DOSSIER DE CANDIDATURE POUR LA PROMOTION

AU GRADE DE :

CHARGE DE COURS

Présenté par

DESOBGO ZANGUE Steve Cary

Assistant

Comité Consultatif des Institutions Universitaires (CCIU)

Commission VI : Sciences de l'Ingénieur

Section 2

Sous-section 2 : Biologie et Biochimie appliquées

1- IDENTIFICATION

DESOBGO ZANGUE Steve Carly ; Ph.D ; Assistant

UNIVERSITE DE NGAOUNDERE; INSTITUT UNIVERSITAIRE DE TECHNOLOGIE

Département de Génie Alimentaire et Contrôle Qualité

B. P. 455 – Ngaoundéré, CAMEROUN ; Tel : 77909391/95134285 ; E-mail : desobgo@yahoo.fr/stevecarly@hotmail.fr

A Monsieur le Ministre de l'Enseignement Supérieur

S/c

Monsieur le Secrétaire Permanent du CCIU

S/c

Monsieur le Recteur de l'Université de Ngaoundéré

S/c

Monsieur le Directeur de l'IUT

Objet : Candidature au grade de Chargé de Cours

Monsieur le Ministre,

J'ai l'honneur de soumettre, pour examen à la prochaine session du Comité Consultatif des Institutions Universitaires (CCIU), mon dossier de candidature au grade de Chargé de Cours des Universités du Cameroun.

Je suis Assistant au département Génie Alimentaire et Contrôle Qualité de l'IUT de l'Université de Ngaoundéré, depuis le 16 avril 2008 (voir Décision N° 2008/229/UN/R/VR-EPDTIC/SG/DAAC/DEPE/SSPE).

Vous remerciant par avance de l'intérêt que porteront vos services à l'examen de mon dossier, et me tenant à votre disposition pour toute information complémentaire, Je vous prie de croire, Monsieur le Ministre, à mes sentiments les plus respectueux.

P.J. : Sept exemplaires du dossier de candidature dont 1 original et 6 copies.

DESOBGO ZANGUE Steve Carly

COMITE CONSULTATIF DES INSTITUTIONS UNIVERSITAIRES
FICHE DE SYNTHESE DE CANDIDATURE

UNIVERSITE : *NGAOUNDERE*
ETABLISSEMENT : *INSTITUT UNIVERSITAIRE DE TECHNOLOGIE*
DEPARTEMENT : *GENIE ALIMENTAIRE ET CONTROLE QUALITE*
GRADE ACTUEL : *ASSISTANT* **depuis le 16/04/2008**

GRADE POSTULE : *CHARGE DE COURS*
SOUS-SECTION 2 : *BIOLOGIE ET BIOCHIMIE APPLIQUEES*
SECTION : 2
COMMISSION VI : *SCIENCES DE L'INGENIEUR*

Identification du candidat	Cursus et Titres Universitaires	Expérience Professionnelle/Publications	Avis motivé du département	Avis de la commission
Nom : DESOBGO ZANGUE **Prénom : Steve Carly** **Né le : 02 Janvier 1977** **A : Douala** **Matricule : 652117-M** **Pièces fournies** Sept exemplaires de dossier de changement de grade dont 1 original comprenant chacun : 1. Demande de changement de grade 2. Curriculum Vitae 3. Photocopie de l'acte de naissance 4. Photocopie des diplômes depuis le BACC 5. Actes Administratifs 6. Actes Académiques 7. Liste des productions scientifiques 8. Une liste d'encadrement des thèses, mémoires et rapports 9. Une liste des experts 10. Sept (07) exemplaires de publications avec comité de lecture Rapport pédagogique (07) Rapport administratif (07)	**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)	**2008 à ce jour :** Assistant / IUT de Ngaoundéré **Publications dans le grade d'assistant** 5 publications dans des journaux à comité de lecture Participation à la 13 ième chair J. DE CLERCK. 2008 Participation aux 1ères Journées Scientifiques (JS) du réseau Génie des Procédés Appliqués à l'Agro-alimentaire (GP3A). 2008 **Encadrement** • Vingt-sept (27) DUT soutenus • Six (6) Licences Professionnelles soutenues		
			Avis motivé de l'établissement	**Avis du Comité**
			Avis motivé de la Sous-section	**Avis du Conseil d'Université**
			Avis motivé de la Section	**Décision du Conseil d'Administration**

DESOBGO ZANGUE Steve Carly

(Assistant à l'IUT de l'Université de Ngaoundéré, CAMEROUN)

Curriculum Vitae

Doctorat/Ph.D en Génie des Procédés
DEA en Génie des Procédés
Ingénieur en Industries Agricoles et Alimentaires
DUT en Génie Agro-Industriel

Information personnelle

Adresse(s)	BP : 455 IUT Ngaoundéré, Cameroun
Téléphone(s)	Portable : 77 90 93 91 / 95 13 42 85
Courrier(s) électronique(s)	desobgo@yahoo.fr / stevecarly@hotmail.fr
Nationalité(s)	Camerounais
Date et lieu de naissance	02 Janvier 1977 à Douala
Sexe	Masculin
Statut Matrimonial	Marié et père de 2 enfants

Domaine de compétence

Génie des Procédés et Ingénierie

Expérience professionnelle

Dates	2008- A nos jours
Nom de l'employeur	Université de Ngaoundéré/Institut Universitaire de Technologie (IUT)
Type ou secteur d'activité	Enseignement supérieur
Fonction ou poste occupé	Enseignant-Chercheur
Activités et responsabilités	- Responsable Pédagogique - Enseignements, Travaux Dirigés, Travaux Pratiques, Recherches

Dates	2006-2007
Nom de l'employeur	Collège de Mazenod (Ngaoundéré)
Type ou secteur d'activité	Education (Enseignement secondaire)
Fonction ou poste occupé	Vacataire
Activités et responsabilités	- Enseignant de Mathématiques - Enseignant d'Informatique
Activités et responsabilités	- TP Génie et Procédés des Industries Alimentaires - TP Biodynamique

Dates	2004-2005
Nom de l'employeur	ENSAI de Ngaoundéré
Type ou secteur d'activité	Agro-alimentaire
Fonction ou poste occupé	Moniteur
Activités et responsabilités	- TP Génie et Procédés des Industries Alimentaires - TP Biodynamique

Dates	Mai – Septembre 2001
Nom de l'employeur	CAM Assistance
Type ou secteur d'activité	Agro-alimentaire (Fabrication des machines, mise au point des nouveaux produits)
Fonction ou poste occupé	Stagiaire (Stage Fin d'Etudes Ingénieur)
Activités et responsabilités	- Elaboration des procédés de fabrication des jus et vins d'ananas - Mise au point des jus et vins d'ananas - Elaboration d'un test de dégustation - Rétention du meilleur procédé pour le jus et le vin d'ananas

Dates	Juin – Août 2000
Nom de l'employeur	Cameroon Development Corporation (CDC)

Type ou secteur d'activité	Agro-alimentaire (Hévéa, Thé,…….)
Fonction ou poste occupé	Stagiaire (Stage Agent de Maîtrise 2)
Activités et responsabilités	- Investigations sur les problèmes de qualité du caoutchouc - Mise au point d'un équipement permettant l'évacuation des eaux issues du lavage du latex coagulé vers les égouts pour prétraitement
Dates	Juillet – Août 1999
Nom de l'employeur	Société des Provenderies du Cameroun
Type ou secteur d'activité	Agro-alimentaire (Provenderies)
Fonction ou poste occupé	Stagiaire (Stage Agent de Maîtrise 1)
Activités et responsabilités	- Contribution à la fabrication de la provende pour volaille, porcs, lapins,…. - Contribution à la gestion de stocks de provende et maintenance
Dates	Juin – Septembre 1998
Nom de l'employeur	Guinness Cameroon S.A.
Type ou secteur d'activité	Agro-alimentaire (Brasseries)
Fonction ou poste occupé	Stagiaire (Stage Fin d'Etudes Technicien Supérieur)
Activités et responsabilités	- Analyse des casses bouteilles au service Packaging - Investigation sur les points critiques des chocs thermiques et des casses de bouteilles sur la chaîne d'embouteillage plus défauts de fabrication. - Evaluation des casses de bouteilles et coûts
Dates	Juin – Juillet 1997
Nom de l'employeur	Guinness Cameroon S.A.
Type ou secteur d'activité	Agro-alimentaire (Brasseries)
Fonction ou poste occupé	Stagiaire (Stage Ouvrier)
Activités et responsabilités	- Apprentissage du fonctionnement de la brasserie, responsable stagiaire

Education et formation

Dates	2001 – 2003
Intitulé du diplôme délivré	Diplôme d'Etudes Approfondies en Génie des Procédés (D.E.A - GP)
Compétences couvertes	**Domaine général :** Génie des Procédés, Mathématique, Statistiques **Domaine spécifique :** Modélisation, Optimisation des procédés des Industries Agricoles et Alimentaires (Boissons)
Établissement	École Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Ngaoundéré, Cameroun
Dates	1998 – 2001
Intitulé du diplôme délivré	Diplôme d'Ingénieur
Compétences couvertes	**Domaine général** : Génie des Procédés et chimique, Ingénierie, Boissons
Etablissement	Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Ngaoundéré, Cameroun
Dates	1996 – 1998
Intitulé du diplôme délivré	Diplôme Universitaire de Technologie (DUT)
Compétences couvertes	**Domaine général** : Génie Agro-Industriel
Établissement	Institut Universitaire de Technologie (IUT), Ngaoundéré, Cameroun
Dates	1995 – 1996
Intitulé du diplôme délivré	Baccalauréat de l'Enseignement Secondaire (Série D)
Compétences couvertes	**Domaine général** : Mathématiques et Sciences de la nature
Etablissement	Institut Privé Polyvalent de Bonamoussadi (IPPB), Douala, Cameroun

Aptitudes et compétences personnelles

Langue(s) maternelle(s) **Français**

Auto évaluation

	Comprendre		**Parler**		**Écrire**
	Écouter	Lire	conversation	oralement	
Anglais	Bien	Bien	Assez Bien	Assez Bien	Bien

Aptitudes sociales

- Bon esprit d'équipe, adaptation facile aux environnements multiculturels
- Bonnes capacités de communication

Aptitudes organisationnelles

- Responsable Pédagogique de la mention Génie Biologique niveau 2 (2009-2011)
- Intérimaire régulier du chef de département Génie Alimentaire et Contrôle Qualité (GACQ) en cas de déplacement.
- Président de la mutuelle des Moniteurs et Doctorants de l'Université de Ngaoundéré (2005)
- Moniteur à l'ENSAI de Ngaoundéré 2004/2005 et 2005/2006
- Membre du Club Junior Entreprise section de Ngaoundéré (1998 – 2000)
- Secrétaire général de la Commission des Projets dans l'Association des Etudiants en Formation Doctorale de l'ENSAI de Ngaoundéré (2001-2004)
- Commissaire aux comptes du Club Génie Agro-industriel, IUT (1996-1997)

Compétences informatiques

- Bonne Maîtrise des logiciels : Word, Excel, PowerPoint, Publisher, SigmaPlot, Statistica, Mathcad, Matlab, Mathematica, Scientific Workplace, Statgraphics, SPSS, Systat, S Plus 2000, Derive 5, Adobe Photoshop, fortran, etc…

Compétences artistiques

- Pratique du dessin et de la poésie

Autres compétences

- Pratique du Football, tennis, tennis de table

Permis de conduire

- Permis B

Complément

- Distinction Spéciale décernée pour avoir validé toutes les Unités de Valeur en session normale par le Recteur Pr. Maurice TCHUENTE (Recteur de l'Université de Ngaoundéré) (1998)
- Major de la Promotion DEA Génie des Procédés (2003)

Thèses, Mémoires

- Optimisation de l'action des hydrolases sur quelques caractéristiques physico-chimiques des moûts de deux cultivars de sorgho (2012). Thèse de Doctorat Ph.D en Génie des Procédés. ENSAI de l'Université de Ngaoundéré.
- Etude de l'effet de quelques hydrolases sur la filtrabilité des moûts a base de sorgho (*Madjeru, Safrari, S.35*) non maltés (2003). Mémoire de DEA en Génie des Procédés. ENSAI de l'Université de Ngaoundéré.
- Mise au point du jus et du vin d'ananas pour une unité semi artisanale (2001). Mémoire de fin d'études Ingénieur. ENSAI de l'Université de Ngaoundéré.
- Investigation sur les problèmes de caoutchouc (2000). Rapport de stage Agent de Maîtrise II. ENSAI de l'Université de Ngaoundéré.
- Contribution à la fabrication de la provende pour volaille, porcs, lapins (1999). Rapport de stage Agent de Maîtrise I. ENSAI de l'Université de Ngaoundéré.

- Analyse des casses de bouteilles au service packaging chaîne n°2 (1998). Mémoire de fin d'études DUT. IUT de l'Université de Ngaoundéré.
- Rapport de stage ouvrier (1997). IUT de l'Université de Ngaoundéré.

Publications et colloques

- **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.
- **Desobgo, Z.S.C.,** Nso, E.J. & Tenin, D., 2011. Use of the response surface methodology for optimizing the action of mashing enzymes on wort reducing sugars of the *Madjeru* sorghum cultivar. *African Journal of Food Science*, 5**,** 91-99.
- **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.
- **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.
- **Desobgo, Z.S.C.,** Nso, E.J., Tenin, D. & Kayem, G.J., 2010. Modelling and optimizing of mashing enzymes-effect on yield of filtrate of unmalted sorghum by use of response surface methodology. *Journal of the Institute of Brewing*, 116**,** 62-69.
- The polyphenol paradox in alcoholic beverages (a beer and wine paradox ?). 13th Chair J. DE CLERCK. 7-10 september 2008. Louvain-la-Neuve, Belgium. www.declerckchair.com
- **Desobgo Z.S.C., 2008**. Modélisation et optimisation de l'effet des enzymes industrielles sur le rendement en filtrat des maïsches de sorgho. 1ères Journées Scientifiques (JS) du réseau Génie des Procédés Appliqués à l'Agro-alimentaire (GP3A). 11 et 12 septembre 2008, Louvain-la-Neuve, Belgique. http://www.gp3a.auf.org

DEPARTEMENT
DIVISION du Wouri

REPUBLIQUE UNIE DU CAMEROUN
Paix—Travail—Patrie

UNITED REPUBLIC OF CAMEROON
Peace—Work—Fatherland

ARRONDISSEMENT
SUBDIVISION de Douala I

CENTRE D'ETAT CIVIL
CIVIL STATUS REGISTRATION CENTRE

de - of Deido et Akwa Nord

Acte de Naissance N° 9/77
BIRTH CERTIFICATE

Nom de l'enfant Desobgo Zuangue Steve Carly
Name of the child

Le - On the Deux Janvier mil neuf cent soixante dix sept

Est né à Douala Cameroun
Was born at

Nom de l'enfant Desobgo Zuangue Steve Carly
Name of the child

De sexe - Sex masculin

De - Of Sobgo Gabriel

Né à - Born at Bafou Dschang

Le - On the 1er Août 1947

Domicilié à Douala
Resident at

Profession - Occupation Vendeur d'Automobiles

Et de - And of Guepi Teindjio Emilienne

Née à - Born at

Le - On the 13 Mai 1955

Domiciliée à Douala
Resident at

Profession - Occupation Ménagère

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

Sur la déclaration de Mlle 2° de Mme A.
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 Ekoula [illegible], Officier d'Etat Civil du Centre spécial de Deido
By Us,

Le Déclarant,
The declarant,

Signature de l'Officier de l'Etat-Civil
Signature of Registrar.

2- DIPLOMES

Ministère de l'Education 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 MATHEMATIQUES ET SCIENCES DE LA NATURE

est conféré à M. DESOBGO ZANGUE STEVE CARLY Matricule 509807

né(e) le 02/01/1977 à DOUALA Mention PASSABLE

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

Signature du titulaire

Fait à Yaoundé le 23 Novembre 2001

Pour expédition conforme
Le Directeur de l'Office du Baccalauréat du Cameroun

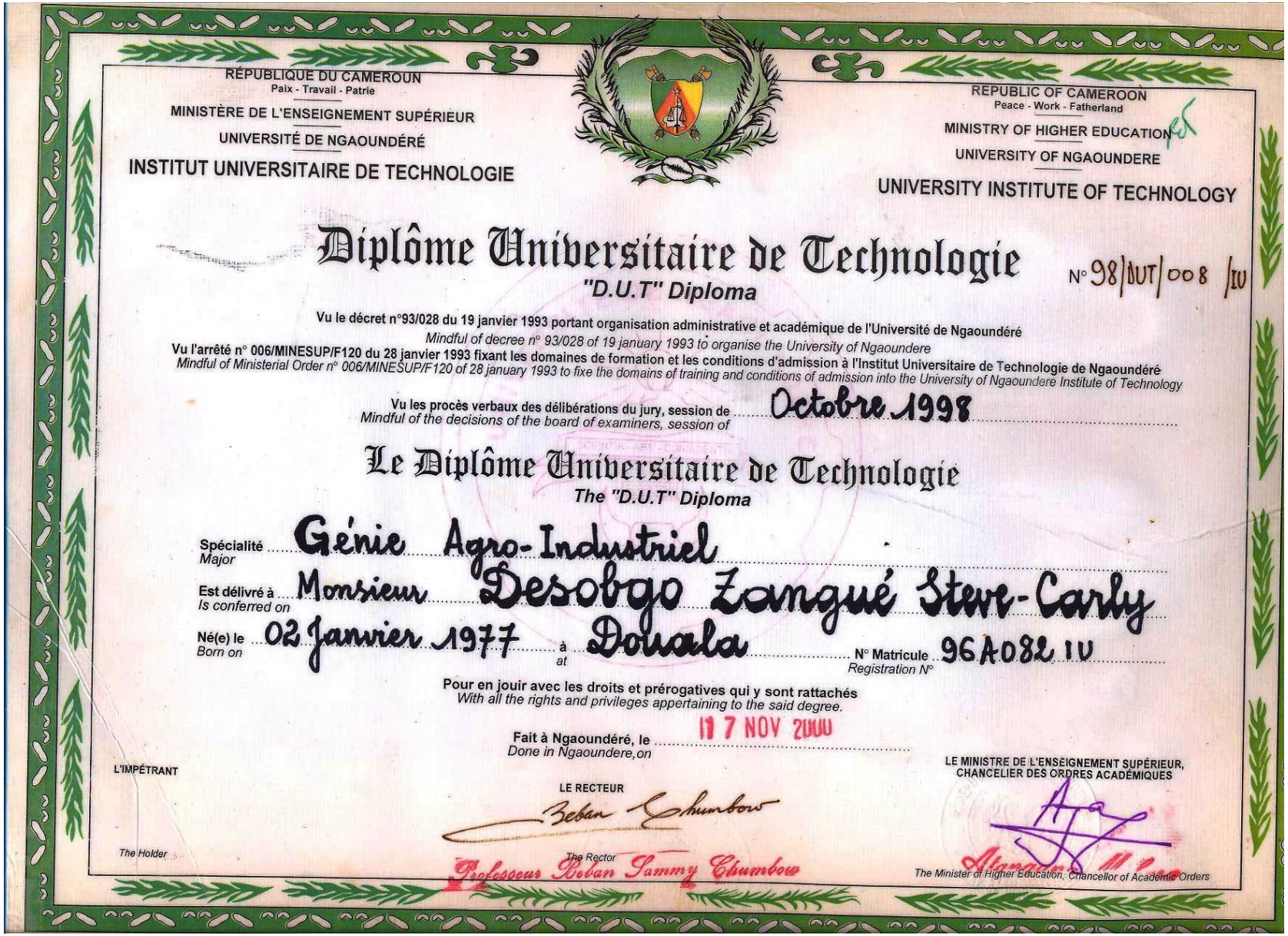
Le Ministre de l'Education Nationale

Le Ministre de l'Enseignement Supérieur
Chancelier des ordres académiques

Matsogo Zacharie

Joseph OWONA

Atangana Mebara



RÉPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR

UNIVERSITÉ DE NGAOUNDÉRÉ

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/DUT/008/IV

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 Ngaoundere

Vu l'arrêté n° 006/MINESUP/F120 du 28 janvier 1993 fixant les domaines de formation et les conditions d'admission à l'Institut Universitaire de Technologie de Ngaoundéré
Mindful of Ministerial Order n° 006/MINESUP/F120 of 28 january 1993 to fixe the domains of training and conditions of admission into the University of Ngaoundere Institute of Technology

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

Le Diplôme Universitaire de Technologie

The "D.U.T" Diploma

Spécialité / *Major* : Génie Agro-Industriel

Est délivré à / *Is conferred on* : Monsieur Desobgo Zangué Steve-Carly

Né(e) le / *Born on* : 02 Janvier 1977 à / *at* Douala N° Matricule / *Registration N°* : 96A082 IV

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

L'IMPÉTRANT
The Holder

LE RECTEUR
Beban Chumbow
The Rector
Professeur Beban Sammy Chumbow

LE MINISTRE DE L'ENSEIGNEMENT SUPÉRIEUR, CHANCELIER DES ORDRES ACADÉMIQUES
The Minister of Higher Education, Chancellor of Academic Orders

RÉPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR

UNIVERSITÉ DE NGAOUNDÉRÉ

ÉCOLE NATIONALE SUPÉRIEURE DES SCIENCES AGRO-INDUSTRIELLES

REPUBLIC OF CAMEROON
Peace - Work - Fatherland

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

NATIONAL ADVANCED SCHOOL OF AGRO-INDUSTRIAL SCIENCES

Diplôme d'Ingénieur
Master of Engineering Degree

Nº 01/ING/028/EN

Vu le décret nº 92/074 du 13 avril 1992 portant transformation des Centres Universitaires de Buea et de Ngaoundéré en Universités
Mindful of decree nº 92/074 of 13 April 1992 to transform Buea and Ngaoundere University Centres into Universities
Vu le décret nº 93/028 du 19 janvier 1993 portant organisation administrative et académique de l'Université de Ngaoundéré
Mindful of decree nº 93/028 of 19 January 1993 to organise the University of Ngaoundere
Vu le décret nº95/061 du 03 avril 1995 portant organisation de l'Ecole Nationale Supérieure des Sciences Agro-Industrielles de l'Université de Ngaoundéré
Mindful of decree nº 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 Octobre 2001
Mindful of the decision of the board of examiners, session of

SCIENTIA - ARS - CONSCIENTIA

Le Diplôme d'Ingénieur
The Master of Engineering Degree

Spécialité Industries Agricoles et Alimentaires
Speciality

Spécialisation
Specialization

Est délivré à Desobgo Zangue Stève-Carly
Is conferred on

Né(e) le 02-01-1977 **à** Douala-Cameroun **Nº Matricule** 98A005EN
Born on at Registration Nº

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 Yaoundé, le 19 MARS 2004
Done in Yaounde, on

L'IMPÉTRANT
Holder

LE RECTEUR
Pr. Amvam Zollo Paul Henri
Rector

LE MINISTRE DE L'ENSEIGNEMENT SUPÉRIEUR, CHANCELIER DES ORDRES ACADÉMIQUES
Maurice TCHUENTE
Minister of Higher Education, Chancellor of Academic Orders

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-iut.minesup.gov.cm

DIPLOME D'ETUDES APPROFONDIES EN GENIE DES PROCEDES

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,

Pr. Kamga Richard

Le Directeur,

3- ACTES ADMINISTRATIFS

REPUBLIQUE DU CAMEROUN
******
Paix – Travail - Patrie
****
UNIVERSITE DE NGAOUNDERE
****
BP: 454
****
RECTORAT
****
VICE -RECTORAT CHARGE DES ENSEIGNEMENTS, DE LA PROFESSIONNALISATION ET DU DEVELOPPEMENT DES TECHNOLOGIES DE L'INFORMATION ET DE LA COMMUNICATION
****
DIRECTION DES AFFAIRES ACADEMIQUES ET DE LA COOPERATION

REPUBLIC OF CAMEROON
******
Peace-Work-Fatherland
****
THE UNIVERSITY OF NGAOUNDERE
****
P.O Box : 454
****
RECTOR'S OFFICE
****
THE VICE RECTORATE IN CHARGE OF TEACHING, PROFESSIONALIZATION AND DEVELOPMENT OF INFORMATION AND COMMUNICATION TECHNOLOGIES
****
DIRECTION OF ACADEMIC AFFAIRS AND CO-OPERATION

2008/229

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.

Ministère des Finances
Direction Générale du Budget
Reçu après de l'Université de NGAOUNDERE
09 JUIL 2008
VISA BUDGETAIRE

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.

09 JUIL. 2008
Ngaoundéré, le .....

REPUBLIQUE DU CAMEROUN
REPUBLIC OF CAMEROON
UNIVERSITY OF NGAOUNDERE
UNIVERSITE DE NGAOUNDERE
Le Recteur

LE RECTEUR

Pr. Amvam Zollo Paul Henri

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

1

REPUBLIQUE DU CAMEROUN
****
Paix – Travail - Patrie
****
UNIVERSITE DE NGAOUNDERE
****

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

Le Directeur

Ngaoundéré, le 16 AVR. 2008

N° 2008/011/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. Mi Alimard

REPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie
UNIVERSITE DE NGAOUNDERE
BP 454
RECTORAT

REPUBLIC OF CAMEROON
Peace - Work - Fatherland
THE UNIVERSITY OF NGAOUNDERE
P.O. Box 454
RECTOR'S OFFICE

2010/687

DECISION N°———————/UN/R/VR-EPDTIC /SG/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.

MINISTERE DES FINANCES
DIRECTION GENERALE DU BUDGET
CONTRÔLE FINANCIER
C.F.S. auprès Univ. de Ngaoundéré
VISA BUDGETAIRE
14 NOV 2010

DÉCIDE :

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

Le Recteur

Amvam Zollo Paul Henri

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

4- ACTES ACADEMIQUES

4-1) Note de présentation soulignant la contribution à l'enseignement, la recherche, au développement de la science et de la culture ainsi qu'à la gouvernance de l'Université, de l'Enseignement Supérieur et de la Nation

A. Contribution à l'Enseignement

Je suis enseignant à l'Université de Ngaoundéré depuis le 16 avril 2008, date de ma prise de service à l'Institut Universitaire de Technologie (IUT). Depuis lors, sous instruction de l'administration de l'IUT de Ngaoundéré, j'ai contribué aux enseignements. Dans un premier temps, j'ai été employé à la réalisation et à l'exécution des travaux pratiques pour finir par obtenir des cours au courant de l'année académique suivante. J'ai été mis en contribution dans les cours de technologies alimentaires au niveau du département Génie Alimentaire et Contrôle Qualité (GACQ). Ces cours étaient globalement dans le domaine des boissons et des céréales et amidonnerie. Par la suite, il m'a été confié des cours dans le domaine de l'huilerie, la sucrerie et fruits et légumes.

J'ai été choisi pour être responsable pédagogique sur deux ans. En effet, la tache consistait en l'élaboration des emplois de temps et à m'assurer tenue effective des enseignements. J'ai également assuré plusieurs intérims ponctuels du Chef de Département GACQ lors de ses déplacements pour les colloques. J'ai également participé en tant qu'encadreur lors des déplacements des étudiants dans le cadre des voyages d'études (visites d'entreprises)

Par la suite, j'ai été associé à la commission de refonte des programmes du département Génie Alimentaire et Contrôle Qualité dans le cadre de la cohérence des programmes du LMD. Depuis plusieurs années déjà, je participe à la recherche des stages académiques auprès des industriels, et également aux encadrements mémoires de fins d'études DUT et Licence Professionnelle.

B. Contribution à la Recherche

Le point focal de la recherche que j'ai menée gravite autour de la valorisation du sorgho camerounais dans le domaine brassicole.

Le sorgho est la céréale par excellence de substitution à l'orge or, paradoxalement au Cameroun, l'orge reste exclusivement la céréale utilisée pour la fabrication de la bière. Pourtant, une politique ambitieuse de culture intensive du sorgho permettrait, sur le plan économique une rentrée de devises dans la mesure où cette matière première pourra être utilisée dans le secteur brassicole et des spiritueux. Politique que le Nigéria a impulsée depuis 1985.

B-1) Les Problématiques du sorgho pour la fabrication de la bière

Le sorgho est indéniablement la céréale prépondérante dans la région de l'extrême nord du Cameroun. L'une des utilisations de cette céréale est la filière *Bili-Bili* (bière traditionnelle de sorgho) qui, prend des ampleurs de plus en plus importantes. Ainsi, la culture de cette céréale n'a plus pour objectif une alimentation sous forme de couscous et autres. Il a été démontré que cette filière *Bili-Bili* est une source indéniable de revenu pour les femmes qui sont à la base de la prolifération de la filière. Les qualités d'une telle boisson seraient indéniablement liées à la qualité du malt, du moût et de la fermentation. Malgré le fait que les schémas traditionnels soient suffisamment éprouvés, les faibles rendements et les qualités approximatives des bières qui y sont produites traduisent des lacunes dans lesquelles, la science pourrait apporter des éléments de solution. Il est donc apparu au vue de la maltabilité insuffisante de certains cultivars qu'un pan de la recherche sur l'amélioration de la filtrabilité de ces maïsches doit être fait. Par la suite, il a paru nécessaire de modéliser et optimiser l'utilisation des enzymes pour pallier à cette carence de synthèse enzymatique au cours du maltage, afin d'obtenir des moûts de qualité.

B-2) Actes posées

Afin de mieux cerner les contours liés à la production du *Bili-Bili*, j'ai effectué dans le cadre d'un groupe de travail des descentes sur le terrain, à la rencontre des productrices de cette boisson dans les villes de Ngaoundéré et Garoua. Le travail de recherche se poursuit dans l'utilisation des plantes amères pour améliser les bières à base de ces céréales locales. La publication de 5 articles est la parfaite illustration du travail déjà entamé.

C. Résumé des publications dans le grade d'Assistant

1. **Desobgo, Z. S. C.**, Nso, E. J., Tenin, D. and Kayem, G. J.,

   Modelling and optimizing of mashing enzymes-effect on yield of filtrate of unmalted sorghum by use of response surface methodology. ***Journal of the Institute of Brewing*****, 2010, 116, 62-69.**

The effect of commercial enzymes on liquefaction of starch from unmalted sorghum was studied. The effects which these enzymes had on rates of filtration were evaluated. Models were developed, validated and optimized to establish the actions of enzymes, either alone or in combination. Preliminary studies on the sorghum cultivars *Safrari*, *Madjeru* and *S.35* showed that α-amylase was the backbone enzyme for starch liquefaction among the enzymes used (α-amylase, Filtrase, protease and β-amylase). Models confirmed this observation as α-amylase individually in its first order (X_1) contributed 25, 11 and 17 %, and in its sum of first and second orders ($X_1 + X_1^2$) contributed a 29, 31 and 36 % yield of filtrate for *Safrari*, *Madjeru* and *S.35* respectively. The ease of starch liquefaction, assessed by summing the first and second orders of individual intervention of all enzymes, was found to be in the order of *Madjeru*, *S.35* and *Safrari* (79, 70 and 56 % of yield of filtrate respectively). The importance of the enzyme combination in starch liquefaction in *Safrari*, *S.35* and *Madjeru* was shown to be 44, 30 and 21 % respectively. Enzyme combinations giving maximal starch liquefaction, as identified from a Doehlert experimental matrix, displayed a similar yield of filtrate (*Safrari*: 85 ml, *Madjeru*: 84 ml and *S.35*: 81 ml) after filtration of a 130 ml mash during 1 h. Validation of the models revealed the model developed for *Madjeru* was the most reliable (R^2 = 0.994), while those developed for *Safrari* (R^2 = 0.987) and *S.35* (R^2 = 0.976) were slightly less reliable. Model optimization gave theoretical enzyme (Brewers Amyliq TS, Filtrase NLC, Brewers Protease and β-amylase) combinations of 25 mg, 5.68 mg, 100 mg and 67.4 U for *Safrari*, 15.06 mg, 0.51 mg, 24.32 mg and 53.8 U for *Madjeru* and 19.01 mg, 6.36 mg, 58.76 mg and 43.48 U for *S.35*, with a resulting yield of filtrate of 94, 87.7 and 83.8 ml respectively.

Key words: Mashing enzymes, model validation, modeling, optimization, unmalted sorghum, yields of filtrates.

2. **Zangué Desobgo S. C.**, Emmanuel Nso J and Dzudie Tenin;

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*, 2011 Vol. 2(1) 5-15.

A three factor Doehlert design was used to develop a statistical model to optimize the action of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) on reducing sugars content of the worts of unmalted and malted *Safrari* sorghum. The response surface methodology revealed that increasing amounts of Hitempase considerably increased reducing sugars content during mashing of unmalted *Safrari* sorghum grist to about 90 g/L. Limited amounts of reducing sugars were obtained with increasing concentrations of both Bioglucanase (≈ 40 g/L) and Brewers Protease (≈ 30 g/L). The milling process facilitated the dissolution of about 10 g/L in yields of reducing sugars for the unmalted *Safrari* sorghum mash type without the help of enzyme. None of the three enzymes as sole mashing enzyme appeared to be of use in mashing malted *Safrari*, as reducing sugars yields were at maximum (168 g/L) after dissolving the grist in water and rather decreased with increasing amounts of enzyme supplements. Optimizing the concerted actions of the three enzymes for reducing sugars content of unmalted *Safrari* sorghum mash gave a combination of 2163 U, 937.5 BGU and 0 mg for Hitempase, Bioglucanase and Brewers Protease respectively. This gave a maximal reducing sugars content of 126.57 g/L. This combination was 0 U, 137.48 BGU and 0 mg for malted *Safrari* sorghum mash, giving a maximal reducing sugars yield of 168.56 g/L.

Key words: Response surface methodology, optimization, mashing enzymes, *Safrari*, reducing sugars

3. **Zangué Desobgo S. C.**, Emmanuel Nso J and Dzudie Tenin;

Use of the response surface methodology for optimizing the action of mashing enzymes on wort reducing sugars of the *Madjeru* sorghum cultivar. *Journal of Brewing and Distilling*, 2011 Vol 2(1), 5-15.

A three factor Doehlert design was used to develop a statistical model to optimize the action of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) on reducing sugars content of the worts of unmalted and malted *Madjeru* sorghum. The response surface methodology revealed that increasing amounts of Hitempase considerably increased reducing sugars content during mashing of unmalted and malted *Madjeru* sorghum grist to about 105.39 g/L and 132.25 g/L respectively. The milling process contributed to about 22 g/L and 54 g/L for the unmalted and malted mash types respectively. Increasing amounts of Bioglucanase was virtually insignificant, while for Brewers protease, reducing sugar yields rather decreased to nil for both the unmalted and malted mash types. Optimization of the concerted actions of the three enzymes for reducing sugars content of unmalted *Madjeru* sorghum mash gave a combination of 1995 U, 89.31 BGU and 28.86 mg for Hitempase, Bioglucanase and Brewers Protease respectively. This gave a maximal reducing sugars content of 108.78 g/L. This combination was 3000 U, 0 BGU and 49.69 mg for malted *Madjeru* sorghum mash, giving a maximal reducing sugars yield of 153.15 g/L.

Key words: Response surface methodology, optimization, mashing enzymes, Madjeru, reducing sugars.

4. **Zangué Desobgo S. C.**, Emmanuel Nso J and Dzudie Tenin;

Modeling the action of technical mashing enzymes on extracts and free-amino nitrogen yields of the Madjeru sorghum cultivar. ***Journal of Brewing and Distilling*****, 2011 Vol 2(3), 29-44.**

The action of three technical mashing enzymes (Hitempase 2XL, Bioglucanase-TX and Brewers Protease) on yields of extract and free amino nitrogen (FAN) of the worts of mashes of unmalted and malted *Madjeru* sorghum was modeled and analyzed using the response surface methodology. The analysis showed that increasing amounts of Hitempase 2XL considerably increased yields of extract during mashing of unmalted *Madjeru* sorghum grist. The use of Bioglucanase-TX was not indispensable, while Brewers Protease contributed very little. Increasing amounts of Hitempase contributed approximately 45 % of the free amino nitrogen, while Brewers Protease influence amounted to not more than 15 %. Bioglucanase's action was globally nil. Addition of the three enzymes into malted *Madjeru* sorghum mashes had no significant effect on the yields of extracts and FAN, but the milling operation singularly liberated more than 50 % of FAN for both mash types. Optimization of the concerted actions of the three enzymes for extract yield for unmalted *Madjeru* sorghum mash gave a combination of (1960.5 U; 132.61 BGU and 28.86 mg) for Hitempase, Bioglucanase and Brewers Protease respectively). This gave a maximal extract yield of 16.55 °P. This combination was: 2610 U; 0 BGU and 40.44 mg for malted *Madjeru* sorghum mash, giving a maximal extract yield of 16.35 °P. Optimization for free amino nitrogen for unmalted *Madjeru* sorghum mash gave a combination of: 3000 U; 0 BGU and 100 mg for Hitempase, Bioglucanase and Brewers Protease respectively). This gave maximal FAN of 93.55 mg/L. The combination was: 3000 U; 0 BGU and 100 mg for malted *Madjeru* sorghum mash, giving a maximal FAN of 144.48 mg/L.

Key words: Modeling, technical mashing enzymes, yields of extract, free-amino-nitrogen, *Madjeru*, optimization.

5. **Zangué Desobgo S. C.**, Emmanuel Nso J and Dzudie Tenin;

Optimisation of the Action of Commercial Mashing Enzymes on Wort Extracts and Free Amino Nitrogen of the Safrari Sorghum Cultivar. ***MBAA TQ*****, 2011 Vol 48(3), 77-86.**

The influence of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers Protease) used as sole mashing enzymes on yields of extract and free amino nitrogen (FAN) of the worts of the mashes of unmalted and malted *Safrari* sorghum was studied using the response surface methodology. The study revealed that increasing amounts of Hitempase considerably increased yields of extract during mashing of unmalted *Safrari* grist, while the effect of Bioglucanase was smaller, and that of Brewers Protease was insignificant. Extract yields decreased with increasing amounts of the three enzymes during the mashing of malted *Safrari*. This decrease was least expressed in the case of Brewers Protease. Yields in FAN amounted to less than 50 %, with increasing amounts of both Hitempase and Brewers Protease, but constantly decreased to nil for Bioglucanase's action in both unmalted and malted *Safrari* mashes. The milling operation singularly liberated more than 50 % of FAN for both mash types and for each of the enzymes. Optimisation of the concerted actions of the three enzymes for extract yields gave a combination of 2,098.5 U, 937.5 BGU and 0 mg (for Hitempase, Bioglucanase and Brewers Protease, respectively) for unmalted *Safrari* sorghum mash. This gave a maximal extract yield of 18°P. The combination was 0 U, 28.68 BGU and 0 mg for malted *Safrari* sorghum mash, giving a maximal extract yield of 18.82°P. Optimisation for FAN gave a combination of 2,434.5 U, 0 BGU and 100 mg (for Hitempase, Bioglucanase and Brewers Protease, respectively) for unmalted *Safrari* sorghum mash. This gave maximal FAN of 144.77 mg/L. The combination was 2,191.5 U, 0 BGU and 100 mg for malted *Safrari* sorghum mash, giving a maximal FAN of 196.73 mg/L.

Keywords: Commercial mashing enzymes, extract, free amino nitrogen, optimisation, *Safrari*.

Perspectives

Les travaux menés sur l'amélioration des caractéristiques physico-chimiques des moûts montrent des possibilités d'élaboration d'une bière à base de sorgho camerounais. Il sera par la suite question de scruter les aléas liés à la fermentation de ces moûts. On pourra déterminer les profils qualitatifs et quantitatif des sucres et acides aminés de ces moûts. Ceci permettra de pouvoir appréhender les déterminants de cette fermentation.

D. Rayonnement Scientifique et Culturel

Suite à la qualité des travaux effectués, j'ai été invité à la 13^{ième} Chair J. DE CLERCK sur le thème «The polyphenol paradox in alcoholic beverages (a beer and wine paradox ?)» du 7 au 10 september 2008, puis aux 1ères Journées Scientifiques (JS) du réseau Génie des Procédés Appliqués à l'Agro-alimentaire (GP3A), du 11 et 12 septembre 2008 à Louvain-la-Neuve en Belgique où, j'y ai présenté une communication intitulée «Modélisation et optimisation de l'effet des enzymes industrielles sur le rendement en filtrat des maïsches de sorgho.»

4-1) Liste des experts

Carl M. F. MBOFUNG (Pr); (Biochimie et Nutrition) Département de Sciences Alimentaire et Nutrition, ENSAI, Université de Ngaoundéré ;

2. TENIN DZUDIE (pr) ; (Génie Alimentaire) Département de Génie des Procédés et Ingénierie, ENSAI, Université de Ngaoundéré ;

3. Robert NDJOUENKEU (pr); (Chimie et Biochimie Alimentaire) Département de Sciences Alimentaire et Nutrition, ENSAI, Université de Ngaoundéré ;

4. TCHIEGANG Clergé (pr) ; (Biochimie Alimentaire) Département de Sciences Alimentaire et Nutrition, IUT, Université de Ngaoundéré ;

5. ETOA François Xavier (pr) ; (Microbiologie Alimentaire) Département de Biochimie,
FS, Université de Yaoundé 1 ;

6. ESSIA NGANG Jean Justin (MC) ; (Microbiologie Industrielle) Département de Biochimie, FS, Université de Yaoundé 1 ;

7. FONTEH Florence (MC); (Biochimie Alimentaire), FASA, Université de Dschang

4-2) Liste des enseignements dispensés et dûment approuvée par le Chef de Département

4-2) Liste des rapports et mémoires dirigés dûment validée par le Chef de Département

Liste des mémoires de fins d'études DUT et LICENCE PROFESSIONNELLE encadrés par Dr. DESOBGO ZANGUE Steve Carly depuis 2009

N°	Noms et Prénoms	Sujet	Année	Type de Mémoire
1	**LEUGUE Rosine**	Etude des cycles de filtration en vue d'optimiser la consommation du Kieselguhr	2009	DUT/IAB
2	**MOUELLE NGOBO**	Mise sur pied d'une méthode de suivi d'un indicateur de performance (cas des freintes)	2009	DUT/IAB
3	**FADI GOY GOY**	Analyse des facteurs de perte de rendement d'huile à partir des résultats du laboratoire	2009	DUT/IAB
4	**ZENGUE EVINA Laurent Patrice**	Etude de faisabilité pour la création d'une usine de pâtes alimentaires	2009	DUT/IAB
5	**JOUBOUNE NGOMSI Christelle**	Contribution à l'amélioration de la qualité du Palm'or	2009	DUT/IAB
6	**ELOUNGOU ZOGO Salomon**	Etude des freintes sur les chaines de production des jus de fruits «TAMPA» et «FRESCO»	2009	DUT/IAB
7	**NNOMO MANGA Franck**	Gestion de début et fin de filtration de la bière	2009	DUT/IAB
8	**TAWEDI Robert Elvis**	Gestion des déchets : traitement, recyclage et essais de valorisation, cas de la communauté urbaine d'Edéa.	2009	DUT/IAB
9	**TIYOU PADRIK**	Etude de l'effet de quelques hydrolases (a-amylase, b-glucanase, protéase et amyloglucosidase) sur l'extrait et la fermentescibilité des moûts de *Safrari* malté	2009	DUT/IAB
10	**SUH QUEENIVA LUM**	Contribution to the improvement of the physico-chemical characteristics of malted sorghum wort	2009	DUT/IAB

11	**AZOULINE MICHEL**	Influence des auxiliaires technologiques sur le rendement et la qualité de l'huile Diamaor	2010	DUT/IAB
12	**DAOUDA YAOUBA**	Suivi des paramètres physico-chimiques de l'oléine du CCIC en fonction du temps	2010	DUT/IAB
13	**DJIZANNE FOFOU Gérard**	Optimisation du fonctionnement des filtres à maïsche en salles à brasser	2010	DUT/IAB
14	**KANA Simon**	**Etude du process d'extraction du jus de soja, en vue de l'amélioration du rendement et du brix**	**2010**	**DUT/IAB**
15	LEUGOUE TCHAGO	Evaluation de l'efficacité de chaque thimonnier	2010	DUT/IAB
16	**MEMANA**	Evaluation des pertes sur la ligne «Riverr»	2010	DUT/IAB
17	MPON ETSIKE Luc Michel	Evaluation des pertes sur les lignes Bidon et Sachet à ADIC	2010	DUT/IAB
18	**NIDJO SAHA**	Optimisation du filtre MEVRA 2001	2010	DUT/IAB
19	**BONBO DIBE**	Etude sur la gestion des déchets d'une savonnerie et proposition d'amélioration (cas d'ISF)	2010	LICENCE PRO/GEN
20	ELOUNGOU ZOGO SALOMON	Etude comparative du pouvoir fermentaire de trois levures (levures sauvages, *Saccharomyces carlsbergensis*, *CLIB 655*) sur les moûts de *Safrari* malté	2010	LICENCE PRO/IAB
21	**NJIAH SUSAN FOFEYIN**	Comparative study on the fermentation of malted *Madjeru* by the use of three differents yeasts, *Saccharomyces carlsbergensis*, *Clib 655* and Indigenous yeast)	2010	LICENCE PRO/IAB
22	**SUH QUEENIVA LUM**	Proposal of a production unit and the recommendation of the land dimensions for the manufacturing of baby food (kukulu) SERDIF project	2010	LICENCE PRO/IAB

23	**LUM Linda**	An environmental audit of International Soap Factory	2011	DUT/GEN
24	**MENIAPI Berthe**	Suivi de la méthode de gestion du kieselguhr	2011	DUT/GEN
25	**BATCHEP BEKIMA Bill**	Evaluation de la cadence des ensacheuses volumétriques, respectivement sur les lignes de conditionnement «Nouriss» et «Jovino»	2011	DUT/IAB
26	**BOUN LIKENG Joseph**	Suivi de la viabilité des levures types SABC : axes d'amélioration	2011	DUT/IAB
27	**MBELLI Juliet ABONG**	Ameliorating the ageing time of P14 ageing oven using Cameroon Natural Rubber (CNR) 10	2011	DUT/IAB
28	**MBETMI KELLEU Charles Ghislain**	Etude des freintes des boites sur la chaine de production des conserves de haricots verts	2011	DUT/IAB
29	NDOOH Samuel	Optimisation de la production en yaourterie	2011	DUT/IAB
30	**NGNOKAM NZOUKOU Calixte**	Optimisation du temps d'execution aux postes d'analyses physico-chimiques au laboratoire : cas poste de physico-chimie bière	2011	DUT/IAB
31	**TJEEGA Jean Elisée**	Mise au point d'un yaourt aromatisé à la noix de coco	2011	DUT/IAB
32	**YOMI YOMI Brice Herman**	Mise au point d'un Fromage enrichi aux épices locales	2011	DUT/IAB
33	**KENMOGNE YONY Blaise**	Suivi de la fermentation d'une bière de sorgho et essai de clarification à l'aide d'extraits de *Moringa oleifera*	2011	DUT/IAB
34	**MORNADJI Félix**	Etude de la capabilité du filtre à maïsche usine SABC de Garoua	2011	LICENCE PRO/IAB

MINISTERE DE
L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER
EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP: 455 NGAOUNDERE

TEL: (237) 77 51 21 08/ 77 11 22 17/ 74 91 60 57

dstages2004@yahoo.fr

Division des Stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

Filière: Génie Biologique

Option : Industries Alimentaires et Biotechnologies (IAB)

SUJET : ETUDE DES CYCLES DE FILTRATION EN VUE D'OPTIMISER LA CONSOMMATION DE KIESELGUHR

Effectué du 1er **juin au 14 août 2009 à**

La Société Anonyme des Brasseries du Cameroun Agence de l'Ouest

Les Brasseries du Cameroun

Par

LEUGUE POUNGOUE Rosine Arcelle

Encadreurs Industriels

M. MANGA Yves

Adjoint Chef service Fabrication

M. KAMGA Dominique

Contremaître fabrication

Encadreur académique

Ing. DESOBGO Steve

Assistant à l'IUT de Ngaoundéré

Année académique 2008 - 2009

RÉPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie

MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR

UNIVERSITÉ DE NGAOUNDERE

REPUBLIC OF CAMEROON
Peace-Work-Fatherland

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
DE NGAOUNDERE

BP: 455 NGAOUNDERE

TEL: 77512108/77112217/74916057
Dstage2004@yahoo.fr
Division des Stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

MÉMOIRE DE FIN D'ÉTUDE

Filière : Génie Biologique
Option : Industrie Alimentaire et Biotechnologie

MISE SUR PIED D'UNE MÉTHODE DE SUIVI D'UN INDICATEUR DE PERFORMANCE (CAS DES FREINTES)

Effectué du 03 Juin au 29 Août aux Brasseries du Cameroun
Par

MOUELLE NGOBO RÉGINE VALENCIA

Les Brasseries du Cameroun since

ENCADREUR INDUSTRIEL

M. NGANDO EDJENGUELE ACHILLE :

Chef fabrication

ENCADREUR ECOLE

M. DESOBGO ZANGUE STEVE:

Assistant à l'I.U.T.

Année Académique 2008 / 2009

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP: 455 NGAOUNDERE

TEL: (237) 77 51 21 08/ 77 11 22 17/ 74 91 60 57

dstages2004@yahoo.fr

Division des Stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

Filière: Génie Biologique

Option : Industries Alimentaires et Biotechnologies (IAB)

SUJET : ANALYSE DES FACTEURS DE PERTES DE RENDEMENT D'HUILE A PARTIR DES RESULTATS DU LABORATOIRE

Effectué du 03 juin au 28 août 2009 à l'huilerie SODECOTON de Maroua

Par

FADI GOY GOY

Encadreur Industriel

Ing. YOUSSOUFA

Chef d'usine

Encadreur Ecole

Ing. DESOBGO Steve Carly

Assistant à l'IUT de Ngaoundéré

Année académique 2008/2009

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

THE UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
BP: 455 NGAOUNDERE
TEL: (237) 77 51 21 08/ 77 11 22 17/ 74 91 60 57
dstages2004@yahoo.fr
Division des Stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'étude en vue de l'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire de Technologie (IUT)

Filière : Génie Biologie

Option : Industries Alimentaires et Biotechnologies (IAB)

SUJET : ETUDE DE FAISABILITE POUR LA CREATION D'UNE USINE DE PATES ALIMENTAIRES

Effectué du 03 juin au 06 septembre 2009 à l'entreprise de CAMDEV S.A de Yaoundé

Par

ZENGUE EVINA LAURENT PATRICE

Encadreur industriel

David WOUNFACK

Conseiller technique

Encadreur école

M. DESOBGO Steve

Assistant à l'IUT de Ngaoundéré

Année académique 2008 / 2009

REPUBLIQUE DU CAMEROUN
PAIX – TRAVAIL - PATRIE
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROON
PEACE – WORK - FATHERLAND
MINISTERY OF HIGHER EDUCATION
UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
BP: 455 NGAOUNDERE
TEL: 77512108/77112217/74916057
dnstage2004@yahoo.fr
Division des stages, de la formation permanente et des relations avec les milieux professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

Filière : génie biologique

Option : Industries Alimentaires et Biotechnologies (IAB)

Sujet:

Contribution à l'amélioration de la qualité du PALM'OR

Effectué du 06 juillet au 31 aout 2009 à la :

SOCIÉTÉ DES PALMERAIES DE LA FERME SUISSE-PALM'OR

Par

JOUBOUNE NGOMSI Christelle

Encadreur industriel:
ILOGA Mystère Friède
Ingénieur des IAA
Directeur production raffinerie

Encadreur école
M. DESOBGO ZANGUE Steve Carly
Assistant à l'IUT de Ngaoundéré

Année académique 2008/2009

REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROUN
Peace – Work – Fatherland
MINISTRY OF HIGHER EDUCATION
UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
BP: 455 NGAOUNDERE
TEL: 77512108/77112217/74916057
E-mail: *dstages2004@yahoo.fr*
Division de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études

en vue de l'obtention du diplôme

Diplôme Universitaire de Technologie

ETUDE DES FREINTES SUR LES CHAINES DE PRODUCTION DES JUS DE FRUITS « TAMPA » ET « FRESCO »

Stage effectué du 08 Juin *au 08 Aout 2009* **à SOTICAM**

Rédigé et soutenu par :

ELOUNGOU ZOGO SALOMON

Encadreur Industriel :
M. EMESSIENE Richard
Ing des IAA

Encadreur académique :
M. DESOBGO ZANGUE Steve Carly
Ing des IAA : Assistant à l'IUT

Année académique 2008/2009

REPUPLIQUE DU CAMEROUN

PAIX-TRAVAIL-PATRIE

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROON

PEACE-WORK-FATHERLAND

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP: 455 NGAOUNDERE

TEL: 77 51 21 08/ 77 11 22 17/ 74 91 60 57

dstages2004@yahoo.fr

Division des stages, de la Formation Permanente et des Relations avec les milieux Professionnels

MEMOIRE DE FIN D'ETUDES

En vue de l'obtention du Diplôme Universitaire de Technologie (DUT)

Filière : Génie Biologique.

Option : Industries Alimentaires et Biotechnologies (IAB)

Sujet :

GESTION DE DEBUT ET DE FIN DE FILTRATION DE LA BIERE

*Effectué du **08 Juin au 31 Août 2009** à la **S.A. B.C. Direction d'usine De Yaoundé***

Les Brasseries du Cameroun

Par NNOMO MANGA FRANK

Encadreur industriel :

Mr. FELIX MBANGTENG
Chef Service Fabrication

Mlle NATHALIE NOAH
Chef Atelier Caves

Encadreur école :

Mr. DESOBGO STEVE C.
Assistant chargé de cours IUT

Année académique 2008/2009

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION
UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE
DE NGAOUNDERE
B.P. : 455 Ngaoundéré TEL : 77512801/77112217
dstages2004@yahoo.fr
Division des Stages de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

Filière: Génie Biologique

Option : Génie de l'environnement (GEN)

SUJET : GESTION DES DECHETS : TRAITEMENT, RECYCLAGE ET ESSAIS DE VALORISATION, CAS DE LA COMMUNAUTE URBAINE D'EDEA

Effectué du 1er Juillet au 28 Août 2009 à la Communauté Urbaine d'Edéa

Par :

TAWEDI Robert Elvis

Encadreur industriel

M. NYEMBE Roger

Chef service hygiène et assainissement

Encadreur école

M. DESOBGO Steve

Assistant à l'IUT de Ngaoundéré

Année académique : 2008/2009

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP: 455 NGAOUNDERE

TEL: (237) 77 51 21 08/ 77 11 22 17/ 74 91 60 57

dstages2004@yahoo.fr

Division des Stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

Mention: Génie Biologique

Parcours : Industries Alimentaires et Biotechnologies (IAB)

SUJET : Etude de l'effet de quelques hydrolases (α-amylase, β-glucanase, protéase et amyloglucosidase) sur l'extrait et la fermentescibilité des moûts de *Safrari* malté

Effectué du 15 Octobre au 15 Décembre 2009 à l'IUT de Ngaoundéré

Par

TIYOU Jules Padrik

Encadreur Industriel

Ing. DESOBGO ZANGUE Steve C.

Assistant a l'IUT de Ngaoundéré

Encadreur Ecole

Ing. DESOBGO ZANGUE Steve C.

Assistant a l'IUT de Ngaoundéré

Année académique 2008/2009

REPUBLIQUE DU CAMEROUN
PAIX – TRAVAIL – PATRIE

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROON
PEACE-WORK-FATHERLAND

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

- **INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE**
BP: 455 NGAOUNDERE
Email: dstages2004@yahoo.fr
Dvision des stages, de la Formation permanente et des Relations avec les Milieux Professionnelles

END OF COURSE INTERNSHIP REPORT

In partial fulfilment of the requirements for the award of a University Diploma of Technology in Food Industry and Biotechnology (IAB)

CONTRIBUTION TO THE IMPROVEMENT OF THE PHYSICO-CHEMICAL CHARACTERISTICS OF MALTED SORGHUM WORT

Carried out in the University Institute of Technology (IUT) Ngaoundere

From June 19 – September 15, 2009

by

SUH QUEENIVA LUM

Supervisor

Eng. DESOBGO STEVE

Assistant Lecturer IUT Ngaoundere

2008/2009 Academic year

REPUBLIQUE DU CAMEROUN

Paix-Travail-Patrie

MINISTERE DE L'ENSEIGNEMENTSUPERIEUR

Université de Ngaoundéré

REPUBLIC OF CAMEROON

Peace-Work-Fatherland

MINISTRY OF HIGHER EDUCATION

University of Ngaoundéré

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP 455 NGAOUNDERE

Tel: 77512108/77112217/74916057

dstages2004@yahoo.fr

Division des Stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (D.U.T.)

Parcours : Génie Biologie

Mention : Industries Alimentaires et Biotechnologies (I.A.B.)

Sujet :

INFLUENCE DES AUXILIAIRES TECHNOLOGIQUES SUR LE RENDEMENT ET LA QUALITE D'HUILE DIAMAOR

Effectué du 01 juin au 27 Aout 2010 à l'huilerie de la SODECOTON de GAROUA

Par

AZOULINNE MICHEL

Matricule : 08B007IU

Encadreur industriel :

Ing. Bobo Yaya

Encadreur Ecole :

Ing. Desobgo Steve Carly

Année académique 2009 /2010

REPUBLIQUE DU CAMEROUN

PAIX-TRAVAIL-PATRIE

******

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROON

PEACE-WORK-FATHERLAND

******

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
B.P.: 455 NGAOUNDERE
TEL.: 77 51 21 08/77 11 22 17/74 91 60 57
Dstages2004@yahoo.fr
Division des Stages de la Formation Permanente et des Relations avec les Milieux Professionnels

MEMOIRE DE FIN D'ETUDES

En vue de l'obtention du Diplôme Universitaire de Technologie (D.U.T.)

Mention : Génie Biologique

Parcours: Industries Alimentaire et Biotechnologique (I.A.B.)

THEME :

SUIVI DES PARAMETRES PHYSICO-CHIMIQUES DE L'OLEINE DU CCIC EN FONCTION DU TEMPS

Stage effectué du 01er JUIN au 20 AOUT 2010 à l'usine CCIC, Douala

Par
DAOUDA YAOUBA
Matricule : 08B018IU

Encadreur Industriel
M. HAMINOU HALILOU
Chef Service Qualité

Encadreur Ecole
M. DESOBGO ZANGUE Steve Carly
Enseignant à l'IUT de Ngaoundéré

Année Académique : 2009-2010

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION
UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
B.P: 455 NGAOUNDERE
TEL: 77 51 21 08 / 77 11 22 17 / 74 91 60 57
dstages2004@yahoo.fr
Division des stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

Filière : Génie Biologique

Option : Industries Alimentaires et Biotechnologies (IAB)

SUJET : **OPTIMISATION DU FONCTIONNEMENT DES FILTRES À MAISCHE EN SALLES À BRASSER**

Les Brasseries Cameroun

Effectué du 15 octobre au 15 décembre 2009 à l'usine SABC de Koumassi

Par

DJIZANNE FOFOU Gérard

Encadreurs Industriels:

M. ZONTSOP Etienne
Directeur des Usines Adjoint
SABC Littoral

M. NSANGOU ABOUBAKAR
Adjoint au Chef Service Fabrication
à l'usine de Koumassi

Encadreur école:

M. DESOBGO Steve
Assistant
à l'IUT de Ngaoundéré

Année académique : 2008 / 2009

REPUBLIQUE DU CAMEROUN

Paix – Travail – Patrie

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

REPUBLIC OF CAMEROUN

Peace – Work – Fatherland

MINISTRY OF HIGHER EDUCATION

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

B.P: 455 NGAOUNDERE

TEL: 99 85 13 82 / 99 65 56 44

E-mail: dstages2004@yahoo.fr

Division des stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

MEMOIRE DE FIN D'ETUDES PRESENTE EN VUE DE L'OBTENTION DU DIPLOME UNIVERSITAIRE DE TECHNOLOGIE (DUT)

FILIERE : Génie Biologique

OPTION : Industries Alimentaires et Biotechnologique

THEME :

ETUDE DU PROCESS D'EXTRACTION DU JUS DE SOJA, EN VUE DE L'AMELIORATION DU RENDEMENT ET DU BRIX

Camlait

La force du lait

Stage réalisé du 01 juin au 26 Août 2010 A CAMLAIT RIVERR -DOUALA

Par

KANA SIMON EMMANUEL

Encadreur école :

M. DESOBGO STEVE

Enseignant à l'IUT de Ngaoundéré

Encadreur industriel :

M TAGNE SOTER *Ing* IAA

Chef d'usine de Camlait riverr

Année académique 2009 / 2010

REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROUN
Peace – Work – Fatherland
MINISTRY OF HIGHER EDUCATION
THE UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
B.P.: 455 NGAOUNDERE
TEL: 77 51 21 08/ 77 11 22 17/ 74 91 60 57
E-mail: *dstages2004@yahoo.fr*
Division des stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (D.U.T.) de Ngaoundéré

Filière : Génie Biologique

Mention : Industries Agroalimentaires et Biotechnologiques

Thème:

EVALUATION DES PERTES SUR LA LIGNE RIVERR

Stage effectué du 02 juin au 26 Août 2010 à CAMLAIT NDOKOTTI-DOUALA

Camlait
La force du lait

Par

MEMANA OLINGA Salomé Flora

08B044IU

Encadreur industriel

M. TAGNE SOTER Ledoux

Chef d'usine de CAMLAIT NDOKOTTI

Encadreur école

M. DESOBGO ZANGUE Steve Carly

Enseignant à l'I.U.T. de Ngaoundéré

Année académique 2009 / 2010

REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROUN
Peace – Work – Fatherland

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGOUNDERE B.P. 455NGAOUNDERE

TEL 77 51 21 08 / 77 11 22 17 /74 91 60 57

Dstages2004à yahoo.fr

Division des stages de la formation permanente et des relations avec les milieux professionnels

MEMOIRE DE FIN D'ETUDE

En vue de L'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire Technologie (IUT) de Ngaoundere

PARCOURS : Génie Biologique

MENTION : Industrie Alimentaires et Biotechnologies (IAB)

SUJET :

OPTIMISATION DU FILTRE MEURA 2001

Effectué dans

Les Brasseries du Cameroun since

Usine de KOUMASSI

Du 15 juin au 14 août 2010

Par :
NIDJO SAHA Flobert Anderson
Matricule : 08B064IU

Encadreur Industriel :

M. MANGA Yves Mathieu
Adjoint chef service fabrication

Encadreur académique :

M. DESOBGO ZANGUE Steve Carly
Enseignant à l'IUT de Ngaoundéré

Année académique 2009/2010

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP: 455 NGAOUNDERE

TEL: 77 51 21 08 / 77 11 22 17 / 74 91 60 57

Dstages2004@yahoo.fr

Division des Stages de la Formation Permanente et des Relations avec les Milieux Professionnels

DEPARTEMENT DE GENIE BIOLOGIQUE

Mémoire de fin d'études en vue de l'obtention de la licence professionnelle à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

MENTION : Génie Biologique

PARCOURS : Génie de l'Environnement (GEN)

Sujet : ETUDE SUR LA GESTION DES DECHETS D'UNE SAVONNERIE ET PROPOSITION D'AMELIORATION (cas d'ISF)

Effectué du 05 aout au 29 octobre 2010 à International Soap Factory BAMENDA

Par

BONBO DIBE Pierre Pascal

Encadreur industriel :

M.SITCHEU ELI

Directeur Technique d'ISF

Encadreur école :

M.DESOBGO ZANGUE Steve. C

Assistant à l'IUT de Ngaoundéré

Année académique 2009-2010

REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROON
Peace – Work – Fatherland
MINISTRY OF HIGHER EDUCATION
UNIVERSITY OF NGAOUNDERE

UNIVERSITY INSTITUTE OF TECHNOLOGY (UIT)
P.O. BOX 455 NGAOUNDERE
TEL.: 77512108/77112217/74916057/CTF
dstages2004@yahoo.fr
Division of internships, permanent training and relations with the professional milieu

END OF COURSE REPORT

In partial fulfilment of the requirements for the award of a Professional Degree at the University Institute of Technology (IUT) Ngaoundere

FIELD: Genie Biologique
OPTION: Industrial Food Biotechnology (IAB)

THEME:

COMPARATIVE STUDY ON THE FERMENTATION OF MALTED MADJERU BY THE USE OF THREE DIFFERENT YEAST, SACCHAROMYCES CARLSBERGENSIS, CLIB 655 AND INDIGENOUS YEAST.

CARRIED OUT BY:
NJIAH SUSANA FOFEYIN
(06B055IU)
AT
I.U.T NGAOUNDERE

FROM: AUGUST 2nd - OCTOBER 29th 2010

Industrial and Academic Supervisor

Eng. DESOBGO STEVE C.
LECTURER I. U.T

ACADEMIC YEAR 2009/2010

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION
UNIVERSITY
OF NGAOUNDERE

UNIVERSITY INSTITUTE OF TECHNOLOGY
NGAOUNDERE
P.O B: 455 Ngaoundéré: Tel/Fax :(237)225 27 51
Email:dstages@yahoo.fr
Division of internships, permanent training and relations
With the professional milieu

END OF COURSE INTERNSHIP REPORT

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A PROFESSIONAL BACHELOR'S DEGREE
IN
Food Processing and Biotechnology (IAB)

TOPIC

PROPOSAL OF A PRODUCTION UNIT AND THE RECOMMENDATTION OF THE LAND DIMENSIONS FOR THE MANUFACTURING OF BABY FOOD (kukulu) SERDIF/ PROJECT

Done from 5th August to 29th October 2010
AT
SERDIF
Presented by:

SUH QUEENIVA LUM

Industrial Supervisor
Eng .KITO Guy
Technical Director

Academic Supervisor
Eng.DESOBGO STEVE
Lecturer IUT Ngaoundéré

Academic year 2009/2010

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
****
UNIVERSITE DE NGAOUNDERE
****

MINISTRY OF HIGHER EDUCATION
****
UNIVERSITY OF NGAOUNDERE
****

UNIVERSITY OF TECHNOLOGY NGAOUNDERE
P.O.B: 455 NGAOUNDERE
TEL: 77 51 21 08 / 77 11 22 17/ 74 91 60 57
E-mail: dstages2004@yahoo.fr
Division of Internships, Permanent Training and Relations with the Professional Milieu

END OF COURSE DISSERTATION REPORT

SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF THE UNIVERSITY DIPLOMA OF TECHNOLOGY (DUT)
In
ENVIRONMENTAL ENGINEERING

TOPIC

AN ENVIRONMENTAL AUDIT OF INTERNATIONAL SOAP FACTORY

DONE FROM THE 20TH OF JULY TO THE 20TH OF SEPTEMBER 2011

AT:

INTERNATIONAL SOAP FACTORY (ISF) BAMENDA

Written by:

LUM LINDA

(09BO36IU)

Industrial supervisors

M. NDI Paul
Eng. ACHAATAP Jean
In charge of students on internship

Academic supervisor

M. DESOBGO Zangue Steve
Lecturer IUT NGAOUNDERE

2010/2011 Academic year

PAIX-TRAVAIL-PATRIE

MINISTERE DE

L'ENSEIGNEMENT SUPERIEUR

UNVERSITE DE NGAOUNDERE

PEACE-WORK-FATHERLAN

MINISTRY OF HIGER

EDUCATION

UNIVERSITY OF NGAOUNDE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE

NGAOUNDERE

B.P. 455 NGAOUNDERE

DIVISION DES STAGES, DE LA FORMATION PERMANENTE ET DES RELATIONS AVEC LES MILIEUX PROFESSIONNELS

TEL 77 51 21 08/ 77 11 22 17/ 74 91 60 57

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (D.U.T.) à l'Institut Universitaire de Technologie (I.U.T.) de Ngaoundéré

Mention : Génie Biologique

Parcours : Génie de l'Environnement

SUJET : SUIVI DE LA METHODE DE GESTION DU KIESELGUHR

Effectué du 13 Juin au 19 Août 2011 à l'usine SABC DE YAOUNDE

Agence du Centre

Les Brasseries du Cameroun

Par :

MENIAPI Berthe

Matricule : 09B047IU

Encadreur industriel :

M. ALI WANTOUNA Salatou

Agent environnement

Encadreur Académique :

M. DESOBGO Steve

Assistant chargé de cours IUT

Année Académique 2010-2011

REPUBLIQUE DU CAMEROUN
****
Paix-Travail-Patrie
****
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
****
UNIVERSITE DE NGAOUNDERE
****

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

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
BP: 455 NGAOUNDERE
TEL: 77 51 21 08 / 77 11 22 17 / 74 91 60 57
dstages2004@yahoo.fr
Division des stages, de la Formation Permanente et des Relations avec les milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (D.U.T.) à l'Institut Universitaire de Technologie (I.U.T.) de Ngaoundéré

MENTION : Génie Biologique
PARCOURS : Industries Alimentaires et Biotechnologiques (I.A.B.)

SUJET :

EVALUATION DE LA CADENCE DES ENSACHEUSES VOLUMETRIQUES, RESPECTIVEMENT SUR LES LIGNES DE CONDITIONNEMENT NOURISS ET JOVINO

Effectué du 11 juin au 11 septembre 2011 dans la société Camlait ***à Douala, Bassa***

Par :
BATCHEP BEKIMA Bill Donald
Matricule : 09B008IU

Encadreur industriel
M. MONDJO Roger
Chef de quart, CAMLAIT

Encadreur école
M. DESOBGO Steve
Enseignant, IUT de Ngaoundéré

Année académique 2010 / 2011

REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie
MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
UNIVERSITE DE NGAOUNDERE

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

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
B.P: 455 NGAOUNDERE
TEL: 77 51 21 08 / 77 11 22 17 / 74 91 60 57
dstages2004@yahoo.fr
Division des stages, de la Formation Permanente et des Relations avec les milieux Professionnels

MEMOIRE DE FIN D'ETUDES EN VUE DE L'OBTENTION DU DIPLOME UNIVERSITAIRE DE TECHNOLOGIE (DUT) A L'INSTITUT UNIVERSITAIRE DE TECHNOLOGIES (IUT) DE NGAOUNDERE

Mention : Génie Biologique
Parcours : Industries Alimentaires et Biotechnologies(IAB)

SUIVI DE LA VIABILITE DES LEVURES DE TYPE SABC : AXES D'AMELIORATION

Effectué du 15Juillet au 14Septembre 2011
A
L'usine SABC de YAOUNDE

Les Brasseries du Cameroun

Par

BOUN LIKENG Joseph
Matricule : 09B015IU

ENCADREURS INDUSTRIELS

M. MBANGTENG Félix
Chef Service fabrication
M.NDOMBI Robert
Adjoint chef service fabrication
M.NGUIARO Serge
Adjoint chef service fabrication

ENCADREUR ECOLE

M.DESOBGO Steve C.
Assistant
Enseignant à l'IUT

Année académique 2010 / 2011

REPUBLIQUE DU CAMEROUN
Paix - Travail - Patrie

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

REPUBLIC OF CAMEROON
Peace - Work - Fatherland

MINISTRY OF HIGHER EDUCATION

THE UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGY DE NGAOUNDERE
BP: 455 NGAOUNDERE
TEL: 77 51 21 08/77 11 22 17/74 91 60 57
dstages2004@yahoo.fr
Division des stages, de la formation permanente et des Relations avec les Milieux Professionnels

END OF COURSE PROJECT REPORT

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A UNIVERSITY DIPLOMA OF TECHNOLOGY (DUT)
IN
FOOD PROCESSING AND BIOTECHNOLOGY

TOPIC :

AMELIORATING THE AGEING TIME OF P14 AGEING OVEN USING CAMEROON NATURAL RUBBER (CNR) 10

Carried out at:
CDC Central Rubber Factory, Tiko
From the 28th of June to the 30th of August

By:
MBELLI JULIETE ABONG

Industrial Supervisor
Eng. NDIFON JACOB
Worker at CDC Rubber Factory

Academic Supervisor:
Mr. DESOGBO STEVE
Lecturer at I.U.T Ngaoundere

Academic Year: 2010-2011

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR
****
UNIVERSITE DE NGAOUNDERE
****

MINITRY OF HIGHER EDUCATION
****
UNIVERSITY OF NGAOUNDERE
****

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP : 455 NGAOUNDERE
TEL : (+237) 7751-2208 / 7711-2217 / 7491-6057
Email : dstages2004@yahoo.fr
Division des stages, de la formation permanente et des relations avec les milieux professionnels

Mémoires de fin d'études en vue de l'obtention du diplôme universitaire de technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

MENTION : *Génie Biologie*
PARCOURS : *Industries Alimentaires et Biotechnologies (IAB)*

Sujet :

ETUDES DES FREINTES DES BOITES SUR LA CHAINE DE PRODUCTION DES CONSERVES DE HARICOT VERT

Effectué du 15 Juin au 01 Septembre 2011 à l'usine PROLEG S.A

Par
MBETMI Charles Ghislain
Matricule : 09BO42IU

Encadreur industriel
COUAYA Sandrine
Responsable qualité

Encadreur académique
M. DESOBGO Steve
Enseignant à l'I.U.T

Année académique 2010 - 2011

REPUBLIQUE DU CAMEROUN
****
Paix – Travail – Patrie
****
UNIVERSITE DE NGAOUNDERE
****

REPUBLIQUE OF CAMEROON
****
Peace – Work – Fatherland
****
UNIVERSITY OF NGAOUNDERE
****

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
BP: 455 NGAOUNDERE
TEL : 77 51 21 08 / 77 11 22 17/ 74 91 60 57
dstages2004@yahoo.fr
Division des Stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de Technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

MENTION : Génie Biologique

PARCOURS : Industries Alimentaires et Biotechnologies (IAB)

SUJET : OPTIMISATION DU TEMPS D'EXECUTION AUX POSTES DES ANALYSES PHYSICOCHIMIQUES AU LABORATOIRE.
CAS : POSTE DE LA PHYSICOCHIMIE BIERE

Effectué du 13 juin au 09 septembre 2011 à la

SOCIETE ANONYME DES BRASSERIES DU CAMEROUN

Usine de l'Ouest

LES BRASSERIES DU CAMEROUN

Par

NGNOKAM NZOUKOU Claude Calixte

Matricule : 09B059IU

Encadreurs industriels

Mr MAYOH Jacques Fidèle
Chef Laboratoire et Qualité

Mr BIBILA Georges
Chef de groupe

Encadreur école

Ing. DESOBGO Steve
Assistant à l'IUT de Ngaoundéré

Année Académique 2010/2011

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP :455 NGAOUNDERE

TEL : 77 51 21 08/77 11 22 17/74 91 60 57

dstages2004@yahoo.fr

Division des stages,de la formation permante et des relations avec les milieux professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

MENTION: Génie Biologique

PARCOURS: Industries Alimentaires et Biotechnologies (IAB)

SUJET : MISE SUR PIED D'UN YAOURT AROMATISE A LA NOIX DE COCO

Effectue du 23 juin au 23 septembre au laboratoire de technologie alimentaire et post-récolte de l'IRAD Bambui

Par

TJEEGA JEAN ELISEE

Matricule: 09BO75IU

Encadreur industriel

Dr MENDI Stephan

Responsable du laboratoire de technologie Alimentaire et post-récolte

Encadreur école

DESOBGO Steve

Ingénieur en IAA

Charge de cour a l'IUT

Année académique 2010/2011

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE

BP :455 NGAOUNDERE

TEL : 77 51 21 08/77 11 22 17/74 91 60 57

dstages2004@yahoo.fr

Division des stages,de la formation permante et des relations avec les milieux professionnels

Mémoire de fin d'études en vue de l'obtention du Diplôme Universitaire de technologie (DUT) à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

MENTION: Génie Biologique

PARCOURS: Industries Alimentaires et Biotechnologies (IAB)

SUJET : MISE SUR PIED D'UN FROMAGE ENRICHI AUX EPICES LOCALES

Effectue du 23 juin au 23 septembre au laboratoire de technologie laitière et post-récolte du Centre Régional de Recherche Agricole de Bambui

Par

YOMI-YOMI-Brice-Hermann

Matricule : 09B080IU

Encadreur industriel

Dr MENDI Stephan

Responsable du laboratoire de technologie laitière et post-récolte

Encadreur école

Mr DESOBGO Steve Carly

Charge de cour a l'IUT

Année académique 2010/2011

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
BP: 455 NGOUNDERE
TEL: 77 51 21 08 / 77 11 22 17 / 74 91 60 57
dstages2004@yahoo.fr
Division des stages, de la Formation Permanente et des Relations avec les milieux Professionnels.

Mémoire de fin d'études en vue de l'obtention de la licence professionnelle de l'Institut Universitaire de Technologie (IUT) de l'Université de Ngaoundéré.

Mention : Génie Biologique.

Parcours : Industries Alimentaires et Biotechnologiques.

SUJET : # Suivi de la fermentation d'une bière de sorgho et essai de clarification à l'aide d'extraits de *Moringa oleifera*

Effectué du 18 Juillet au 28 Octobre 2011 à l'IRAD de Maroua

Par :

KENMOGNE Yony Blaise
Matricule : 08B038IU
DUT en IAB

Encadreur Industriel :

Dr DJOULDE Roger

Chargé de cours à l'ISS de Maroua

Encadreur école :

Mr DESOBGO Steve

Enseignant à l'IUT de Ngaoundéré

Année académique 2010 / 2011

MINISTERE DE L'ENSEIGNEMENT SUPERIEUR

UNIVERSITE DE NGAOUNDERE

MINISTRY OF HIGHER EDUCATION

UNIVERSITY OF NGAOUNDERE

INSTITUT UNIVERSITAIRE DE TECHNOLOGIE DE NGAOUNDERE
B.P: 455 NGAOUNDERE
TEL: 77 51 21 08 / 77 11 22 17 / 74 91 60 57
E-mail: dstages2004@yahoo.fr
Division des stages, de la Formation Permanente et des Relations avec les Milieux Professionnels

Mémoire de fin d'études en vue de l'obtention de la Licence Professionnelle à l'Institut Universitaire de Technologie (IUT) de Ngaoundéré

MENTION : Génie Biologique
PARCOURS : Industries Alimentaires et Biotechnologies (IAB)

SUJET : ETUDE DE CAPABILITE DU FILTRE A MAISCHE USINE SABC DE GAROUA

Effectué du 18 Juillet 2011 au 28 Octobre 2011 à l'usine SABC de GAROUA

Par
MORNADJI Félix
Matricule : 93A009UT

Encadreur industriel
NGANDO EJENGUELE Achille
Ingénieur IAA
Chef Service Fabrication

Encadreur école
Ing. DESOBGO Steve
Enseignant IUT Ngaoundéré

Année académique 2010 / 2011

4-3) Publications dans le Grade d’Assistant

Modelling and Optimizing of Mashing Enzymes – Effect on Yield of Filtrate of Unmalted Sorghum by Use of Response Surface Methodology

Zangué S. C. Desobgo, Emmanuel J. Nso*, Dzudie Tenin and G. J. Kayem

ABSTRACT

J. Inst. Brew. 116(1), 62–69, 2010

The effect of commercial enzymes on liquefaction of starch from unmalted sorghum was studied. The effects which these enzymes had on rates of filtration were evaluated. Models were developed, validated and optimized to establish the actions of enzymes, either alone or in combination. Preliminary studies on the sorghum cultivars *Safrari, Madjeru* and *S.35* showed that α-amylase was the backbone enzyme for starch liquefaction among the enzymes used (α-amylase, Filtrase, protease and β-amylase). Models confirmed this observation as α-amylase individually in its first order (X_1) contributed 25, 11 and 17%, and in its sum of first and second orders ($X_1+X_1^2$) contributed a 29, 31 and 36% yield of filtrate for *Safrari, Madjeru* and *S.35* respectively. The ease of starch liquefaction, assessed by summing the first and second orders of individual intervention of all enzymes, was found to be in the order of *Madjeru, S.35* and *Safrari* (79, 70 and 56% of yield of filtrate respectively). The importance of the enzyme combination in starch liquefaction in *Safrari, S.35* and *Madjeru* was shown to be 44, 30 and 21% respectively. Enzyme combinations giving maximal starch liquefaction, as identified from a Doehlert experimental matrix, displayed a similar yield of filtrate (*Safrari:* 85 mL, *Madjeru:* 84 mL and *S.35:* 81 mL) after filtration of a 130 mL mash during 1 h. Validation of the models revealed the model developed for *Madjeru* was the most reliable ($R^2 = 0.994$), while those developed for *Safrari* ($R^2 = 0.987$) and *S.35* ($R^2 = 0.976$) were slightly less reliable. Model optimization gave theoretical enzyme (Brewers Amyliq TS, Filtrase NLC, Brewers Protease and β-amylase) combinations of 25 mg, 5.68 mg, 100 mg and 67.4 U for *Safrari,* 15.06 mg, 0.51 mg, 24.32 mg and 53.8U for *Madjeru* and 19.01 mg, 6.36 mg, 58.76 mg and 43.48 U for *S.35,* with a resulting yield of filtrate of 94, 87.7 and 83.8 mL respectively.

Key words: Mashing enzymes, model validation, modeling, optimization, unmalted sorghum, yields of filtrates.

INTRODUCTION

The addition of enzymes to increase fermentable sugars and free amino acids, and to facilitate filtration when mashing with poorly malted or unmalted cereals, is an established practice in beer brewing[1,2,6,7,10,12,15]. One of the most important technological parameters to which brewers pay attention during mashing is the ease with which the mash filters[8,10,17,19,20]. Studies carried out on malts of three sorghum cultivars of North Cameroon showed that, whether malted traditionally or under controlled laboratory conditions to brew the traditional beer *Bili-Bili*, the mashes of *Madjeru* cultivar filtered slower than the cultivars *Safrari* and *S.35*[17]. Although the proportions of fermentable sugars of worts of the three cultivars were comparable, that of maltose was less than 50% for *Madjeru,* when compared with *Safrari* and *S.35*[17]. These observations were partly attributed to limited amounts of starch hydrolyzing enzymes such as α-amylase and β-amylase present in the *Madjeru* malts[17]. This is indeed one of the major problems encountered in mashing with some malted sorghum cultivars[3-5,8,9,13,14,17,18,21]. However, it is known that acceptable worts for beer brewing can be obtained from 100% unmalted sorghum by supplementing the mashes with optimal amounts of thermostable α-amylase, fungal α-amylase and bacterial proteases[11]. In contrast, what is not known is the effect of singular or combined contributions of these enzymes on starch liquefaction of unmalted sorghum grains. In this paper, we report validated and optimised mathematical models for mashing unmalted grains of the *Safrari, S.35* and *Madjeru* sorghum cultivars using as mashing enzymes the following: Brewer Amyliq (α-amylase), Filtrase NLC (β-glucanase and hemicellulase), Brewers protease and β-amylase in order to assess the singular or combined contributions of these enzymes in mash liquefaction.

Ecole Nationale Supérieure des Sciences Agro-industrielles (ENSAI), Université de Ngaoundéré, BP 455 Ngaoundéré, Cameroun.
*Corresponding author. E-mail: nso_emmanuel@yahoo.fr

Publication no. G-2010-0316-1048

MATERIALS AND METHODS

Chemicals

The characteristics of the commercial enzymes used (Brewers Amyliq TS from *Baccillus licheniformis,* Filtrase NLC, Brewers protease from *Bacillus amyloliquefaciens* and β-amylase type II-B from crude barley) are presented in Table I. The amounts used for the first three ranged from 0–25 mg, 0–10 mg and 0–100 mg respectively, while for β-amylase the range used was 0–80 U.

Table I. Characteristics of commercial enzyme preparations used in mashing[a].

	Organism of origin	Activity	Description	Temperature optima	pH optimum	Recommended application level in adjuncts	Form
Brewers Amyliq TS	*Bacillus licheniformis*	27.7 ± 6.5 U/mg of solid	α-amylase	93–95°C	5.5–6.5	0.3 g/Kg	Powder
β-amylase (E 3.2.1.2)	Type raw II-B of barley	23–80 U/mg of protein	β-amylase	*NI[b]	*NI	*NI	Powder
Filtrase NLC	*NI	*NI	β-glucanase and hemicellulase	*NI	*NI	0.15–0.2 g/Kg	Solution
Brewers Protease	*Bacillus amyloliquefaciens*	1842.2 ± 1.8 mg FAN/min/mL	Protease	45–50°C (denatured at 85°C)	6.5–7.5	0.4–2 g/Kg	Solution

[a] All the commercial enzymes used in this study were obtained from DSM Food Specialities, Cedex France, apart from β-amylase which was sourced from SIGMA CHIMIE, Cedex, France.
[b] Not indicated by DSM Food Specialities France/SIGMA.

Table II. Matrices of Doehlert coded and transformed experimental values.

Coded values				Transformed experimental values				*Madjeru*			*Safrari*			*S.35*		
α-amylase	Filtrase	Proteases	β-amylase	α-amylase (mg)	Filtrase (mg)	Proteases (mg)	β-amylase (U)									
X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res	Exp	Theo	Res
1.000	0.000	0.000	0.000	25	5	50	40	68	65	3	85	83	2	76	76.1	–0.1
–1.000	0.000	0.000	0.000	0	5	50	40	24	27	–3	36	38	–2	35	34.9	0.1
0.500	0.866	0.000	0.000	18.75	10	50	40	61	60.405	0.595	67	70.001	–3	77	78.404	–1.4
–0.500	–0.866	0.000	0.000	6.25	0	50	40	64	64.596	–0.59	60	56.999	3.001	60	58.602	1.398
0.500	–0.866	0.000	0.000	18.75	0	50	40	84	83.097	0.903	71	71.5	–0.5	69	67.702	1.298
–0.500	0.866	0.000	0.000	6.25	10	50	40	40	40.905	–0.9	40	39.502	0.498	45	46.304	–1.3
0.500	0.289	0.816	0.000	18.75	6.66	100	40	55	57.088	–2.08	75	75.698	–0.69	80	79.993	0.007
–0.500	–0.289	–0.816	0.000	6.25	3.33	0	40	63	60.885	2.115	59	58.297	0.703	61	60.991	0.009
0.500	–0.289	–0.816	0.000	18.75	3.33	0	40	74	76.885	–2.88	71	69.789	1.211	77	72.579	4.421
0.000	0.577	–0.816	0.000	12.5	8.33	0	40	61	60.29	0.71	64	62.805	1.195	70	69.897	0.103
–0.500	0.289	0.816	0.000	6.25	6.66	100	40	38	35.088	2.912	41	42.19	–1.19	46	50.381	–4.38
0.000	–0.577	0.816	0.000	12.5	1.66	100	40	60	60.685	–0.68	66	67.205	–1.2	69	69.097	–0.09
0.500	0.289	0.204	0.791	18.75	6.66	62.5	80	55	56.459	–1.45	75	73.301	1.699	81	79.483	1.517
–0.500	–0.289	–0.204	–0.791	6.25	3.33	37.5	0	35	33.458	1.542	47	48.7	–1.7	45	46.483	–1.48
0.500	–0.289	–0.204	–0.791	18.75	3.33	37.5	0	63	63.97	–0.97	65	67.693	–2.69	67	72.583	–5.58
0.000	0.577	–0.204	–0.791	12.5	8.33	37.5	0	42	42.372	–0.37	53	51.701	1.299	65	62.394	2.606
0.000	0.000	0.612	–0.791	12.5	5	87.5	0	49	49.078	–0.07	54	50.894	3.106	67	62.491	4.509
–0.500	0.289	0.204	0.791	6.25	6.66	62.5	80	50	48.971	1.029	50	47.293	2.707	70	64.383	5.617
0.000	–0.577	0.204	0.791	12.5	1.66	62.5	80	70	69.565	0.435	62	63.299	–1.29	73	75.592	–2.59
0.000	0.000	–0.612	0.791	12.5	5	12.5	80	76	75.876	0.124	55	58.093	–3.09	72	76.489	–4.48
0.000	0.000	0.000	0.000	12.5	5	50	40	78	77.25	0.75	63	64.25	–1.25	79	78.25	0.75
0.000	0.000	0.000	0.000	12.5	5	50	40	79	77.25	1.75	65	64.25	0.75	77	78.25	–1.25
0.000	0.000	0.000	0.000	12.5	5	50	40	75	77.25	–2.25	63	64.25	–1.25	77	78.25	–1.25
0.000	0.000	0.000	0.000	12.5	5	50	40	77	77.25	–0.25	66	64.25	1.75	80	78.25	1.75

[a] Experimental result values.
[b] Theoretical values (values coming from mathematical models).
[c] Residue.

Sorghum cultivars

The *Safrari, Madjeru* and *S.35* sorghum cultivars were obtained from the Institute of Research and agronomic development (IRAD) Maroua, Cameroon.

Mashing

Sorghum cultivar grains were milled to particle sizes of 0.7 mm or less using a hammer mill Polymix PX-MFC 90D apparatus type (VWR International S.A.S. Le Périgas 201, rue Carnot, 94126 Fontenay-sous-Bois Cedex, France). Twenty five grams of unmalted sorghum was weighed, placed in a 600 mL beaker and 150 mL of distilled water added. The suspension was homogenised at 24°C by stirring with a glass rod. It was then heated to boiling temperature, at which starch gelatinization was allowed to take place for 20 min, with intermittent stirring at intervals of 5 min, before cooling to 60°C. Mashing was carried out at 60°C for 1 h taking into consideration the conditions indicated in Table I. The mash was cooled to 25°C and filtered for 1 h using Whatman grade 1 filter paper.

Mathematical modelling

The establishment of an experimental matrix was necessary to develop the mathematical model. The Doelhert (number of factors, k = 4)[16] matrix was adopted for this work. Transformation of the matrix of coded variables to the experimental matrix was automatic (Table II), while the quantities of mashing enzymes used were maintained at fixed levels. The amounts of selected enzymes ranged

as follows: Brewers Amyliq TS (0–25 mg); Filtrase NLC (0–10 mg); Protease (0–100 mg); and β-amylase (0–80 U) (β-amylase activity was 31 U/mg of solid).

Doelhert's experiment design (having a homogenous distribution in space) was used to establish the experiment matrix (Table II). This matrix has coded variables and must be converted into an experimental matrix[16] having real variables directly usable in the laboratory. The results of the phenomenon to study are then established and the data considered as "y_{exp}". With the help of the matrix of coded variables and "y_{exp}", the coefficients of the model are obtained. The model is obtainable only from the coded variable matrix because, in this case, there is no need for a counterbalancing effect of the factors in study[16]. The coefficients of the model obtained will thus be effectively linked to the impact of each factor. These coefficients and the model were obtained with the help of the Systat version 12 software (Systat Software, Inc., San Jose, USA). This software also gives a statistical analysis on the model. Lastly, the curves are plotted using Sigmaplot version 11 build 11.0.0.77 software (WPCubed, GmbH, Germany).

Validation of the model was conducted after assays using several enzyme combinations found in the experimental domains not explored by the experimental matrix. Another method consisted of tracing the theoretical results against the experimental results, as the coefficient of correlation R^2 gives an appreciation of the reliability of the model.

Model optimization used Mathcad version 14 software (Parametric Technology Corporation, Massachusetts, USA). Optimal combination was obtained by initially entering the model and then specifying the starting point of each factor. The sweeping interval by the software was given for each factor. Once the data was entered, the software gave a response for a maximum or minimum combination as requested for. This theoretical optimum was then explored for the confirmation of the optimal point.

RESULTS AND DISCUSSION

Before establishing the mathematical model for mashing, trials were carried out to investigate the action of each of the enzyme preparations in starch liquefaction

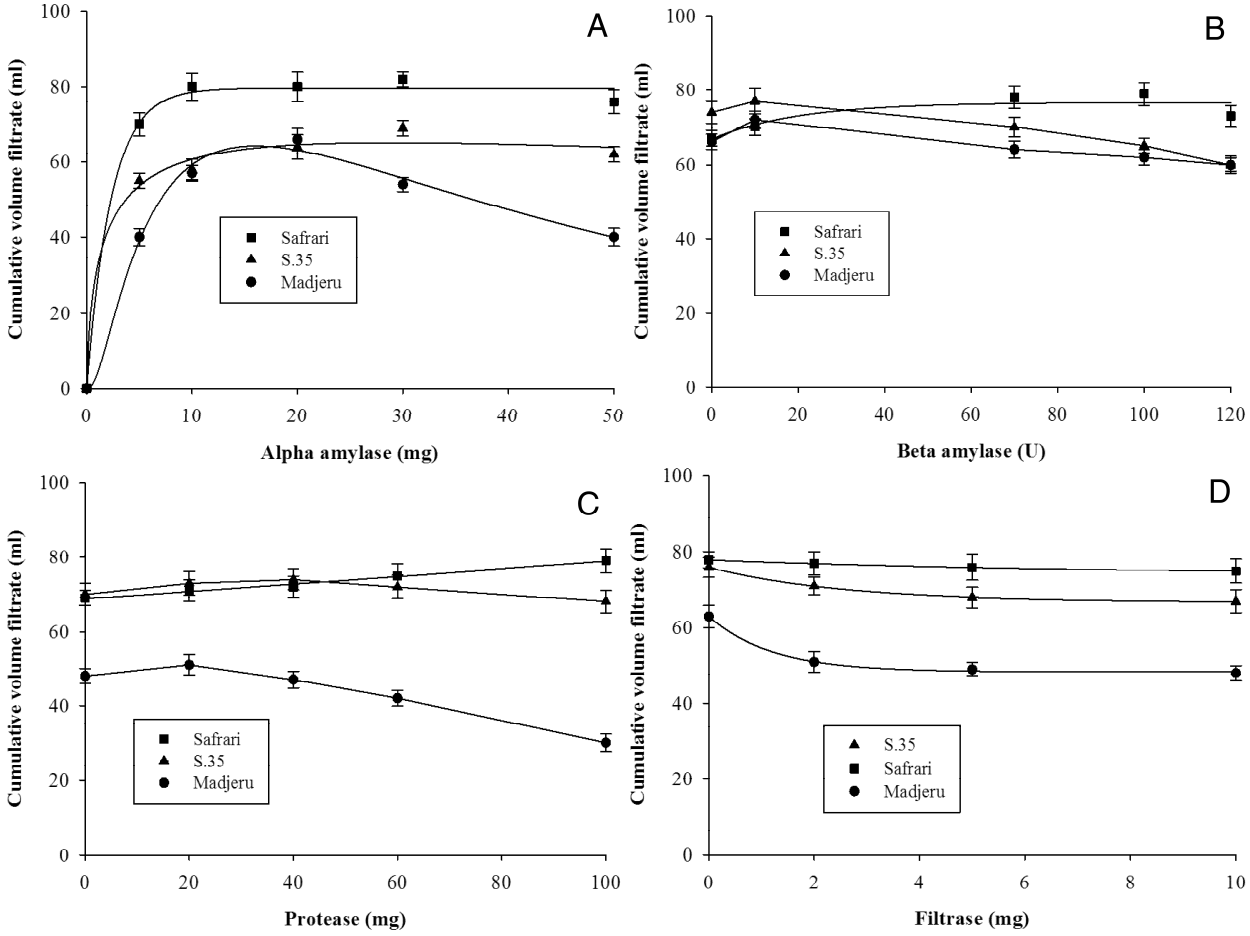


Fig. 1. (A) Effect of α-amylase concentration on filtration of unmalted sorghum mash. The β-amylase, Protease, and Filtrase being constant at 80 U, 60 mg and 10 mg respectively. (B) Effect of β-amylase concentration on filtration of unmalted sorghum mash. The α-amylase, Protease and Filtrase being constant at 20 mg, 60 mg and 10 mg respectively. (C) Effect of Protease concentration on filtration of unmalted sorghum mash. The α-amylase, Filtrase and β-amylase were constant at 20 mg, 10 mg and 80 U respectively. (D) Effect of Filtrase concentration on filtration of unmalted sorghum mash. The α-amylase, Protease and β-amylase were constant at 20 mg, 60 mg and 80 U respectively.

during mashing. Among the enzyme preparations used in this study, it was observed (Fig. 1a) that α-amylase was the principal enzyme of starch liquefaction during mashing[1,7,12]. Figures 1b, 1c and 1d show that the actions of β-amylase, filtrase and protease on starch liquefaction were only noticeable in the presence of α-amylase. This is justified by the fact that filtration was not possible after mashing in the presence of these enzymes, but in the absence of α-amylase (Fig. 1a). These results indicated that mathematical modeling would provide more precise information on the contribution of each enzyme component and combination of enzyme components required to liquefy the mash optimally.

Previous studies on malting and mashing of sorghum cultivars used in this study showed that *Madjeru* mashes filtered slower than those of *Safrari* and *S.35*[17,18]. This slow filtration rate was suggested to be due to the low amounts of α-amylase and β-amylase in *Madjeru* malts compared to *Safrari* and *S.35*. Experimental conditions for the three cultivars in this study were standardized by using unmalted grains and several combinations of the mashing enzymes as shown in Table II. The best enzyme combinations to mash with were identified and the rates of filtration compared under those conditions (Fig. 2). The results showed that the filtration rates, and thus the yields of filtrates for the three varieties were comparable as 85 mL, 84 mL and 81 mL of filtrate was obtained after filtration of 130 mL for 1 h of the *Safrari, Madjeru* and *S.35* mash, respectively. These results confirmed the hypothesis that the slow filtration of mashes of *Madjeru* malts during mashing could be partly attributed to insufficient quantities of α-amylase and β-amylase as suggested previously[17]. A mathematical model was established for each cultivar using a precise matrix. Models obtained by the response surface method established the following equations for mashing the three cultivars.

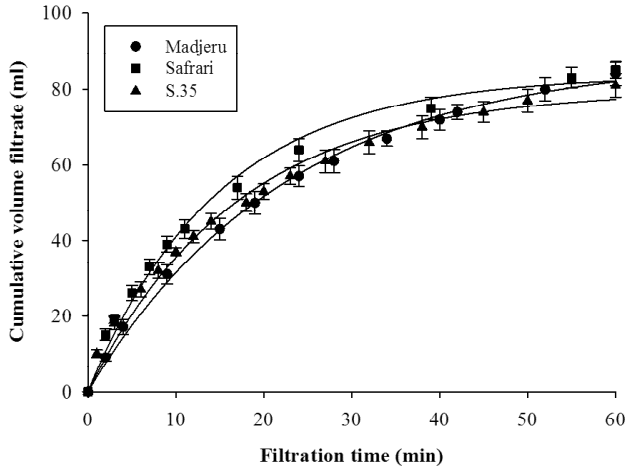


Fig. 2. The effect of best combinations of enzymes on mash filtration: *Madjeru* [α-Amylase (18.75 mg), Filtrase (0 mg), Protease (50 mg), β-amylase (40 U)]; *Safrari* [α-amylase (25 mg), Filtrase (5 mg), Protease (50 mg), β-amylase (40 U)]; *S.35* [α-amylase (18.75 mg), Filtrase (6.66 mg), Protease (62.5 mg), β-amylase (80 U)].

Safrari

$$Y = 64.25 + 22.5X_1 - 5.484X_2 - 1.182X_3 + 3.636X_4 + 9.237X_1X_2 + 10.219X_1X_3 - 1.576X_1X_4 - 5.437X_2X_3 + 3.470X_2X_4 + 8.655X_3X_4 - 3.75X_1^2 - 5.083X_2^2 - 0.169X_3^2 - 8.796X_4^2$$

Madjeru

$$Y = 77.25 + 19X_1 - 13.39X_2 - 9.226X_3 + 9.801X_4 + 0.577X_1X_2 + 3.472X_1X_3 - 15.66X_1X_4 + 3.427X_2X_3 - 0.638X_2X_4 - 14.23X_3X_4 - 31.25X_1^2 - 9.583X_2^2 - 17.96X_3^2 - 23.87X_4^2$$

S.35

$$Y = 78.25 + 20.6X_1 - 0.461X_2 - 0.816X_3 + 8.217X_4 + 13.279X_1X_2 + 6.342X_1X_3 - 13.44X_1X_4 - 1.536X_2X_3 - 0.822X_2X_4 + 1.147X_3X_4 - 22.75X_1^2 - 13.08X_2^2 - 7.692X_3^2 - 8.508X_4^2$$

The three equations were structurally identical and all of second order with interactions. In the models, the factors X_1 and X_4, corresponding to α-amylase and β-amylase respectively had positive coefficients. These indicated that the two enzymes were directly involved in the liquefaction of starch and were thus instrumental contributors to yields of filtrates. Figures 3a, 3b and 3c detail the individual and combined contributions of the various mashing enzyme components on the total yield of filtrates of the liquefied starch. Thus, α-amylase, in its singular expression as first order in the model, contributed 25%, 11% and 17% of the yield of filtrate for the mashes of *Safrari, Madjeru* and *S.35* respectively. Similarly, β-amylase accounted for 4%, 6% and 7% respectively. The factors X_2 and X_3, corresponding to filtrase and protease respectively, had negative coefficients. This suggested that these enzymes were not directly responsible for starch liquefaction, and cannot, a priori, be considered as directly contributing to yield of filtrate during mashing. Filtrase, in its singular expression as first degree in the model, indirectly contributed to 6%, 8% and 1% in yields of filtrates, while protease contributed to 1%, 5% and 1% in *Safrari, Madjeru* and *S.35* mashes respectively. The factors X_1^2, X_2^2, X_3^2 and X_4^2 corresponding to the second order of the components of α-amylase, filtrase, protease and β-amylase respectively, showed that the singular action of all these enzymes expressed to their second order in the model, had a negative coefficient. Their absolute contribution to the yield of filtrate was about 4%, 6%, 0% and 10% for *Safrari,* 20%, 5%, 10% and 14% for *Madjeru* and 19%, 11%, 7% and 7% for S.35 mashes respectively. Table III summarises these results, with the sum totals in percent, of the contribution in yield of filtrate by these enzymes in their first and second orders as expressed by the model. The results corroborated those shown in Fig. 1, wherein α-amylase is evidently the sole enzyme of starch liquefaction during mashing. Indeed, this enzyme in its first and second orders in the model, singularly contributed 29%, 31% and 36% to filtrate yield of the cultivars *Safrari, Madjeru* and *S.35* respectively.

Table III also summarises the results for the contributions of the interactions between various enzyme components used. Thus, the interaction X_1X_2, corresponding to the combined action of α-amylase and filtrase, had a pos-

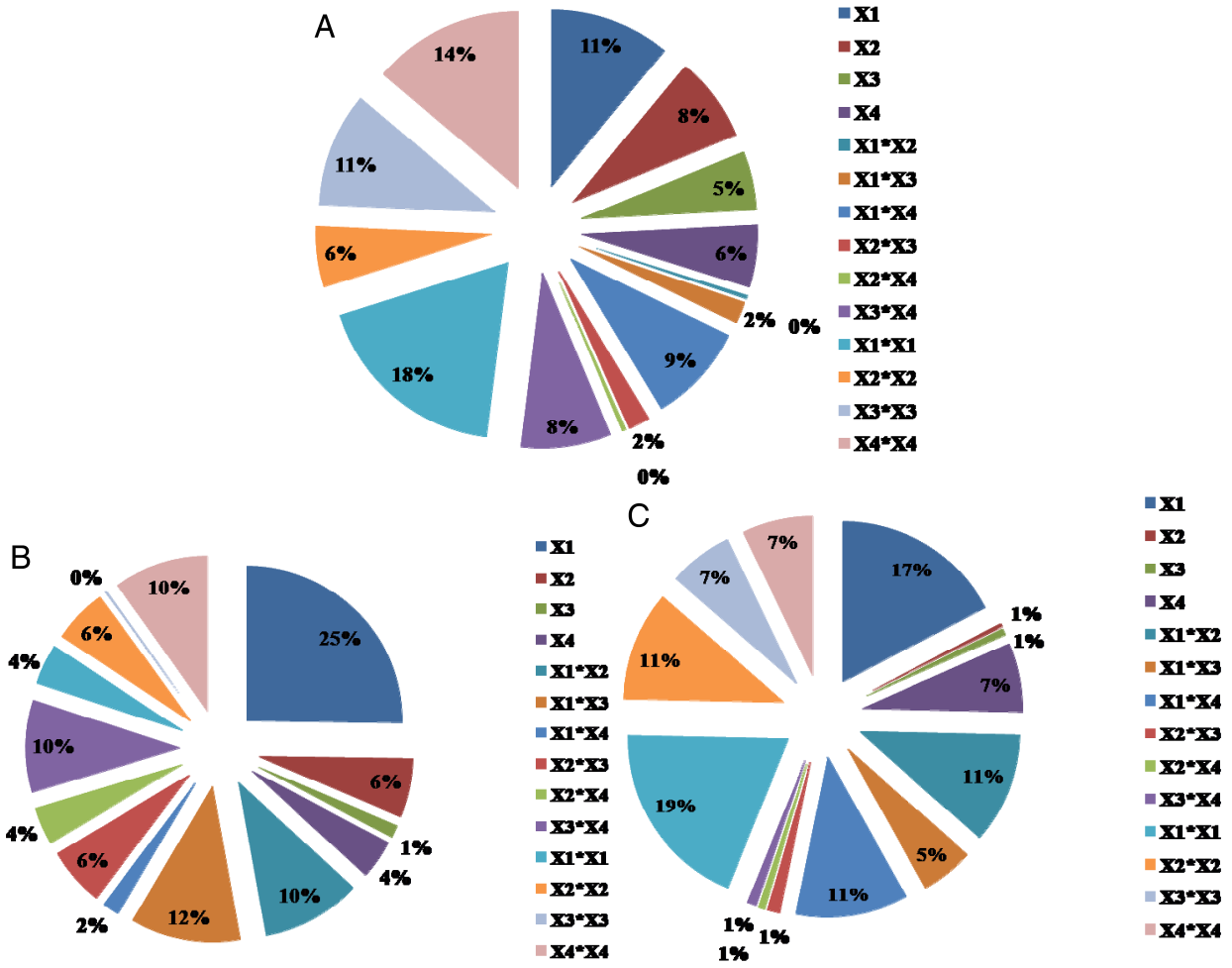


Fig. 3. (A) Contribution of the model factors on the total effect for *Safrari*. (B) Contribution of the model factors on the total effect for *Madjeru.* (C). Contribution of the model factors on the total effect for *S.35*.

itive coefficient, suggesting that the second enzyme contributed to reducing the viscosity of the mash and thus facilitated its filtration. This positive interaction between α-amylase and filtrase roughly contributed to 10%, 0% and 11% in yield of filtrate for *Safrari, Madjeru* and *S.35* mashes respectively. The interaction X_1X_3, corresponding to the combined action of α-amylase and protease, also had a positive coefficient. Similarly to the α-amylase/filtrase interaction, protease facilitates mash hydrolysis by α-amylase. This positive interaction between the two enzymes contributed to about 12%, 2% and 5% in yield of filtrate for *Safrari, Madjeru* and *S.35* mashes respectively. The interaction X_1X_4, corresponding to the combined action of α-amylase and β-amylase had a negative coefficient. Since these two enzymes are starch hydrolysing enzymes, they independently and directly attack free convertible starch granules in suspension without any prerequisites. Whereas starch granules embedded in the protein matrix and surrounded by β-glucano-hemicellulosic cell walls must absolutely be liberated by the action of proteases and β-glucanases in order to allow α-amylase and β-amylase to act on starch[21]. The contribution by this combined effect in yield of filtrate was 2%, 9% and 11% for *Safrari, Madjeru* and *S.35* mashes respectively. Upon adding the contributions of α-amylase's action in its singular first and second orders, to those of its interactions with the other enzymes (α-amylase/filtrase, α-amylase/protease and α-amylase/β-amylase) in the model, we obtained the direct and indirect contribution to starch liquefaction. These contributions amounted to 53%, 42% and 63% for *Safrari, Madjeru* and *S.35* respectively. The interaction X_2X_3, corresponding to the combined action of filtrase and protease, had a negative coefficient for the mashes of the cultivars *Safrari* and *S.35,* but was positive for *Madjeru*. The contribution by this combined effect in yield of filtrate was roughly 6%, 2% and 1% for *Safrari, Madjeru* and *S.35* mashes respectively. These two enzymes are not starch hydrolysing enzymes, and only help to liberate starch granules embedded in the protein matrix and surrounded by β-glucano-hemicellulosic cell walls. The low contribution of this interaction is therefore predictable. Interaction X_2X_4, corresponding to the combined action of filtrase and β-amylase, had a positive coefficient for the cultivar *Safrari,* but was negative for *Madjeru* and *S.35*. The contribution by this combined effect in yield of filtrate was about 4%, 0% and 1% for *Safrari, Madjeru* and *S.35* mashes respectively. Once more, the low contribution of this interaction for the three cultivars suggested that the filtrase/β-amylase combination was not important for filterability. This can be explained by the fact that the

Table III. Contributions of single (1st and 2nd orders), their sums and combined enzyme components to filtrate yields.

Enzyme	Single component as 1st, 2nd degree and sum	Cultivar type *Safrari*	*Madjeru*	*S.35*
α-amylase	X_1	25	11	17
	X_1^2	4	20	19
	$X_1 + X_1^2$	29	31	36
Filtrase	X_2	6	8	1
	X_2^2	6	5	11
	$X_2 + X_2^2$	12	13	12
Protease	X_3	1	5	1
	X_3^2	0	10	7
	$X_3 + X_3^2$	1	15	8
β-amylase	X_4	4	6	7
	X_4^2	10	14	7
	$X_4 + X_4^2$	14	20	14
Total		**56**	**79**	**70**
Enzymes interacting	Combined component	*Safrari*	*Madjeru*	*S.35*
α-amylase / Filtrase	$X_1 X_2$	10	0	11
α-amylase / Protease	$X_1 X_3$	12	2	5
α-amylase / β-amylase	$X_1 X_4$	2	9	11
Filtrase / Protease	$X_2 X_3$	6	2	1
Filtrase / β-amylase	$X_2 X_4$	4	0	1
Protease / β-amylase	$X_3 X_4$	10	8	1
Total		**44**	**21**	**30**

role of filtrase was limited to unmasking starch granules embedded in cells by hydrolysing the β-glucans and hemicelluloses of the walls of these cells, while β-amylase, a saccharifying enzyme, had only limited action on the molecular size of starch material. Finally, the interaction X_3X_4 corresponding to the combined action of protease/β-amylase had a positive coefficient for the cultivars *Safrari* and *S.35,* but negative for the cultivar *Madjeru*. The contribution of this combined effect on yield of filtrate was about 10%, 8% and 1% for *Safrari, Madjeru* and *S.35* respectively. The impact of this enzyme combination on the filtration of mash will depend not only on the extent of hydrolysis of the protein matrix surrounding starch granules, but also on that of the β-amylase component, which will be assessed by the extent of hydrolysis of convertible starch granules. Thus, if proteolysis is effective, but the starch type is more of amylopectin than amylose, the rate of mash filtration would be slower, as the saccharifying action of β-amylase would be reduced. This could explain the disparity in the yield of filtrate observed for these three cultivars of sorghum, with respect to the enzyme combinations.

With respect to the observed effects of the first and second orders as a sum for each of the enzyme components in the model, 56%, 79% and 70% for *Safrari, Madjeru* and *S.35* respectively, it is clear that the ease of mash liquefaction was in the order of *Madjeru, S.35* and *Safrari* (Table III). Also, the model showed that the ease of mash liquefaction on the basis of enzyme combined components was in the order of *Safrari, S.35* and *Madjeru* (44%, 30% and 21% respectively) (Table III). These results suggest that the action of filtrase and protease in liberating protein embedded starch granules surrounded by β-glucano-hemicellulosic cell walls, was of great importance in liquefying and saccharifying starch by α-amylase and β-amylase respectively for *Safrari,* followed by *S.35* and then by *Madjeru*. This also suggests that *Safrari* was richer in proteins and/or β-glucans than *S.35*. This has indeed been shown with respect to the total protein contents of these three cultivars[17].

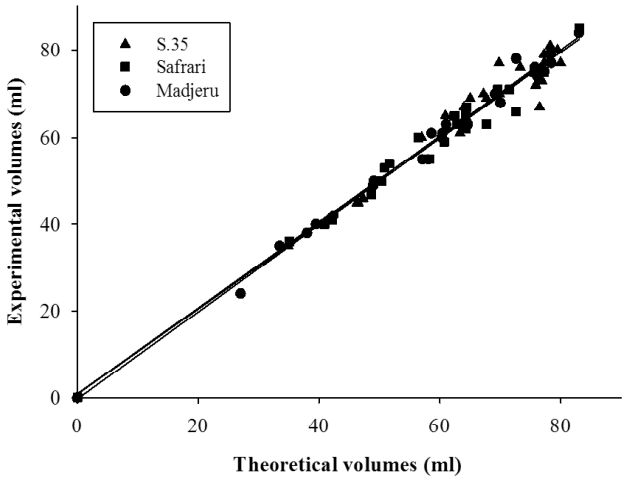


Fig. 4. Validation of mathematical models for *Madjeru, Safrari* and *S.35* with R^2 = 0.994, 0.987 and 0.976 respectively.

The mathematical models were validated using two methods. Firstly, tests were carried out on several combinations of the experimental space which were not explored within the framework of the experimental matrix. The results were then compared with the theoretical results and the errors statistically evaluated. These errors were between 2% and 3.7%, 1.7% and 3%, and 0.1% and 3.4% for *Safrari, Madjeru* and *S.35* respectively. The global error thus was 0.1% to 3.7%. As the highest error limit is approximately 3.7%, it could be concluded that the mathematical models established, satisfactorily describe the observed phenomena. It is however necessary to determine this error and reliability. This can be done by a second method consisting of plotting the theoretical results against the experimental results and determining the

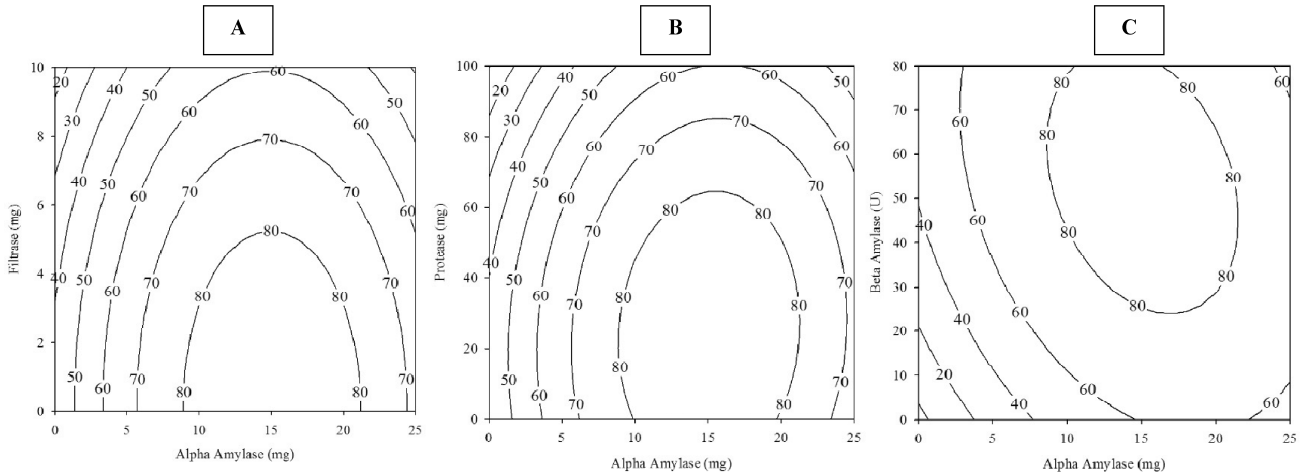


Fig. 5. Response surface curves for *Madjeru* yield in filtrates [all other factors were fixed at optimal quantity: (A) Protease: 24.32 mg; β-amylase: 53.8 U; (B) Filtrase: 0.51 mg; β-amylase: 53.80U; (C) Filtrase: 0.51 mg; Protease: 24.32 mg].

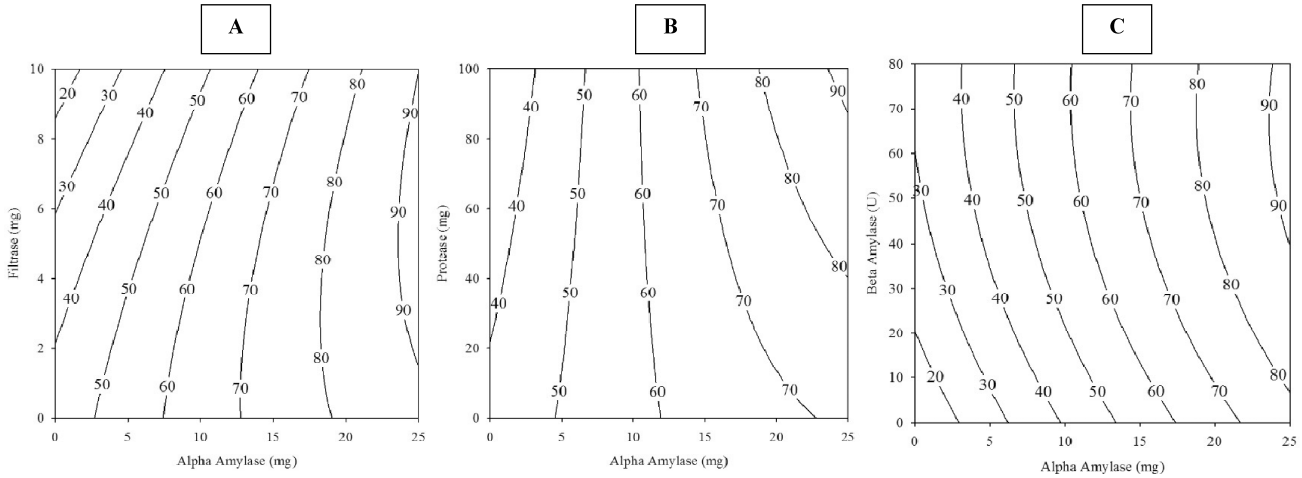


Fig. 6. Response surface curves for *Safrari* yield in filtrates [all other factors were fixed at optimal quantity: (A) Protease: 100 mg; β-amylase: 67.40 U; (B) Filtrase: 5.68 mg; β-amylase: 67.40 U; (C) Filtrase: 5.68 mg; Protease: 100 mg].

coefficient of correlation R^2, in order to appreciate the reliability of the models. The second method of validating the models allowed for the classification of reliability in the following order: first *Madjeru* (R^2 = 0.994), followed by *Safrari* (R^2 = 0.987) and then *S.35* (R^2 = 0.976) (Fig. 4).

Optimization of the outputs in filtrates was logically the final step in this work after validating the models. This was conducted using Mathcad version 14 software (Parametric Technology Corporation, Massachusetts, USA). The theoretical maxima for the combinations in amounts of dispensable mashing enzyme preparations, in order to obtain optimal filtrates in our working conditions, were for α-amylase, filtrase, protease and β-amylase, as follows: 25 mg, 5.68 mg, 100 mg and 67.4 U (or in coded values: 1, 0.118, 0.816, 0.542) respectively for *Safrari,* 15.06 mg, 0.51 mg; 24.32 mg and 53.80 U (or in coded values: 0.205, –0.777, –0.419, 0.273) respectively for *Madjeru* and: 19.01 mg, 6.36 mg, 58.76 mg and 43.48 U (or in coded values: 0.521, 0.236, 0.143, 0.069) respectively for *S.35*. Figs. 5, 6 and 7 show these results in their response surface representations. Thus, the theoretical optimal volumes calculated for the three models were 94, 87.7 and 83.8 mL for the three cultivars respectively. It is however important to note that the optima obtained were with respect to cooking, cooling and then mashing at 60°C as described in the Materials and Methods. The response surface methodology will obviously give different results if other temperature regimes taking into consideration the high thermostability of the α-amylase (temperature optimum at 93–95°C) are applied.

CONCLUSIONS

The α-amylase was the main enzyme component responsible for starch liquefaction during the mashing of unmalted sorghum. The β-amylase, Filtrase and protease served basically as supporting enzymes. The release and hydrolysis (liquefaction) of starch were facilitated by the sequential actions of filtrase, which hydrolysed cell wall materials, and protease, which facilitated the breakdown of released protein materials and thus enhanced starch release. The β-amylase complements the dominant action of α-amylase in starch liquefaction and extract develop-

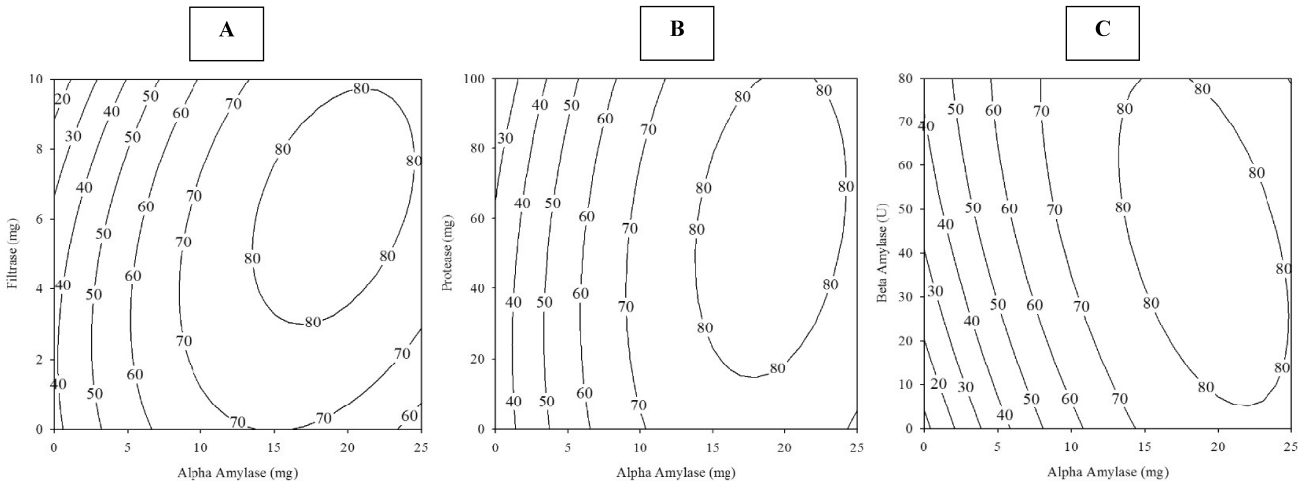


Fig. 7. Response surface curves for *S.35* yield in filtrates [all other factors were fixed at optimal quantity: (A) Protease: 58.76 mg; β-amylase: 43.48 U. (B) Filtrase: 6.36 mg; β-amylase: 43.48 U. (C) Filtrase: 6.36 mg; Protease: 58.76 mg].

ment. In general, the response surface methodology appears to be a very reliable tool in assessing the scope and the actions of mashing enzymes, as single or combined components, in starch liquefaction during mashing. This methodology should also be helpful in predicting the development of important wort parameters such as soluble nitrogen, free α-amino nitrogen, extract content, fermentability and viscosity.

ACKNOWLEDGEMENTS

The authors are grateful to the University of Ngaoundere Research Funding Program (GPRU) for funding this research. They also thank DSM France for supplying the enzyme kits used in this work.

REFERENCES

1. Agu, R. C. and Palmer, G. H., Effect of mashing with commercial enzymes on the properties of sorghum worts. *World J. Microbiol. Biotechnol.,* 1998, **14,** 43-48.
2. Agu, R. C., Ugwu, A. H., Okenchi, M. U., Aneke, I. B. and Anyanwu, T. U., Effect of low kilning temperatures on diastase and cellulase development of Nigerian malted sorghum (Sorghum bicolor). *Process Biochem.,* 1995, **31,** 63-68.
3. Aisien, A. O., Enzymic modification of sorghum endosperm during seedling growth and malting. *J. Sci. Food Agric.,* 1982, **33,** 754-759
4. Aniche, G. N. and Palmer, G. H., Development of amylolytic activities in sorghum and barley malt. *J. Inst. Brew.,* 1990, **96,** 377-379.
5. Arri, B. K., Problems associated with the use of sorghum for lager beer production. Industrial utilization of sorghum: Symposium on the Current Status and Potential of Industrial Uses of Sorghum. ICRISAT: Kano, Nigeria, 1989.
6. Bajomo, M. F. and Young, T. W., Development of profile for the use of microbial enzymes in brewing with raw sorghum (80%) and malted barley of sorghum malt (20%). *J. Inst. Brew.,* 1992, **98,** 515-523.
7. Dale, C. J., Young, T. W. and Omole, T., Small scale mashing experiments with grist containing high proportions of raw sorghum. *J. Inst. Brew.,* 1990, **96,** 403-409.
8. Dufour, J. P. and Melotte, L., Sorghum malts for the production of a lager beer. *J. Am. Soc. Brew. Chem.,* 1992, **50,** 110-119.
9. EtokApan, O. U. and Palmer, G. H., Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. *World J. Microbiol. Biotechnol.,* 1990, **6,** 408-417.
10. Goode, L. D. and Arendt, E. K., Pilot scale production of lager beer from grist containing 50% unmalted sorghum. *J. Inst. Brew.,* 2003, **109,** 208-217.
11. Goode, L. D., Halbert, C. and Arendt, E. K., Mashing studies with unmalted sorghum and malted barley. *J. Inst. Brew.,* 2002, **108,** 465-473.
12. Goode, L. D., Halbert, C. and Arendt, E. K., Optimisation of mashing conditions when mashing with unmalted sorghum and commercial enzymes. *J. Am. Soc. Brew. Chem.,* 2003, **61,** 69-78.
13. Hallgren, L., Lager beers from sorghum. In: Sorghum and millets. Chemistry and Technology DAV Dendy, American Association of Cereal Chemists: Minneapolis, 1995, pp. 283-297.
14. Little, B. T., Alternative cereals for beer production. *Ferment,* 1994, **7,** 163-168.
15. MacFadden, D. P. and Clayton, M., Brewing with sorghum-use of exogenous enzymes. *Brew. Bev. Ind. Int.,* 1989, **1,** 71-81.
16. Mathieu, D., Feneuille, D. and Phan-Tan-Luu, R., Methodologie de la recherche expérimentale: Etude des surfaces de réponse, Laboratoire de Prospective Reactionnelle et d'Analyse de l'Information, IUT de l'Université d'Aix-Marseille, 1977.
17. Nso, E. J., Ajebesone, P. E., Mbofung, C. M. and Palmer, G. H., Properties of three sorghum cultivars used in the production of Bili-Bili beverage in Northern Cameroon. *J. Inst. Brew.,* 2003, **109,** 245-450.
18. Nso, E. J., Nanadum, M. and Palmer, G. H., The effect of formaldehyde on enzyme development in sorghum malts. *Tech. Q. Master Brew. Assoc. Am.,* 2006, **43,** 177-182.
19. Obliana, A. T., Sorghum seed production and grain utilisation in the brewing industries: the Nigerian experience, Proceedings of 2nd Workshop on Seed Production and Utilisation, Lusaka, Zambia, 1990.
20. Okafor, N., Prospects and challenges of the use of local cereals and starchy substrates in brewing. National symposium on the Brewing Industry, Now and the 21st Century. Owerri. Nigeria, 1985, pp. 22-25.
21. Palmer, G. H., Cereals in malting and brewing. In: Cereal Science and Technology. G. H. Palmer Ed., Aberdeen University Press: Aberdeen, Scotland, 1989, pp. 61-462.

(Manuscript accepted for publication February 2010)

Journal of Brewing and Distilling Vol. 2(1) pp. 5-15, February 2011
Available online http://www.academicjournals.org/JBD
ISSN 2141-2197 ©2011 Academic Journals

Full Length Research Paper

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

Zangué S. C. Desobgo[2], Emmanuel J. Nso[1]* and Dzudie Tenin[1]

[1]Department of Process Engineering, National School of Agro- Industrial Sciences (ENSAI) of Ngaoundere, Cameroon.
[2]Department of Food Processing and Quality Control, University Institute of Technology (IUT) of Ngaoundere, Cameroon.

Accepted 14 January, 2011

A three factor Doehlert design was used to develop a statistical model to optimize the action of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) on reducing sugars content of the worts of unmalted and malted *Safrari* sorghum. The response surface methodology revealed that increasing amounts of Hitempase considerably increased reducing sugars content during mashing of unmalted *Safrari* sorghum grist to about 90 g/L. Limited amounts of reducing sugars were obtained with increasing concentrations of both Bioglucanase (≈ 40 g/L) and Brewers Protease (≈ 30 g/L). The milling process facilitated the dissolution of about 10 g/L in yields of reducing sugars for the unmalted *Safrari* sorghum mash type without the help of enzyme. None of the three enzymes as sole mashing enzyme appeared to be of use in mashing malted *Safrari*, as reducing sugars yields were at maximum (168 g/L) after dissolving the grist in water and rather decreased with increasing amounts of enzyme supplements. Optimizing the concerted actions of the three enzymes for reducing sugars content of unmalted *Safrari* sorghum mash gave a combination of 2163 U, 937.5 BGU and 0 mg for Hitempase, Bioglucanase and Brewers Protease respectively. This gave a maximal reducing sugars content of 126.57 g/L. This combination was 0 U, 137.48 BGU and 0 mg for malted *Safrari* sorghum mash, giving a maximal reducing sugars yield of 168.56 g/L.

Key words: Response surface methodology, optimization, mashing enzymes, *Safrari*, reducing sugars

INTRODUCTION

Sorghum (*Sorghum bicolor (L.) Moench)* is a vital calorie-based food component in human nutrition in some parts of Africa (Taylor, 2004). Besides this fundamental function, sorghum is used in the production of beer, traditional opaque beer and non-alcoholic drinks in developing countries, and also industrial beer (Palmer, 1989; Taylor and Dewar, 2001). The poor developmental profile of the principal hydrolytic enzymes during malting of this cereal is however a limiting factor to easy mashing of its malts as compared to barley malt (EtokAkpan and Palmer, 1990; EtokAkpan, 1992). This was ascribed to the malting procedures and varietal types of sorghum used. Work on some popular sorghum cultivars of Northern Cameroon used in brewing the traditional beer Bili-Bili confirmed that the profile of hydrolytic enzymes and the levels of fermentable sugars during mashing were indeed cultivar-dependent. The *Safrari* and *S.35* sorghum cultivars were shown to be poorer in starch than the *Madjeru* cultivar, but had higher hydrolytic enzymes profiles and fermentable sugars potentials (Nso et al., 2003; Nso et al., 2006). The use of commercial enzymes when mashing with sorghum in order to obtain better wort specifications for beer brewing has however become a common practice (MacFadden and Clayton, 1989;Dale et al., 1990; Bajomo and Young, 1992; Agu and Palmer, 1998; Goode et al., 2002; Goode and Arendt, 2003; Goode et al., 2003). However, it is not clear whether the use of mashing enzyme supplements in *Safrari* sorghum

*Corresponding author. E-mail: nso_emmanuel@yahoo.fr.

cultivar mashes are indispensable in alleviating the levels of fermentable sugars in its worts. In this work, the action of three principal commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers Protease) on the reducing sugars content of worts from the *Safrari* sorghum cultivar (unmalted and malted) was modeled and optimized using the response surface methodology (RSM) to verify the necessity of mashing enzymes in mashing with this cultivar.

MATERIALS AND METHODS

Enzymes

The characteristics of the commercial mashing enzymes used, hitempase 2xl®, a thermo stable α-amylase from Baccillus licheniformis, Brewers protease from bacillus amyloliquefaciens and bioglucanase tx, from an enzymatic composition of β-glucanase and hemicellulases from trichoderma reesei) and their sources are presented in Table 1.

Sorghum cultivar

The *Safrari* sorghum cultivar was obtained from the Institute of Research and Agronomic Development (IRAD) Maroua, Cameroon.

Modeling

Modeling was carried out as previously described (Desobgo et al., 2010). A Doehlert matrix design of 3 factors representing Hitempase 2XL (X_1), Bioglucanase TX (X_2) and Brewers Protease (X_3) at ranges of [0 to 3000 U], [0 to 937.5 BGU] and [0 to 100 mg] respectively, was used. The transformed matrix of coded variables to an experimental matrix and the desired response (reducing sugars) are shown in Table 2. Mathematical models describing the relationships among the process dependent variable and the independent variables in a second-order equation were developed (Giovanni, 1983). Design-based experimental data were matched according to the following second-order polynomial Equation [1].

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \quad [1]$$

where, I and j, are linear and quadratic coefficients respectively, while 'β', the regression coefficient, k the number of factors studied and optimized in the experiment and 'ε' is random error.

The coefficients of the models and the models were obtained using the Systat version 12 software (Systat Software, Inc., San Jose, USA). This software also gives a statistical analysis on the model. Lastly, the curves were plotted using Sigmaplot version11 build 11.0.0.77 software (WPCubed, GmbH, Germany).

Validation of models

The quality of fit of the second order equations was expressed by the coefficient of determination R^2. The models were validated using two differents methods. The first method was the Absolute Average Deviation (AAD) method (Bas and Boyac, 2007), while the second method consisted in applying the bias factor and accuracy factor (Ross, 1996; Baranyi et al., 1999).

Malting

One kilogram of Safrari sorghum cultivar grains was washed three times using 3 L of distilled water to remove dirt and foreign bodies. The grains were steeped in 3 L of distilled water for 48 h at room temperature (≈25°C) with 3 changes of water at intervals of 12 h before steep out. Germination was carried out for 4 days in a Heraeus type incubator (D-63450 Hanau, Germany) at a temperature of 25°C with water sprinkled on the grains on daily basis. The malt was then air dried at 40°C for 4 days using a CKA 2000 AUF-type dryer Ngaoundere; Cameroon. The malt was rubbed-off of its rootlets and stored in plastics sachets at -18°C until further use.

Mashing

Two hundred and fifty millilitres of distilled water were put into a 600 ml beaker and 50 g of sorghum (malted or unmalted) flour (Ø< 1 mm) added with continuous stirring until a homogenous mixture was obtained. This mixture was incubated at 45°C for 1 h in a water bath with intermittent stirring at intervals of 5 min. The mix was allowed to decant and 50 ml of the supernatant was withdrawn and kept aside. The temperature of the mash was then raised to boiling so as to gelatinize sorghum starch for 40 min with intermittent stirring at intervals of 5 min before cooling to 65°C. The 50 ml of supernatant to the which commercial enzyme/s is/are added according to the Doehlert matrix design of 3 factors, were added to the mash and allowed to incubate (at 65°C) for 1 h 30 min with intermittent stirring at intervals of 10 min. The mash was filtered at 25°C for one 1 h 30 min using a Whatmann paper NO 42.

Determination of the content of Reducing Sugars

The reducing sugars content was determined using DNS reagent (Miller, 1959).

Optimization of models

Models were optimized as previously described (Desobgo et al., 2010). The intersection of the curves, representing the optimal zone, was highlighted.

RESULTS AND DISCUSSION

Modeling and validation of results

Optimisation of the action of mashing enzymes on the reducing sugars yields was carried out by modeling the experimental design required for laboratory purposes. Table 2 shows the results obtained after mashing unmalted and malted *Safrari* using the commercial mashing enzymes Hitempase (α-amylase), Bioglucanase (β-glucanase) and Brewers Protease (Protease). The mathematical models obtained for reducing sugars after mashing unmalted and malted *Safrari* were as follows respectively:

Table 1. Characteristics of commercial enzyme preparations used in mashing.

	Organism of origin	Activity	Description	Optimum temperature	Optimum pH	Recommended application level in adjuncts	Form
Hitempase 2XL	*Bacillus licheniformis*	4416.29 ± 19.34 U/ml	α-amylase	60 – 95°C	4 – 8	60 U/g	Solution
BioglucanaseTX	*Trichodermareesei*	750 BGU/ml	β-glucanase	60 °C	4.5 – 6.5	0.01 et 0.025 % (v/w)	Solution
Brewers protease	*Bacillus amyloliquefaciens*	1842.2 ± 1.8 mg FAN/min/ml	Protease	45 – 50°C (denatured at 85°C)	6.5 – 7.5	0.4 – 2 g/Kg	Solution

Hitempase 2XL and Bioglucanase TX were obtained from Kerry Bioscience; Kilnagleary, Carrigaline, Co. Cork, Ireland. Brewers Protease was obtained from DSM Food Specialities, Cedex France.

Table 2. Matrices of Doehlert coded and transformed experimental values.

Coded values			Transformed experimental values			Reducing sugars content (*Safrari* wort cultivar)					
Hit	Bio	Brew Prot	Hit (U)	Bio (BGU)	Brew Prot (mg)	Unmalted			Malted		
X_1	X_2	X_3	X_1	X_2	X_3	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res
0.000	0.000	0.000	1500	468.75	50	99.00	99.80	-0.80	130.50	131.98	-1.48
1.000	0.000	0.000	3000	468.75	50	94.00	84.36	9.64	110.50	109.91	0.59
0.500	0.866	0.000	2250	937.5	50	110.00	110.47	-0.47	108.73	104.90	3.83
-0.500	-0.866	0.000	750	0.00	50	71.00	63.88	7.12	108.91	113.31	-4.40
0.500	-0.866	0.000	2250	0.00	50	89.54	89.97	-0.43	107.93	105.45	2.48
-0.500	0.866	0.000	750	937.5	50	80.00	78.54	1.46	75.90	78.35	-2.45
0.500	0.289	0.816	2250	615.18	100	95.00	96.45	-1.45	141.50	146.27	-4.77
-0.500	-0.289	-0.816	750	312.32	0.0	79.62	68.95	10.67	161.67	157.74	3.93
0.500	-0.289	-0.816	2250	312.32	0.0	96.71	101.72	-5.01	92.50	95.54	-3.04
0.000	0.577	-0.816	1500	781.07	0.0	114.00	113.95	0.05	109.13	110.48	-1.35
-0.500	0.289	0.816	750	615.18	100	77.00	71.20	5.80	68.50	65.38	3.12
0.000	-0.577	0.816	1500	156.43	100	92.00	94.86	-2.86	109.07	107.42	1.65
0.000	0.000	0.000	1500	468.75	50	96.20	99.80	-3.60	131.00	131.98	-0.98
-1.000	0.000	0.000	0.000	468.75	50	24.00	26.35	-2.35	90.50	91.23	-0.73
-1.000	-0.866	-0.816	0.000	0.0	0.0	05.00	06.21	-1.21	166.98	166.53	0.45
0.000	0.000	0.000	1500	468.75	50	98.70	99.80	-1.10	133.05	131.98	1.07
0.000	0.000	0.000	1500	468.75	50	99.35	99.80	-0.45	134.00	131.98	2.02

$$Y_{SafSRed}(X_1, X_2, X_3) = 99.804 + 29.007X_1 + 10.153X_2 - 4.522X_3 + 3.376X_1X_2 - 5.800X_1X_3 - 13.731X_2X_3 - 41.448X_1^2 - 3.968X_2^2 - 0.818X_3^2 \quad [2]$$

$$Y_{SafMSRed}(X_1, X_2, X_3) = 131.976 + 9.344X_1 - 10.252X_2 - 9.124X_3 + 19.873X_1X_2 + 80.639X_1X_3 + 10.301X_2X_3 - 31.406X_1^2 - 31.500X_2^2 - 11.548X_3^2 \quad [3]$$

with: $Y_{SafSRed}(X_1, X_2, X_3)$, representing the mathematical model for unmalted *Safrari*; $Y_{SafMSRed}(X_1, X_2, X_3)$, the model for malted *Safrari*; X_1, Hitempase; X_2, Bioglucanase and X3

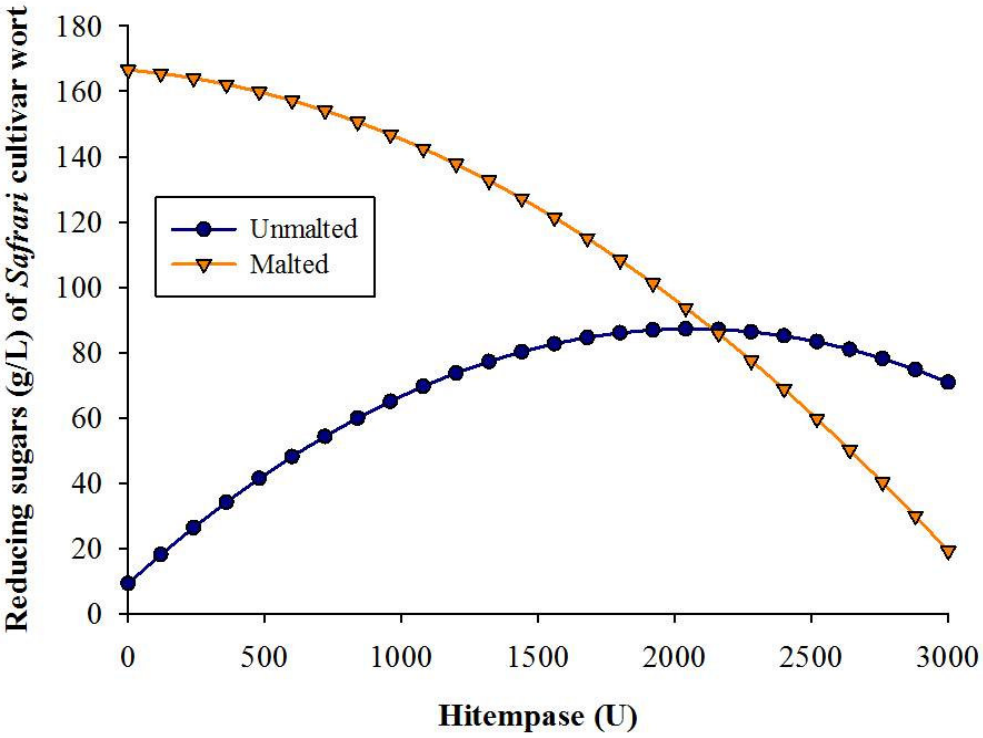


Figure 1A. Effect of concentration of Hitempase (α-amylase) as sole mashing enzyme on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

Brewers Protease.

These mathematical models are polynomials having several variables with correlation coefficients R2 = 0.975 for unmalted Safrari and R2 = 0.990 for malted Safrari. These coefficients, coupled to AAD values of 0.101 and 0.021 (Bas and Boyac, 2007) for unmalted and malted Safrari respectively, allowed for the validation of the models for the wort reducing sugars yields. In addition, bias factors of 1.04 and 1.00 for unmalted and malted Safrari respectively, coupled to accuracy factors of 1.08 and 1.02 for unmalted and malted Safrari respectively, also allowed for validation of the models according to the method described (Ross, 1996; Baranyi et al., 1999). The factors of the models were linear or of first degree (X1, X2 and X3), quadratic or of the second degree (X12, X22 and X32) and of interacting form (X1X2, X1X3, X2X3). They were statistically considered significant or not if the probability (P) of increasing reducing sugars yields was ≤ 0.05 or ≥ 0.05 respectively (Table 3).

Effect of Hitempase 2XL on reducing sugars production

The impact of Hitempase as the sole mashing enzyme on the yields of reducing sugars of unmalted and malted Safrari is shown in Figure 1A. Reducing sugars yields increased with increasing amounts of enzyme for unmalted Safrari mash to attain a maximum level (≈ 90 g/L) at about 2000 U, followed by a slight and steady decrease thereafter. The reducing sugars yield was maximal (≈ 168 g/L) even in absence of Hitempase for malted Safrari mash. Indeed, increasing concentrations of enzyme instead resulted into a steady decrease of reducing sugars content for malted Safrari mash. The maximal reducing sugars content observed for malted Safrari mash in absence of Hitempase was however attributed to the natural virtues that the malting process imparts to mashing cereals in terms of synthesis and development of hydrolytic enzymes. This explains why though known to be the principal mashing enzyme, supplements of Hitempase (α-amylase) seemed to be of no use in producing reducing sugars as the natural enzyme synthesized during malting seemed to be in sufficient amounts to ensure starch hydrolysis. When the mathematical models were applied to predict the impact of supplements of Bioglucanase and Brewers Protease at concentrations of 400 BGU and 60 mg respectively as accompanying mashing enzymes to Hitempase, it was observed that the profile of reducing sugars increased with increasing amounts of Hitempase for malted *Safrari* mash though the amounts in the absence of enzyme dropped by roughly two folds (Figure 1B). In both mash types, the milling operation facilitated the dissolution of reducing sugars as could be seen in Figure 1A. This

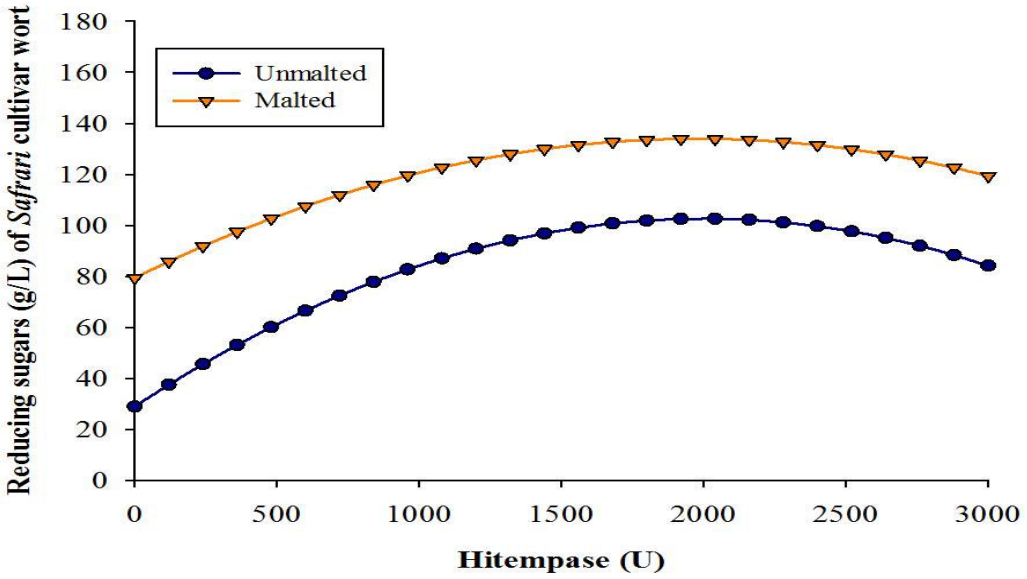


Figure 1B. Effect of concentration of Hitempase (α-amylase) in the presence of fixed concentrations of Bioglucanase (400 BGU) and Brewers protease (60 mg) on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

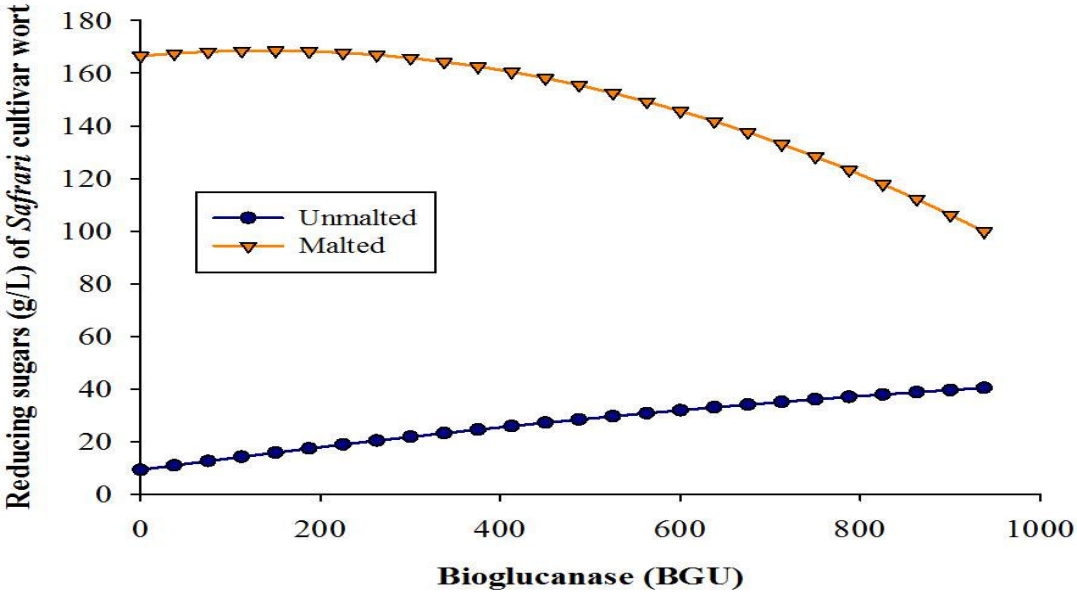


Figure 2A. Effect of concentration of Bioglucanase as sole mashing enzyme on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

dissolution accounted for total reducing sugars contents (168 g/L) for malted *Safrari* mash and for 10 g/L for unmalted Safrari mash. Starch is indeed the main macromolecule of cereals and the main substrate of α-amylase. It is therefore expected that Hitempase contributes to the greatest amounts of reducing sugars in resulting worts due to its action on starch (Goode et al., 2003; Phiarais et al., 2006; Desobgo et al., 2010). The steady decrease in yields of reducing sugars of malted mash could be attributed to Milliard reactions between soluble nitrogenous compounds and reducing sugars obtained earlier due to the action of hydrolytic enzymes synthesized during malting (Figure 1A) (Hough et al., 1982). From the mathematical models, it was shown that

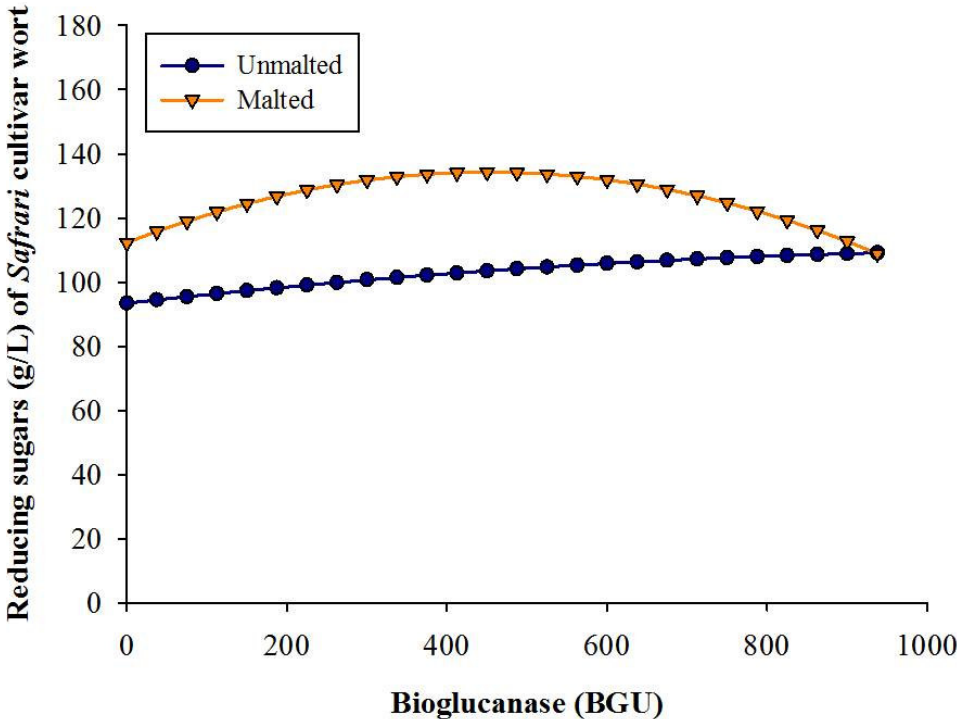


Figure 2B. Effect of concentration of Bioglucanase (β-glucanase) in the presence of fixed concentrations of Hitempase (2000 U) and Brewers protease (60 mg) on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

in its linear form (X_1), Hitempase's impact on reducing sugars yields was significant (P = 0.000 and 0.002) (Table 3) for both unmalted and malted *Safrari* mash types. This action contributed to 26and 4% for unmalted and malted *Safrari* respectively (Table 3). In its quadratic form (X_1^2), Hitempase's action remained statistically significant for both mash types (P = 0.000) (Table 3). Its contribution to increasing reducing sugar yields in this quadratic form (X_1^2) (excess of α-amylase in principle) is indeed 37 and 15% for unmalted and malted *Safrari* respectively (Table 3).

Effect of Bioglucanase TX on reducing sugars production

Figure 2A shows the effect of mashing unmalted and malted *Safrari* using Bioglucanase as only mashing enzyme on reducing sugars yields. There was a slight but constant increase in reducing sugars yield (≈ 40 g/L) as enzyme concentration increased for unmalted *Safrari* mash. This small yield of reducing sugars due to Bioglucanase's action on unmalted *Safrari* mash can be attributed to its ability to hydrolyse β-glucans into glucose and other soluble carbohydrates. Contrary to Hitempase, Bioglucanase was therefore not a backbone enzyme for reducing sugars production during mashing. The reducing sugars yield for malted *Safrari* mash was maximal even in the absence of Bioglucanase, and steadily decreased with increase in enzyme concentration. A similar application of the mathematical models as carried out above for Hitempase's action, using 60 mg of Brewers Protease and 2000 U Hitempase as accompanying enzymes, predicted that supplements of these two key mashing enzymes could provide similar results in reducing sugar yields for both unmalted and malted *Safrari* mashes (Figure 2B). Hitempase once more demonstrated that it was the backbone enzyme contributing to most of the reducing sugars. Figure 2B indeed showed that in the absence of Bioglucanase, but in the presence of Hitempase, yields of reducing sugars became comparable for both unmalted and malted *Safrari* mashes. In addition, the facilitator role of dissolving reducing sugars by the milling process was considerably accentuated. These observations were all statistically confirmed. Indeed, in its linear form (X_2), Bioglucanase's action was significant (P = 0.018 and 0.001 for unmalted and malted *Safrari* respectively (Table 3)). Table 3 showed that this enzyme contributed for 9 and 5% of reducing sugars for unmalted and malted *Safrari* respectively. In its quadratic form (X_2^2) (excess of enzyme in principle), Bioglucanase contributed for 3and 15% of reducing sugars for unmalted and malted *Safrari* respectively (Table 3). These contributions were not statistically significant for unmalted *Safrari* mash, but were for malted *safrari* mash (P = 0.492 and 0.000)

Table 3. Estimation of regression coefficients and enzyme contributions for the reducing sugars content of *Safrari.*

Effects	Coefficients		Standard Error		t		Probability		Enzyme Contributions (%)	
	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted
Constant	99.804	131.976	3.197	1.938	31.217	68.108	0.000	0.000		
X_1	29.007	9.344	3.289	1.993	8.82	4.688	0.000	0.002	26	4
X_2	10.153	-10.252	2.868	1.738	3.066	-5.108	0.018	0.001	9	5
X_3	-4.522	-9.124	2.71	1.642	-1.362	-4.533	0.216	0.003	4	4
X_1^2	-41.448	-31.406	5.638	3.417	-7.351	-9.19	0.000	0.000	37	15
X_2^2	-3.968	-31.5	4.103	2.487	-0.725	-9.499	0.492	0.000	3	15
X_3^2	-0.818	-11.548	3.54	2.145	-0.154	-3.584	0.882	0.009	1	5
X_1*X_2	3.376	19.873	6.437	3.901	0.454	4.411	0.663	0.003	3	9
X_2*X_3	-13.731	10.301	5.665	3.434	-1.713	2.12	0.130	0.072	12	5
X_1*X_3	-5.8	80.639	6.707	4.065	-0.706	16.186	0.503	0.000	5	38

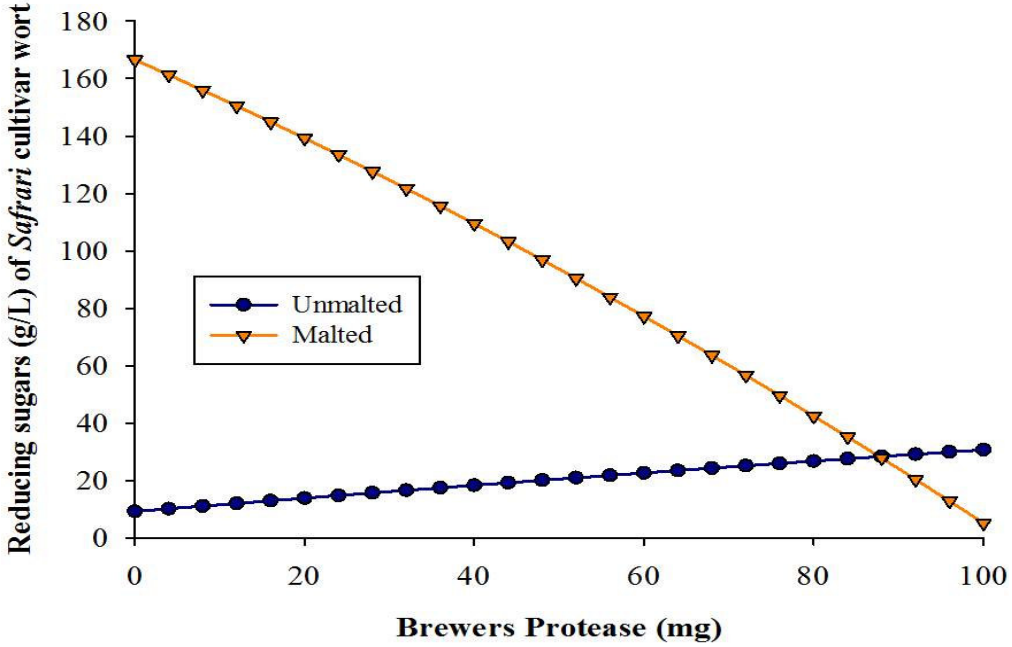


Figure 3A. Effect of concentration of Brewers Protease as sole mashing enzyme on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

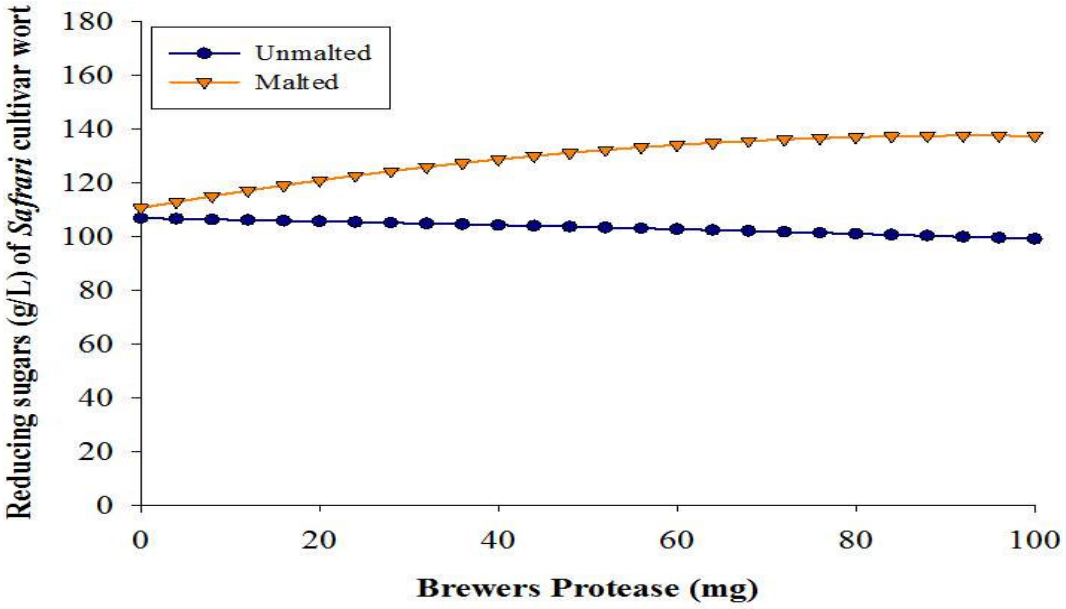


Figure 3B. Effect of concentration of Brewers protease in the presence of fixed concentrations of Hitempase (1875 U) and Bioglucanase (750 BGU) on reducing sugars content (g/L) of the worts of *Safrari* sorghum cultivar.

(Table 3). This 1 to 5 ratio difference in reducing sugars content between the unmalted and malted *Safrari* mash types could once more be attributed to the natural virtues of the malting process.

Effect of Brewers Protease on reducing sugars production

The effect of mashing unmalted and malted *Safrari* on yields of reducing sugars using sole mashing enzyme, Brewers Protease, is shown in Figure 3A. A small but constant increase in reducing sugars content with increasing enzyme concentrations was observed (≈ 30 g/L) for unmalted *Safrari* mash. The milling process once more contributed to about 10 g/L of these reducing sugars amounts. As observed for Bioglucanase, the maximal reducing sugars amounts were obtained when mashing with malted *Safrari* in the absence of supplements of Brewers Protease. This could once more be attributed to the virtues known to the malting process as earlier explained above. A high rate of decrease of reducing sugars content with increasing concentrations of Brewers Protease for the malted *Safrari* mash was observed. This could be explained by the fact that this enzyme released into the medium soluble nitrogenous materials (amino acids and others) which reacted with the reducing sugars (produced during the malting process), displaying as such the rapid decrease in the amounts of the sugars as observed in Figure 3A (Hough et al., 1982). The mathematical models were once more used to predict the yield in reducing sugars as carried out above for Hitempase and Bioglucanase actions. Thus, using 2000 U of Hitempase and 400 BGU of Bioglucanase as accompanying mashing enzymes with increasing amounts of Brewers Protease, similar results in reducing sugars yields for both unmalted and malted *Safrari* mashes were once more observed (Figure 3B). These observations were statistically confirmed. In its first degree form (X_3), the impact of Brewers Protease was not significant for unmalted *Safrari* mash but significant for malted *Safrari* mash (P = 0.216 and 0.003 respectively) (Table 3). Its contribution to reducing sugars was barely 4% for both mash types (Table 3). The impact of the enzyme in its quadratic form (X_3^2), was not significant for unmalted *Safrari* mash but significant for malted *Safrari* mash (P = 0.882 and 0.009 respectively) (Table 3). Its contribution to reducing sugars was 1 and 5% respectively (Table 3).

Effect of enzyme interactions on the production of reducing sugars

The models were further exploited to predict the impacts of the interactions (X_1X_2, X_1X_3 and X_2X_3) of these enzymes on yields of reducing sugars. The results are shown in Table 3. Globally, they were statistically not significant for unmalted *Safrari* mashes (P = 0.339), but were for malted *Safrari* mashes (P = 0.000) (Table 4). The interaction X_1X_2 (Hitempase/Bioglucanase) had no significant impact on unmalted *Safrari* mash, but had for

Table 4. ANOVA for the reducing sugars content of *Safrari.*

Source	dl	Sum square		Mean square		F		Probability	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	12650.765	11623.844	1405.641	1291.538	30.804	77.047	0.000	0.000
Linear	3	8527.165	2482.605	2842.388	827.535	62.29	49.367	0.000	0.000
Quadratic	3	3941.669	583.306	1313.89	194.435	28.793	11.599	0.000	0.004
Interactions	3	181.932	8557.933	60.644	2852.644	1.329	170.175	0.339	0.000
Residual error	7	319.423	117.341	45.632	16.763				
Total error	16	12970.188	11741.185						

Table 5. ANOVA for comparing reducing sugars content of unmalted and malted *Safrari* worts.

Source	dl	Sum square	Mean square	F	P
Inter-groups	1	9200.22	9200.22	11.91	0.0016
Intra-groups	32	24711.4	772.23		
Total	33	33911.6			

malted *Safrari* (P = 0.663 and 0.003 respectively (Table 3). It contributed for merely 3% of reducing sugars for unmalted *Safrari* and 9% for malted *Safrari* mash (Table 3). The interaction X_1X_3, corresponding to the couple Hitempase/Brewers Protease, also had no significant impact on yields of reducing sugars of unmalted *Safrari,* but had a significant impact on those of malted *Safrari* mashes (P = 0.503 and 0.000 respectively) (Table 3). Its contribution to reducing sugar yields was merely 5% for unmalted *Safrari*, but as much as 38% for malted *Safrari* (Table 3). This remarkable difference was once more to be attributed to the virtues of the malting process and not simply to supplements of the Hitempase/Brewers Protease couple as such. Efficient starch hydrolysis by α-amylase indeed occurs only after the breakdown of cereal grain cell walls by β-glucanase, followed by liberation of starch granules due to proteolysis of the protein matrix enrobing them. This should be the natural sequence of events which accounted for the results obtained for the malted sample type as compared to the unmalted *Safrari* sample type. The interaction Bioglucanase/Brewers Protease (X_2X_3) had no significant impact on reducing sugars yields for both the unmalted *Safrari* and malted *Safrari* mashes (P = 0.130 and 0.072 respectively) (Table 3). Its contribution to reducing sugars yields was 12 and 5% respectively for both mash types (Table 3). The Bioglucanase/Brewers Protease combination plays a supporting role in starch hydrolysis during mashing (Desobgo et al., 2010). Table 4 statistically confirmed the observation that *Safrari* sorghum malted type samples were more potential mashing materials than unmalted *Safrari* sorghum adjuncts to which commercial enzymes are supplemented for the production of worts of higher reducing sugars yields (P = 0.001).

Optimization of the concerted mashing enzymes' action on the production of reducing sugars

The results obtained for the action of the enzymes on reducing sugars yields after mashing on the basis of the models, were optimized to define a satisfactory domain of compromise for the action of the mashing enzymes. This domain was obtained for a reducing sugars content ≥ 90 g/L. The theoretical optimal combination of enzyme action for unmalted *Safrari* gave the following triplet of coded variables for reducing sugars content: 0.442, 0.866 and – 0.816 (2163 U, 937.5 BGU and 0 mg real variables) for Hitempase, Bioglucanase and Brewers Protease respectively.

This triplet allowed for a maximal reducing sugars content of 126.57 g/L. The triplet for malted *Safrari* was – 1, – 0.612 and – 0,816 (0 U, 137.48 BGU and 0 mg real variables). It allowed for a maximal reducing sugars content of 168.56 g/L. The optimal enzyme combinations were thus different and gave different results for the two mash types. These results showed that maximal reducing sugars contents could be obtained using Hitempase and Bioglucanase but not Brewers Protease supplements when mashing unmalted *Safrari*, whereas only Bioglucanase, and in limited amounts, could be used for mashing malted *Safrari*. The significant difference (P = 0.001) between reducing sugars content of unmalted and malted *Safrari* worts is shown in Table 5. Minimal reducing sugars content (90 g/L) could be obtained under these conditions permitting the highlight of the optimal domain (Figure 4).

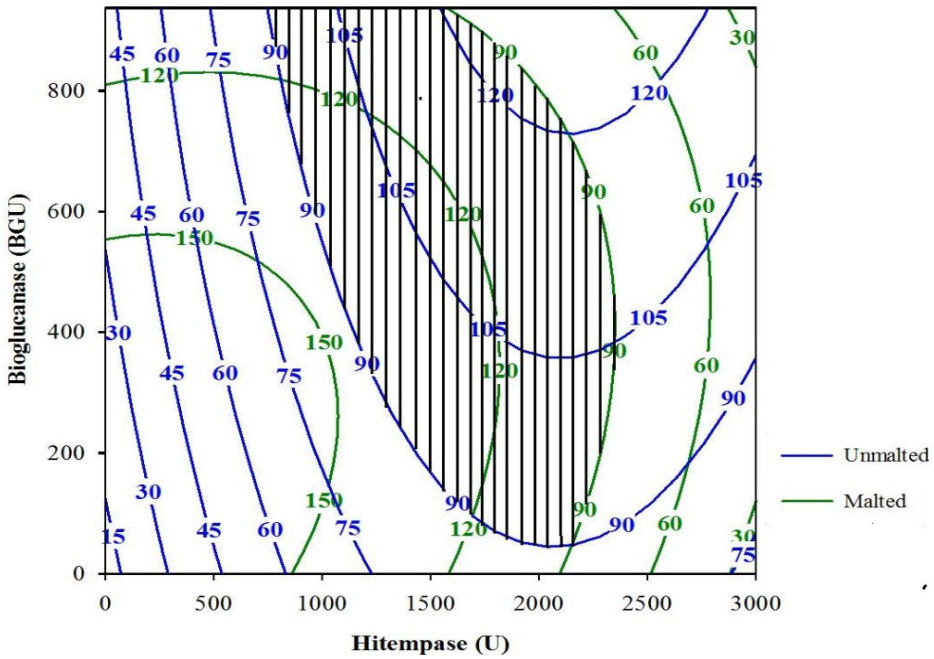


Figure 4. Response surface curves for the enzyme combinations providing for optimal reducing sugars content (g/L) for unmalted and malted sorghum worts of the cultivar *Safrari*.

Conclusion

This work clearly showed that though Hitempase 2XL was the most important enzyme component for producing significant amounts of reducing sugars during mashing of unmalted *Safrari* grist, it appeared to be indispensable for mashing malted *Safrari* grist. Bioglucanase TX and Brewers Protease were merely supporting enzymes to Hitampase 2XL only for mashing unmalted *Safrari* grist, as they too showed little or no participatory role in increasing reducing sugars amounts when used as supplements when mashing with malted *Safrari* grist - in general, the malting process seems to be auto-sufficient in accounting for the required natural mashing enzymes in terms of both amounts and quality, for producing the amounts of reducing sugars obtainable when mashing malted *Safrari* grist.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Process Engineering, National School of Agro- Industrial Sciences (ENSAI) of University of Ngaoundere (Cameroon) for providing necessary facilities for the successful completion of this research work.

REFERENCES

Agu RC, Palmer GH (1998). Effect of mashing with commercial enzymes on the properties of sorghum worts. World J. Micro-biol. Biotechnol., 14: 43-48.

Bajomo MF, Young TW (1992). Development of a mashing profile for the use of microbial enzymes in brewing with raw sorghum (80%) and malted barley or sorghum malt (20%). Journal of the Institute of Brewing, 98: 515-523.

Baranyi J, Pin C, Ross T (1999). Validating and comparing predictive models. International Journal of Food Microbiology 48:159-166.

Bas D, Boyac IH (2007). Modeling and optimization I: Usability of response surface methodology. J. of Food Engin, 78: 836-845.

Dale CJ, Young TW, Omole, T (1990). Small scale mashing experiments with grist containing high proportions of raw sorghum. J. Inst. Brew., 96: 403–409.

Desobgo ZSC, Nso EJ, Tenin D, Kayem GJ (2010). Modelling and optimizing of mashing enzymes-effect on yield of filtrate of unmalted sorghum by use of response surface methodology. J. Inst. Brew, 116: 62-69.

EtokAkpan OU (1992). Amylolytic potentials and wort fermenting components of nigerian sorghums and barley. World J. Microbiol. Biotechnol., 8: 287-289.

EtokAkpan OU, Palmer GH (1990). Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. World J. Micro-biol. Biotechnol., 6:408-417.

Giovanni M (1983). Response surface methodology and product optimization. Food Technol., 37:41-45.

Goode DL, Arendt EK (2003). Pilot scale production of lager beer from grist containing 50% unmalted sorghum. J. Inst. Brew, 109: 208-217.

Goode DL, Halbert C, Arendt EK (2002). Mashing studies with unmalted sorghum and malted barley. J. of the Institut of Brew 108:465-473.

Goode DL, Halbert C, Arendt EK (2003). Optimisation of mashing conditions when mashing with unmalted sorghum and commercial enzymes. J. Am. Soc. Brew Chem., 61:69-78.

Hough JS, Briggs DE, Stevens R, Young TW (1982). In: Malting and brewing science. London: Chapman and Hall.MacFadden DP, Clayton M (1989). Brewing with sorghum – Use of exogenous enzymes. Brewing and Beverage Industry International 1:71–81.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428.

Nso EJ, Ajebesone PE, Mbofung CM, Palmer GH (2003). Properties of three sorghum cultivars used for the production of *Bili-Bili* beverage

in Northern Cameroon. J. Inst. Brewing, 109: 245-250.
Nso EJ, Nanadoum M, Palmer GH (2006). The effect of formaldehyde on enzyme development in sorghum malts. MBAA TQ 43:177-182.
Palmer GH (1989). Cereal in malting and brewing. In: Cereal science and technology(Palmer, G., ed) Aberdeen, Scotland: Aberdeen University Press.
Phiarais NB, Schehl BD, Oliveira JC, Arendt EK (2006). Use of surface response methodology to investigate the effectiveness of commercial enzymes on buckwheat malt for brewing purpose. J. Inst. Brew, 112: 324-332.
Ross T (1996). Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol., 81: 501-508.
Taylor JRN (2004). Overview: In importance of sorghum in Africa. Department of Food Science, Pretoria, South Africa: University of Pretoria.
Taylor JRN, Dewar J (2001). Developments in sorghum food technologies. In: Advances in Food and Nutrition Research(Taylor, J., ed), pp. 217-264 San Diego, USA: Academic Press.

African Journal of Food Science Vol. 5(2), pp. 91 - 99 February 2011
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2011 Academic Journals

Full Length Research Paper

Use of the response surface methodology for optimizing the action of mashing enzymes on wort reducing sugars of the *Madjeru* sorghum cultivar

Zangué Desobgo S. C.[2], Emmanuel Nso J.[1]* and Dzudie Tenin[1]

[1]Department of Process Engineering, National School of Agro- Industrial Sciences (ENSAI) of Ngaoundere, Cameroon.
[2]Department of Food Processing and Quality Control, University Institute of Technology (IUT) of Ngaoundere, Cameroon.

Accepted 24 January, 2011

A three factor Doehlert design was used to develop a statistical model to optimize the action of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) on reducing sugars content of the worts of unmalted and malted *Madjeru* sorghum. The response surface methodology revealed that increasing amounts of Hitempase considerably increased reducing sugars content during mashing of unmalted and malted *Madjeru* sorghum grist to about 105.39 g/L and 132.25 g/L respectively. The milling process contributed to about 22 g/L and 54 g/L for the unmalted and malted mash types respectively. Increasing amounts of Bioglucanase was virtually insignificant, while for Brewers protease, reducing sugar yields rather decreased to nil for both the unmalted and malted mash types. Optimization of the concerted actions of the three enzymes for reducing sugars content of unmalted *Madjeru* sorghum mash gave a combination of 1995 U, 89.31 BGU and 28.86 mg for Hitempase, Bioglucanase and Brewers Protease respectively. This gave a maximal reducing sugars content of 108.78 g/L. This combination was 3000 U, 0 BGU and 49.69 mg for malted *Madjeru* sorghum mash, giving a maximal reducing sugars yield of 153.15 g/L.

Key words: Response surface methodology, optimization, mashing enzymes, *Madjeru*, reducing sugars.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an important tropical cereal-bearing monocotyledonous plant found in the semi-arid areas of the world. It is a vital calorie-based food component in humane nutrition in some parts of Africa (Taylor, 2004).

Besides this fundamental function, sorghum is used in the production of traditional opaque beer and non-alcoholic drinks in developing countries and, until recently, in the production of industrial beer (Palmer, 1989; Taylor and Dewar, 2001). The low synthesis and development of principal hydrolytic enzymes during malting of this cereal is however a limiting factor for easy mashing of its malts as compared to barley malt (EtokAkpan and Palmer, 1990; EtokAkpan, 1992). This was ascribed to the malting procedures and varietal types of sorghum used. Work on some popular sorghum cultivars of Northern Cameroon used in brewing the traditional beer *Bili-Bili* confirmed that the profile of hydrolytic enzymes and the levels of fermentable sugars during mashing were indeed cultivar-dependent. The *Madjeru* sorghum cultivar was shown to be richer in starch than the *Safrari* and *S.35* cultivars, but had poorer hydrolytic enzymes and fermentable sugar potentials (Nso et al., 2003; 2006).

The use of commercial enzymes when mashing with sorghum in order to obtain better wort specifications for

*Corresponding author. E-mail: nso_emmanuel@yahoo.fr.

Table 1. Characteristics of commercial enzyme preparations used in mashing.

	Organism of origin	Activity	Description	Optimum temperature	Optimum pH	Recommended application level in adjuncts	Form
Hitempase 2XL	*Bacillus licheniformis*	4416.29 ± 19.34 U/ml	α-amylase	60 – 95°C	4 – 8	60 U/g	Solution
BioglucanaseTX	*Trichodermareesei*	750 BG U/ml	β-glucanase	60°C	4.5 – 6.5	0.01 et 0.025 % (v/w)	Solution
Brewers Protease	*Bacillus amyloliquefaciens*	1842.2 ± 1.8 mg FAN/min/mL	Protease	45 – 50°C (denatured at 85°C)	6.5 – 7.5	0.4 – 2 g/Kg	Solution

Hitempase 2XL and Bioglucanase TX were obtained from Kerry Bioscience; Kilnagleary, Carrigaline, Co. Cork, Ireland. Brewers Protease was obtained from DSM Food Specialities, Cedex France.

beer brewing has however become common practice (Bajomo and Young, 1992, Agu and Palmer, 1998; Goode et al., 2002; 2003; Goode and Arendt, 2003,).

It is not however clear how far mashing enzyme supplements in *Madjeru* sorghum cultivar mashes could help alleviate the levels of fermentable sugars in its worts. In this work, the action of three principal commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers Protease) on the reducing sugars content of worts from the *Madjeru* sorghum cultivar (unmalted and malted) was modeled and optimized using the response surface methodology (RSM).

MATERIALS AND METHODS

Enzymes

The characteristics of the commercial enzymes used (Hitempase 2XL, a thermo stable α-amylase from *Baccillus licheniformis,* Brewers Protease from *Bacillus amyloliquefaciens* and Bioglucanase TX, from an enzymatic composition of β-glucanase and hemicellulases from *Trichoderma reesei*) and sources are presented in Table 1.

Sorghum cultivar

The *Madjeru* sorghum cultivar was obtained from the Institute of Research and Agronomic Development (IRAD) Maroua, Cameroon.

Modeling

Modeling was carried out as previously described (Desobgo et al., 2010). A Doehlert matrix design of 3 factors representing Hitempase 2XL (X_1), Bioglucanase TX (X_2) and Brewers Protease (X_3) at ranges of [0-3000 U], [0-937.5 BGU] and [0-100 mg] respectively, was used. The transformed matrix of coded variables to an experimental matrix and the desired response (reducing sugars) are shown in Table 2. Mathematical models describing the relationships among the process dependent variable and the independent variables in a second-order equation were developed (Giovanni, 1983).

Design-based experimental data were matched according to the following second-order polynomial Equation 1.

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

Where, I and j, are linear and quadratic coefficients respectively, while 'β', the regression coefficient, k the number of factors studied and optimized in the experiment and 'ε' is random error. The coefficients of the models and the models were obtained using the Systat version 12 software (Systat Software, Inc., San Jose, USA). This software also gives a statistical analysis on the model. Lastly, the curves were plotted using Sigmaplot version11 build 11.0.0.77 software (WPCubed, GmbH, Germany).

Validation of models

The quality of fit of the second order equations was expressed by the coefficient of determination R^2. The models were validated using two differents methods. The first method was the absolute average deviation (AAD) method (Bas and Boyac, 2007), while the second method consisted in applying the bias factor and accuracy factor (Ross, 1996; Baranyi et al., 1999).

Malting

One kilogram of *Madjeru* sorghum cultivar grains was washed three times using 3 L of distilled water to remove dirt and foreign bodies. The grains were steeped in 3 L of distilled water for 48 h at room temperature (≈25°C) with 3 changes of water at intervals of 12 h before steep out. Germination was carried out for 4 days in a Heraeus type oven (D-63450 Hanau, Germany) at a temperature of 25°C with water sprinkled on the grains on daily basis. The malt was then air dried at 40°C for 4 days using a CKA 2000

Table 2. Matrices of Doehlert coded and transformed experimental values.

Coded values			Transformed experimental values			Reducing sugars content(g/L)(*Madjeru* wort cultivar)					
Hit	Bio	Brew Prot	Hit (U)	Bio (BGU)	Brew Prot (mg)	Unmalted			Malted		
X_1	X_2	X_3	X_1	X_2	X_3	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res
0.000	0.000	0.000	1500	468.75	50	100.81	102.57	-1.76	124.00	123.08	0.92
1.000	0.000	0.000	3000	468.75	50	85.55	78.61	6.94	137.59	132.66	4.93
0.500	0.866	0.000	2250	937.5	50	87.56	87.51	0.05	106.21	109.81	-3.60
-0.500	-0.866	0.000	750	0.00	50	80.34	73.74	6.60	76.30	77.63	-1.33
0.500	-0.866	0.000	2250	0.00	50	104.64	105.30	-0.66	140.30	142.90	-2.60
-0.500	0.866	0.000	750	937.5	50	76.98	76.93	0.05	99.27	96.23	3.04
0.500	0.289	0.816	2250	615.18	100	79.65	82.49	-2.84	114.47	118.89	-4.42
-0.500	-0.289	-0.816	750	312.32	0.0	88.61	75.79	12.82	93.99	96.95	-2.96
0.500	-0.289	-0.816	2250	312.32	0.0	94.01	100.67	-6.66	115.48	117.55	-2.07
0.000	0.577	-0.816	1500	781.07	0.0	89.28	90.00	-0.72	88.89	87.90	0.99
-0.500	0.289	0.816	750	615.18	100	70.97	65.23	5.74	63.37	60.65	2.72
0.000	-0.577	0.816	1500	156.43	100	80.02	82.93	-2.91	79.34	77.66	1.68
0.000	0.000	0.000	1500	468.75	50	97.32	102.57	-5.25	126.00	123.08	2.92
-1.000	0.000	0.000	0.000	468.75	50	31.20	36.47	-5.27	47.65	53.82	-6.17
-1.000	-0.866	-0.816	0.000	0.0	0.0	17.40	22.85	-5.45	58.95	54.94	4.01
0.000	0.000	0.000	1500	468.75	50	100.03	102.57	-2.54	125.00	123.08	1.92
0.000	0.000	0.000	1500	468.75	50	104.39	102.57	1.82	123.00	123.08	-0.08

[a]Experimental result values. [b]Theretical values (values coming from mathematical models). [c] Residue. Hit: Hitempase 2XL, Bio: Bioglucanase TX, Brew Pro: Brewers Protease.

AUF-type dryer Ngaoundere, Cameroon. The malt was rubbed-off of its rootlets and stored until further use.

Mashing

Two hundred and fifty (250) ml of distilled water were put into a 600 ml beaker and 50 g of sorghum (malted or unmalted) flour (Ø < 1 mm) added with continuous stirring until a homogenous mixture was obtained. This mixture was incubated at 45°C for 1 h in a water bath with intermittent stirring at intervals of 5 min. The mix was allowed to decant and 50 ml of the supernatant withdrawn and kept aside. The temperature of the mash was then raised to boiling so as to gelatinize sorghum starch during 40 min with intermittent stirring at intervals of 5 min before cooling to 65°C. The 50 ml of supernatant to the which commercial enzyme/s is/are added according to the Doehlert matrix design of 3 factors, were added to the mash and allowed to incubate for 1 h 30 min with intermittent stirring at intervals of 10 min. The mash was filtered at 25°C during 1h 30 min using Whatmann paper N° 42.

Determination of reducing sugars contents

The reducing sugars content was determined using DNS reagent (Miller, 1959).

Optimization of models

Models were optimized as previously described (Desobgo et al., 2010). The intersection of the curves, representing the optimal zone, was highlighted.

RESULTS AND DISCUSSION

Optimization of the action of mashing enzymes on reducing sugars was carried out by modeling the experimental design required for manipulation in laboratory. Table 2 shows the results obtained for reducing sugars after mashing unmalted and malted *Madjeru* using Hitempase 2XL (α-amylase), Bioglucanase TX (β-glucanase) and Brewers Protease (Protease).

Modeling and validation of results

The mathematical models (Equations 2 and 3) obtained for reducing sugars after mashing unmalted and malted *Madjeru* were as follows respectively:

$$Y_{MadSRed}(X_1, X_2, X_3) = 102.573 + 21.070X_1 - 4.217X_2 - 7.312X_3 - 12.118X_1X_2 - 0.378X_1X_3 + 5.688X_2X_3 - 45.031X_1^2 - 7.261X_2^2 - 16.534X_3^2 \quad (2)$$

$$Y_{MadMSRed}(X_1, X_2, X_3) = 123.077 + 39.421X_1 - 4.181X_2 - 9.230X_3 - 29.849X_1X_2 + 33.635X_1X_3 + 28.583X_2X_3 - 29.835X_1^2 - 11.971X_2^2 - 34.318X_3^2 \quad (3)$$

Table 3. Estimation of regression coefficients and enzyme contributions for the reducing sugars content of *Madjeru*.

Effects	Coefficients		Standard error		t		Probability		Enzyme contributions(%)	
	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted	Unmalted	malted
Constant	102.573	123.077	3.79	2.304	27.066	53.413	0.000	0.000		
X_1	21.07	39.421	3.898	2.37	5.405	16.631	0.001	0.000	18	18
X_2	-4.217	-4.181	3.399	2.067	-1.074	-1.752	0.318	0.123	3	2
X_3	-7.312	-9.23	3.212	1.953	-1.858	-3.856	0.106	0.006	6	4
X_1^2	-45.031	-29.835	6.683	4.064	-6.738	-7.342	0.000	0.000	38	13
X_2^2	-7.261	-11.971	4.864	2.958	-1.12	-3.036	0.300	0.019	6	5
X_3^2	-16.534	-34.318	4.196	2.551	-2.624	-8.957	0.034	0.000	14	16
X_1*X_2	-12.118	-29.849	7.63	4.639	-1.375	-5.572	0.211	0.001	10	14
X_2*X_3	5.688	28.583	6.715	4.083	0.599	4.947	0.568	0.002	0	15
X_1*X_3	-0.378	33.635	7.951	4.834	-0.039	5.677	0.970	0.001	5	13

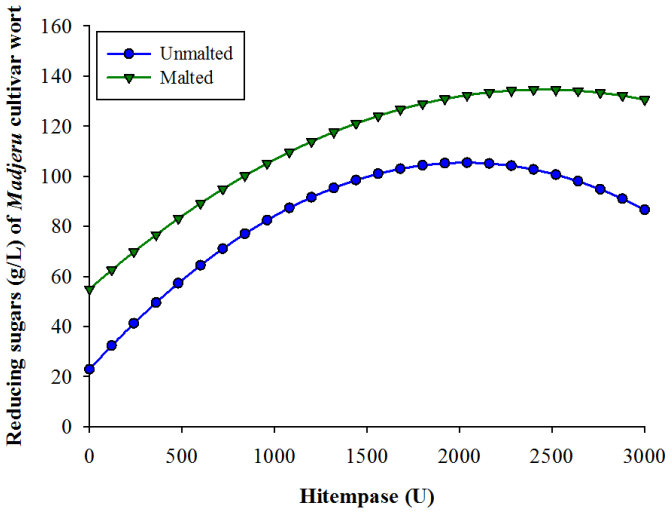


Figure 1A. Effect of concentration of Hitempase (α-amylase) as sole mashing enzyme on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

With :$Y_{MadSRed}(X_1, X_2, X_3)$ representing the mathematical model for unmalted *Madjeru*; $Y_{MadMSRed}(X_1, X_2, X_3)$, the model for malted *Madjeru*: X_1, Hitempase; X_2, Bioglucanase and X_3, Brewers Protease.

These mathematical models are polynomials having several variables with correlation coefficients R^2 = 0.951 for unmalted *Madjeru* and R^2 = 0.987 for malted *Madjeru*. These coefficients, coupled to AAD values of 0.067 and 0.032 obtained by the method (Bas and Boyac, 2007) for unmalted and malted *Madjeru* respectively, allowed for the validation of the models for the wort reducing sugars. In addition, a bias factor (1.01 and 1 for unmalted and malted *Madjeru*), coupled to accuracy factors of 1.07 and 1.03 for both unmalted and malted *Madjeru* respectively, also allowed for validation of the models according to the method described (Ross, 1996; Baranyi et al., 1999). The factors of the models were linear or of first degree (X_1, X_2 and X_3), quadratic or of the second degree (X_1^2, X_2^2 and X_3^2) and of interaction form (X_1X_2, X_1X_3, X_2X_3). They were statistically considered significant or not if the probability (P) of increasing reducing sugars content was ≤ 0.05 or ≥ 0.05 respectively (Table 3).

Effect of Hitempase 2XL on reducing sugars production

The impact of Hitempase 2XL (α-amylase) as sole mashing enzyme on the reducing sugars content of unmalted and malted *Madjeru* is shown in Figure1A. Reducing sugars content increased with increasing concentration of enzyme for unmalted and malted *Madjeru* mash types to attain maxima of 105.39 g/L and 132.25 g/L respectively, at an enzyme concentrations of 2031 U and 2451 U respectively. This was followed by a slight and steady decrease thereafter for the unmalted *Madjeru* mash. The measurable reducing sugar contents could not however be ascribed entirely to the action of Hitempase supplements, as in the absence of the enzyme, Figure 1A clearly showed that the contents for the unmalted and malted *Madjeru* mash types were about 22 g/L and 54 g/L respectively. These amounts of reducing sugars could be attributed to the milling process in the case of the unmalted *Madjeru* mash type, and to milling and malting processes for the malted *Madjeru* mash type. When the mathematical models were applied to predict the impact of a combination of the three enzymes (Bioglucanase and Brewers protease added at fixed amounts of 750 BGU and 60 mg, respectively) on the reducing sugar contents, the same trends as described above in Figure 1A were observed with increasing amounts of Hitempase for both *Madjeru* mash types

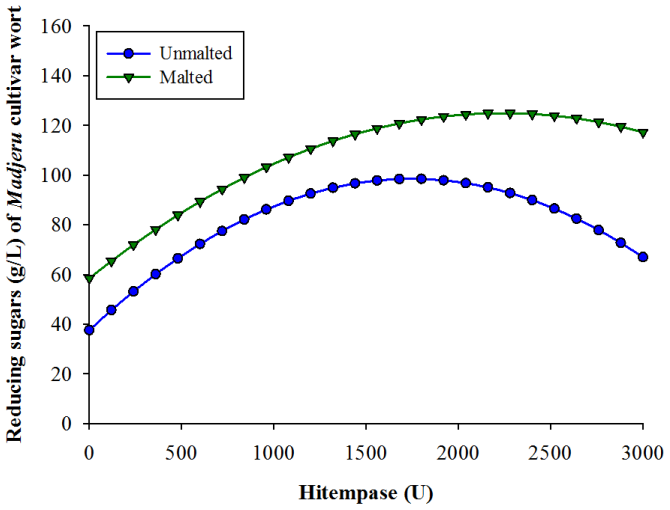


Figure 1B. Effect of concentration of Hitempase (α-amylase) in the presence of fixed concentrations of Bioglucanase (750 BGU) and Brewers protease (60 mg) on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

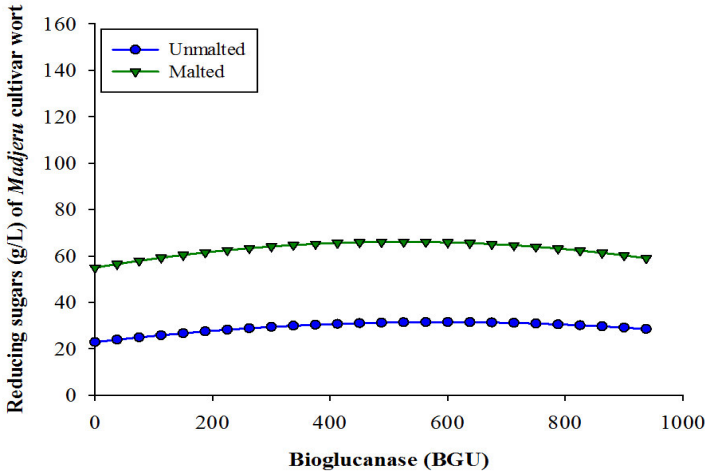


Figure 2A. Effect of concentration of Bioglucanase as sole mashing enzyme on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

(Figure 1B). In fact, starch is the main macromolecule of sorghum and the main substrate of α-amylase. It could therefore be expected that Hitempase contributes for the greatest amount of soluble materials in the form of reducing sugars that could be found in resulting worts due to its action on starch (Goode et al., 2003, Phiarais et al., 2006, Desobgo et al., 2010). The reducing sugars content for the unmalted *Madjeru* mash type slightly reduced beyond the maxima concentrations of the enzyme (Figures 1A and 1B). This could be explained by the fact that traces of amino-acids and other soluble nitrogenous materials present in the wort reacted with some of the reducing sugars (Hough et al., 1982), thus decreasing the measurable content. These results also demonstrated that the presence of the key mashing enzyme (α-amylase) developed during malting boosted the mashing efficiency of this cereal (Figure 1A). From the mathematical model, it was shown that in its linear form (X_1), the action of Hitempase contributed for 18% of reducing sugars for both unmalted *Madjeru* and malted *Madjeru* (Table 3). Statistical analyses also showed that this contribution was significant (P = 0.001 and 0.000 for the two mash types, respectively) (Table 3). In its quadratic form (X_1^2), the action of Hitempase remained statistically significant (P = 0.000) for both unmalted and malted *Madjeru*. This confirmed the above biological observation according to which supplements of this enzyme in both the unmalted and malted mashes of *Madjeru* are important. Its contribution to reducing sugars in its quadratic form (X_1^2) (excess of α-amylase in principle) is indeed 38 and 13% for unmalted and malted *Madjeru* respectively (Table 3).

Effect of Bioglucanase TX on reducing sugars production

Figure 2A shows the effect of mashing unmalted and malted *Madjeru* using Bioglucanase TX as sole mashing enzyme on reducing sugars content. There was no significant effect of Bioglucanase on the reducing sugars content of both unmalted and malted *Madjeru* mashes (Table 3). Reducing sugars were present at 22.85 and 54.94 g/L for the two mash types respectively in the absence of this mashing enzyme. Almost all of the measurable soluble reducing sugars therefore seemed to be due to the milling operation. In the presence of Bioglucanase, reducing sugars contents were maximal at 22.86 and 54.95 g/L at concentrations of 590.24 and 521.74 BGU for unmalted and malted *Madjeru* respectively with increasing enzyme concentration. The small reducing sugars yields due to Bioglucanase's action can be attributed to its hydrolysis of β-glucans into glucose and other soluble carbohydrates. Bioglucanase was therefore not a backbone enzyme for production of reducing sugars during mashing (Phiarais et al., 2006, Desobgo et al., 2010). A similar application of the mathematical models as carried out above for Hitempase's action, using 60 mg of Brewers protease and 1875 U of Hitempase, predicted that supplementing these two key mashing enzymes could provide higher reducing sugars content for both unmalted and malted *Madjeru* mashes (Figure 2B). Hitempase once more demonstrated that it was the backbone enzyme that contributed most in reducing sugars' yields. Figure 2B indeed showed that even in the absence of Bioglucanase, but in the presence of Hitempase, the reducing sugars content increased 5 folds for unmalted

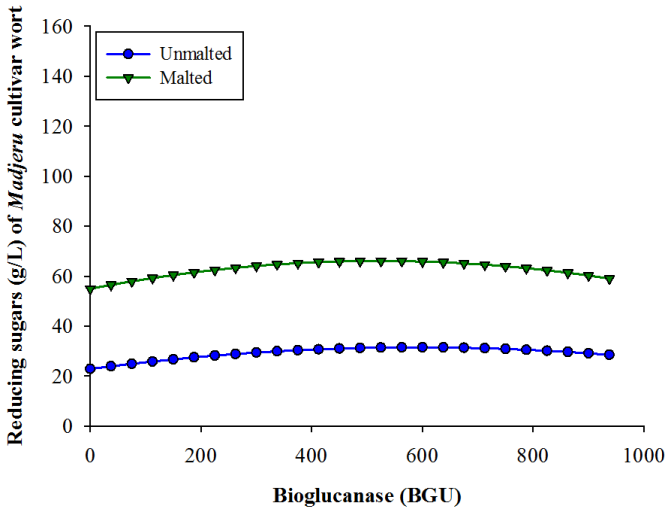


Figure 2A. Effect of concentration of Bioglucanase as sole mashing enzyme on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

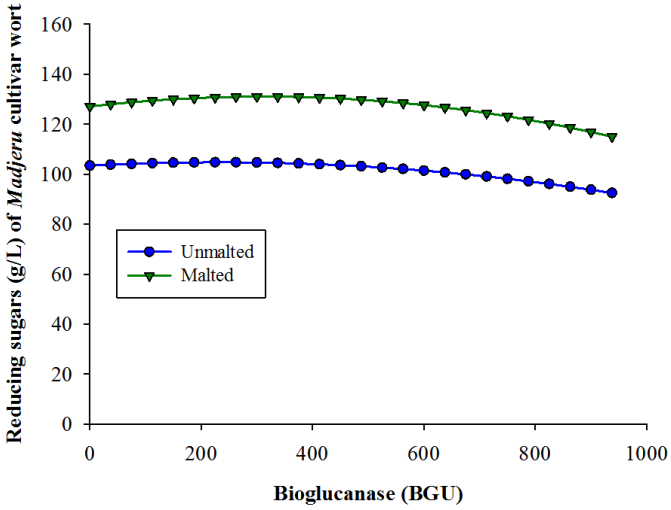


Figure 2B. Effect of concentration of Bioglucanase (β-glucanase) in the presence of fixed concentrations of Hitempase (1875 U) and Brewers protease (60 mg) on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

Madjeru mashes and 3 folds for malted *Madjeru* mashes. These observations were all statistically confirmed. Indeed, in its linear form (X_2), Bioglucanase's action was not significant (P = 0.318 and 0.123 for unmalted and malted *Madjeru* respectively) (Table 3). Table 3 showed that this enzyme contributed to merely 3 and 2% of reducing sugars content for unmalted and malted *Madjeru* mashes respectively. In its quadratic form (X_2^2) (excess of enzyme in principle), Bioglucanase contributed to 6 and 5 % of reducing sugars for unmalted and malted *Madjeru* mashes respectively (Table 3). This contribution was not statistically significant for unmalted *Madjeru* mash, but significant for malted *Madjeru* mash (P = 0.300 and 0.019) (Table 3).

Once more, the highest values of reducing sugars obtained for malted *Madjeru* mash, as compared to unmalted *Madjeru* mash, could be attributed to the impact of hydrolytic enzymes developed during malting.

Effect of Brewers protease on reducing sugars production

The effect of mashing unmalted and malted *Madjeru* on reducing sugars content using as sole mashing enzyme, Brewers protease, is shown in Figure 3A. There was no contribution of reducing sugars for both unmalted and malted *Madjeru* by this enzyme.

All the measurable soluble reducing sugars seemed to be due to the milling operation for the unmalted mash type, and the malting and milling operations, for the malted mash type. The decrease of yields of reducing sugars with increase of Brewers protease for unmalted and malted *Madjeru* mashes could be attributed to reactions between nitrogenous functions and some of the reducing sugars (Hough et al., 1982). The mathematical model was once more used to predict the reducing sugars content in the presence of fixed amounts of the two other key mashing enzymes. Thus, using 1875 U of Hitempase and 750 BGU of Bioglucanase with increasing amounts of Brewers protease, the results showed that higher amounts of reducing sugars could be obtained for both unmalted and malted *Madjeru* mashes (Figure 3B). These observations were statistically confirmed using the mathematical model. In its first degree form (X_3), the impact of Brewers protease was not significant for unmalted *Madjeru* mash but significant for malted *Madjeru* mash (P= 0.106 and 0.006 respectively) (Table 3). Its contribution to reducing sugars content was 6 and 4% respectively for both mashes (Table 3). The impact of the enzyme in its quadratic form (X_3^2), was significant for both mashes (P= 0.034 and 0.000 respectively). Its contribution to reducing sugars content was 14 and 16% respectively (Table 3).

Effect of enzymes interactions on reducing sugars production

The models were further exploited to predict the impacts of the interactions (X_1X_2, X_1X_3 and X_2X_3) of these enzymes on reducing sugars content. The results are shown in Table 3. They were statistically not significant

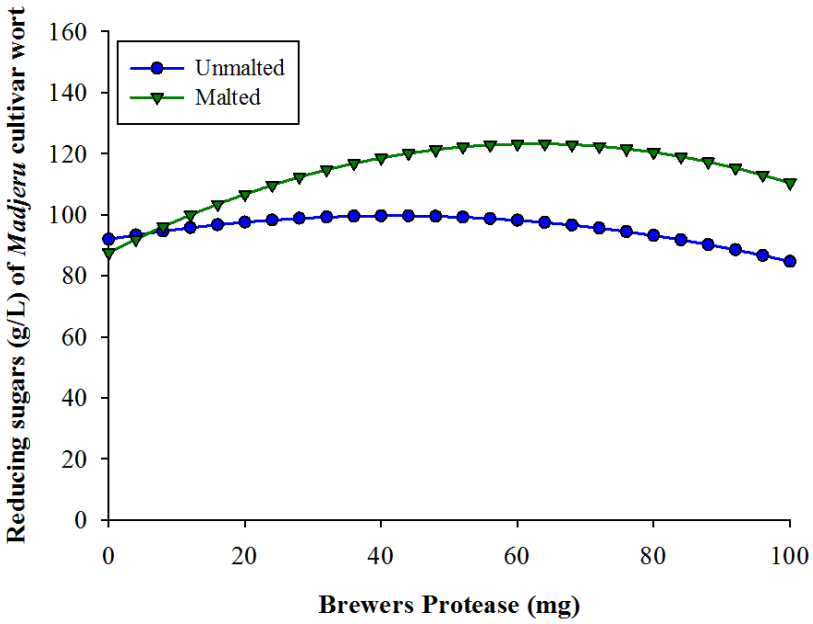


Figure 3B. Effect of concentration of Brewers protease in the presence of fixed concentrations of Hitempase (1875 U) and Bioglucanase (750 BGU) on reducing sugars content (g/L) of sorghum wort cultivar *Madjeru*.

Table 4. ANOVA for the reducing sugars content of *Madjeru*.

Source	DF	Sum square		Mean square		F		P	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	8675.21	12655.782	963.912	1406.198	15.034	59.323	0.001	0.000
Linear	3	4174.58	8636.535	1391.53	2878.845	21.704	121.45	0.001	0.000
Quadratic	3	4337.98	2342.886	1446	780.962	22.553	32.946	0.001	0.000
Interactions	3	162.641	1676.361	54.214	558.787	0.846	23.574	0.511	0.000
Residual error	7	448.802	165.928	64.115	23.704				
Total error	16	9124.01	12812.71						

DF: Degree of freedom

for unmalted *Madjeru* mashes (P = 0.511), but were for malted *Madjeru* mashes (P=0.000) (Table 4). The interaction X_1X_2 (Hitempase/Bioglucanase) had no significant impact on unmalted *Madjeru* mash but had for malted *Madjeru* (P = 0.211 and 0.001) respectively (Table 3). It contributed to 10% of reducing sugars content for unmalted *Madjeru* mash and to 14% for malted *Madjeru* mash (Table 3). Though known to be the backbone starch hydrolyzing enzyme, the action of Hitempase is best exploited when the cell walls of cereal grains are broken down by β-glucanases, hemicellulases and cellulases, to liberate starch granules. This sequence of events during malting was confirmed by the mathematical models above.

The interaction X_1X_3, corresponding to the couple Hitempase/Brewers protease, also had no significant impact on the reducing sugars content of unmalted *Madjeru* mash (P = 0.970), but had a significant impact on the reducing sugars content of malted *Madjeru* mash (P = 0.001) (Table 3). Its contribution to reducing sugars content was 0 and 15% respectively (Table 3). This result was once more in conformity with the biological sequence occurring during malting. Efficient starch hydrolysis by α-amylase indeed occurs only after the breakdown of cell walls by β-glucanase, followed by liberation of starch granules due to proteolysis of the protein matrix enrobing them. The interaction Bioglucanase/Brewers protease (X_2X_3) had no significant impact on reducing sugars content for unmalted *Madjeru* mashes, but had for malted *Madjeru* mashes (P = 0.568 and 0.002 respectively)

Table 5. ANOVA for comparing reducing sugars content of unmalted and malted *Madjeru* worts.

Source	DF	Sum square	Mean square	F	P
Inter-groups	1	3224.25	3224.25	4.7	0.037
Intra-groups	32	21945.7	685.804		
Total	33	25170			

DF : Degree of freedom.

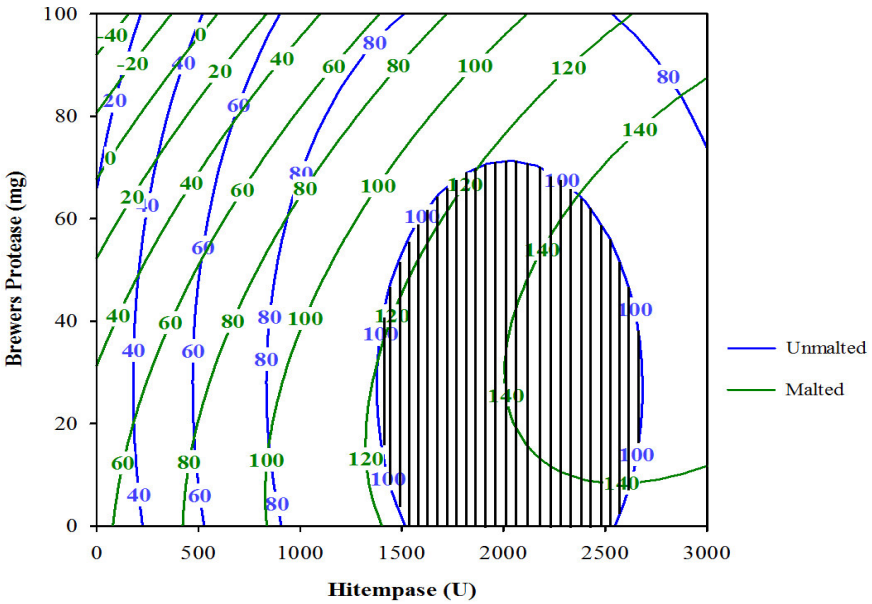


Figure 4. Response surface curves for the enzyme combinations providing for optimal reducing sugars content (g/L) for unmalted and malted sorghum wort cultivar *Madjeru*.

(Table 3). Its contribution to reducing sugars content was 5 and 13% respectively for both mash types (Table 3). These low contributions by the couple (Bioglucanase/ Brewers protease) were expected, as the two enzymes only play a supporting role in starch hydrolysis during mashing (Desobgo et al., 2010).

Optimization of the concerted mashing enzymes action on the production of reducing sugars

The results obtained for the action of the enzymes on reducing sugars yields after mashing on the basis of the models, were optimized to define a satisfactory domain of compromise for the action of the mashing enzymes. This domain was obtained for a reducing sugars content ≥ 100 g/L. The theoretical optimal combination of enzyme action for unmalted *Madjeru* gave the following triplet of coded variables for reducing sugars content: 0.330, – 0.701 and – 0.345 (1995 U, 89.31 BGU and 28.86 mg real variables) for Hitempase, Bioglucanase and Brewers protease respectively. This triplet allowed for a maximal reducing sugars content of 108.78 g/L. The triplet for malted *Madjeru* was 1, – 0.866 and – 0.005 (3000 U, 0 BGU and 49.69 mg real variables). It allowed for a maximal extract of 153.15 g/L. The optimal enzyme combinations were thus different and gave different results. These results once more confirmed the fact that commercial enzymes supplements for mashing of unmalted and malted *Madjeru* were necessary to obtain maximum reducing sugars contents. The significant difference (P = 0.037) between reducing sugars content of unmalted and malted *Madjeru* worts is shown in Table 5. By fixing Bioglucanase concentration at 0 BGU (according to the optimization), the minimal reducing sugars content (100 g/L) could be obtained and the necessary enzyme combination could therefore permit highlight the optimal domain (Figure 4).

Conclusion

Optimizing the amounts of the enzyme needed for alleviating the levels of reducing sugars during mashing

demonstrated that Hitempase 2XL was the most important enzyme component in terms of both amounts and efficiency for the unmalted and malted mash types of the *Madjeru* sorghum cultivar. Optimizing studies also showed that Brewers Protease was the next important enzyme in increasing reducing sugars yields, as its need in both mash types was demonstrated. Bioglucanase TX was not indispensable for the malted mash type as shown by the optimal triplet of mashing enzymes obtained for this sample. The role of the milling process also independently facilitated the dissolution of some of these reducing sugars during mashing of both unmalted and malted *Madjeru* grist. Finally, the response surface methodology used in this work permitted to confirm the specific virtues always allotted to the malting process as an unavoidable route in providing the best malt types that can be used in mashing and beer brewing.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Process Engineering, National School of Agro- Industrial Sciences (ENSAI) of University of Ngaoundere (Cameroon) for providing necessary facilities for the successful completion of this research work.

REFERENCES

Agu RC, Palmer GH (1998). Effect of mashing with commercial enzymes on the properties of sorghum worts. World J. Micro-biol. Biotechnol., 14: 43-48.

Bajomo MF, Young TW (1992). Development of a mashing profile for the use of microbial enzymes in brewing with raw sorghum (80%) and malted barley or sorghum malt (20%). J. Inst. Brew., 98: 515-523.

Baranyi J, Pin C, Ross T (1999). Validating and comparing predictive models. Int. J. Food Microbiol., 48: 159-166.

Bas D, Boyac IH (2007). Modeling and optimization I: Usability of response surface methodology. J. Food Eng., 78: 836-845.

Desobgo ZSC, Nso EJ, Tenin D, Kayem GJ (2010). Modelling and optimizing of mashing enzymes-effect on yield of filtrate of unmalted sorghum by use of response surface methodology. J. Inst. Brew., 116: 62-69.

EtokAkpan OU (1992). Amylolytic potentials and wort fermenting components of nigerian sorghums and barley. World J. Microbiol. Biotechnol., 8: 287-289.

EtokAkpan OU, Palmer GH (1990). Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. World J. Microbiol. Biotechnol., l 6:408-417.

Giovanni M (1983). Response surface methodology and product optimization. Food Technol., 37:41-45.

Goode DL, Arendt EK (2003). Pilot scale production of lager beer from grist containing 50% unmalted sorghum. J. Institute of Brew., 109: 208-217.

Goode DL, Halbert C, Arendt EK (2002). Mashing studies with unmalted sorghum and malted barley. J. Institute of Brewing 108:465-473.

Goode DL, Halbert C, Arendt EK (2003). Optimisation of mashing conditions when mashing with unmalted sorghum and commercial enzymes. J. Am. Soc. Brew. Chem., 61: 69-78.

Hough JS, Briggs DE, Stevens R, Young TW (1982). In: Malting and brewing science. London: Chapman and Hall.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem., 31: 426-428.

Nso EJ, Ajebesone PE, Mbofung CM, Palmer GH (2003). Properties of three sorghum cultivars used for the production of *Bili-Bili* beverage in Northern Cameroon. J. Inst. Brew., 109: 245-250.

Nso EJ, Nanadoum M, Palmer GH (2006). The effect of formaldehyde on enzyme development in sorghum malts. MBAA TQ 43:177-182.

Palmer GH (1989). Cereal in malting and brewing. In: Cereal science and technology(Palmer G ed) Aberdeen, Scotland: Aberdeen University Press.

Phiarais NB, Schehl BD, Oliveira JC, Arendt EK (2006). Use of surface response methodology to investigate the effectiveness of commercial enzymes on buckwheat malt for brewing purpose. J. Institute of Brew., 112: 324-332.

Ross T (1996). Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol., 81: 501-508.

Taylor JRN (2004). Overview: Importance of sorghum in Africa. In: Department of Food Science, Pretoria, South Africa: University of Pretoria. p. 21

Taylor JRN, Dewar J (2001). Developments in sorghum food technologies. In: Advances in Food and Nutrition Research (Taylor, J., ed), San Diego, USA: Academic Press, pp. 217-264.

Journal of Brewing and Distilling Vol. 2(3), pp. 29-44, May 2011
Available online http://www.academicjournals.org/JBD
ISSN 2141-2197 ©2011 Academic Journals

Full Length Research Paper

Modeling the action of technical mashing enzymes on extracts and free-amino nitrogen yields of the *Madjeru* sorghum cultivar

Zangué S. C. Desobgo[2], Emmanuel J. Nso[1]* and Dzudie Tenin[1]

[1]Department of Process Engineering, National School of Agro- Industrial Sciences (ENSAI) of Ngaoundere, Cameroon.
[2]Department of Food Processing and Quality Control, University Institute of Technology (IUT) of Ngaoundere, Cameroon.

Accepted 18 April, 2011

The action of three technical mashing enzymes (hitempase 2XL, bioglucanase-TX and brewers protease) on yields of extract and free amino nitrogen (FAN) of the worts of mashes of unmalted and malted *Madjeru* sorghum was modeled and analyzed using the response surface methodology. The analysis showed that increasing amounts of hitempase 2XL considerably increased yields of extract during mashing of unmalted *Madjeru* sorghum grist. The use of bioglucanase-TX was not indispensable, while Brewers' protease contributed very little. Increasing amounts of hitempase contributed approximately 45% of the free amino nitrogen, while Brewers' protease influence amounted to not more than 15%. Bioglucanase's action was globally nil. Addition of the three enzymes into malted *Madjeru* sorghum mashes had no significant effect on the yields of extracts and FAN, but the milling operation singularly liberated more than 50% of FAN for both mash types. Optimization of the concerted actions of the three enzymes for extract yield for unmalted *Madjeru* sorghum mash gave a combination of (1960.5 U; 132.61 BGU and 28.86 mg) for hitempase, bioglucanase and brewers protease respectively). This gave a maximal extract yield of 16.55 °P. This combination was: 2610 U; 0 BGU and 40.44 mg for malted *Madjeru* sorghum mash, giving a maximal extract yield of 16.35 °P. Optimization for free amino nitrogen for unmalted *Madjeru* sorghum mash gave a combination of: 3000 U; 0 BGU and 100 mg for hitempase, bioglucanase and brewers protease respectively). This gave maximal FAN of 93.55 mg/L. The combination was: 3000 U; 0 BGU and 100 mg for malted *Madjeru* sorghum mash, giving a maximal FAN of 144.48 mg/L.

Key words: Modeling, technical mashing enzymes, yields of extract, free-amino-nitrogen, *Madjeru*, optimization.

INTRODUCTION

Sorghum in its malted form or as adjuncts has become a potential brewing cereal particularly in the tropics where barley is not grown (Taylor, 1983; Aisien and Muts, 1987; Arri, 1989; Palmer, 1989; Adejemilua, 1995). Its low contents of potential mashing enzymes (Aisien, 1982; EtokAkpan and Palmer, 1990; Nso et al., 2003, 2006) due to their poor development during malting has often triggered the use of technical mashing enzymes as supplements (Agu and Palmer, 1998; Goode et al., 2002, 2003; Goode and Arendt, 2003) to achieve higher yields in extracts and other important wort specifications in beer brewing. Modeling and optimization approaches to ameliorate wort properties of sorghum grist and buckwheat malts has recently thrown more light into the precise role played by supplements of technical mashing enzymes (Goode et al., 2003, Phiarais et al., 2006, Desobgo et al., 2010). It is not however clear whether the use of technical mashing enzymes to obtain optimal mashing and brewing specifications for worts when using malted

*Corresponding author. E-mail: nso_emmanuel@yahoo.fr. Tel: + 237 79 82 88 00.

sorghum is indispensable. A series of work using two popular sorghum cultivars used in northern Cameroon for the production of the traditional beer Bili-Bili is currently taking place. This will help to have a clear understanding on the necessity of applying or not, technical enzymes when mashing with these cultivars. Some of the findings were recently reported for yields of reducing sugars for these cultivars (Desobgo et al., 2011a, b).

As a follow up, the effect of the same three technical mashing enzymes (Hitempase 2XL, Bioglucanase B-10L and Brewers protease) were modeled and optimized in this work for yields of extracts and free amino nitrogen during mashing of *Madjeru,* one of the popular sorghum cultivars of Northern Cameroon, using the response surface methodology (RSM).

MATERIALS AND METHODS

Enzymes

The characteristics of the technical enzymes used (Hitempase 2XL, a thermo stable α-amylase from *Baccillus licheniformis,* Brewers protease from *Bacillus amyloliquefaciens* and Bioglucanase TX, an enzymatic composition of β-glucanase and hemicellulases from *Trichodermareesei*) and their sources are presented in Table 1.

Sorghum cultivar

The *Safrari* sorghum cultivar was obtained from the Institute of Research and agronomic development (IRAD) Maroua, Cameroon.

Modelling

Modelling was carried out as previously described (Desobgo et al., 2011a, b).

Validation of models

The models were validated as previously described (Desobgo et al., 2011a, b).

Mashing

Mashing was carried out as described (Desobgo et al., 2011a, b).

Determination of extract

Extract was determined as described in analytica-EBC, 1998.

Determination of free amino nitrogen

Free amino nitrogen (FAN) was determined as described in analytica-EBC, 1998.

Optimization of models

Models were optimized as previously described (Desobgo et al., 2011a, b).

RESULTS AND DISCUSSION

The modeling and optimization of the action of mashing enzymes on the two key wort properties: extract and free amino nitrogen (FAN), was carried out for the experimental design required for manipulation in the laboratory. Table 2 shows the results obtained for extracts and free amino nitrogen (FAN) after mashing unmalted and malted *Madjeru* using the technical enzymes hitempase 2XL (α-amylase), bioglucanase TX (β-glucanase) and brewers protease (protease).

Modeling and validation of results of yields of extract

The mathematical models obtained for extracts after mashing unmalted and malted *Madjeru* were as follows respectively:

$$Y_{MadEX}\ (X_1, X_2, X_3) = 15.134 + 4.477X_1 - 1.397X_2 - 1.729X_3 + 0.980X_1X_2 - 2.155X_1X_3 + 0.296X_2X_3 - 7.507X_1^2 - 0.964X_2^2 - 3.732X_3^2 \quad (1)$$

$$Y_{MadMEX}\ (X_1, X_2, X_3) = 15.566 + 1.036X_1 - 0.197X_2 - 0.304X_3 - 0.842X_1X_2 + 0.784X_1X_3 + 0.623X_2X_3 - 1.11X_1^2 - 0.14X_2^2 - 0.843X_3^2 \quad (2)$$

With: $Y_{MadEX}\ (X_1, X_2, X_3)$ representing the mathematical model for unmalted *Madjeru*; $Y_{MadMEX}\ (X_1, X_2, X_3)$, the model for malted *Madjeru;* X_1, Hitempase; X_2, Bioglucanase and X_3 Brewers Protease.

The mathematical models were polynomials having several variables with coefficients of determination R^2 = 0.940 for unmalted *Madjeru* and R^2 = 0.980 for malted *Madjeru*. These coefficients, coupled to AAD values of 0.091 and 0.006 for unmalted and malted *Madjeru* respectively, allowed for the validation of the models for yields of extract of the worts. In addition, a bias factor of 1.05 and 1 for unmalted and malted *Madjeru* respectively, coupled to exactitude factors of 1.19 and 1.01 for both unmalted and malted *Madjeru* respectively, also allowed for validation of the models according to the method described (Ross, 1996). The factors of the models were linear or of first degree (X_1, X_2 and X_3), quadratic or of the second degree (X_1^2, X_2^2 and X_3^2) and of interaction form (X_1X_2, X_1X_3, X_2X_3). They were statistically considered significant or not if the probability (P) of increasing yields of extracts was ≤ 0.05 or ≥ 0.05 respectively (Table 3).

Effect of hitempase 2XL on yields of extract

The impact of hitempase 2XL as sole mashing enzyme on the yield of extract of unmalted and malted *Madjeru* is shown in Figure 1A. Extract yield increased from 2 °P with increasing concentration of enzyme for unmalted *Madjeru* mash to attain a maximum level (15.72°P) at

Table 1. Characteristics of commercial mashing enzyme preparations.

Commercial mashing enzyme	Organism of origin	Activity	Description	Temperature optima (°C)	pH optima	Recommended application level in adjuncts	Form
Hitempase 2XL	*Bacillus licheniformis*	4416.29 ± 19.34 U/ml	α-amylase	60 – 95	4 – 8	60 U/g	Solution
Bioglucanase TX	*Trichoderma reesei*	750 BGU/ml	β-glucanase	60	4.5 – 6.5	0.01 et 0.025% (v/w)	Solution
Brewers Protease	*Bacillus amyloliquefaciens*	1842.2 ± 1.8 mg FAN/min/mL	Protease	45 – 50 (denatured at 85)	6.5 – 7.5	0.4 – 2 g/Kg	Solution

Hitempase 2XL and bioglucanase TX were obtained from Kerry bioscience; Kilnagleary, Carrigaline, Co. Cork, Ireland. Brewers protease was obtained from DSM Food Specialities, Cedex France.

Table 2. Matrices of Doehlert coded and transformed experimental values.

Coded value			Transformed experimental value			*Madjeru*											
Hit	Bio	Brew Prot	Hit (U)	Bio (BGU)	Brew Prot (mg)	Unmalted						malted					
						Extract (°P)			FAN (mg/L)			Extract (°P)			FAN (mg/L)		
X_1	X_2	X_3	X_1	X_2	X_3	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res	Exp	Theo	Res	Exp	Theo	Res
0.000	0.000	0.000	1500	468.75	50	15.07	15.13	-0.06	64.34	63.50	0.84	15.60	15.57	0.03	94.00	94.81	-0.81
1.000	0.000	0.000	3000	468.75	50	13.93	12.10	1.83	71.79	71.17	0.62	15.70	15.49	0.21	107.70	107.32	0.38
0.500	0.866	0.000	2250	937.5	50	13.10	13.99	-0.89	98.00	94.42	3.58	15.08	15.17	-0.09	147.00	141.67	5.33
-0.500	-0.866	0.000	750	0.00	50	13.61	11.93	1.68	65.38	64.31	1.07	14.56	14.47	0.09	98.10	95.82	2.28
0.500	-0.866	0.000	2250	0.00	50	15.09	15.56	-0.47	83.08	83.01	0.07	16.12	16.24	-0.12	126.00	125.54	0.46
-0.500	0.866	0.000	750	937.5	50	9.05	8.66	0.39	81.79	82.30	-0.51	14.97	14.86	0.11	122.70	123.85	-1.15
0.500	0.289	0.816	2250	615.18	100	10.00	10.45	-0.45	84.78	86.08	-1.30	15.14	15.27	-0.13	130.00	130.96	-0.96
-0.500	-0.289	-0.816	750	312.32	0.0	11.25	9.60	1.65	44.00	35.71	8.29	14.97	14.85	0.12	66.00	53.61	12.39
0.500	-0.289	-0.816	2250	312.32	0.0	14.14	15.55	-1.41	48.00	48.97	-0.97	15.39	15.49	-0.10	72.00	73.27	-1.27
0.000	0.577	-0.816	1500	781.07	0.0	13.20	12.79	0.41	49.07	52.58	-3.51	14.76	14.80	-0.04	73.60	78.48	-4.88
-0.500	0.289	0.816	750	615.18	100	8.74	7.45	1.29	68.86	68.53	0.33	13.93	13.84	0.09	103.30	103.07	0.23
0.000	-0.577	0.816	1500	156.43	100	10.73	11.58	-0.85	73.80	72.84	0.96	14.56	14.53	0.03	110.70	109.97	0.73
0.000	0.000	0.000	1500	468.75	50	14.56	15.13	-0.57	62.27	63.50	-1.23	15.60	15.57	0.03	93.40	94.81	-1.41
-1.000	0.000	0.000	0.000	468.75	50	1.52	3.15	-1.63	40.88	40.35	0.53	13.20	13.42	-0.22	61.30	59.78	1.52
-1.000	-0.866	-0.816	0.000	0.0	0.0	1.20	1.86	-0.66	33.31	37.13	-3.82	13.52	13.52	0.00	50.00	56.24	-6.24
0.000	0.000	0.000	1500	468.75	50	14.90	15.13	-0.23	62.00	63.50	-1.50	15.60	15.57	0.03	93.00	94.81	-1.81
0.000	0.000	0.000	1500	468.75	50	15.08	15.13	-0.05	60.00	63.50	-3.50	15.46	15.57	-0.11	90.00	94.81	-4.81

[a]Experimental result values. [b]Theoretical values (values coming from mathematical models). [c] Residue.

about 2038 U, followed by a slight and steady decrease thereafter. It was already high (13.52 °P) in the absence of Hitempase for malted *Madjeru* mash and only increased slightly to attain a maximal level (16.04 °P) at 2268 U of enzyme concentration. When the mathematical models were applied to predict the impact of supplements of bioglucanase and brewers protease at concentrations of 750 BGU and 60 mg respectively and in the presence of Hitempase, it was observed that the profile of extract yields remained similar to that in Figure 1a (compare with

Table 3. Estimation of regression coefficients for the extracts of umalted and malted *Madjeru*.

Effect	Coefficient		Std. deviation		t-statistics		P-value	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Constant	15.134	15.566	0.766	0.08	19.768	195.176	0.000	0.000
X_1	4.477	1.036	0.788	0.082	5.684	12.63	0.001	0.000
X_2	-1.397	-0.197	0.687	0.072	-1.762	-2.387	0.121	0.048
X_3	-1.729	-0.304	0.649	0.068	-2.174	-3.67	0.066	0.008
X_1^2	-7.507	-1.11	1.35	0.141	-5.56	-7.895	0.001	0.000
X_2^2	-0.964	-0.14	0.983	0.102	-0.736	-1.027	0.486	0.339
X_3^2	-3.732	-0.843	0.848	0.088	-2.932	-6.357	0.022	0.000
X_1*X_2	0.98	-0.842	1.541	0.161	0.55	-4.543	0.599	0.003
X_2*X_3	0.296	0.623	1.357	0.141	0.154	3.115	0.882	0.017
X_1*X_3	-2.155	0.784	1.606	0.167	-1.095	3.823	0.310	0.007

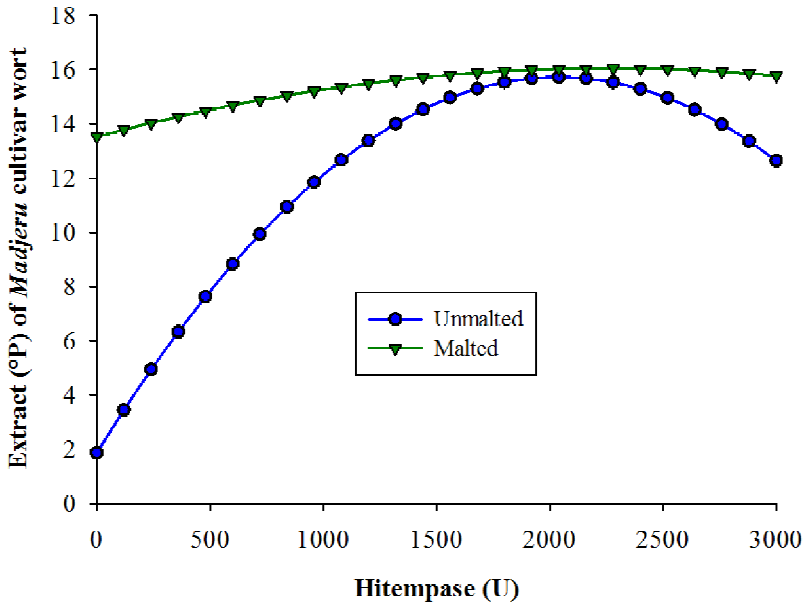


Figure 1a. Effect of concentration of hitempase (α-amylase) as sole mashing enzyme on yield of wort extract of sorghum cultivar *Madjeru*.

Figure 1b). Starch is indeed the main macromolecule of cereals and the main substrate of α-amylase. It is therefore expected that this enzyme contributes to the greatest amount of soluble materials that could be found in resulting worts due to its action on starch (Goode et al., 2003, Phiarais et al., 2006, Desobgo et al., 2010). Figures 1 A and B also showed that supplements of hitempase in unmalted *Madjeru* mash was by far more useful than for the malted *Madjeru* mash type. The important role of milling in obtaining instantaneous dissolution of soluble materials at the beginning of mashing was better displayed for the malted *Madjeru* mash type than for the unmalted *Madjeru* mash type (93 and 14% of extract yields respectively). The soluble nitrogenous compounds and reducing sugars (Hough et al., 1982) in the medium (Figures 1a and b).

From the mathematical models, it was shown that in its linear form (X_1), hitempase contributed 19 and 18% of extract yields for unmalted and malted *Madjeru* respectively (Figures 4a and b). This more or less equitable contribution of extracts in the worts of the two mash types, pairs with the observations made earlier whereby extract levels were virtually the same for both. Moreover, statistical analyses also showed that this contribution was significant (P = 0.001 and 0.000 for unmalted and malted *Madjeru* respectively (Table 3). In its quadratic form (X_1^2), hitempase remained statistically significant for mashing both unmalted *Madjeru* and malted *Madjeru* (P = 0.001 and 0.000 respectively). This confirmed the earlier biological observation according to which supplements of this

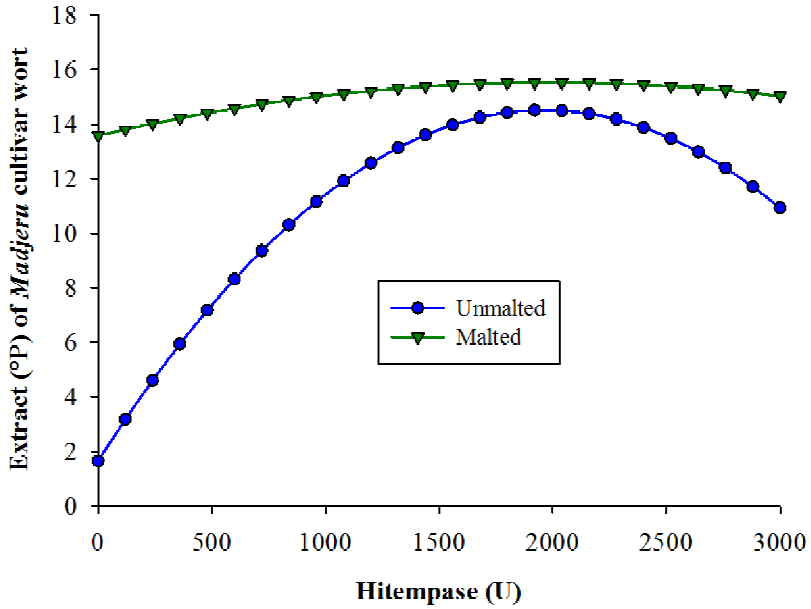


Figure 1b. Effect of concentration of hitempase (α-amylase) in the presence of fixed concentrations of bioglucanase (750 BGU) and brewers protease (60 mg) on yield of wort extract of sorghum cultivar *Madjeru*

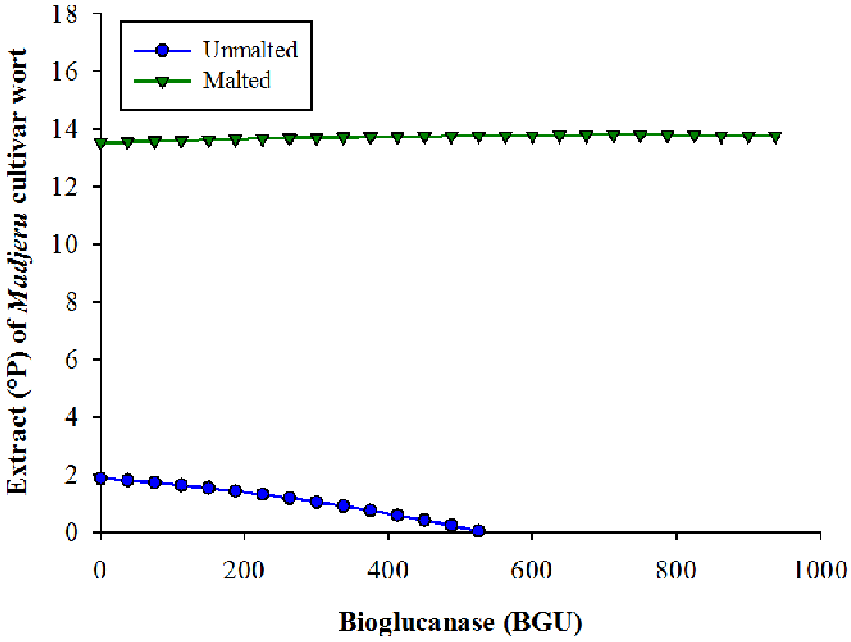


Figure 2a. Effect of concentration of bioglucanase (β-glucanase) as sole mashing enzyme on yield of wort extract of sorghum cultivar *Madjeru*.

enzyme in malted mashes of *Madjeru,* was also necessary. Its contribution in increasing extract yields in its quadratic form (X_1^2) (excess of α-amylase in principle) was indeed 33 and 19% for unmalted and malted *Madjeru* respectively (Figures 4a and b).

Effect of bioglucanase TX on yields of extract

Figure 2a shows the effect of mashing unmalted and malted *Madjeru* using bioglucanase, as sole mashing enzyme, on yields of extract. There was a progressive

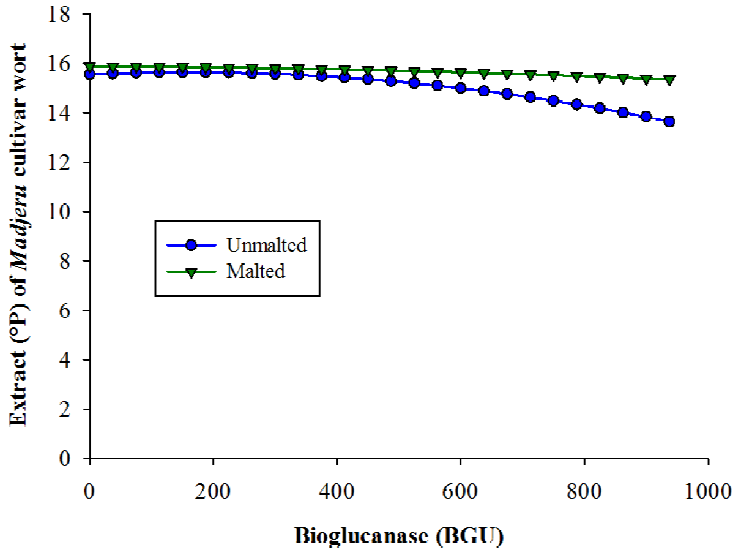


Figure 2b. Effect of concentration of bioglucanase (β-glucanase) in the presence of fixed concentrations of hitempase (1875 U) and brewers protease (60 mg) on yield of wort extract of sorghum cultivar *Madjeru*.

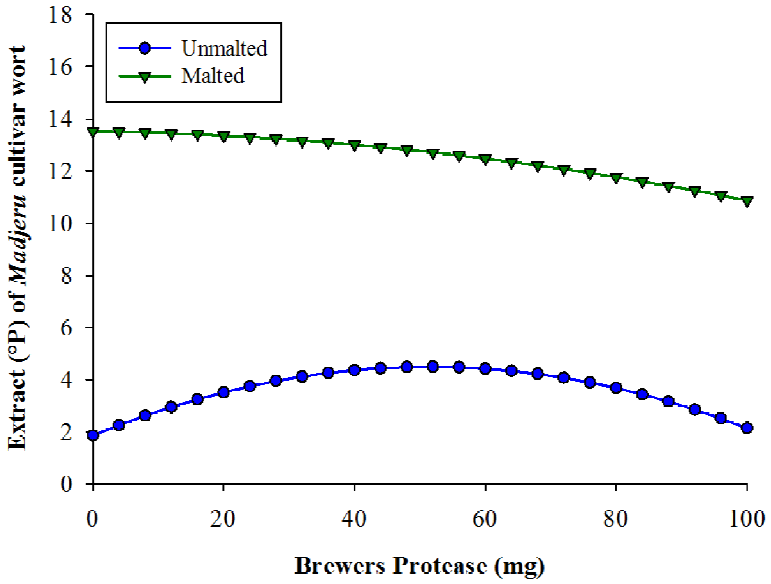


Figure 3a. Effect of concentration of brewers protease as sole mashing enzyme on yield of wort extract of sorghum cultivar *Madjeru*.

decrease of yield of extract from 1.86 °P to nil as enzyme concentration increased for unmalted *Madjeru* wort. This figure also shows that the extract obtained (1.86 °P) was probably completely due to milling, suggesting that the enzyme plays no important role in production of extract from the grist of the unmalted *Madjeru* cultivar during mashing. The yield of extract for malted *Madjeru* mash was virtually at its maximal level (13.78 °P) even in the absence of bioglucanase and remained virtually constant with increasing enzyme concentration. Bioglucanase was therefore not a backbone enzyme for extract production during mashing. A similar application of the mathematical models as carried out earlier for hitempase's action, using 60 mg of brewers protease and 1875 U hitempase, predicted that the supplementation of these two mashing enzymes could provide similar results in extract yields for both unmalted and malted *Madjeru* mashes (Figure 2B). Hitempase once more revealed that it was the real

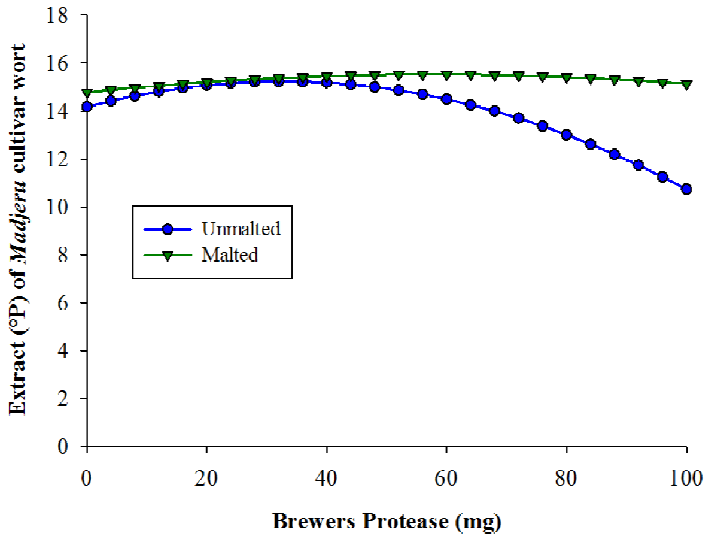


Figure 3b. Effect of concentration of brewers protease in the presence of fixed concentrations of hitempase (1875 U) and bioglucanase (750 BGU) on yield of wort extract of sorghum cultivar *Madjeru*.

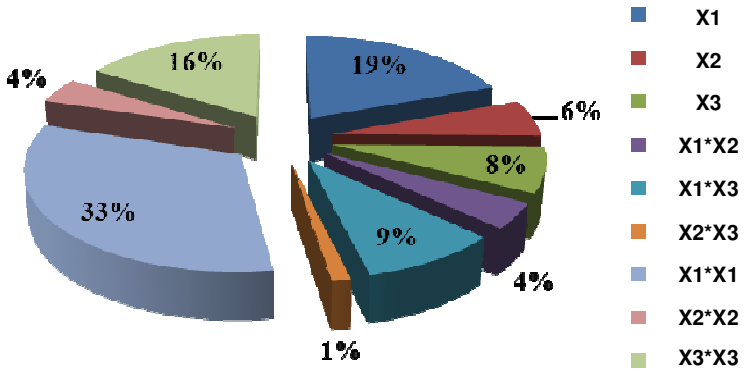


Figure 4a. Contribution to yield of wort extract (°P) of each factor in its linear, quadratic and interaction (combined) forms for unmalted sorghum cultivar *Madjeru*.

backbone enzyme that contributed for most of the yields of extract. The level of extract yields reached almost 15.54 °P for unmalted *Madjeru* and 15.87 °P for malted *Madjeru* mash types. From the two figures, it is also important to underline the natural virtues of the malting procedure in rendering the Madjeru grains potentially mash-able to reasonable extract yields in the absence of all these enzymes. These observations were all statistically confirmed. Indeed, in its linear form (X_2), bioglucanase's action was not significant (P = 0.121) for unmalted but significant for malted *Madjeru* (P = 0.048) (Table 3).

Figures 4A and B showed that this enzyme contributed barely for 6 and 3% of extract yields for unmalted and malted *Madjeru* respectively. In its quadratic form (X_2^2) (excess of enzyme in principle), bioglucanase contributed 4 and 3% of extract yield for unmalted and malted *Madjeru* respectively (Figures 4a and b). Similarly, these contributions were statistically not significant for both unmalted and malted *Madjeru* mash types (p = 0.486 and 0.0.339 respectively) (Table 3).

Effect of brewers protease on yields of extract

The effect of mashing unmalted and malted *Madjeru* on yields of extract using as sole mashing enzyme, "brewers protease", is shown in Figure 3A. Yields of extracts

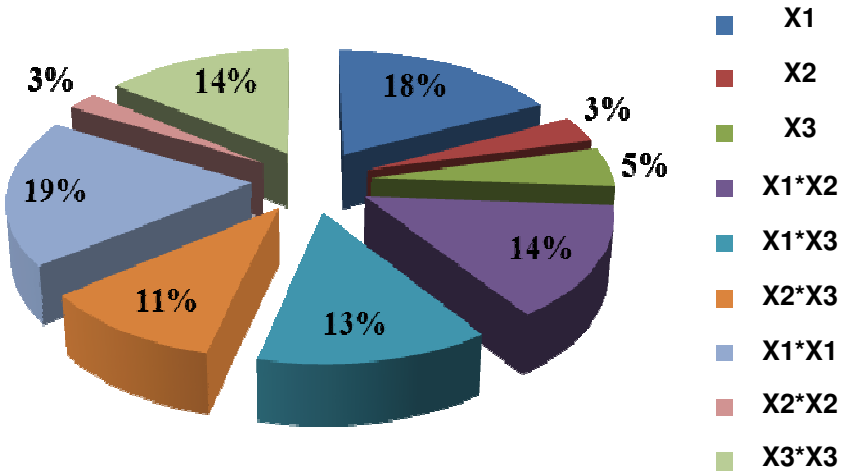


Figure 4b. Contribution to yield of wort extract (°P) of each factor in its linear, quadratic and interaction (combined) forms for malted sorghum cultivar *Madjeru*.

Table 4. ANOVA for the extracts of umalted and malted *Madjeru*.

Source	Ddl	Sum square		Mean square		F-value		P-value	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	285.91	9.965	31.768	1.107	12.14	38.993	0.002	0.000
Linear	3	156.256	6.507	52.085	2.169	19.905	76.379	0.001	0.000
Quadratic	3	126.512	2.496	42.171	0.832	16.116	29.299	0.002	0.000
Interactions	3	3.141	0.963	1.047	0.321	0.400	11.302	0.757	0.004
Residual error	7	18.317	0.199	2.617	0.028				
Total error	16	304.227	10.164						

gradually increased from 1.86 °P with increasing amounts of enzyme to reach a maximum of 4.48 °P at enzyme concentration of 51.39 mg. This small yield of extract due to the action of this enzyme could be attributed to its capacity of hydrolyzing proteins into soluble amino acids and peptides (Briggs et al., 2004) during which additional soluble sugars could also be liberated. Figure 3a however shows once more that in the absence of supplements of "brewers protease", maximal extract was obtained when mashing with malted *Madjeru*. This could be once more attributed to the virtues well known to the malting process as explained earlier. These results confirmed earlier observations (Desobgo et al., 2011a, b). The gradual and slight decrease of extract yields with increasing amounts of "brewers protease" for malted *Madjeru* and unmalted *Madjeru* (as from 51.39 mg concentration) mash types could once more be attributed to reactions between soluble nitrogenous functions and some of the soluble sugars. The mathematical models were once more used to predict the yield in extract as carried out earlier for hitempase and bioglucanase actions. Thus, using 1875 U hitempase and 750 BGU of bioglucanase with increasing amounts of "brewers protease", the models once more showed that the adding of these mashing enzymes could provide similar results in extract yields for both unmalted and malted *Madjeru* mashes (Figure 3b). The aforementioned observations were statistically confirmed using the mathematical model. In its first degree form (X_3), the impact of "brewers protease" was not significant for unmalted *Madjeru* mash but was for malted *Madjeru* mash (P = 0.066 and 0.008 respectively) (Table 3). Its contribution to extract yield was 8 and 5% respectively for both unmalted and malted mashes (Figures 4a and b).

The impact of the enzyme in its quadratic form (X_3^2) was significant for both unmalted and malted mashes (P = 0.022 and 0.000 respectively) (Table 3). Its contribution to extract yield was 16 and 14% respectively (Figures 4a and 4).

Effect of enzymes' interactions on yields of extract

The models were further exploited to predict the impacts of enzyme interactions (X_1X_2, X_1X_3 and X_2X_3) on yields of extract. The results are shown in Figures 4a and b. They were globally not statistically significant for unmalted *Madjeru* mashes (P = 0.757), but were for malted *Madjeru* mashes (P = 0.004) (Table 4). The interaction X_1X_2 (hitempase/bioglucanase) had no significant impact on unmalted *Madjeru* mash, but had for malted *Madjeru* (P = 0.599 and 0.003 respectively (Table 3). It contributed

Table 5. ANOVA for comparing extracts of unmalted and malted *Madjeru* worts.

Source	DF	Sum of squares	Mean of squares	F-value	P-value
Inter-groups	1	102.344	102.344	10.420	0.003
Intra-groups	32	314.391	9.824		
Total	33	416.735			

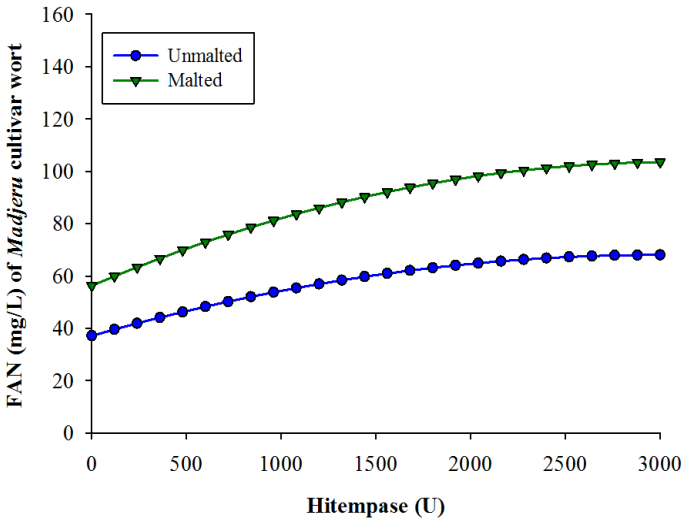


Figure 5a. Effect of concentration of hitempase (α-amylase) as sole mashing enzyme on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

for merely 4% of extract for unmalted *Madjeru* mash, but up to 14% for malted *Madjeru* mash (Figures 4a and b). It is however significant to underline that this important contribution could be attributed to the intrinsic virtues that malting offers when mashing with malted *Madjeru* and not to the hitempase/bioglucanase interaction as such. Though known to be the backbone starch hydrolyzing enzyme, the action of hitempase is best exploited when the cell walls of cereal grains are broken down by β-glucanases, hemicellulases and cellulases to liberate starch granules. This sequence of events during malting was confirmed by the mathematical models aforementioned (Desobgo et al., 2010). The interaction X_1X_3 corresponding to the couple hitempase/brewers protease, also had no significant impact on extract yields of unmalted *Madjeru* mash (P = 0.310), but had on malted *Madjeru* mash (P = 0.007) (Table 3). Its contribution to extract yield was 9 and 13% respectively (Figures 4a and b). This result was once more in conformity with the biological sequence occurring during malting. Efficient starch hydrolysis by α-amylase indeed occurs only after the breakdown of cell walls by β-glucanase followed by liberation of starch granules due to proteolysis of the protein matrix enrobing them. The interaction bioglucanase/brewers protease (X_2X_3) had no significant impact on extract yields for unmalted *Madjeru* mash (P = 0.882), but had for malted *Madjeru* mashes (P = 0.017) (Table 3). Its contribution to extract yields was 1 and 11% respectively for both mash types (Figures 4a and b). These low contributions by the couple (bioglucanase/brewers protease) were expected, as the two enzymes only play a supporting role in starch hydrolysis during mashing (Desobgo et al., 2010).

Table 5 statistically confirms the observation that malted *Madjeru* worts associated with the technical enzymes have better yields of extracts than unmalted *Madjeru* worts (P = 0.003).

Modeling and validation of results of free amino nitrogen (FAN)

The mathematical models obtained for free amino nitrogen (FAN) for mashing unmalted and malted *Madjeru* were as follows respectively:

$$Y_{MadAAL}(X_1, X_2, X_3) = 63.504 + 15.411X_1 + 8.487X_2 + 18.418X_3 - 3.798X_1X_2 + 3.976X_1X_3 + 7.807X_2X_3 - 7.747X_1^2 + 25.922X_2^2 - 8.636X_3^2 \quad (3)$$

$$Y_{MadMAAL}(X_1, X_2, X_3) = 94.807 + 23.771X_1 + 12.745X_2$$

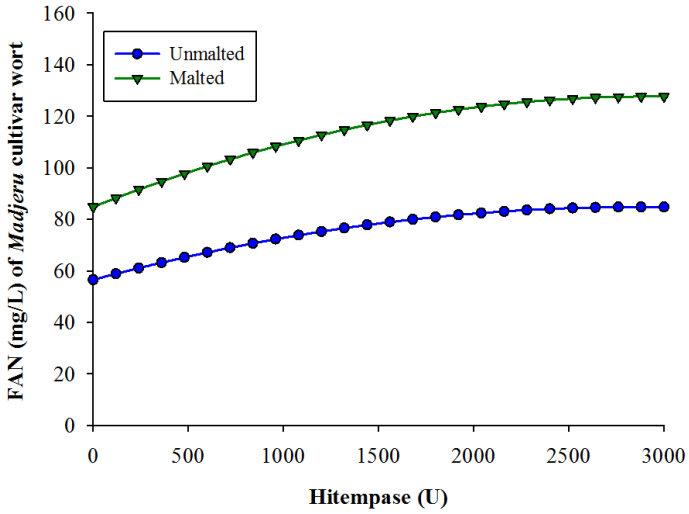


Figure 5b. Effect of concentration of hitempase (α-amylase) in the presence of fixed concentrations of bioglucanase (750 BGU) and brewers protease (60 mg) on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

Table 6. Estimation of regression coefficients for free amino nitrogen of umalted and malted *Madjeru*.

Effects	Coefficient		Std. deviation		t-statistics		P-value	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Constant	63.504	94.807	2.047	3.024	31.026	31.347	0.000	0.000
X_1	15.411	23.771	2.106	3.111	7.319	7.641	0.000	0.000
X_2	8.487	12.745	1.836	2.713	4.003	4.069	0.005	0.005
X_3	18.418	28.311	1.735	2.563	8.663	9.012	0.000	0.000
X_1^2	-7.747	-11.26	3.61	5.334	-2.146	-2.111	0.069	0.073
X_2^2	25.922	36.639	2.627	3.882	7.4	7.658	0.000	0.000
X_3^2	-8.636	-11.98	2.266	3.348	-2.538	-2.383	0.039	0.049
X_1*X_2	-3.798	-6.875	4.121	6.089	-0.798	-0.978	0.451	0.361
X_2*X_3	7.807	12.315	3.627	5.359	1.521	1.624	0.172	0.148
X_1*X_3	3.976	7.478	4.294	6.345	0.756	0.962	0.475	0.368

$$+28.311X_3 - 6.875X_1X_2 + 7.478X_1X_3 + 12.315X_2X_3 - 11.261X_1^2 + 39.639X_2^2 - 11.983X_3^2 \quad (4)$$

With: Y_{MadAAL} (X_1, X_2, X_3) representing the mathematical model for unmalted *Madjeru*; $Y_{MadMAAL}$ (X_1, X_2, X_3) for malted *Madjeru;* X_1, hitempase; X_2, bioglucanase and X_3, brewers protease (protéase). These mathematical models were once more polynomials having several variables with determination coefficients of R^2 = 0.973 for unmalted *Madjeru* and R^2 = 0.974 for malted *Madjeru*. These coefficients, coupled to AAD values of 0.037 and 0.036 for unmalted and malted *Madjeru* respectively, allowed for the validation of the models for assessment of the wort free amino nitrogen content. In addition, a bias factor of 1 for both unmalted and malted *Madjeru* mash types, coupled to exactitude factors of 1.00 for both mash types, also allowed for validation of the models according to the method described (Ross, 1996). The factors of the models were once more linear or of first degree (X_1, X_2 and X_3), quadratic or of the second degree (X_1^2, X_2^2 and X_3^2) or of interaction form (X_1X_2, X_1X_3, and X_2X_3). They were statistically considered significant or not if the probability (P) in increasing yields of FAN was ≤ 0.05 or ≥ 0.05 respectively (Table 6).

Effect of hitempase 2XL on yields of free amino nitrogen (FAN)

The impact of hitempase as sole mashing enzyme on wort FAN for unmalted and malted *Madjeru* is shown in Figure 5a. Free amino nitrogen content of wort gradually increased from 37.12 mg/L with increasing enzyme

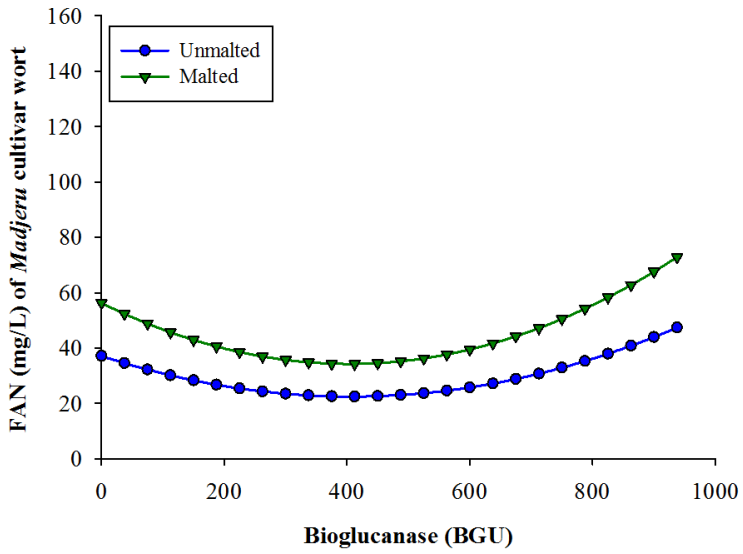


Figure 6a. Effect of concentration of bioglucanase (β-glucanase) as sole mashing enzyme on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

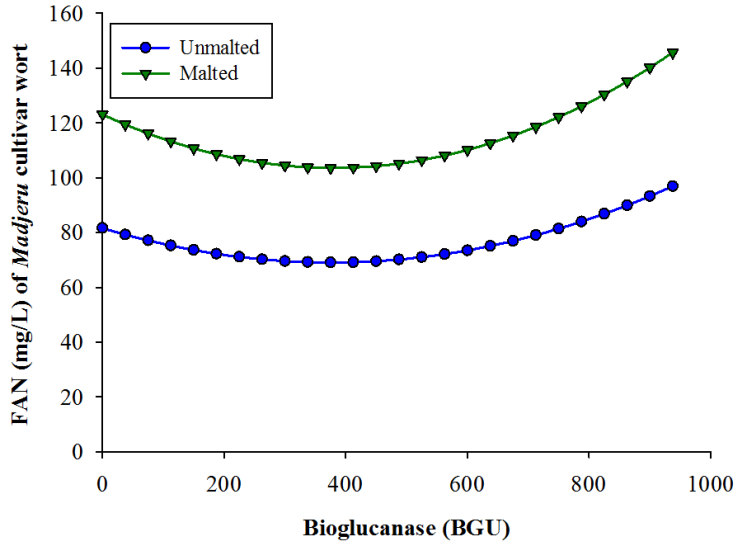


Figure 6b. Effect of concentration of bioglucanase (β-glucanase) in the presence of fixed concentrations of hitempase (1875 U) and brewers protease (60 mg) on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

concentration to reach a maximum of 68.04 mg/L for both unmalted mashes and from 56.26 mg/L to reach a maximum of 103.51 mg/L for malted *Madjeru* mashes. The Figure also showed that for both mash types, the yields in FAN in the absence of the enzyme represented more than 50% of the final yields. This suggests that the milling operation was at the basis of the instantaneous dissolution of these considerable amounts of free amino nitrogen at the start of mashing. Although hitempase is not a protein hydrolyzing enzyme, it exposes more free amino nitrogen functions upon acting on globular proteins and starch granules. This could explain the increase in FAN observed with increase in enzyme concentration. The higher FAN content for malted *Madjeru* mash compared to unmalted *Madjeru* mash is once more to be attributed to the natural virtues that the grains incur during

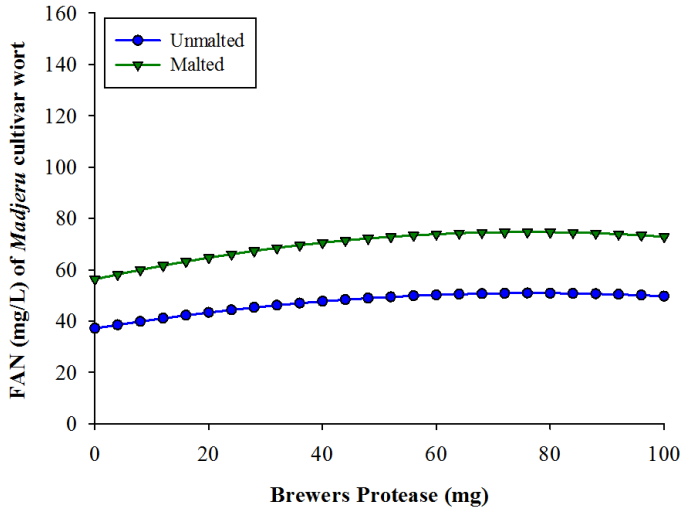


Figure 7a. Effect of concentration of brewers protease as sole mashing enzyme on yield of wort free amino nitrogen of sorghum cultivar *Madjeru.*

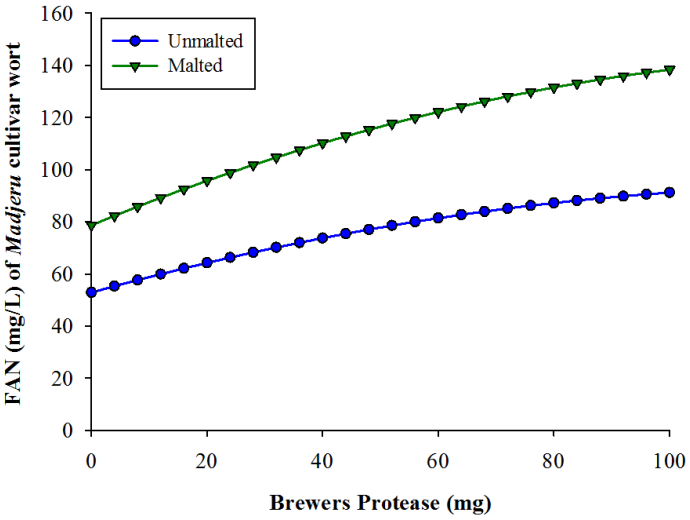


Figure 7b. Effect of concentration of brewers protease in the presence of fixed concentrations of hitempase (1875 U) and bioglucanase (750 BGU) on yield of wort free amino nitrogen of sorghum cultivar *Madjeru*.

during the malting process. Use of the models to predict the profile of FAN content of worts if the mashing enzymes bioglucanase (at 750 BGU) and brewers protease (at 60 mg) were coupled to hitempase's action, showed a profile of increases in yields of FAN similar to that observed in Figure 5b with increments contributing to amounts equivalent to roughly 15%. The models also showed that hitempase (X_1), in its first degree form, contributed for 15% of the FAN content of both the unmalted and malted *Madjeru* mashes (Figures 8a and b). This contribution was statistically significant for the two mash types (P = 0.000) (Table 6).

Similarly, in its quadratic form (X_1^2), hitempase's effect was not significant for the two mash types (P = 0.069 and 0.073 respectively) (Table 6). Its contribution in this form was 8 and 7% respectively (Figures 8a and b).

Effect of bioglucanase TX on yields of free amino nitrogen (FAN)

Figure 6a shows the effect of bioglucanase on the FAN content as sole mashing enzyme in unmalted and malted *Madjeru*. This content initially decreased with increasing enzyme concentration in both mash types, dropping from

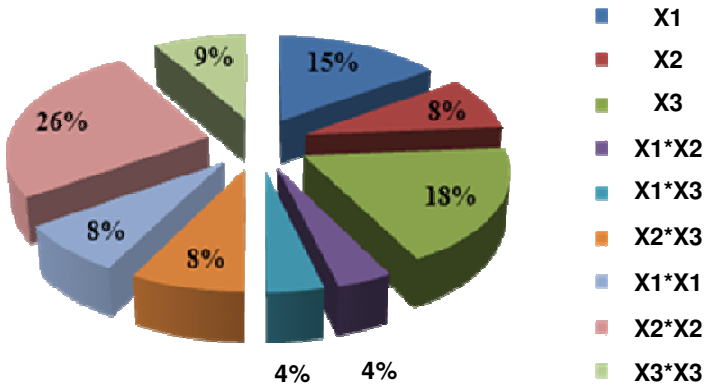


Figure 8a. Contribution to yield of wort free amino nitrogen (FAN) of each factor in its linear, quadratic and interaction (combined) forms for unmalted sorghum cultivar *Madjeru*.

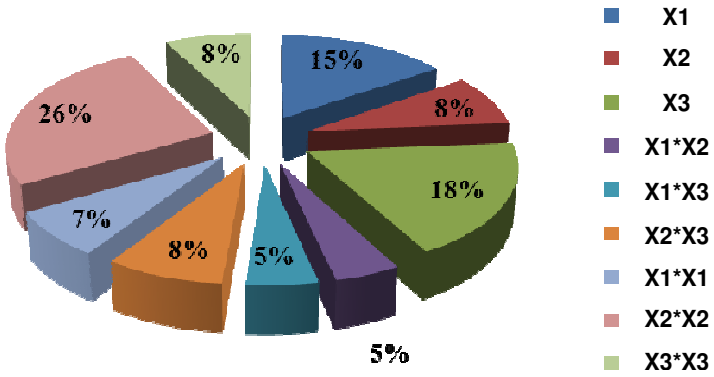


Figure 8b. Contribution to yield of wort free amino nitrogen (FAN) of each factor in its linear, quadratic and interaction (combined) forms for malted sorghum cultivar *Madjeru*.

37.12 to a minimal of 22.47 mg/L for unmalted *Madjeru*, and from 56.26 to 34.25 mg/L for malted *Madjeru,* and henceforth gradually increased with increases in enzyme concentration as from 407 BGU. This observation indicated that bioglucanase is not a protein hydrolyzing enzyme. The initial decrease in FAN content, followed by a subsequent increase, could be explained by the fact that, the free amino nitrogen instantaneously dissolved in the mash after the milling process, reacted with soluble sugars, after which the hydrolyzing action of cell wall components by bioglucanase, kinetically become perceptible to permit observing the liberation of extra FAN molecules. Upon using the model to predict the amounts of FAN if mashed in the presence of hitempase (at 1875 U) and brewers protease (at 60 mg), the same profile as in Figure 6A were observed, but with the levels of free amino nitrogen contents for both mash types increasing 2 times as compared to the original contents (Figure 6b). This confirmed the need to have all mashing enzymes present in appropriate proportions during mashing to permit obtaining substantial amounts of FAN in worts. These observations once more displayed the role of the malting process in guaranteeing worts of higher brewing properties.

According to the models, bioglucanase (X_2), in its linear form, contributed to 8% of the FAN content for both the unmalted and malted *Madjeru* mash types (Figures 8A and B). This contribution was statistically significant for the two mash types (P = 0.005) (Table 6). Similarly, in its quadratic form (X_2^2), the effect of the enzyme remained significant for both mash types (P = 0.000) (Table 6). Its contribution in this form was 26% for both mash types (Figures 8a and b).

Effect of brewers protease on yields of free amino nitrogen (FAN)

The effect of brewers protease as sole mashing enzyme on FAN content for unmalted and malted *Madjeru* is presented in Figure 7A. Free amino nitrogen content increased very slightly from 37.12 to 49.66 mg/L for unmalted *Madjeru* worts and from 56.26 to 72.86 mg/L for malted *Madjeru* worts with increasing enzyme concentrations. These levels were maintained virtually constant as from about 60 mg of enzyme input thereof. The models predicted that coupling hitempase (at 1875 U)

Table 7. ANOVA for free amino nitrogen of umalted and malted *Madjeru*.

Source	DF	Sum of squares		Mean of squares		F-value		P-value	
		Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
Regression	9	4697.665	10845.612	521.963	1025.068	27.908	29.51	0.000	0.000
Linear	3	3323.582	7688.072	1107.861	2562.691	59.235	62.756	0.000	0.000
Quadratic	3	1311.862	2987.332	437.287	995.777	23.381	24.385	0.001	0.000
Interactions	3	62.221	170.208	20.74	56.736	1.109	1.389	0.407	0.323
Residual error	7	130.919	285.848	18.703	40.835				
Total error	16	4828.585	11131.46						

Table 8. ANOVA for comparing free amino nitrogen of unmalted and malted *Madjeru* worts.

Source	DF	Sum of squares	Mean of squares	F-value	P-value
Inter-group	1	8813.2	8813.2	17.67	0.000
Intra-group	32	15960	498.751		
Total	33	24773.2			

and bioglucanase (at 750 BGU) to the action of brewers protease would induce a steady increase in FAN content (Figure 7b). This once more indicates the need of having all mashing enzymes present in order to obtain higher FAN yields. The additional FAN content observed for malted *Madjeru* mash as compared to unmalted *Madjeru* mash could once more be attributed to the natural virtues that the malting process contributed to the grains used. The mathematical model statistically showed that the action of brewers protease was in its linear form significant for both mash types (P = 0.000 for both (Table 6). Its contribution to FAN content in this form (X_3) was 18% for both the unmalted and malted *Madjeru* worts (Figures 8a and b).

The effect of the enzyme in its quadratic form (X_3^2) however remained significant for both mash types (P = 0.039 and 0.049 respectively) (Table 6). Its contribution was 9% for unmalted *Madjeru* worts and 8% for malted *Madjeru* worts (Figures 8a and b).

Effect of enzymes interactions on yields of free amino nitrogen (FAN)

The global action of the enzymes' interaction or as coupled forms (X_1X_2, X_1X_3 and X_2X_3) on the FAN content was statistically not significant (P = 0.407 for unmalted *Madjeru* and P = 0.323 for malted *Madjeru*) (Table 7). Their contributions of FAN content are shown in Figures 8a and b. The effect of the X_1X_2 (hitempase 2XL/bioglucanase TX) interaction was not significant for both wort types (P = 0.451 for unmalted *Madjeru* and P = 0.361 for malted *Madjeru*) (Table 6). Its contribution of FAN content in both mash types was 4 and 5% respectively) (Figures 8a and b). Similarly, the action of the couple X_1X_3 (hitempase 2XL/brewers protease) was also not significant for both mash types (P = 0.475 for unmalted *Madjeru* and P = 0.368 for malted *Madjeru*) (Table 6). Its contribution of FAN content in both mash types was 5 and 4% respectively (Figures 8a and b). Finally, for the couple X_2X_3 (BIOGLUCANASETX/brewers protease), its action was also not significant for both wort types (P = 0.172 for unmalted *Madjeru* and P = 0.148 for malted *Madjeru*) (Table 6). The contribution of FAN content in both wort types was 8% for each (Figures 8a and b).

Table 8 statistically confirmed the biological assertion that malted type samples were more potential raw materials for mashing in terms of FAN contents than the combination of unmalted grains and commercial enzymes (P = 0.000).

Optimization of the concerted mashing enzymes' action on yields of extracts and free amino nitrogen

The results obtained for the action of the enzymes on extract and free amino nitrogen yields after mashing on the basis of the models were optimized to define satisfactory domains of compromise for the mashing enzymes. These domains were obtained for the two key brewing parameters by fixing the wort conditions at: extract ≥ 12 °P and free amino nitrogen ≥ 80 mg/L. The theoretical optimal combination of enzyme action for unmalted *Madjeru* gave the following triplet of real variables for extract: 1960.5 U; 132.61 BGU and 28.86 mg for hitempase 2XL, Bioglucanase TX and brewers protease respectively. This triplet allowed for a maximal extract of 16.55 °P. The triplet for malted *Madjeru* was: 2610 U; 0 BGU and 40.44 mg. It allowed for a maximal extract of 16.35 °P. The optimal enzyme combinations were thus different particularly with regards to

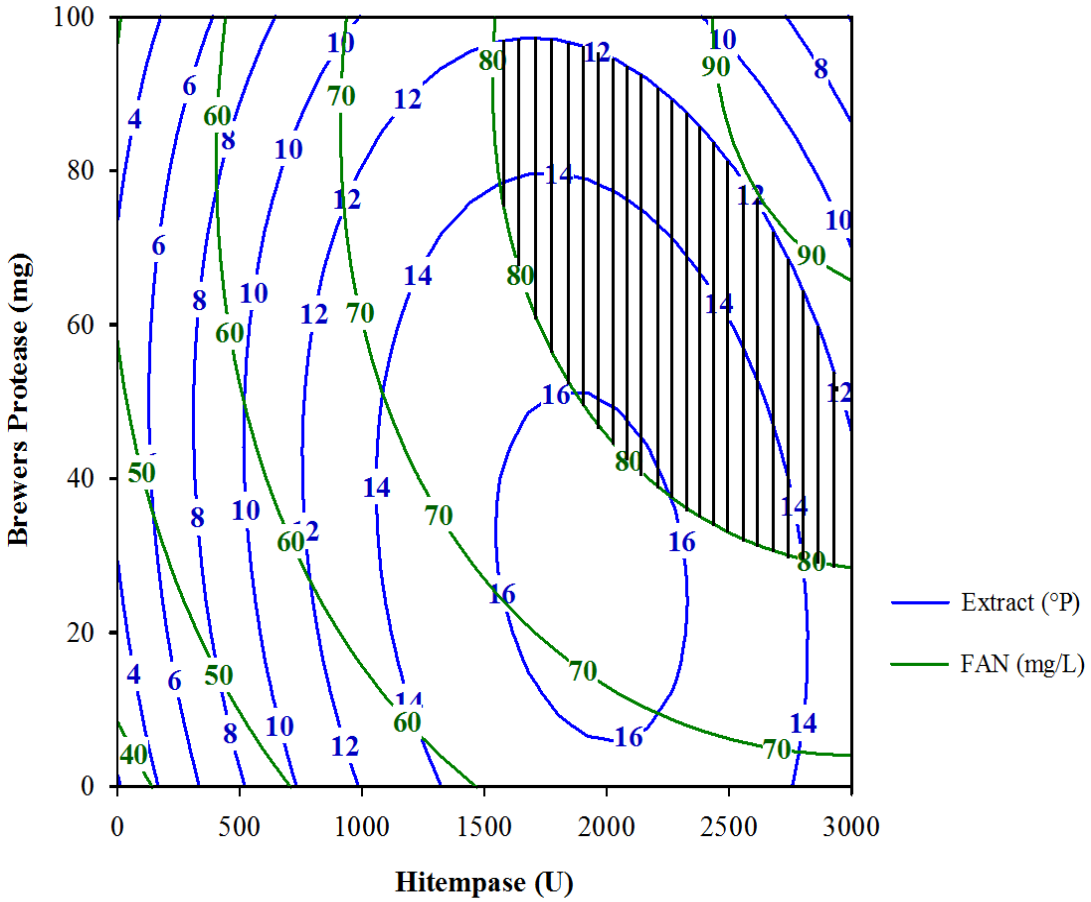


Figure 9a. Response surface curves for the enzyme combinations providing for optimal yields in extract and free amino nitrogen for unmalted sorghum cultivar *Madjeru*.

bioglucanase TX but both gave comparable yields of extracts. These results show that bioglucanase TX supplements for mashing malted *Madjeru* is not indispensable for obtaining required yields in extract, whereas the three enzymes are needed as supplements when mashing unmalted *Madjeru*. Figure 9a shows the response surface areas exploitable for efficient mashing capable of giving optimal results of yields of extract. For free amino nitrogen, the theoretical optimal combination of enzymes' action for unmalted *Madjeru* gave as triplet of real variables: 3000 U; 0 BGU and 100 mg for hitempase 2XL, bioglucanase TX and brewers protease respectively. This triplet allowed for maximal free amino nitrogen of 93.55 mg/L. The triplet for malted *Madjeru* was: 3000 U; 0 BGU and 100 mg. It allowed for maximal free amino nitrogen content of 144.48 mg/L. These results show that bioglucanase TX is not an indispensable mashing enzyme when seeking for appreciable amounts of free amino nitrogen in the worts of *Madjeru*, be it malted or not.

Figure 9b shows the response surface areas exploitable for efficient mashing giving optimal results of free amino nitrogen content.

Conclusions

The effects of three technical mashing enzymes (hitempase 2XL, bioglucanase TX and brewers protease) on yields of extract and free amino nitrogen were studied during the mashing of unmalted and malted *Madjeru* grist. Hitempase 2XL was principally responsible for extract yields in unmalted *Madjeru* mash but its impact on malted *Madjeru* mash type was mild. Bioglucanase TX played no role, while brewers protease showed limited contributions to yields of extract. Hitempase 2XL and brewers protease individually contributed to yields in free amino nitrogen in both unmalted and malted *Madjeru* mashes, though the milling operation contributed to FAN yields for more than 50% in both mashes. This study shows that proper malting and mashing of this sorghum cultivar could lead to satisfactory worts properties in terms of extract and free amino nitrogen for brewing purposes. Supplements of technical mashing enzymes to boost their yields of extract in particular, are thus not indispensable when mashing with malted *Madjeru.* Optimization of mashing properties through models clearly describing the actions of individual technical mashing

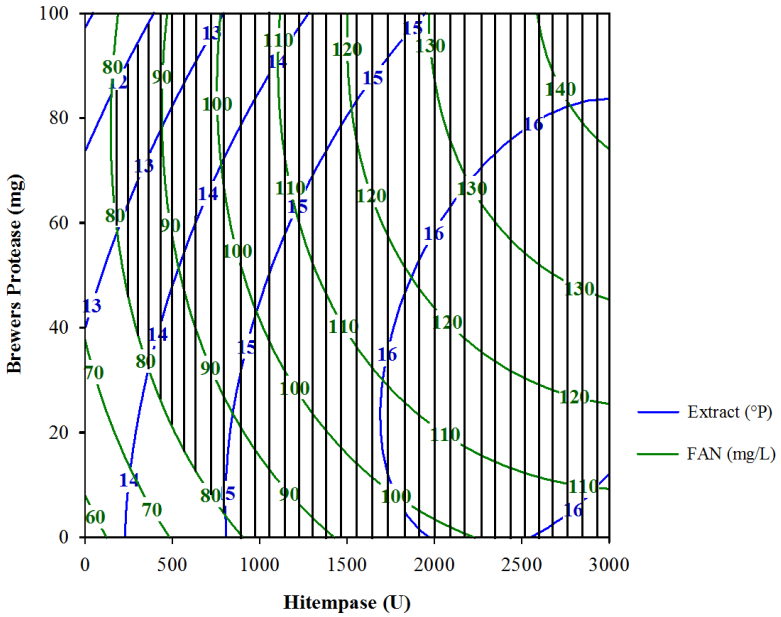


Figure 9b. Response surface curves for the enzyme combinations providing for optimal yields in extract and free amino nitrogen for malted sorghum cultivar *Madjeru.*

enzymes, as displayed in this study using the response surface methodology is however of interest particularly when mashing with high amounts of sorghum adjuncts. Further studies on the fermentability of worts obtained after such studies would be of importance in order to assess the exploitability of the results for improved brewing practices with this sorghum cultivar.

REFERENCES

Adejemilua F (1995). Processing and profile of sorghum malt. Tech. Q. Master Brew. Assoc. Am., 32:15-18.

Agu RC, Palmer GH (1998). Effect of mashing with commercial enzymes on the properties of sorghum worts. World J. Micro-biol Biotechnol., 14: 43-48.

Aisien AO (1982). Enzymic modification of sorghum endosperm during seedling growth and malting. J Sci. Food Agric., 33: 754-759.

Aisien AO, Muts GCJ (1987). Micro-scale malting and brewing studies of some sorghum varieties. J. Instit. Brew., 93:328-333.

Arri BK (1989). Problems associated with the use of sorghum for lager beer production. Industrial Utilization of sorghum. In: Symposium on the Current Status and Potential of Industrial Uses of Sorghum Kano, Nigeria: ICRISAT.

Briggs DE, Boulton CA, Brookes PA, Stevens R (2004). Brewing science and practice. Cambridge: Woodhead Publishing Limited and CRC Press LLC.

Desobgo ZSC, Nso EJ, Tenin D (2011a). 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. J. Brew. Distill., 2:5-15.

Desobgo ZSC, Nso EJ, Tenin D (2011b). Use of response surface methodology for optimizing the action of mashing enzymes on wort reducing sugars of the *Madjeru* sorghum cultivar. Afr. J. Food Sci., 5: 91-99.

Desobgo ZSC, Nso EJ, Tenin D, Kayem GJ (2010). Modelling and optimizing of mashing enzymes-effect on yield of filtrate of unmalted sorghum by use of response surface methodology. J. Instit. Brew., 116: 62-69.

EtokAkpan OU, Palmer GH (1990). Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. World J. Microbiol. Biotechnol, 6: 408-417.

Goode DL, Arendt EK (2003). Pilot scale production of lager beer from grist containing 50% unmalted sorghum. J.Instit. Brew., 109: 208-217.

Goode DL, Halbert C, Arendt EK (2002). Mashing studies with unmalted sorghum and malted barley. J. Instit.Brew., 108: 465-473.

Goode DL, Halbert C, Arendt EK (2003). Optimisation of mashing conditions when mashing with unmalted sorghum and commercial enzymes. J. Am. Soc. Brew. Chem., 61: 69-78.

Hough JS, Briggs DE, Stevens R, Young TW (1982). Malting and brewing science. London: Chapman and Hall

Nso EJ, Ajebesone PE, Mbofung CM, Palmer GH (2003). Properties of three sorghum cultivars used for the production of *Bili-Bili* beverage in Northern Cameroon. J. Instit. Brew., 109: 245-250.

Nso EJ, Nanadoum M, Palmer GH (2006). The effect of formaldehyde on enzyme development in sorghum malts. MBAA TQ, 43:177-182.

Palmer GH (1989). Cereal in malting and brewing. In: Cereal science and technology (Palmer, G., ed) Aberdeen, Scotland: Aberdeen University Press.

Phiarais NB, Schehl BD, Oliveira JC, Arendt EK (2006). Use of surface response methodology to investigate the effectiveness of commercial enzymes on buckwheat malt for brewing purpose. J. Instit. Brew., 112: 324-332.

Ross T (1996). Indices for performance evaluation of predictive models in food microbiology. J. Appli.Bacteriolog., 81:501-508.

Taylor JRN (1983). Effect of malting on the protein and free amino nitrogen composition of sorghum. J. Sci. Food Agric., 34: 885-892.

MBAA TQ vol. 48, no. 3 • 2011 • pp. 77–86

Optimisation of the Action of Commercial Mashing Enzymes on Wort Extracts and Free Amino Nitrogen of the *Safrari* Sorghum Cultivar

Zangué S. C. Desobgo, Emmanuel J. Nso*, and Dzudie Tenin

Ecole Nationale Supérieure des Sciences Agro-industrielles (ENSAI), Université de Ngaoundéré, BP 455 Ngaoundéré, Cameroon

ABSTRACT

The influence of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) used as sole mashing enzymes on yields of extract and free amino nitrogen (FAN) of the worts of the mashes of unmalted and malted *Safrari* sorghum was studied using the response surface methodology. The study revealed that increasing amounts of Hitempase considerably increased yields of extract during mashing of unmalted *Safrari* grist, while the effect of Bioglucanase was smaller, and that of Brewers protease was insignificant. Extract yields decreased with increasing amounts of the three enzymes during the mashing of malted *Safrari*. This decrease was least expressed in the case of Brewers protease. Yields in FAN amounted to less than 50%, with increasing amounts of both Hitempase and Brewers protease, but constantly decreased to nil for Bioglucanase's action in both unmalted and malted *Safrari* mashes. The milling operation singularly liberated more than 50% of FAN for both mash types and for each of the enzymes. Optimisation of the concerted actions of the three enzymes for extract yields gave a combination of 2,098.5 U, 937.5 BGU and 0 mg (for Hitempase, Bioglucanase and Brewers protease, respectively) for unmalted *Safrari* sorghum mash. This gave a maximal extract yield of 18°P. The combination was 0 U, 28.68 BGU and 0 mg for malted *Safrari* sorghum mash, giving a maximal extract yield of 18.82°P. Optimisation for FAN gave a combination of 2,434.5 U, 0 BGU and 100 mg (for Hitempase, Bioglucanase and Brewers protease, respectively) for unmalted *Safrari* sorghum mash. This gave maximal FAN of 144.77 mg/L. The combination was 2,191.5 U, 0 BGU and 100 mg for malted *Safrari* sorghum mash, giving a maximal FAN of 196.73 mg/L.

Keywords: commercial mashing enzymes, extract, free amino nitrogen, optimisation, *Safrari.*

SÍNTESIS

Se estudiaron, utilizando el método de superficie de respuesta, tres enzimas comerciales de maceración (Hitempase 2XL, Bioglucanase TX y "Brewers protease") con respecto al rendimiento de extracto y de amino nitrógeno libre (FAN) en mostos de macerados de sorgo *Safrari* malteado y no malteado. El estudio reveló que un aumento en la cantidad de Hitempase aumentó considerablemente el rendimiento de extracto de un macerado de *Safrari* no malteado, mientras que el efecto de de la Biogluconase era menor y el efecto de la "Brewers protease" era insignificante. El rendimiento de extracto disminuyó con un aumento en la cantidad de las tres enzimas en el macerado de *Safrari* malteado, siendo menor la disminución con el "Brewers protease". El rendimiento de FAN fue menor del 50 por ciento con el aumento en las cantidades tanto de Hitempase y de "Brewers protease" y con Biogluconase se fue bajando hasta cero, tanto en macerados de *Safrari* malteado como no malteado. La molienda liberó por sí solo más de 50 por ciento del FAN para los dos tipos de macerado y para cada uno de las enzimas. La optimización del uso conjunto de las tres enzimas para aumentar el rendimiento de extracto resultó en la combinación 2.098,5 U, 937,5 BGU y 0 mg (para Hitempase, Bioglucanase y "Brewers protease", respectivamente) para macerados de sorgo *Safrari* no malteado, resultando en un extracto máximo de 18°P. La combinación óptima para *Safrari* malteado fue de 0 U, 28,68 BGU y 0 mg dando un extracto máximo de 18,82°P. La combinación óptima para FAN fue de 2.434,5 U, 0 BGU y 100 mg para *Safrari* no malteado, dando un FAN máximo de 144,77 mg/L, mientras que para *Safrari* malteado fue de 2.191,5 U, 0 BGU y 100 mg, para un FAN máximo de 196,73 mg/L.

Palabras claves: enzimas comerciales de maceración, extracto, amino nitrógeno libre (FAN), optimización, sorgo *Safrari.*

Dr. Emmanuel Nso is a senior lecturer and head of the Department of Process Engineering in Ecole Nationale Supérieure des Sciences Agro-industrielles (ENSAI) of the University of Ngaoundéré in Cameroon. He earned a masters degree in applied chemistry and bioindustries specializing in food technology, a masters degree in brewing science and technology and a PhD in enzymology, all from the Catholic University of Louvain – Louvain-la-Neuve in Belgium. He lectures enzymology, brewing science and technology, biochemical engineering and industrial microbiology in ENSAI. His main domains of research are brewing science, enzyme technology in food processing (mainly in beer brewing and processing of tropical fruits into juice), enzyme extraction and purification, and production of biogas from biodegradable domestic wastes.

* Corresponding author. E-mail: nso_emmanuel@yahoo.fr

doi:10.1094/TQ-48-3-0829-01

Introduction

Sorghum in its malted form or as adjuncts has become a potential brewing cereal, particularly in the tropics where barley is not commonly grown[6,14,24,25,26,30,31,32,33,34]. Its low contents of potential mashing enzymes due to their poor development during malting has often triggered the use of commercial mashing enzymes as supplements to achieve higher yields in extracts and other important wort specifications in beer brewing[1,2,3,4,5,7,8,16,17,19,20,28,29,37,38]. The inclusion of commercial mashing enzymes when mashing sorghum malts and adjuncts is important[9,10,11,14,15,22,23,24,27], but the amount of enzymes needed for optimal mashing may vary from one sorghum cultivar to the next. It is also not clear whether the use of commercial mashing enzymes for optimal mashing of malted sorghum is indispensable. In this work, the influence of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and

Table 1. Characteristics of commercial mashing enzyme preparations.

Commercial mashing enzyme	Organism of origin	Activity	Description	Temperature optima	pH optima	Recommended application level in adjuncts	Form
Hitempase 2XL[a]	*Bacillus licheniformis*	4,416.29 ± 19.34 U/ml	α-amylase	60–95°C	4–8	60 U/g	Solution
Bioglucanase TX[a]	*Trichodermareesei*	750 BGU/ml	β-glucanase	60°C	4.5–6.5	0.01 and 0.025% (v/w)	Solution
Brewers protease[b]	*Bacillus amyloliquefaciens*	1,842.2 ± 1.8 mg FAN/min/ml	Protease	45–50°C (denatured at 85°C)	6.5–7.5	0.4–2 g/kg	Solution

[a] Hitempase 2XL and Bioglucanase TX were obtained from Kerry Bioscience; Kilnagleary, Carrigaline, Co. Cork, Ireland.
[b] Brewers protease was obtained from DSM Food Specialities, Cedex France.

Brewers protease) was modelled and optimised for yields of extracts and free amino nitrogen during mashing of unmalted and malted *Safrari* sorghum cultivar using the response surface methodology (RSM).

Materials and Methods

Enzymes

The characteristics of the commercial enzymes used (Hitempase 2XL, a thermo stable α-amylase from *Baccillus licheniformis;* Bioglucanase TX, an enzymatic composition of β-glucanase and hemicellulases from *Trichodermareesel;* and Brewers protease from *Bacillus amyloliquefaciens*) are presented in Table 1.

Sorghum Cultivar

The *Safrari* sorghum cultivar was obtained from the Institute of Research and Agronomic Development (IRAD) Maroua, Cameroon.

Modelling

Modelling was carried out as previously described[15]. A Doehlert matrix design with three factors representing Hitempase (X_1), Bioglucanase (X_2) and Brewers protease (X_3) at ranges of 0–3,000 U, 0–937.5 BGU and 0–100 mg, respectively, was used. The transformed matrix of coded variables to an experimental matrix and desired responses (extract and free amino nitrogen) are shown in Table 2. The coefficients of the models and the models were obtained using the Systat version 12 software (Systat Software, Inc., San Jose, USA). This software also gives a statistical analysis on the model. Lastly, the curves were plotted using Sigmaplot version 11 build 11.0.0.77 software (WPCubed, GmbH, Germany).

Validation of Models

The models were validated using two procedures. The first consited of coupling the method earlier described[15] to the absolute average deviation (AAD) method[13]. The second procedure consisted of applying the method described[12,36].

Malting

About 1 kg of *Safrari* sorghum cultivar grains were washed three times using 3 litres of distilled water to remove dirt and other foreign bodies. The grains were steeped in 3 litres of distilled water for 48 h at room temperature (≈25°C) with three changes of water at intervals of 12 h before steep out. Germination was carried out for 4 days in a Heraeus type oven (D-63450 Hanau, Germany) at a temperature of 25°C with water sprinkled on the grains on daily basis. The malt was then air dried at 40°C for 4 days using a CKA 2000 AUF-type dryer (Ngaoundere, Cameroon). The malt was rubbed-off of its rootlets and stored until further use.

Mashing

Two hundred and fifty ml of distilled water were put into a 600 ml beaker and 50 g of sorghum (malted or unmalted) flour (Ø < 1 mm) added with continuous stirring until a homogenous mixture was obtained. This mixture was incubated at 45°C for 1 h in a water bath with intermittent stirring at intervals of 5 min. The mix was allowed to decant and 50 ml of the supernatant withdrawn and kept aside. The temperature of the mash was then raised to boiling so as to gelatinise sorghum starch during 40 min with intermittent stirring at intervals of 5 min before cooling to 65°C. The 50 ml of supernatant, to which commercial enzymes are added according to the Doehlert matrix design of three factors, were added to the mash and mashing continued for 1 h and 30 min with intermittent stirring at intervals of 10 min. The mash was cooled and filtered at 25°C for 1 h and 30 min using Whatmann paper no. 42.

Determination of Extract

Extract was determined as described by analytica-EBC[18].

Determination of Free Amino Nitrogen

Free amino nitrogen (FAN) was determined using the Ninhydrin method as described by analytica-EBC[18].

Optimisation of Models

Models were optimised as previously described[15]. The optimal zone of intersection of the curves was highlighted.

Results and Discussion

Optimisation of the action of mashing enzymes on the two key mashing parameters, extract and FAN, was carried out by modelling the experimental design required for laboratory purposes. Table 2 shows the results obtained for extracts and free amino nitrogen (FAN) after mashing unmalted and malted *Safrari* using the commercial mashing enzymes Hitempase (α-amylase), Bioglucanase (β-glucanase) and Brewers protease (protease).

The mathematical models obtained for extracts after mashing unmalted and malted *Safrari* were as follows, respectively:

$$Y_{SafEX}(X_1, X_2, X_3) = 14.62 + 5.478X_1 + 1.226X_2 - 0.881X_3 + 0.527X_1X_2 + 0.589X_1X_3 - 2.008X_2X_3 - 6.569X_1^2 - 0.935X_2^2 - 0.496X_3^2$$

$$Y_{SafMEX}(X_1, X_2, X_3) = 16.566 + 1.076X_1 - 1.377X_2 - 0.469X_3 + 4.774X_1X_2 + 2.338X_1X_3 - 1.326X_2X_3 - 0.901X_1^2 - 3.117X_2^2 - 0.180X_3^2$$

With: $Y_{SafEX}(X_1, X_2, X_3)$ representing the mathematical model for unmalted *Safrari;* $Y_{SafMEX}(X_1, X_2, X_3)$, the model for

Table 2. Matrices of Doehlert coded and transformed experimental values.

Coded values			Transformed experimental values			*Safrari*											
						Unmalted						Malted					
						Extract			FAN			Extract			FAN		
Hit X_1	Bio X_2	Brew prot X_3	Hit (U) X_1	Bio (BGU) X_2	Brew prot (mg) X_3	Exp[a]	Theo[b]	Res[c]	Exp	Theo	Res	Exp	Theo	Res	Exp	Theo	Res
0.000	0.000	0.000	1,500	468.75	50	14.81	14.62	0.19	95	91.78	3.22	16.43	16.57	–0.14	140	136.97	3.03
1.000	0.000	0.000	3,000	468.75	50	14.21	13.53	0.68	88	90.76	–2.76	16.64	16.74	–0.10	132	131.32	0.68
0.500	0.866	0.000	2,250	937.5	50	15.89	16.31	–0.42	110	97.91	12.09	15.60	15.42	0.18	165	151.42	13.58
–0.500	–0.866	0.000	750	0.00	50	8.75	8.70	0.05	100	106.23	–6.23	16.02	16.72	–0.70	149	154.06	–5.06
0.500	–0.866	0.000	2,250	0.00	50	13.40	13.73	–0.33	135.60	128.72	6.88	14.56	13.67	0.89	189	182.34	6.66
–0.500	0.866	0.000	750	937.5	50	10.73	10.37	0.36	63	70.41	–7.41	9.36	10.21	–0.85	94.50	101.93	–7.43
0.500	0.289	0.816	2,250	615.18	100	14.29	14.79	–0.50	118.33	124.00	–5.67	16.64	17.05	–0.41	177.50	186.44	–8.94
–0.500	–0.289	–0.816	750	312.32	0.0	9.98	10.04	–0.06	66.33	51.87	14.46	17.16	17.53	–0.37	99.50	77.78	21.72
0.500	–0.289	–0.816	2,250	312.32	0.0	14.55	14.88	–0.33	61.67	66.12	–4.46	14.56	15.32	–0.76	92.50	100.32	–7.82
0.000	0.577	–0.816	1,500	781.07	0.0	16.44	16.35	0.09	38.33	43.54	–5.21	16.33	15.62	0.71	57.50	64.42	–6.92
–0.500	0.289	0.816	750	615.18	100	9.06	8.68	0.38	91.92	88.27	3.65	13.52	12.69	0.83	137.88	131.22	6.66
0.000	–0.577	0.816	1,500	156.43	100	13.61	13.50	0.11	126	123.99	2.01	16.02	16.44	–0.42	178	175.72	2.28
0.000	0.000	0.000	1,500	468.75	50	14.50	14.62	–0.12	85.33	91.78	–6.45	16.64	16.57	0.07	128	136.97	–8.97
–1.000	0.000	0.000	0	468.75	50	1.80	2.57	–0.77	45	40.78	4.22	14.56	14.59	–0.03	55	53.55	1.45
–1.000	–0.866	–0.816	0	0.0	0.0	1.02	0.72	0.30	54	58.80	–4.80	19.24	18.81	0.43	81	87.97	–6.97
0.000	0.000	0.000	1,500	468.75	50	14.60	14.62	–0.02	91.67	91.78	–0.11	16.64	16.57	0.07	137.50	136.97	0.53
0.000	0.000	0.000	1,500	468.75	50	15.00	14.62	0.38	88.33	91.78	–3.45	17.16	16.57	0.59	132.50	136.97	–4.47

[a] Experimental result values.
[b] Theoretical values (values coming from mathematical models).
[c] Residue.

Table 3. Estimation of regression coefficients for the extracts of umalted and malted *Safrari.*

Effects	Coefficient		Std. deviation		t-statistics		P-value	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
CONSTANT	14.62	16.566	0.271	0.396	54.039	41.882	0.000	0.000
X_1	5.476	1.076	0.278	0.407	19.683	2.644	0.000	0.033
X_2	1.226	–1.377	0.243	0.355	4.374	–3.361	0.003	0.012
X_3	–0.881	–0.469	0.229	0.335	–3.136	–1.141	0.016	0.291
X_1^2	–6.569	–0.901	0.477	0.698	–13.768	–1.291	0.000	0.238
X_2^2	–0.935	–3.117	0.347	0.508	–2.020	–4.604	0.083	0.002
X_3^2	–0.496	–0.18	0.300	0.438	–1.103	–0.274	0.307	0.792
X_1*X_2	0.527	4.774	0.545	0.796	0.839	5.192	0.429	0.001
X_2*X_3	–2.008	–1.326	0.479	0.701	–2.960	–1.336	0.002	0.223
X_1*X_3	0.589	2.338	0.568	0.83	0.846	2.299	0.425	0.055

malted *Safrari;* X_1, Hitempase; X_2, Bioglucanase and X_3 Brewers protease.

These mathematical models are polynomials having several variables with correlation coefficients $R^2 = 0.993$ for unmalted *Safrari* and $R^2 = 0.930$ for malted *Safrari*. These coefficients, coupled to AAD values of 0.026 and 0.031 for unmalted and malted *Safrari,* respectively, allowed for the validation of the models for the wort extract yields. In addition, a bias factor of 1, coupled to exactitude factors of 1.06 and 1.03 for both unmalted and malted *Safrari,* respectively, also allowed for validation of the models according to the method described[12,36]. The factors of the models were linear or of first degree (X_1, X_2 and X_3), quadratic or of the second degree (X_1^2, X_2^2 and X_3^2) and of interacting form (X_1X_2, X_1X_3 and X_2X_3). They were statistically considered significant or not if the probability (P) of increasing yields of extracts was ≤0.05 or ≥0.05, respectively (Table 3).

The impact of Hitempase as sole mashing enzyme on the yields of extract of unmalted and malted *Safrari* is shown in Figure 1A. Extract yield increased with increasing concentration of enzyme for unmalted *Safrari* mash to attain a maximum level (≈13°P) at about 2,000 U, followed by a slight and steady decrease thereafter. It was already maximal (≈18.5°P) even in absence of Hitempase for malted *Safrari* mash. Indeed, increasing amounts in enzyme concentration instead induced a steady decrease of extract yield for malted *Safrari* mash. However, when the mathematical models were applied to predict the impact of supplements of Bioglucanase and Brewers protease at concentrations of 400 BGU and 60 mg, respectively, it was observed that the profile of extract remained fairly constant with increasing amounts of Hitempase for malted *Safrari* mash (Fig. 1B). Starch is indeed the main macromolecule of cereals and the main substrate of α-amylase. It is therefore expected that this enzyme contributes the greatest amount of soluble materials that could be found in resulting worts due to its action on starch[15,35]. Figures 1A and B also showed that supplements of Hitempase in malted *Safrari* mash was of no importance. The steady decrease in yields of extract of malted *Safrari* mash could be attributed to Maillard reactions between soluble nitrogenous compounds and reducing sugars earlier produced during malting (Fig. 1A). These results also indicated that efficient mashing was obtainable in the presence of the key mashing enzymes developed during malting (Fig. 1A). From the mathematical model, it was shown that in its linear form (X_1), Hitempase contributed 29% and 7% of extract yields for unmalted and malted *Safrari,* respectively (Figs. 2A and B). Statistical analyses also showed that this contribution was significant (P = 0.000 and 0.033 for unmalted and malted

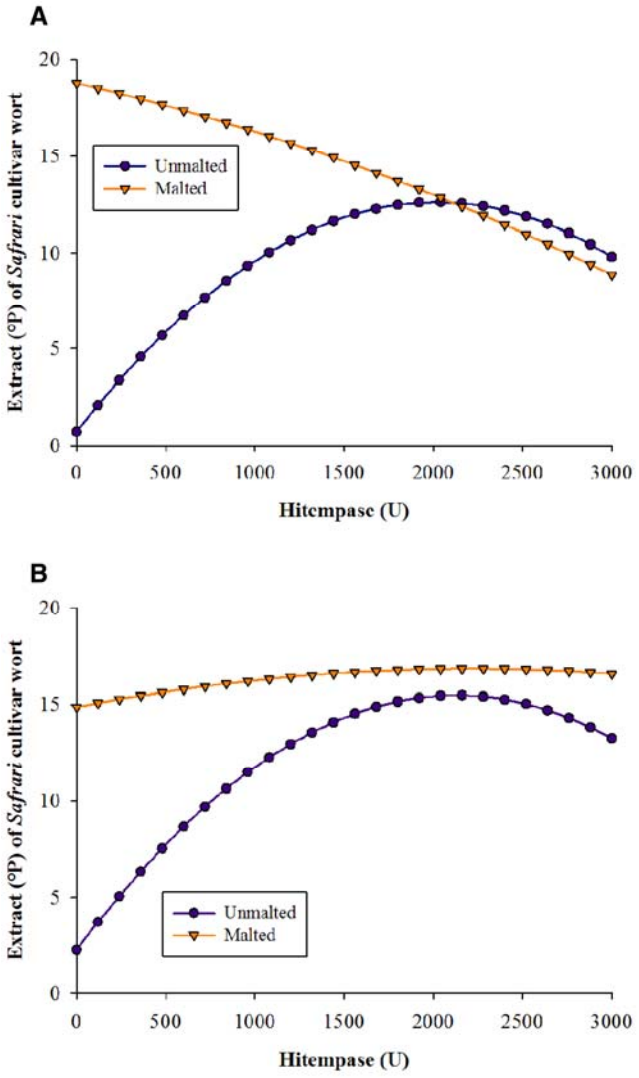


Figure 1. A, Effect of concentration of Hitempase (α-amylase) as sole mashing enzyme (concentrations of Bioglucanase and Brewers protease set at 0) on yield of wort extract of sorghum cultivar *Safrari.* **B,** Effect of concentration of Hitempase (α-amylase) in the presence of fixed concentrations of Bioglucanase (400 BGU) and Brewers protease (60 mg) on yield of wort extract of sorghum cultivar *Safrari.*

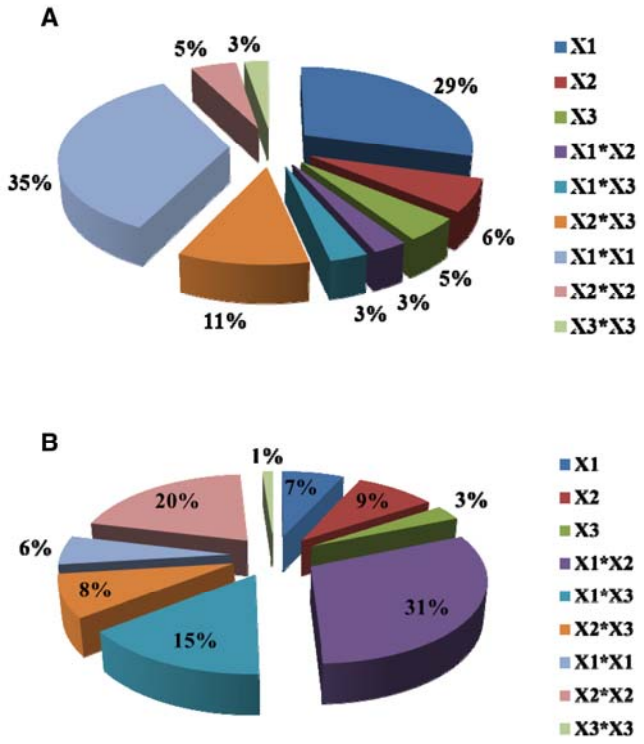


Figure 2. A, Contribution to yield of wort extract (°P) of each factor in its linear, quadratic and interaction (combined) forms for unmalted sorghum cultivar *Safrari*. **B,** Contribution to yield of wort extract (°P) of each factor in its linear, quadratic and interaction (combined) forms for malted sorghum cultivar *Safrari*.

Safrari, respectively) (Table 3). In its quadratic form (X_1^2), Hitempase remained statistically significant for mashing unmalted *Safrari,* but not for malted *Safrari* (P = 0.000 and 0.238, respectively). This confirmed the above biological observation according to which supplements of this enzyme in malted mashes of *Safrari* were of no importance. Its contribution to increasing extract yields in its quadratic form (X_1^2) (excess of α-amylase in principle) is indeed 35% and 6% for unmalted and malted *Safrari,* respectively (Figs. 2A and B).

Figure 3A shows the effect of mashing unmalted and malted *Safrari* using Bioglucanase as sole mashing enzyme on yields of extract. There was a slight and constant increase in yields of extract as enzyme concentration increased for unmalted *Safrari* mash. The yields of extract for malted *Safrari* mash was maximal even in the absence of Bioglucanase and steadily decreased with an increase in enzyme concentration. The small yields of extract due to Bioglucanase action in unmalted *Safrari* mash can be attributed to its ability to hydrolyse β-glucans into glucose and other soluble carbohydrates. Bioglucanase was therefore not a backbone enzyme for extract production during mashing. A similar application of the mathematical models as carried out above for Hitempase's action, using 60 mg of Brewers protease and 2,000 U of Hitempase, predicted that supplements of these two key mashing enzymes could provide similar results in extract yields for both unmalted and malted *Safrari* mashes (Fig. 3B). Hitempase once more demonstrated that it was the backbone enzyme contributing to most of the extract yields. Figure 3B indeed showed that in the absence of Bioglucanase, but in the presence of Hitempase, yields of extract became comparable for both unmalted and malted *Safrari* mashes. These observations were all statistically confirmed. Indeed, in its linear form (X_2), Bioglucanase's action was significant (P = 0.003 and 0.012 for unmalted and malted *Safrari,* respectively) (Table 3). Figures 2A and B showed that this enzyme contributed for 6% and 9% of extract yield for unmalted and malted *Safrari,* respectively. In its quadratic form (X_2^2) (excess of enzyme in principle), Bioglucanase contributed for 5% and 20% of extract yield for unmalted and malted *Safrari,* respectively (Figs. 2A and B). These contributions were not statistically significant for unmalted *Safrari* mash, but were for malted *Safrari* mash (P = 0.083 and 0.002) (Table 3). Once more, the observed 1:4 ratio difference of extract yield between unmalted *Safrari* mashes as to malted *Safrari* mashes could be attributed to the impact of enzymes developed during malting and not to Bioglucanase supplements as such.

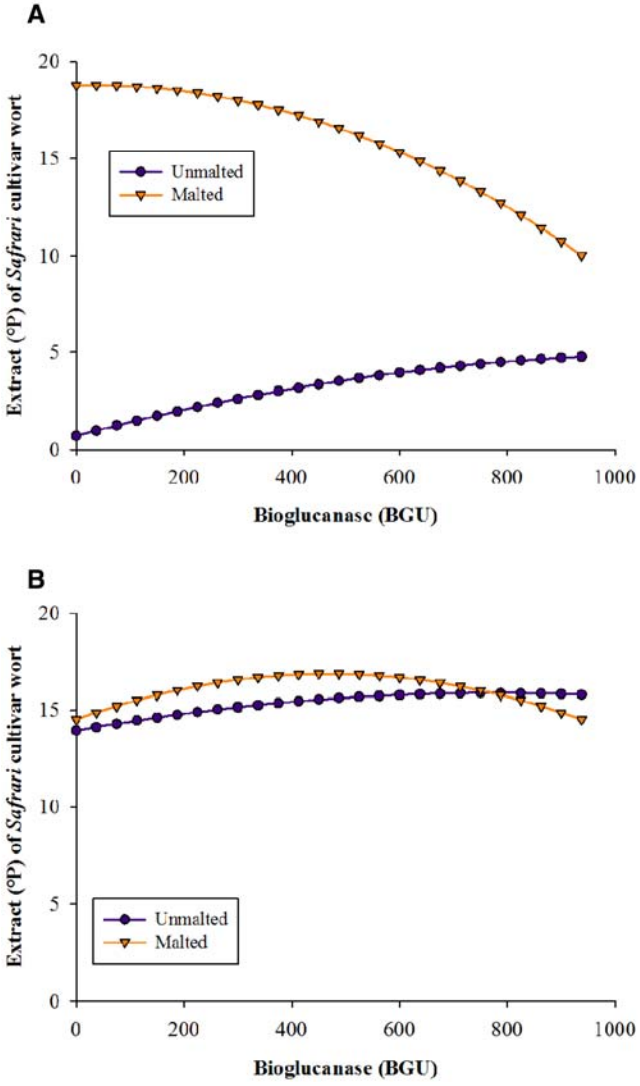


Figure 3. A, Effect of concentration of Bioglucanase (β-glucanase) as sole mashing enzyme (concentrations of Hitempase and Brewers protease set at 0) on yield of wort extract of sorghum cultivar *Safrari*. **B,** Effect of concentration of Bioglucanase (β-glucanase) in the presence of fixed concentrations of Hitempase (2,000 U) and Brewers protease (60 mg) on yield of wort extract of sorghum cultivar *Safrari*.

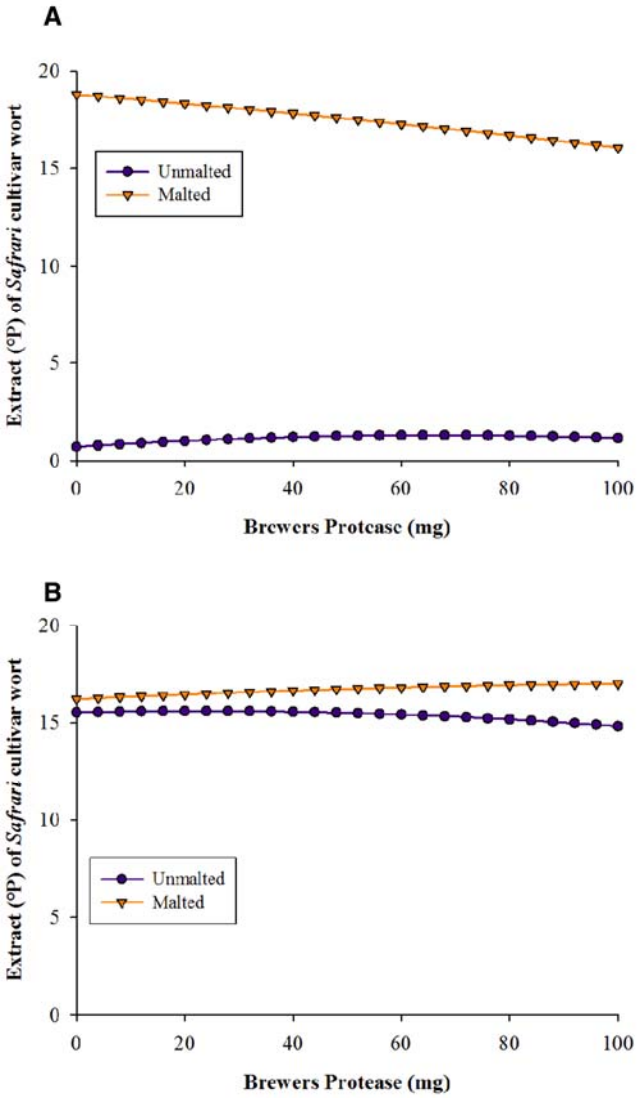


Figure 4. A, Effect of concentration of Brewers protease as sole mashing enzyme (concentrations of Bioglucanase and Hitempase set at 0) on yield of wort extract of sorghum cultivar *Safrari.* **B,** Effect of concentration of Brewers protease in the presence of fixed concentrations of Hitempase (2,000 U) and Bioglucanase (400 BGU) on yield of wort extract of sorghum cultivar *Safrari*.

The effect of mashing unmalted and malted *Safrari* on yields of extract using as sole mashing enzyme, Brewers protease, is shown in Figure 4A. This enzyme contributed little or no extracts in mashes of unmalted *Safrari* as compared to Hitempase and even Bioglucanase. Once more, the maximal extract observed when mashing with malted *Safrari* even in the absence of Brewers protease supplements is due to the virtues known to the malting process as explained earlier. These results confirmed earlier observations[15]. The steady and slight decrease of extract yield with increase of Brewers protease for the malted *Safrari* mash could once more be attributed to reactions between nitrogenous functions and some of the soluble sugars resulting from the malting process. The mathematical model was once more used to predict the yield in extract as carried out above for Hitempase and Bioglucanase actions. Thus, using 2,000 U of Hitempase and 400 BGU of Bioglucanase with increasing amounts of Brewers protease, the results once more showed that adding these mashing enzymes could provide similar results in extract yields for both unmalted and malted *Safrari* mashes (Fig. 4B). These observations were statistically confirmed using the mathematical model. In its first degree form (X_3), the impact of Brewers protease was significant for unmalted *Safrari* mash but not for malted *Safrari* mash (P = 0.016 and 0.291, respectively) (Table 3). Its contribution to extract yields was barely 5% and 3%, respectively, for both mash types (Figs. 2A and B). The impact of the enzyme in its quadratic form (X_3^2) was not significant for both mashes (P = 0.307 and 0.792, respectively) (Table 3). Its contribution to extract yield was 3% and 1%, respectively (Figs. 2A and B).

The models were further exploited to predict the impacts of the interactions (X_1X_2, X_1X_3 and X_2X_3) of these enzymes on yields of extract. The results are shown in Figures 2A and B. Globally, they were statistically not significant for unmalted

Table 4. ANOVA for the extracts of umalted and malted *Safrari.*

		Sum square		Mean square		F-value		P-value	
Source	**DDL**	**Unmalted**	**Malted**	**Unmalted**	**Malted**	**Unmalted**	**Malted**	**Unmalted**	**Malted**
Regression	9	333.038	65.429	37.004	7.27	113.241	10.408	0.000	0.003
Linear	3	240.289	18.625	80.096	6.208	245.112	8.888	0.000	0.009
Quadratic	3	89.659	7.736	29.886	2.579	91.459	3.692	0.000	0.070
Interactions	3	3.090	39.069	1.030	13.023	3.152	18.645	0.095	0.001
Residual error	7	2.287	4.889	0.327	0.698				
Total error	16	335.326	70.319						

Table 5. ANOVA for comparing extracts of unmalted and malted *Safrari* worts.

Source	**DF**	**Sum of squares**	**Mean of squares**	**F-value**	**P-value**
Inter-groups	1	130.693	130.693	9.21	0.004
Intra-groups	32	454.199	14.193		
Total	33	584.892			

Safrari mashes (P = 0.095), but were for malted *Safrari* mashes (P = 0.001) (Table 4). The interaction X_1X_2 (Hitempase/Bioglucanase) had no significant impact on unmalted *Safrari* mash, but did for malted *Safrari* (P = 0.429 and 0.001, respectively) (Table 3). It contributed merely 3% of extract for unmalted *Safrari* mash but up to 31% for malted *Safrari* mash (Figs. 2A and B). However, it is important to underline that this significant contribution could be attributed to the intrinsic virtues that malting offers when mashing with malted *Safrari,* and not to the Hitempase/Bioglucanase interaction as such. Though known to be the backbone starch hydrolysing enzyme, the action of Hitempase is best exploited when the cell walls of cereal grains are broken down by β-glucanases, hemicellulases and cellulases to liberate starch granules. This sequence of events during malting was confirmed by the mathematical models above. The interaction X_1X_3, corresponding to the couple Hitempase/Brewers Protease, also had no significant impact on both the extract yields of unmalted *Safrari* and malted *Safrari* mashes (P = 0.425 and 0.055, respectively) (Table 3). Its contribution to extract yields was 3% and 15%, respectively (Figs. 2A and B). This result was once more in conformity with the biological sequence occurring during malting. Efficient starch hydrolysis by α-amylase indeed occurs only after the breakdown of cell walls by β-glucanase, followed by liberation of starch granules due to proteolysis of the protein matrix enrobing them. The interaction Bioglucanase/Brewers protease (X_2X_3) had a significant impact on extract yields for unmalted *Safrari* mash but not for malted *Safrari* mashes (P = 0.002 and 0.223, respectively) (Table 3). Its contribution to extract yields was 11% and 8%, respectively, for both mash types (Figs. 2A and B). These low contributions by the couple (Bioglucanase/Brewers protease) were expected, as the two enzymes only play a supporting role in starch hydrolysis during mashing[15]. Table 5 statistically confirmed the observation that *Safrari* sorghum malted type samples were more potential mashing materials than unmalted *Safrari* sorghum adjuncts to which commercial enzymes are supplemented for the production of worts of higher extract yields (P = 0.004).

The mathematical models obtained for FAN for mashing unmalted and malted *Safrari* were as follows, respectively:

$$Y_{SafAAL}(X_1, X_2, X_3) = 91{,}781 + 24{,}992X_1 - 19{,}234X_2 + 35{,}693X_3 + 2{,}892X_1X_2 + 12{,}142X_1X_3 + 14{,}822X_2X_3 - 26{,}014X_1^2 + 20{,}723X_2^2 - 11{,}921X_3^2$$

$$Y_{SafMAAL}(X_1, X_2, X_3) = 136{,}969 + 38{,}884X_1 - 23{,}975X_2 + 51{,}247X_3 + 12{,}243X_1X_2 + 15{,}682X_1X_3 + 31{,}393X_2X_3 - 44{,}536X_1^2 + 28{,}806X_2^2 - 17{,}579X_3^2$$

With: Y_{SafAAL} (X_1, X_2, X_3) representing the mathematical model for unmalted *Safrari;* $Y_{SafMAAL}$ (X_1, X_2, X_3) for malted *Safrari;* X_1, Hitempase; X_2, Bioglucanase and X_3, Brewers protease. These mathematical models were once more polynomials having several variables with correlation coefficients R^2 = 0.944 for unmalted *Safrari* and R^2 = 0.955 for malted *Safrari*. These coefficients, coupled to AAD values of 0.073 and 0.060 for unmalted and malted *Safrari,* respectively, allowed for the validation of the models for assessment of the wort free amino nitrogen content. In addition, a bias factor of 1, coupled to exactitude factors of 1.08 and 1.06 for both unmalted and malted *Safrari,* respectively, also allowed for validation of the models according to the method described[12,36]. The factors of the models were once more linear or of first degree (X_1, X_2 and X_3), quadratic or of the second degree (X_1^2, X_2^2 and X_3^2) or of interaction form (X_1X_2, X_1X_3 and X_2X_3). They were statistically considered significant or not if the probability (P) of increasing yields of FAN was ≤0.05 or ≥0.05, respectively (Table 6).

The impact of Hitempase as sole mashing enzyme on yields of FAN for unmalted and malted *Safrari* is shown in Figure 5A. FAN content of wort gradually increased with increasing enzyme concentration to reach maxima of about 85 and 150 mg/L at about 1,750 U for both unmalted and malted *Safrari* mashes, respectively. This was followed by slight decreases for the unmalted *Safrari* mash as compared with malted *Safrari* mash. The curves showed that either for unmalted *Safrari* or malted *Safrari* mash types, the FAN content at origin was well above zero (60 mg/L and 90 mg/L, respectively). This suggests that the milling operation was at the basis of a good amount of the FAN present at the beginning of mashing. Although Hitempase is not a protein hydrolyzing enzyme, it exposes more free amino nitrogen functions upon acting on starch granules. This could explain the slight increase in FAN observed with increase in enzyme concentration. The higher FAN content for malted *Safrari* mash as compared to unmalted *Safrari* mash is once more to be attributed to the natural virtues that the grains incur during the malting process. Use of the models to predict the profile of FAN content of worts if the mashing enzymes Bioglucanase (at 400 BGU) and Brewers protease (at 60 mg) were coupled to Hitempase's action showed no remarkable difference. A balance between the amounts of enzymes needed to obtain maximal FAN contents and its disappearance in the medium due to reactions with sugars needs to be known to clearly understand this indifference. The models also showed that Hitempase (X_1), in its first degree form, contributed 15% of the FAN content of both the unmalted and malted *Safrari* mashes (Figs. 6A and B). This contribution was statistically significant for the two mash types (P = 0.001 and 0.000), re-

Table 6. Estimation of regression coefficients for free amino nitrogen of umalted and malted *Safrari.*

Effects	Coefficient		Std. deviation		T-statistics		P-value	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
CONSTANT	91.781	136.969	4.75	6.148	19.321	22.278	0.000	0.000
X_1	24.992	38.884	4.886	6.325	5.115	6.148	0.001	0.000
X_2	–19.234	–23.975	4.261	5.515	–3.909	–3.765	0.006	0.007
X_3	35.693	51.247	4.026	5.211	7.234	8.025	0.000	0.000
X_1^2	–26.014	–44.536	8.377	10.843	–3.105	–4.107	0.017	0.005
X_2^2	20.723	28.806	6.097	7.891	2.549	2.738	0.038	0.029
X_3^2	–11.921	–17.579	5.259	6.807	–1.509	–1.72	0.175	0.129
X_1*X_2	2.892	12.243	9.563	12.378	0.262	0.857	0.801	0.420
X_2*X_3	14.822	31.393	8.418	10.895	1.244	2.036	0.253	0.081
X_1*X_3	12.142	15.682	9.966	12.899	0.994	0.992	0.353	0.354

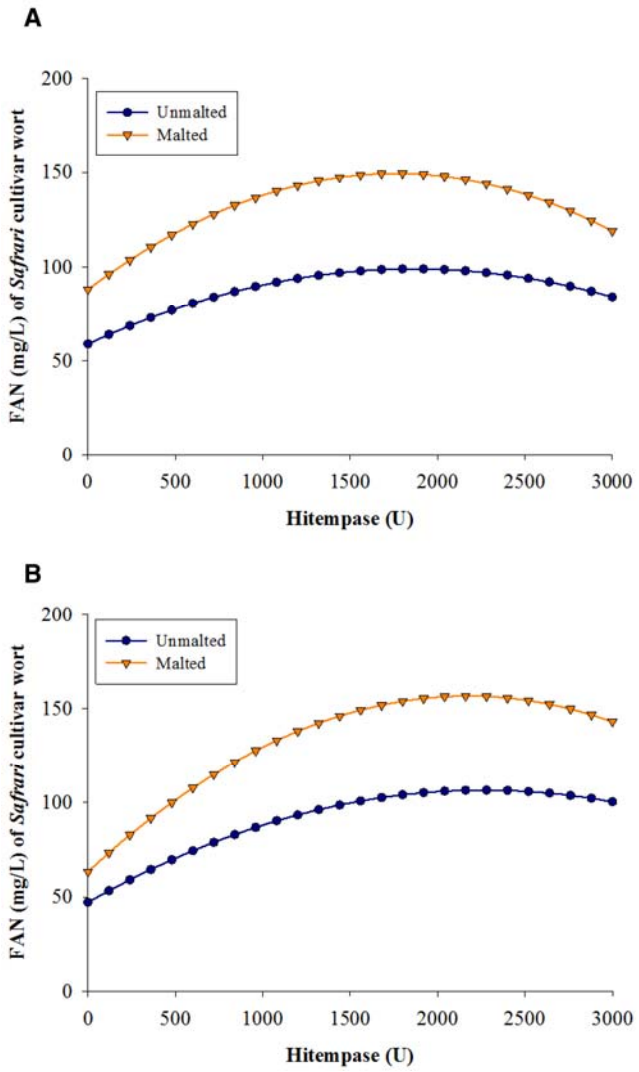


Figure 5. A, Effect of concentration of Hitempase (α-amylase) as sole mashing enzyme (concentrations of Bioglucanase and Brewers protease set at 0) on yield of wort free amino nitrogen of sorghum cultivar *Safrari.* **B,** Effect of concentration of Hitempase (α-amylase) in the presence of fixed concentrations of Bioglucanase (400 BGU) and Brewers protease (60 mg) on yield of wort free amino nitrogen of sorghum cultivar *Safrari*.

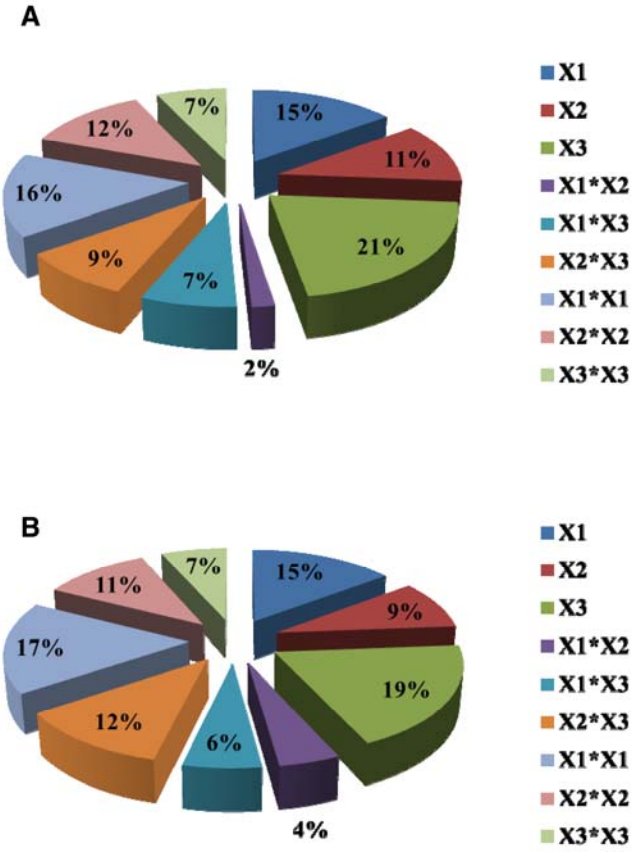


Figure 6. A, Contribution to yield of wort free amino nitrogen (FAN) of each factor in its linear, quadratic and interaction (combined) forms for unmalted sorghum cultivar *Safrari*. **B,** Contribution to yield of wort free amino nitrogen (FAN) of each factor in its linear, quadratic and interaction (combined) forms for malted sorghum cultivar *Safrari*.

spectively (Table 6). Similarly, in its quadratic form (X_1^2), Hitempase's effect remained significant for the two mash types (P = 0.017 and 0.005, respectively) (Table 6). Its contribution in this form was 16% and 17%, respectively (Figures 6A and B). The small difference in FAN content contribution (±2%) between its linear and quadratic (enzyme supplemented in excess) forms suggests that supplementing the enzyme in excess is of no technological use.

Figure 7A shows the effect of Bioglucanase on the FAN content as sole mashing enzyme in unmalted and malted *Safrari*. This content decreased to zero with increasing enzyme concentration in the mashes, indicating that Bioglucanase is not a protein hydrolysing enzyme. Mashing with the enzyme rather seemed to be of no use as the FAN was exposed to reactions with reducing sugars. Upon using the model to predict the amounts of FAN if mashed in the presence of Hitempase (at 2,000 U) and Brewers protease (at 60 mg), it was observed

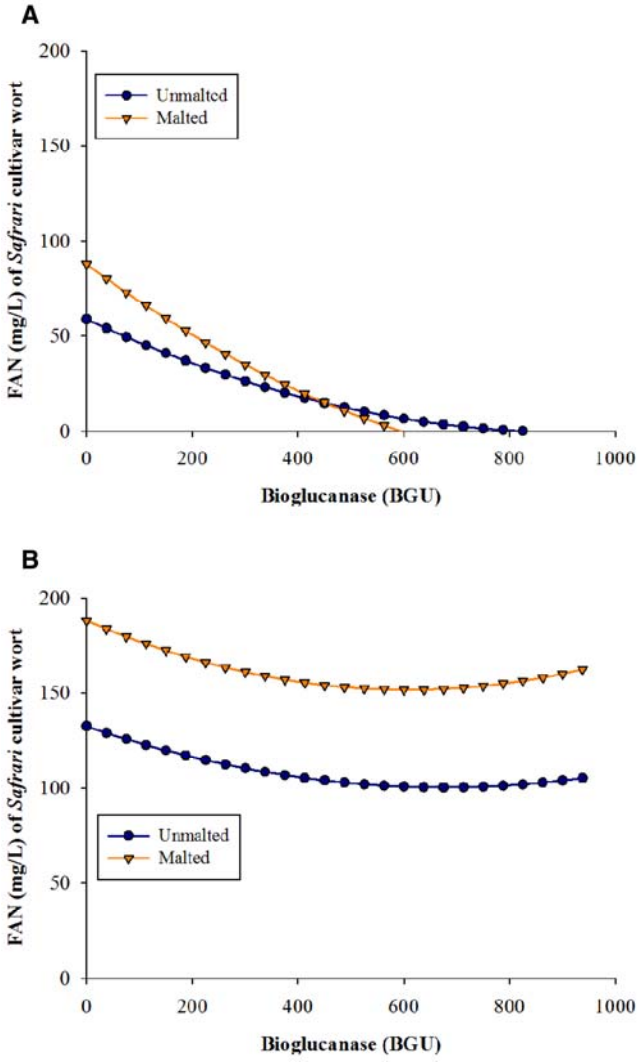


Figure 7. A, Effect of concentration of Bioglucanase (β-glucanase) as sole mashing enzyme (concentrations of Hitempase and Brewer's Protease set at 0) on yield of wort free amino nitrogen of sorghum cultivar *Safrari*. **B,** Effect of concentration of Bioglucanase (β-glucanase) in the presence of fixed concentrations of Hitempase (2000 U) and Brewers protease (60 mg) on yield of wort free amino nitrogen of sorghum cultivar *Safrari*.

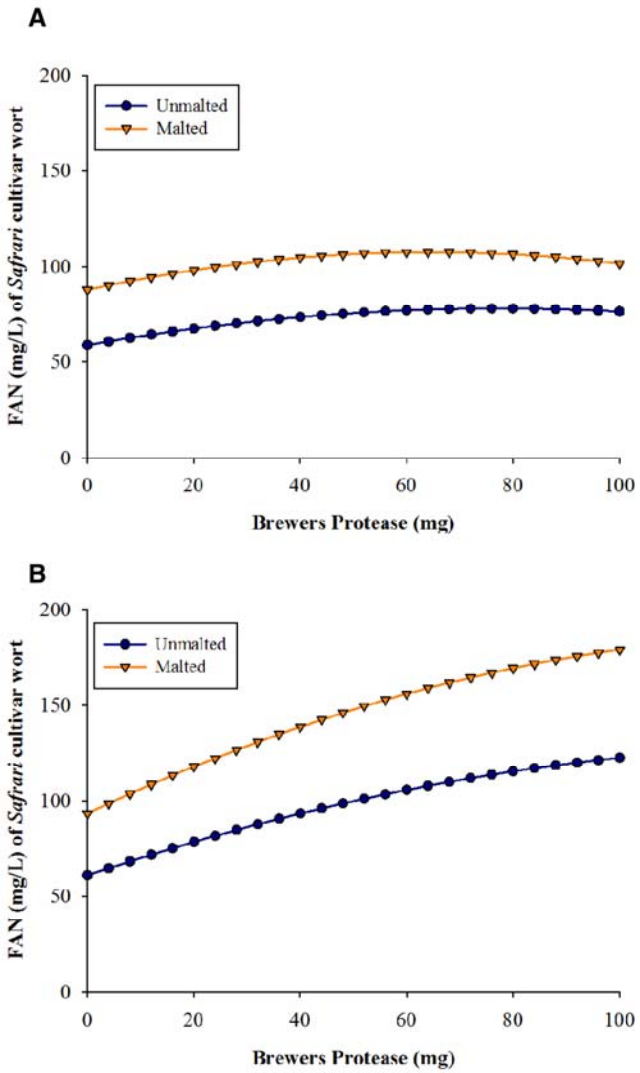


Figure 8. A, Effect of concentration of Brewers protease as sole mashing enzyme (concentrations of Bioglucanase and Hitempase set at 0) on yield of wort free amino nitrogen of sorghum cultivar *Safrari*. **B,** Effect of concentration of Brewers protease in the presence of fixed concentrations of Hitempase (2000 U) and Bioglucanase (400 BGU) on yield of wort free amino nitrogen of sorghum cultivar *Safrari*.

that the decrease of FAN levels to zero could be stopped and controlled (Fig. 7B). This confirms the need to have all mashing enzymes present in appropriate proportions during mashing to permit obtain sustainable amounts of FAN contents. According to the models, Bioglucanase (X_2), in its linear form, contributed 11% of the FAN content of the unmalted *Safrari* mash and 9% of the malted *Safrari* mash (Figures 6A and B). This contribution was statistically significant for the two mash types (P = 0.006 and 0.007, respectively) (Table 6). Similarly, in its quadratic form (X_2^2), the effect of the enzyme remained significant for the two mash types (P = 0.038 and 0.029, respectively) (Table 6). Its contribution in this form was 12% and 11%, respectively (Figures 6A and B).

The effect of Brewers protease as sole mashing enzyme on FAN content for unmalted and malted *Safrari* is presented in Figure 8A. Free amino nitrogen content increased very slightly from 60 mg/L to 75 mg/L for unmalted *Safrari* mash and from 90 mg/L to 110 mg/L for malted *Safrari* mash with an increase in enzyme concentration. This level was maintained constant from about 60 mg of enzyme input thereof. The model predicted that coupling Hitempase (at 2,000 U) and Bioglucanase (at 400 BGU) to the action of Brewers protease induced a steady increase in FAN content (Fig. 8B). This once more indicates the need of all mashing enzymes in order to obtain higher FAN yields. The additional FAN content observed for malted *Safrari* mash as compared to unmalted *Safrari* mash could once more be attributed to the natural and additional virtues of the malting process. The mathematical models statistically showed that the action of Brewers protease was in its linear form significant for both mash types (P = 0.000 for both) (Table 6). Its contribution to FAN content in this form (X_3) was 21% and 19% for both the unmalted and malted *Safrari* mashes, respectively (Figs. 6A and B). The effect of the enzyme in its quadratic form (X_3^2) is however not significant for the two mash types (P = 0.175 and 0.129, respectively) (Table 6). Its contribution decreased to as low as 7% for both

Table 7. ANOVA for free amino nitrogen of umalted and malted *Safrari.*

		Sum of squares		Mean of squares		F-value		P-value	
Source	**DF**	**Unmalted**	**Malted**	**Unmalted**	**Malted**	**Unmalted**	**Malted**	**Unmalted**	**Malted**
Regression	9	11,814.795	25,319.50	1,312.755	2,813.278	13.032	16.671	0.001	0.001
Linear	3	9,794.014	20,169.46	3,264.671	6,723.156	32.408	39.841	0.000	0.000
Quadratic	3	1,577.067	3,427.379	525.689	1,142.46	5.218	6.77	0.033	0.018
Interactions	3	443.714	1,722.655	147.905	574.218	1.468	3.403	0.303	0.083
Residual error	7	705.153	1,181.253	100.736	168.75				
Total error	16	12,519.94	26,500.75						

Table 8. ANOVA for comparing free amino nitrogen of unmalted and malted *Safrari* worts.

Source	**DF**	**Sum of squares**	**Mean of squares**	**F-value**	**P-value**
Inter-groups	1	13,916.3	13,916.3	11.41	0.001
Intra-groups	32	39,020.7	1,219.4		
Total	33	52,937			

mash types (Figs. 6A and B). Excess doses of this enzyme during mashing therefore appear to be of no technological importance.

The global action of these enzymes in their interaction or coupled forms (X_1X_2, X_1X_3 and X_2X_3) on the FAN content was statistically not significant (P = 0.303 for unmalted *Safrari* and P = 0.083 for malted *Safrari*) (Table 7). Their contributions of FAN content are shown in Figures 6A and B. The effect of the X_1X_2 (Hitempase/Bioglucanase) interaction was not significant for both mash types (P = 0.801 for unmalted *Safrari* and P = 0.420 for malted *Safrari*) (Table 6). Its contribution of FAN content in both mash types was 2% and 4%, respectively. Similarly, the action of the couple X_1X_3 (Hitempase/Brewers protease) was also not significant for both mash types (P = 0.353 for unmalted *Safrari* and P = 0.354 for malted *Safrari*) (Table 6). Its contribution of FAN content in both mash types was 7% and 6%, respectively (Figs. 6A and B). Finally, for the couple X_2X_3 (Bioglucanase/Brewers protease) (P = 0.253 for unmalted *Safrari* and P = 0.081 for malted *Safrari*) (Table 6), the contribution of FAN content in both mash types was 9% and 12%, respectively (Figs. 6A and B). Once more, Table 8 statistically confirmed the observation that malted *Safrari* sorghum type samples were more potential mashing materials than unmalted *Safrari* sorghum adjuncts to which commercial enzymes were supplemented for production of worts of higher FAN content (P = 0.001).

The results obtained for the action of the enzymes on extract and FAN yields after mashing on the basis of the models were optimised to define satisfactory domains of compromise for the mashing enzymes. These domains were obtained for the two key brewing parameters by fixing the wort conditions at extract ≥12°P and FAN ≥ 80 mg/L. The theoretical optimal combination of enzyme action for unmalted *Safrari* gave the following triplet of coded variables for extract: 0.399, 0.866 and –0.816 (2,098.5 U, 937.5 BGU and 0 mg real variables) for Hitempase, Bioglucanase and Brewers protease, respectively. This triplet allowed for a maximal extract of 18°P. The theoretical optimal enzyme combination for maximal contribution of FAN for unmalted *Safrari* mash gave as triplet of coded variables: 0.623, –0.866 and 0.816 (2,434.5 U, 0 BGU and 100 mg real variables). This triplet allowed for maximal free amino nitrogen of 144.77 mg/L (Fig. 9A). This triplet for maximal extract yields for malted *Safrari* was –1, –0.813 and –0.816 (0 U, 28.68 BGU and 0 mg real variables) for Hitempase, Bioglucanase and Brewers protease, respectively. It allowed for a maximal extract of 18.82°P. The optimal enzyme combinations were thus different for the two mash types, but both gave comparable yields of extracts. The triplet for maximal FAN content for malted *Safrari* was 0.461, –0.866 and 0.816 (2,191.5 U, 0 BGU and 100 mg real variables). It allowed for maximal FAN of 196.73 mg/L (Fig. 9B). These results once more confirmed

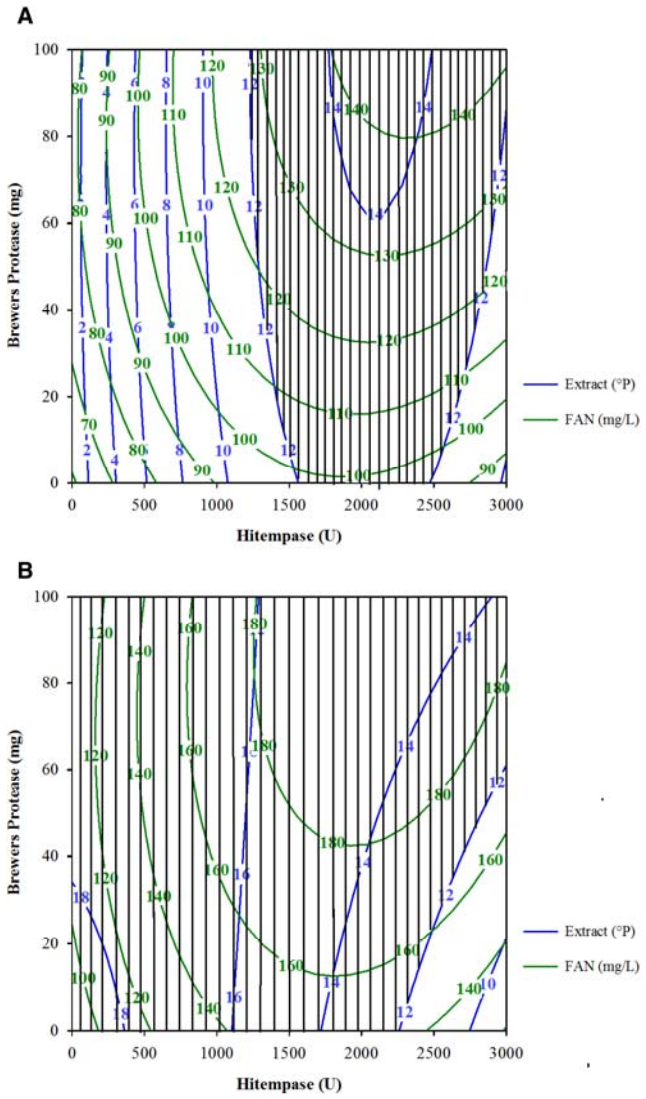


Figure 9. A, Response surface curves for the enzyme combinations providing for optimal yields in extract and free amino nitrogen for unmalted sorghum cultivar *Safrari.* **B,** Response surface curves for the enzyme combinations providing for optimal yields in extract and free amino nitrogen for malted sorghum cultivar *Safrari.*

that commercial enzyme supplements for mashing malted *Safrari* are not indispensable for obtaining maximal yields of extract as compared to unmalted *Safrari*. The enzymes supplements however proved useful for obtaining maximal FAN.

Conclusions

The effects of three commercial mashing enzymes (Hitempase 2XL, Bioglucanase TX and Brewers protease) on yields of extract and FAN were studied during the mashing of unmalted and malted *Safrari* grist. Hitempase 2XL was principally responsible for extract yields in unmalted *Safrari* mash but had no impact on the malted *Safrari* mash type. Bioglucanase TX barely played a supporting role in these yields, while Brewers protease showed no significant role. Hitempase 2XL and Brewers protease individually contributed to yields in FAN in both unmalted and malted *Safrari* mashes, though the milling operation contributed to FAN yields for more than 50% in both mashes. This study shows that proper malting and mashing of this sorghum cultivar could lead to satisfactory wort properties in terms of extract and FAN for brewing purposes. Supplements of commercial mashing enzymes to boost their yields of extract in particular are thus not indispensable when mashing with malted *Safrari*. Optimisation of mashing properties through models clearly describing the actions of individual commercial mashing enzymes, as displayed in this study using the response surface methodology, is however of interest, particularly when mashing with high amounts of sorghum adjuncts. Further studies on the fermentability of worts obtained after such studies would be of importance in order to assess the exploitability of the results for improved brewing practices with this sorghum cultivar.

REFERENCES

1. Adejemilua, F. (1995). Processing and profile of sorghum malt. Tech. Q. Master Brew. Assoc. Am. 32:15-18.
2. Agu, R. C. and Palmer, G. H. (1998a). Enzymic modification of endosperm of barley and sorghum of similar total nitrogen. Brewers' Dig. August:30-35.
3. Agu, R. C. and Palmer, G. H. (1998b). Effect of mashing with commercial enzymes on the properties of sorghum worts. World J. Microbiol. Biotechnol., 14:43-48.
4. Agu, R. C., Ugwu, A. H., Okenchi, M. U., Aneke, I. B. and Anyanwu, T. U. (1995). Effect of low kilning temperatures on diastase and cellulase development of Nigerian malted sorghum (*Sorghum bicolor*). Process Biochem. 31:63-68.
5. Aisien, A. O. (1982). Enzymic modification of sorghum endosperm during seedling growth and malting. J. Sci. Food Agric., 33:754-759.
6. Aisien, A. O. and Muts, G. C. J. (1987). Micro-scale malting and brewing studies of some sorghum varieties. J. Inst. Brew. 93:328-333.
7. Aniche, G. N. and Palmer, G. H. (1990). Development of amylolytic activities in sorghum and barley malt. J. Inst. Brew. 96:377-379.
8. Arri, B. K. (1989). Problems associated with the use of sorghum for lager beer production. Industrial utilization of sorghum: Symposium on the Current Status and Potential of Industrial Uses of Sorghum. ICRISAT: Kano, Nigeria.
9. Bajomo, M. F. and Young, T. W. (1992). Development of a mashing profile for the use of microbial enzymes in brewing with raw sorghum (80%) and malted barley or sorghum malt (20%). J. Inst. Brew. 98:515-523.
10. Bajomo, M. F. and Young, T. W. (1993). The properties, composition and fermentabilities of worts made from 100% raw sorghum and commercial enzymes. J. Inst. Brew. 99:153-158.
11. Bajomo, M. F. and Young, T. W. (1994). Fermentation of worts made from 100% raw sorghum and enzymes. J. Inst. Brew. 100:79-84.
12. Baranyi, J., Pin, C. and Ross, T. (1999). Validating and comparing predictive models. Int. J. Food Microbiol. 48:159-166.
13. Bas, D. and Boyac, I. H. (2007). Modeling and optimization I: Usability of response surface methodology. J. Food Eng. 78:836-845.
14. Dale, C. J., Young, T. W. and Omole, T. (1990). Small scale mashing experiments with grist containing high proportions of raw sorghum. J. Inst. Brew. 96:403-409.
15. Desobgo, Z. S. C., Nso, E. J., Tenin, D. and Kayem, G. J. (2010). Modelling and optimizing of mashing enzymes-effect on yield of filtrate of unmalted sorghum by use of response surface methodology. J. Inst. Brew. 116:62-69.
16. Dufour, J. P. and Melotte, L. (1992). Sorghum malts for the production of a lager beer. J. Am. Soc. Brew. Chem. 50:110-119.
17. EtokApan, O. U. and Palmer, G. H. (1990). Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. World J. Microbiol. Biotechnol. 6:408-417.
18. European Brewery Convention. Analytica-EBC, Fachverlag Hans Carl: Nürnberg, 1998.
19. Evans, D J. and Taylor, J. R. N. (1990a). Extraction and assay of proteolytic activities in sorghum malt. J. Inst. Brew. 96:201-207.
20. Evans, D. J. and Taylor, J. R. N. (1990b). Influence of cultivar and germination conditions on proteolytic activities in sorghum malt. J. Inst. Brew. 96:399-402.
21. Goode, L. D. and Arendt, E. K. (2003). Pilot scale production of lager beer from grist containing 50% unmalted sorghum. J. Inst. Brew. 109:208-217.
22. Goode, L. D., Halbert, C. and Arendt, E. K. (2002). Mashing studies with unmalted sorghum and malted barley. J. Inst. Brew. 108:465-473.
23. Goode, L. D., Halbert, C. and Arendt, E. K. (2003). Optimisation of mashing conditions when mashing with unmalted sorghum and commercial enzymes. J. Am. Soc. Brew. Chem. 61:69-78.
24. Hallgren, L. (1995). Lager beers from sorghum. In: Sorghum and millets. Chemistry and Technology DAV Dendy, pp. 283-297. American Association of Cereal Chemists: Minneapolis.
25. Jayatissa, P. M., Pathirana, R. A. and Sivayogasundaram, K. (1980). Malting quality of Sri Lankan varieties of sorghum. J. Inst. Brew. 86:18-20.
26. Little, B. T. (1994). Alternative cereals for beer production. Ferment 7:163-168.
27. MacFadden, D. P. and Clayton, M. (1989). Brewing with sorghum-use of exogenous enzymes. Brew. Bev. Ind. Int. 1:71-81.
28. Nso, E. J., Ajebesone, P. E., Mbofung, C. M. and Palmer, G. H. (2003). Properties of three sorghum cultivars used for the production of Bili-Bili beverage in Northern Cameroon. J. Inst. Brew. 109:245-250.
29. Nso, E. J., Nanadoum, M. and Palmer, G. H. (2006). The effect of formaldehyde on enzyme development in sorghum malts. Tech. Q. Master Brew. Assoc. Am. 43:177-182.
30. Obilana, A. T. (1990). Sorghum seed production and grain utilisation in the brewing industries: the Nigerian experience. Proceedings of the 2nd Workshop on Seed Production and Utilisation, Lusaka, Zambia.
31. Ogbonna, A. C. (1992). Developments in the malting and brewing trials with sorghum. World J. Microbiol. Biotechnol. 8:87-91.
32. Okafor, N. (1985). Prospects and challenges of the use of local cereals and starchy substrates in brewing. In: National Symposium on the Brewing Industry, Now and the 21st Century, pp. 22-25. Owerri, Nigeria.
33. Owuama, C. I. (1999). Brewing beer with sorghum. J. Inst. Brew. 105:23-34.
34. Owuama, C. I. and Okafor, N. (1990). Use of unmalted sorghum as a brewing adjunct. World J. Microbiol. Biotechnol. 6:318-322.
35. Phiarais, N. B., Schehl, B. D., Oliveira, J. C. and Arendt, E. K. (2006). Use of surface response methodology to investigate the effectiveness of commercial enzymes on buckwheat malt for brewing purpose. J. Inst. Brew. 112:324-332.
36. Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol. 81:501-508.
37. Taylor, J. R. N. (1983). Effect of malting on the protein and free amino nitrogen composition of sorghum. J. Sci. Food Agric. 34:885-892.
38. Taylor, J. R. N. (1991). Proteolysis in sorghum maltings. In: Proceedings of the Third Scientific and Technical Convention, Institute of Brewing, Central and South African Section, Johannesburg, pp. 18-24.