ISSN 0975-4393 (Print)
2349-2988 (Online)
DOI: 10.5958/2349-2988.2018.00035.9

Vol. 10 |Issue-04|
October–December | 2018

Available online at
www.anvpublication.org

Research Journal of Science and Technology
Home page *www.rjstonline.com*

RESEARCH ARTICLE

A Screening approach for The Expression of Total Amylase activity during Malting of *Safrari* Sorghum Cultivar

Nguemogne A. C.[1], Desobgo Z. S.C.[2*], Nso E. J.[1]
[1]Department of Process Engineering, National School of Agro-Industrial Sciences (ENSAI) of The University of Ngaoundere, Cameroon
[2]Department of Food Processing and Quality Control of University Institute of Technology (UIT) of The University of Ngaoundere, P.O. Box 455 UIT, Cameroon
*Corresponding Author E-mail: **desobgo.zangue@gmail.com**

ABSTRACT:
A fractional factorial design with a level of confidence of $P<0.05$ was carried out to determine the factors which have an influence on total amylase activity of a Cameroonian sorghum cultivar *Safrari*. An equation relating total amylase activity to the factors such as grains disinfection, alkaline steeping, renewal of steeping liquor, steeping time and temperature, steeping with air-rest periods, germination time and temperature, kilning time and temperature and finally grinding malt with roots and shoots, gave a good fit to first order equation ($R^2=0.98$). Among the eleven factors screened, the following five have shown a significant impact on total amylase activity of *Safrari* sorghum malting process: the renewal of the steeping liquor, germination time, kilning time and temperature and grain disinfection. Grain disinfection being the only one with a highly negative impact, meaning that the use of a disinfectant is detrimental for starch hydrolysing enzyme synthesis during malting of *Safrari* sorghum.

KEYWORDS: *Safrari* sorghum; Malting; Screening; Total amylase; Modeling.

1. INTRODUCTION:
Malting is a process which involves cereal grains germination under controlled aeration, moisture and temperature conditions. These germination conditions lead to the development and activation of enzyme systems (amylolytic and proteolytic) necessary for the breakdown of proteins and carbohydrates of malt and adjuncts during brewing (Gibson, 2001; Briggs *et al.*, 2004). However, the most important feature of the brewing quality of malt is the level of total amylolytic power which develops during malting (Taylor and Dewar, 2000; Evans, 2001). Whereas barley malt is the traditionally chosen raw material in the manufacturing of European-type lager beers, in Africa, the use of local cereals, in particular sorghum have attracted much attention (Briggs *et al.*, 2004). This is attributed to the fact that sorghum is available and a low-cost source; while some of its qualities during brewing are not comparable to those of barley malt (Anderson, 2000; Briggs *et al.*, 2004; Ogbonna, 2011; Lyumugabe *et al.*, 2012). Most of the problems highlighted in the malting and brewing with sorghum are incomplete saccharification due to low starch digestibility, and low wort filtration (Taylor *et al.*, 2006). Low starch digestibility has been solely attributed to high gelatinization temperature of sorghum malt (67–81 °C) compared to barley malt **(**51–60 °C) (Agu and Palmer, 1997; Okolo *et al.*, 1997), but several studies also showed that the low activity of starch degrading enzymes contributes significantly to low digestibility of sorghum starch (Dicko *et al.*, 2006; Desobgo *et al.*, 2011). Essays of improvement of the brewing properties of sorghum were led for malting and mashing process with the aim of enabling the development of starch hydrolysing enzymes by (i) grain disinfection (Ezeogu and Okolo, 1994; Beta *et al.*, 1995; Ogbonna, 2011) (ii) steeping the sorghum grains in alkaline solution before germination (Okolo and

Ezeogu, 1996; Dewar *et al.*, 1997; Owuama, 1999; Taylor *et al.*, 2006); (iii) steeping with air-rest periods and water renewal (Ezeogu and Okolo, 1994; Okolo and Ezeogu, 1995; Dewar *et al.*, 1997); (iv) the use of higher temperatures during steeping and germination (25–30 °C) (Agu and Palmer, 1997; Dewar *et al.*, 1997; Igyor *et al.*, 1998; Nso *et al.*, 2003; Irakoze *et al.*, 2010; Ijasan *et al.*, 2011). One can notice that these authors have each focused on one or more aspect of the intrinsic property of the grain to optimise malting of sorghum without considering them as a whole and without modelling. However, considering some particular factors to optimise the malting process instead of all of them may be due to their large number which becomes difficult to manage when an optimisation is to be carried out. Moreover, the lack of experimental design in these works might have hidden some details which could be foreseen without laboratory assays. Thus, the interest of modelling using an experimental screening design, to identify among several factors those which have a statistically significant influence on a particular variable (Goupy and Creighton, 2006). In addition to that, sorghum is a cereal with a remarkable genetic variability leading to the variability of malt produced according to the sorghum variety, cultivar and growth conditions (Aniche and Palmer, 1990; Antinori, 1995; Wang *et al.*, 2006; Okoli *et al.*, 2010). Considering this variability and the limitations of the previous trials to use an experimental design to screen factors which may influence amylolytic enzymes synthesis on sorghum during malting, we aimed in this study to use a screening experimental design to sort out factors which have a statistically significant impact on total amylase activity during malting of a Cameroonian grown sorghum cultivar.

2. MATERIALS AND METHODS:

2.1. Biological material

The sorghum (*Sorghum bicolor* (L.) Moench) cultivar used was *Safrari*, grown in the northern part of Cameroon and obtained from IRAD *(Agronomic Institute of Research for Development*) Maroua, Cameroon.

2.2. Chemicals:

All reagents were of analytical grade. Soluble starch and sodium hydroxide were obtained from SIGMA-ALDRICH (Spuce Street, St. Louis, MO 63103 USA); Sodium acetate was from NORMAPUR (France); Potassium iodide was from BURGOYNE (India); Iodine was obtained from Thermo Fisher Scientific, Inc. Geel–Belgium; Acetic acid (Riedel-de Haën, AG D-3016 Seelze 1); Sulphuric acid from Fisher Scientific (Fair Lawn, New Jersey 07410); Kjeldahl catalyst (Riedel-de Haën, AG D-3016 Seelze 1); Acetyl acetone Sharlab S.L reagent (Gato Perez, 33-P. I. Mas d'En Cisa. 08181 Sentmenat SPAIN); Formaldehyde, RECTAPUR reagent from PROLABO (12, rue Pelée 75011 Paris)

2.3. Apparatus:

The grade filter papers Whatman No. 1 (Ø 90 mm) were obtained from Whatman, GE Healthcare UK Ltd., UK; pH meter Model: Consort C863, type: multi parameter analyser; Belgium; Oven; mark: Heraeus-kendro laboratory products, Model: D-63450 type: T6, fabrication n^{o} 20001046, Germany; Electronic balance (Mark: Denver instrument, Model: APX-3202, max 3200, d=0.01g); Convective oven dryer (Mark: Memmert); Electrical grinder: Culatti typ MFC (Nr 7131757).

2.4. Moisture content:

Water content of sorghum was determined using a milled sample by loss in mass on oven drying at 105 °C for 24 hours (Analytica-EBC, 1998). The difference in masses before introducing samples (in triplicate) into the oven and after removing samples from the oven was recorded. Then, reporting as a percentage of the masses before oven drying.

2.5. Total protein content:

Nitrogen content was estimated by standard method (Devani *et al.*, 1989), after samples mineralization using sulphuric acid in presence of a catalyst (selenium), with a conversion coefficient of nitrogen into total protein of 6.25 (AOAC, 1980).

Nitrogenous compounds in sorghum sample were digested with concentrated sulphuric acid in the presence of Kjeldahl catalyst in hot to give ammonia. The ammonia is measured according to Devani et al. (Devani *et al.*, 1989). The measurement is based on the ammonia reaction with formaldehyde and acetyl acetone in an aqueous milieu to obtain 3, 5–diacetyl- 1,4-dihydrolutidine (a yellow complex). The absorbance of this complex is read at 412 nm. The protein content is obtained by multiplying the ammonia content by a conventional factor of 6.25.

2.6. Germinative capacity:
Three batches of 200 grains of sorghum were immersed in 200 mL of hydrogen peroxide solution at 7.5 g/L at room temperature (25±2 °C) for 2 days. After this time, the hydrogen peroxide solution was replaced by a fresh solution (at the same concentration) for an additional 24 hours after which the solution was poured. Seeds that developed roots and shoots were then counted and the percentage was recorded (Analytica-EBC, 1998).

2.7. Germinative energy:
Germinative energy was determined using triplicate samples of 100 grains of sorghum placed on 2 Whatman No. 1 filter paper layers, in petri dishes having a diameter of 9.0 cm. Distilled water (4.0 mL) was added and the dishes were placed in a germination chamber at 25 C and 90 % relative humidity. Germinated grains were eliminated after 24 and 48 h and a final count was made after 72 h and the percentage was recorded (Analytica-EBC, 1998).

2.8. Water sensitivity:
Water sensitivity was estimated the same as described for germinative energy, except that 8.0 mL of distilled water was added to each petri dish (Analytica-EBC, 1998). The water sensitivity is the value obtained from the difference between the germinative energy tests of 4 mL and 8 mL.

2.9. Malting:
Sorghum grains were manually cleaned to remove stones, broken or holed grains and other contaminants. Thereafter, samples (150 g) were washed twice with tap water and rinsed with distilled water. The operations of steeping, germination and drying were carried out using a screening experimental design with the levels of variation of factors detailed in table 1.

Tables1: Malting program

Operations	**Factors**	**Abbreviation**	**Variation of factors**	
			Low level	**High level**
Steeping	1- Time	St	20 h	48 h
	2- Temperature	ST	25°C	35°C
	3- Air-rest	Ar	No	Yes
	4- Water renewal	Wr	No	Yes
	5- Alkaline steeping (0.1 % NaOH)	Al	No	Yes
Germination	6- Temperature	GT	25°C	35°C
	7- Time	Gt	1 day	5 days
Kilning	8- Temperature	KT	30°C	55°C
	9- Time	Kt	16 h	48 h
Pre-treatment 1 (before steeping)	10- Grains desinfection (1 % NaOCl / 20 min)	Gd	No	Yes
Pre-treatment 2 (after drying)	11- Grinding with roots	GR	No	Yes

During germination, grain was watered twice daily with a set 18 mL of water. The dried roots and shoots were removed by hand in a sieve. The grains were milled using the electrical Culatti grinder incorporated with sieve (Ø≤ 1 mm). The obtained flour was then used for analyses.

For the qualitative operations: grains disinfection, air-rest, water renewal, alkaline steeping and grinding with roots, "No" means that the concerned operation was not carried out while "Yes" means that the concerned operation was carried out.

The air-rest periods were as follow: 1 hour 40 minutes of air-rest period after 10 hours of steeping, for a total of 20 hours of steeping; 4 hours of air-rest period after 24 hours of steeping, for a total of 48 hours of steeping.

2.10. Enzyme extraction:
This was done as described by Dicko (Dicko, 2006) with slight modification. Milled malt samples, 5 g each, were extracted for 5 minutes at room temperature (30±2 °C) with 20 mL of 50 mM acetate buffer pH 5.5. The suspensions were centrifuged at 6000 g for 30 minutes at 4 °C to obtain the clear supernatant which served as the enzyme source.

2.11. Total amylase assay procedure:
The determination of total amylase activity was done by measuring the initial velocities of enzyme-substrate reactions as a decrease of starch-iodine colour with time (Dicko, 2006).

0.2 mL of starch solution (1 mg/mL) was mixed with acetate buffer (2.5 mL; pH 5.5) and I_2/KI solution (0.2 g of I_2 dissolved in 100 mL of KI at 2 % w/v) and homogenized; then the enzyme extract (0.2 mL) was added to the mixture and absorbance at 580 nm was immediately red after every 10 seconds till 3 minutes or till there were no more reduction of the absorbance with time.

The initial velocities were determined graphically as the slopes of the linear parts (at initial conditions) of the plots of starch-iodine absorbance against time.

One unit of total amylase activity is defined as the quantity of enzyme which causes hydrolysis of 1 mg of starch (0.1 %) per minute at pH 5.5 and 30 ± 2 °C.

2.12. Experimental design, modelling and validation of the model:
Table 1 shows factors whose effects were to be determined on the total amylase activity of the *Safrari* sorghum malt. We have a total of 11 factors with their domains of variation.

A screening fractional factorial design with the general form $n=2^{k-r}$ was chosen to conduct experiments; where n is the number of experiments; k is the number of factors; r is the number of experiments subtracted to the corresponding complete factorial design and 2 is the number of levels of the factors.

The two levels of variation of each factor considered here are 1 and -1, which are the codded values for the high level and low level respectively.

We have then chosen for this study a screening fractional factorial design 2^{11-7} to conduct a total of 16 experiments detailed in table 2.

Table 2: Experimental design for malting

Factor	St/X_1	ST/X_2	Ar/X_3	Wr/X_4	Al/X_5	GT/X_6	Gt/X_7	KT/X_8	Kt/X_9	Gd/X_{10}	GR/X_{11}
Experiment	hours	°C				°C	hours	°C	hours		
1	-1	1	-1	-1	1	1	-1	1	-1	-1	1
2	1	-1	-1	-1	1	-1	1	1	-1	-1	-1
3	1	1	1	1	1	1	1	1	1	1	1
4	1	-1	1	-1	-1	1	-1	1	1	-1	1
5	1	1	1	-1	1	-1	-1	-1	-1	1	1
6	1	-1	1	1	-1	-1	1	-1	-1	-1	1
7	-1	1	1	-1	-1	-1	1	1	1	-1	-1
8	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1
9	-1	-1	-1	1	-1	1	1	1	-1	1	1
10	-1	-1	1	-1	1	1	1	-1	-1	1	-1
11	1	-1	-1	1	1	1	-1	-1	1	-1	-1
12	-1	-1	1	1	1	-1	-1	1	1	1	-1
13	1	1	-1	1	-1	-1	-1	1	-1	1	-1
14	1	1	-1	-1	-1	1	1	-1	1	1	-1
15	-1	1	-1	1	1	-1	1	-1	1	-1	1
16	-1	1	1	1	-1	1	-1	-1	-1	-1	-1

Where, X_1: steeping time (St); X_2: steeping temperature (ST); X_3: air-rest period (Ar); X_4: steeping water renewal (Wr); X_5: alkaline steeping (Al); X_6: germination temperature (GT); X_7: germination time (Gt); X_8: kilning temperature (KT); X_9: kilning time (Kt); X_{10}: grain disinfection (Gd); X_{11}: grinding with roots (GR). Also, 1 and -1 for the qualitative factors mean that the concerned operation was carried out for the former and not for the latter.

Mathematical model describing the relationship between dependent variable (total amylase activity) and the independent variables (factors) of the process was developed in a first-order equation without interactions (Goupy, 1999; Goupy and Creighton, 2006) and expressed as:

$$y = \beta_0 + \sum \beta_i x_i \qquad \text{eq 1}$$

Were Y = total amylase activity; βi=regression coefficients; Xi are the studied factors
The coefficients of the models were obtained using Statgraphics Centurion XV.II. This software had also given a statistical analysis (ANOVA) of the model.

Validation of the model was done by the verification of model fitting. This was achieved by an evaluation of the resultant least squares regression coefficient R^2 which is obtained by comparing the observed and fitted values. A model is validated with a value of R^2 between 0.92 and 1 (Goupy, 1999; Goupy and Creighton, 2006).

3. RESULTS AND DISCUSSION:

3.1. Characterization of sorghum grains:

The results of the characterization of sorghum grains with the aim of estimating their viabilities were presented in table 3. The sorghum cultivar investigated had good germinative property as revealed in the high viability with no tendency of dormancy. Dormancy is defined as the difference between germinative capacity and 4 mL germinative energy tests (Briggs, 1998). The sorghum used is thus able to germinate rapidly and uniformly.

Table 3: Characteristics of *Safrari* sorghum

Properties	***Safrari* sorghum**	**Standard (Analytica-EBC, 1998)**
Germinative energy (%) (4 mL)	93	60-99
Germinative energy (%) (8 mL)	83	40-99
Water sensitivity (%)	10	
Germinative capacity (%)	94	92-100
Moisture content (%)	11.1	≤13 %
Total nitrogen (%)	1.6	1.5 – 2.1
Total proteins (%)	10	

Results here are averages of triplicates.

Though being almost not dormant, the lot used was very water-sensitive as indicated by the high value of water sensitivity (10 %). This means that the amount of water in 8 mL germinative energy test is in excess and contributes to reduce germination. In fact, gains in the presence of large amounts of water remain covered with a film of moisture which presents a diffusion barrier to oxygen reaching the living tissues (Briggs, 1998). Consequently, if steeped for prolonged period, these grains will require "air-resting" period or out of steep periods to allow the take up of the surface film of water and the respiration of the grains before re-immersing them so that they reach the desired moisture content to germinate normally (Briggs, 1998).

3.2. Model validation and factors influencing total amylase activity during malting:

A fractional factorial design 2^{11-7} was used to determine if grains disinfection (Gd), alkaline steeping (Al) and renewal of steeping water (Wr), steeping time (St) and temperature (ST), steeping with air-rest periods (Ar), germination time (Gt) and temperature (GT), kilning time (Kt) and temperature (Kt) and finally grinding malt with roots and shoots (GR), significantly influence total amylase production and activity during malting of *Safrari* sorghum cultivar. In table 4, the experimental design with the different enzyme activities are shown.

Table 4: Experimental design with total amylase activities

Factor	**St**	**ST**	**Ar**	**Wr**	**Al**	**GT**	**Gt**	**KT**	**Kt**	**Gd**	**rM**	**Total amylase activity**
Experiment	**h**	**°C**				**°C**	**h**	**°C**	**h**			**mU**
1	20	35	no	no	yes	35	24	55	16	no	yes	24
2	48	25	no	no	yes	25	120	55	16	no	no	36
3	48	35	yes	yes	yes	35	120	55	48	yes	yes	30
4	48	25	yes	no	no	35	24	55	48	no	yes	24
5	48	35	yes	no	yes	25	24	30	16	yes	yes	12
6	48	25	yes	yes	no	25	120	30	16	no	yes	36
7	20	35	yes	no	no	25	120	55	48	no	no	36
8	20	25	no	no	no	25	24	30	48	yes	yes	12
9	20	25	no	yes	no	35	120	55	16	yes	yes	35
10	20	25	yes	no	yes	35	120	30	16	yes	no	18
11	48	25	no	yes	yes	35	24	30	48	no	no	30
12	20	25	yes	yes	yes	25	24	55	48	yes	no	24
13	48	35	no	yes	no	25	24	55	16	yes	no	18
14	48	35	no	no	no	35	120	30	48	yes	no	24
15	20	35	no	yes	yes	25	120	30	48	no	yes	42
16	20	35	yes	yes	no	35	24	30	16	no	no	18

St: steeping time; S: steeping temperature; Ar :air-rest priod; Wr: change of steep water; Al: alcaline steeping ; GT: germination temperature; Gt: germination time; KT: kilning temperature; Kt: kilning time; Gd: grain disinfection; GR: grinding with roots and shoots; h: hour; mU: milli-Unit.

After experimentations, the obtained model was as follow:

$$\textit{Total amylase activity} = 25.875 + 0.375St - 0.375ST - 1.125Ar + 2.625Wr + 1.125Al - 1.125GT + 5.625Gt + 1.875KT + 1.875Kt - 4.875Gd + 0.375GR \quad \text{eq 2}$$

The resultant least squares regression coefficient R^2 was 0.98. This regression coefficient being closer to 1, the model is then validated; that is the measured and calculated enzyme activities showed a good fit.

It should be noticed from equation 1 that a coefficient with a positive sign (+) shows a positive influence of the concerned factor and a coefficient with a negative sign (-) shows a negative influence of the concerned factor. But to know their impacts and the extent of these impacts on enzyme activity, the ANOVA table (Table 5) was drawn.

Table 5: Analysis of variance for total amylase activity

Source of variation	Sum of squares	Degree of Freedom	Mean square	Fisher (f-test)	Significance (P<0.05)
St	6,25E-10	1	6,25E-10	0,33	0,5946
ST	6,25E-10	1	6,25E-10	0,33	0,5946
Ar	5,625E-9	1	5,625E-9	3,00	0,1583
Wr	3,0625E-8	1	3,0625E-8	16,33	0,0156
Al	5,625E-9	1	5,625E-9	3,00	0,1583
GT	5,625E-9	1	5,625E-9	3,00	0,1583
Gt	1,40625E-7	1	1,40625E-7	75,00	0,0010
KT	1,5625E-8	1	1,5625E-8	8,33	0,0447
Kt	1,5625E-8	1	1,5625E-8	8,33	0,0447
Gd	1,05625E-7	1	1,05625E-7	56,33	0,0017
GR	6,25E-10	1	6,25E-10	0,33	0,5946
Total Error	7,5E-9	4	1,875E-9		
Total (corr.)	3,34375E-7	15			

As well as sorghum malt quality is concerned, the total amylase activity is of primary importance, because these are hydrolytic enzymes required to hydrolyse starch from the malt and adjunct to fermentable sugars when used to brew beer (Briggs, 1998; Gibson, 2001; Briggs *et al.*, 2004). These enzymes are synthesized during the malting process which includes steeping, germination and drying/kilning.

Among the eleven factors identified in the literature which have been demonstrated to have a significant impact on starch hydrolytic enzymes production during malting of cereals in general and sorghum in particular, it can be observed from table 5 that only five factors showed a statistically significant influence *(P<0.05)* on total amylase activity of *Safrari* sorghum during malting. These are: steep water renewal (Wr), germination time (Gt), kilning time (Kt) and temperature (KT) and finally grain disinfection (Gd).

From equation 2 and table 5, table 6 was drawn, which highlights the direction of the influence (positive or negative) and the degree of influence of the five factors which have presented a significant impact on total amylase activity.

Table 6: Factors influencing total amylase activity during malting

Operation	Factor	Sign	Probability	Degree of influence (%)
Steeping	1. Change of steep liquor (Wr)	+	0.0156	12.3
Germination	2. Time (Gt)	+	0.001	26.3
Kilning	3. Time (Kt)	+	0.0447	8.8
	4. Temperature (KT)	+	0.0447	8.8
Pre-treatment	5. Grains desinfection (Gd)	-	0.0017	22.8

3.2.1. Steeping:

Steeping was carried out at two temperatures: 25 °C and 35°C and two lengths of duration: 20 h and 48 h with and without air-rest periods. These steeping conditions had no significant effect on total amylase activity of sorghum malting. The indifference of steeping conditions to temperature (25 °C and 35°C) suggests that there is no significant difference between steeping at 25°C and 35°C on total amylase synthesis. Also, the change of the steep liquor had a significant (P = 0.0156) and positive effect. This is in accordance with the findings of some authors (Ezeogu and Okolo, 1994; Okolo and Ezeogu, 1995; Dewar *et al.*, 1997). As Okolo and Ezeogu (1996) stated, the

renewal of the steep liquor might have helped to improve the biochemical modifications of the grains, and thus enzyme synthesis.

3.2.2. Germination:
In the germination process which included time and temperature, only time showed a high significant (P = 0.001) and positive impact on amylolytic enzyme synthesis during malting. The positive influence of time demonstrated the increase of enzyme synthesis and activity with increase of germination time as stated by Owuama (1999), and similar to the results of Okoli *et al*., 2010; Bekele *et al*., 2012; Archibong *et al*., 2015; Abuajah *et al*., 2016. This can be explained by the fact that during germination, cellular metabolites are mobilised for the synthesis of new compounds among which hydrolytic enzymes (Lewis and Bamforth, 2006; Schmitt *et al*., 2013). The indifference of germination temperatures (25 °C and 35°C) to amylase synthesis and activity suggests that there is no significant difference between germinating at 25°C and 35°C.

3.2.3. Kilning:
Kilning time and temperature had a significant (P = 0.04) and positive impact on enzyme synthesis during malting (table 6). Knowing the inactivation of enzymes with higher temperatures, the slight increase of amylolytic activity with kilning time (10 % influence) and temperature (10 % influence) can be explained by the fact that during the first hours of kilning, when there is still free water in the kernels and the temperature of the kilning medium being bellow the inactivation temperature of the malts enzymes, modifications continued and seeds might have even shown an increase in biological activities (Hämäläinen and Reinikainen, 2007). However, this increase in enzyme activity with kilning may be due to the increase in α-amylase activity at normal kilning temperatures (up to 80 °C) as stated by Guido and Moreira (2014).

3.2.4. Grains disinfection:
The influence of grain disinfection was highly (98 %) and negatively significant (P = 0.0017). This means that grain disinfection highly contribute to decrease enzyme activity during malting of *Safrari* sorghum cultivar. This result is in sync with the works of Nso et al. (2006) on the same sorghum cultivar, in that the treatment of sorghum grains with disinfectants as formaldehyde reduced the enzyme potential of the obtained malt. Sodium hypochlorite used in this work as disinfectant might have had the same effect. In fact, sodium hypochlorite is a strong oxidant which can decompose to give either chloride or oxygen which might have oxidised grains constituents including enzymes (Ditommaso and Nurse, 2004).

3.2.5. Crushing with roots and shoots:
The removal of roots and shoots or not had no influence of total amylase activity of sorghum malt. This might be advantageous in time and energy saving with no extra work of removing them in malting process. In addition, they are most of the time not removed for the manufacture of African opaque beers, in contrary to European lager beers (Briggs *et al.*, 2004). Their contribution to the free amino nitrogen content of the malt has also been demonstrated (Dewar *et al.*, 1997; Briggs *et al.*, 2004).

4. CONCLUSIONS:
The results of this study show that total amylase activity of *Safrari* sorghum is influenced principally by the following five factors: (i) grain disinfection, which has contributed to decrease amylase activity on one hand and (ii) the renewal of the steep liquor, (iii) germination time, (iv) kilning time and (v) kilning temperature which have contributed to enhance amylase activity on the other hand. There are some discrepancies with the literature and this could be attributed to the variability of malt quality with variety, cultivar, growth conditions and storage conditions.

Abbreviations:
X1: steeping time (St);
X2: steeping temperature (ST);
X3: air-rest period (Ar);
X4: steeping water renewal (Wr);
X5: alkaline steeping (Al);
X6: germination temperature (GT);
X7: germination time (Gt);
X8: kilning temperature (KT);

X9: kilning time (Kt);
X10: grain disinfection (Gd);
X11: grinding with roots (GR).

5. ACKNOWLEDGEMENTS:

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

6. REFERENCES:

1. Abuajah C. I., Ogbonna A. C., Onwuka N. U., Umoren P. E. and Ojukwu, M. 2016. Effect of varietal differences and germination period on some malting and brewing potentials of new improved sorghum varieties (SAMSORG17, SAMSORG14, and SAMSORG40) from Nigeria. International Food Research Journal 23; 1600-1608.
2. Agu, R. C. and Palmer, G. H. 1997. The effect of temperature on the modification of sorghum and barley during malting. Process Biochemistry 32(6): 501-507.
3. Analytica-EBC. 1998. European brewery convention. Nürnberg: Fachverlag Hans Carl.
4. Anderson, R. G. 2000. Current practice in malting, brewing and distilling. In Peter, C. M. and James, H. B. (Eds.) Cereal biotechnology. Cambridge, England.
5. Aniche, G. N. and Palmer, G. H. 1990. Development of amylolytic activities in sorghum and barley malt. Journal of the Institute of Brewing 96: 377-379.
6. Antinori, U. 1995. La valorisation industrielle et commerciale des matières premières agricoles d'origine locale: L'expérience de l'onudi en afrique: Le cas du sorgho. In Menyonga, J. M., Bezuneh, T., Nwasike, C. C., Sedogo, P. M. and Tenkouano, A. (Eds.) Transformation et utilisation industrielle du sorgho et céréales assimilées en afrique, p. 19-27. Ouagadougou, Burkina Faso: OUA/CSTR – SAFGRAD.
7. AOAC. 1980. Official methods of analysis. Association of Official Analytical Chemists, Washington, DC.
8. Archibong, E. J., Irondi, C. R., Ezemba, C. C., Awah, N. S. and Ozuah, C. L. 2015. Extraction of Enzymes from Improved Sorghum Cultivars (SSV98001, SSV98002 and SK5912) and their Applications in the Mashing Studies of a Nigerian Local White Sorghum Variety. International Journal of Current Research in Biosciences and Plant Biology 2; 20-28.
9. Bekele A., Bultosa G. and Belete K. 2012. The effect of germination time on malt quality of six sorghum (Sorghum bicolor) varieties grown at Melkassa, Ethiopia. Journal of the Institute of Brewing 118; 76–81.
10. Beta, T., Rooney, L. W. and Waniska, R. D. 1995. Malting characteristics of sorghum cultivars. Journal of Cereal Chemistry 72: 533-538.
11. Briggs, D. E. 1998. Malts and malting. 1 Ed. London: Blackie Academic & Professional.
12. Briggs, D. E., Boulton, C. A., Brookes, P. A. and Stevens, R. 2004. Brewing science and practice. Cambridge: Woodhead Publishing Limited and CRC Press LLC.
13. Desobgo, Z. S. C., Nso, E. J. and 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(3): 77-86.
14. Devani, M. B., Shishoo, C. J., Shah, S. A. and Suhagia, B. N. 1989. Microchemical methods: Spectrophotometric methods for microdetermination of nitrogen in kjeldahl digest. Journal Association of Official Analytical Chemistry 72: 953-956.
15. Dewar, J., Taylor, J. R. N. and Berjak, P. 1997. Effect of germination conditions, with optimised steeping, on malt quality with a particular reference to free amino nitrogen. Journal of the Institute of Brewing 103(3): 171-175.
16. Dicko, M. H. 2006. Travaux pratiques de biochimie structurale et d'enzymologie. Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles (CRSBAN), Université de Ouagadougou.
17. Dicko, M. H., Gruppen, H., Traoré, A. S., Voragen, A. J. G. and Van Berkel, W. J. H. 2006. Sorghum grain as human food in africa; relevance of content of starch and amylase activities. African Journal of Biotechnology 5: 384-395.
18. Ditommaso, A. and Nurse, R. E. 2004. Impact of sodium hypochlorite concentration and exposure period on germination and radicle elongation of three annual weed species. Journal of Seed Science and Technology 32; 377-391.
19. Evans, E. J. 2001. Cereal production method. In Owens, G. (Ed.) Cereal processing technology, p. 7 – 26. Cambridge, England: Woodhead Publishing Limited and CRC Press LLC.
20. Ezeogu, L. I. and Okolo, B. N. 1994. Effects of final warm steeping and air-rest cycles on malt quality parameters of three improved nigerian sorghum cultivars. Journal of the Institute of Brewing 100: 335-338.
21. Gibson, G. 2001. Malting. In Owens, G. (Ed.) Cereal processing technology, p. 173-203. Cambridge, England: Woodhead Publishing Limited and CRC Press LLC.
22. Goupy, J. 1999. Plans d'expériences pour surfaces de réponse. Dunod.
23. Goupy, J. and Creighton, L. 2006. Introduction aux plans d'expériences. 3 Ed. Paris: Dunod.
24. Guido, L. F. and Moreira, M. M. 2014. Malting. In Taylor & Francis Group LLC Engineering aspects of cereal and cereal based products. p. 51 – 70.
25. Hämäläinen, J. J. and Reinikainen, P. 2007. A simulation model for malt enzyme activities in kilning. Journal of the Institute of Brewing 113(2): 159-167.
26. Igyor, M. A., Ogbonna, A. C. and Palmer, G. H. 1998. Effect of malting temperature and time on enzyme development and sorghum wort properties. Journal of the Institute of Brewing 104: 101-104.
27. Ijasan, B., Goodfellow, V., Bryce, J. H., Bringhurst, T. A., Brosnan, J. M., Jack, F. R. and Agu, R. C. 2011. Quality assessment of a sorghum variety malted commercially under tropical conditions and controlled tropical temperatures in the laboratory. Journal of the Institute of Brewing 117: 206-211.
28. Irakoze, P. C., Haihua, Z., Qin, L., Zhou, K. and Huiming, Z. 2010. Optimized conditions of steeping and germination and their effect on sorghum [sorghum bicolor (l.) moench] composition. Pakistan Journal of Nutrition 9(7): 686-695.
29. Lewis, M. J. and Bamforth, C. W. 2006. Essays in Brewing Science. Springer Science+Business Media, LLC; Davis, California, USA.

30. Lyumugabe, F., Gros, J., Nzungize, J., Bajyanga, E. and Thonart, P. 2012. Characteristics of african traditional beers brewed with sorghum malt: A review. African Journal of Biotechnology 16(4): 509-530.
31. 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. Journal of the Institute of Brewing 109(3): 245-250.
32. Nso, E. J., Nanadoum, M. and Palmer, G. H. 2006. The effect of formaldehyde on enzyme development in sorghum malts. Technical Quarterly Master Brewers Association of the Americas 43(3): 177-182.
33. Ogbonna, A. C. 2011. Current development in malting and brewing trials with sorghum in nigeria: A review. Journal of the Institute of Brewing 117(3): 394-400.
34. Ogbonna, A. C., Obi, S. K. C. and Okolo, B. N. 2003. Protein modification in malting sorghum. World J. Microbiol. Biotechnol. 19: 495-503.
35. Okoli, E. V., Okolo, B. N., Moneke, A. N. and Ire, F. S. 2010. Effects of cultivar and germination time on amylolytic potential, extract yield and wort fermenting properties of malting sorghum. Asian journal of biotechnology 2(1): 14-26.
36. Okolo, B. N. and Ezeogu, L. I. 1995. Effects of air rest periods on malting sorghum response to final warm water steep. Journal of the Institute of Brewing 101: 39-45.
37. Okolo, B. N. and Ezeogu, L. I. 1996. Promoting sorghum reserve protein mobilisation by steeping in alkaline liquor. Journal of the Institute of Brewing 102: 277-284.
38. Okolo, B. N., Ezeogu, L. I. and Ugwuanyi, K. E. 1997. Amylolysis of sorghum starch as influenced by cultivar, germination time and gelatinisation temperature. Journal of the Institute of Brewing 103(6): 371-376.
39. Owuama, C. I. 1999. Brewing beer with sorghum. Journal of the Institute of Brewing 105: 23-34.
40. Schmitt, M. R., Skadsen, R. W. and Budde, A. D. 2013. Protein mobilization and malting-specific proteinase expression during barley germination. Journal of Cereal Science 58; 324-332.
41. Taylor, J. and Dewar, J. 2000. Developments in sorghum food technologies. In Taylor, J. (Ed.) Adv. Food nutr. Res., p. 217-264. San Diego, USA: Academic Press.
42. Taylor, J. R. N., Schober, J. T. and Bean, S. R. 2006. Novel food and non-food uses for sorghum and millets. Journal of Cereal Science 44(3): 252-271.
43. Wang, X., Yang, J. and Zhang, G. 2006. Genotypic and environmental variation in barley limit dextrinase activity and its relation to malt quality. Journal of Zhejiang University SCIENCE B 7(5): 386-392.

Received on 22.05.2018 Modified on 29.07.2018
Accepted on 10.08.2018
Research J. Science and Tech. 2018; 10(4):244-252.
DOI: 10.5958/2349-2988.2018.00035.9