. Guava contains several types of phenolic compounds: anthocyanins, flavonoids, and proanthocyanidins. These compounds contain several hydroxyl groups (at least 3) likely to be involved in several types of reactions (Flores et al., 2015).

3.3.6. Effect of enzymatic treatment on the antioxidant capacity of guava juice

The antioxidant capacity of the initial juice is $94.671 \pm 0.325\%$. Figure 10 shows that the enzymatic treatment does not possess a significant effect on the antioxidant capacity of the samples compared to that of the untreated juice. The antioxidant capacity is that exerted by the soluble compounds in the ethanol used for the precipitation of the particles. The antioxidant capacity would be a function of the phenolic compounds soluble in alcohol and ascorbic acid which would be one of the major contributors.

The phenolic compounds that contribute to the antioxidant capacity would be those that are free since those complexed in particulate form would be removed by centrifugation. Twenty-one (21) phenolic compounds have previously been identified in guava cultivars. Among these compounds are two anthocyanins, ten flavonoids, two proanthocyanidins, two sesquiterpenoids, and five triterpenes. All of these compounds would have abilities to donate hydrogen atoms or to transfer electrons; thus, contributing to the antioxidant capacity (Flores et al., 2015).

A strong positive correlation was observed between antioxidant capacity, phenolic compounds, and ascorbic acid showing that these two families of compounds are essential contributors to the antioxidant capacity of guava. This correlation is characteristic of fruit with high levels of ascorbic acid such as guava and orange (Thaipong et al., 2006).

3.4. Analysis of samples by Field Emission Scanning Electron Microscopy (FESEM)

To visualize the different suspension, these were analyzed by Field Emission Scanning Electron Microscopy (FESEM). This technique enabled to obtain images of the dehydrated samples. The images allowed the samples to be analyzed for the particles they contain, the distribution of these particles, and the homogeneity of the samples. It also makes it possible to compare these samples with untreated puree (Figure 11A).

Figure 11A shows particles of different sizes that can vary between 60 nm and 5200 nm. The particle distribution is heterogeneous. Some particles are in the form of clusters with irregularities (asperities). Other particles, on the other hand, have a round shape; however, their magnification (Figure 11B) reveals that they are also provided with several asperities. These particles represent a cluster of tissues consisting of several polysaccharides and proteins forming a complex network. Pectin is the major constituent of this network in the primary cell wall (35% w/w). It tends, because of its solubility in water, as well as proteins, to form a gel around the other constituents of the wall and thus acting as a binder (Prasanna et al., 2007).

Figure 12 shows that the enzymatic treatment causes the disintegration of the particles observed on the images of the raw sample (Figure 12A) with the consequence of reducing the sizes of these particles. It also shows that juice samples after 8 min of treatment for all enzyme concentrations show an almost identical particle distribution. This treatment time is not fit to highlight the differences due to the variation of the enzyme concentration. For a given enzyme concentration, the increase in the treatment duration is accompanied by a gradual

appearance of homogeneous layers surrounding the particles. These layers represent the liquid phase, showing that the enzymatic treatment is accompanied by liquefaction of the mixture due to the release of water molecules. This release of the water molecules would occur during the hydrolysis of pectin (a compound of high-water retention capacity and hydraulic radius compared to the monomers that constitute them) with the consequent appearance of a liquid phase leading to lower viscosity (Akesowan and Choonhahirun, 2013). The hydrolysis of pectin would also be accompanied by a destruction of the macromolecule network with the result of a release of these in the medium. Images from 90 min samples show a higher proportion of the liquid phase. Analysis of the 90 min samples reveals that the one corresponding to the 0.1% w/w concentration has a higher proportion of the liquid phase. This sample has fewer particles and has better homogeneity. This can be explained by the fact that this sample corresponds to the combination of maximum values of treatment time and enzyme concentration. Samples corresponding to all enzyme concentrations still show particles after 90 min of treatment. However, the size of the particles, their distribution or their grouping vary with the increase of the enzyme concentration. With the enzyme concentration, the particle size decreases, the latter is more dispersed.

4. Conclusion

In sum, this paper examined the effect of the enzyme treatment of guava puree on the physicochemical parameters of the juice. The enzymatic treatment of the puree resulted in a significant decrease in the viscosity of the juice from the third minute of treatment. Viscosity decreased with increasing enzyme concentration. This enzyme treatment generated a significant increase in galacturonic acid content over time. Parameters such as pH, protein, and polyphenol levels decreased significantly during treatment; while others such as conductivity and color increased. However, enzyme treatment did not have a significant effect on the antioxidant capacity of the samples. Images of the dried extracts from the samples showed the decay of the particles and liquefaction of the mixture during enzymatic treatment. Images also showed that increased enzyme concentration led to an increase in the proportion of the liquid phase and improved homogeneity of the samples. It was also observed that particle size decreased during treatment and, with increased processing time, particles were more dispersed.

Declarations

Author contribution statement

Kombele Aime Ninga: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Zangue Steve Carly Desobgo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sirshendu De, Emmanuel Jong Nso: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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V.9) Optimization of Extraction
Conditions of Phenolic Compounds
from *Cymbopogon citratus* and
Evaluation of Phenolics and Aroma
Profiles of Extract

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Research article

Optimization of extraction conditions of phenolic compounds from *Cymbopogon citratus* and evaluation of phenolics and aroma profiles of extractWiyeh Claudette Bakisu Muala^a, Zangué Steve Carly Desobgo^{b,*}, Nso Emmanuel Jong^a^a Department of Process Engineering, National School of Agro-Industrial Sciences (ENSAI), University of Ngaoundere, P.O. Box 455, Adamaoua, Cameroon^b Department of Food Processing and Quality Control of University Institute of Technology (UIT) of The University of Ngaoundere, P.O. Box 455, Cameroon

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ABSTRACT

Decoction extraction procedure was implemented to regain phenolic compounds from *C. citratus* leaves. The extraction variables, solid/liquid ratio (2–5 g/100 mL), temperature (85–95 °C), and time (5–10 min) were assessed by central composite design for process optimization. Antioxidant activity (DPPH) and total polyphenol content (TPC) were monitored as responses. The TPC and DPPH were 71.98 ± 0.33 mg GAE/100 mL extract and 80.63 ± 0.49 mg TE/100mL extract respectively under optimal conditions (solid/liquid ratio = 5, temperature = 93.8 °C and time 11.3 min). The evaluation of phenolic compounds and volatile compounds of *C. citratus* extract at conditions for optimum extraction revealed that caffeic (20.81 ± 0.003 mg/100mL) and syringic acids (18.63 ± 7.390 mg/100mL) were the main phenolic compounds while citral and geraniol were the primary volatile compounds. The results achieved herein suits the potential use of *C. citratus* extract as natural source of antioxidant and aroma compounds that can be employed in different industrial sectors.

Practical application: Lemongrass obtained at the optimal extraction conditions is a good source of antioxidants and the extract has organic acids and a lemon scent due to the presence of citral. This extract can thereby be incorporated in the production of beverages which can help aromatize the beverage and also contribute in the addition of the antioxidant property of the beverage. It is also rich in organic acids, the main being propionic acid, which is known to have antimicrobial activity primarily against bacteria and mold. The lemongrass extract can therefore, extend the shelf life of the beverage they are incorporated in and also the citral present in lemongrass has antimicrobial properties.

1. Introduction

Cymbopogon citratus (lemongrass) is a tall perennial grass of the family Poaceae commonly cultivated in humid subtropical and tropical regions of the world (Olorunnisola et al., 2014). Lemongrass is rich in minerals, vitamins, and macronutrients (carbohydrate, protein, and small amounts of fat). These leaves also are good source of various bioactive compounds including alkaloids, terpenoids, flavanoids, phenols, saponins and tannins that confer *C. citratus* leaves pharmacological properties such as anti-cancer, antihypertensive, anti-mutagenicity, anti-diabetic, anti-oxidant, anxiolytic, anti-nociceptive and anti-fungi (Balakrishnan et al., 2014; Olorunnisola et al., 2014). In this new era, the search of food ingredients rich in bioactive components is increasing due to the outbreak of COVID-19 caused by the SARS-CoV-2 virus. Foods rich in bioactive compounds are advantageous because they boost the immune

system and natural polyphenols have exhibited properties as inhibitors of COVID-19 main protease (Galanakis, 2020).

Lemongrass has been used either as fresh leaves, dried powdered concentrated extract, or essential oil depending on the application. Several conventional and non-conventional methods are used in the extraction of bioactive components from plants (Zinoviadou et al., 2015). Non-conventional methods like ultrasound, microwave-assisted extraction, high pressure combined with thermal processing, supercritical carbon dioxide (SC-CO₂), pulsed electric fields assisted processing (Deng et al., 2014; Roselló-Soto et al., 2015a,b; Siewe et al., 2019, 2021) have been employed in the extraction of polyphenols from different plant sources. These emerging separation techniques are advantageous because they use limited extraction time and solvent, polyphenol yield is high (Jovanovic et al., 2017) and they also have minimal impact on sensorial and nutritional properties (Zinoviadou et al., 2015; Siewe et al., 2019). However, the extraction of these bioactive components depends

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on the matrix and solvent used and the emerging techniques are used in combination with other techniques for better yield (Jovanovic et al., 2017; Siewe et al., 2019). Up to date, the lemongrass extract from infusion method remains the widely employed methods owing to its lower cost and simplicity. Some process parameters, like, process time, water/substrate ratio, and temperature has been declared as the principal factors for efficient extraction of bioactive components from plants (Obboh et al., 2010; Uma et al., 2010; Roseiro et al., 2013; Thangam et al., 2014). The application of unsuitable conditions during the extraction procedure could lead to the degradation of target compounds and reduce extraction efficiency. Therefore, optimizing the extraction process can aid in choosing suitable process conditions for improving the extraction yield of bioactive compounds. Response surface methodology (RSM), an assembly of statistical and mathematical methods, is widely used in all sectors to optimize operating conditions of processes. With RSM, there is a limited number of experimental runs, which facilitates its application to the development, improvement, and optimization of operating conditions for a process or product (Dean et al., 2017). Thangam et al. (2014) used Box-Behnken design, a three-level factorial design, to survey the extraction of hot water-soluble polysaccharides (HWSPs) from *Cymbopogon citratus* via decoction and at the end did not evaluate the various polyphenols in the extract. The Box-Behnken design is efficient and economical but lacks accuracy (Lundstedt et al., 1998). However, central composite design is a full factorial or fractional factorial design with axial points in which experimental points are at a distance α from its center, the design is studied at five levels and has additional center points (Bezerra et al., 2008). Enough replication of center points, allow for a test for model lack of fit (Dean et al., 2017). Hence, the objective of this present study was to optimize the polyphenol and antioxidant extraction from lemongrass by decoction method using central composite design. In addition, the aroma profile and organic acid content of the lemongrass extract at optimum conditions were determined.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

HPLC grade phenolic standards, were obtained from Sigma-Aldrich. HPLC grade acetonitrile, acetic acid, methanol were from Merck, HCl (hydrochloric acid) and Folin-ciocalteu reagent were purchased from Merck. HPLC grade organic acid standards were from Sigma-Aldrich. Sodium carbonate, DPPH, Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) were supplied from Sigma-Aldrich (St. Louis, MO, USA). All chemicals utilised were of analytical grade.

2.1.2. Biological materials

C. citratus leaves were collected from a plantation in Bini-dang, Ngaoundere, Adamawa region, Cameroon in the month of October, 2018. They were washed with running tap water, cut into 2 cm cuts and oven-dried at 60 °C for 3h. Dried leaves were ground, sieved with a 1 mm sieve, packaged and stored at -40 °C in order to preserve its quality until use.

2.2. Methods

2.2.1. Determination of proximate and bioactive composition of lemongrass leaves

Moisture, ash, crude fiber, protein, and fat contents were estimated by procedures provided by the Association of Official Analytical Chemists (AOAC, 2005). The phytochemical content of *C. citratus* leaves was extracted in 80% methanol, and TPC, FRAP, and DPPH analyzed in the extract.

2.2.2. Decoction method

The lemongrass powder was extracted with distilled water at lemongrass to water ratio (2–5 g/100mL), temperature between (85–95 °C) and time (5–10 min). In brief, the water bath (Julabo TW8, Germany)

was set at the temperature, as imposed by the design of experiments. The conical flask containing distilled water was deposited in the water bath until it reached the set temperature. The lemongrass powder was then introduced in the conical flask and agitated at 150 rpm. After extraction, samples were cooled immediately in ice water to reach room temperature (28–30 °C). The slurry was further filtrated using Whatman paper N° 4. The filtrate was rinsed three times with distilled water, and all three extracts were mixed. The volume of the total extract was adjusted to 100 mL with distilled water and referred to as lemongrass extract.

2.2.3. Experimental design, modelling, validation of model, and optimization

The orthogonal quadratic central composite design (CCD) was utilized to scrutinize the decoction process. Table 2 presents the factors and their coded levels utilized for the CCD. The independent factors studied were lemongrass powder to water ratio (x_1), decoction temperature (x_2), and extraction time (x_3). The range of factors were; x_1 , 2–5g/100 mL; x_2 , 85 to 95 °C, and x_3 , 5–10 min. The CCD consisted of 20 trials, and each trial was done in triplicate, and the average responses (TPC and DPPH) were reported. The mathematical model employed was a second-degree polynomial model with linear, quadratic, and interaction terms (equation 1).

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

Where Y is the response, β_0 is the constant term, β_i are the linear coefficient terms, β_{ii} are the quadratic coefficient terms, β_{ij} are the interaction coefficient terms and x_i and x_j the factors.

From the coded variables, Eq. (2) was used to transform them into real values to realize experiments in the laboratory. The equation is as follows:

$$X_i = X_{0i} + x_i \Delta X_i \quad (2)$$

The value of α and the number of experiment N (Eqs. (3) and (4) respectively) were calculated in order to respect the orthogonality criterion and using the formulas:

$$\alpha = \left(\frac{2^k (\sqrt{2^k + 2k + n_0} + \sqrt{2^k})^2}{4} \right)^{\frac{1}{4}} \quad (3)$$

$$N = k^2 + 2k + n_0 \quad (4)$$

Where: k is the number of variables, n_0 is the number of trials in the centre.

The CCD matrix was obtained by Minitab 19.2 (2019 Minitab, LLC, USA). R-square (R^2), R-square adjusted ($\text{adj-}R^2$), absolute average deviation (AAD) (equation 5), the bias factor (B_f) (equation 6), and the accuracy factor (A_f) (equation 7) were utilized to validate the models.

$$AAD = \frac{\left[\sum_{i=1}^N \left(\frac{|Y_{i,\text{exp}} - Y_{i,\text{cal}}|}{Y_{i,\text{exp}}} \right) \right]}{N} \quad (5)$$

$$B_f = 10^{\frac{1}{N} \sum_{i=1}^N \log \left(\frac{Y_{i,\text{cal}}}{Y_{i,\text{exp}}} \right)} \quad (6)$$

$$A_f = 10^{\frac{1}{N} \sum_{i=1}^N \left| \log \left(\frac{Y_{i,\text{cal}}}{Y_{i,\text{exp}}} \right) \right|} \quad (7)$$

Where $Y_{i,\text{exp}}$ is the responses, $Y_{i,\text{cal}}$ the calculated responses, and N is the number of experiments used in the calculation.

The statistical analyses of the experimental design data and plotting of surface plot were realized using the softwares, Minitab 19.2 and OriginPro 2019b (9.6.5.169, OriginLab Corporation). ANOVA test was utilized to obtain the statistical significance of the regression coefficient on the level of significance declared at $p \leq 0.05$.

Lastly, optimization was executed in one hand using Minitab 19.2 (2019 Minitab, LLC, USA). The conditions fixed were to maximize both total polyphenol content and DPPH Radical Scavenging Activity. A composite optimal was considered for the two responses and the responses at the composite optimal verified.

2.3. Physicochemical analyses

2.3.1. Determination of total polyphenol content

The TPC of the lemongrass extract was analyzed according to the method mentioned by Marigo (1973) with modifications. 20 μ L of the extract was mixed with 680 μ L of distilled water and 100 μ L of 1N folin-ciocalteu reagent (FCR). The mixture was kept for 5 min, and 200 μ L of 20% Na_2CO_3 was introduced in the test tube. The whole mixture was agitated and incubated at 40 °C for 20 min in the dark. The blue complex absorbance was read at 725 nm using TECAN microplate reader (Infinite M200 PRO, Tecan Austria). Standard gallic acid (0.001–0.008 g/L) was used to construct the calibration curve and the results were expressed as mg of gallic acid equivalent (GAE)/100 mL extract. All samples were analyzed in triplicate and an average taken.

2.3.2. Estimation of DPPH radical scavenging activity

The DPPH radical scavenging activity was performed as mentioned by Shimada et al. (1992) with some modifications. Briefly, 30 μ L of lemongrass extract was reacted with 150 μ L of 0.1 mM DPPH in 96-well microplate. The mixture was homogenized and incubated at 37 °C for 30 min. The absorbance was then measured at 517 nm and a standard curve was constructed using standard Trolox in the range of 0–50 μ g/mL. The activity was expressed as mg Trolox equivalents (mg TE)/100 mL of extract.

2.3.3. Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was assessed with respect to the method of Benzie and Strain (1996) with slight modifications. FRAP solution was prepared fresh daily by mixing 0.3 M acetate buffer (pH 3.6), 0.01 M TPTZ in 40 mM HCl, and FeCl_3 (20 mM) in the ratio 10:1:1 respectively. Then, 30 μ L of the extract was mixed with 200 μ L of FRAP solution (freshly prepared). The mixture was homogenized properly and incubated for 30 min at 37 °C in dark conditions. The ferrous tripyridyltriazine complex formed was measured at 595 nm against a blank prepared in the same manner using distilled water instead of the sample. A standard curve was prepared using Trolox in the range of 0–50 μ g/mL, and the activity was expressed as mg TE/100 mL extract.

2.3.4. Analysis of phenolic compounds

HPLC analyses for polyphenol compounds of lemongrass extract was performed on LC-10AS (Shimadzu, Kyoto, Japan) system equipped with Shimadzu LC-10AT pumps, and CBM-20A communication bus module. Chromatography was carried out in a gradient system using 250 $\times$ 4.60 mm, 5 μ m C18 column (Gemini, Phenomenex, California, USA). A flow rate of 1 mL/min and the injection volume of 10 μ L was employed. The mobile phases consisted of A (99.9% acetonitrile and 0.1% acetic acid) and B (0.1% acetic acid in MQ water). The gradient elution was 0–15min (8% A and 92% B), 30min (22% A and 78% B), 45min (78% A and 22% B), 55min (8% A and 92%B), and 60min (8% A and 92% B). A UV-Visible DAD detector was used, and the wavelengths detected at 280 and 320nm. Pure standards were injected at different concentrations and the calibration curves obtained. Polyphenols were identified by comparing their retention times with those of pure standards and the polyphenol concentration of each compound calculated with respect to the calibration curves of the standards (Sonmezdag et al., 2017).

2.3.5. Analysis of organic acids

The organic acid profile was obtained following the method of Sharma and Devi (2018) with slight modifications. Organic acids were determined using LC-8A HPLC (Shimadzu, Kyoto, Japan) equipped with

SPD-M10A VP diode array detector. A C18 (250 $\times$ 4.60 mm, 5 μ m) column (Gemini, Phenomenex, California, USA) was used, and the mobile phase was 8 mM H_2SO_4 . Ten microliters of the extract were injected, and the separation of organic acids was carried out at a flow rate of 1 mL min^{-1} for 30 min. Standards organic acids were run for identification and quantification of individual organic acids.

2.3.6. Volatile analysis

Extraction and analysis of volatile compounds in lemongrass extract was done according to the method described by Siewe et al. (2020) with some modifications. The solid-phase microextraction (SPME) method was used in extraction of volatile compounds from lemongrass extract. 5 mL of lemongrass extract in well-sealed glass vials (20 mL) were heated at 50 °C for 20 min in a water bath. The aroma compounds trapped in the headspace of the vials were adsorbed for 30 min with the 2 cm, 50/35 μ m Carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) fiber (Supelco Inc., Bellefonte, USA). The adsorbed fiber was directly introduced into a 7890B Agilent GC injector port (Agilent Technologies, Santa Clara, California, USA) at 250 °C for 3 min to desorb the volatile compounds. The RT-WAX capillary column (60 $\times$ 0.25 mm, 0.25 μ m; J & W Scientific, Folsom, CA) assisted in separating the volatile compounds. The program of the GC column was set at 40 °C for 3 min, then the temperature was increased with an increment of 5 °C/min to 235 °C, and maintained for 10 min. The carrier gas, helium was used at a flow rate of 1.8 mL/min. The temperature and electron voltage of the mass spectrometric detector was operated at 230 °C and 70 eV respectively with the transfer line temperature of 250 °C. The chromatogram was recorded in the range of 40–450 amu of the total ion current.

The mass spectra of the volatile compounds obtained were identified by comparing with the mass spectra database of the US National Institute of Standards and Technology (NIST). Calculations of the relative percentage (% area) were based on the ratio between the peak area of each compound and the sum of areas of all compounds (Pino and Barzola-Miranda, 2020).

3. Results and discussion

3.1. Proximate composition, polyphenol content and DPPH activity of *C. citratus* extract

The proximate and phytochemical composition of lemongrass is presented on Table 1. Lemongrass leaves contain nutrients (proteins, carbohydrate and fibers) which explains its use. The moisture content of dried lemongrass, $10.09 \pm 0.06\%$, lower than 11.35% determined by Uraku et al. (2016) shows it is desirable for longer periods of storage and less attack of microorganisms.

The value of ash content $8.06 \pm 0.05\%$ indicates the presence of minerals in lemongrass leaves Asaolu et al. (2009). The protein content

Table 1. Proximate composition of *C. citratus* L. extract.

Proximate	
Component	Content (g/100 g DW sample)
Moisture content	10.09 ± 0.06
Ash	8.06 ± 0.05
Fat	4.45 ± 0.1
Proteins	8.40 ± 0.05
Crude fibre	30.32 ± 0.65
Total carbohydrate	44.16 ± 0.77
Polyphenol content (mg/g DW sample)	
TPC	16.56 ± 0.05
Antioxidant activities (mg TE/100 g DW sample)	
DPPH	1261.30 ± 3.28
FRAP	1920.92 ± 2.59

Table 2. Central composite design coded values, real values and experimental responses.

Run	Coded variables			Real variables			Experimental responses	
	Solid/liquid ratio (w/v)	Decoction temperature (°C)	Extraction time (min)	Solid/liquid ratio (w/v)	Decoction temperature (°C)	Extraction time (min)	DPPH (mg TE/100 mL extract)	TPC (mg GAE/100 mL extract)
19	0	0	0	3.5	90	7.5	58.73 ± 0.18	52.62 ± 0.24
10	1.52	0	0	5.78	90	7.5	85.26 ± 0.15	65.92 ± 0.03
2	1	-1	-1	5	85	5	77.13 ± 0.49	59.39 ± 0.16
8	1	1	1	5	95	10	77.01 ± 0.01	68.09 ± 0.07
1	-1	-1	-1	2	85	5	38.09 ± 0.05	32.29 ± 0.62
9	-1.52	0	0	1.22	90	7.5	19.10 ± 0.06	23.32 ± 0.41
13	0	0	-1.52	3.5	90	3.7	63.69 ± 0.17	43.78 ± 0.30
11	0	-1.52	0	3.5	82.4	7.5	51.09 ± 0.51	44.15 ± 0.11
14	0	0	1.52	3.5	90	11.3	55.93 ± 0.01	55.04 ± 0.05
18	0	0	0	3.5	90	7.5	59.08 ± 0.04	56.17 ± 0.10
3	-1	1	-1	2	95	5	36.92 ± 0.03	29.57 ± 0.11
17	0	0	0	3.5	90	7.5	61.01 ± 0.01	55.08 ± 0.55
12	0	1.52	0	3.5	97.6	7.5	54.04 ± 0.53	54.33 ± 0.51
4	1	1	-1	5	95	5	79.95 ± 0.04	60.05 ± 0.65
6	1	-1	1	5	85	10	73.40 ± 0.38	64.96 ± 0.23
5	-1	-1	1	2	85	10	25.50 ± 0.33	28.80 ± 0.49
7	-1	1	1	2	95	10	34.43 ± 0.17	38.46 ± 1.13
15	0	0	0	3.5	90	7.5	55.93 ± 0.24	55.92 ± 0.85
16	0	0	0	3.5	90	7.5	58.49 ± 0.10	56.41 ± 0.57
20	0	0	0	3.5	90	7.5	58.12 ± 0.53	53.70 ± 0.85

(8.40 ± 0.05%) was higher compared to 4.56% reported by Asaolu et al. (2009) and in agreement with 8.51% reported by Ojo (2017). The crude fiber content (30.32 ± 0.65%) indicates lemongrass leaves are an adequate source of crude fiber compared to other conventional plant leaves (Asaolu et al., 2009; Nambiar and Matela, 2012). The carbohydrate content of 44.16 g/100 g sample indicates it is a good energy source. The variations in the composition of lemongrass leaves with literature could be due to differences in maturity stage and geographical location of the plant (Ranjah et al., 2019).

Polyphenol compounds are one of the vital group of compounds acting as main antioxidants which contributes to the medicinal value of various plants (Kouassi et al., 2017). The total polyphenol content of the methanol extract of *C. citratus* was found to be 16.56 ± 0.05 mg GAE/g DW. The antioxidant activities, DPPH and FRAP activities were found to be 1261.30 ± 3.28 and 1920.92 ± 2.59 mg TE/100 g DW, respectively. The total

polyphenol content and antioxidant activities of *C. citratus* leaves were different from those stated in literature (Kouassi et al., 2017; Nambiar and Matela, 2012; Sah et al., 2012). The values of 118.14 ± 1.05 mg GAE/g and 178.069 ± 1.57 mM TE/mL for TPC and antioxidant was observed by Kouassi et al. (2017); 1324.9 ± 31.06 mg %, 15.96 ± 0.53 and 23.40 ± 1.19 µmol TE/g DW for TPC, DPPH and FRAP respectively was realized by Nambiar and Matela (2012). The variations in the polyphenol content and antioxidant activities of *C. citratus* leaves compared to that of literature could be as a result of the maturity stage, geographical location and extraction method (Adeyemo et al., 2018; Ranjah et al., 2019).

3.2. Mathematical modelling

The results of the 20 experimental runs with their responses are presented in Table 2, while the ANOVA results are put forward in Table 3.

Table 3. Analysis of variance for the regression models of TPC and DPPH.

Source	TPC (mg GAE/100 mL extract)			DPPH (mg TE/100 mL extract)		
	Sum of squares	F-value	P-value	Sum of squares	F-value	P-value
Model	3239.34	62.97	<0.0001	6169.45	213.63	<0.0001
X ₁	2804.15	1210.94	<0.0001	5909.96	2203.64	<0.0001
X ₂	54.35	23.47	0.0047	27.65	10.31	0.0237
X ₃	103.35	44.63	0.0011	89.13	33.24	0.0022
X ₁ X ₂	1.25	0.54	0.4960	0.22	0.081	0.7878
X ₁ X ₃	8.44	3.65	0.1145	8.85	3.298	0.1290
X ₂ X ₃	27.57	11.91	0.0182	14.77	5.507	0.0658
X ₁ ²	163.71	70.698	0.0004	58.40	21.774	0.0055
X ₂ ²	39.07	16.87	0.0093	50.38	18.786	0.0075
X ₃ ²	36.14	15.61	0.0108	10.04	3.74	0.1107
Residual	57.16			32.09		
Lack of fit	45.58	3.94	0.0794	18.68	1.39	0.3625
Pure error	11.58			13.41		
Cor total	3296.50			6201.54		

X₁, X₂, and X₃ represents the linear effects (solid/liquid ratio, temperature and time respectively); X₁₂, X₁₃ and X₂₃ are the different interactions and X₁², X₂², and X₃² the quadratic effects.

Table 4. Model validation parameters.

Model	R ²	R _{adj}	AAD	B _f	A _f
TPC	0.9827	0.9671	0.0011	1.0004	1.0034
DPPH	0.9948	0.9902	0.0016	1.0012	1.0218

The significance of the model terms and model equations were validated with respect to the p -value ($p \leq 0.05$). The models of both responses were highly significant ($p < 0.0001$). The linear coefficients (x_1 , x_2 , x_3), the interaction (x_2x_3) and the quadratic terms (x_1^2 , x_2^2 , x_3^2) were the significant model terms ($p \leq 0.05$) for TPC while for DPPH scavenging activity, the linear terms (x_1 , x_2 , x_3), and the quadratic terms (x_1^2 , x_2^2) were significant ($p \leq 0.05$). The model validation terms, lack of fit, coefficient of determination (R^2), adjusted R^2 (adj- R^2), AAD, B_f and A_f are presented in Table 4. The lacks of fit for both models were not significant ($p > 0.05$), revealed that no considerable improvement was achieved by the inclusion of the statistically parametric values.

The coefficient of determination R^2 were 0.9829 and 0.9948 for TPC and DPPH, respectively; indicating that both mathematical models can explain 98.29% and 99.48% (respectively for TPC and DPPH) experimental observations as a function of independent variables. Besides, adj- R^2 of both models (0.9674 and 0.9900 for TPC and DPPH, respectively) were within close range to their respective coefficient of determination indicating that the variability of each response can be explained by the independent variables involved in the process. Joglekar and May (1987) suggested that R^2 should at least be 80% for model fit; therefore, the empirical models of TPC and DPPH fits the actual data models. Baranyi et al. (1999) and Ross (1996) stated, in addition to R^2 , other validation model terms, AAD, bias and accuracy factors are of great interest to be considered. They measure the relative average deviation of predicted and observed responses. An AAD of 0 and a bias factor and accuracy factor of 1 indicate model adequacy. In this study, all the validation terms fell within the accepted range of model validity which affirms the validity of the model (Table 4). The empirical equations developed for TPC (equation 8) and DPPH (equation 9) activity are as follows:

$$TPC = 54.735 + 9.806x_1 + 1.365x_2 + 1.883x_3 - 1.692x_1^2 - 0.827x_2^2 - 0.795x_3^2 - 0.171x_1x_2 + 0.445x_1x_3 + 0.803x_2x_3 \quad (8)$$

$$DPPH = 58.377 + 14.237x_1 + 0.974x_2 - 1.748x_3 - 1.011x_1^2 - 0.939x_2^2 + 0.419x_3^2 - 0.071x_1x_2 + 0.455x_1x_3 + 0.588x_2x_3 \quad (9)$$

3.2.1. Singular and quadratic effects on TPC and DPPH

In this case, to view the effect of a singular factor, the other factors were fixed at their minimal value to lessen their contribution. That means for lemongrass powder/water ratio (x_1), for decoction temperature (x_2) and extraction time (x_3), the respective fixed values were: 1.22; 82.4 °C and 3.7 min.

3.2.1.1. Effect of lemongrass powder/water ratio (x_1). The lemongrass powder/water ratio (x_1) has a significant impact on TPC and DPPH (Table 3). It is observed from Figure 1 that, at an initial ratio value of 1.22 g/100 mL, the values of 29.72 mg GAE/100 mL and 36.62 mg TE/100 mL were obtained for TPC and DPPH respectively.

An increase of that ratio until 5.78 g/100 mL, generated a significant increase of TPC and DPPH respectively to 58.27 mg GAE/100 mL and 78.13 mg TE/100 mL. At a fixed decoction temperature of 82.4 °C and a fixed extraction time of 3.7 min, an increase in ratio led to increase in the amounts of TPC and DPPH activity in the extract.

The solid/liquid ratio was found to be the essential factor ($p \leq 0.0001$) that affected the yield of TPC and DPPH activity of the lemongrass leaves extract. As the solid/liquid ratio increased, the TPC and DPPH activity increased significantly. However, when the solid/liquid ratio was extended beyond the critical limit (quadratic effect), a marked decline in TPC was observed while the DPPH activity was constant. Indeed, increasing the mass with a constant solvent volume caused molecule congestion which decreased mass transfer hence, decrease in the extraction of total polyphenols.

3.2.1.2. Effect of decoction temperature (x_2). The decoction temperature has a significant positive and negative impact on TPC and DPPH (Table 3). Firstly, it is observed (Figure 2) that, at an initial decoction

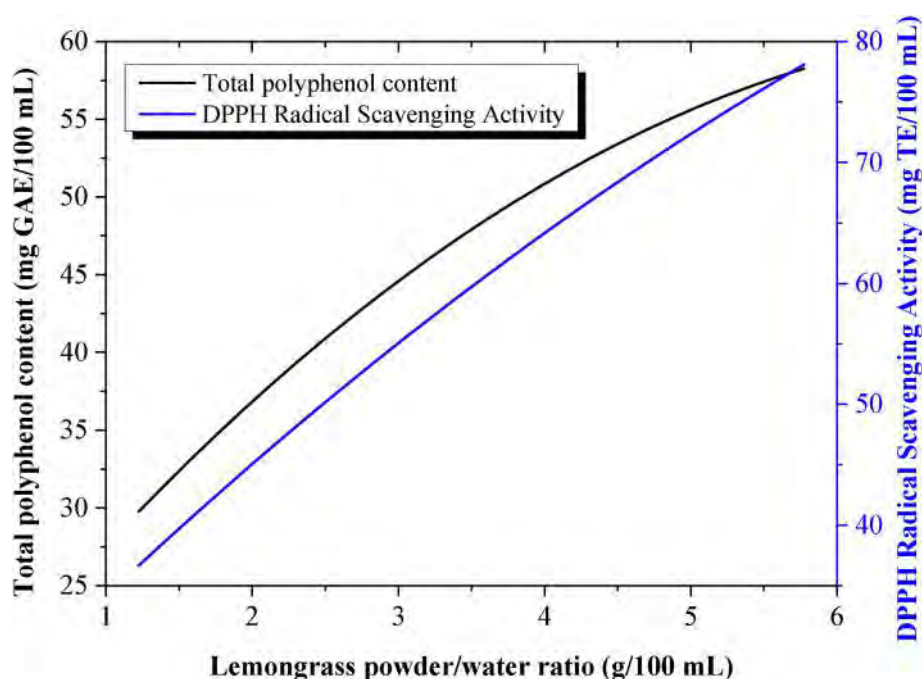


Figure 1. Evolution of Total polyphenol content and DPPH radical scavenging activity as a function of lemongrass powder/water ratio. Decoction temperature and extraction time respectively fixed at 82.4 °C and 3.7 min.

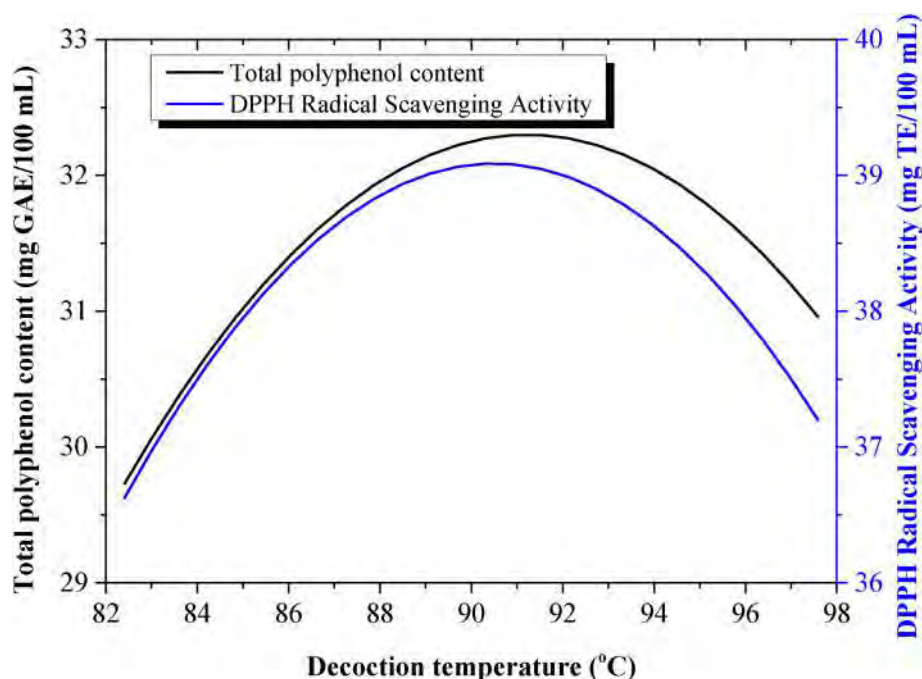


Figure 2. Evolution of Total polyphenol content and DPPH radical scavenging activity as a function of decoction temperature. Lemongrass powder/water ratio and extraction time respectively fixed at 1.22 and 3.7 min.

temperature of 82.4 °C, the values of 29.72 mg GAE/100 mL and 36.62 mg TE/100 mL were obtained for TPC and DPPH respectively. These values increased to reach a maximum of 32.29 mg GAE/100 mL for TPC at 91.2 °C and, 39.08 mg TE/100 mL for DPPH at 90.5 °C. After that, a significant decrease was obtained up to 30.95 mg GAE/100 mL for TPC and 37.19 mg TE/100 mL for DPPH, when increasing the decoction temperature to 97.6 °C.

The TPC and DPPH activity increased with the rise of extraction temperature and then level off at high temperature. The increment in TPC and DPPH activity is due to the fact that high temperatures soften

cell wall tissue and hydrolyse the phenolic compounds present thereby enhancing the solubility of polyphenols into the solvent (Cacace and Mazza, 2003; Irakli et al., 2018). The solvent thereby penetrates the plant matrix and results in the mass transfer of compounds from the matrix into the solvent (Jovanovic et al., 2017). However, a further increase of the temperature led to the decrease of both TPC and DPPH activity suggesting that high temperature may have caused the degradation of phenolic compounds resulting in a decrease in DPPH activity.

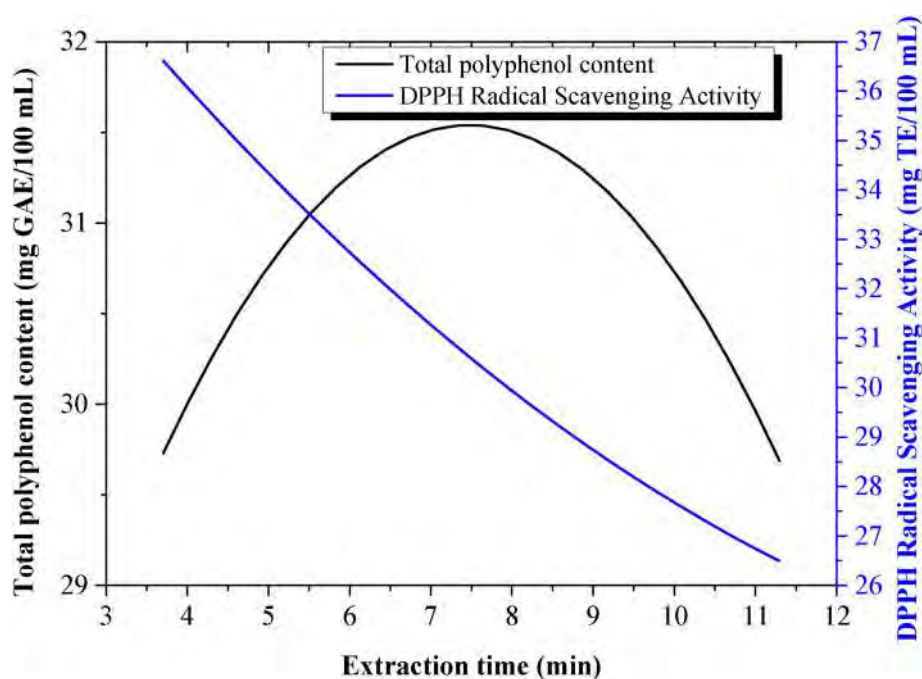


Figure 3. Evolution of Total polyphenol content and DPPH radical scavenging activity as a function of extraction time. Lemongrass powder/water ratio and decoction temperature respectively fixed at 1.22 and 82.4 °C.

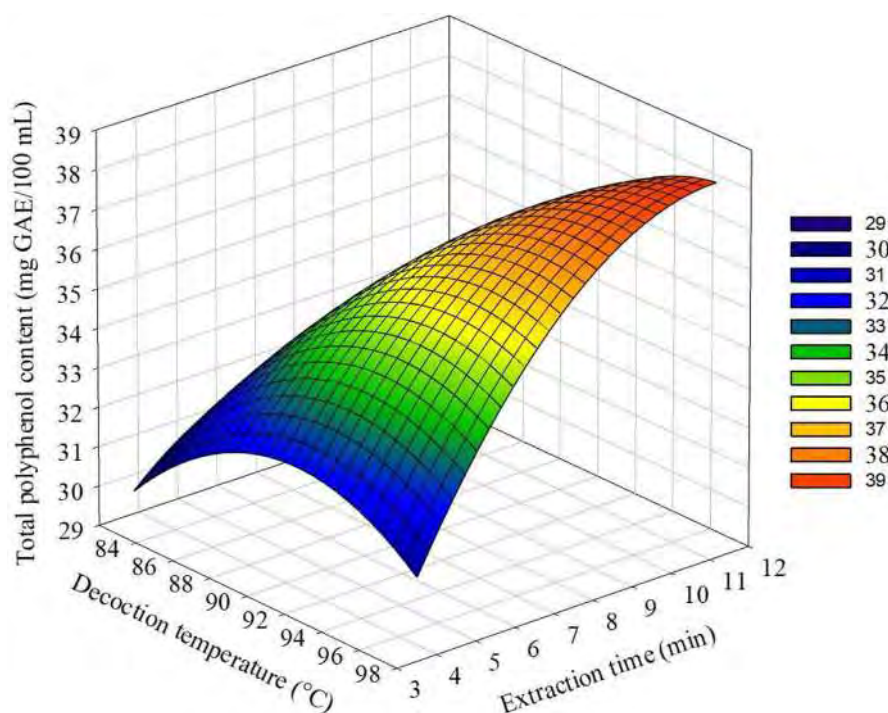


Figure 4. Mesh plot of the evolution of Total polyphenol content as a function of decoction temperature and extraction time. Lemongrass powder/water ratio fixed at 1.22.

3.2.1.3. Effect of extraction time (x_3). The extraction time has a negative significant impact on DPPH while, it is noted in the first hand a positive significant and after a significant negative impact on TPC (Figure 3). For TPC, the value of 29.72 mg GAE/100 mL was obtained at 3.7 min extraction time and increased with a rise in extraction time to reach a max of 31.54 mg GAE/100 mL at 7.47 min extraction time. After that, TPC decreased to 29.68 mg GAE/100 mL at 11.3 min extraction time.

This could be explained by that, as the lemongrass get in to contact with the hot liquid, there is immediate dissolution of the phenolic compounds, and diffusion of target analytes from materials to outside solvent. A relatively lengthy extraction time contributed to a positive influence on the TPC. However, extended extraction time led to degradation of polyphenols and lowering of DPPH activity due to the thermolabile feature of phenolic compounds. This finding is in accord with that of Jeszka-Skowron and Zgola-Grzeskowiak (2014) who realized that extended extraction time of *Camellia sinensis* caused a decrease in rutin and chlorogenic acid content.

3.2.2. Effect of interaction decoction temperature/extraction time (x_2x_3) on TPC

The synergistic effect of temperature and time generated a positive impact (the increase) on the TPC. With increase in temperature at a short time (Figure 4), the plant matrix is fragilized and, the solvent enters the cell leading to a mass transfer of soluble compounds from the matrix into the solvent (Jovanovic et al., 2017).

3.2.3. Determination of optimal conditions

Composite desirability was effectuated to find the composite optimum by maximizing the TPC and DPPH (Table 5). Lemongrass was

therefore extracted at the composite optimum (solid/liquid ratio; 5g/100 mL, temperature: 93.8 °C and time: 11.3 min) and analyzed. The experimental values of TPC and DPPH obtained at the optimal conditions were 71.98 ± 0.33 mg GAE/100 mL of extract and 80.63 ± 0.49 mg TE/100 mL of extract, respectively. Compared to the predicted response of 73.00 mg GAE/100 mL extract for polyphenol and 87.75 mg TE/100mL extract for DPPH, the experimental data was in conformity.

Comparing to the results of Obboh et al. (2010), they obtained a total polyphenol content of 0.5 mg GAE/g and DPPH radical scavenging activity of 70% for hot water extracts of lemongrass at conditions (lemongrass concentration 10 g/100 mL, temperature 100 °C and time 10 min). The differences could be as a result of the different extraction conditions used and the temperature of extraction has a major effect on the polyphenol content as described above. Due to the importance of natural polyphenols, many works are done in recovering polyphenols from different sources and with different solvents (Galanakis et al., 2013; Rahmanian et al., 2014) for their applications in food and cosmetics.

3.2.4. Characterisation of *C. citratus* extract obtained at optimum conditions

3.2.4.1. Phenolic compounds of *C. citratus* extract. Phenolic acid compound content of the lemongrass leaf extract is presented in Table 6. Caffeic (20.816 ± 0.003 mg/100mL) and syringic (18.635 ± 7.390 mg/100mL) acids were the prominent phenolic acid compounds in the lemongrass extract. Other phenolic compounds (Gallic acid, dihydroxybenzoic acid, catechin, vanillic acid, epicatechin, *p*-coumaric acid, trans-ferulic acid, quercetin, and trans-cinnamic acid) were present in small amounts. Caffeic acid was also reported the main phenolic acid compound present in lemongrass infusion (Coelho et al., 2016). Other

Table 5. Composite desirability of total polyphenol and DPPH activity of *Cymbopogon citratus* extract.

Solid/liquid ratio	Temperature (°C)	Time (min)	Predicted TPC (mg GAE/100 mL)	Experimental TPC (mg GAE/100 mL)	Predicted DPPH (mg TE/100 mL)	Experimental DPPH (mg TE/100 mL)	Desirability value
5	93.8	11.3	73.00	71.98	87.75	80.63	0.92

Table 6. Phenolic acid content of *C. citratus* L. extract.

Compound	Concentration (mg/100 mL)
Gallic acid	3.932 ± 0.515
Dihydroxybenzoic acid	3.411 ± 0.121
Catechin	9.433 ± 5.493
Vanillic acid	5.979 ± 2.172
Caffeic acid	20.816 ± 0.003
Syringic acid	18.635 ± 7.390
Epicatechin	3.765 ± 1.243
<i>p</i> -coumaric acid	0.881 ± 0.394
Trans ferulic acid	0.582 ± 0.173
Quercetin	6.068 ± 0.326
Trans cinnamic acid	0.251 ± 0.010

phenolic acids (chlorogenic acid, *p*-coumaric acid, ferulic acid, quercetin, rosmarinic acid) that can arouse the antioxidant activities of lemongrass extract were also present in small amounts.

Otherwise, [Rodrigues et al. \(2015\)](#) instead detected a high quantity of chlorogenic acid in hot and cold extracts of lemongrass. [Kouassi et al. \(2017\)](#) also detected the presence of protocatechuic acid, caffeic acid, rutin, *p*-coumaric acid, ferulic acid, quercetin, kaempferol in ethanol and methanol extracts of lemongrass. Harvest region and seasons might account for the differences in profile and content in phenolic compounds ([Costa et al., 2016](#)). Likewise, the difference in profile and content might be the main factors that drive the difference in antioxidant activities of lemongrass extract.

Due to the antioxidant properties of natural polyphenols, they have gained applications in several fields. They have been applied in chicken patties ([Ibrahim and Abu Salem, 2013](#)), in herbal cookies ([Thorat et al., 2017](#)), in meat products ([Siewe et al., 2015](#); [Galanakis, 2018](#)), chicken sausage ([Boeira et al., 2018](#)) ([Boeira et al., 2018](#)), and as UV booster in cosmetics ([Galanakis et al., 2018](#)).

3.2.4.2. Organic acids of lemongrass extract. An organic acid is an organic compound with acidic properties known to affect particularly taste formation and many physiological functions ([Theron and Lues, 2010](#)). Organic acids content of lemongrass extract are displayed in [Table 7](#).

Propionic acid was the principal organic acid with a concentration of 20.137 ± 0.163 mg/mL, followed by glutaric acid, succinic acid, citric acid, tartaric acid, citric acid, malic acid, oxalic acid in descending order. Organic acids are traditionally employed as food preservatives because they exhibit antimicrobial inhibitory activities and also act as acidulants. Propionic acid, the main acid found in lemongrass is known to have antimicrobial activity primarily against molds and bacteria ([Theron and Lues, 2010](#)), this explains the antibacterial effect of lemongrass ([Balakrishnan et al., 2014](#); [Ekpenyong et al., 2015](#)). Succinic acid, citric acid, malic acid and tartaric acid are employed in industry as acidulants to modulate the taste of juice. Citric acid is the primal acid found in fruits and it possess a fresh acidic flavor and a pleasant taste. Malic acid has a smooth lingering taste, tart taste but not as sharp as that of citric acid ([Theron and Lues, 2010](#)).

Table 7. Organic acid content of *C. citratus* extract.

Organic acid	Concentration (mg/mL)
Oxalic	0.009 ± 0.002
DL-Tartaric	0.131 ± 0.022
L-malic	0.038 ± 0.009
Isocitric acid	0.217 ± 0.025
Citric	0.059 ± 0.008
Succinic	0.259 ± 0.007
propionic	20.137 ± 0.163
Glutaric	0.459 ± 0.106

Table 8. Volatile compounds content (%) of *C. citratus* extract.

Composition	RT	% composition
Hydrocarbons		4.13 ± 0.31
Undecane	5.431	2.38 ± 0.73
Naphthalene, decahydro-1,6-dimethyl-	25.664	1.75 ± 0.37
Esters		11.35 ± 3.44
Oxalic acid, allyl ethyl ester	3.64	0.92 ± 0.21
Acetic acid, butyl ester	5.14	1.54 ± 0.44
Linalyl acetate	24.058	8.74 ± 2.21
Methyl 2-undecynoate	39.794	0.15 ± 0.00
Alcohols		10.70 ± 0.71
2-Nonanol	5.641	1.46 ± 0.51
6-methyl-5-Hepten-2-ol	20.367	1.04 ± 0.08
Verbenol	24.493	0.88 ± 0.04
cis-Verbenol	24.678	3.12 ± 0.03
5,8,10-Undecatrien-3-ol	26.944	0.73 ± 0.12
cis-p-mentha-1(7),8-dien-2-ol	31.091	1.02 ± 0.50
3,7-dimethyl-6-Octen-1-ol	32.116	1.45 ± 0.38
Selina-6-en-4-ol	37.924	1.01 ± 0.17
Aldehydes		38.33 ± 14.06
3,7-dimethyl-2,6-Octadienal	22.502	0.61 ± 0.06
2-Decenal, (E)	27.119	0.38 ± 0.20
Citral	30.306	35.77 ± 4.21
2-Undecenal	30.986	0.30 ± 0.13
2,4-Decadienal	32.496	1.26 ± 1.11
Acids		5.48 ± 0.34
Acetic acid	19.481	0.49 ± 0.14
Hexanoic acid	33.391	1.11 ± 0.20
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl,	34.162	1.14 ± 0.90
Octanoic acid	36.698	1.00 ± 0.36
Nonanoic acid	37.928	0.66 ± 0.16
n-Decanoic acid	39.044	0.19 ± 0.04
Benzoic acid	40.439	0.28 ± 0.11
Dodecanoic acid	41.025	0.60 ± 0.18
Terpenes and terpenoids		19.74 ± 7.17
Citronellol	31.861	2.38 ± 0.45
2,6,10-trimethyl-Dodecane	32.852	0.69 ± 0.09
Geraniol	33.632	16.67 ± 2.83
Ketone		7.51 ± 3.19
6-methyl-5-Hepten-2-one	13.974	6.95 ± 3.70
6-methyl-3,5-Heptadien-2-one	25.133	0.57 ± 0.26
Others		2.76 ± 0.57
Tetrahydro-3-Furanol	4.815	0.33 ± 0.05
1,1'-oxybis- Octane	31.491	1.69 ± 0.30
4-Acetonilcycloheptanone	39.399	0.75 ± 0.19

3.2.4.3. Aroma profile of lemongrass extract. Lemongrass extract is widely used in perfumery and beverages owing to its desirable aroma ([Haque et al., 2018](#)). A total of 35 aroma compounds were identified in the *C. citratus* extract ([Table 8](#)). Amongst the group, aldehydes were the most prominent that made up to 38.33 ± 14.06%. The principal aldehyde identified was citral (35.77 ± 4.21%), reporting to be the main compound that accounts for scent and antimicrobial properties of lemongrass ([Fattah et al., 2010](#); [Li et al., 2018](#)). Terpenes and terpenoids were also present in appreciable amounts (19.74 ± 7.17%). In this group, geraniol (16.67 ± 2.83%) was the main component, followed by citronellol (2.38 ± 0.45%) and 2,6,10-trimethyl-dodecane (0.69 ± 0.09%), respectively. [Coelho et al. \(2016\)](#) also stated that citral and geraniol were the major volatile compounds of lemongrass extract. Through biological activities and flavouring properties of lemongrass, it has been used in beverages like yoghurt ([Fattah et al., 2010](#)), soy ice cream ([Natisri et al., 2014](#)), and ice cream ([Chamchan et al., 2017](#)). The essential oils of lemongrass are applied in perfumes and cosmetics ([Wifek et al., 2016](#)).

4. Conclusion

Nowadays, consumers are increasingly choosing food products formulated with natural additives due to the comprehension of the relationship between health and diet. Therefore, it is important for the industrial food sector to find novel sources and efficient extraction methods of bio-based ingredients, including natural antioxidant and aroma. In this study, the RSM was successfully used to optimize the decoction conditions of lemongrass leaves. The optimal conditions generated were: solid/liquid ratio (5g/100 mL), temperature (93.8 °C) and time (11.3 min). This yielded a TPC of 71.98 ± 0.33 mg GAE/100 mL of extract and 80.63 ± 0.49 mg TE/100 mL for TPC and DPPH, respectively. The achieved experimental data were successfully fitted to the theoretical models used to determine the optimal extraction conditions. Caffeic (20.816 ± 0.003 mg/100mL) and syringic (18.635 ± 7.390 mg/100mL) acids were the most abundant phenolic acid compounds found in lemongrass extract. In addition, citral and geraniol were detected as the essential volatile compounds of lemongrass extract. This extract could therefore be employed in beverages.

Declarations

Author contribution statement

Wiyeh Claudette Bakisu Muala: Performed the experiments; Wrote the paper.

Zangué Steve Carly Desobgo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nso Emmanuel Jong: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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V.10) Antagonistic Effects of Raffia Sap with Probiotics against Pathogenic Microorganisms

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Antagonistic effects of raffia sap with probiotics against pathogenic microorganisms

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Abstract:

Introduction. Probiotics are known for their beneficial properties. Numerous studies have been conducted to find advantages that probiotics can provide. This study aimed to evaluate the functional properties of raffia sap, a Cameroonian drink, fermented with probiotics by investigating its antagonistic activity against pathogenic bacteria.

Study objects and methods. The study objective was raffia sap fermented by *Lactobacillus fermentum* and *Bifidobacterium bifidum*. Box-Behnken design with four factors (seeding rates of *L. fermentum* and *B. bifidum*, temperature, and incubation time) was used to generate mathematical models. The disc diffusion method was used to evaluate an antagonistic effect of the probiotics against four pathogenic bacteria (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp., and *Bacillus cereus*). An optimization of mathematical models of the inhibition diameters allowed to determine the optimal conditions of antagonistic effect.

Results and discussion. The experimental data showed that zones of inhibition were 0–21 mm for *Salmonella* sp., 0–23 mm for *E. coli*, 0–20 mm for *L. monocytogenes*, and 0–22 mm for *B. cereus*. ANOVA results and the mathematical models obtained showed that *L. fermentum* was effective against *B. cereus* and *B. bifidum* against *Salmonella* sp., *E. coli*, and *B. cereus*. The optimization of the models revealed maximum zones of inhibition at the seeding rates of *L. fermentum* and *B. bifidum* of 2 and 10%, respectively, incubation time of 48 h, and temperature of 37°C.

Conclusion. Raffia sap fermented by *L. fermentum* and *B. bifidum* demonstrated antagonistic effect against pathogenic bacteria such as *E. coli*, *L. monocytogenes*, *Salmonella* sp., and *B. cereus*.

Keywords: Probiotics, antagonistic activity, pathogenic bacteria, response surface methodology, mathematical model

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INTRODUCTION

Probiotics are defined as microorganisms that, when ingested in sufficient quantity, effect beneficially the host [1, 2]. The beneficial effects resulting from the consumption of foods enriched with probiotics have been known for millennia [3]. At the beginning of the 20th century, Mechnikov, a winner of the Nobel Prize, suggested replacing the dangerous germs by useful bacteria [4]. Additionally, *Bifidobacterium* spp. was recommended against infantile diarrhea [3, 5]. Despite the scientists' research, the idea of eating certain bacteria to improve the health of the digestive system was ignored. Taking into account the different technical issues related to the production of foods with probiotics,

attention must be focused on their beneficial effects on health [6].

The latest studies in this area have shown that probiotic bacteria are able to stimulate the immune system and inhibit the adhesion and multiplication of pathogenic bacteria [7, 8]. Since pathogenic microorganisms are becoming resistant to antibiotics, probiotics are a new alternative to be studied in the search for new molecules and/or antibacterial organisms [9].

Such an antimicrobial or antibacterial effect is generally called an antagonistic effect. Factors responsible for the antagonistic effect of one microorganism against another one are: production

of organic acids or hydrogen peroxide that lower pH, competitive exclusion, immune system modulation, stimulation of defence systems, as well as production of antimicrobials such as bacteriocins and antioxidants [10]. Lactic, acetic, benzoic and other organic acids are the antimicrobial substances generally produced by beneficial microorganisms. The most produced bacteriocins are plantaricin, enterolysin, lactacin, lactocin, reuterin, pisciolin, enterocin, and pediocin [11].

Many probiotics have a broad spectrum of action and can be effective against diseases caused by food contaminated with certain pathogenic strains such as *Listeria monocytogenes*, *Escherichia coli*, *Bacillus cereus* and *Salmonella*. These four bacteria are the most common pathogens causing food-borne diseases [12]. Generally, there are difficulties in selecting an appropriate strain, substrate, as well as in determining optimal conditions for probiotic effectiveness.

In this context, researchers use local Cameroonian raw materials, including raffia sap. Raffia sap is a widespread drink in sub-Saharan Africa and particularly in Cameroon. Raffia sap undergoes wild fermentation and produces raffia wine that is difficult to keep. In 10 h after the harvest, alcohol produced during the primary fermentation transforms into acid, which seriously compromises the organoleptic characteristics appreciated by consumers.

In our previous research, we developed a probiotic beverage with raffia sap fermented by *Lactobacillus fermentum* and *Bifidobacterium bifidum* [13]. In the current research we studied an antagonistic potential of raffia sap inoculated by probiotics. The study was aimed to use Response Surface Methodology (RSM) to evaluate and optimize the effectiveness of *L. fermentum* and *B. bifidum* against *E. coli*, *L. monocytogenes*, *Salmonella* sp., and *B. cereus*.

STUDY OBJECTS AND METHODS

Raffia sap harvesting. The fresh sap of less than eight hours was harvested in a 25 L container and transferred to the laboratory. The sap then was immediately dispensed into 1 L bottles and sterilized in a water bath at 65°C for 30 min. The bottles were cooled and stored at 4°C.

Bacteria and probiotics. Pathogenic bacteria (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp. and *Bacillus cereus*) were provided by the Food Microbiology Laboratory of Ngaoundere University. Probiotics (*Lactobacillus fermentum* and *Bifidobacterium bifidum*) were prepared using KwikStik™ lyophilized microorganism.

Revitalization and multiplication of probiotics. To revitalize and multiply probiotic cells contained in the freeze-dried products, 1 g of lyophilisate of each strain was rehydrated as recommended by the manufacturer.

First, the powder was rehydrated in 10 mL of dilute saline solution (DS) consisting of 0.85% NaCl and 0.1% peptone in distilled water and stirred for 10 min until maximum recovery was reached. The solution was then transferred into 1 L of MRS broth previously prepared and sterilized. After incubation at 42°C for 48 h, MRS broth with probiotics was centrifuged at 6500 g and 4°C for 15 min.

The supernatant was removed, the pellet was washed in the saline solution without being resuspended and then recentrifuged as above. The supernatant was discarded and the pellet was finally resuspended in 10 mL of DS first and then transferred into 250 mL of DS. The concentration of probiotics in this solution was obtained by serial dilutions. The dilutions were spread on MRS petri dishes and incubated at 42°C for 24 h, then the colonies were counted [14].

Antagonistic effect of raffia sap fermented. To evaluate the antagonistic effect of the fermented raffia sap, we used the disc method described by Tadesse *et al.*, with some modifications [5]. Mueller-Hinton agar was seeded with pathogenic bacteria (*L. monocytogenes*, *B. cereus*, *E. coli* and *Salmonella* sp.) and incubated at 37°C for 30 min. Sterile discs (5 mm) then were placed on the agar surface incubated at 37°C for 24 h. Each disk was impregnated with 100 µL of raffia sap fermented by probiotics according to the experimental design (Table 1). The inhibition of pathogenic bacteria resulted in the formation of clear zones around the discs. The zone of these inhibition zones was measured, which was used as the main response of the trial.

Experimental design for sap fermentation process and data analysis. Fermentation was done following a four factor Box-Behnken design. The factors were seeding rates of *L. fermentum* (X_1) and *B. bifidum* (X_2), temperature (X_3), and incubation time (X_4). The levels of each factor were chosen after prior testing (Table 1).

The Box-Behnken experimental matrix in coded variables (−1; 0; +1) was generated with the Minitab 18 software. This coded variable matrix consisted of 28 trials, four of which enabled a better evaluation of the experimental error; each trial was repeated three times. The experimental matrix applicable to the laboratory was obtained by transforming the matrix into the coded variables with the EXCEL software using the following formula:

Table 1 Range of variation and factor levels

Variable		Level of factor		
		−1	0	+1
Seeding rate of <i>Lactobacillus fermentum</i> , % (v/v)	X_1	0	5	10
Seeding rate of <i>Bifidobacterium bifidum</i> , % (v/v)	X_2	0	5	10
Temperature, °C	X_3	37.0	39.5	42.0
Incubation time, h	X_4	2	25	48

$$X_j = \frac{U_j - U_{j0}}{\Delta U_j} \quad (1)$$

where X_j is a value of the coded variable j ; U_j is a value of the real variable j ; U_{j0} is a value of real variable j at the center, and ΔU_j is called a “step” of variation.

Modelling and optimization. The zones of inhibition zones obtained after the application of the various tests of the experimental matrix were analysed on Minitab 18. The obtained models were in the form of:

$$y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum_{i < j} \beta_{ij} x_i x_j \quad (2)$$

where y is the model of the inhibition zones of the strain concerned, $\beta_{(i,j)}$ are model coefficients and $x_{(i,j)}$ are the factors. The data was analysed at the level of 10%, including the maximum of significant factors on each model. Response Surface Methodology was used for the three-dimensional graphical representation of the models of each inhibition zone after setting temperature and incubation time at constant values. Sigmaplot 12 software was used to plot the curves. Optimization was done on Minitab with the specifications for maximizing the inhibition zones of each pathogen.

RESULTS AND DISCUSSION

The results of the measurements of the inhibition zones of pathogenic bacteria (*Salmonella* sp., *Escherichia coli*, *Listeria monocytogenes*, and *Bacillus cereus*) obtained after the implementation of the four factor Box-Behnken experimental matrix showed that the zones of inhibition ranged from 0 to 21 mm for *Salmonella* sp., 0 to 23 mm for *E. coli*, 0 to 20 mm for *L. monocytogenes*, and 0 to 22 mm for *B. cereus*.

Raffia sap without probiotics did not demonstrated an inhibitory activity against pathogenic bacteria

(inhibition zone = 0). However, the study conducted by Ojo and Agboola displayed different results [15]. The authors evaluated the antagonistic activity of bacteria isolated from Palm wine (*Raphia vinifera* L.) towards *Salmonella typhi*. The study revealed that raffia sap, due to its own microbial flora, was antagonistic against several pathogenic bacteria, including *Salmonella* sp. This also could be explained by pasteurization of fresh sap to avoid any interaction between the natural microflora of the sap and added probiotics, as well as wild fermentation.

Thus, seeding rates of *Lactobacillus fermentum* and *Bifidobacterium bifidum* played an important role in the antagonistic effect of the drink against the pathogenic bacteria tested, but statistical analysis was performed for a better demonstration of these effects (Table 2).

Effect of factors on microbial inhibition.

According the data in Table 2, *B. bifidum* did not show a strong antagonistic effect on *E. coli*, *L. monocytogenes*, and *Salmonella* sp., but it was effective against *B. cereus* ($P \leq 0.1$). *L. fermentum* had a significant antagonistic effect on *Salmonella* sp., *E. coli*, and *B. cereus* with probabilities of 0.060, 0.040 and 0.072, respectively. Moreover, the incubation time significantly increased all the zones of inhibition ($P = 0.000$).

Effect of incubation time on inhibition of pathogenic bacteria. The curves of inhibition zone of *E. coli*, *L. monocytogenes*, *B. cereus*, and *Salmonella* sp. as a function of time were obtained after fixing seeding rates of *B. bifidum* and *L. fermentum* at 0 in coded variables (5% in real variables) and the temperature at 0 in coded variable (39.5°C in real variable). Under these conditions, these curves (Fig. 1) showed that the inhibition zones of *E. coli* ranged from 8 mm (2 h of incubation) to 20 mm (48 h).

Table 2 ANOVA results and coefficients of mathematical model of inhibition zones for *Lactobacillus fermentum* and *Bifidobacterium bifidum* on *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus*, and *Salmonella* sp.

Terms	<i>Escherichia coli</i>		<i>Listeria monocytogenes</i>		<i>Bacillus cereus</i>		<i>Salomonella sp.</i>	
	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P
Constant	13.750	0	15.750	0	14.250	0	15.000	0
X_1	0.917	0.233	0.333	0.722	1.500	0.058	1.167	0.173
X_2	1.667	0.041	1.083	0.259	1.417	0.072	1.667	0.060
X_3	-0.500	0.507	0.333	0.722	0	1.000	0.417	0.615
X_4	6.417	0	5.750	0	6.083	0	6.583	0
X_1+X_1	-1.290	0.235	-1.580	0.244	-1.750	0.110	-1.040	0.379
X_2+X_2	-1.170	0.281	-3.460	0.019	-0.630	0.551	-2.540	0.045
X_3+X_3	-0.170	0.875	0.170	0.900	0.750	0.476	1.080	0.361
X_4+X_4	0.710	0.506	-0.710	0.594	0.370	0.719	-0.920	0.438
X_1+X_2	-3.750	0.011	-2.750	0.107	-4.000	0.007	-2.750	0.072
X_1+X_3	-0.250	0.847	-1.000	0.540	0.250	0.845	-1.000	0.488
X_1+X_4	0.250	0.847	0.250	0.877	-0.250	0.845	-0.250	0.861
X_2+X_3	0	1.000	0.250	0.877	1.750	0.185	2.250	0.133
X_2+X_4	0.250	0.847	-1.250	0.446	-0.500	0.696	0.500	0.727
X_3+X_4	-0.750	0.565	-0.750	0.645	-1.000	0.438	-1.000	0.488

where X_1 and X_2 are seeding rates of *L. fermentum* and *B. bifidum*, respectively, X_3 is temperature, and X_4 is incubation time

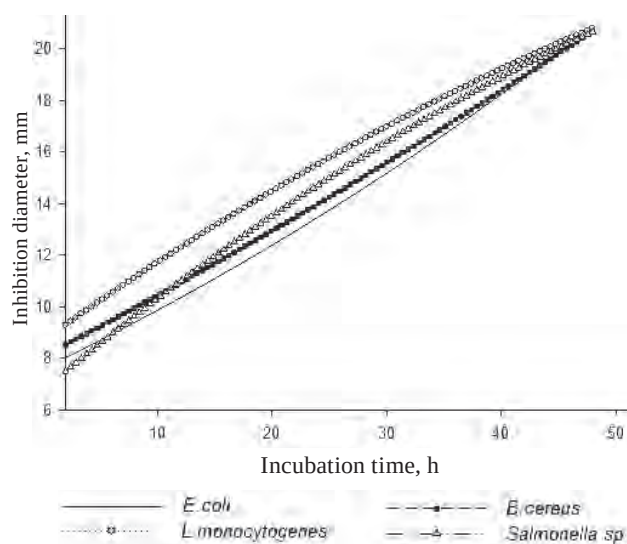


Figure 1 Effect of incubation time on inhibition of pathogenic bacteria

The inhibition zones of *B. cereus*, *L. monocytogenes*, and *Salmonella* sp. varied from 8.5, 9.0 and 7.3 mm in 2 h of incubation, respectively. In 48 h, the zones reached 20 mm in all the samples. The inhibition zones measured for each pathogenic strain as a function of the incubation time demonstrated that time is an essential factor to assess the antagonistic effect of probiotic drink based on raffia sap fermented with *L. fermentum* and *B. bifidum*. In fact, *B. bifidum* and *L. fermentum* need time to synthesize acids and other antimicrobial compounds contributing to antagonist effect against pathogenic bacteria [16, 17].

Individual effect of *B. bifidum* on *E. coli*, *B. cereus* and *Salmonella* sp. To obtain the inhibition curves of *E. coli*, *B. cereus*, and *Salmonella* sp. (Fig. 2) as a function of the seeding rate of *B. bifidum*, the seeding rate, incubation temperature, and incubation time of

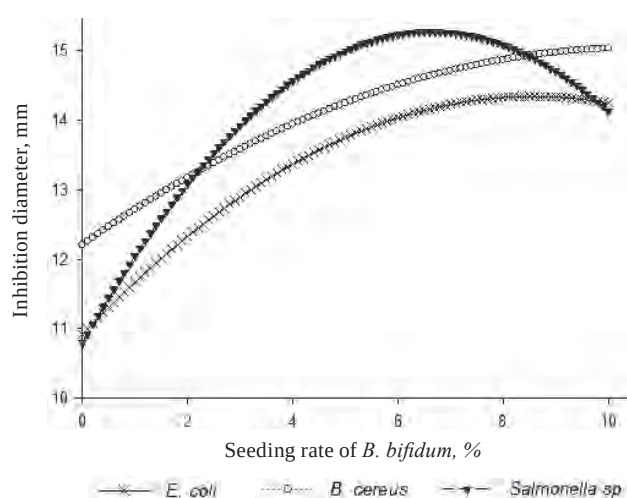


Figure 2 Individual effect of *Bifidobacterium bifidum* on *Escherichia coli*, *Bacillus cereus*, and *Salmonella* sp.

L. fermentum were set at 0 in coded variable – 5%, 39.5°C, and 25 h in real variables, respectively.

The inhibition curve of *Salmonella* sp. as a function of the seeding rate of *B. bifidum* showed that the maximum zone of inhibition of *Salmonella* sp. (15 mm) was obtained when the seeding rate of *B. bifidum* was 6%. The curve of the inhibition zone of *B. cereus* demonstrated that the inhibition zone depended directly on the seeding rate of *B. bifidum*. The inhibition zones of *B. cereus* ranged from 12.1 to 14.2 mm for the seeding rates of 0 and 10%. As for *E. coli*, its curve of the inhibition increased and then decreased, with a peak of 13.3 mm when the seeding rate of *B. bifidum* was 6.6%.

According to Luquet and Corrieu, bifidobacteria promote better absorption of milk lactose in adults with intestinal lactase deficiency [18]. In our study, these probiotics (in particular *B. bifidum*) in raffia sap also played an important antagonistic role against *E. coli*, *B. cereus*, and *Salmonella* sp. In addition, some invitro studies showed that bifidobacteria and their metabolites stimulated IgA production, phagocytic activity, and growth [19]. These metabolites produced in raffia sap as well as the *B. bifidum* strain itself can therefore be a natural way to stimulate the immune system, to inhibit pathogenic strains such as *E. coli*, *B. cereus* and *Salmonella* sp., and to balance intestinal flora.

Individual effect of *L. fermentum* on *B. cereus*. Figure 3 shows the curve of the inhibition zone of *B. cereus* as a function of the seeding rate of *L. fermentum*. This curve increased then decreased, with the inhibition zone peak of 14.3 mm at the seeding rate of 6.5%. This curve was obtained by setting the seeding rate of *B. bifidum*, incubation temperature, and incubation time at 0 in coded variables – 5%, 39.5°C, and 25 h in real variables, respectively.

Thus, if it were necessary to optimize the antagonistic properties of our probiotic drink by referring only to an ability to inhibit the *B. cereus*

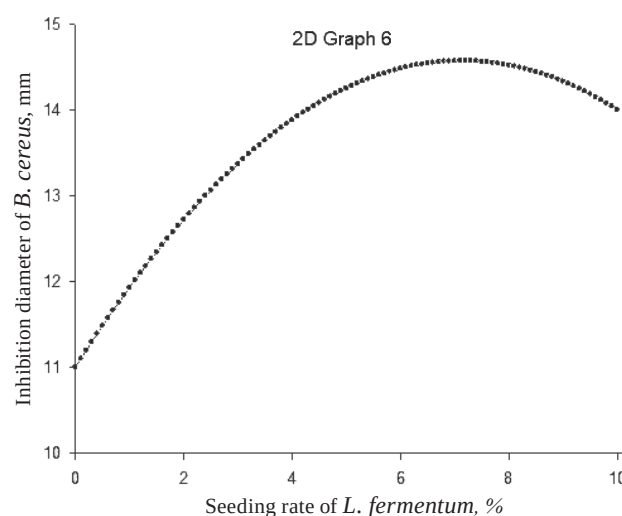


Figure 3 Individual effect of *Lactobacillus fermentum* on *Bacillus cereus*

strain, the seeding rates of *L. fermentum* and *B. bifidum* would be 5% and 5%, respectively, with an incubation temperature of 39.5°C and an incubation time of 25 h. Under these conditions, this probiotic drink could eventually be used as a means of combating infectious diseases which can be caused by *B. cereus*. *B. cereus* is a group of bacteria that can be pathogenic for humans. The infections they can cause are generally infrequent and not serious. However, ingestion of these bacteria, and their toxins in particular, can lead to infections characterized by vomiting or diarrhea [20].

In spite of the fact that our results were obtained *in vitro*, it is clear that *L. fermentum* introduced into raffia sap had a significant antagonistic effect on *B. cereus*. However, further research should be carried out *in vivo* to take into account factors that could affect the drink properties such as its passage through the intestinal tract, the survival of strains and the bioavailability of antibacterial compounds, as well as their direct or indirect effect on the body.

Effects produced by combination of *L. fermentum* and *B. bifidum* in raffia sap on the pathogens tested.

The response surface methodology was applied to represent the mathematical models obtained by holding temperature and incubation time at 0 in coded variables –39.5°C and 25 h in real values, respectively.

Figure 4 presents the response surface of the mathematical model of inhibition zone of *L. fermentum* and *B. bifidum* against *Salmonella* sp. An increase in the seeding rate of *B. bifidum* and a simultaneous increase in the seeding rate of *L. fermentum* and *B. bifidum* considerably increased the antagonistic effect, with the inhibition zone of 16 mm.

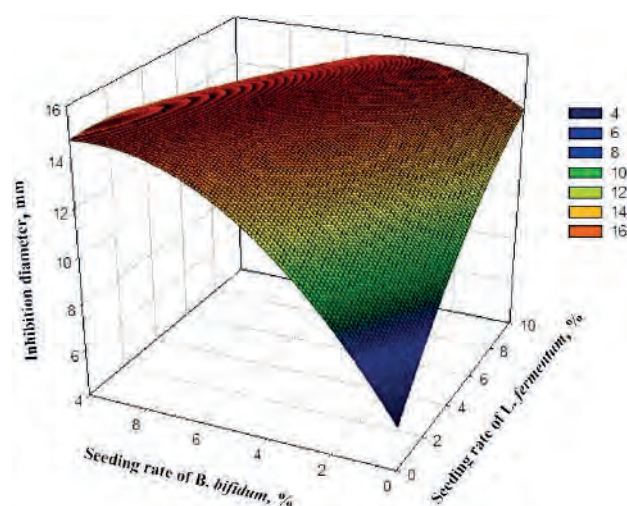


Figure 4 Response surface model of inhibition zone of *Lactobacillus fermentum* and *Bifidobacterium bifidum* against *Salmonella* sp. with seeding rates of *Lactobacillus fermentum* (X_1) and *Bifidobacterium bifidum* (X_2), incubation temperature (X_3), and incubation time (X_4) at temperature 0 (39.5°C) and time 0 (24 h)

However, only *B. bifidum* had a significant antagonistic effect on *Salmonella* sp. ($P = 0.060$, Table 2) at a 10% probability level. Indeed, lactic acid produced by *B. bifidum* lowers the pH by creating an unfavorable conditions for pathogenic microorganisms such as *Salmonella* sp. [21, 22]. Garcia *et al.* and Callaway *et al.* reported that bifidobacteria can prevent or reduce diseases caused by pathogens, protecting thus consumers' health [16, 23]. Based on our study results, raffia sap fermented by *B. bifidum* can be effective against salmonellosis due to *Salmonella* proliferation.

Figure 5 demonstrates the response surface of *L. fermentum* and *B. bifidum* against *E. coli*. As in the case with *Salmonella* sp., only *B. bifidum* showed a significant antagonistic effect on *E. coli* ($P = 0.041$, Table 2) at a 10% probability level. An increase in the seeding rate of *B. bifidum* considerably increased the antagonistic effect, with the maximum inhibition zone of 18 mm.

Indeed, lactic acid bacteria exert a strong antagonistic activity against several microorganisms, including those causing the deterioration of food and pathogenic microbes such as *E. coli* [4, 24]. In addition, the antimicrobial effect of some probiotic extends the shelf life of food [25]. This effect is mainly due to the production of organic acids (lactic acid) and also the production of antimicrobial compounds such as hydrogen peroxide, diacetyl, acetaldehyde, amino acid isomers and bacteriocins [19, 26].

It is important to remember that *E. coli* is a Gram-negative mammalian intestinal bacterium that makes up about 80% of the aerobic intestinal flora in humans [27, 28]. However, some strains of *E. coli* can be pathogenic, resulting in gastroenteritis, urinary tract infections, meningitis, or sepsis. Therefore, consumption of raffia

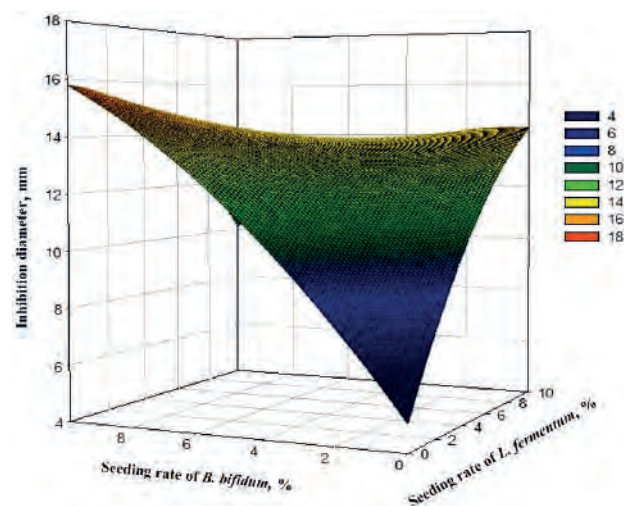


Figure 5 Response surface model of inhibition zone of *Lactobacillus fermentum* and *Bifidobacterium bifidum* against *Escherichia coli* with seeding rates of *Lactobacillus fermentum* (X_1) and *Bifidobacterium bifidum* (X_2), incubation temperature (X_3), and incubation time (X_4) at temperature 0 (39.5°C) and time 0 (24 h)

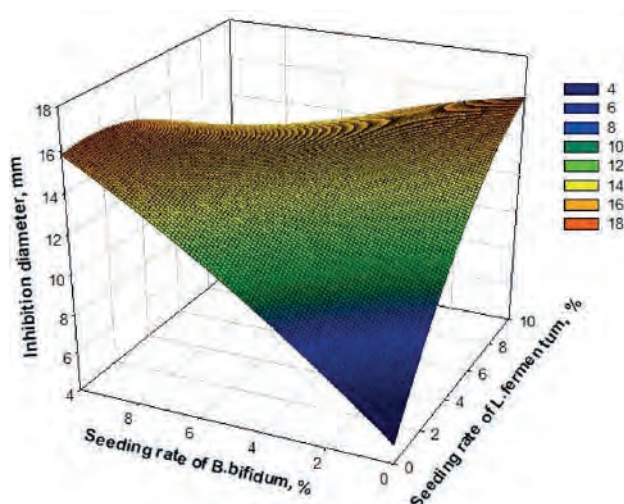


Figure 6 Response surface model of inhibition zone of *Lactobacillus fermentum* and *Bifidobacterium bifidum* against *Bacillus cereus* with seeding rates of *Lactobacillus fermentum* (X_1) and *Bifidobacterium bifidum* (X_2), incubation temperature (X_3), and incubation time (X_4) at temperature 0 (39.5°C) and time 0 (24 h)

sap fermented by *B. bifidum* can prevent and control the pathogenicity of *E. coli*.

Figure 6 presents the response surface of the mathematical model of inhibition zone of *L. fermentum* and *B. bifidum* against *B. cereus*. Both *L. fermentum* and *B. bifidum* individually had a significant antagonistic effect ($P = 0.058$ and 0.072 , respectively, Table 2), whereas their combination was a highly effective ($P = 0.007$). *B. cereus* had similar sensitivities to both probiotics in raffia sap (Fig. 6). Inhibition zones reached 18 mm when the seeding rates of *L. fermentum* and *B. bifidum* were maximum. The acids and antimicrobial compounds secreted by *L. fermentum* and *B. bifidum* in raffia sap are thus a pathway to be exploited to treat diseases, although rare, due to consumption of *B. cereus*-infected foods. *B. cereus* is a well-known food-borne pathogen that is ubiquitously distributed in nature and is frequently responsible for food poisoning [20].

Effect of *L. fermentum* and *B. bifidum* on *L. monocytogenes* and optimization of the antagonistic effect. In the case of *L. monocytogenes*, response surface curves were not required because neither of the probiotic bacteria in raffia sap had a significant antagonistic effect ($P = 0.722$ for

L. fermentum and $P = 0.259$ for *B. bifidum*, Table 2). This can be explained by the greater resistance of this bacterium to acidity [29]. Probably, the fermentation time should be increased to enhance the antagonistic properties of the raffia sap drink, but it would make the drink more acidic and hence undrinkable. It would be better to exploit this hypothesis in the context of the synthesis, isolation and production of biologically active compounds from raffia sap fermented by *L. fermentum* and *B. bifidum*.

In conclusion, the optimization of the antagonistic effect was done on the basis of specifications that aimed to maximize the inhibition zones. Thus, an optimal antagonistic effect would be given by seeding rates of *L. fermentum* and *B. bifidum* of 2 and 10%, respectively, incubation time of 48 h, and temperature of 37°C.

CONCLUSION

The results obtained in this study revealed that raffia sap fermented by probiotics (*Lactobacillus fermentum* and *Bifidobacterium bifidum*) had antibacterial properties against bacteria such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* sp., and *Bacillus cereus* which can sometimes be pathogenic. However, further studies should be carry out to determine the mechanism of action of this finding and to confirm its beneficial effect in animal models.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism. The idea and analysis belongs to S.C.Z. Desobgo. M.J.A. Mbarga and L.N. Tatsadjieu collected the data, performed the analysis and wrote the paper. L. Kalisa and N. Kavhiza translated and edited the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests related to this article.

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V.11) A Field Survey to Assess the
Consumption of Nkang for
Standardization and Valorization in
the North-West Region of Cameroon

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A Field Survey to Assess the Consumption of *Nkang* for Standardization and Valorization in the North-West Region of Cameroon

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Abstract

In African communities, traditional beer drinking remains a unifying factor within its populations both socially, commercially, culturally, politically, in health and nutrition and for some ritual practices. In this research a field survey was carried out to investigate the consumption of corn beer and in particular *Nkang* in the North-West Region of Cameroon. The tools that were employed to carry out these investigations included face-to-face interviews and the use of properly designed questionnaires. Results from the survey showed that three types of maize-based beverages are drunk in the North-West Region of Cameroon, which are locally called *Kwacha* (whitish, most viscous and most turbid), *Sha-ah* (cream white, viscous and turbid) and *Nkang* (dark brown, least viscous and least turbid) in terms of colour, viscosity and turbidity. The percentage awareness of the existence of these beers from the sampled population gave the following values; 60.9% for *Kwacha*, 100% for *Sha-ah* and 89.1% for *Nkang*. Nonetheless, 54.5% of the 60.9% of those who were aware of the existence of *kwacha* had at least tasted it. Also 98.2% out of the 100% for *Sha-ah* and 85.5% out of 89.1% for *Nkang* had tasted them, too. *Nkang* was found to be the most preferred to *Sha-ah* then *Kwacha* in that order by the consumers since *Nkang* is very tasteful, least alcoholic, least turbid, least viscous, has the most attractive colour than the others, has a significant impact on the culture of some localities in this region and as well as it is natural and nutritious. However, *Nkang* as well as the other two has varying organoleptic properties, unsatisfactory conservation and short shelf-life. Hence are consumed within a short period of time from their production. Because of the low alcoholic content of *Nkang*, the beverage is consumed by both adults (most elderly), children, those who have health problems and it is mostly preferred by some Christians though not frequently

seen in the markets. It was equally observed that the little quantity of *Nkang* found in the markets is of poor quality which keeps dropping everyday thus an indication of its risk becoming extinct. Therefore, if *Nkang* is clarified and its quality improved, the problems can be solved as even attested by the consumers who say they will buy at even a higher price if clarify. As well as those who want it for their cultural reasons do not want it to face out too.

Keywords

Corn Beer, *Kwacha*, *Sha-ah*, *Nkang*, Alcoholic Content, Least Viscous, Shelf-Life

1. Introduction

Indigenous fermented products play an important role in the diet of most people in the developing countries [1]. An example of these indigenous fermented products is beer which has become a staple part of the diet in many cultures [2]. The traditional African sorghum beers are very rich in calories, B-group vitamins including thiamine, folic acid, riboflavin, and nicotinic acid, and essential amino acids such as lysine [3] [4]. Beer has been found to increase the plasma high-density lipoprotein (HDL) which is a scavenger of cholesterol thus reducing risk of cardiovascular diseases, gallstones and stomach ulcers and protection of brain against mental decline due to aging [5]. It is also a tonic, nutritive, digestive, appetizer, soothing and sedative beverage [6]. It is galactogene thus a small quantity is good for pregnant and lactating women. The beers are consumed at various festivals and African ceremonies such as marriages, births, baptism ceremonies, the handing over of a dowry, are very important for political, social and for economic interactions *inter alia* as well as constituting a source of economic return for the female beer producers [7]. Beer differs in its significance, acceptability and importance from culture to culture. Other examples of these African traditional beers include; *ikigage* in Rwanda [8], *tchoukoutou* in Benin and Togo [9], *dolo* in Burkina Faso [10], *pito* or *burkutu* in Nigeria and Ghana and *amgba* in Cameroon as well as maize-based beers (generally called corn beer) of various types such as *Nkang* which are often consumed in the North West Region of Cameroon. *Nkang* which is one of the corn beer types has some cultural affiliations to the people of Bali, one of the ethnic groups found in the North-West Region of Cameroon pointing out the degree of cultural attachment to *Nkang* by the people of this locality. However, traditional African beers are generally “opaque” beers [11], less attractive than Western beers brewed with barley malt because of their relatively poor hygienic quality, low ethanol content (usually less than 5% v/v), organoleptic variation and unsatisfactory conservation [8] [12]. *Nkang* and related locally made alcoholic beverages suffer the same fate. To overcome these constraints, some of these African traditional beers have been industrialized by standardizing their production

processes, for example Star Lager (Nigerian Beer), Tusker Lager (Kenyan Beer), Castle Lager (from South Africa), Casablanca, (Moroccan Beer) [10]. In the same light, because of its cultural significant, if *Nkang* beer production process can be standardized and documented as no literature is found on, this will go a long way to prolong its shelf-life [13]. To standardize such a process, a survey on its consumption from those who drink it is sine qua none to upgrading the beer in terms of production, shelf-life enhancement and quality enhancement and assurance and thus standardizing the product, since some sensory qualities and preferential appreciations can be investigated [14] [15]. This study was aimed at carrying out a field survey on the consumption of corn beer and in particular *Nkang* beer in the North-West Region of Cameroon and using the results to attempt to design a standardization scheme for *Nkang*.

2. Materials and Method

2.1. Materials

Questionnaires were used for data collection. Software Sphinx V5 was used to format the questionnaires, treat and analyzed the data collected from the field.

2.2. Study Area

The survey was carried out in four Divisions (Bui, Mezam, Momo, Ngoketunjia), with co-ordinates in degree, minutes, seconds as 10°0'0" to 11°0'0"E Latitudes and 6°0'0"to 6°30'0"N Longitude using the WJS 84 system of projection, chosen from the seven Divisions of the North-West Region of Cameroon considered to be the prime areas where *Nkang* is drunk most going by the number of people involved in such activities and the population of these areas.

2.3. Methods

In order to deduce the quality of corn beer the inhabitants of the North West are exposed to, one hundred and ten (110) corn beer consumers were identified and interviewed by administering questionnaires within the chosen study area. Eighty-six (86) questions each per questionnaire were developed with the idea of checking on the quality of corn beer types and the consumption sustainability of the corn beer with emphasis on *Nkang*. The questions were grouped under five sub-sections:

2.3.1. Socio-Economic Status

To know the type of people who drink corn beer and particularly *Nkang*, their age group, sex, and occupation to evaluate the preference given to domestic products and to see how many people love adhere to tradition or prefer the so called modernism.

2.3.2. Consumers General Knowledge on Corn Beer and the Various Types

✓ Here we wish to know whether the present generation knows the type of local

beers that existed before and how many do exist.

- ✓ To verify if those who know these beers have even tasted any.
- ✓ To see if they know the starting raw material for corn beer production?
- ✓ Degree of awareness with Age group, to see whether those who are aware and have tasted these corn beers do vary with the age group.
- ✓ To rate the drinking of corn beer with age group thus identify which age group drinks highest.
- ✓ To have knowledge of the sensory attributes of the corn beer types. To see if the consumers can identify these corn beer types in terms of colour, taste, cloudiness (turbidity) and viscosity.
- ✓ Preference for these corn beer types; to know which type do they prefer and why?
- ✓ Reasons for not drinking any of these corn beers; to know why some consumers do not drink all of the three types of corn beer and to know the qualities of the type of beer they want.
- ✓ Comparing their love for corn beer to other available industrial beers in the market and why?

2.3.3. Particular Knowledge and Quality of *Nkang* and Its Marketability

- ✓ How many people have actually tasted *Nkang* and their reactions and to identify those aspects which they like or dislike about *Nkang*.
- ✓ Some preferred attributes of *Nkang* by the consumers.
- ✓ Other quality aspects of *Nkang*; to know whether the consumers like clarified *Nkang* and why?
- ✓ Desire Qualities of *Nkang*; to know which aspects of *Nkang* will they like the producers to work on so as to improve on its quality?
- ✓ To see if *Nkang* is frequently found in the market?
- ✓ To rate whether the purchasing power of the consumers depend on the price or quality of the beer? How much they can offer for a liter of *Nkang*?
- ✓ To check on whether their preference for one bar to another depends on the quality or price of the beer per bottle?
- ✓ To verify if *Nkang* is clarified, will they buy it or not?

2.3.4. Knowledge on *Nkang* Production from Consumer's View

- ✓ To see if there are some consumers who know how to produce *Nkang*.
- ✓ To know who taught them? (*i.e.* source of technology transfer)
- ✓ To investigate why some do not know how to produce *Nkang* though it is locally made? (*i.e.* to see the degree of negligent on their custom and culture)

2.3.5. Some Cultural Aspects of *Nkang*

- ✓ To see if *Nkang* has an impact on their culture and to what extent?
- ✓ Do they wish this tradition of *Nkang* production for cultural reasons to continue?
- ✓ What is their opinion on *Nkang* if it is substituted with red wine or Whiskey?

3. Results and Discussions

3.1. Socio-Economic Status

A demographic representation of Corn Beer consumers in the North West Region of Cameroon is as shown on **Table 1**.

From **Table 1**, corn beer is consumed by both men and women with a percentage of 53.6% and 46.4% respectively in conformity with the report of [16]. Considering the age groups, from the highest to the least age group in terms of drinking corn beer, we have from 30 - 50, 50 - 65, 65 and above, 20 - 30 and then to 15 - 20 showing that the younger generation does not like drinking it as shown by a 0.9% for those between 15 - 20 years and 12.7% for those between 20 - 30 years, indicating a gradual shift from culture to modernism with age [17]. It can also be observed from the table that the highest consumers are from Momo

Table 1. Demographic characteristics of consumers of corn beer.

Variable	Group	Percentage (%)
Sex	Female	46.4
	Male	53.6
Age	15 - 20	0.9
	20 - 30	12.7
	30 - 50	44.5
	50 - 65	25.5
	65 and above	16.4
Division of Origin	Momo	48.2
	Mezam	30.9
	Ngoketunjia	13.6
	Bui	4.5
	Menchun	1.8
Level of Education	None scholar	8.2
	Primary	50
	Standard	4.5
	Secondary	26.4
	University	10.9
Occupation	Farmer	44.6
	Civil servant	12.3
	Non-public Workers	2.3
	Private worker	13.8
	Business	16.2
	Non-Workers	6.2
	Retired Civil servant	4.6

origin, most of them having primary school level of education and are mostly farmers, showing the population settlement at the local level.

3.2. Consumer's General Knowledge on Corn Beer and the Various Corn Beer Types

The general knowledge of the various types of corn beer is depicted in terms of percentage of sampled population by **Figure 1**.

From **Figure 1**, three types of corn beer (*kwacha*, *sha-ah* and *Nkang*) do exist with the percentage awareness by consumers of 60.9% for Kwacha, 89.1% for Nkang and 100% for Sha-ah. These show that *Sha-ah* is the most popular and available than *Nkang* and then *Kwacha*. However, 54.5% of the 60.9% of those who are aware of the existence of *Kwacha* has at least tasted it, same as 98.2% out of the 100% for *Sha-ah* and 85.5% out of 89.1% for *Nkang* as shown on **Figure 2**. From this observation we can say that, the actual Population of those who have at least tasted any of these three types of corn beer is lower than those who are aware of their existence indicating that not everybody that knows about the existence of these beers has tasted it. Therefore, the present generation at least has an idea of locally produced corn beer. As for the starting raw material for corn beer production, 65.4% say that it is corn, 23.1% talk of germinated corn thus they are aware that the beer is from corn. This is also an indication of some degree of preference for domestic beverages [18].

3.3. Those Who Have at Least Tasted Any of These Three Types of Corn Beer

From **Figure 2**, *Sha-ah* is the most popular corn beer type in the study area and closely followed by *Nkang* and then Kwacha showing how the knowledge on Nkang and Kwacha is dying down thus a point of concern, see **Figure 3**.

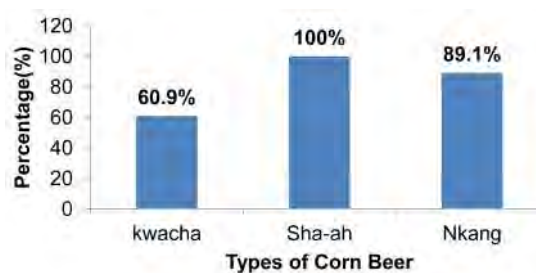


Figure 1. Percentage awareness of corn beer types.

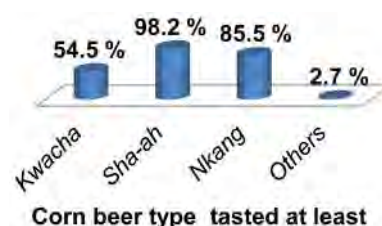


Figure 2. Percentages of those who have at least tasted any of these three corn beer types.

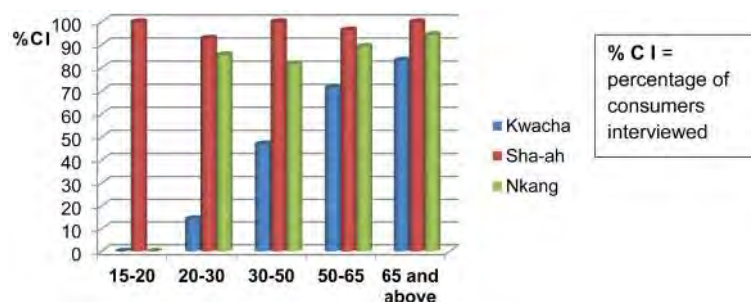


Figure 3. Awareness of consumers and their knowledge on corn beer types versus age group.

3.4. Degree of Awareness with Age Group

Figure 3, shows that the degree of awareness by the consumers of *kwacha* and *Nkang* increases from the age group 20 - 30 up to 65 and above, with *no person* within the age group 15 - 20 years knowing *Kwacha* and *Nkang*. This is an indication of the ebbing awareness of *Nkang* and *Kwacha* as generation pass on. On the other hand, all age groups interviewed have an idea of *sha-ah* meaning that it is the only corn beer type known by all at moment.

3.5. Rate of Drinking Corn Beer with Age Group

The actual Population that have at least tasted any of these corn beers within each group with the highest being those of the age group 30 to 50 years is as shown on **Figure 4**. It can also be observed from the figure that the drinking ability decreases on both sides of the age group 30 - 50, per each beer type showing that it is the active age group that drinks the highest. Hence if *Nkang* and related corn beers are standardized, they will be demanded and consumed by the active population which exceeds the other age groups [19].

3.6. Knowledge on Some Sensory Attributes of the Corn Beer Types

It can be observed from **Figure 5**, that *Kwacha* is whitish in colour, *Sha-ah* is cream white and *Nkang* is dark brown. As for the taste attributes, *Kwacha* is not sweet, *Sha-ah* is sweet and *Nkang* is very sweet. While *kwacha* is very viscous, *Sha-ah* is less viscous and *Nkang* is least viscous. Considering turbidity, *Kwacha* is very turbid, *Sha-ah* is turbid and *Nkang* is least turbid [20]. Thus prospect increase in *Nkang*'s shelf life as filtration is improved and faster throughput [21]. This shows that the consumers can easily identify these beers types from their sensory attributes [22].

3.7. Preference for These Corn Beer Types

Table 2, shows the various percentages for the reasons why the consumers prefer drinking each of the three types of corn beer. 100% of those who drink *Kwacha* prefer it because it serves as food (makes their stomach full) and, 57.1% say it is more alcoholic than the other two and 57.1% also say they have the habit of

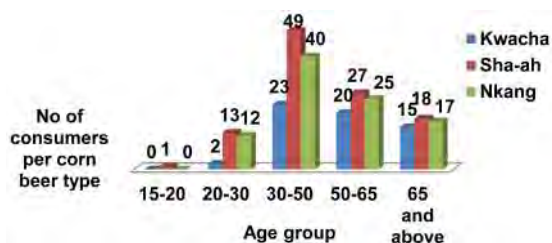


Figure 4. Number of interviewers who have at least tasted the various types of corn beer within each age group.

Table 2. Preference for each corn beer type by the consumers.

Corn Beer Type	Preference for each corn beer type	Percentage affirmative
Kwacha	It is not sweet	14.30%
	More alcoholic than others	57.10%
	It is a habit	57.10%
	It's the cheapest	14.30%
	The heaviest(most viscous and turbid)	42.90%
	Serves as food	100.00%
	Does not cause running stomach	14.30%
Sha-ah	Sweeter than Kwacha too sweet as Nkang	37.50%
	Less alcoholic than Kwacha	41.10%
	More alcoholic than Nkang	16.10%
	Good taste	76.80%
	Has an attractive colour	53.60%
	It is a habit	69.60%
	It is available (easy to find)	78.60%
	Good for my system	41.10%
	Serves as food	85.70%
	It's Cheap	46.40%
	Knows how to produce it	19.60%
Nkang	Due to scarcity of Nkang in the market	19.60%
	It is very sweet	66.70%
	Has a very good taste	96.10%
	least alcoholic than all	94.10%
	It is clear(least turbid)	92.20%
	Least viscous	94.10%
	Has an attractive colour	90.20%
	Satisfactory as food	72.50%
	Quenches thirst	54.90%
	Gives pleasure	52.90%
	Knows how to produce it	19.60%
	Do not causes any health problems	62.70%

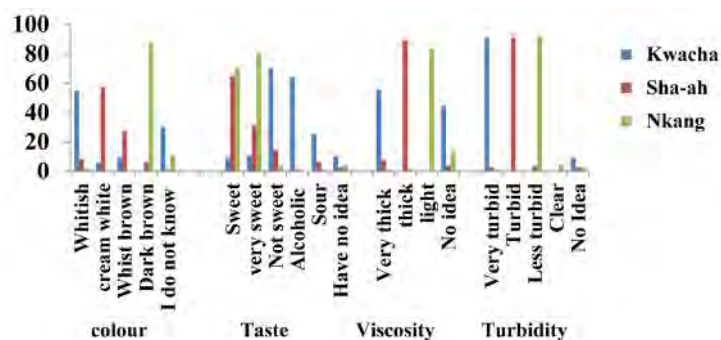


Figure 5. Some sensory attributes of the three types of corn beer.

drinking it. While 42.90% prefer drinking *Kwacha* because it is more viscous and turbid. These are indications to show that *Kwacha* is the most turbid, viscous and most alcoholic amongst the three types of corn beer confirming the information on Figure 5 for *Kwacha*. Different physical with attendant chemical properties leads varied preferences for locally brewed drinks and is a function of demand for the products [23]

Considering those who like drinking *Sha-ah*, 85.70% like drinking it because it serves as food 'so it fills their stomach' but not as *kwacha* does. 78.60% say *Sha-ah* is more available indicating the most popular and the most available than the others as also indicated on Figure 1, 76.80% says it has a good taste *i.e.* good mouth-feel, 69.60% says it is a habit, 41.10% says it is good for their system, 41.10% says it is less alcoholic than *Kwacha*, 37.50% say it is sweeter than *Kwacha* and not too sweet as *Nkang* meaning that *Sha-ah* is not too sweet and has moderate alcoholic content from the other two.

As to those who prefer drinking *Nkang*, 96.10% says it has a very good taste, 94.10% says it is less alcoholic, 94.10% says it is least viscous, 92.20% says it is clear that is least turbid, thus it easy to drink as they do not feel particles at the throat during drinking as its the case with the other two, 90.20% say it has an attractive colour, 66.70% says it is very sweet, these imply that *Nkang* has a very good taste, nice colour and is sweet. 19.60% know how to produce it showing that only a small population knows the *Nkang* production technology.

3.8. Reasons for Not Drinking Any of These Corn Beer

Table 3 shows why some people will not like to drink any or all of these corn beer types. We can observe that, 66.90% of the consumers do not like drinking *Kwacha* and 64.80% for *Sha-ah* because they feel particles at the throat during drinking, 45.60% for *Kwacha* and 42.60% for *sha-ah* talk of them to be too heavy and thick as food not as a drink (*i.e.* they are viscous and turbid) as they have to chew before swallowing. 42.70% say *Kwacha* needs to be heated before drinking showing how viscous *kwacha* is while 25.40% say *Nkang* is least viscous as it can be compared to water [24]. 42.70% say they do not know *Kwacha* with 15.30% not also knowing *Nkang* and 33.90% say *Nkang* is not available indicating their scarcity in the markets. From these observations we can say that *kwacha* and

Table 3. Deterrents from corn beer products.

Corn beer type	Reasons for not drinking any of the corn beer type	Percentages disapproval
Kwacha	Too heavy & thick as food not as a drink	45.60%
	Colour and taste not appreciable	34.00%
	High alcoholic content than others	43.70%
	Feel particles not easy to drink	66.90%
	Not sweet	36.90%
	Need to be heated before drinking	42.70%
	Don't know it	42.70%
Sha-ah	Heavy & thick as food not as a drink	42.60%
	Colour and taste not appreciable	46.30%
	High alcoholic content	42.60%
	Feel particles not easy to drink	64.80%
	Not sweet	27.80%
	Provokes vomiting	13.00%
Nkang	Least viscous can be liken to water	25.40%
	Has less alcoholic content	8.50%
	Too sweet	22.00%
	Not available (cannot easily be seen)	33.90%
	Don't know it	15.30%
	Poor quality	10.20%

Nkang are scare in the markets and that the population want beer that is least turbid and least viscous indicating that a clarify corn beer will be preferred as confirmed by **Figure 8**. On the other hand they population wish that corn beer should have moderate alcoholic content as can be deduced from the following observations; 43.70% says *kwacha* has a high alcoholic content than others, 42.60% say same for sha-ah while a very small percentage of 8.5% say *Nkang* has the least alcoholic content with 22.0% saying that Nkang is too sweet. Therefore the consumers dislike corn beer that has high alcoholic content, viscous and turbid, but are comfortable with that which is sweet with moderate alcoholic content [25].

3.9. Relative Desire for Corn Beer

From **Figure 6**; 87.30%, 86.40%, 84.50%, 77.30%, 71.80%, 69.10%, 69.10%, 68.20% of the population says that they love drinking corn beer to industrial beer because it serves as food, more nutritive, natural, cheaper, has good taste, low alcoholic content, know its raw materials and to consume local products respectively. While 31.80% which falls below average says that they drink corn beer because it is available meaning that corn beer is scare in our markets. Also,

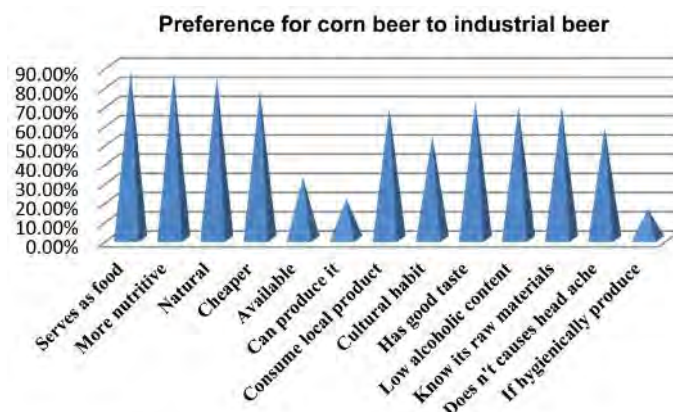


Figure 6. Preference for corn beer to industrial beer.

only 20.90% of the consumers know how to produce any of the corn beer type meaning that there are lesser producers reason why the product is rare in the market. 15.50% says if corn beer is hygienically produced, they will like it meaning that corn beer has some unhygienic aspects but has upgrading potentials.

From **Figure 7**, 50.0%, 49.10%, 49.10%, 47.30%, 46.40% of the Population interviewed love industrial beer because it looks more hygienic, well made, of high quality, less turbid, less viscous and well packaged respectively than corn beer. While 70.90% say they do not prefer industrial beer to corn beer meaning they still love corn beer even if it does not have the above mentioned qualities as the industrial beer. This indicates that though many people love industrial beer because of its quality, they still have an attachment of great love for traditionally made products. This is corroborated by the low 14.50% for those who love industrial beer because it is modern.

3.10. Particular Knowledge and Quality of *Nkang* and Its Marketability

85.5% of the consumers have actually tasted *Nkang* as from **Figure 2**, with a 67.3% and 42.7% haven't tasted it from the market and their homes respectively.

Nkang is most preferred by the consumers as can be seen from **Table 2** (with the highest percentages) and **Table 3** (with the lowest percentages) with their preference for *Nkang* to sha-ah and kwacha as shown on **Table 4**.

3.11. *Nkang* Clarification, Standardization and Valorization

95% of the population wish that *Nkang* should be clarified according to **Figure 8** because it will look attractive as 96.8% consumers affirmed, 87.3% says it will improve its quality, 81.0% say it would be conserved easily, 79.4% says clarification will make it portable as it can be bottled and 90.5% talk of increase in its shelf life thus standardizing *Nkang* and hence quality control [26].

Considering **Figure 9** with blocks A, B, C; 73.60% say they cannot produce *Nkang* with just 21.80% that can, thus technology not known by all. From block B, 70.80% of the 21.80% that knows how to produce *Nkang* learnt it from their

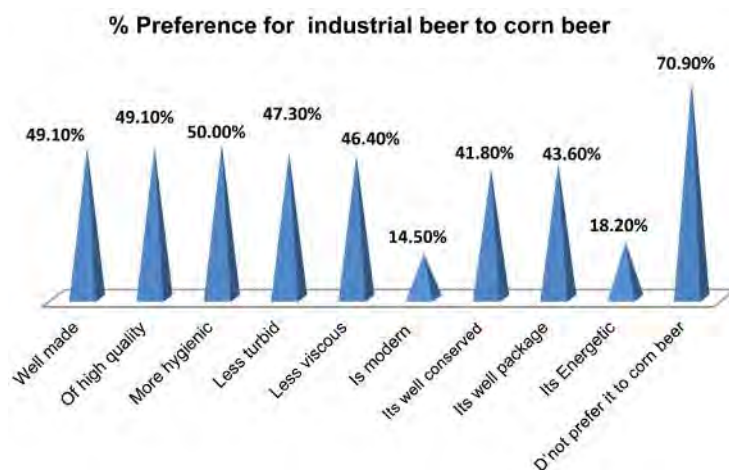


Figure 7. Preference for industrial beer to corn Beer.

Should Nkang beer be Clarified

■ Yes ■ No ■ I have nothing to say

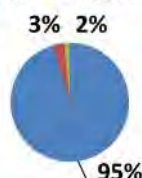


Figure 8. Acceptability for *Nkang* to be clarified in percentages.

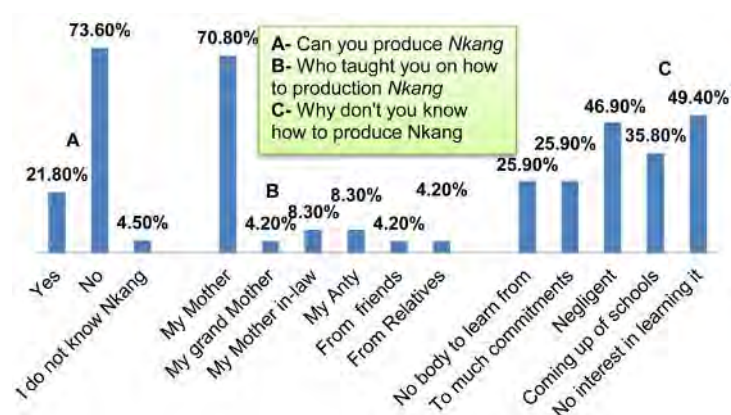


Figure 9. Consumer's production know-how and source of technology transfer.

Table 4. Preference for *Nkang* to *Sha-ah* and *Kwacha*.

Preference for <i>Nkang</i> to <i>sha-ah</i> and <i>kwacha</i>	Score in Percentage (%)
Very tasteful	54.6
Least alcoholic	55.5
Least turbid	54.6
Least viscous	55.5
Has an attractive colour	51.8
Has an impact on the culture	60.0

mothers, 8.30% from mother in-law and 4.20% from their grandmothers indicating that mostly women are involved in the production of *Nkang* [7] [27] and that the technology is a hand down process from parent to children without documentation. 49.40% have no interest in learning on how to produce *Nkang*, 46.90% are negligent, 35.80% says with the coming up of schools they do not have time to learn it as well as those who are too committed with other things that make up 25.90% and no body to learn from that make up 25.90%, too. This is an indication that the technology is dying out thus need to be valorize.

Although *Nkang* is not frequently found in the markets with a 77.3% as shown on **Figure 10**, some consumers that make up 72.90% from **Figure 11**, point out that this is due to less producers and a 61.20% saying that they believe that the technology is not well transferred to the children as those who say it is due to poor quality thus drop in the market make up 37.60%, However, 22.40% of the consumers attribute it to the death of most of the producers which can be confirmed with the 36.5% who says that the *Nkang* beer is mostly produced by older women. Nonetheless, 58.80% say it is due to the coming up of schools and 54.10%, talk of coming up of many other types of drinks especially sweet drinks but it seems the present generation has no interest in its production as shows on **Figure 11**, with a 7.10%.

3.12. Purchasing Power with the Price or the Quality of the Beer

According to **Figure 12**, 86.40% of the consumers say that, their purchasing power is a function of the price/quality ratio of the beer. 85.50% say they drink *Nkang* not because of the price but because of the quality of the beer that is the overall sensory attributes and its nutritious nature. With respect to bar preference, 89.10% say that they like one bar to another depending on the quality, 83.60% like the quality of services, 80.00% cleanness and neatness of the bar and the shop owner, 63.60% say it depends on the availability of the beer. Thus their purchasing power and drinking habit is a function of the quality and not the price [28].

3.13. Cultural Attachment to *Nkang*

We can observe from **Figure 13** that, 92.40% of the consumers say *Nkang* drinking should continue so as to preserve their cultural values. While 80.30%, 66.70%, 50.00% also attest that *Nkang* drinking, should be promoted because it serves as a unifying factor, source of entertainment and to maintain their tradition, respectively [29]. 13.60% says that according to their tradition and custom

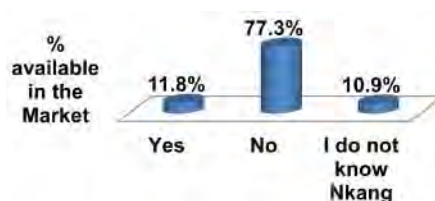


Figure 10. Percentage availability of *Nkang* in the Markets.

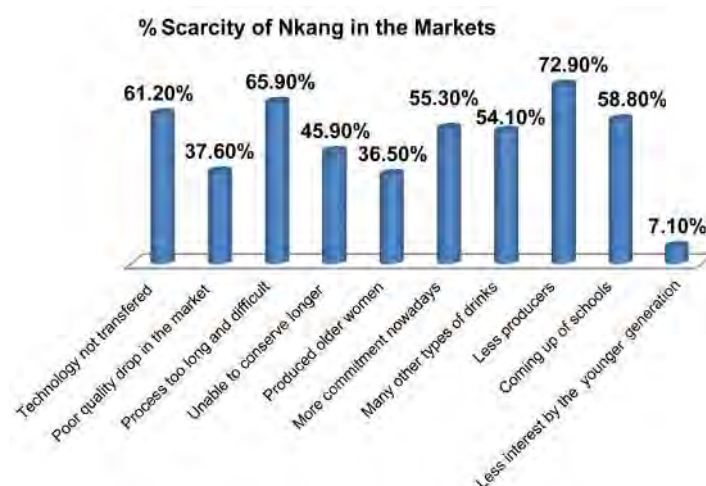


Figure 11. Why *Nkang* is scarce in the market in percentages.

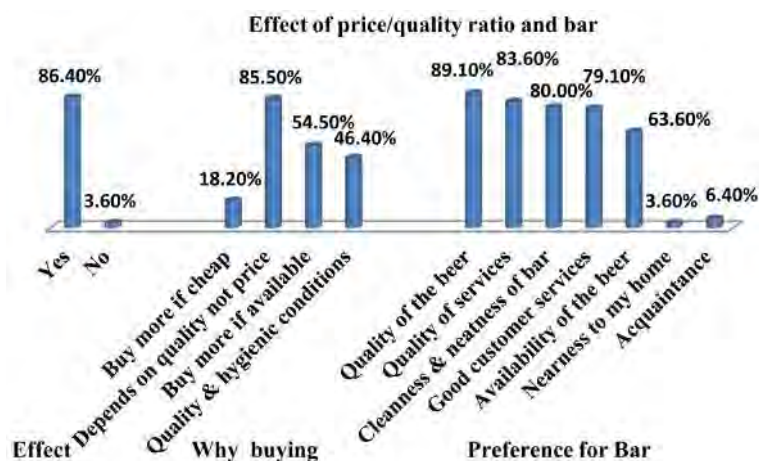


Figure 12. Effect of price/quality ratio and bar with their drinking habit.

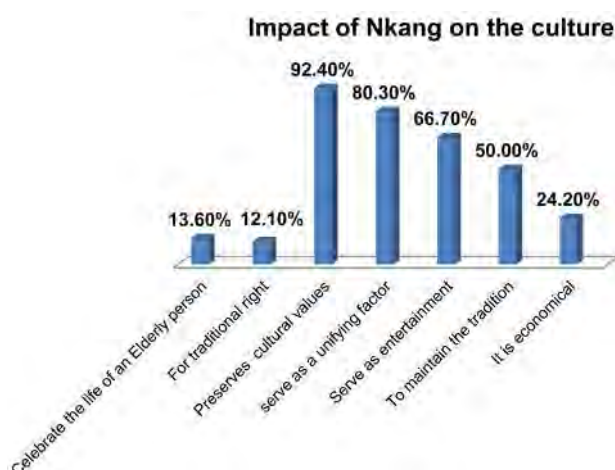


Figure 13. Cultural impact of Nkang.

Nkang must be prepared when an elderly person of rightful age dies and 12.10% uses *Nkang* for traditional rights.

Further investigation was made to see if the consumers will like *Nkang* to be replaced with red wine or whiskey for rituals and there was a 100.0% dislike by the consumers. When asked why; the following responses were got; 98.2% saying that they have to promote their cultural, 85.5% says its dignity be preserved for continuity, 83.6% says it is natural, 83.6% says it can serve many people at a time, 81.8% say it is produced locally, 69.1% says their culture must be conserved, 60.0% talk of *Nkang* being nutritious and 58.2% less alcoholic than the red wine.

4. Conclusion

Results from the survey show that corn beer locally made beer, as well as palm wine and industrially produced beer from barley are consumed in the North-West Region of Cameroon. Three types of corn beer *kwacha*, *sha-ah* and *Nkang* do exist with *Sha-ah* being the most popular, most available but with *Nkang*, the most appreciated by the consumers. On the other hand, *Kwacha* and *Nkang* are not frequently seen in the markets. This is an indication of them running the risk of extinction. *Kwacha* is more turbid and viscous than *Sha-ah* and then *Nkang*. *Kwacha* is whitish, *Sha-ah* is cream white and *Nkang* is dark brown in colour. *Nkang* is the most preferred to *Sha-ah* and then *Kwacha* by the consumers due to the fact that *Nkang* is very tasteful, least alcoholic, least turbid, least viscous, has the most attractive colour, has a significant impact on the culture of some localities in this region, *Nkang* is also natural and nutritious as the others. But it has a short shelf-life like the other two and is consumed within a short period of its production. Thus people tend to consume it more when freshly made because most households cannot afford cooling devices to extend the keeping quality of the *Nkang* which could result in further fermentation with final sour taste. Because of its low alcoholic content, the beverage is consumed by both adults (the most elderly), children, those who have health problems and mostly preferred by some Christians. However, it is less popular as comparing with *Sha-ah*, not available as it is not frequently seen in the markets. The consumers complain that the little found in the markets is of poor quality which keeps dropping everyday, thus an indication of it disappearing and risk becoming extinct. Consumers also attest that if *Nkang* is clarified and its quality improved, then they will be happy and can even buy it at a higher price. While those who want it for cultural reason say that, they do not want the culture to die down so its production process can be improved so as to have quality *Nkang* which can sustain their culture. Hence further works can be carried out on how to improve its quality and conservative method so as to make it sustainable.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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V.12) Optimization of Pectinase-Assisted Extraction of *Annona muricata* L. Juice and the Effect of Liquefaction on its Pectin Structure

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Optimization of pectinase-assisted extraction of *Annona muricata* L. juice and the effect of liquefaction on its pectin structure

Calister Wingang Makebe,^{a,b} Zangue Steve Carly Desobgo,^c Wilson Agwanande Ambindei,^a Abraham Billu,^b Emmanuel Jong Nso^a and P Nisha^{b*} 



Abstract

BACKGROUND: Soursop (*Annona muricata* L.) is an underutilized tropical and subtropical fruit with high nutritional and therapeutic benefits. This fruit is faced with enormous post-harvest losses due to its high perishability. This work was aimed to optimize the pectinase-assisted extraction conditions of soursop juice using Doehlert design and to study the effect of pectinase on its pectin structure.

RESULTS: The predicted models were validated for all the responses studied and the regression coefficients ranged from 0.905 to 0.987 ($P \leq 0.05$). An incubation time of 172 min, enzyme concentration of 0.04% (w/w) and incubation temperature at 42.9 °C were found to be the optimal conditions for soursop juice extraction, which resulted in 75.20%, 3.74, 7.35 °Brix, 87.06%T, and 0.44% MAE for soursop juice yield (%), pH, total soluble solids (TSS) (°Brix), clarity (%T) and titratable acidity (% malic acid equivalent, MAE), respectively. Morphologically, untreated soursop pulp presented a non-uniform spherical surface; enzyme hydrolyzed soursop exhibited ruptured and wrinkled surface; meanwhile for the different pectin obtained, untreated soursop pectin depicted porous surface and enzyme hydrolyzed soursop pectin showed whirling rough surface. Fourier-transform infrared (FTIR) confirmed the presence of similar chemical group stretching and vibrations in commercial pectin and soursop pectin.

CONCLUSION: Under the optimum conditions, the numerical predictions were similar to the experimental data obtained, thus confirming the validity of the models. Application of enzyme treatment caused the breakdown of pectin structure as illustrated by scanning electron microscopy (SEM) and FTIR analyses.

Supporting information may be found in the online version of this article.

Keywords: *Annona muricata* L.; juice; pectinase; optimization; liquefaction; pectin

1 INTRODUCTION

Annona muricata L., commonly called soursop, is a tasty fruit widely grown in the tropical and subtropical regions of the world, including South America, Africa, Asia, and Australia.^{1,2}

As a result of the increasing information on the high nutritional profile and content of health-protective phytochemicals as well as the peculiar flavor of this exotic fruit, it has received considerable research attention during the last decade.³ The fruit is reported to be a good source of fibers, minerals [phosphorus (P), iron (Fe), and calcium (Ca)], vitamins (B1, B2, B3, and C) and amino acids [methionine (Met), lysine (Lys) and tryptophan (Trp)].⁴ Previous findings revealed that *A. muricata* L. is a rich source of bioactive phenolic compounds with antidiabetic and antihypertensive potentials⁵ and anticarcinogenic properties.⁶ Antioxidant activity and inhibition against α -amylase, α -glucosidase, and angiotensin-I converting enzyme (ACE) of *A. muricata* L. pericarp extract was also reported.⁵ Unfortunately, the highly perishable nature of soursop fruit aggravated by the lack of appropriate post-harvest techniques leads to its post-harvest losses at about 76% of its total

production.⁴ Towards curbing post-harvest losses, several processing techniques such as spray drying of soursop pulp,⁷ and processing into products such as puree,^{8,9} nectar,¹⁰ jam,¹¹ and juice^{12–14} have been reported.

Soursop pulp is a suitable feedstock for juice production. However, the processing of this fruit pulp into juice is difficult due to its pectinaceous nature, which prevents the diffusion of solutes

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during the extraction process.^{9,15} Previous studies reported that depectinization improves the yield of extraction via increase liquefaction, reducing viscosity,¹⁶ higher release of phenolic and nutritional components,¹⁷ reduce efforts to press,⁹ enhances clarification¹⁷ and increases quality¹⁶ of several fruit juices such as banana,^{16,18} guava¹⁹ and mango.²⁰ The use of enzymes for the extraction of soursop has been reported earlier, and the enzyme-treated soursop could be used as juice,¹⁵ puree,⁹ and spray-dried powder.²¹ Chang *et al.*⁹ studied the impact of enzyme concentration (pectinase, α -amylase, and cellulase) and incubation time on soursop puree extraction. In a study carried out by Yusof and Ibrahim,¹⁵ the authors evaluated the quality of soursop juice after treatment with varying concentrations of pectinase and for different time intervals at a fixed temperature. The optimum conditions of treatment were found to be 0.075% of pectinase for an incubation time of 2 h, which improved the juice yield by 41%. However, the earlier studies did not consider the impact of liquefaction temperature on the extraction yield and the quality of soursop juice. As extraction temperature is an important parameter with respect to enzyme activity and the extraction yield, it is important to optimize the extraction conditions with respect to enzyme concentration, incubation time and temperature.

Response surface methodology (RSM) is a modeling technique used in the analysis of scientific problems by grouping mathematical and statistical systems, wherein a given outcome/response is impacted by many variables.²² RSM is employed to optimize the outcome/response by adopting an appropriate experimental design. Optimization is the improvement of a process efficiency so as to maximize the outcome.²² In 1970, Doehlert proposed a plan with a uniform distribution of the experimental points in the experimental space.²³ Compared to the other experimental designs, this design is practical, economical, more uniform, has few experimental application points and higher efficiency.¹⁶ With this background, this study aimed to understand the interactions between the extractions conditions of soursop juice, namely enzyme concentration, incubation time, and temperature, using pectinase-assisted extraction, and to determine the optimal extraction conditions using the Doehlert design (DD). The study also aims to evaluate the effect of liquefaction on its pectin structure.

2 MATERIALS AND METHODS

2.1 Raw material

Fully fresh mature soursop fruits, yellowish-green in color were purchased directly from a farmer in Penja, Littoral region of Cameroon. The fruits were washed under a running tap and then immersed in 2% hypochlorous acid, drained and allowed to ripen at ambient temperature (25 ± 2 °C) for 72 h. After ripening, the fruits were peeled, seeds were removed and then crushed (to increase the surface area for the action of pectinase) intermittently at an interval of 2.5 min for 5 min using an electric mixer (MG 218; Zodiac Preethi, Chennai, India). The crushed soursop pulp was stored in sealed plastic bags at -20 °C until use.

2.2 Enzyme and chemical reagents

Pectinase from *Aspergillus niger* with the enzymatic activity of 1.11 unit/mg (pH 4.0 and 50 °C) was purchased from Sigma-Aldrich, Vandtårnsvej 62A, 2860 Søborg, Denmark.

Sodium hydroxide, Folin Ciocalteu's phenol reagent, sodium carbonate, and sulfuric acid were purchased from Himedia, Bengaluru, India. Carbazole, D-(+)-galacturonic acid monohydrate,

commercial citrus pectin, gallic acid standard, sodium potassium tartrate, potassium ferrocyanide, and zinc acetate were purchased from Sigma-Aldrich, Steinheim, Germany. All chemicals were of analytical grade.

2.3 Soursop juice extraction

The crushed soursop pulp was thawed at 4 °C for 12 h prior to extraction. The crushed soursop pulp (150 g) was weighed into a beaker, followed by the addition of water to obtain a final water-to-substrate ratio of 1:1 v/w. It was placed in a temperature-controlled water bath at the appropriate temperature then pectinase was added to give a final enzyme-to-substrate ratio, as mentioned for each trial of the DD. Hydrolysis was carried out with continuous shaking at an interval of 15 min using a Remi Motor agitator (RQ 122; Elektrotechnik Ltd, Kolkata, India).

After liquefaction, the pectinase was inactivated by heating the sample at 95 °C in a water bath for 5 min. Sample was cooled at room temperature and centrifuged at $6000 \times g$ for 15 min at 25 °C using a centrifuge (7780; Kubota, Bunkyo-ku, Tokyo, Japan). The juice extract (supernatant) was carefully separated from the pellet and used for further analysis.

2.4 Modeling and optimization

The DD was used as an RSM to model the factors and obtain optimal conditions for the extraction of soursop juice. Based on literature,^{19,24–26} preliminary studies were carried out to define the experimental domain of independent variables, which were as follows: incubation time (30–180 min), enzyme concentration (0.01–0.1%), and incubation temperature (35–55 °C). The independent variables were studied at three levels ($-1, 0, +1$), with a total of 17 trials with five center points. The dependent variables (responses) were: yield (%), pH, total soluble solids (TSS) (°Brix), clarity (%T) and titratable acidity (% malic acid equivalent, MAE). Once the optimal conditions were obtained using DD, soursop juice extraction was carried out under these conditions to validate the accuracy of the model, and the extract was further analyzed.

For predicting the optimal point, a mathematical model was fitted to correlate the independent variables and the dependent variables. The mathematical model employed was a second-degree polynomial model with linear, quadratic, and interaction terms, as represented in Eqn (1).

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

where, Y_i is the response, X_i and X_j are the variables, β_0 is a constant, β_i is the coefficient of the linear term ($\beta_1, \beta_2, \beta_3$), β_{ii} is the coefficient of the quadratic terms ($\beta_{11}, \beta_{22}, \beta_{33}$) and β_{ij} is the coefficient of the interaction term ($\beta_{12}, \beta_{13}, \beta_{23}$).

To this effect, the mathematical model equations were analyzed by ANOVA (analysis of variance) using Minitab 19.1.1 (2019 Minitab, LLC, State College, PA, USA) software; meanwhile, response surface curves were obtained using OriginPro 9.0.0 (OriginLab Corporation, Northampton, MA, USA), to describe the individual and interactive effects of the factors on the responses.

Mathematical model validation is valuable for the prediction of responses in a given domain studied; hence, in the present study, the experimental and theoretical values given by the models were compared. Furthermore, the linear regression coefficient, absolute average deviation (AAD), bias factor (B_f), and exactitude factor (A_f) were used to validate the models.²⁷

2.5 Physicochemical analysis

Moisture, crude fiber, crude fat, crude protein, ash and carbohydrate contents of the crushed soursop pulp were determined by the official methods of the Association of Official Analytical Chemists (AOAC).²⁸

2.5.1 Yield

The soursop juice yield (% v/w) was evaluated as a percentage of the volume of juice extracted (supernatant) and obtained after centrifugation from the initial mass of the pulp, as presented in Eqn (2):

$$\text{Yield (\%v/w)} = \frac{\text{Volume of extract (supernatant)}}{\text{mass of pulp}} \times 100 \quad (2)$$

2.5.2 pH

The pH value of soursop juice was read using a pH-meter (pH 700; Eutech, Ayer Rajah Crescent, Singapore). The pH-meter was calibrated as stipulated in the pH-meter operating manual by dipping the electrode for a specified time in the neutral buffer (7.01), acid buffer (4.01) and basic buffer (10.01).

2.5.3 Total soluble solids (TSS)

The total soluble solids (TSS) content of soursop juice was obtained using a hand refractometer (0–32% Brix; ERMA, Nashik, Maharashtra, India). The refractometer was calibrated using distilled water, and °Brix of soursop juice was then measured.

2.5.4 Titratable acidity

Titrate acidity was measured according to the standard method.²⁸ To 10 mL of soursop juice aliquot was added a few drops of phenolphthalein indicator then titrated against standardized 0.1 N sodium hydroxide to a pink color change which persisted for at least 30 s. The results were expressed in percentage of malic acid equivalent (%MAE).

2.5.5 Clarity

Clarity of the soursop juice was measured at 660 nm by noting the percentage transmittance (%T) using a spectrophotometer (UV-2600; Shimadzu, Kyoto, Japan) according to Sagu *et al.*¹⁶

2.6 Pectin analysis

2.6.1 Galacturonic acid determination

Pectin quantification in terms of galacturonic acid content in crushed soursop pulp before and after enzymatic hydrolysis was obtained using the method described by Ninga *et al.*¹⁹ with slight modifications. Sample (1 g) was extracted with 0.05 N sodium hydroxide for 30 min. Then, 6 mL of concentrated sulfuric acid was added to the above extract. The mixture was homogenized and heated at 95 °C for 10 min in a water bath and then cooled in an ice bath. The mixture was further added with 0.5 mL of 0.15% carbazole and incubated for 15 min at 25 °C to observe a color change. Absorbance was read at 520 nm using a spectrophotometer (UV-2600; Shimadzu). After enzymatic action, pure (95.6%) ethanol was added to the hydrolyzed soursop at the ratio of 1:3 (w/w) while agitating, and the mixture was kept at 4 °C overnight, followed by centrifugation at 10 000 × *g* for 15 min at 4 °C, which was aimed at precipitating the pectin. The supernatant was discarded, and the pellet was washed successively with 50%, 75%, and pure (95.6%) ethanol to eliminate any monosaccharides and disaccharides present in the pellet before further analysis. The

value of galacturonic acid was determined and expressed as gram per kilogram of sample.

2.6.2 Pectin extraction

Pectin was extracted as described by Ranganna²⁹ with some modifications. Soursop sample was diluted in distilled water, and the pH was reduced to 2.0 using 0.5 mol/L hydrochloric acid and was allowed to boil for 1 h at 90 °C while stirring every 15 min. Sample was rapidly filtered using a muslin cloth, and absolute ethanol was added to the liquid phase at the ratio of 1:3 (v/v) and centrifuged at 5000 × *g* for 10 min at 4 °C. The pellet was rinsed with acetone to remove impurities and then freeze-dried.

2.6.3 Equivalent weight, methoxyl content, total anhydrouronic acid content and degree of esterification

After soursop pulp pectin extraction, the pectin was tested for the absence of amide as described by Ranganna.²⁹ The equivalent weight analysis was carried out by mixing 0.5 g of the pectin with 5 mL of ethanol, sodium chloride (1 g), and 100 mL of distilled water. Complete dissolution of the pectin was ensured by stirring before the addition of six drops of red phenol indicator then titration with standardized 0.1 N sodium hydroxide. To the neutral solution of equivalent weight, 25 mL of 0.25 N sodium hydroxide was added, shaken thoroughly, and kept at ambient temperature (25 ± 2 °C) for 30 min. To this, 25 mL of 0.25 N hydrochloric acid was added then titrated to purple color. The anhydrouronic acid content and the degree of esterification were calculated using the formula developed by Ranganna²⁹ and expressed in percent.

2.7 Scanning electron microscopy (SEM)

A scanning electron microscope (EVO 18; Zeiss, Oberkochen, Germany) was used to observe the morphological change of the treated sample. Untreated soursop, hydrolyzed soursop residue, soursop pectin, and hydrolyzed soursop pectin were lyophilized, cut/spread on the metallic plate and, coated with a thin layer of gold for an hour. Micrographs were obtained at a magnification of 20 000× with 15 kV of acceleration.

2.8 Fourier-transform infrared (FTIR) spectroscopy

The Fourier-transform infrared (FTIR) spectra of commercial pectin, pectin from untreated soursop, and hydrolyzed soursop were analyzed using an attenuated total reflectance-Fourier-transform infrared (ATR-FTIR) spectrometer (IR spectrophotometer; Bruker Optik GmbH, Ettlingen, Germany). The sample was deposited on the surface of a diamond crystal and pressed with a system press tip flap. Spectra were registered in transmission mode within the wavenumber range of 4000–500 cm⁻¹ with 32 scans per spectrum at a resolution of 8 cm⁻¹.

3 RESULTS AND DISCUSSION

3.1 Proximate composition and physicochemical properties of soursop

The proximate composition and physicochemical properties of soursop are presented in Table 1 alongside the values reported in the literature. The soursop fruit investigated in the current study has higher crude fat value than that reported by Sanusi and Bakar.³ Similarly, the values of ash and crude fiber were higher than those pointed out by Ndife *et al.*¹⁴ Meanwhile, the carbohydrate, protein, and moisture contents showed similarities to the findings of Badrie and Schauss,⁴ Ndife *et al.*¹⁴ and Pinto *et al.*³⁰ Some researchers termed this fruit to be a high caloric

Table 1 Proximate composition and physicochemical properties of soursop pulp

Parameter	Values	Literature values
Moisture content (g/kg)	822.7 ± 5.10	82.8 g/100 g ⁴
Ash content (g/kg)	24.4 ± 0.01	1.83 ± 0.15% ¹⁴
Crude fat content (g/kg)	15.0 ± 0.01	0.74–0.97 ± 0.06% ^{4,12,14}
Crude fiber content (g/kg)	39.96 ± 0.01	2.26 ± 0.10% ¹⁴
Carbohydrate content (g/kg)	173.3 ± 9.40	17.25 ± 0.1 g/100 g ³⁰
Protein content (g/kg)	50.8 ± 2.50	5.35 ± 0.24% ¹⁴
pH	3.66 ± 0.010	3.68 ± 0.16 ⁹
Color		
L*	82.08 ± 0.005	74.44 ± 0.60 ⁹
a*	0.09 ± 0.005	−0.94 ± 0.16 ⁹
b*	16.64 ± 0.005	10.19 ± 0.27 ⁹
Titrateable acidity (% MAE)	4.13 ± 0.630	1.50 ± 0.05 (%) ¹⁴
Reducing sugars (g/kg)	131.1 ± 3.00	9.91 ± 0.04 (g/100 g) ³¹
Total polyphenol content (g GAE/kg)	0.8 ± 0.040	34.63 ± 1.21 (µg/100 g) ¹³
Total flavonoid content (g quercetin/kg)	0.01 ± 0.000	NA
Total soluble solids (°Brix)	11.12 ± 0.300	11.0 ± 0.4 ⁸

MAE, malic acid equivalent; GAE, gallic acid equivalent; NA, not available.

value fruit because of the high carbohydrate content.³⁰ These discrepancies in the proximate composition of soursop pulp compared to other studies could be due to the dissimilarities in geographical conditions, agronomical practices, fruit variety and maturity stage.

The physicochemical properties of the soursop pulp revealed that the values of pH and TSS were similar to those reported in other works.^{8,21} Nevertheless, higher values were obtained in the present study for color, reducing sugars, titrateable acidity and total polyphenol content compared to those previously reported in similar studies (shown in Table 1).^{9,13,14,31} The variation in the physicochemical properties of the soursop pulp is attributed to fruit varietal differences, horticultural practices, and climatic conditions,³⁰ meanwhile other findings associated these discrepancies to the ripening processing typical of *Annonaceae* fruits due to the decomposition of complex carbohydrates and organic acids.³¹

3.2 Pectin analysis

Pectin yield, equivalent weight, methoxyl content, anhydrouronic acid content, degree of esterification, and galacturonic acid values are represented in Table 2. The pectin yield is in accordance with other findings, which proposed that the pectin in soursop could

be applied as an important by-product.³⁰ The equivalent weight represents an index of pectin's jelly-forming potential under suitable conditions. Soursop pectin was of the low methoxyl type since the value of degree of esterification was below 50%. Hence, it exhibited a very slow gel set and the possible formation of thermo-reversible gels at low pH and calcium ions. However, soursop pectin has the capacity to form gel even in the presence of lower sugar concentration. The degree of esterification obtained in this study was similar to that reported by Liew *et al.*,³² who obtained a degree of esterification of 41.67% for passion fruit peel pectin extracted at pH 3.3 for 120 min. The anhydrouronic acid content of pectin was below 65%, which showed that it contained some impurities like proteins, starches, and sugars. This anhydrouronic acid result is in agreement with those obtained from banana peel pectin that displayed an anhydrouronic acid content ranging from 55.61 to 58.77%.³³

3.3 Statistical analysis and model fitting of DD

Incubation time, enzyme concentration, and incubation temperature were computed for the optimization of yield, pH, clarity, TSS, and titrateable acidity by the use of a DD. The experimental matrix of the DD (both coded and real values) and the responses are presented in Table 3 meanwhile, the coefficient of models (Y_1 , Y_2 , Y_3 , Y_4 , and Y_5) and their respective P -values are shown in Table 4. The coefficients of models were validated based on the P -values, with P -values less than or equal to 0.05. However, the mathematical models in this study were valid upon consideration of R^2 , adjusted R^2 , AAD, A_f and B_f .²⁷ A model was claimed workable if the R^2 was greater than or equal to 0.8, R^2 -adjusted was greater than or equal to 0.7, AAD was around 0, and finally, the A_f and B_f were closed to 1.²⁷ As shown in Table 4, the coefficients of linear terms (x_1 , x_2 , and x_3) and quadratic terms (x_1^2 , x_2^2 and x_3^2) were shown to be significant for yield, clarity, TSS, titrateable acidity and pH. The interaction x_1x_2 term was found to be significant on clarity and titrateable acidity, while interaction x_1x_3 was found to be significant for yield, clarity, and titrateable acidity, and finally, the pH, TSS, and titrateable acidity were affected significantly by the interaction x_2x_3 . The strong significance of interactions in this study justifies

Table 2 Soursop pectin extraction and characterization

Parameter	Values
Yield (%)	9.60 ± 0.001
Galacturonic acid (g/kg)	5.96 ± 0.370
Galacturonic acid after hydrolysis (g/kg)	15.42 ± 0.978
Amide test	Negative
Equivalent weight	559.67 ± 17.240
Methoxyl content (%)	2.86 ± 0.540
Total anhydrouronic acid content (%)	48.0 ± 1.310
Degree of esterification (%)	33.95 ± 4.320

Table 3 Doehlert design of coded, real variables and experimental responses

Coded values			Real values			Responses				
Incubation time x_1	Enzyme concentration x_2	Incubation temperature x_3	Incubation time (min) X_1	Enzyme concentration (%) X_2	Incubation temperature (°C) X_3	Yield (%)	pH	Clarity (%T)	Total soluble solids (TSS) (°Brix)	Titrateable acidity (% MAE)
0	0	0	105	0.055	45.0	73.67 ± 0	3.76 ± 0	69.8 ± 4.95	7.4 ± 0.14	0.447 ± 0
0	0	0	105	0.055	45.0	73 ± 0.47	3.775 ± 0.01	71.51 ± 4.30	7.5 ± 0.28	0.447 ± 0
0	0	0	105	0.055	45.0	73.33 ± 0	3.77 ± 0.01	67.68 ± 2.47	7.4 ± 0.14	0.447 ± 0
0	0	0	105	0.055	45.0	72.66 ± 0	3.77 ± 0.01	70.68 ± 1.67	7.4 ± 0.14	0.467 ± 0
0	0	0	105	0.055	45.0	74 ± 1.64	3.76 ± 0.01	68.98 ± 3.70	7.5 ± 0.18	0.447 ± 0.03
-1	0	0	30	0.055	45.0	71.5 ± 1.41	3.825 ± 0.01	62.8 ± 2.78	7.1 ± 0	0.424 ± 0.03
1	0	0	180	0.055	45.0	75 ± 0	3.74 ± 0.01	78.82 ± 2.30	7.4 ± 0	0.469 ± 0
-0.5	-0.866	0	67.5	0.01513	45.0	71.67 ± 0	3.77 ± 0	48.69 ± 3.67	7.1 ± 0.14	0.411 ± 0.03
0.5	-0.866	0	142.5	0.01513	45.0	74 ± 0.94	3.74 ± 0.01	77.47 ± 1.06	7.3 ± 0.28	0.467 ± 0.06
-0.5	0.866	0	67.5	0.09487	45.0	74.67 ± 0	3.775 ± 0.01	83.21 ± 3.52	7.4 ± 0	0.447 ± 0
0.5	0.866	0	142.5	0.09487	45.0	75 ± 1.77	3.72 ± 0.04	84.43 ± 0.41	7.5 ± 0.14	0.409 ± 0
-0.5	-0.289	0.816	67.5	0.041995	53.16	72.63 ± 0.47	3.77 ± 0.01	64.19 ± 3.25	7.3 ± 0	0.500 ± 0.03
0	0.577	0.816	105	0.080965	53.16	74.5 ± 0	3.755 ± 0.01	77.15 ± 4.64	7.2 ± 0.28	0.438 ± 0.31
0.5	-0.289	0.816	142.5	0.041995	53.16	72.5 ± 1.89	3.75 ± 0	63.95 ± 5.34	7.4 ± 0	0.469 ± 0.03
-0.5	0.289	-0.816	67.5	0.068005	36.84	71.67 ± 0.94	3.78 ± 0	70.15 ± 1.92	7.1 ± 0	0.424 ± 0.03
0	-0.577	-0.816	105	0.029035	36.84	73 ± 0.94	3.77 ± 0	72.52 ± 2.49	7.1 ± 0.14	0.433 ± 0.06
0.5	0.289	-0.816	142.5	0.068005	36.84	74.33 ± 1.88	3.735 ± 0.01	87.94 ± 6.14	7.3 ± 0.14	0.502 ± 0

Table 4 Coefficients of the second order polynomial models for the responses (yield, pH, clarity, TSS, titratable acidity), *P* values and validation tools (*R*², *R*²-adjusted, AAD, *A_f*, and *B_f*)

Source	Yield (%)		pH		Clarity (%T)		TSS (°Brix)		Titratable acidity (% MAE)	
	Coefficient	<i>P</i> Values	Coefficient	<i>P</i> Values	Coefficient	<i>P</i> Values	Coefficient	<i>P</i> Values	Coefficient	<i>P</i> Values
<i>x</i> ₀	73.332	NA	3.767	NA	69.73	NA	7.44	NA	0.451	NA
<i>x</i> ₁	1.524	0.0001	−0.04	0.0001	9.95	0.0010	0.15	0.0107	0.019	0.0001
<i>x</i> ₂	1.126	0.0006	−0.005	0.1420	11.61	0.0004	0.10	0.0432	−0.007	0.0036
<i>x</i> ₃	0.129	0.4198	−0.002	0.5629	−5.17	0.0161	0.0817	0.0831	0.0098	0.0148
<i>x</i> ₁₁	−0.082	0.7685	0.0155	0.0655	1.08	0.6824	−0.19	0.0451	−0.0045	0.0580
<i>x</i> ₂₂	0.667	0.0484	−0.025	0.0060	4.39	0.1376	−0.086	0.2812	−0.0208	0.0023
<i>x</i> ₃₃	−0.487	0.1145	−0.008	0.1599	3.02	0.2754	−0.24	0.0161	0.0213	0.0056
<i>x</i> ₁₂	−1.129	0.0192	−0.014	0.1168	−15.55	0.0051	−0.0564	0.5376	−0.053	0.0003
<i>x</i> ₁₃	−2.109	0.0026	0.010	0.2792	−16.56	0.0068	−0.081	0.4423	−0.0856	0.0001
<i>x</i> ₂₃	1.105	0.0331	0.019	0.0695	3.47	0.3950	−0.213	0.0798	−0.0484	0.0003
<i>R</i> ²	0.938		0.974		0.969		0.901		0.959	
<i>R</i> ² -adjusted	0.941		0.928		0.930		0.775		0.906	
AAD	0.003		0.001		0.023		0.007		0.021	
<i>A_f</i>	1.003		1.001		1.023		1.007		1.022	
<i>B_f</i>	1.000		1.000		1.001		1.000		0.988	

x is the coefficient of the equations for each mathematical model; *x*₀ is the constant term, *x*₁, *x*₂, and *x*₃ are the linear effects (1, 2, 3 for incubation time, enzyme concentration and incubation temperature respectively), *x*₁₁, *x*₂₂, and *x*₃₃ are quadratic effects and *x*₁₂, *x*₁₃, and *x*₂₃ are the interactions. TSS, total soluble solids; AAD, absolute average deviation; *A_f*, exactitude factor; *B_f*, bias factor; MAE, malic acid equivalent; NA, not available.

the use of RSM as an optimization tool since the one-variable-at-a-time method does not allow the investigation of the contribution of interactive effects, which makes it difficult to determine optimum values. It is worth noting that as for all the responses, incubation time (*x*₁) had the most significant effect, followed by enzyme concentration (*x*₂) and incubation temperature (*x*₃), respectively.

The high *R*² (coefficient of determination) for yield, pH, clarity, TSS and titratable acidity of 0.938, 0.974, 0.969, 0.901 and 0.959 indicated that the variables successfully explained 93.8%, 97.4%, 96.9%, 90.1% and 95.9% of the variation in the models respectively, denoting the models are practical. Nonetheless, the value of each *R*² was close to that of its respective *R*²-adjusted value, hence corroborating the high explanatory power of the regression models used in this study. The results represented in Table 4 also revealed that the five models were significant and suitable for the adequate prediction of yield, pH, clarity, TSS, and titratable acidity responses within the variable ranges chosen in this study. Deleting all non-significant terms, the regression models for yield, pH, clarity, TSS, and titratable acidity are listed as follows:

$$Y_1 = 73.332 + 1.524x_1 + 1.126x_2 + 0.667x_2^2 - 1.129x_1x_2 - 2.109x_1x_3 + 1.105x_2x_3 \quad (3)$$

$$Y_2 = 3.767 - 0.04x_1 - 0.025x_2^2 \quad (4)$$

$$Y_3 = 69.73 + 9.95x_1 + 11.61x_2 - 5.17x_3 - 15.55x_1x_2 - 16.56x_1x_3 \quad (5)$$

$$Y_4 = 7.44 + 0.15x_1 + 0.1x_2 - 0.19x_1^2 - 0.2414x_3^2 \quad (6)$$

$$Y_5 = 0.451 + 0.019x_1 - 0.007x_2 + 0.009x_3 - 0.020x_2^2 + 0.002x_3^2 - 0.053x_1x_2 - 0.085x_1x_3 - 0.048x_2x_3 \quad (7)$$

where, *Y*₁ = yield (%), *Y*₂ = pH, *Y*₃ = clarity (%T), *Y*₄ = TSS (°Brix), *Y*₅ = titratable acidity (% MAE), *x*₁ = incubation time, *x*₂ = enzyme concentration and *x*₃ = incubation temperature.

3.4 Analysis of RSM

Table 4 shows that incubation time had a significant impact on all the responses. An increase in incubation time resulted in a significant increase in the yield, clarity, and titratable acidity and a significant decrease in the pH (*P* < 0.05). Results shown in Fig. 1(a) were obtained by fixing enzyme concentration (*x*₂) at 0.015% and the incubation temperature (*x*₃) at 36.84 °C, while varying the incubation time (*x*₁); Fig. 1(b) by fixing incubation time (*x*₁) at 30 min and incubation temperature (*x*₃) at 36.84 °C while varying enzyme concentration (*x*₂) and Fig. 1(c) was obtained by fixing the incubation time (*x*₁) at 30 min and enzyme concentration (*x*₂) at 0.015% with a variation in incubation temperature (*x*₃).

3.4.1 Effect of variables on yield

An increase of incubation time from 30 to 180 min, led to a significant increase from 68.90 to 77.35% for yield (Fig. 1(a)). Juice yield while increasing the incubation time was paired with a more pronounced action of the pectinase. Indeed, pectinase acts by cleavage of the pectin constituting the cell wall of the soursop pulp at the α-1,4-glucosidic bonds into monomers of galacturonic acid, resulting in the breakdown of cell walls and a release of intracellular liquids containing solutes that migrate from intracellular environment to extracellular environment and hence increased the extraction yield of juice.¹⁸ It has been reported that prolonged incubation resulted in a higher extraction yield of fruit juice.^{15,18} Increase of enzyme concentration from 0.015% to 0.095% induced a significant increase (Fig. 1(b)) in yield from 68.90% to 71.25%. Since the enzyme concentration is very low compared to that of the substrate, the reaction rate is directly proportional to the concentration of the enzyme. That is, the reaction rate increased as enzyme concentration increased. In other words, the significant increase in yield could be related to the rate of hydrolysis of the pectin following the increase in pectinase concentration. Similar observations have been reported^{15,34} for pectin-based pulps treated with pectinase. The impact of

interaction x_1x_3 (incubation time/incubation temperature) as shown in Fig. 1(f) revealed that the higher the incubation time and incubation temperature within the experimental range, the better and faster the pulp hydrolysis resulting in an increase in the juice extraction yield because of pulp degradation by pectinase followed by juice release.¹⁵

3.4.2 Effect of variables on pH

There was a significant decrease in pH value from 3.81 to 3.74 when the incubation time was increased from 30 to 180 min, which could be attributed to the liberation of the carboxyl groups and other organic acids following the enzymatic hydrolysis of pectin.³⁵ This decrease in pH with an increase in extraction time is similar to the findings of Makebe *et al.*²⁷ and Yusof and Ibrahim.¹⁵ It is observed from Fig. 1(i) that the impact of the interaction x_2x_3 (enzyme concentration/incubation temperature) was significant on pH. A simultaneous increase in the values of both factors contributed to a decrease in the pH value, and this is obviously due to the release of galacturonic and other organic acids.³⁵

3.4.3 Effect of variables on clarity

Increasing the incubation time (30 to 180 min) led to a significant increase in clarity from 35.76 to 100% (Fig. 1(a)). During enzymatic treatment, pectinases hydrolyze pectin molecules over time, facilitating the formation of protein–pectin complexes, and the elimination of these colloidal particles in juice contributes to an

increase of juice clarity.^{15,34} This result is similar to that reported^{16,26} whereby the effect of pectinase on the extraction yield of banana juice was studied. Increasing the concentration of pectinase (Fig. 1(b)) could increase the clarity of juice, which in this study, increased from 35.77%T to 77.90%T when enzyme concentration was increased from 0.015% to 0.095%, thus suggesting the formation of larger aggregates as earlier explained and thereby settling,³⁴ eventually increasing the clarity of the juice. The increase in juice clarity from 35.77%T to 49.52%T with incubation temperature (36.84 to 53.16 °C) (Fig. 1(c)) is explained by the fact that the optimal temperature of pectinases is between 40 and 55 °C.³⁶ An increase in temperature in this range allows a more efficient pectinase action upon the hydrolysis of pectin, and thus an increasingly clear juice. The significant impact of interaction x_1x_2 (incubation time/enzyme concentration) is presented in Fig. 1(d) with a significant increase in clarity (from 35.77%T to 97.89%T), indicating a synergistic effect between both factors. This is quite normal because incubation time and pectinase concentration are considered as key parameters for pectin hydrolysis. Knowing the product of the reaction of pectinase on pectin and the consequences on the juice clarity, it is therefore evident to obtain an increase of both responses. This was obtained at the same incubation temperature of 36.84 °C. As shown in Fig. 1(g), interaction x_1x_3 (incubation time/incubation temperature) had a significant increase in clarity, and this could be due to the better decantation of trub.³⁵

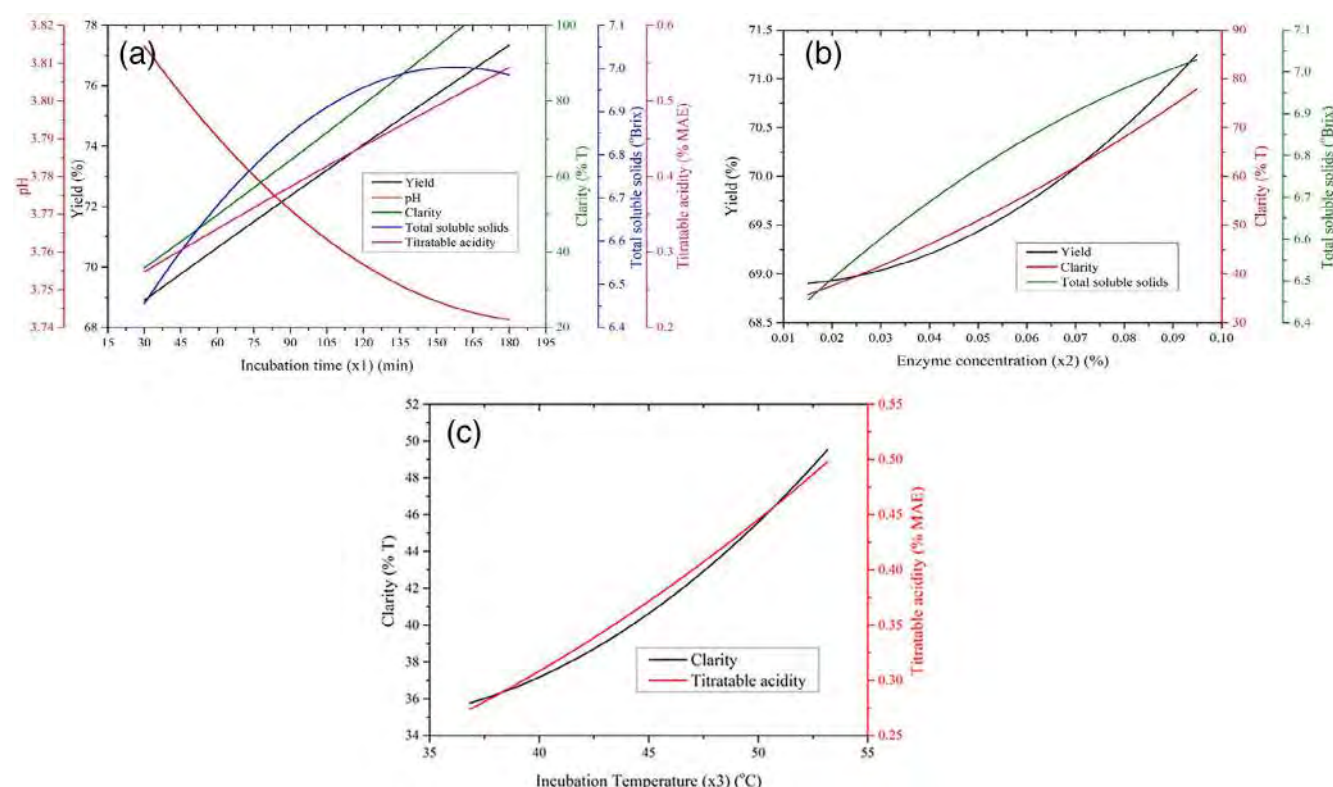


Figure 1 Evolution of responses as a function of factors: (a) incubation time (enzyme concentration and incubation temperature fixed respectively at 0.015% and 36.84 °C); (b) enzyme concentration (incubation time and incubation temperature fixed respectively at 30 min and 36.84 °C); (c) incubation temperature (incubation time and enzyme concentration fixed respectively at 30 min and 0.015%); mesh plot of clarity (d) and titratable acidity (e) as a function of incubation time (x_1) and enzyme concentration (x_2) (incubation temperature fixed at 36.84 °C); yield (f), clarity (g) and titratable acidity (h) as a function of incubation time (x_1) and incubation temperature (x_3) (enzyme concentration fixed at 0.015%); pH (i), total soluble solids (j) and titratable acidity (k) as a function of enzyme concentration (x_2) and incubation temperature (x_3) (incubation time fixed at 30 min).

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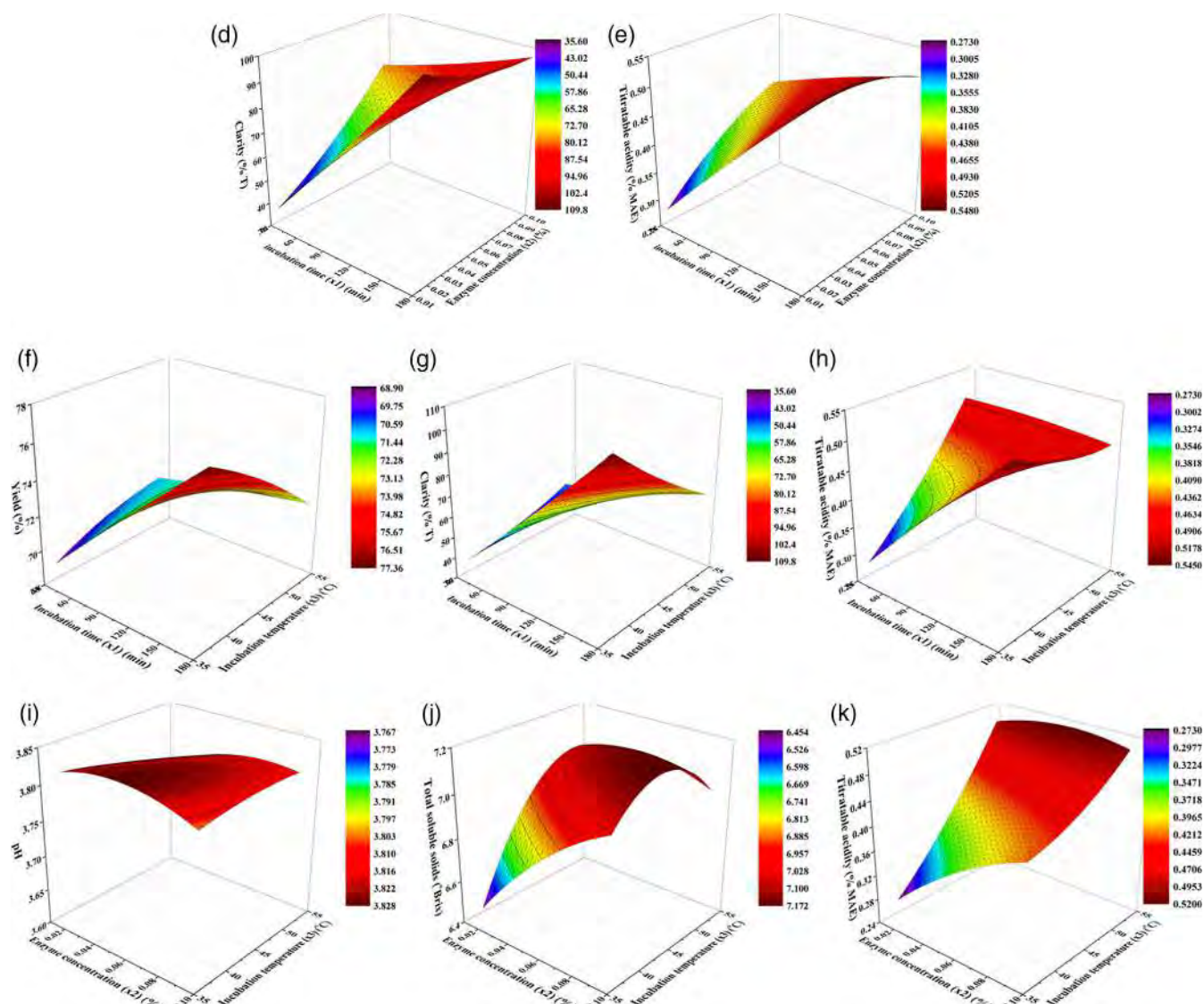


Figure 1 (Continued)

3.4.4 Effect of variables on TSS

As for TSS, an increase in incubation time from 30 to 52.66 min (Fig. 1(a)), contributed to an increase in the TSS value from 6.45 °Brix to 7.00 °Brix, meanwhile further increase in incubation time from 52.66 to 180 min yielded constant TSS (Fig. 1(a)). This could be explained by the extensive hydrolysis of cellular pectin, resulting in increased release of compounds such as sugars³⁵ and other components that are soluble solids. A plateau was observed for the TSS towards the end of the prolonged incubation time. An increase in TSS from 6.45 °Brix to 7.03 °Brix with an increase in enzyme concentration (Fig. 1(b)) could be attributed to the release of significant soluble solids components into the medium following hydrolysis.²⁷ The impact of the interaction x_2x_3 (enzyme concentration/incubation temperature) represented in Fig. 1(j) was significant on TSS, which was due to the accumulation of the soluble solids formed upon hydrolysis.³⁵

3.4.5 Effect of variables on titratable acidity

A significant increase in titratable acidity from 0.27 to 0.55% MAE was observed with an increase of incubation time from 30 to 180 min (Fig. 1(a)). Commercial pectinase is a mixture of several enzymes, including polygalacturonase and pectin methylesterase. Hydrolysis of pectin during long incubation time led to the release of galacturonic acids and other organic acids due to the action of these two enzymes, thus increasing the titratable acidity of the juice. Several authors have observed this phenomenon.^{15,27,37} Organic acids play a relevant role in the processing of fruit juice. It serves as a mild preservative, contributes to the flavor development via a balanced sugar/acid ratio, and stimulates saliva secretion through a thirst-quenching effect.^{9,14,38} The increase in titratable acidity (from 0.274% MAE to 0.498% MAE) is justified by the fact that further hydrolysis of pectin by pectinase with increasing incubation temperature (Fig. 1(c)) permitted

a greater release of galacturonic acids and other organic acids in the juice. The interaction x_1x_2 (incubation time/enzyme concentration) as presented in Fig. 1(e) contributed to a significant increase in the titratable acidity from 0.273% MAE to 0.508% MAE meanwhile, for the interaction x_1x_3 (incubation time/incubation temperature) shown in Fig. 1(h) and the interaction x_2x_3 (enzyme concentration/incubation temperature) represented in Fig. 1(k), it was observed that the factors concerned with the interactions increased the titratable acidity simultaneously because of the release of galacturonic acid and other organic acids.³⁵

3.5 Optimization and verification of the predicted optimum

The optimum conditions for juice yield, pH, clarity, TSS, and titratable acidity were found to be different. Therefore, there was a need to find a predicted composite optimum from the models, which should take into account all responses (yield, pH, clarity, TSS, and titratable acidity). The model simulation generated theoretical optimum conditions for the extraction of soursop juice as follows: 172.22 min, 0.0398%, and 42.15°C for incubation time, enzyme concentration, and incubation temperature, respectively. Under theoretical conditions, the predictive responses were 75.23%, 3.746, 87.06%T, 7.35 °Brix, and 0.44% MAE for juice yield, pH, clarity, TSS, and titratable acidity, respectively. Using the optimum conditions, the following experimental results were

obtained; 77.33%, 3.64, 94.95%T, 7.5 °Brix, and 0.46% MAE for juice yield, pH, clarity, TSS and titratable acidity, respectively. These results were close to that predicted by the model simulation, which implies that each model was quite precise in its prediction and also confirmed the validation done using statistical tools.

3.6 Morphological analysis

In Fig. 2(a), soursop presented a non-uniform spherical surface, and this was ascribed to the breeding of ice in intermolecular spaces of the soursop during lyophilization at the point of freezing of the free water, limiting the molecular movement of polysaccharides linked to bound water. A similar observation was also reported for guava structure.³⁹ In contrast, hydrolyzed soursop (Fig. 2(b)) presented a ruptured, coarse, and wrinkled surface compared to unhydrolyzed soursop (Fig. 2(a)), which had all its fibrous structures maintained. The damaged structure of hydrolyzed soursop is linked to pectinase hydrolysis of pectic substances like polygalacturans, which make up the middle lamella and are bound by α -1-4-glucosidic bonds of galacturonic acid units.⁴⁰ The hydrolysis of pectic substances caused the collapse of the middle lamella and the loss of structural matrix of the cell walls resulting in the release of more solutes from soursop and increase of yield of soursop juice extraction. Therefore, the destruction of soursop cell walls with the help of pectinase was

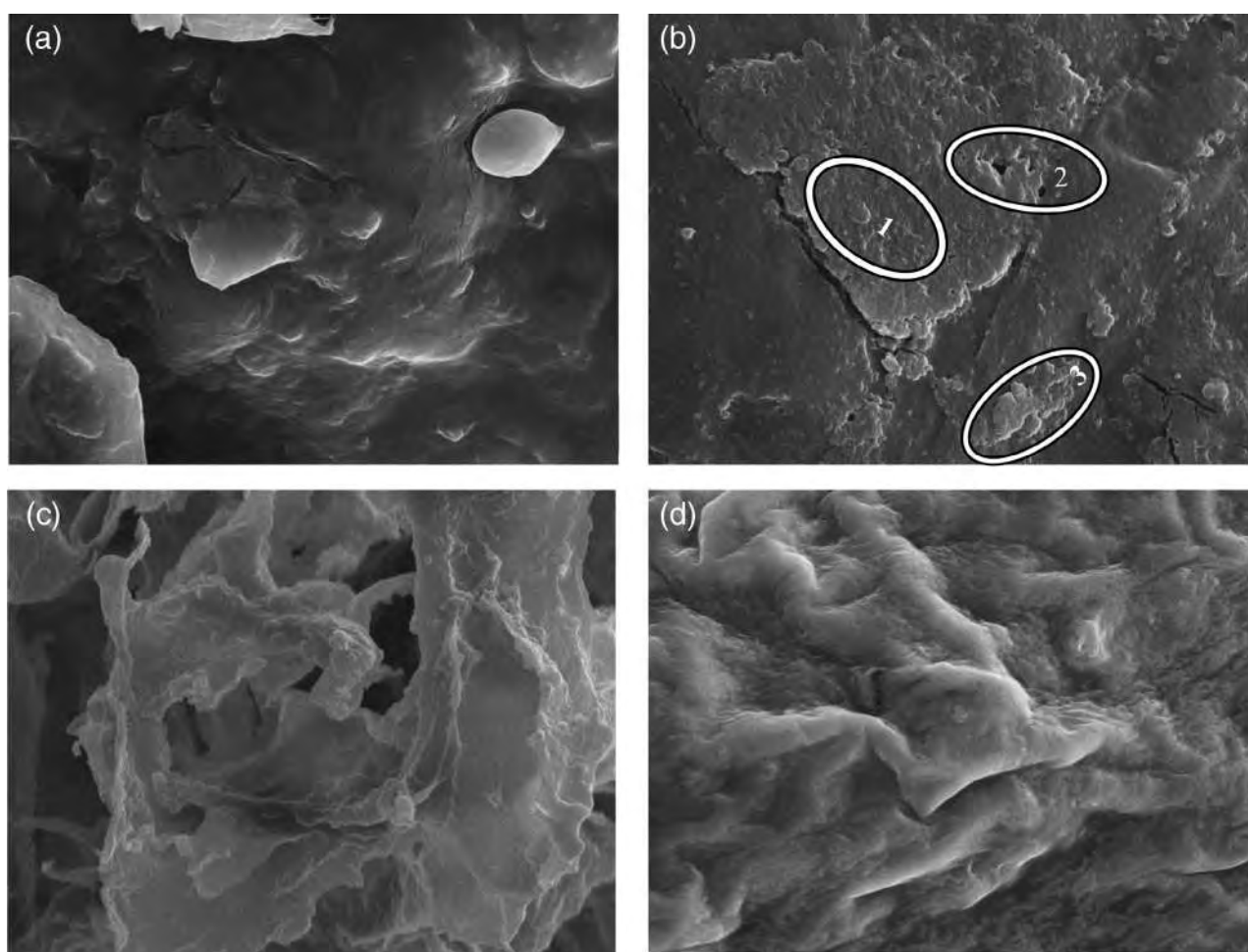


Figure 2 Micrographs of untreated soursop (a), hydrolyzed soursop (b), soursop pectin (c) and hydrolysed soursop pectin (d). 1, coarse; 2, ruptured; 3, wrinkled.

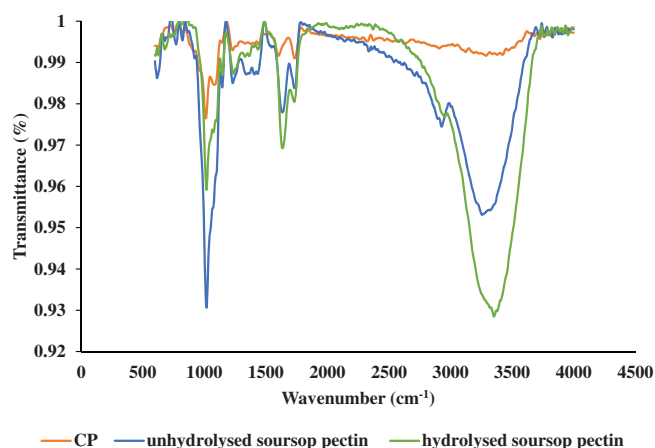


Figure 3 FTIR spectra for unhydrolyzed soursop pectin, hydrolyzed soursop pectin and commercial citrus pectin.

beneficial for releasing solutes, which were previously detained in the plant cell structure.

The image of pectin extracted from untreated soursop (Fig. 2(c)) was found to be porous. This is presumed to be as a consequence of the high incubation temperature (90°C), which generates a disintegration in the structure, leading to a thinner surface.⁴¹ Pectin from hydrolyzed soursop is of the low methoxyl type, and its destruction is responsible for the whirling rough surface presented in Fig. 2(d). The morphology, as mentioned earlier, is predicted to be a result of the removal of methyl esters leading to degradation of the galacturonic acid linkages and calcium-mediated crosslinks between pectins, which corroborates to total structural rupture.⁴²

3.7 Structural analysis

The FTIR spectra of commercial pectin, pectin from untreated soursop pulp and hydrolyzed soursop pulp are shown in Fig. 3. FTIR spectra of different pectin samples have characteristic peaks at 3390.6, 2939.0, 1749.0, and 1052.1 cm^{-1} corresponding to $-\text{OH}$, $-\text{CH}$, $\text{C}=\text{O}$ of ester, and acid, and $-\text{COC}-$ stretching of galacturonic acid (Supplementary Table 1).^{43,44} The intensity of bands between 3400–2900 cm^{-1} was higher in pectin from hydrolyzed soursop, confirming the hydrolysis of glycosidic bonds and exposition of free $-\text{OH}$ to a greater extent. This is in agreement with the findings of Xu *et al.*,⁴⁵ who obtained broad absorption peaks at 3410 cm^{-1} (hydroxyl groups) and weak bands at 2920 cm^{-1} ($\text{C}-\text{H}$ stretching) from jackfruit pectin. Likewise, Manrique and Lajolo⁴⁶ also reported the same stretching at 3400 cm^{-1} and 2930 cm^{-1} bands for pectin isolated from ripening papaya fruit. The results obtained in this work for commercial pectin and untreated soursop pectin are in accordance with the findings of Xu *et al.*⁴⁵ whose FTIR data confirmed that methyl-esterified forms existed predominantly in pectin samples. Other studies revealed the assignment of $\text{C}=\text{O}$ stretching vibration of the methyl-esterified carboxyl groups of bands at 1750 cm^{-1} for soy hull pectin,⁴³ at 1746 cm^{-1} for jackfruit pectin⁴⁵ and 1737 cm^{-1} for the cell wall pectin fraction of ripe strawberry fruit.⁴⁷ FTIR spectra of both samples showed a good match with the spectrum of commercial pectin. The relatively weak intensity of the Raman bands at 1470, 1183, and 1165 cm^{-1} evidenced that pectins from hydrolyzed soursop and untreated soursop were acetylated (Fig. 3). The

region of 1200 to 1000 cm^{-1} contained skeletal $\text{C}-\text{O}$ and $\text{C}-\text{C}$ vibration bands of glycosidic bonds and pyranoid ring, and this is in agreement with the works of Kalapathy and Proctor.⁴³ The band intensity of ring vibrations and $\text{C}-\text{O}$ stretching was accentuated in pectin from hydrolyzed soursop. Moreover, the intensity of band of glycosidic bonds was higher in commercial and untreated soursop pectin, evidencing the breakdown of glycosidic bonds by pectinase during liquefaction. Therefore, FTIR spectra revealed evidence of the breakdown of soursop pulp pectin during liquefaction.

4 CONCLUSION

The current study optimized the pectinase-assisted extraction of soursop juice. The optimum conditions of the soursop juice extraction process obtained were 172 min of incubation time, 0.04% (w/w) of enzyme concentration, and incubation temperature at 42.9 °C. The combination of these optimal conditions resulted in 75.2% yield, pH of 3.75, TSS of 7.35 °Brix, 87.06% clarity, and titratable acidity of 0.46% MAE. With the optimal conditions, the numerical predictions were similar to the experimental data obtained, which ranged from 0.905 to 0.987, thus confirming the validity of the models. The morphological analysis using scanning electron microscopy (SEM) revealed that pectinase hydrolyzed the pectin in soursop and improved the juice extraction process. The FTIR spectra of pectin from untreated and hydrolyzed soursop indicated bands between 1790 and 1610 cm^{-1} , representing spectral identification of galacturonic acid with a higher intensity seen for the hydrolyzed soursop pectin. This structural variation was attributed to the hydrolysis of the soursop pectin. The study provided the optimized conditions for pectinase-assisted extraction of soursop juice, which could be one of the promising methods for the value addition of soursop.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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V.13) Optimisation de l'Extraction
de la Dextrinase Limite de la
Variété de Sorgho Camerounais
Safrari

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ORIGINAL ARTICLE

Optimisation de l'Extraction de la Dextrinase Limite de la Variété de Sorgho Camerounais *Safrari*

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Résumé

La méthodologie des surfaces de réponses, utilisant un plan de Doehlert à 4 facteurs, a été utilisée pour optimiser l'extraction de la dextrinase limite du malt de sorgho *Safrari*. Les facteurs étudiés ont été la température, le pH, le ratio masse de malt/volume de tampon (m/v) et le temps. Le modèle généré reliant l'activité enzymatique de la dextrinase limite aux précédents facteurs a été validé. L'analyse de variance a montré que trois effets simples (température, ratio et temps), une interaction (pH/temps d'extraction) et tous les effets quadratiques influencent significativement ($P < 0,05$) l'activité enzymatique de la dextrinase limite pendant l'extraction. Tous les effets significatifs à l'exception de l'interaction pH/temps ont contribué à baisser l'activité enzymatique de la dextrinase limite. Les valeurs trouvées pour une extraction optimale sont 23 °C, 10 h, 5,0 et 5/32 respectivement pour la température, le temps, le pH et le ratio masse de malt/volume de tampon. L'expérimentation de cette condition a conduit à une activité enzymatique optimale en dextrinase limite de 140 mU/mL.

Application pratique

La dextrinase limite obtenue à partir de la variété de sorgho camerounais *Safrari*, peut être utilisée pour réduire la turbidité des moûts et principalement, lors du brassage avec les céréales qui conduisent à des bières opaques. Ceci est assez important car aucune des enzymes utilisées par les usines brassicoles au Cameroun sont d'origine locale.

Mots clés : *Safrari*, Extraction, Optimisation, Dextrinase limite, Activité enzymatique.

Abstract

The response surface methodology, using a 4-factor Doehlert design, was used to optimize the extraction of limit dextrinase from *Safrari* sorghum malt. The factors studied were temperature, pH, mass ratio of malt / volume of buffer (m / v) and time. The generated model linking dextrinase limit activity to the previous factors has been validated. The analysis of variance showed that three simple effects (temperature, ratio and time), an interaction (pH / extraction time) and all the quadratic effects significantly influence ($P < 0.05$) the activity of limit dextrinase during extraction. All of the significant effects except the pH / time interaction contributed to lower activity of limit dextrinase. The values found for optimal extraction are 23 °C, 10 h, 5.0 and 5/32 respectively for temperature, time, pH and the ratio mass of malt / volume of buffer. The experimentation with this condition led to an optimal enzymatic activity in limit dextrinase of 140 mU/mL.

Practical Application

Limit dextrinase obtained from the Cameroonian sorghum variety *Safrari* can be used to reduce the turbidity of worts especially when brewed with cereals which lead to opaque beers. This is quite important because none of the enzymes used by brewing factories in Cameroon are from local origin.

Keywords: *Safrari*; Extraction, Optimization, Limit dextrinase, Enzyme activity



1. Introduction

L'extraction enzymatique à partir de sources végétales a pour but soit de déterminer l'activité enzymatique dans un extrait brut, soit de purifier des quantités relativement grandes d'enzymes (Piggott, 2002). Ce potentiel est exploitable par optimisation d'une part des conditions de synthèse de celles-ci, et d'autre part des conditions d'extraction. L'extraction de la dextrinase limite à partir des malts de céréales a fait l'objet de plusieurs travaux ayant abouti dans la plupart des cas à sa purification, notamment dans l'orge (Kristensen, Planchot, Abe, & Svensson, 1998; MacGregor, Macri, Schroeder, & Bazin, 1994a; Manners & Yellowlees, 1971), le sorgho (Hardie, Manners, & Yellowlees, 1976), l'avoine (Dunn & Manners, 1975; Yamada, 1981b), le maïs (Beatty et al., 1999; Li et al., 2009; Wu, Colleoni, Myers, & James, 2002), le riz (Li et al., 2009; Yamasaki, Nakashima, & Konno, 2008) et le blé (Nethrphan, 2002; Repellin, Båga, & Chibbar, 2008). L'approche méthodologique des plans d'expériences s'avère donc inexistante dans les processus d'extraction d'enzymes des céréales. L'optimisation par plans d'expériences a fait ses preuves dans divers secteurs notamment en brasseries (Desobgo, Nso, & Tenin, 2011c, 2011b, 2011a, 2011d; Desobgo, Nso, Tenin, & Kayem, 2010; Evans, Li, & Eglinton, 2010), en malterie (Adefila, Bakare, & Adewale, 2012; Claver, Zhang, Li, Zhou, & Zhu, 2010; Usansa et al., 2011; Zarnkow et al., 2007) et en enzymologie (Anuradha & Valli, 2010; Karmakar & Ray, 2011; Rodríguez-Nogales, Ortega, Perez-Mateos, & Busto, 2007), entre autres. En effet, l'utilisation de la méthodologie de surface de réponses serait plus efficace et satisfaisante que les méthodes classiques d'un facteur à la fois, dans la mesure où plusieurs

variables sont étudiées simultanément avec un nombre minimum d'expériences, permettant de gagner en temps et en coût de procédé (Karmakar & Ray, 2011). Les facteurs majeurs généralement considérés dans les procédés d'extraction enzymatique sont: la température, le pH, la teneur en eau et la force ionique du milieu. Ceux-ci sont choisis de manière à préserver l'activité enzymatique et la stabilité de l'enzyme à extraire (Amersham-Biosciences, 2001; Dennison, 2002). Il s'agira donc dans ce travail, de déterminer les conditions optimales d'extraction de la dextrinase limite de la variété de sorgho *Safrari*.

2. Matériel et Méthodes

2.1. Matériel végétal

Il est utilisé ici le malt vert de sorgho *Safrari* germé dans les conditions optimales définies par Nguemogne et al. (2018). Cette variété cultivée dans le grand Nord du Cameroun a été obtenu auprès de l'IRAD (Institut de Recherche Agronomique pour le Développement) de Maroua, Cameroun.

2.2. Méthodes

2.2.1. Procédé de maltage

Le maltage a été conduit dans les conditions optimales obtenues par Nguemogne et al. (2018). Spécifiquement, des lots de 150 g de grains de sorgho ont été trempés à 25 °C dans 450 mL de solution de NaOH (0,1 %) pendant 24 h avec changement de la solution de trempage après 12 h. Une fois le trempage (à 25 °C) terminé, les grains ont été étalés sur des sacs perforés en plastique, puis recouverts et disposés dans le noir à l'étuve à 25 °C pendant 5 jours pour permettre le processus de la germination. Le malt vert obtenu a été broyé avec ses radicules à l'aide du

broyeur manuel (Victoria, Colombie).

2.2.2. Plan d'expériences pour l'extraction

Un plan d'expérience de Doehlert à quatre facteurs a été utilisé pour effectuer l'extraction. Les facteurs choisis et leur domaine de variation sont regroupés dans le tableau 1. Le choix des facteurs et leurs bornes ont été fait à partir de leur variabilité dans la bibliographie (Heisner & Bamforth, 2008; Kristensen et al., 1998; MacGregor, Macri, Schroeder, & Bazin, 1994b; Nguemogne, Desobgo, & Nso, 2017) et de leur importance dans le procédé d'extraction d'enzymes.

Les opérations de transformation utilisées pour passer des valeurs codées aux valeurs réelles ont été celles de Mathieu & Phan-tan-luu (1997) données par:

$$x_j = \frac{U_j - U_j^0}{\Delta U_j}$$

$$\Delta U_j = \left| \frac{U_j^{\min} - U_j^0}{x_j^{\min}} \right| = \left| \frac{U_j^{\max} - U_j^0}{x_j^{\max}} \right|$$

$$U_j^0 = \frac{U_j^{\max} + U_j^{\min}}{2}$$

Tableau 1: Facteurs d'optimisation de l'extraction et domaines de variation

Facteur	Nom	Abréviation	Unité	Domaine de variation réel	Domaine de variation codé
1	Température	T	°C	[4 – 50]	[-1 ; +1]
2	Potentiel d'hydrogène	pH		[3,5 – 6,5]	[-0,866 ; +0,866]
3	Ratio m/v		g/mL	[1/10 – 1/3]	[-0,816 ; +0,816]
4	Temps d'extraction	t	heures	[1 – 24]	[-0,791 ; +0,791]

Ce plan requiert un nombre d'expériences N:

$$N = k^2 + k + N_0$$

Avec k, le nombre de facteurs et N0 le nombre de répétitions au centre. Ayant quatre facteurs, il a été choisi une matrice de Doehlert à 24 expériences consignées dans le tableau 2.

Ainsi, U_j qui est la variable réelle d'intérêt est :

$$U_j = x_j \times \Delta U_j + U_j^0$$

Avec: x_j = valeur de la variable codée; U_j = valeur de la variable réelle; U_j^0 = valeur de la variable réelle au centre du domaine; $U_{j\min}$ = valeur de la variable réelle à la borne inférieure du domaine; $U_{j\max}$ = valeur de la variable réelle à la borne supérieure du domaine; ΔU_j = pas de variation.

Tableau 2: Matrice d'expériences de Doehlert

N°essai	Matrice d'expériences (valeurs codées)				Matrice d'expériences (valeurs réelles)			
	X1	X2	X3	X4	X1'	X2'	X3'	X4'
1	1	0	0	0	50	5	6,5	12,5
2	-1	0	0	0	4	5	6,5	12,5
3	0,5	0,866	0	0	38,5	6,5	6,5	12,5
4	-0,5	-0,866	0	0	15,5	3,5	6,5	12,5
5	0,5	-0,866	0	0	38,5	3,5	6,5	12,5
6	-0,5	0,866	0	0	15,5	6,5	6,5	12,5
7	0,5	0,289	0,816	0	38,5	5,5	10,0	12,5
8	-0,5	-0,289	-0,816	0	15,5	4,50	3,0	12,5
9	0,5	-0,289	-0,816	0	38,5	4,50	3,0	12,5
10	0	0,577	-0,816	0	27	6,00	3,0	12,5
11	-0,5	0,289	0,816	0	15,5	5,50	10,0	12,5
12	0	-0,577	0,816	0	27	4,00	10,0	12,5
13	0,5	0,289	0,204	0,791	38,5	5,50	7,4	24
14	-0,5	-0,289	-0,204	-0,791	15,5	4,50	5,6	1
15	0,5	-0,289	-0,204	-0,791	38,5	4,50	5,6	1
16	0	0,577	-0,204	-0,791	27	6,00	5,6	1
17	0	0	0,612	-0,791	27	5	9,1	1
18	-0,5	0,289	0,204	0,791	15,5	5,50	7,4	24
19	0	-0,577	0,204	0,791	27	4,00	7,4	24
20	0	0	-0,612	0,791	27	5	3,9	24
21	0	0	0	0	27	5	6,5	12,5
22	0	0	0	0	27	5	6,5	12,5
23	0	0	0	0	27	5	6,5	12,5
24	0	0	0	0	27	5	6,5	12,5

Avec $X1=X1'$ = Température ; $X2=X2'$ = pH ; $X3=X3'$ = Ratio m/v ; $X4=X4'$ = Temps

Le plan d'expériences généré a conduit à l'obtention d'un modèle de second degré obtenu grâce au logiciel Statgraphics XV.II. Ce modèle est quadratique:

$$y = \beta_0 + \sum_{j=1}^k \beta_j x_j + \sum_{j=1}^k \beta_{jj} x_j^2 + \sum_{i < j} \beta_{ij} x_i x_j$$

Où : y = réponse mesurée ; x = facteurs ; β_0 = constante ; k = nombre de facteurs ; β_j = coefficients des effets linéaires ; β_{jj} = coefficients des effets quadratiques ; β_{ij} = coefficients des interactions. Les valeurs moyennes des réponses des essais (en triplicat) ont servi à l'établissement du modèle.

2.2.3. Validation du modèle mathématique

Trois méthodes ont permis la validation du modèle obtenu: La détermination du coefficient de détermination R^2 et du coefficient de détermination ajusté Ra^2 grâce à Statgraphics XV.II; Le calcul de l'analyse absolue de déviation moyenne (AADM), des facteurs polarisé (Bf) et d'exactitude (Afl) grâce à Excel.

Les valeurs acceptables de R^2 , de AADM, de Bf et de Afl signifient que l'équation du modèle décrit le comportement réel du système et qu'elle peut être utilisée pour l'interpolation dans le domaine expérimental.

2.2.4. Procédé d'extraction et test d'activité enzymatique de la dextrinase limite

Ce procédé ainsi que les conditions d'extraction sont résumés à la figure 1. L'activité enzymatique de la dextrinase limite a été déterminée par dosage des sucres réducteurs issus de l'action de la dextrinase limite sur le pullulan, avec la méthode au DNS de Fischer & Stein (1961). Les essais ont été faits en triplicat.

L'activité enzymatique enzymatique ici est définie comme étant la quantité d'enzyme qui libère une μmol de maltose équivalent du pullulan en une minute à 37 °C et s'exprime en U/mL.

2.2.5. Effets de l'EDTA, le DTT, le CaCl_2 , le SAB et l'acide ascorbique sur l'extraction et l'activation de la dextrinase limite

Dans ce travail, cinq composés ont été introduits au cours de l'extraction de l'enzyme (figure 2) et, en fonctions des doses recommandées dans la bibliographie. Il s'agit notamment:

-Des antioxydants: le dithiothréitol (DTT) à 1 mM et l'acide ascorbique à 5 mM ;

-Un inhibiteur de métaux: l'acide éthylène diamine tétra-acétique (EDTA) à 1 mM ;

-Un cofacteur: les ions Ca^{2+} dans le chlorure de calcium (CaCl_2) à 5 mM ;

-Un agent stabilisateur: le sérum albumine bovine (SAB) à 10 mg/mL.

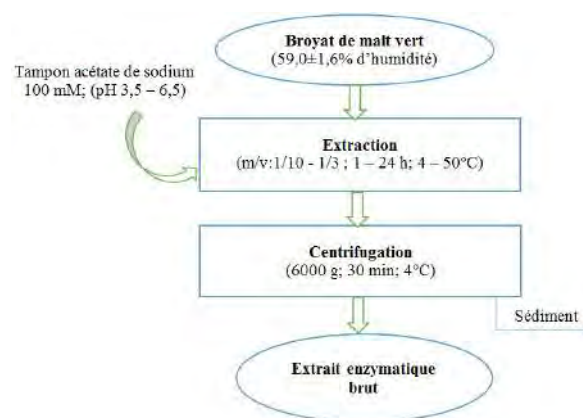


Figure 1: Procédé d'optimisation de l'extraction de la dextrinase limite

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2.2.6. Logiciels

Le logiciel Statgraphics version XV.II (StatPoint, Inc., Virginia 20171, USA), a été utilisé pour la planification expérimentale et les analyses statistiques ; tandis que le logiciel sigmaplot version 12.5 (Systat Software, Inc., Point Richmond, CA 94804-2028, USA) et Excel ont servi aux tracés des courbes.

3. Résultats et Discussion

3.1. Modélisation

Les résultats du tableau 3 ont servi à l'établissement du modèle mathématique (Yact) traduisant l'activité enzymatique de la dextrinase limite en fonction de la température, du pH, du ratio m/v et du temps d'extraction.

Tableau 3: Matrice d'expérimentation pour l'extraction de la dextrinase limite

Expériences	Matrice d'expériences (valeurs codées)				Matrice d'expériences (valeurs réelles)				Réponse (Activité enzymatique en dextrinase limite (mU/mL))
	X1	X2	X3	X4	X1'	X2'	X3'	X4'	
1	1	0	0	0	50	5	6,5	12,5	10,8±0,0
2	-1	0	0	0	4	5	6,5	12,5	94,2±6,0
3	0,5	0,866	0	0	38,5	6,5	6,5	12,5	5,8 ±0,5
4	-0,5	-0,866	0	0	15,5	3,5	6,5	12,5	12,4±0,0
5	0,5	-0,866	0	0	38,5	3,5	6,5	12,5	1,9±0,0
6	-0,5	0,866	0	0	15,5	6,5	6,5	12,5	35,1±0,0
7	0,5	0,289	0,816	0	38,5	5,5	10,0	12,5	1,2±0,0
8	-0,5	-0,289	-0,816	0	15,5	4,50	3,0	12,5	64,1±4,4
9	0,5	-0,289	-0,816	0	38,5	4,50	3,0	12,5	42,8±0,0
10	0	0,577	-0,816	0	27	6,00	3,0	12,5	29,5±1,4
11	-0,5	0,289	0,816	0	15,5	5,50	10,0	12,5	58,7±0,0
12	0	-0,577	0,816	0	27	4,00	10,0	12,5	8,1±0,0
13	0,5	0,289	0,204	0,791	38,5	5,50	7,4	24	22,0±0,0
14	-0,5	-0,289	-0,204	-0,791	15,5	4,50	5,6	1	99,2±14,2
15	0,5	-0,289	-0,204	-0,791	38,5	4,50	5,6	1	80,3±0,0
16	0	0,577	-0,204	-0,791	27	6,00	5,6	1	13,5±0,0
17	0	0	0,612	-0,791	27	5	9,1	1	74,9±0,0
18	-0,5	0,289	0,204	0,791	15,5	5,50	7,4	24	35,9±0,0
19	0	-0,577	0,204	0,791	27	4,00	7,4	24	0,4±0,0
20	0	0	-0,612	0,791	27	5	3,9	24	56,0±0,0
21	0	0	0	0	27	5	6,5	12,5	127,4±0,0
22	0	0	0	0	27	5	6,5	12,5	134,3±0,0
23	0	0	0	0	27	5	6,5	12,5	143,2±0,0
24	0	0	0	0	27	5	6,5	12,5	134,7±0,0

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Le modèle obtenu s'écrit comme suit:

$$Y_{act} = 0,135 - 0,031X_1 - 0,001X_2 - 0,014X_3 - 0,024X_4 - 0,085X_1^2 - 0,011X_1X_2 - 0,020X_1X_3 + 0,012X_1X_4 - 0,133X_2^2 + 0,015X_2X_3 + 0,055X_2X_4 - 0,098X_3^2 - 0,031X_3X_4 - 0,076X_4^2$$

Avec X1 : Température ; X2 : pH ; X3 : Ratio m/v ; X4 : Temps

Ce modèle est polynomial d'ordre 2 avec interactions. Les paramètres de validation du modèle 'Yact' rentrant dans les plages standards (tableau 4), ce modèle est valide (Baş & Boyaci, 2007; Dalgaard & Jørgensen, 1998; Goupy & Creighton, 2006; Joglekar & May, 1987) et reflèterait l'évolution réelle de l'activité enzymatique de la dextrinase limite en fonction des facteurs dans leur plage d'étude. Pour une meilleure évaluation et interprétation de l'influence des facteurs et des interactions entre facteurs sur la réponse, une analyse statistique a été effectuée.

effets quadratiques des quatre facteurs étudiés (temps, température, pH et ratio) et l'interaction pH-temps d'extraction.

L'on peut déduire du tableau 5 que tous les facteurs qui ont une influence significative (à l'exception de l'interaction pH-temps d'extraction) le sont dans le sens négatif (signe des coefficients), ce qui signifie que leur augmentation (effets simples X1, X3, X4) ou leur excès (effets quadratiques X1X1, X2X2, X3X3, X4X4) concoure à inhiber l'extraction et/ou l'activation de la dextrinase limite ; tandis que l'interaction pH-temps d'extraction (X2X4) a l'effet inverse.

Tableau 4: Paramètres de validation du modèle "Y_{act}"

	Abréviation	Valeur obtenue	Plages acceptables
Coefficient de détermination	R ²	96,92%	≥ 92 % (Goupy & Creighton, 2006)
Coefficient de détermination ajusté	R ² a	92,30%	≥80 % (Joglekar & May, 1987)
Analyse Absolue de Déviation Moyenne	AADM	0,04	[0-0,3] (Baş & Boyaci, 2007)
Facteur de Biais	Bf	1,02	[0,75-1,25] (Dalgaard & Jørgensen 1998)
Facteur d'exactitude	Af ₁	1,03	[0,75-1,25] (Dalgaard & Jørgensen 1998)

La table d'ANOVA (tableau 5), renseigne sur les probabilités d'influence des facteurs et de leur interaction au niveau de confiance de 95 %. D'après les valeurs des probabilités de la table d'ANOVA, 08 effets influencent significativement le process au niveau de confiance de 95 % (P < 0,05). Il s'agit de: la température, le ratio et le temps d'extraction, les

3.1.1. Effet de la température d'extraction (X1)

On observe à la figure 2 d'une part, une augmentation non significative d'activité enzymatique de la dextrinase limite avec l'augmentation de la température d'extraction puis d'autre part, une diminution significative (tableau 5) d'activité enzymatique suite à l'augmentation continue de la température. En

effet, on part d'une activité enzymatique de 0,081 U/mL à 4 °C pour atteindre la valeur maximale de 0,137 U/mL à 22,4 °C; suivi d'une diminution d'activité enzymatique jusqu'à 0,019 U/mL à 50 °C.

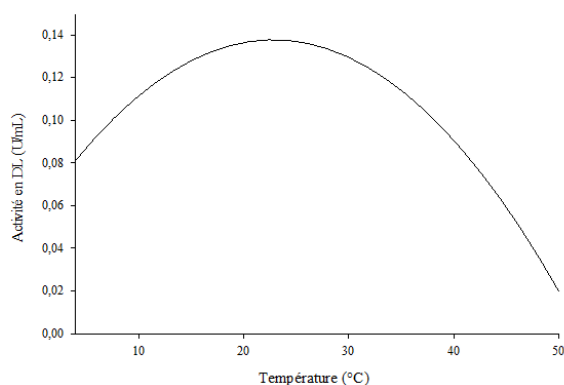


Figure 2: Evolution de l'activité enzymatique de la dextrinase limite en fonction de la température d'extraction (ratio: 1/6, 5; temps: 12, 5 h; pH 5, 0)

L'accroissement d'activité enzymatique avec l'augmentation de la température serait dû à l'augmentation de la quantité de dextrinase limite extraite avec l'élévation de température. En effet, elle accroît l'énergie cinétique des molécules (Hejnaes, Matthiesen, & Skriver, 1998) et par ricochet, la vitesse des échanges entre le solvant (solution tampon) et le soluté (malt). Plus il y a échange, plus la dextrinase limite serait extraite avec pour conséquence directe l'augmentation de sites actifs disponibles pour la fixation du substrat, d'où l'accroissement d'activité enzymatique.

Par contre, la chute d'activité enzymatique observée par la suite serait due à la dénaturation progressive de l'enzyme extraite à ces températures. En effet, bien que la vitesse des échanges soit améliorée avec l'accroissement de la température, l'augmentation des vibrations

moléculaires générée conduirait à la rupture des liaisons de faible énergie telles les liaisons ioniques, les liaisons hydrogènes, et même des liaisons covalentes tels les ponts disulfures ; celles-ci rentrant dans la préservation de la structure tertiaire des enzymes (donc de leur activité enzymatique) et même de leur stabilité (Hejnaes *et al.*, 1998; Kotzia *et al.*, 2012). De ce fait, même si l'enzyme est extraite aux fortes températures, elle serait dénaturée et donc non fonctionnelle. Ce résultat est similaire à ceux de plusieurs auteurs qui ont montrés que pendant que la température augmente, la vitesse des réactions enzymatiques croît également, mais en même temps, il y a une inactivation progressive de l'enzyme, qui est de plus en plus prononcée au fur et à mesure que la température augmente (Hejnaes *et al.*, 1998; Rajagopal, Ramakrishnan, & Indhumathi, 2009).

3.1.2. Effet du ratio masse de malt (g)/volume de tampon (mL) (X3)

L'activité enzymatique de la dextrinase limite augmente de façon non significative avec la diminution du ratio jusqu'à un seuil au-delà duquel la diminution continue du ratio entraîne également une diminution cette fois-ci significative (tableau 5) de l'activité enzymatique pendant l'extraction (figure 3). En effet, on part d'une activité enzymatique de 0,080 U/mL au ratio 1/3 pour atteindre la valeur maximale de 0,135 U/mL au ratio 1/6 ; suivi d'une diminution d'activité enzymatique jusqu'à 0,058 U/mL au ratio 1/10.

De ce fait, malgré l'importance du rapport solide/liquide dans les procédés d'extraction, des rapports extrêmes sont défavorables pour le cas particulier des extractions enzymatiques. En effet, les ratios élevés limitent la vitesse de transfert de matière (Tucker, 1995).

Tableau 5: Analyse de la variance

Source	Coefficients	Somme des carrés	ddl	Moyenne quadratique	Rapport F	Probabilité
X ₁ :Temp	-0,03	0,00471325	1	0,00471325	26,80	0,0006
X ₂ :pH	-0,001	0,000006331	1	0,000006331	0,04	0,8537
X ₃ :Ratio	-0,014	0,000935196	1	0,000935196	5,32	0,0465
X ₄ :temps	-0,024	0,00294938	1	0,00294938	16,77	0,0027
X ₁ X ₁	-0,085	0,00952569	1	0,00952569	54,17	0,0000
X ₁ X ₂	-0,011	0,0000931129	1	0,0000931129	0,53	0,4853
X ₁ X ₃	-0,020	0,000246747	1	0,000246747	1,40	0,2665
X ₁ X ₄	0,012	0,0000865582	1	0,0000865582	0,49	0,5007
X ₂ X ₂	-0,133	0,0237315	1	0,0237315	134,96	0,0000
X ₂ X ₃	0,015	0,000131867	1	0,000131867	0,75	0,4090
X ₂ X ₄	0,055	0,00167828	1	0,00167828	9,54	0,0129
X ₃ X ₃	-0,098	0,0143274	1	0,0143274	81,48	0,0000
X ₃ X ₄	-0,031	0,000523437	1	0,000523437	2,98	0,1186
X ₄ X ₄	-0,076	0,00970069	1	0,00970069	55,17	0,0000
Erreur totale		0,00158257	9	0,000175841		
Total (corr.)		0,0513865	23			

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Par ailleurs, il y a également saturation du milieu en soluté, avec une augmentation de la quantité de matière en suspension, ce qui rend difficile la solubilisation des protéines avec pour conséquence l'agrégation de celles-ci et donc l'absence d'activité enzymatique (GE-Healthcare, 2010; Hejnaes *et al.*, 1998).

De très faibles ratios par contre ont un effet déstabilisateur sur l'enzyme (Tucker, 1995). Par ailleurs, un excès de solvant diminue la concentration enzymatique dans le milieu; et ceci limite à son tour la probabilité de rencontre de l'enzyme avec le substrat (Hejnaes *et al.*, 1998).

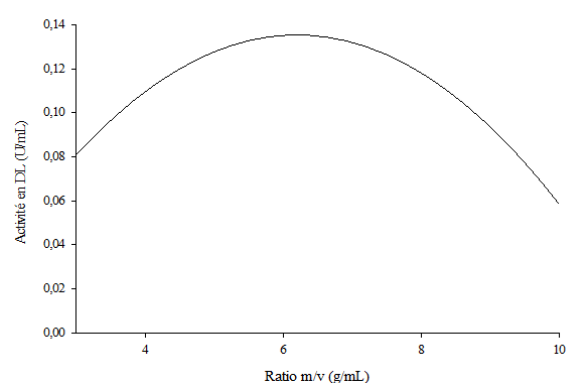


Figure 3: Activité enzymatique de la dextrinase limite en fonction du ratio m/v d'extraction (température: 27 °C; pH 5, 0; temps: 12, 5 h)

Dans une autre mesure, une extrême dilution de l'enzyme dans le milieu réactionnel peut conduire à une perte d'activité enzymatique due soit à l'instabilité de l'enzyme suite à une faible concentration en protéines, soit à la dissociation de ses sous-unités, ce qui la rend inactive (Fiechter, 1983; GE-Healthcare, 2010; Scopes, 1987; Tipton, 2002). Une autre conséquence de l'effet de dilution c'est la dissociation des cofacteurs, indispensables à l'activité enzymatique de l'enzyme (Tipton, 2002). Ces résultats sont similaires à ceux de Mehrnough et al. (2014), sur l'extraction de l'amylase des épiluchures du dragonnier (*Hylocereus polyrhizus*) et, sont également compatibles avec le principe de transfert de matière où la force de transmission durant ce transfert est le gradient de concentration de soluté entre le solide et le liquide (Ibarz & Barbosa-Cánovas, 2002).

3.1.3. Effet du temps d'extraction (X4)

Il ressort du tableau 5 que le temps d'extraction et son effet quadratique ont un impact significatif et négatif. Par conséquent, de longues durées d'extraction ne seraient pas favorables au maintien de l'activité enzymatique de la dextrinase limite extraite. En effet, de longues durées d'extraction peuvent causer la perte d'activité enzymatique en raison soit de la technique d'extraction qui peut être dénaturante pour les enzymes avec le temps (agitation, chauffage, oxydation en présence d'oxygène), soit à la libération progressive des protéases dans le milieu (Scopes, 1987). Cependant, à la figure 4, l'on peut observer qu'il existe une plage pour laquelle l'augmentation du temps d'extraction accroît de façon non significative l'activité enzymatique de la dextrinase limite.

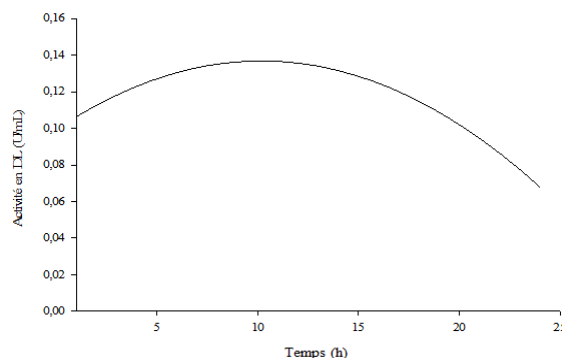


Figure 4: Activité enzymatique de la dextrinase limite en fonction du temps d'extraction (température: 27 °C; pH 5, 0; ratio : 1/6,5)

Ceci peut s'expliquer par le fait que l'augmentation du temps accroît la durée de contact entre le malt et le tampon, ce qui favoriserait davantage l'extraction. Cependant, cette plage reste très minime par rapport à celle où il y a baisse d'activité enzymatique avec le temps; d'où l'effet global négatif dû au temps d'extraction. En effet, l'on part d'une activité enzymatique de 0,106 U/mL après 1 h d'extraction, pour atteindre la valeur maximale de 0,136 U/mL après 10 h d'extraction ; suivi d'une diminution d'activité enzymatique jusqu'à 0,068 U/mL après 24 h d'extraction. Des résultats similaires ont été obtenus par Mehrnough et al. (2014), sur l'extraction de l'amylase des épiluchures du dragonnier (*Hylocereus polyrhizus*).

3.1.3. Effet de l'interaction pH-temps d'extraction (X2X4)

Bien que le pH n'ait pas un impact significatif ($P > 0,05$) sur l'activité enzymatique de la dextrinase limite pendant l'extraction, son impact devient significatif en prenant en compte le temps d'extraction, ceci de façon positive ($P <$

0,05, tableau 5). Ce qui signifie que la synergie entre ces deux facteurs accroît l'activité enzymatique de la dextrinase limite au cours de l'extraction (figure 5).

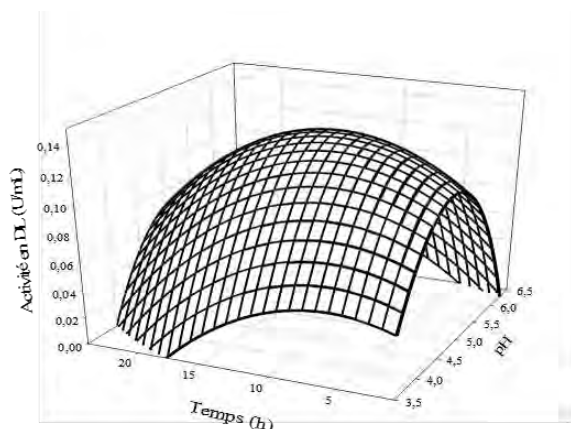


Figure 5: Courbe de surface de réponse de l'activité enzymatique en DL en fonction du pH et du temps (ratio : 1/6,5 ; température : 27 °C)

Il ressort de cette figure que l'augmentation simultanée, de même que la diminution simultanée de ces deux facteurs (jusqu'à 4,9 pour le pH, et jusqu'à 10,2 h pour le temps) contribuent à augmenter l'activité enzymatique en dextrinase limite.

Ces observations peuvent s'expliquer par le fait que le temps peut être un facteur favorable à l'extraction de la dextrinase limite si les conditions de pH, entre autres, propices à l'extraction et à la stabilisation de l'enzyme sont réunies d'une part, et d'autre part, le temps peut être à la défaveur du maintien de l'activité enzymatique et de la stabilité de l'enzyme si les conditions de pH entre autres sont dénaturantes et/ou déstabilisantes pour l'enzyme. Les enzymes en effet sont des molécules amphotères renfermant des groupements acides et basiques chargés. Ces charges varient en fonction du pH

environnant (Hejnaes *et al.*, 1998; Andrés Illanes, Altamirano, & Wilson, 2008; Mohan, Long, & Mutneja, 2013). Les variations de charge avec le pH affectent l'activité enzymatique de l'enzyme, sa stabilité structurale et même sa solubilité. Par ailleurs, des pH extrêmes pour l'enzyme causeront avec le temps des dénaturations irréversibles (Hejnaes *et al.*, 1998; Andrés Illanes *et al.*, 2008).

3.2. Optimisation de l'extraction de la dextrinase limite

Le logiciel Statgraphics version XV.II, a permis de faire ressortir les conditions d'extraction optimales de la dextrinase limite pour chaque facteur étudié tel que présentées dans le tableau 6. On observe dans ce tableau que les conditions optimales de température, de pH, de ratio masse de malt/volume de tampon et de temps pour l'extraction non dénaturante et maximale de la dextrinase limite du malt de sorgho *Safrari* sont respectivement de 23 °C, pH 5, ratio 5/32 pour un temps de 10 h. Ceci conduit à une activité enzymatique maximale théorique de 0,140 U/mL.

La vérification de l'activité enzymatique optimale théorique en dextrinase limite qui est de 0,140 U/mL aux points optimaux, a donné la valeur expérimentale de $0,106 \pm 0,001$ U/mL. Près de 76 % d'activité enzymatique sont retrouvées expérimentalement. Cette différence avec l'activité enzymatique optimale théorique serait due aux équipements et autres appareils de mesures.

L'optimum de température de 23 °C obtenu se rapproche de 24 °C utilisée pour l'extraction de la dextrinase limite dans le sorgho de variété Kafficorn et de l'orge (Hardie *et al.*, 1976; Heisner & Bamforth, 2008; Kristensen *et al.*, 1998).

Tableau 6: Points optimaux des facteurs pour l'extraction de la dextrinase limite

Facteur	Niveau bas	Niveau haut	Optimum en valeurs codées	Optimum en valeurs réelles
Température (°C)	-1,0	1,0	-0,189868	22,63 ≈ 23
pH	-0,866	0,866	-0,0352808	4,93 ≈ 5
Ratio m/v (g/mL)	-0,816	0,816	0,0242621	1/6,4 = 5/32
Temps d'extraction (Heure)	-0,791	0,791	-0,182701	9,84 ≈ 10
Activité enzymatique de la dextrinase limite (U/mL)				
Optimum théorique		Optimum expérimental		
0,140		0,106 ± 0,001		

Par ailleurs, cette température optimale de 23 °C n'est pas tout aussi éloignée des températures ambiantes qui se trouvent généralement autour de 24 °C. Par conséquent, l'extraction de la dextrinase limite du malt de sorgho *Safrari* pourrait être menée à température ambiante (voir même entre 20 et 30 °C d'après la figure 2) sans nécessité d'apport ou d'enlèvement d'énergie, ce qui réduit le coût du procédé.

L'optimum de pH d'extraction s'est situé à 5,0, valeur qui s'accorde avec les résultats de la bibliographie pour la dextrinase limite du sorgho Kafficorn, de l'orge et d'autres malts de céréales (Hardie et al., 1976; Kristensen et al., 1998; Longstaff & Bryce, 1993; McCleary, 1992). L'optimum obtenu ici en conditions naturelles (avec le pullulan naturel comme substrat) intègre les plages de pH de brassage de bières en brasserie, comprises entre 5,0 et 5,6 (Briggs, 1998; Macgregor, 1996; Sissons, Lance, & Sparrow, 1992). Aussi, l'utilisation de substrat naturel va en droite ligne des conditions

d'exploitation potentielle du malt de sorgho *Safrari* tant comme matière première brassicole, que comme source d'enzymes dans les procédés de transformation de l'amidon dont les plages de pH sont de 4,5-5,5 (Synowiecki, 2007).

Le ratio optimal de 5/32 se rapproche de la valeur de 1/5 utilisée par Heisner et Bamforth (2008) avec l'orge. L'extraction optimale de la dextrinase limite du malt de sorgho *Safrari* imposerait donc une dilution modérée. Ce ratio est également comparable à ceux employés pour le brassage des bières de sorgho compris entre 1/5 (Desobgo et al., 2011c, 2011a, 2011b) et 1/6 (Igyor, Ogbonna, & Palmer, 2001; Nso, Ajebesone, Mbofung, & Palmer, 2003).

Toutefois, pour des applications technologiques, il serait plus intéressant d'avoir des plages de valeurs de facteurs permettant d'obtenir une activité enzymatique relativement acceptable; d'où l'importance de la courbe d'iso-réponses en fonction de l'interaction entre le pH et le temps (seule interaction significative ici),

mais cette fois-ci aux optima de ratio et de température respectivement de 5/32 et 22, 63 °C (figure 6).

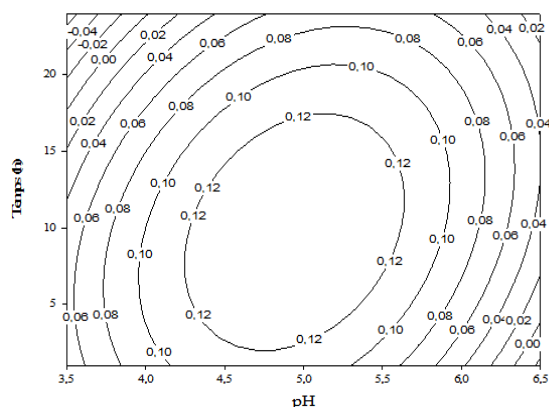


Figure 6: Courbe d'iso-réponse de l'activité enzymatique en dextrinase limite en fonction du pH et du temps (ratio : 5/32 ; température : 22,63 °C)

Il en ressort que pour avoir une activité enzymatique en dextrinase limite supérieure ou égale à 0,10 U/mL, le temps d'extraction et le pH doivent se situer dans l'aire définie à sa limite par l'iso réponse de 0,10 U/mL (Figure 6), ceci pour le ratio m/v optimum de 5/32 et la température optimale de 22,63 °C.

3.3. Effet de quelques additifs sur l'extraction de la dextrinase limite

Certains composés peuvent soit favoriser l'extraction complète de la dextrinase limite, soit inhiber l'enzyme au cours du processus d'extraction. L'effet de quelques-uns d'entre eux ont été étudié à savoir l'acide ascorbique et le dithiotréitol (DTT) comme agents antioxydants, l'acide éthylène diamine tétra-acétique (EDTA) comme inhibiteur de métalloenzymes, le chlorure de calcium comme cofacteur, et le sérum albumine bovine comme stabilisateur.

La figure 7 présente les résultats, par comparaison à l'extrait sans additifs (contrôle). On peut remarquer sur cette figure qu'aucun additif ne permet d'améliorer l'extraction de la dextrinase limite; au contraire, une baisse est notoire et varie de 12 % à 63 %.

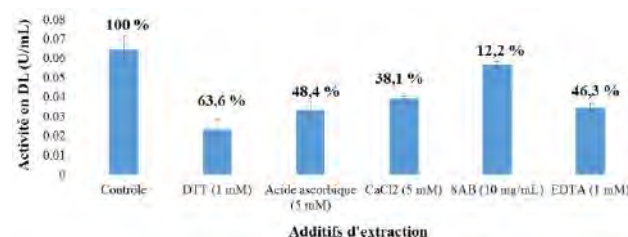


Figure 7: Effet de certains additifs sur l'extraction de la dextrinase limite

D'après cette figure, que ce soit des antioxydants ou stabilisateur ou cofacteur ou chélateur de métaux, tous seraient inhibiteurs pour l'extraction de la dextrinase limite.

En effet, les agents réducteurs (antioxydants) aident à maintenir les protéines à l'état réduit en empêchant l'oxydation des groupements sulfhydriles chez les enzymes qui en possèdent (Amersham-Biosciences, 2001; Laing & Christeller, 2004). Il semblerait que, soit la dextrinase limite du malt de sorgho *Safrari* n'en possède pas, soit celle-ci ne serait pas en partie liée à des inhibiteurs par des ponts disulfures où un environnement réducteur permettrait la libération de la dextrinase limite par rupture de ces ponts (Longstaff & Bryce, 1993; Yamada, 1981a) et donc son activation. Par conséquent, la dextrinase limite du malt de sorgho *Safrari* ne serait pas une enzyme sulfhydrile. La baisse d'activité enzymatique en dextrinase limite observée pourrait s'expliquer par le fait de l'inhibition par rupture des ponts disulfures des cys-protéinases (qui seraient synthétisées

pendant la germination) par des composés sulfhydriles (agents réducteurs utilisés ici) en présence d'oxygène (Scopes, 1987). Les cystoprotéinases permettraient l'activation de la dextrinase limite par des modifications protéolytiques (Longstaff & Bryce, 1993). Aussi, un éventuel effet inhibiteur dû à la technique de test d'activité enzymatique enzymatique (utilisation du pullulan naturel comme substrat et dosage des sucres réducteurs formés par la méthode au DNS) employée dans ces conditions n'est pas à exclure.

La baisse d'activité enzymatique en présence d'EDTA voudrait signifier que la dextrinase limite nécessiterait pour son activation pendant l'extraction, la présence d'ions métalliques divalents. Cependant, les ions calcium ne seraient pas indiqués en raison de la baisse d'activité enzymatique observée également en leur présence. Par ailleurs, il a été mentionné qu'il est parfois nécessaire de baisser la concentration en calcium en deçà de 10^{-7} mol.L⁻¹ dans la mesure où des concentrations au-delà peuvent faire varier l'activité enzymatique de certaines enzymes (Rothe, 1994); cela serait le cas ici (concentration de CaCl₂ de 5 mM) où la variation aurait consisté en la diminution d'activité enzymatique pendant l'extraction de la dextrinase limite. Il a été également observé une baisse d'activité enzymatique de la dextrinase limite de 15 % pendant l'extraction avec l'orge maltée en présence d'EDTA (Kristensen et al., 1998).

Il est reconnu pour ce qui est du SAB d'avoir un effet stabilisateur pour certaines enzymes; mais cela ne fut pas le cas ici où il y a eu plutôt baisse d'activité enzymatique de l'ordre de 12 % pendant l'extraction. Heisner et Bamforth (2008) ont également observé une baisse d'activité

enzymatique de la dextrinase limite en présence de SAB avec l'orge, d'environ 25 %.

4. Conclusion

Il a été question dans cet article d'utiliser la MSR pour optimiser les paramètres d'extraction de la dextrinase limite du malt de sorgho *Safrari*. Il en est ressorti que les conditions optimales d'extraction ont été comparables à celles de bien d'auteurs avec d'autres céréales et même le sorgho. Par ailleurs, avec la MSR, l'on a également pu montrer que l'on pouvait avoir pour les facteurs étudiés, des plages de variation permettant d'atteindre un optimum d'activité enzymatique. Le secteur brassicole a été tout particulièrement prisé pour l'exploitation de ces différentes plages, ce qui a démontré encore l'intérêt d'utilisation du sorgho dans ce secteur d'activité enzymatique. Aussi, la non-nécessité à utiliser des additifs d'extraction limite les coûts du procédé.

Conflit d'intérêt

Les auteurs déclarent qu'ils n'ont pas de conflits d'intérêt.

Éthique

Cette étude n'est pas faite sur des hommes ou des animaux

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19 Winemaking: Control, Bioreactor and Modelling of Process

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1. Introduction

Agribusiness is embedded in production systems; hence the many current procedures subject to in-depth studies on methods to develop better systems for safety and quality. It is the way to ensure the quality and respect for the requirements of security, the system costs and natural effect. Among the classification (specifically structuring, preservation, separation and bioconversion) of nourishment forms, bioconversion incorporates most likely the biggest class of procedures. It is focal in the generation of maturing foods with some outstanding cases from the winemaking factory. Winemaking is customarily considered to be beginning with the blending of the berries and the introduction of yeast to realise fermentation. Innovation, smashing and regular squeezing could be viewed as the final stages of the vineyard operation. The control of quality of wine is basically essential. Institution study has accentuated the necessity to examine vulnerability to oxygen, extraction management of the substances from the skin, temperature monitoring amid fermentation, observing of sugar depletion and monitoring of microbes and malo-lactic fermentation. At present, the search is on for better quality items, keeping in mind that the general utilisation of wine is reducing, interest in astounding wines is expanding. Buyers are drinking less though 'better'. Wine producers throughout the world are consolidating winemaking techniques of centuries with new methodologies and thoughts, to satisfy purchaser's interest for better item quality and a maintainable and healthy lifestyle.

This chapter presents an overview of winemaking, monitoring, safety and quality control, which display the activities concerning each unit operation, the bioreactor characteristics and uses and finally, innovative approaches aimed at optimising the process efficiency.

2. Overview of Winemaking

2.1 Juice Extraction

As soon as grapes are received in the winery, they ought to be destemmed as well as squeezed with the particular ultimate objective to extract the juice (Soufleros, 1997a; 1997b). Likewise, care should be taken to keep the seeds in place. At the point when the outer securing seed shell is cracked, the huge measures of phenolic substances that the seeds hold, will concede to the wine and impart it an astringent taste (Ough, 1992). In the wake of stemming and pulverising, the juice moves into either a device used for draining, or a significant holding vessel or the concerned red grapes inside a fermenter. Within white cultivars, the prompt expulsion of juice in the peels and seeds is basic, as there are important measures of tannin-like substances in the peels. The touch between peel and juice (in the wake of pulverising) outperforms at 12 hours as the usual basement temperature might be destructive to the consequent wine and the degree of sensory characteristics that are involved (Ough, 1992). The must, to be transformed for white wine, is expelled from the skins, as they remain an important wellspring of regular microbial action and the level of phenol removal from the skins is restricted (Boulton *et al.*, 1996). Mash should be right away removed

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from the factory as it rapidly attracts dreadful little animals and diverse aggravations (Vine *et al.*, 1997). Of course, the juice from red grapes is less fragile than the white must and is not trailed by the systems, previously fermentation, since they are performed prior to it. Besides, the contact between peel and juice is appealing in red wines since the phenolic substances should be expelled from the seeds and should persist in the completed wine. The slurry is sent for pressing and the squeezed juice is incorporated in the key squeeze must and the crushed slurry ought to be separated and sent to the winery.

White juice is cleaned in the wake of pressing with a particular but ultimate objective to diminish the suspended grape compounds. Universally, a depletion of particles to less than 0.5 per cent is appropriate. This methodology can be reached by crisp falling or using mechanical methods. Sometimes, the extension or addition of pectolytic enzymes can help in clarification of juice. Starting late, there has been a more noteworthy use of them with a particular objective to quicken the wine clarification, subsequent to fermentation. It is perfect to incorporate the catalysts at this advanced step because the high ethanol contents achieved following fermentation tend to repress the activity of the enzyme (Tucker and Woods, 1996). Particles in juice provide a site to mature yeasts for CO₂ and ethanol release. Extraordinary refining of juice reduces the number of cells in the normal yeast concentration, reducing or annihilating their duty regarding ethanol (Wood, 1998).

Juices are much of the time put away to be utilised as a refreshing part or to raise the time of fermentation. Capacity states must be checked (PCO₂ < 3.5 atm, pH: 3.0 to 3.5, T < 2 °C) to hinder the improved decay of microbial cells (Fugelsang, 1997). Juices should be set up in one of the following ways: sulphating, chilling, juice concentration and cross-stream microfiltration (Boulton *et al.*, 1996).

2.2. Juice Preparation

Modification of the must before fermentation engages the winemaker to begin fermenting with every juice part. This process much of the time requires no less than one of the backing tasks: nutrient, SO₂ and catalyst incorporation, acidity, oxidation of juice (Boulton *et al.*, 1996). The fitness of the additional substances and their estimations need to be mastered (Tartaric acid: 0.5 to 1 g/L, Tannin: 5 g/L, CaCO₃: 0.5 to 1 g/L,) (Soufleros, 1997a).

At the point when the juice stabilises, the fermenters are loaded with juice having enough SO₂ and the yeast inoculum is incorporated. Care must however, be taken for the estimation of sulphur dioxide (<200 µg/mL juice), as over-the-top measure of it can realise yeast restraint and give a sulphur dioxide odour to the completed wine (Fugelsang, 1997). It is a direct but essential operation to incorporate an active yeast strain of *Saccharomyces* in the form of inoculum, to finish the alcoholic fermentation instead of relying upon the local microbial population (Lea and Piggott, 1995). The yeast strains might be observed imperceptibly to ensure that the ferments are of the vital sort and not that oxidative ferments and organisms are present (Fugelsang, 1997).

2.3. Fermentation

Fermentation ought to be the 'centre' of winemaking as the sugars of grape are transformed into alcohol by *Saccharomyces cerevisiae*. The nearness of increased heat (10-30°C) in the midst of fermentation can provoke destruction of the yeast inoculum and the impact of the all the more thermo-resistant microorganisms to finish the fermentation and plan unwanted side effects (Fugelsang, 1997). Berries, not strongly treated with pesticides in the farm can in addition be a source of the problem (Sala *et al.*, 1996). The extraordinary contamination with moulds, lactic and acetic acid organisms on grapes previously accumulated can convey some components that may ruin or obstruct yeast development in the midst of ethanolic fermentation (Wood, 1998). Also, an extreme extension of SO₂ can eradicate the predominant piece of appealing and unwanted cell and present a damaging impact on the wine flavour (Fugelsang, 1997). The nearness of ethyl carbamate concentration (<30 ppb) is a creation threat that might be viewed as critical as it is accepted to be cancer-causing (Fleet, 1994). That chemical is conveyed in the midst of the fermentation when having high heat in the direction of the completion of fermentation. Ferment strains that make a little estimation of urea are introduced and the farm is arranged vivaciously with characteristic compounds (Boulton *et al.*, 1996).

2.4. Malo-lactic Fermentation (MLF)

After ethanolic fermentation, wine regularly encounters the malo-lactic fermentation (MLF), that continues around 14 days to a month. Lactic acid organisms, occupant in the wine, are responsible for the MLF; however, various winemakers enable this deacidification by incorporation with strains of *Leuconostoc oenos*. The MLF realises a reduction in the taste of wine and an increase in its pH by around 0.3-0.5 units. That fermentation, however, is not helpful to every wine. Wines made of grapes developed in more hotter environments showed the tendency of being not so much acidic (pH > 3.5) and additionally fall in acidity should be malevolent to sensory characteristics. Also, the growth of MLF increases their pH to levels where crumbling microorganisms are more prone to develop (Wood, 1998). In cold climates, deacidification by MLF is desired so as to make the wine drinkable (Lea and Piggott, 1995).

At the point, when aging bubbles have crossed out of a direct recurrence, the novel wine might be racked off the gross stay in clean storage tanks (Vine *et al.*, 1997). Beginning hereon, for the term of the wine life, it should be essential to ensure that it is secured in compartments which are finished fully. Preventing air contact with the wine diminishes the first experience with oxygen which is a section for oxidation and the advanced deterioration of living cells (Soufleros, 1997b).

The common tried guidelines in mixing of wine are tantamount to imparting the assorted characteristics of distinctive wines in the wine getting a more extensive and fulfilling solicitation. Wines might be stirred prior to stabilisation in view of the fact that the various components required for steadiness, once in a while result in stable wines combined to shape a balanced wine (Vine *et al.*, 1997).

Just prior to fermenting and packing of wines, they are cleared up by utilising more than one method, which consolidates extension of fining materials (bentonite, egg whites, isinglass, gelatine) layer filtration and centrifugation (Ough, 1992). Frost stabilisation could then begin. It is finished by putting away the wine in a chiller at 3-2°C for not less than 21 days. This operation can generally be reduced by the incorporation of potassium bitartrate powder, that should be separated by using filtration into residue (Vine *et al.*, 1997). Wine precariousness can be expedited by numerous substances (synthetic, microbiological) and negatively affects wine quality. Moreover, Cu and the high iron compound can raise medical issues and need to be watched and cut down (Cu < 3 µg/mL wine, Fe < 12 µg/mL wine) by mixing. The genuine issue is metal in wine after the fermentation (Soufleros, 1997b). It can happen from components settling in the wine or vessel comprising these metals. Thus, it is essential to screen the metal substance (Pb < 0.3 µg/mL wine, As < 0.01 µg/mL wine) (Soufleros, 1997a).

2.5. Maturation

White wines start to mature when the yeast ends fermentation. When these yeasts are eliminated, wines are close to being drinkable. Certain white wines are matured in barrels. This generally is on the side of wines which will be traded at high expenses. Red wine maturation is more expanded than white (Ough, 1992). During maturation, each and every wooden vessel loses a little wine content because of absorption and leakage. It is basic to avoid air entrance to the wine in containers, especially to maintain a strategic distance from oxygen-devouring microbial growth. Each barrel might be precisely analysed at the minimum of four weeks in order to make up to the full capacity of wine. A couple of winemakers prefer ‘wet-bung’ barrels prior to filling as a means to remember the ultimate objective of diminishing the risk of bacterial contamination in barrels via the bung hole (Vine *et al.*, 1997). Exactly as soon as red wines begin developing in barrels (or something else), the mind should be applied to inspecting for unwanted microbes and changes in shade, smell or flavour. Mix-ups in too little sulphur dioxide can generate wine spoilage by acetic acid bacteria and yeast (Ough, 1992).

Barrels are hard to clean and routinely hard to sanitise in case they appear to be corrupted with unwanted microbes and could be ousted from the winery. However, it is inconceivable since it could be damaging due to ethyl carbamate formation that is tumour-causing. Plus, ethyl carbamate could be formed in the midst of maturation as soon as there are urea build-ups which elevate the temperature. In this way, it must be measured prior to wine bundling (Gump and Pruett, 1993).

2.6. Packaging

The packing procedure is usually a wonder among the most joyful processes. During winemaking, genuine consideration must be given to the observance of hygienic practices (Vine *et al.*, 1997). The

bundled wine is relied upon to be free of residual microbes. Wines are clarified through depth filters prior to their entry into the bundling vessel. Almost all issues arose due to the wrong utilisation of hygienic filtration procedure. The filter and notwithstanding the bundling line might be cleaned before the wine output. Air expulsion or counteractive action of the packing line is basic. The essential site where the wine is in connection with air is at the level of the filler bowl. The volume above the liquid in the bottle, in order to cover, could be blown with carbon dioxide or N₂ ahead of plugging (Ough, 1992). New bottles should be flushed frequently with extremely hot water and used jugs ought to have been sanitised when they were depleted, and held topsy turvy. Apparatus could be steam-sanitised. The filling equipment was seen to be the best explanation behind tainting, alongside the corking (Vine *et al.*, 1997).

The stopper ought to be accurately treated and the neck of the jug should be of the most ideal size. Attention should be paid that the plugs don't have a bit of fragment or striations which could create spillage. The moistness of the plug is pivotal and might be 5-7 per cent (Ough, 1992). If it is bigger, it will be airtight too quickly. Stopper pollution is regarded as a noteworthy imperfection in packed wine. Stopper disease lead to a foul and off-flavour. It is seen that the most ideal approach to reduce the event of plug imperfection is to restrain the extreme conditions for microbial growth on the stopper by regulating the water activity (Fleet, 1994). Up-to-date stopper suppliers furnish sterile stoppers. In case of vulnerability, plugging might be done prior to being used in a solution of sulphur dioxide concentration of 10 g/hL (Marriott, 1994). Labels are a straightforward piece of trading wine. The paper should be rub-confirm, water-safe and preserved through fast bundling lines. Beyond this, it is basic to be coded in the event that there is an issue in the packed wine (Ough, 1992). Transport and storage of wines are normally not beneath the winemaker's mastery. Noteworthy harm is expected in packed wine as soon as it is predisposed to over abundance of heat or chill (Ough, 1992).

Just before the red wine is involved, de-stemming is vital to be executed cautiously in the production of red wine, as the stalk stores are not cancelled prior to the completion of fermentation and thus have a detrimental impact on the sensory attributes. At whatever point, the stalks are withdrawn from the contact with the pounded grapes by using a broadened time of less than two hours, a 'stemmy' off-nature might occur in wine. Also, the methodologies of skin dissociation and wet mash crushing are realised by following the fermentation method utilised for white wine production (Ough, 1992).

Wine is unreasonably acidic due to the development of pathogens. Various life forms don't survive in lesser pH medium (Speck, 1984). Microbes giving rise to wine deterioration are predominantly primitive yeasts and other bacteria. Basic deterioration yeasts include *Candida*, *Pichia* and different *Saccharomyces* spp. that cause development of films on the top of the wine. Wine-crumbling microorganisms are fundamentally lactic acid and acetobacters bacteria (Forsythe and Hayes, 1998). The best control method is keeping the perishing grapes away.

3. Monitoring Safety and Quality Control

3.1. Gathering/Harvesting

Grape gathering is a Critical Control Point (CCP1) accommodating chemical and physical risks (Table 1). Materially, grapes might be gathered in the absence of ruined parts, principally oxidation and pollution from microorganisms which can quickly grow.

In this way, gathering might be dovetailed with the best feasible precautions and a systematic contamination control method must be executed (Ellison *et al.*, 1998; Dibble and Steinke, 1992). Pesticides play an unequivocal part in disturbance administration; in any case they should be taken care of deliberately in the light of the chemical dangers they present (Maner and Stimmann, 1992). At the season of gathering, the grapes should have accomplished proper development when acidity levels and Brix display matureness of fruit. Because chemical sediments on top of the berries represent chemical risks, studies suggest a quick and fundamental gas chromatographic technique for their estimation (Oliva *et al.*, 1999). The best progress borders for insecticides in wines and grapes are granted by the Codex Alimentarius (Codex, 1998) and OIV (Organisation International du Vin) (OIV, 1994). In the end, mass receptacles utilised for grapes transferral must be successfully sanitised to stay away from any microbial contamination.

Table 1. Activities Concerning Security and Quality Control for Harvesting

		Quality	Safety
Harvesting/ Gathering (CCP 1)	Risks/Cause	• Untimely grapes gathering • Overripe grapes gathering • The hurt of grapes due to lack of precautions • Piteous gathering techniques • Mould contagion from affected grapes • <i>Penicillium</i> and <i>Aspergillus</i> infection of grapes • Growing of <i>Acetobacter</i> on grapes • Traces of iprodione, vinclozolin, procymidone in the grapes	• Pesticide trace • Unwanted substance from the soil • Infection of gathering equipment
	Precaution measures	• Quotidian precaution amid gathering • Mature grapes gathering • Gathering workers with experience • Conscientious compliance of MRLs • Control of sugars and acid concentration in grapes • Acceptable cleaning of the gathering machines • Use SO₂ for mould contamination of grape	• Apply attention during gathering • Harvest uncontaminated grapes • Scrupulous conformity of MRLs • Cleanliness rehearses application to stay away from the pollution of grapes
	Severe factors/ limits/ Controls	• Determination of grapes density • Determination of grapes acidity • Checking on grapes integrity • Determination of insecticide residue content of grapes • Check-up of grapes amid harvesting • Investigation of cleanliness application amid collecting	• Determination of insecticide residue concentration • Auditing of hygienic methods amid gathering • Inspection of harvesting equipment hygiene

Source: Adapted from Kourtis and Arvanitoyannis, 2001

3.2. Stemming

Stemming (CCP 2, Table 2) considers the elimination of leaves, grape stalks and stems prior to beating. This system has a few purposes of interest since the entire volume of the disposed items falls by 30 per cent, as needs demand bringing about littler tanks and subsequently increasing the ethanol concentration. Regardless of this, the completion of fermentation and the ethanol concentration of the completed wine rely generally on the sugar concentration of grapes. Stemmers mostly comprise a pierced cylinder for berries to experience yet preserve the area of stems and stalks.

3.3. Blending

Blending/crushing (CCP 3, Table 3) ordinarily instantly takes place in the wake of stemming. Released juice is especially prone to browning due to oxidation and microorganism pollution. The immensely acknowledged squeezing forms incorporate pressing the fruits in contact with a punctured equipment or directing the berries via rollers. It is fundamental to go without pulverising the seeds to protect against polluting the must with oils from the seed. Its oxidation could produce unwanted smells and represent an unpleasant fountainhead of acrid tannins. Essentially, it is fundamental to have the most ideal treatment of the product, since wrong arranging may incite an unexpected start of ethanolic fermentation and therefore lead to high temperature of fermentation. though a recess may generate microbe pollution and browning (Zoecklein *et al.*, 1994).

Table 2. Activities Concerning Security and Quality Control for Stemming

		Quality	Safety
Stemming (CCP 2)	Risks/Cause	• Stem residue in grapes (red winemaking) • <i>Botrytis cinera</i> infection of grapes • Grapes contamination by foreign matter coming from equipment	• Infection of grapes coming from bad cleaning • Foreign matter of grapes coming from equipment
	Precaution measures	• Destemming maintenance of equipment • Manual elimination of external material from grapes • Prevention of grapes degradation using SO₂ • The utilisation of cold water for adequate cleaning of destemmer	• GMP (Good Manufactured Practice) and sanitation amid destemming • Equipment and environment sanitation in the winery
	Severe factors/limits/controls	• GMP control (red winemaking) • Convenient expulsion of mold tainted grapes • Sulphur dioxide (40 mg/L) estimation of the grapes • Monitoring of destemmer refreshing	• Mastery of GMP and hygiene amid destemming • Mastery of hygienic techniques for traces of microorganisms and traces of clearing in the destemmer

Source: Adapted from (Kourtis and Arvanitoyannis, 2001)

Table 3. Activities Concerning Security and Quality Control for Crushing/Blending

		Quality	Safety
Crushing/ blending (CCP 3)	Risks/Cause	• Oxidation of must • The increment in the measure of mass in the must • Contamination of must with metal compound coming from apparatus	• Infection of must via insufficient clearing (deposits of microbes, traces of chemicals) • Must contamination by external matter coming from apparatus
	Precaution measures	• Application of GMP during crushing • Sufficient space between crushing cylinders • The absence of air amid squashing	• GMP and sanitation amid crushing • Utilisation of authorised cleaning agents
	Severe factors/limits/controls	• Monitoring and application of GMP amid crushing • The environment of crushing air control • Crushing equipment cleaning and control	• Control of GMP and hygiene amid destemming • Must supply time in the blenders <2 h • Cleaning of blending equipment at the end of two days • Dissociation of blending equipment: maximum possible • Sanitation mastery and GMP request amid blending

Source: Adapted from Kourtis and Arvanitoyannis, 2001

3.4. Maceration/Squeezing/Pressing

Maceration is the dislocation of grapea by mashing them. As long as maceration is continually required in the underlying period of red wine fermentation, the long-time practice has led to less soaking in the manufacture of white wine. Span and temperature of mashing depend on wine and grape cultivar.

Conventionally for rose wines and white wines, the time of maceration is below 24 hours – red scheduled for early use, is macerated during three to five days and red for fermentation, is soaked between 120 hours to 21 days. Fermentation all the more regularly occurs in the midst of this or in the direction of the termination of maceration. The quantity of the antimicrobes to be utilised, generally in addition to the musts of white wine which is most sensible to oxidation, relies on the gathering prosperity and maceration heat. SO₂ has an extraordinary favoured viewpoint above alternative antimicrobial substances, as a consequence of the comparative passiveness of the wine ferments to its activity. Notwithstanding this, it is moreover deadly, or hindering, to nearly all yeasts and microorganisms (*Hansenula*, *Pichia* and *Candida*) in little amounts (Farkas, 1984) and has a fairly reduced retentiveness limit following the clarification stage (Gnaegi *et al.*, 1983). The juice is permitted to stay in the press for a time, in the midst of which juice flows out under its own gravity. Being dependent on the press, the obtained must and wine portions differ in regards to their physico-chemical characteristics. Joining various wine parts, the winemaker influences the wine character. In any case, a potential peril might exist in the reaction of oxidation if there is an interference in the procedure (Lichine, 1985).

3.5. Ethanolic Fermentation

Ethanolic fermentation is ordinarily completed by *Saccharomyces cerevisiae* strains since this type is particularly resistant to the large amounts of sugar, ethanol and SO₂ and besides, to lesser pH (3.2-4) for grape juice. The strains of *Saccharomyces cerevisiae* are one constituent of the endogenous microbial population or might be to some degree included in attaining a density of approximately 10⁵-10⁶ cells/mL in the juice (CCP 4, Table 4) (Constanti *et al.*, 1997).

Feasible pollution of juice with 'killer' yeasts (quality generally displayed in undomesticated *Saccharomyces* strains, furthermore in the alternative genus of yeast, for instance, *Cryptococcus*, *Torulopsis*, *Pichia*, *Kluyveromyces*, *Hansenula*, *Debaryomyces* and *Candida*,) could lead to bad fermentation (Van-Vuuren and Jacobs, 1992). Thought should be paid to the extra measure of SO₂ (175-225 µg/mL for white and red wine, independently) remembering the true objective is to prevent, if not to eradicate, the majority of wild yeast masses of grapes (Sudraud and Chauvet, 1985) and furthermore acidity management, and to Brix and tannin amount of the must. In fermentation, the accomplished chemical risks contain toxic metals (As <0.2 µg/mL, Cd <0.01 µg/mL, Cu <1 µg/mL, Pb <0.3 µg/mL), methanol amounts (300 µg/mL and 150 µg/mL for red wine and white wine, exclusively), EC amounts, insecticide traces and detergents (non-attendance) and ethylene glycol (non-appearance).

Attention should be paid with respect to the EC amount, in light of the fact that there is no enactment opposed to it in Europe, but it is so in USA (<60 ppb and <15 ppb for dessert and table wines, exclusively). The latter is confirmed from the chemical reaction of ethanol with materials rich in amino acids, basically, amino acids and urea like citrulline and arginine. Its management including gas chromatography (GC) measurement and evasion can be done by preventing concentrated fertiliser treatment of vines, elevated temperatures for conclusion or after ethanolic fermentation, utilising yeast strains for smaller ethyl carbamate and urea creation, using an enzyme and testing urea when prolonged storage is required.

The temperature of fermentation is noteworthy among the most basic factors affecting the metabolism of yeast, both clearly and in a roundabout way. For red and white wines, the appealing temperature vacillates to the extent of 8-15°C and 25-28°C, separately. Any existence of leftover sugars (fructose, glucose, sucrose) before the completion of fermentation is a risk that may create microbial destabilisation of wine.

The system of fermentation needs no oxygen. Nonetheless, residual oxygen towards the beginning of the exponential stage of yeast development quickens the fermentation, considering that the yeast cell number rises and the ordinary cell gets viability augmented. The pH (<3.0) may impact the procedure exactly at extraordinary levels where the advancement of fermentation yeasts is quelled (Zoecklein *et al.*, 1994).

At long last, the fungicide in the must may accept improvement of yeast inhibition and hinder the sensory characteristics of wine by affecting biosynthetic metabolisms (Pilone, 1986; Cabras *et al.*, 1988; Fatichenti *et al.*, 1984).

Table 4. Activities Concerning Security and Quality Control for Fermentation

		Quality	Safety
Fermentation (CCP 4)	Risks/Cause	• Development of troublesome bacteria in the fermenters • Stuck fermentation • Loss of wine aroma profile • Acetic acid and H₂S production • Oxidation because of air entrance in the fermenters • Sugar fermentation into lactic acid • Glycerol lactic fermentation • Tartaric acid lactic fermentation • Augmentation of wine viscosity • Abnormal proceeding amid fermentation • Fermentors breaking because of high temperature or CO₂	• EC production • Cleaning chemicals residues in fermentors • Other residues from pre-fermentation phases (yeasts, bentonite) • Extreme injection of SO₂ in the fermented must
	Precaution measures	• Use of SO₂ to prevent wine spoilage • Application of authorised SO₂ limitations • Injection of favoured ferment strains into preceding inoculation • Introduction of yeasts nutrients • Maintain fermentation range temperature by utilising the automatic cooling system • Clean fermentors • Stabilise fermentation temperature • Pump-over process (for the manufacture of red wine)	• GMP and sanitation amid destemming • Insertion of sulphur dioxide < 200 µg/mL fermented juice • Sanitation of fermenters • Setup of small temperature in bioreactors • injection of special yeasts inside the preceding inoculation
	Severe factors/limits/controls	• White wine fermentation temperature: 10-21°C • Red wine fermentation temperature: 20-30°C • Must aeration amid the first two days of fermentation • Monitor yeast injection • Authorised SO₂ < 200 mg/L must • Juice gravity control amid fermentation • Monitoring of pump-over process	• Mastery of GMP and cleaning amid destemming • EC content: <30 ppb in the fermented must • Authorised sulphur dioxide < 200 µg/mL fermented juice • Fermentor cleaning management • Yeast purity control and safety • Temperature control amid fermentation

Source: Adapted from Kourtis and Arvanitoyannis, 2001

3.6. Malo-lactic Fermentation (MLF)

Early beginning and achievement of MLF incite development of SO₂ and stockpiling at chill temperatures and clearing. It is driven, using lactic acid (LA) organisms (*Oenococcus oenos*) that clearly decarboxylate the L-malic acid to L-lactic acid. This change achieves acidity reduction and pH increase, that are linked to the extended drinkability and creaminess of red wines (Davis *et al.*, 1985; Guzzo *et al.*, 1998). The underlying pH, the sulphite capture (Vaillant *et al.*, 1995), the anthocyanin and the phenolic amount

(Vivas *et al.*, 1997) of must/wine unambiguously impact in the case, giving rise of MLF. Phages could truly interrupt MLF by affecting the *Oenococcus oenos* along these lines, creating destabilisation of wine microflora (Gnaegi and Sozzi, 1983). In this way, to ensure the progression of MLF, winemakers inject the fermented juice with no less than one *Oenococcus oenos* strains (CCP3, Table 5) (Nielsen *et al.*, 1996; Nault *et al.*, 1995). After fermenting, the wine's accepted total acidity is believed to differ inside to the extent of 0.55-0.85 per cent. At any point, total acidity beats the breaking points and fermentation and deacidification techniques are set up (Jackson, 1998).

Table 5. Activities Concerning Security and Quality Control for MLF

		Quality	Safety
Malolactic fermentation (CCP 5)	Risks/Cause	• Augmentation of wine pH • Reduction of wine acidity • Degradation of wine taste	• Microbiological contamination
	Precaution measures	• Injection (inoculation) of malolactic yeasts	• Certified suppliers, strictly following instructions
	Severe factors/limits/controls	• Controlling the pH of the wine • Controlling the acidity of the wine • Wine pH <3.5	• Microbial analysis

Source: Adapted from Kourtis and Arvanitoyannis, 2001

3.7. Maturation/Aging

The maturation step regularly keeps going from six months to one year in oak barrels. Amid maturation, a score of chemical and physical interactions occur inside the barrel, the encompassing environment and the wine in maturation, prompting a change of savour and characteristics of wine (Martinez *et al.*, 1996). At this level, we have a CCP (CCP 6, Table 6) regarding the oak vessel, which is expected to be flaw-free and ought to have been subjected to disinfecting processing.

The wood likewise should be exempted from noticeable or unpleasant smells, which pollute the fermented must (Mosedale and Puech, 1998). During the aging period, a few compounds of the wood are deleted to tannin of wine (Viriote *et al.*, 1993; Towey and Waterhouse, 1996). Since oak tannins could essentially increase wine savour, white wines are generally aged in oak for a smaller time than red wines and in prepared oak containers to discharge a smaller amount of extractable tannin (Popock *et al.*, 1984; Quinn and Singleton, 1985).

One more CCP is marked with the restraint of air infiltration along wood or amid racking and inspection of wine. In spite of the fact that a little oxidation is alluring, a more substantial one could generate different sensory modifications, for example, oxidised smell, browning, colour loss in red wines, yeast activation and bacteria spoilage, ferric casse development and tannin precipitation (Ranken *et al.*, 1997). Restrains on free and total sulphur dioxide amounts in completed wine vary from nation to nation.

3.8. Clarification

Clarification includes physical methods for evacuating the floating particles. Must clarification by filtration, centrifugation, or racking frequently enhances the savour improvement in white wine and supports the avoidance of spoilage by microorganisms. Assuming that an adequate period is given, fining and racking could create stable, completely clear wines; however, now that premature packaging in months or 14 days following fermentation is utilised, filtration and centrifugation are done to facilitate the required clearance amount (Ribereau-Cayon *et al.*, 1998). Pollution of wine by microorganisms amidst the previously stated techniques causes a possible issue for its steadiness (Ubeda and Briones, 1999). Racking is likewise powerful on insecticide traces and lessening of wine (Gennari *et al.*, 1992).

Table 6. Activities Concerning Security and Quality Control for Maturation/Aging

		Quality	Safety
Aging/ Maturation (CCP 6)	Risks/ Cause	• Wine sensory characteristics modification • Barrel flavour in the wine • Oxidation of wine • <i>Dekkera</i>, <i>Brettanomyces</i>, <i>Pechia</i>, <i>Candida</i>, development in the fermented must • <i>Acetobacter</i> development in the fermented must	• DMDG wine residue • EC in wine • Wine contamination by the development of microorganisms in barrels • Wine contamination from the dirty winery
	Precaution measures	• Prevention of wine spoilage by adding SO₂ • The use of N₂ to remove O₂ from wine • The barrels must be kept totally full • Barrels must be carefully cleaned • Tight-bunged barrel • Maturation of wine using always wetted bung • Maturing wine temperature (<12°C)	• Attentive barrel clearing • Utilisation of new oak vessels • Attentive winery clearing • Keeping small temperature amid maturation
	Severe factors/ limits/ controls	• Monitoring of SO₂ concentration (>3 µg/mL wine) • Monitoring of keeping temperature (<12°C) • Monitor odour of empty barrels • Monitor oxygen absence amid wine maturation • Monitoring spoilage bacteria in wine • Monitoring barrel cleaning methods	• EC in wine measurement • Monitoring of barrel clearing methods • Mastery of the suitability of the barrels • Monitoring of winery cleaning methods • SO₂ measurement in wine (>3 mg/L)

Source: Adapted from Kourtis and Arvanitoyannis, 2001

3.9. Fining/Stabilisation

The purpose behind fining is the creation of a lastingly clear and flavoured flaw-free wine. Nearly all essential strategies incorporate a) fining using tartrate by cooling the matured wine to close to its freezing temperature and afterward filtration or centrifugation is executed to evacuate the solids, b) protein fining with fixing, neutralisation, or degradation by bentonite is carried out (Blade and Boulton, 1988), c) polysaccharide expulsion is done with enzymes which hydrolyse the macromolecule, perturbing its defensive colloidal activity and membrane stopping characteristics (Ribereau-Cayon *et al.*, 1998), and d) stabilisation of metal casse (Fe, Cu) (CCP 7, Table 7) is initiated.

Ferric casse is monitored using the expansion of bentonites and proteins by adjusting the aggregation of ferric complexes which are insoluble, though wines with Cu content more noteworthy than 0.5 µg/mL are especially vulnerable to Cu casse development (Langhans and Schlotter, 1985). Legitimate remaining Cu levels in completed wines fluctuate and not all strategies for Cu evacuation are authorised in all the countries.

3.10. Bottling

Wine is packed in glass containers covered with stopper. The container might pass a sanitising stage and an examination to ensure the non-appearance of any inadequacy (CCP 8, Table 8) and the steadiness of the wine until its gobbling (Cooke and Berg, 1984).

The stopper must be well sized, 6-7 mm higher than the inside neck diameter of the bottle, to abstain from any feasible leaks. In packaging, all three risks might be found. In special, stopper microorganism, heavy metals traces, SO₂, insecticides and detergents, and non-appearance of cracks, scrapes and fissures in the lute speak for physical, chemical and microbiological risks.

Table 7. Activities Concerning Security and Quality Control for Stabilisation/Fining

		Quality	Safety
Fining/ Stabilization (CCP 7)	Risks/Cause	• Fining chemical residue in the wine • The residue of lees in the wine • Wine over fining • Agents of adsorption in the wine • Brown cloudiness of wine • Cloudiness of wine due to microbes • Cloudiness of wine due to Fe^{2+} • The turbidity of wine due to Cu^{2+} • The turbidity of wine due to colloidal substances	• Impure addition compounds in the wine • Traces of stabilisation chemicals in the wine • Traces of poisonous metals in wine • Residues of chemical substances in wine
	Precaution measures	• Use of dosage pump to add a fining agent • Dissolution of fining chemical in water • Quick withdrawal of lees residues from wine • Introduction in chilly weather environment of the fining agent • Prevention of deterioration by adding SO_2 • Storage of wine far from sun and air • Addition of bentonite	• Authorized substances addition according to legislation • Addition of authorised substances for wine stabilisation • Authorised substances for addition
	Severe factors/limits/controls	• Monitoring of the addition of agent solution in the wine • Monitoring of lees traces in the wine • Control of over treatment of trub in the wine • Control of weather environments amid fining • Monitoring of fining chemical traces in the wine • Oxidase measurement in wine • Microscopic check-up of wine for microbes • $\text{Fe}^{2+} < 12 \mu\text{g/mL}$ wine • $\text{Cu}^{2+} < 3 \mu\text{g/mL}$ wine	• Monitor the purity of fining agents • Monitor the authorised substances • Monitor the residues of fining chemicals in the wine • Monitoring of authorised additives • Metal limits estimation (As $< 0.01 \mu\text{g/mL}$, Cu $< 0.1 \mu\text{g/mL}$, Pb $< 0.3 \mu\text{g/mL}$ wine)

Source: Adapted from Kourtis and Arvanitoyannis 2001

Although cork is important for its non-reactive property when touching the wine, it could generate unwanted flavours when polluted (Simpson *et al.*, 1986; Simpson, 1990) or when winemakers are not executing functional quality control (Neel, 1993). The control for the stopper is non-appearance of yeast and LAB and which could be verified by microbial test. When a long maintenance period of wine is predicted, higher and denser stoppers are favoured since a long exposure bit by bit influences the stopper integrity. When forcing the cork into the bottle neck, attention should be paid to stop the development of microbes inside the equipment (Malfeito-Ferreira *et al.*, 1997; Ubeda and Briones, 1999) and the lead transfer to wine through the wine-stopper-capsule method (Eschner 1986), and the oxidation during packing by washing out the glass containers with CO_2 . Stopper placing might also happen under vacuum. The empty space occupied by oxygen could impact the item quality by giving rise to the disease of the 'bottle'. The containment limit for sulphur dioxide is 175-225 $\mu\text{g/mL}$ respectively for red wine and white wine. Nearly $< 0.2 \mu\text{g/mL}$, Cd $< 0.01 \mu\text{g/mL}$, Cu $< 1 \mu\text{g/mL}$, Pb $< 0.3 \mu\text{g/mL}$ traces of insecticides and pesticides in the completed item, are given by Office International de la Vigne et du Vin (OIV, 1994).

Table 8. Activities Concerning Security and Quality Control for Bottling

		Quality	Safety
Bottling (CCP 8)	Risks/Cause	• Deterioration microbes in wine bottles • The growth of moulds in wine bottles • Leakage of wine from bottles • Oxidation of wine enhancing loss of sensory characteristics • Foreign substances in wine coming from bottles	• Undesirable substances in the wine and coming from glass containers and filling equipment • Residues of clearing agents in the fermented must • Pollution of wine from the winery • Development of microbes in glass containers polluting the wine • Development of microbes in filling equipment pollute the wine
	Precaution measures	• Mechanical and chemical cleaning of bottles • Bottling as stated to legislation • SO₂ addition in wine before bottling • Removal of air in wine using N₂	• Cleaning of bottles • Sanitation of bottles line • Sanitation of winery
	Severe factors/limits/controls	• Monitoring of wine cleaning methods • Control of bottles visually and microbiologically • GMP monitoring application during the bottling of wine	• Cleaning of bottles methods • Monitoring of GMP during the bottling of wine • Hygiene control measurement for bottles line, bottles and environment • Microbial control measurement for bottles line, bottles and environment

Source: Adapted from Kourtis and Arvanitoyannis, 2001

3.11. Storage

Storage and shipping of wine at high temperatures could induct fast modifications in wine flavour and colour. Straight subjection to sunlight reflects the influence of hot storage temperature. It impacts the reaction speeds implicated in maturation, for instance, the speeding up of the terpene fragrance loss and aromatic ester hydrolysis (De-la-Presa-Owens and Noble, 1997). Temperature can influence the volume of wine, alleviating the stopper seal, generating oxidation, leakage and eventually microbial growth due to bottled wine spoilage (CCP 9, Table 9).

Table 9. Activities Concerning Security and Quality Control for Storage

		Quality	Safety
Storage (CCP 9)	Risks/Cause	• Alteration of cartons and labels of wine bottles due to a humid area • High temperature provoking wine leakage	
	Precaution measures	• Storage in low humidity environment • Storage at environment temperature between 12-15°C	
	Severe factors/limits/controls	• Control of storage condition	

Source: Adapted from Kourtis and Arvanitoyannis, 2001

4. Bioreactors Typology, Technology and Uses

Winemaking innovation has seen amazing progressions all through the most recent 20 years, upgrading the nature of wines and furthermore formulating it to deliver wines with an extensive extent of traits. On this ground, some innovative progressions like enzymatic actions, use of picked yeasts, modification of microbe starters and immobilisation are of key importance (Fig. 1). These headways have influenced all aspects of winemaking, with wine remaining the last consequence of a mechanical chain that joins the handling and must treatment, fermentation, aging and packing. This inventive advance has upgraded the nature of the wines made. Quality wine is evaluated by intensity, fineness, advancement in smell and taste, and physico-chemical and microbiological stability (Dubourdieu, 1986; Noble, 1988; Rapp and Mandey, 1986; Schreier, 1979).

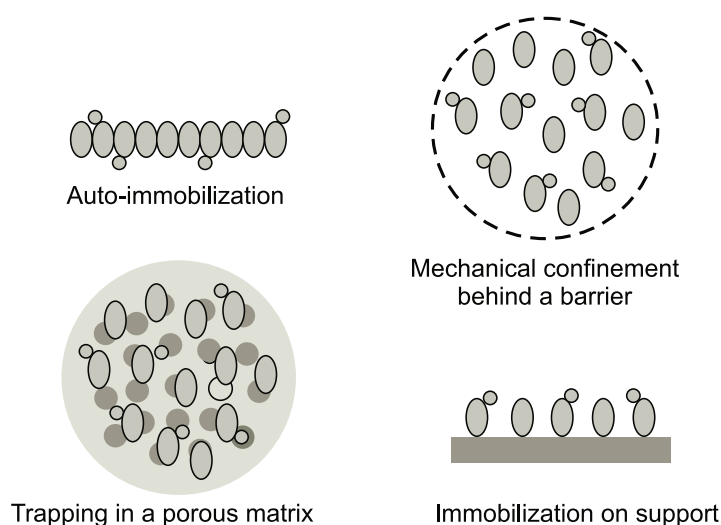


Figure 1. Yeast immobilisation system

4.1. Bioreactors Shape and Size

Fermenters of a broad collection of forms are straight-tube, barrel, V, square vessels, external forms etc. are utilised for wine manufacture (Boulton *et al.*, 1996). Forms of different fermenters used in fermented beverages (Maule, 1986; Moresi, 1989) are shown in Fig. 2. In most of the fermenters, floor inclining is done in the direction of the front. Bioreactors with domed or hemispherical bases are used in winemaking. Despite positive conditions in mash discharge in red winemaking, the usage of the funnel-shaped-based bioreactors has not sharpened. One of the most exorbitant and freshest advancements in the fermentation of red wine are the turning stainless-steel bioreactors that are uncommonly gainful with respect to the degree of energy and time anticipated that would build perfect skin introduction in the aging wine and inconsequential oxygen open to deterioration microbes. Despite having focal points, the usage of rolling and barrel-shaped vessels as a differentiating alternative to standard fermenters has found affirmation in red wines (Peyron and Feuillat, 1985).

There is a noteworthy distinction in measure, shape, outline and advancement materials used as a piece of fermentation tank in the manufacture of wine, inciting a fluctuated grouping of matured wines (Moresi, 1989). Any non-porous and non-perilous tank could be used as a bioreactor. Each tank could be ordered in two fundamental classes (tanks and vats). Vats are open at the top, although tanks are closed at the top. Earlier vats were used for red wine manufacture in view of the fact that a prompt access to the highest point of skins and seeds is desired in the midst of aging. White wines could be manufactured in tanks and are in a position to disallow air from the maturing juice. Most of the bioreactors outlined are rudimentary and a problem in planning occurs since their volume is extended. The development in volume moreover decreases the surface area for heat transfer. In red wine manufacturing, the unpredictability of the tank technology depends on the method used for the top submersion. Usually, the batch fermentation technique is used in wineries. Continuous bioreactors are moreover open but rarely used. As of now,

fermentations used to be finished in 2.25-2.28 hL tanks or 6-12 hL vats (Diviès, 1988). The wooden or concrete vessels used earlier have now been replaced with especially planned stainless-steel bioreactors. A diagram demonstrating particular fragments of a stainless-steel bioreactor is shown in Fig. 2. Small fermenters are often used in red wine aging as a result of the problem in achieving an agreeable top submersion (Jackson, 1999). Business wineries are using bioreactors of 20 m³ or more prominent limits. They are sensible to the extent of capital cost, computerisation and automation.

4.2. Types of Bioreactors

Efficiency in the fermentation of wine can be increased by utilising elevated yeast cell density by expanding the operative cell density or size by accumulation or cell immobilisation on a certain support. These methods are called high-cell-density reaction techniques. In addition, these methods are insensitive to unforeseen changes in working conditions or other characteristics of the must. Thus the total amount of organisms is maintained, the fermentation activity being restored once the problem is solved. The flow propels the procedures of immobilisation and have led to the improvement of proficient immobilised fermenters to completely make use of the benefits of biocatalysts and cell immobilisation (Fig. 2). The utilisation of the procedure of immobilisation for fermentation of wine accordingly, needs the improvement of a deliberately planned and reasonably constructed fermenter. Non-stop alcohol fermentation methods utilising immobilised cells have been widely reviewed (Gôdia *et al.*, 1987) and inferred that immobilised systems have many preferences over the customary suspended cell systems. In the bioreactors, the collection of ethanol inhibits the productivity (Goma, 1978). Thus, it is useful to complete in consecutive bioreactors the fermentation or in a gradient of concentration in reactors. A continuous procedure for the must fermentation to utilise serially associated fermenters was licensed in USA (Epchtein, 1984). A bioreactor with multistage systems, utilising expendable fixed bioplates, has likewise been produced for fermentation of wine in a continuous way (Ogbonna *et al.*, 1989). An overview of bioreactor technologies created demonstrated that developments in the last vious couple of years has occurred primarily in three zones: outlines, double phasic responses and environmental fermenters (Deshusses *et al.*, 1997). There are two noteworthy methods (heterogeneous and homogeneous) for immobilisation cell or limiting biomass (Diviès *et al.*, 1994). The harmonised method comprises identical dispensation of biomass as

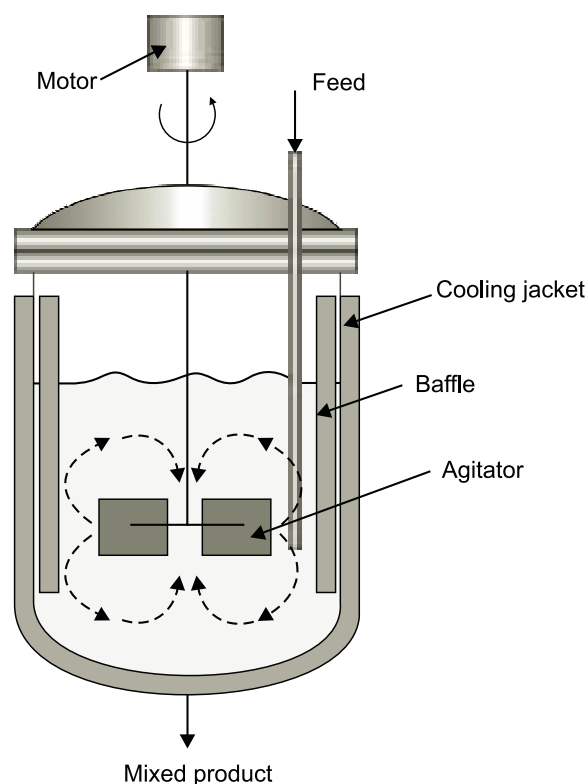


Figure 2. Classical agitated bioreactor

free organisms in the milieu. Rehashed utilisation of weight of organisms could be done by flocculation, centrifugation of yeast with outside or inside decanter or membrane bioreactor where the cells are introduced. Then again, the heterogeneous technique has two different stages, like fluid milieu that is supposed to be changed and a particulate phase having the cells. In this technology, biomass is restricted by way of support, auto flocculation and entrapment in gels.

4.2.1. Heterogeneous Bioreactors

In heterogeneous bioreactors, microbes are immobilised by using bonding. The essential and vital thing to do is to augment the concentration of cells and keep their life in recycled or in a continuous method.

4.2.1.1. Continuous Stirred Tank Bioreactor (CSTB)

Stirred tank bioreactors (Fig. 2) comprise of a stirred tank where crisp milieu is continuously introduced and compared, the volume of the fluid substance is evacuated. They are well blended by the utilisation of impellers. The fluid component of the reactor is equivalent in the constitution, like the convergence of the surge. With the immobilisation of cells, high liquid speeds are expected to accomplish a steady provision of product and substrate expulsion. CSTBs or back-mix bioreactors, as they are occasionally named, are inexpensive, adaptable and particularly satisfactory when fluid phase reaction is required. The gas provision, temperature and pH control are simple. New agents can be effectively introduced to the tank and particulate substrate materials can be endured without a problem. In any case, the moderately strong-power input needed to give effective stirring in CSTB is obviously a weakness and it might cause erosion or destruction of the immobilised cell in view of the high cutting forces at the impellor surface.

In any case, the continuous stirred tank bioreactor provides the finest blending qualities and air exchange. The medium density in a continuous stirred tank bioreactor is normally smaller than the fluidised bed and packed bed bioreactors used in smaller average speeds. But bringing down the concentration of substrate might be favourable for hindered organism culture. Quick mixing speeds in the continuous stirred tank bioreactor in elevated shear stresses increase organism spillage from alignate (Margaritis and Wallace, 1982), or carrageenan beads (Jain *et al.*, 1985), cell separation from ion exchange resins (Bar *et al.*, 1987) and floc disruption (Fein *et al.*, 1983).

4.2.1.2. Packed Fixed Bed Bioreactor (PBB)

The packed (fixed) bed bioreactor is oftentimes utilised in immobilisation of the cell reactor for alcohol production (Gôdia *et al.*, 1987). The immobilised cells are loaded in a column at its most extreme density between which the milieu solution moves and the level of conversion of the substrate increments with the length of the column occurs (Fig. 3).

If the fluid momentum profile is completely flat, the packed (fixed) bed bioreactor works as a seal-flow bioreactor, which has a perfect behaviour. The efficiency of the packed (fixed) bed bioreactor for a specific biocatalyst relies upon the kind of fixation. High cell loadings are frequently accomplished by entrapment, bringing about enhanced productivity. The particle size for cell attachment likewise impacts efficiency. On a basic level, it is conceivable to accomplish full transformation into an item so that these bioreactors are perfect where full expulsion of a medium is required (detoxification).

The packed (fixed) bed bioreactor has the benefit of effortlessness operation and low cost-effective flow through the bed. It additionally can be flimsy amid long-term procedures in the light of non-stop biomass amassing, mass exchange restrictions and CO₂ holdup resulting in channelling and formation of dead spaces (Ghose and Bandyopadhyay, 1980) and even matrix disruption (Webb *et al.*, 1990). Gas developed may likewise lead to back blending, bringing a deviation from perfect seal-flow trend. Horizontal packed (fixed) bed bioreactor was utilised to help gas evacuation. Consequently, decreased channelling and gas fixation occur in the non-stop system (Shiotani and Yamane, 1981). The horizontal packed



Figure 3. Packed bed bioreactor with the counter current flow

(fixed) bed bioreactor has been 1.5 times more profitable than the vertical packed (fixed) bed bioreactor. A lessening in channelling by CO₂ can likewise be acquired by partitioning the column into isolated stages with perforated plates (Grote *et al.*, 1980).

4.2.1.3. Fluidised Bed Bioreactor (FBB)

Fluidised bed bioreactor gives conditions which are middle to the one of the CSTB and packed (fixed) bed bioreactor. Blending is of higher quality in the packed (fixed) bed bioreactor yet it brings down amounts of shear when contrasted with CSTB. FBB comprises a column in which the cell particles are kept suspended in respect to every other by a non-stop flow of the medium or gas at the highest flow speeds (Fig. 4). The benefits of FBB can be seen in numerous studies. The high concentration of yeasts that aggregate in the reactor makes the system suitable for working at high productivities. As indicated by the literature, the fluid flow (must) at the inlet of the FBB might be near that of the outlet and is regulated by the working conditions utilised. This can be viewed as an extraordinary favourable advantage, particularly for reactions repressed by the product, as for alcoholic fermentation (Gôdia *et al.*, 1987; Gôdia and Solâ, 1995; Viegas *et al.*, 2002).

The reduced pressure of the liquid flow underpins the mass of the bed. The FBB provides higher efficiency than CSTB in light of the fact that fluid estimates plug flow-like the packed (fixed) bed bioreactor. But, the FBB is more profitable for fermentation with medium hindrance than the packed (fixed) bed bioreactor as a result of the blending created by liquid flow. These reactors advance great mass exchange. The dead organisms are expelled in the process (Andrews, 1988) and expansive volumes of CO₂ are discharged without channelling (Keay *et al.*, 1990) and limits pressure decrease. Fluidisation abstains from such issues as pollution, damage of shear and constraints to scaling up, related to impellor shafts and sharp edges in mixed tanks (Dempsey, 1990). FBB can extend to suit developing organism mass so that they are less sensible to plugging and more helpful for cultures where oxygenation is required. FBB needs less energy (four times) contribution than a mechanically-agitated bioreactor. However, it is more energy consuming than the packed (fixed) bed bioreactor (Brodelius and Vandamme, 1987).

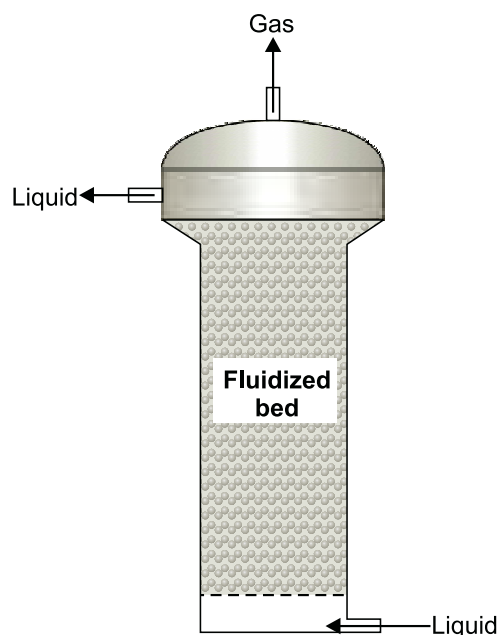


Figure 4. Fluidised bed bioreactor (tower fermenter)

4.2.1.4. Rotating Disc Bioreactor (RDB)

It is made up of fixed cell units, for example, polyurethane froth sheets (Amin and Doelle, 1990) or fibre discs (Parekh *et al.*, 1989) appended to a pivoting shaft (Fig. 5). It is gradually blended, thus permitting complete blending and expulsion of dead organisms, residues and the developed CO₂. The energy needed for RDB is not as much as that for STB as a result of its moderate blending speed. This bioreactor can take industrial media-holding particle suspensions to attain high efficiency. No problem occurs with the elevated solid milieu in this sort of reactor (Parekh *et al.*, 1989).

4.2.1.5. Air (gas) Lift Bioreactor (ALB)

In ALB (Fig. 6), the liquid volume of the tank is separated into two joined areas by means of a bewilderer – one area is sparged with air and another area that gets no gas is called down-comer. Bubbles convey the fluid, causing a lessening in fluid specific gravity. Gas runs away from the summit and the fluid falls in the down-comer. An outer loop method might substitute the internal bewilderer for the distribution of fluid in some reactors. Stirring in the ALB because of gas move derives little shear with effective blending and mass transfer. The dimension of the bewilderer impacts the hydrodynamics of the bioreactor. ALB are exceedingly energy productive in respect to mixed bioreactors.

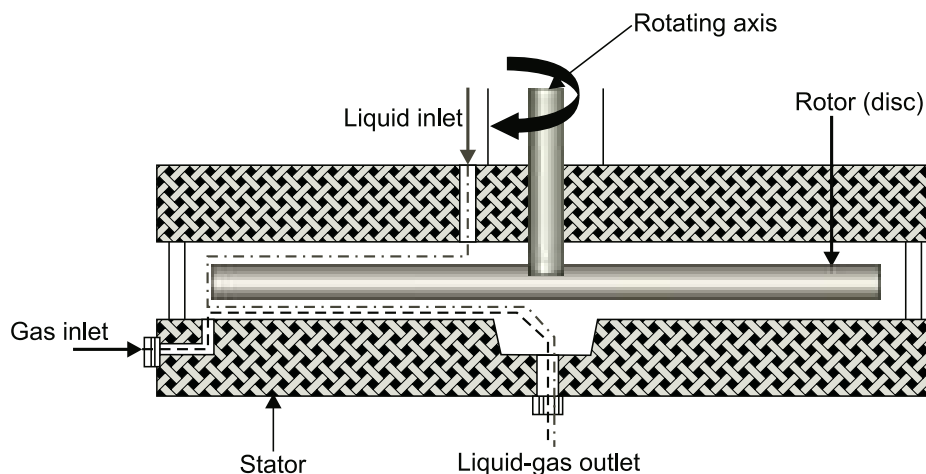


Figure 5. Classical rotating disc bioreactor

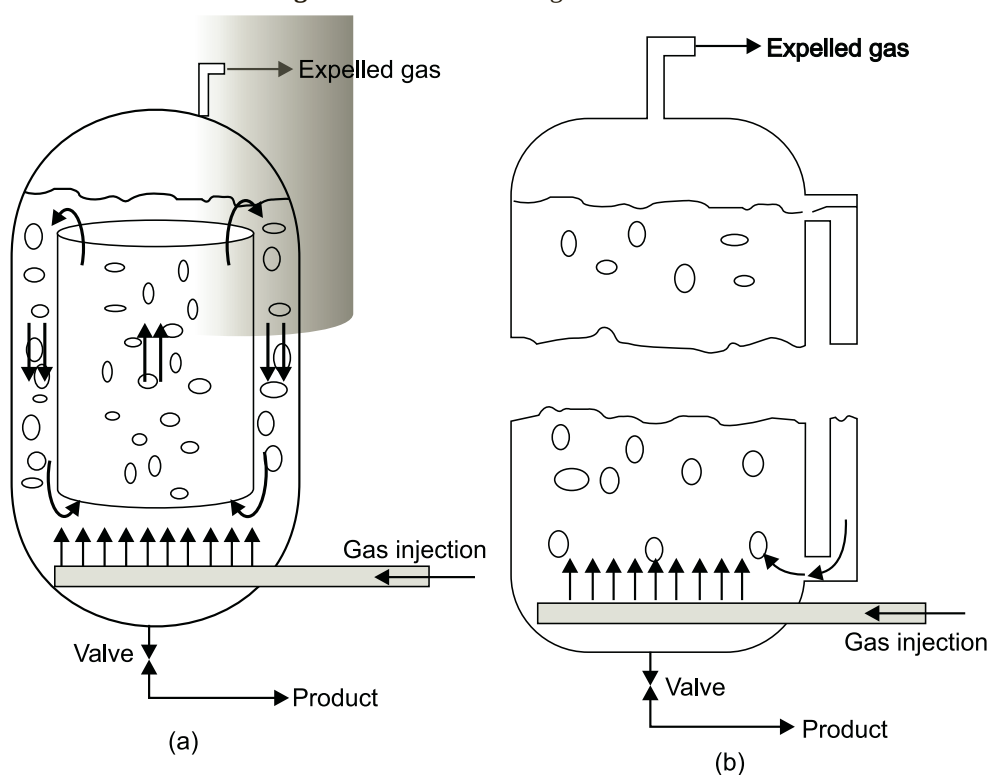


Figure 6. Airlift bioreactor: (a) Classical; (b) External-loop

4.2.2. Cell Recycle Batch Bioreactors (CRBB)

The CRBB (Fig. 7), whose main development is the numerous progressive utilisation of the identical microbe starter in various batch fermentations remains the roughly adequate non-ordinary method in wine production. Not like continuous, the CRBB doesn't need the entire modifications in the winery methods nor undertakes it in the new machine. Indeed, yeast to get reused can be recuperated via natural settling or by membrane separation or centrifugation with equipment effectively existing in nearly all wineries. Five distinct procedures were studied (Guidoboni, 1984); the majority of them utilise a centrifugation stage which accompanies a unique bioreactor for reuse of yeast.

Increase in cell weight and also in efficiency was attained by utilising a fractional vacuum technique (Cysenski and Wilke, 1978). The principal disservices of centrifugation method are the reduction in life of the microbial biomass because of the tension as they are exposed to centrifugation equipment. The utilisation of membrane reactor is an optional technique to realise centrifugation in CRBB, where the yeast cells are held in the fermenter possessing a membrane with pore size of under 0.45 mm.

The medium is directed by the membrane reactor and the changed item moves downstream from the membrane. The productivity of the change can be expanded by reusing the item by the reactor (Diviès

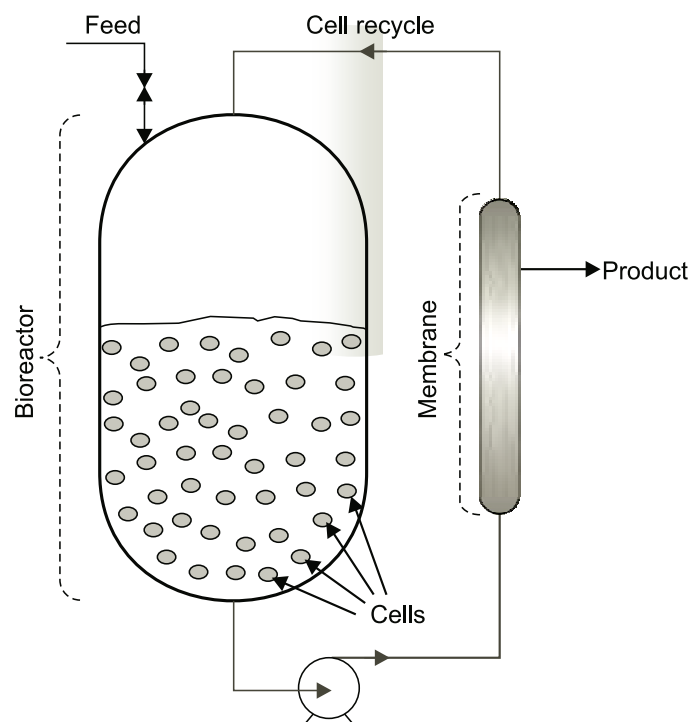


Figure 7. Cell recycle batch bioreactors

et al., 1994). The principal restriction of this is the stopping and the membrane unclogging (Mehaia and Cheryan, 1990).

In order to examine the general legitimacy of nearly all proficient methods of fermentation to diminish the wine production costs, an off-skin fermentation of clear *Trebbianotoscano* juice was done by utilisation of a non-regular cell reuse batch fermentation system (Rosini, 1986). The procedure decreased the fermentation length and also changed the ethanol efficiency and yield. It can be advantageously used in the production of conventional table wines.

Numerous investigations have exhibited the likelihood of controlling MLF by utilising a bioreactor with cell immobilisation or enzymes. Utilisation of bioreactor introduces various benefits in the ordinary wine deacidification. Starter microbes could be reutilised. The diminished development of auxiliary fermentation and products could be ended and initiated at the right moment by the wine producer. But, contamination by phage, a transient decline in activity and a little change in the sensory characteristics of the treated wine cannot be precluded always (Maicas, 2001).

The utilisation of cell immobilisation in fermentation procedures over the utilisation of free organisms offers a few benefits, like increment in efficiency or giving a more protective condition and enhancing the resulting separation of the cell. The concentration of catalytic activity in a decreased volume enables the winemakers to lessen the dimension of the fermenters and recoup the final items more effectively in batch or continuous production methods. In spite of the fact that the characteristics of cell immobilisation were similar to the one of free cells, however, the immobilised cells are interestingly simple to recoup and reuse. The fixation and attachment/adsorption are the two principal immobilisation systems used to prompt MLF in wine. However, entrapment is a well-known strategy because of utilisation of non-dangerous chemicals in agreement with food manufacture (Cassidy *et al.*, 1996). The change of immobilisation strategies for deacidification of wine was long examined by utilising alginates (Shieh and Tsay, 1990), polyacrylamide (Clementi, 1990), $\hat{e}$ -carrageenan (Crapisi *et al.*, 1987; Crapisi, *et al.*, 1990) and κ -carrageenan, etc. For example, an increase was noted in the operational stability of immobilised cells of *Lactobacillus* sp. in a κ -carrageenan matrix (Crapisi, *et al.*, 1987). The combined use of bentonite silica and this polymer has produced an effective bioreactor to develop the MLF of wine. The immobilised cells have shown great efficacy in decreasing L-malic acid, the conversion rate and reduction of titratable acidity being about 60 per cent. These studies have been extended to several species of lactic acid bacteria, including *O. oeni* and *Lactobacillus* (Crapisi, *et al.*, 1987).

The decision of the immobilised matrix must be done as per the long-term protection of cell life and for beverage manufacture because of its acknowledgment as GRAS (Generally Recognized As Safe). Alginate is just an appropriate matrix in both the considerations, has the size to reduce the diffusion limitation for the media and the items, and to increase the biomass dissemination. However, it is reversible and the existence of chelating compounds in the milieu could prompt leakage and incomplete matrix dissolution of the packed biomass. Bioreactor technologies comprising the high density of MLF microorganisms immobilised in alginate supports or carrageenan or packed between membranes was developed (Colagrande *et al.*, 1994). In MLF microscopic organisms react as biocatalyst and without development, quickly convert malic acid to lactic acid in wine that has gone through the bioreactor on a non-stop basis (Gao and Fleet, 1994).

4.2.3. Continuous Bioreactors for Winemaking

The continuous reactor is 'open'. There is a constant flow consisting of entry of the substrate on one hand and output of the product on the other. The main specificity of the continuous reactor is the opportunity to achieve dynamic equilibrium, that is to say that the system operates on the basis of the equilibrium state. Continuous reactors are widely used in chemical and food industries among others. Most operating reactors are multiphase, including fixed bed, fluidised bed, bubble column and to lift air (Verbelen *et al.*, 2006). Multiphase reactors are structured in three phases: gas (air or other), solid (support) and liquid (the medium). In terms of the production of wine, inert gas (CO₂ or N₂) may replace the air to avoid oxidation of the wine. The continuous fermentation technique appears as an option that would reduce manufacturing costs and increase the ethanol yield (Ribéreau-Gayon *et al.*, 2006). According to the literature, it is proposed to use higher levels of SO₂ in continuous fermenters to stop contamination. The advantages of continuous fermentation are higher and faster substrate conversion rate; increased homogeneity of the wine; lower losses; best environmental management practices; better control of fermentation; and consistency in the quality of finished wines (Clement *et al.*, 2011; Genisheva *et al.*, 2014; Ribéreau-Gayon *et al.*, 2006).

5. Optimising Winery Unit Operation

Wineries nowadays are confronted with the increasing expenses of trading. In the course of recent years, the cost of gas and electricity for manufacturing has expanded and this expanding pattern is probably going to proceed. These expanded utility costs put additional pressure on business. The outcome is an industry confronting more tightly net revenues and an increased significance on the selection of procedures and technologies that empower quality wine to be manufactured at lower cost. Optimisation should be therefore a business mentality concentrating on executing procedures and innovations to lessen costs, increase speed and improve asset utilisation. Numerous enterprises have effectively embraced optimisation as a foundation for staying focused in nearby and worldwide markets.

5.1. Computerisation

Computerisation can take different structures inside a winery. It can be as essential as computerising areas of a refrigeration framework, or as elaborate as a completely mechanized winery. Computerisation is useful as it permits the change of each of the procedure productivity measures, i.e. production, work, materials, water and energy. It is accomplished by advancing procedure gear, permitting round-the-clock operations, enhancing quality and decreasing human mistakes. The computerisation can optimise the process in the winery by the means shown in Table 10.

5.2. Cross Flow Filtration

Cross stream (flow) filtration has developed as a productive filtration method, with differing application possibilities for both the quick moving customer merchandise enterprises and the wine factory. Several wineries have actualised this innovation; however many others have not executed this innovation yet. Cross-stream filtration is customised and can include various applications inside a winery. It can be basically more energy proficient than conventional winery filtration while permitting fast-filtration

Table 10. Optimisation Aspects of Computerisation

<i>Process optimisation</i>	<i>Effect of chance on process effectiveness measure</i>
Production rate	Critical increments to fabrication speed and improvement of process apparatus
Work	Reductions difficult work enabling staff to be used all the more fittingly
Materials	Gives more noteworthy process control and accordingly decreases material waste
Energy	Gives large amounts of control and can fundamentally lessen energy— particularly in the systems of refrigeration for wineries
Water	Computerisation can give extra water productivity relying upon the particular use of the robotisation system

speeds. Cross-stream filtration can enhance material effectiveness by removing the requirement for added substances (e.g. filter aid) and lessening wine misfortune caused by development through numerous filtration exercises. The way cross-stream (flow) filtration could optimise the filtration efficiency as summarised in Table 11.

Table 11. Optimisation Aspects of Cross-flow Filtration

<i>Process optimisation</i>	<i>Effect of chance on process effectiveness measure</i>
Production rate	Augment filtration speeds by enabling numerous filtration operations to be embraced in one wine development. Can finished filtration in one stage rather than numerous means.
Materials	Can diminish wine development and decline wine waste. Can recuperate wine from dregs expanding production. Can diminish the utilisation of bentonite
Energy	Cross stream filtration is to a greater extent energy-saving per litre of wine obtained in contrasted to other filtration procedures
Water	Cross-stream filtration can be utilised to purify and recover process water

5.3. Frosty Adjustment Methods

A typical issue in the wine factory amid vintage is the absence of enough fermentation vessels. A quicker essential fermentation rate enables more tanks to be reutilised amid vintage. Moderate or blocked fermentation not just decreases the manufacture speed, it can expand material use by expecting added substances to ‘restart’ aging and require extra energy for the control of temperature. Expanding fermentation productivity includes both grape juice attributes, remedying for any basic differences (i.e. potassium accessibility, pH, etc.) and choosing yeast strains that are most appropriate to the juice characteristics. This guaranteed fermentation is attempted in a controlled and optimised manner, increasing the manufacture efficiency.

5.4. Continuous Processing

Continuous manufacture systems have more prominent efficiencies in contrast to batch procedure systems. The batch procedure system requires an extensive manufacture chain to stop until the the group bottleneck is prepared for the following cycle. In the wine factory, batch squeezing delays the destemming, receival and pulverising stages. This can prompt temperature changes and extended oxidation. Screw squeezing permits the receival procedure motion to progress from a batch procedure to a continuous procedure. This can reduce bottlenecks all through the receival chain. Presses using screws have gradually been supplanted by press systems utilising membranes because of their capacity to diminish extraction of phenolic substances. Innovation in a screw press, for example, utilising bigger screw blades and moderating upheavals, can lessen quite a bit of this phenolic compound while optimising the speed of production.

6. Conclusion and Future Thrust

Winemakers are confronting increasing rivalry due to the enlarging gap between wine manufacture and wine utilisation, the trend for customer inclination far from fundamental ware wine to top quality wine and financial globalisation. Thus, there is a requirement for a global transformation in the realm of wine. One of the requirements is the usage of HACCP method in the beverages factory that has been of a colossal help. Despite the fact that alcoholic drinks are relatively more secure than different foods and beverages due to their high ethanol content, recognition of potential dangers and recommencement of inhibitory and restorative activities (at whatever point needed) are of essential significance. Foundation of basic control restrains in co-occurrence with suitable and viable checking methods completed by capable staff have figured out how to limit the episodes of occurrences that are perilous and malicious for human well-being. The way towards changing the wine factory from a manufacture to an oriented market industry is an increased reliance on biotechnological advancements and HACCP method. A great part of the procedure proficiency technology utilised in agro-industry factories is promptly accessible for use in the wine factory. A few wineries have executed this innovation while yet others are yet to do as such. Specifically, cross-flow filtration and computerisation-procedure efficiencies are yet being executed. These deferrals in usage are not because of the accessibility of innovation, but rather are expected partially to capital accessibility, the absence of information on proficient practices, or vulnerability regarding what the advantageous prices are for expanding the process productivity. In spite of a substantial number of results on wine, the characteristic course of the fermentation of wine is not completely investigated and fermentation procedures are not really completely managed to require the comprehension of the biochemical conduct of yeast and other organisms in the wine milieu. The overall spread of wine manufacture has prompted novel vineyards delivering quality wine by receiving tried systems, like centrifugation, filtration, stainless steel tanks for fermentation, monitoring of temperature and chosen yeasts, and so forth. A portion of the biotechnological developments, like specific yeasts, change of starters, treatment using enzymes, bioreactor planning and cell immobilisation are of key significance in wine production. The improvements in reactor innovation as for fruit wines other than grape are rare. Mastering ongoing technique efficiencies ought to be viewed as key to enhancing business productivities. Assuring these means can prompt noteworthy cost reserve funds for the business, especially with respect to assets, materials and manufacture rates. This will need an adjustment in worldview for some wineries, yet it is important to guarantee that they stay beneficial in the changing business condition.

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VI) AUTRES

VI.1) Participation aux jurys de soutenance de thèse de Doctorat PhD

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**POTENTIEL TECHNOLOGIQUE DES AMANDES DE MANGUES (*MANGIFERA INDICA L.*)
EN PANIFICATION POUR LA REDUCTION DU RASSISSEMENT DU PAIN**

THESE

Présentée en vue de l'obtention du diplôme de **DOCTORAT/Ph.D.** en Sciences de l'Ingénieur

Mention : Génie des Procédés

Spécialité : Génie Alimentaire et Bioprocédés

Par :

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TENIN DZUDIE (décédé)	Professeur	Université de Ngaoundéré	Directeur

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VI.2) Participation aux séminaires, colloques nationaux et internationaux



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The wort boiling techniques and energy requirements: A Review

Les techniques d'ébullition du moût et les besoins énergétiques : État des lieux

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ABSTRACT:

For several decades, the developers of equipment and technologies intended for wort boiling have declared for commercial and technical reasons any kind of performance of their systems in order to interest the breweries. For most of them, there was not enough information in literature to verify their claim. Thus, the aim of this paper was to conduct a review of some of these technologies and estimate the specific energy requirements during wort boiling. The comparison between theoretical calculation results obtained and some manufacturer data for their wort boiling equipment has revealed a slight difference. Thus, at the end of this work, and under equal conditions (without taking into account the recovery of energy), Ecostripper Meura system could be considered to be the most economical in terms of energy requirements and considering only usual boiling equipment. More generally, the theory of the abolition of wort boiling could be considered to be the most economical and with some adjustments should be taken into account by manufacturers and brewers.

Keywords: Wort, Boiling, Stripping, Energy requirements, Calculation, Equipment

RÉSUMÉ :

Depuis plusieurs décennies, les développeurs d'équipements et de technologies destinés à l'ébullition du moût ont déclaré pour des raisons commerciales et techniques tout type de performance de leurs systèmes afin d'intéresser les brasseries. Pour la plupart d'entre eux, il n'y avait pas suffisamment d'informations dans la littérature pour vérifier leur affirmation. Ainsi, le but de cet article était de mener une revue de certaines de ces technologies et d'estimer les besoins énergétiques spécifiques pendant l'ébullition du moût. La comparaison entre les résultats de calculs théoriques obtenus et certaines données des fabricants pour leur équipement d'ébullition du moût a révélé une légère différence. Ainsi, à l'issue de ces travaux, et à conditions égales (sans tenir compte de la récupération d'énergie), le système Ecostripper Meura pourrait être considéré comme le plus économique en termes de besoins énergétiques et en ne considérant que les équipements d'ébullition usuels. Plus généralement, la théorie de l'abolition de l'ébullition du moût pourrait être considérée comme la plus économique et avec quelques ajustements devrait être prise en compte par les fabricants et les brasseurs.

Mots clés : Moût, Ébullition, Volatilisation, Besoins énergétiques, Calcul, Équipement



COLLOQUE / CONFERENCE



PROGRAMME

Mardi 10/05/2022	
	- Arrivée des conférenciers à l'aéroport international de Douala (Transferts vers l'Hotel Tchero sis à Bonanjo)
	- Arrivée des participants nationaux
Mercredi 11/05/2022	
8:00 – 10:00	- Accueil des participants + Ouverture des inscriptions (Laboratoire GLT, IUT de Douala)
10:00 – 14:00	- Cérémonie d'ouverture du colloque Innovation Scientifique et Technologique (Amphi 200 Pr EBANJA)
10:00 – 10:15	o Mise en place des participants
10:15 – 10:45	o Arrivée des autorités (Invités spéciaux, Chefs d'établissements, Recteur de l'Université de Douala et sa suite)
10:45 – 10:55	o Exécution de l'hymne national (Chorale de l'IUT)
10:55 – 11:05	o Mot de bienvenue de Monsieur le Directeur de l'IUT de Douala
11:05 – 11:15	o Intermède musicale (Chorale de l'IUT)
11:15 – 11:30	o Discours d'ouverture du Colloque par Monsieur le Recteur/Représentant
11:30 – 12:00	o Plénière 1 Intervenant : HAMOUTI Najib Thème : Enjeux de la pédagogie active pour les pays en voie de développement. Modérateur : Pr Djanna Koffi Francis Lénine Rapporteurs : Njocke Martin, Magne Nestorine, Tchoudja Jimmy, Assimizele Brice
12:00 – 12:30	o Plénière 2 Intervenant : Abate André Modeste Thème : Mutualisation des processus d'achat et d'utilisation des équipements de laboratoires et d'ateliers dans les établissements de l'enseignement supérieur au Cameroun Modérateur : Pr Djanna Koffi Francis Lénine Rapporteurs : Njocke Martin, Magne Nestorine, Tchoudja Jimmy, Assimizele Brice
12:30 – 12:45	o Photo de famille
12:45 – 13:00	o Interview presse
13:00 – 14:30	o Pause Déjeuner
Travaux en ateliers	
	Atelier : Génie Civil et Géosciences Salle : Salle des Actes IUT
	Rapporteurs : Kouske Arnaud, Akoumbon Vishiti, Njiteu Cyrille, Tome Sylvain, Kibong Marius
Mercredi 11/05/2022	
	Session GC 1 Modérateur : Pr Owona Sébastien
14:30 – 15:00	Conférence 1 Intervenant : Guillaume Marie Thème : Maitrise des risques côtiers pour un développement durable des zones côtières
15:00 – 15:30	Présentation orale N°GC-GS 12 Intervenant : Dzoujo T. Hermann Thème : Synthesis of poly (ferro-sialate-siloxo)-biochar eco-adsorbents derived from pozzolan and sugarcane bagasse for methylene blue sequestration in aqueous medium
15:30 – 16:00	Présentation orale N°GC-GS 84

	Session SS 7 Modérateur : Pr Tchoumboungang François
10:30 – 11:00	Présentation orale <u>N°S-S 167</u> Intervenant : Man-Ikri Bertin / Pr. Desobgo Zangué Steve Carly Thème : Study of the impact of <i>Grewia mollis</i> on the clarification of Mbayeri sorghum cultivar worts
11:00 – 11:30	Présentation orale <u>N°S-S 13</u> Intervenant : Koloko Brice Landry Thème : <i>Fumaria indica</i> extracts alleviate carbon tetrachloride-induced hepatotoxicity by modulating TGF- β signaling pathway: <i>In vivo</i> and <i>in vitro</i> study
11:30 – 12:00	Présentation orale <u>N°S-S 168</u> Intervenant : Sinkam Nana Sofiane Emeline Thème : Development of a new cocoa-based product: Dark chocolate enriched with maize grains (<i>Zea mays</i>)
12:00 – 13:00	Pause Déjeuner
	Session SS 8 Modérateur : Pr Ndom Jean Claude
13:00 – 13:30	Présentation orale <u>N°S-S 19</u> Intervenant : Tchabong Samuel Raymond Thème : Chemical composition and antifungal potential of the essential oils of <i>Ocimum gratissimum</i> L, <i>Ocimum suave</i> L, <i>Aframomum albulaceum</i> Ridley and <i>Zingiber officinale</i> Roscoe against two molds associated with the alteration of smoked fish <i>ethmalosafimbriatabowdich</i>
13:30 – 14:00	Présentation orale <u>N°S-S 170</u> Intervenant : Nkoubag Nkuimi Dorcas Chanelle Thème : Formulation d'une pâte a tartiner epicee a base de safou (<i>Dacryodes edulis</i>)
14:00 – 14:30	Présentation orale <u>N°S-S 173</u> Intervenant : Mbede junior Thème : Développement d'un procédé de conservation des larves de hannetons (<i>Rynchophorus phoenicis</i>) : influence sur leur valeur nutritionnelle et leur conservabilité.
14:30 – 15:00	Pause café
	Session SS 9 Modérateur : Pr Wansi Jean Duplex
15:00 – 15:30	Présentation orale <u>N°S-S 174</u> Intervenant : Essomba Nkouamo stephane Thème : Formulation et caractérisation d'une margarine à base d'huile des larves de hannetons (<i>Rynchophorus phoenicis</i>)
15:30 – 16:00	Présentation orale <u>N°S-S 78</u> Intervenant : Stève Olugu Voundi Thème : Formulation des bioplastiques à base d'épices du Cameroun aux propriétés anti-oxydantes et inhibitrices des bactéries sporogènes du genre <i>Bacillus</i>
16:00 – 16:30	Présentation orale <u>N°S-S 185</u> Intervenant : Ambassa LM, Thème : Saponines au Service de la Sécurité Alimentaire (3SA)
16:30 – 17:00	Restitution des présentations par le secrétariat
	Atelier : Industrie et Environnement Salle : Salle de lecture bibliothèque
	Rapporteurs : Mouzong Marcelin, MOUNGOUTOU Inoussah, Ndam Max, Mandeng Jean-Jacques, Nkapkop Jean De Dieu, Onguene Raphael
Mercredi 11/05/2022	
	Session IE 1 Modérateur : Pr Edoun Marcel
14:30 – 15:00	Conférence 1 Intervenant : Jean François Bissonnette Thème : Gouvernance des mangroves et développement durable de la ville de Douala
15:00 – 15:30	Présentation orale <u>N°IE34</u> Intervenant : Tiadjoue Benjamin

VI.3) Membre de Sociétés savantes

April 27, 2015

Food Processing and Quality Control University Institute of Technology (University of Ngaoundere), Cameroon.

Dear Dr. Desobgo Zangue Steve Carly,

Appointment as an Editorial Board Member

I am pleased to inform you that your application for membership of the editorial board of the **African Journal of Biotechnology (AJB)** has been evaluated and accepted.

Your responsibilities will be:

- To act as a reviewer for submitted manuscript
- To evaluate and determine if reviewed and revised manuscript(s) are to be accepted, modified or rejected.
- To communicate, issues affecting the growth and development of the journal directly with her editorial office and to resolve any conflict(s) that may arise after the publication of a given manuscript as well as evaluating the relative importance of the issues raised by reviewers and giving recommendations to authors.

Please find attached:

1. COPE - Code of Conduct and Best Practice Guidelines for Journal Editors
2. COPE - Code of Conduct for Journal Publishers
3. Memorandum of Understanding for Editors and Editorial Board Members

Kindly read the attached documents and return a signed copy of the Memorandum of Understanding for Editors and Editorial Board Members to us.

Also provide us with your ORCID ID and Scopus ID (if available).

Your appointment takes effect from April 27, 2015. Kindly note that yours is a voluntary position and there will be no financial remuneration.

As soon as we receive your response, we will update the journal's website with your details and start forwarding manuscripts to you for evaluation.

Thank you once again for your interest in serving on the board of our journal.

You can login to our portal as an editorial board member ms.academicjournals.org

User name: desobgo@yahoo.fr

Password: deso2015

Accept my congratulations.

Yours faithfully,



Clement Onou
Academic Journals

ASBC Membership

Boîte de réception x



Cheryl Kruchten <CKruchten@scisoc.org>

27/06/
2017

À moi

anglais
français

[Traduire le message](#)

[Désactiver pour : anglais](#)

Dear Dr. Desobgo Zangue,

Thank you for joining the ASBC! Your membership application has been processed. We are pleased that you have made the decision to become a part of a member-driven organization focused on scientific and technical research in the brewing and related industries.

The ASBC Website is where you can access and update your member record, access your subscription to the *Journal of the ASBC*, register for an event, purchase a book or online product, or search for a colleague or contact in the membership directory. The ASBC Website is designed to interface directly with our database. By signing in, the system will know who you are and will automatically provide corresponding member pricing and options. All transactions are completely safe, secure, and instantaneous.

Please use the link below to access the ASBC website.

www.asbcnet.org

CERTIFICATE

OF EDITORIAL BOARD MEMBERSHIP

DESOBGO ZANGUE Steve Carly

has been appointed as one of the

Editorial Board Members

In

SCIREA Journal of Food

<http://www.scirea.org/journal/Food>

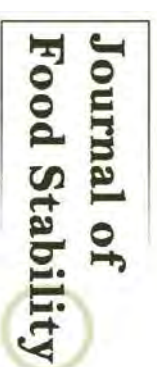
Science Research Association

Date: March 2019

Arvind Chhabra

Editorial Board Membership Certificate

THIS IS TO CERTIFY THAT



Dr. Desobgo Zangué Steve Carly

IS A HONORABLE ASSOCIATE EDITOR OF THE
JOURNAL OF FOOD STABILITY

FTD RESOURCES PUBLISHER

JOURNAL OF FOOD STABILITY

www.foodstability.com/editorial.php

VI.4) Reviewer pour les journaux



Certificate No: BPI/PR/Cert/12050.2F/PRO

B P International



Certificate of Excellence in Peer-Reviewing

awarded to

Prof Desobgo Zangue Steve Carly

University Institute of Technology of the University of Ngaoundere, Cameroon

in recognition of an outstanding contribution to the quality of the book.

Date: 8-Mar-2024

(Dr. M. Basumondal)
Chief Managing Editor
B P International

B P International
Offices:
India: Guest House Road, Street no - 1/6, Hooghly, West Bengal, India (Reg. Address),
Diamond Heritage Building, 16, Strand Road, Kolkata, 700001 West Bengal, India (Corporate Address),
Tele: +91 743901 6438 | +91 9748770553, Email: director@bopki.org (Headquarters)
UK: 27 Old Gloucester Street London WC1N 3AX, UK, Fax: +44 20-3031-1429,
Email: director@bopki.org (Branch office)
Website: www.bopki.org

em Journal of the American Society of Brewing Chemists Desobgo Zengué Steve Carly Logout Home Main Menu Submit a Manuscript About Help									
View Reviewer Comments View Attachments Send Email	2	JASBC-2021-0047	Research Article	Evaluation of commercial strains of <i>Torulaspora delbrueckii</i> in beer production	Accept	Jul 03, 2021	Jul 04, 2021	Jul 25, 2021	Jul 2, 2021
View Reviewer Comments View Attachments Send Email	2	JASBC-2019-0067R1	Research Article	Statistically significant differences between aroma profiles of beer brewed from sorghum and malt	Withdrawn	Oct 17, 2020	Oct 17, 2020	Nov 07, 2020	Oct 3, 2020

Gmail

review thank yo

Thank you for reviewing for Heliyon

Boîte de réception x Notifications x

J

Heliyon <em@editorialmanager.com>

20 déc. 2023 08:50

À moi

Traduire en français

x

Manuscript Number: HELIYON-D-23-48684

Flavor assessment of a lacto-fermented vinegar described in Japanese books from the Edo period (1603–1867)

Dear Pr. Desobgo,

Thank you for reviewing the above referenced manuscript for Heliyon, an open access journal that is part of the Cell Press family. I greatly appreciate your contribution and time, which not only assisted me in reaching my decision, but also enables the author(s) to disseminate their work at the highest possible quality. Without the dedication of reviewers like you, it would be impossible to manage an efficient peer review process and maintain the high standards necessary for a successful journal.

I hope that you will consider Heliyon as a potential journal for your own submissions in the future.

Kind regards,

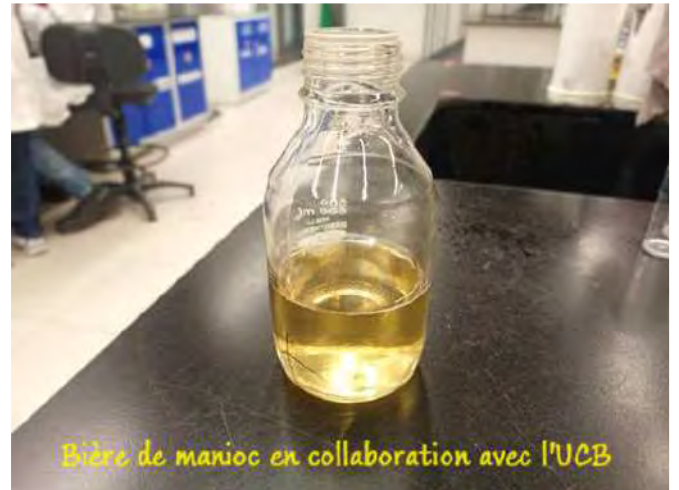
Wing-Fu Lai
Associate Editor - Food and Nutrition
Heliyon

VI.5) Expertises diverses

Quelques exemples de produits élaborés avec les étudiants dans le cadre des stages académiques de fins d'études (IUT/ENSAI)



Quelques exemples de réalisations des boissons avec les entrepreneurs du Made In Cameroon



Bière de manioc en collaboration avec l'UCB



Bière de malt d'orge amérisée au
Khaya senegalensis



N° **23 - 0000599** /APME/DG/GU/II



Yaoundé, le **12 8 SEPT 2023**

Le Directeur Général

À

Professeur Steve Carly DESOBGO

IUT - Douala

Tel : 697160004/ 672790222/ 222251660

Email : Desobgo.zangue@gmail.com;
desobgo@yahoo.fr ; stevedesobgo@gmail.com

Objet : Sollicitation d'une prestation intellectuelle

Professeur,

Dans le cadre du Programme de Valorisation et de Transformation des Produits Agricoles et Agro-alimentaires (TRANSFAGRI) mis en œuvre avec le financement de l'Agence Française de Développement (AFD) via le troisième Contrat Désendettement-Développement, l'Agence de Promotion des Petites et Moyennes Entreprises (APME) organise les 11 et 12 octobre 2023, à la salle des fêtes d'Akwa- Douala, la quatrième édition des journées de réseautage des PME/OP.

Cet événement qui a pour but de favoriser la construction d'une plateforme d'échanges entre acteurs de différents bassins agroalimentaires du Cameroun, aura pour thème principal : **« L'impact de la qualité des produits agroalimentaires sur la performance des PME/OP et leur contribution à l'import substitution et à la sécurité alimentaire au Cameroun ».**

Afin d'introduire efficacement les travaux des participants et stimuler leurs réflexions qui porteront sur la qualité des produits agroalimentaire et son impact sur la performance des PME/OP, la sécurité alimentaire et l'import substitution.

J'ai l'honneur de solliciter votre expertise pour la délivrance de la leçon inaugurale de l'événement suivant les termes de référence ci-joints.

Veuillez agréer, **Professeur**, l'expression de ma considération distinguée. /-

Pièce jointe : TdR de la présentation souhaitée



REPUBLIQUE DU CAMEROUN
PAIX – TRAVAIL – PATRIE

AGENCE DE PROMOTION
DES PETITES ET MOYENNES ENTREPRISES

DIRECTION GENERALE

COMITE JNR4

COMITE SCIENTIFIQUE



REPUBLIC OF CAMEROON
PEACE – WORK – FATHERLAND

SMALL AND MEDIUM SIZED ENTERPRISES
PROMOTION AGENCY

GENERAL DIRECTORATE

JNR4 COMITEE

**QUATRIEME EDITION DES JOURNEES NATIONALES DE RESEAUTAGE
(JNR) DES ATEURS DE LA CHAINE DE VALEURS AGROALIMENTAIRES**

AGENDA DES TRAVAUX

HORAIRES	ACTIVITES	INTERVENANTS
PREMIERE JOURNEE		
7h30-8h30	Enregistrement des participants	Secrétariat
8h30	Début des travaux	Modérateur des travaux
8h35-10h05	Présentation des bassins ✓ Evaluation des JNR3 (par les chefs d'antennes) (30mn) ✓ 'succès story' d'une PME/SAE/réseau en rapport avec la thématique (1h)	Chefs d'antennes PMEA/SAE Choisi dans les bassins
10h05-10h30	Echanges et recommandations	Participants et secrétariat
10h 30	Début de la cérémonie protocolaire	Impresario
10h30-10h35	Mot de bienvenue du Maire de la ville de Douala	Maire de la ville
10h35-10h45	Discours d'introduction du DG APME	DG APME
10h45-11h	Leçon Inaugurale	Pr Professeur Steve Carly DESOBGO, IUT – Douala
11h-11h10	Discours d'ouverture	Gouverneur de la Région du Littoral
11h10-11h30	Photo de famille, visite des stands et interviews	Gouverneur, DG et sa suite
12h-12H45	PAUSE DEJEUNER ET FIN DE LA PHASE PROTOCOLAIRE	APME
12H45	REPRISE DES TRAVAUX	
12H45-13H	Présentation de l'agenda des travaux	Modérateur des travaux
13H-13H30	Exposés d'introduction aux trois sous-thématiques par les Experts : 1. La qualité des produits agroalimentaires comme levier de l'import substitution par Monsieur ETOUNDI Jean Martin ; 2. La qualité des produits agroalimentaires comme facteur de la sécurité alimentaire par Madame GOUETH Anne Stéphane ;	

	3. La qualité des produits agroalimentaires : source de performance des PME par Monsieur Robert NDOUMBE MOUKOKO.	
13h30-14h 25	Contributions complémentaires par les institutions clés (ANOR ; Lanacome ; CTA ; Centre pasteur ; CNCC ; IUT/Ecole Polytechnique de Douala ; SGS ; grand Mall ; Santa lucia ; Made in Cameroon)	Les différents intervenants (en raison de 5 mn par intervenant)
14h25-14h35	Présentation de quelques recommandations à l'issue de ces contributions	Secrétariat de l'atelier
14h35-15h	Constitution des groupes de travail en atelier, des échanges B to B et présentation des thématiques à échanger	Modérateur des Travaux
15h-17h	✓ travaux en atelier sur les thématiques définies ✓ Réseautage (B to B)	participants
17 heures	Mot de fin de la journée et rappel des objectifs attendus de la deuxième journée	Modérateur des travaux
17h05	Pause-café et fin de la journée	APME
DEUXIEME JOURNEE		
7H30-8h30	pause-café	APME
8h30-12h00	-B to B -suite des travaux en commission	Participants
12h30-14h00	restitution des travaux en commission	Participants
14h00-14h30	Synthèse des recommandations	Secrétariat de l'atelier
14h 30	Cérémonie de clôture	Impréssario
14h30-15h30	Mot des participants de l'atelier	participants
15h30-16h	Présentation des recommandations de l'atelier	Secrétariat de l'atelier
16h-16h20	discours de clôture du DG	DG APME
16h20	Pause Déjeuner et fin des JNR4	APME



N° REF 0007031
ANOR/SG/D/CPA

Yaoundé le, 12 SEP 2023

LE DIRECTEUR GÉNÉRAL

A

MONSIEUR DESOBGO ZANGUE STEVE Casley
IUT/INSAI
697 16 00 04
-DOUALA-

Objet : *Invitation à prendre part au café de la normalisation*

Dans la perspective de renforcer l'inscription des PME locales dans une démarche qualité, gage d'amélioration de leur processus de production et de leur compétitivité,

J'ai l'honneur de vous inviter à bien vouloir prendre part, au café de la normalisation organisé pour sensibiliser les entrepreneurs locaux sur les questions de normes et de qualité.

L'objectif de cette session de sensibilisation est de rappeler, aux promoteurs des Petites et Moyennes Entreprises (PME) et des Très Petites Entreprises (TPE), l'importance de la prise en compte des normes d'hygiène et de fabrication, pour réussir leur processus de certification et ainsi renforcer la confiance de leurs clients et leurs partenaires.

Ce séminaire de sensibilisation se tiendra le **jeudi 19 octobre 2023** dès **9 heures** à l'**Hôtel la Falaise de Bonanjo (Douala)**.

Veuillez agréer, l'expression de ma parfaite considération./-





CAFÉ DE LA NORMALISATION

THÈME :

**ENJEUX DE LA NORMALISATION DANS LE SECTEUR DE LA PRODUCTION LOCALE
DE BOISSONS ET SPIRITUEUX**

JEUDI 19 OCTOBRE 2023

DOUALA

PROGRAMME

Heures	Activités	Responsables
9h00-10h00	Accueil des participants et enregistrement	Protocole
10h00-10h15	Mot de bienvenue de Monsieur le Directeur Général de l'Agence des Normes et de la Qualité (ANOR) ou son représentant	ANOR
10h15-10h30	Exposé N°1 : L'Agence des Normes et de la Qualité (ANOR), missions et activités	ANOR
10h30-11h00	Exposé N°2 : L'exigence d'application des normes, des bonnes pratiques d'hygiène et des bonnes pratiques de fabrication pour une entreprise en quête de qualité	ANOR
11h00-11h30	Exposé N°3 : L'importance de la certification pour garantir la qualité des produits issus de la transformation locale et faciliter leur accès au marché	ANOR
11h30-12h30	Échanges, discussions et partage d'expériences avec les responsables des PME/TPE	Modérateur
13h00-14h00	Fin des travaux et cocktail de clôture	Protocole



DECISION N° UD 25 UD0/VRRCRME/D-ENSPD/CEP du _____

Portant création, organisation et désignation des membres du Comité Scientifique du « Projet de Développement et de Modernisation des Procédés de Transformation, de Conservation et de Conditionnement Agroalimentaires » (PDEMOPTRACCA) à l'Ecole Nationale Supérieure Polytechnique de Douala.

LE RECTEUR DE L'UNIVERSITE DE DOUALA

- VU la Constitution ;
VU la loi n° 2007/006 du 26 décembre 2007 portant régime financier de l'Etat ;
VU la loi n° 2018/011 du 11 juillet 2018, portant code de transparence et de bonne gouvernance dans la gestion des finances publiques au Cameroun ;
VU la loi n° 2018/012 du 11 juillet 2018 portant régime financier de l'Etat et des autres entités publiques ;
VU la loi n° 2022/020 du 27 décembre 2022, portant loi des Finances de la République du Cameroun pour l'exercice 2023 ;
décembre 2020 portant loi de finances de la République du Cameroun ;
VU Le décret 77/41 du 03 février 1977 fixant les attributions et l'organisation des contrôles financiers
VU Le décret n° 93/026 du 19 Janvier 1993, portant création des Universités ;
VU Le décret n° 93/027 du 19 janvier 1993, portant dispositions communes aux Universités, modifié et complété par le décret n° 2005/342 du 10 septembre 2005 ;
VU Le décret n° 93/030 du 19 Janvier 1993, portant organisation administrative et académique de l'Université de Douala ;
VU Le décret n° 98/231 du 18 septembre 1998 portant organisation du Ministère de l'Enseignement Supérieur ;
VU Le décret n° 2005/383 du 17 octobre 2005 fixant les règles financières applicables aux universités ;
VU Le décret 2013/159 du 15 mai 2013 fixant régime particulier du Contrôle administratif des finances publiques ;
VU Le décret n° 2019/374 du 11 juillet 2019, portant nomination du Recteur de l'Université de Douala ;
VU Le décret n° 2020/231 du 22 avril 2020, portant nomination des responsables dans certaines universités d'Etat ;
VU Le décret n° 2020/232 du 22 avril 2020, portant nomination des responsables dans certaines universités d'Etat ;
VU Le décret 2020/272 du 11 Mai 2020 portant transformation de la Faculté de Génie Industriel en Ecole Nationale Supérieure Polytechnique de Douala ;
VU Le décret 2020/273 du 11 Mai 2020 portant organisation de l'Ecole Nationale Supérieure Polytechnique de Douala ;
VU Le décret n° 2020/303 du 08 juin 2020, portant nomination des responsables dans certaines universités d'Etat ;
VU Le décret n° 2021/300 du 26 mai 2021, portant nomination des responsables dans certaines universités d'Etat ;
VU Le décret n° 2021/301 du 26 mai 2021, portant nomination des responsables dans certaines universités d'Etat ;
VU L'arrêté n° 0381/CAB/PR du 29 août 2000, portant création des contrôles financiers auprès des Ministères établissements publics administratifs ;
VU L'arrêté n° 06/0130 MINESUP/DDES du 18 octobre 2006 portant création de la Faculté de Génie Industriel de Douala ;
VU L'arrêté n° 007/MINFI du 19 sept 2018 portant nomination de responsables au Ministère des Finances ;
VU L'arrêté n° 17-0079/MINESUP du 22 avril 2020, portant nomination des responsables dans certaines Universités d'Etat ;
VU L'arrêté n° 00000208/MINFI du 11 juin 2020, portant nomination des responsables dans les services déconcentrés du Ministère des Finances ;
VU L'arrêté n° 21-00005/Minesup du 05 juillet 2021, portant nomination des responsables dans les Universités d'Etat ;
VU L'arrêté n° 202255/MINESUP du 09 novembre 2022, portant nomination des responsables dans les Universités d'Etat ;
VU La décision n° 23-0015/R/UD0/SG du 17 janvier 2023, portant nomination des Chef de Bureaux à l'Ecole Nationale Supérieure Polytechnique de Douala ;
VU La décision n° 23-00113/UD/D-ENSPD du 07 mars 2023, Habilitant Monsieur le Directeur de l'Ecole Nationale Supérieure Polytechnique de Douala à signer le **Protocole d'Accord de Subvention** avec L'Institut de la Francophonie pour le Développement Durable (IFDD) ;
VU le **Protocole d'Accord de Subvention N° BURADM-IFDD/CMP/LNS/kbe/992/2023** signé entre l'Ecole Nationale Supérieure Polytechnique de Douala et l'Institut de la Francophonie pour le Développement Durable (IFDD) pour la mise en œuvre du « *Projet de développement et de modernisation des procédés de transformation de conservation et de conditionnement agroalimentaires par des solutions durables endogènes et innovantes* » ;
VU La circulaire n° 00000006/C/MINFI du 30 décembre 2022, portant instructions relatives à l'exécution des Lois de Finances, au suivi et au Contrôle de l'Exécution du Budget de l'Etat et des Autres Entités Publiques pour l'exercice 2023 ;
VU Les prévisions budgétaires de l'exercice 2023 ;
Considérant les nécessités de services.



DECIDE :

Article 1 : Il est créé à l'Université de Douala un Comité Scientifique chargé de l'évaluation des candidatures soumises, et de l'accompagnement à la maturation des projets qui seront sélectionnés ; dans le cadre du projet PDTIE-PDEMOPTRACCA à l'Ecole Nationale Supérieure Polytechnique de Douala.

Article 2 : (1) Pendant la phase d'évaluation des candidatures le comité sera chargé de :

- Évaluer la faisabilité scientifique et technique de chaque projet ;
- Déterminer la durée nécessaire à sa maturation ;
- Déterminer le montant nécessaire à la maturation de chaque projet ;
- Evaluer le potentiel marché
- Évaluer l'impact socioéconomique de chaque projet.

(2) Pendant le processus de maturation le comité sera chargé de :

- Optimiser la faisabilité scientifique et technique de chaque projet par un accompagnement approprié ;
- Définir les meilleures options pour améliorer le potentiel marché de chaque projet ;
- Rechercher les meilleures hypothèses devant optimiser l'impact socioéconomique de chaque projet.

Article 3 : Le Comité Scientifique est composé de :

- **Superviseur :** Pr. Ruben MOUANGUE
- **Coordonnateur :** M. Patrick Serges NKENE ZOGO II
- **Président :** Pr. KEDE Charles, chimiste ; Université de Douala
- **Vice-Président :** Dr. FOGAN Bienvenu, biotechnologie alimentaire ; Université de N'Gaoundéré
- **Rapporteurs :**
 - N°1 Dr. KOUTEU Alain, génie de procédés, **Université de Douala**
 - N°2 Dr MBOG MBOG Séverin, biotechnologie environnement, **Université de Douala**
- **Membres :**
 - Pr EDIMA Helene Carole, qualité ; **Université d'Ebolowa** ;
 - Pr Henriette ZANGUE ADJIA, chimie alimentaire ; **Université de N'Gaoundéré** ;
 - Pr. KANA SOP Marie Modestine, Nutrition, ; **Université de Douala** ;
 - Pr. KORO KORO Francioli, microbiologiste, **Université de Douala** ;
 - Pr MINYAKA Emile, Biotechnologie ; **université de Douala** ;
 - Pr. NDOMOU Mathieu, Toxicologiste, **Université de Douala** ;
 - Pr GOUDOUM Augustin, sciences et technologies post récolte, **Université de Maroua** ;
 - **Pr DESOBGO ZANGUE Steve Carly**, Génie des Procédés Alimentaires/ Génie Alimentaire et Qualité **Université de N'Gaoundéré**
 - Pr ONOMO Cyrille, finance, **Université de Douala**
 - Dr. Fabrice ABUNDE NEBA, Biochimiste, Professionnel ;
 - Dr BEDZEME Thierry, économie agricole, **Université de Yaoundé II**
 - Dr MASSODA Dieudonné, Marketing des marchés **Université de Douala**
 - Dr NDZANA ELOUNDOU Martin Jaurès, économie de l'innovation, **Université de Douala**
 - M. FOKENG NGUETEMY Fabrice, Chimiste, Professionnel ;
 - M. BELLA ODEN Martial, Chimiste, Professionnel.

Secrétariat technique :

- Mme. Diane Rosine NOUMBISSIE
- M. KETCHAKOU Jules César

Article 4 : (1) le comité rend sa copie au plus tard le **mardi 30 mai 2023** pour ce qui est de la phase d'évaluation des candidatures.

(2) un cahier de charges fixera les conditions et définira le processus de maturation des projets qui seront sélectionnés.

Article 5 : Les fonctions de président, de vice-président, de rapporteur, de membre statutaire, de membres du secrétariat technique du Comité Scientifique sont gratuites. Toutefois, les indemnités de sessions prélevées sur le budget de l'ENSPD pourront être allouées conformément à la réglementation en vigueur.

Article 6 : La présente décision sera enregistrée et publiée partout où besoin sera.

DOUALA, le _____

AMPLIATIONS :

- SG
- DAAF
- C.F
- A.C
- D/ENSPD

POUR LE RECTEUR
et par délégation
Le Directeur

LE RECTEUR,

[Signature]

[Signature]

Pr. Ruben M. Mouangue

APPEL A CONTRIBUTIONS SCIENTIFIQUES

INTRODUCTION

L'économie des trois régions septentrionales (Adamaoua, Nord et Extrême-Nord) repose essentiellement sur l'agriculture et l'élevage pratiqués surtout en zone rurale. Une activité qui permet de subvenir aux besoins de près de 80% de la population de ces localités. À cette activité agricole de première génération, il faut ajouter l'industrie agro-alimentaire bien présente et qui se développe au fil des années grâce aux initiatives des investisseurs privés de premier rang et des pouvoirs publics. Ces trois régions apportent une contribution forte au développement et à la production agro-pastorale au Cameroun. Un potentiel qui constitue également l'une des principales ressources de développement des Collectivités Territoriales Décentralisées du Grand-Nord.

PROBLEME

Ces dernières années, malgré les innombrables efforts des pouvoirs publics pour développer le secteur agro-pastoral et préserver la souveraineté alimentaire, les crises sécuritaires notamment dans l'Extrême-Nord, les crises sanitaires et les effets néfastes du climat et des intempéries ont sérieusement impacté le potentiel de production agro-pastoral et le développement dans ce secteur, mettant en péril la souveraineté alimentaire dans le Septentrion et l'ensemble du pays.

OBJECTIFS

Le Salon international des industries et techniques agro-pastorales du Septentrion qui se tiendra à Garoua du 10 au 16 juin 2024, a été conçu pour apporter une réponse concrète au processus de réflexion qui vise à trouver des solutions adéquates pour faire face à cette situation. Cette première édition qui se tient à Garoua sous le thème « Industrie agro-alimentaire et renforcement de la sécurité alimentaire dans le Septentrion », vise à permettre aux acteurs et opérateurs des différentes filières agro-pastorales de saisir toutes les opportunités d'affaires, de nouer des partenariats multiformes techniques et financiers, afin de valoriser leur savoir-faire, d'améliorer leur compétitivité sur les marchés de la sous-région et à l'international.

ATTENTES ET RENDUS

Les grands défis en matière de politique agricole et d'élevage au Cameroun ont désormais une boussole : la politique d'import substitution. Dans ce cadre, il est légitime de se poser quelques questions :

- Quelle sera la contribution d'un salon spécifiquement dédié aux industries et techniques agro-pastorales du Septentrion ?
- Quelles dynamiques sociétales, sont susceptibles d'impacter positivement l'agriculture et l'élevage au Nord Cameroun dans un contexte d'implémentation de la politique d'import substitution ?
- Quelle sera la place pour nos terroirs et peuplades dans cette grande dynamique ?

Pour arriver à démêler l'écheveau, nous proposons une série de conférences débats hybrides et interdisciplinaires le long du salon qui permettra à travers une analyse globale et intégrée de comprendre les interactions complexes susceptibles de booster la production agricole et l'économie des régions septentrionales du Cameroun.

Cette démarche basée sur la co-construction entre spécialistes des disciplines connexes à l'agronomie venant d'horizons divers et de tout milieu socio professionnel, cherchera à fédérer des idées autour de la thématique suivante : « Industrie agro-alimentaire et

renforcement de la sécurité alimentaire dans le Septentrion ». Ceci dans l'optique de relever les grands problèmes, les grands défis de développement et leur contribution à la politique d'import substitution prônée par le Chef de l'Etat.

Cinq grandes conférences-débats prévues au cours de cette édition du SIAGROS 2024 devront se situer dans l'une des thématiques suivantes :

- Attractivité des métiers de l'agriculture et des filières alimentaires pour garantir la sécurité alimentaire ;
- Promotion de l'entrepreneuriat dans le secteur agroindustriel et agro-pastoral : rôles et contributions de la CCIMA ;
- Les politiques gouvernementales de développement des chaînes de valeur à fort potentiel : cas du coton et l'anacarde ;
- Les stratégies gouvernementales pour l'amélioration de la compétitivité des produits locaux ;
- Le développement de l'Agro-industrie dans le Septentrion, enjeux et facteurs favorables.

COMITE SCIENTIFIQUE

Nom Et Prénoms	Qualité	Spécialité
Dr Abdoul Nasser Ousmanou	Commissaire Général	Economiste
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Dr Oumar Mahamat Oumar	Membre	Ecologie/Botanique
Tchuenga Seutchueng Thiery	Membre	Agro-Climatologie
Wang-Baa Christophe	Membre	Production animales
Maboune Tetmoun Suzanne Abeline	Membre	Agroalimentaire
Bama Andrée Victoire	Membre	Expert en norme et qualité

NB : Vos propositions de contributions structurées en trois parties dont un résumé, une introduction, le corps du texte et une conclusion (éventuellement une bibliographie) sont attendues par mail à l'adresse : siagros2024@gmail.com ou par Whatsapp 699861764/ Avant le 30 Avril 2024.