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RESEARCH ARTICLE

STUDIES ON THE WORT PROPERTIES OF SOME IMPROVED SORGHUM VARIETIES USING PARTIALLY PURIFIED ALPHA AMYLASE PRODUCED BY *ASPERGILLUS ORYZAEUSING* BREWERS' SPENT GRAINS

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ABSTRACT

Solid-state fermentation (SSF) was carried out to produce α-amylase from *Aspergillus oryzae* using spent brewing grains (SBG) as substrate. The extracted enzyme was subjected to partial purification by ammonium sulphate fractionation. Maximum specific activity was obtained at 70% fraction. It was optimally active at pH 5 and 50°C by using 1% starch as substrate concentration. The partially purified α-amylase loses activity rapidly above 50°C but was retained in the presence of Ca2+ at an optimum temperature of 60°C.Improved Nigerian sorghum cultivars were malted and their brewing qualities evaluated. Their percentage protein and moisture content decreased with increase in days of germination. Statistical analysis showed a significant difference at (P-value <0.05) in theiramyloytic activity of the cultivars from first to the fifth day of germination. Mashing results obtained from the second temperature programmed mashing regime used with partially purified enzyme from *Aspergillus oryzeaand* calcium ion addition produced more reducing sugars, improved wort color, reduced viscosity and dextrin compared to that of first temperature programmed mashing and the single decoction regime with enzyme in the absences of calcium ion. Showing that the brewing spent grains can served as a good sources of hydrolysing enzyme, of which the presence of Ca2+ultimately stabilize and enhance its activity during mashing to produce improved results during brewing operations.

INTRODUCTION

In tropical brewing, the use of sorghum as a replacement for barley in beer brewing has been effective (Archibong *et al.,* 2009). Potentially, sorghum holds a high economic value for the people living in the above mentioned regions and the nation at large due to its low cost of cultivation and an emerging market for its use in the brewing industry as it constitutes cheaper source of extracts for that industry and therefore, offers potential employment to all stakeholders along the supply value chain (i.e.) farmers, marketers, maltstars, brewers for both alcoholic and non-alcoholic. In Africa, the cereal sorghum is malted widely to provide an important raw material (amylolytic enzymes that hydrolyse the starchy endosperm of the sorghum grain into soluble sugars) used in brewing (Esslinger *et al.,* 2005). Since the ban on the importation of barley malt by the Nigerian government in the mid-1980s to 1999, there has also been growing interest in the use of malted sorghum in the brewing of clear lager type beers (Michael and Nzeka, 2012). The potential of sorghum as an alternative substrate for beer brewing has long been recognized over six decades ago (Odibo *et al.,* 2002). Research studies with sorghum as a brewing raw material are progressing rapidly and making a great impart in brewing despite the earlier misunderstanding that malted sorghum produces insufficient hydrolytic enzymes (Agu, 2005). Varietal difference, malting and mashing temperatures employed in the studies of sorghum in the past were major contributory factor that complicated the understanding on the physiological behaviour of sorghum during malting and brewing (Dufour and Melotte, 1992). Malting known as one of the preliminary steps involved in grain preparation for brewing purposes reduces the viscosity of starch slurry, for this reason it is referred to as the controlled germination of cereal grains to produce complement of enzymes which are able to convert cereal starch to fermentable sugars. During malting a number of hydrolytic enzymes develop to degrade the nutrient reserve of the endosperm; such enzyme development ultimately depends on malting and mashing

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conditions, varietal differences coupled with the grains structural and physiological properties. Various enzymes with different specificities are involved in the hydrolysis of starch. α-amylase (endo–1,4,α-D-glucan glucohydorlase, EC 3.2.1.1) is an extracellular enzyme that randomly cleaves the 1,4-α-D-glucosidic linkages between adjacent glucose units in the linear amylase chain. Although amylases can be obtained from several sources, such as plants, animals and microorganisms, the enzymes from microbial sources generally meet industrial demand (Pandey *et al.,* 2000). Amylases find potential application in a number of industrial processes such as in food, textile and paper industries. Microbial amylases have successfully replaced chemical hydrolysis of starch in starch processing industries. Fungal α -amylase is preferred for use in formulations for human or animal consumption involving applications under acidic conditions and around 37°C (Karuki and Imanaka, 1995). Similarly, due to its biocompatibility, fungal amylases are preferred in baking and food processing. With the advent of new frontiers in biotechnology, the spectrum of amylase application has expanded into many other fields, such as clinical, medical and analytical chemistry (Pandey *et al.,* 2000). During the last decade, an increased attention was paid to use various agro industrial wastes for value addition using solid-state fermentation (SSF) by filamentous fungi as the major conversion technology (Pandey *et al.,* 1999). Spent brewing grain (SBG) consists of the residual seed hull and fiber of barley after the malting process of the brewing industry. The potential of SBG to be used as a substrate for the production of enzymes including cellulase and xylanase under SSF has been analysed (Sim *et al.,* 199*0*). The filamentous fungus, *Aspergillus oryzae* is extensively used to produce several industrial enzymes including amylases (Barbesgaard *et al.,* 1992). This research work tends to assess the malt and wort properties of improved sorghum varieties using different mashing regime with partially purified hydrolyzing enzyme produced from *Aspergillus oryzae*by solid-state fermentation (SSF).

MATERIALS AND METHODS

The improved sorghum varieties SSV98001, SSV98002and SK5912were obtained from Institute of Agricultural Research, Ahmadu Bello University, Samaru, Zaria, Nigeria. The crude enzyme from *Aspergillus oryzae*(IFO-30103) was obtained from Federal Institute of Industrial Research Oshodi, maintained on potato dextrose agar (PDA) slant at 4°C and used for the enzyme production. All reagents used were of analytical grades.

Malting, steeping, germination

They were screened by hand to remove broken or damaged kernels and foreign material since damaged grains cause microbial infection during germination (Agu and Palmer, 1999). Five hundred grams (500g) of each of the sorted grains were surface-sterilized by immersion, for 40 minutes, in sodium hypochlorite solution having 1% (v/v) available chlorine to reduce microbial contamination in steeping process (Ogbonna *et al.,* 2003). The grains were subsequently drained and washed four (4) times in tap water. A 24h steeping regime was used (12h water steep, 2h air-rest, 10h water steep) using a steeping ratio of 1:2 (grain : water) at 25ºC.Germination was carried out at 30ºC for five (5) days in dark germination chambers with twelve (12) hourly spray of 10ml water to prevent drying out. Germinated samples were collected every day for kilning and subsequent analysis. Germinated grains were kilned in an oven at 50ºC for 24h.

Grain and malt analyses

Germinative capacity

This was determined according to the Recommended Methods of Analysis of the Institute of Brewing (IOB), 1977 – Hydrogen Peroxide and Peeling Reference Method. Briefly, Two lots of sorted 200 corns were obtained using a sample divider. Each lot of 200 corns was steeped for two (2) days in 200ml fresh Hydrogen Peroxide solution at 18 – 21^{o}C. The steep liquor was strained off and replaced with 200ml of fresh Hydrogen Peroxide solution at 18 – 21^{o}C for one (1) day. The steep liquor was strained off and the corns which have not developed both root and acrospires growth were counted and separated. The outer covers of the embryos (husk of barleys) were peeled from the corns which have not developed root and acrospires growth. The tip of a dissecting needle was inserted under the cover at the side of the germ and swept around to allow the piece of covering over the germ to be peeled back and off. The skin covering the germ was removed by rubbing with the finger to expose the pure white germ. The peeled corns were incubated on a moist filter paper or moist sand contained in a closed petri dish for 1 day at 18-21^{o}C.

The corns showing either root or acrospires growth were counted (d)

The corns which do not show root or acrospires growth were noted (n).

Germinative Capacity Percentage (Hydrogen Peroxide) =
$\frac{200}{2} - n\ \%$ or $\frac{500}{5}$

Including $\frac{d}{2}$ damaged

Germinative Energy/Water Sensitivity

This was determined according to the recommended methods of analysis of the Institute of Brewing (IOB), 1997. Two filter papers were placed in the bottom of the petri dish and either 4ml or 8ml of water were accurately added. 100 corns were counted from the sample, obtained with the divider and placed on the paper so that each grain made good contact. In the 8ml test, the ventral side of each grain only was allowed to touch the paper to avoid drowning the embryo. The petri dishes were covered with their lids ensuring good seals. The dishes were incubated in the cabinet and chited grains were removed at 24h, 48h and 72h from the beginning of steeping. The percentage of corn chited as the germinative energy or water sensitivity were calculated.

Germinative energy = G.E. (4ml)%
Water Sensitivity = W.S. (8ml)%

Determination of thousand (1000) corn weight: This was carried out according to the Recommended Methods of Analysis of the Institute of Brewing (IOB), 1991.

Determination of specific gravity

This was determined according to the official methods of Association of Official Analytical Chemists A.O.A.C, (1980). Procedurally, 50 ml specific gravity bottle was thoroughly cleaned with distilled water, dried in an oven for 1½h at 50^{o}C, then cooled in a desiccator. The weight of the cooled, dried bottle (W_1) was determined to 0.001g. The dried bottle was filled with deionized water and allowed to stand for 20min at 20^{o}C in a temperated water bath. The surface of the bottle was dried with what mass No.1 filter paper. The bottle with water was weighed correct to 0.001g (W_2). The bottle was emptied and cleaned twice with 10ml of sample extract, after which the bottle was filled to the brim with sample and immersed in water bath at 20^{o}C for 20mins. The bottle was dried with Whatmman no.1 filter paper and weighed (W_3). The procedure was repeated for accuracy.

The specific gravity was calculated as:

$$S.G = \frac{S}{W}$$

Where $S = W_3 - W_1$ and $W = W_2 - W_1$

Determination of malting loss and cold water extract (CWE): According to the recommended methods of analysis of the Institute of Brewing (IOB), revised 1991.

Determination of hot water extract (HWE) and Estimation of -amylase and diastatic activity: This was carried out according to the decantation method of Etokakpan (1992) and the simple diamylase procedure described by Etokakpan and Palmer (1990) respectively.

Determination of reducing sugar and crude protein: Determination of reducing sugar was done using the method of Miller (1959) and crude protein performed using the micro-Kjeldahl method.

Inoculum Development

The fully sporulated PDA slant culture was mixed with 10ml of distilled water containing 0.1% tween-80 to dislodge the spores and determine total viable count using serial dilution. About 5g of brewing spent grain moisture with salt solution of the following concentrating was added to form a solid state fermentation media accordingly: KH_2PO_4, 5; NH_4NO_3, 5; Nacl, 1; and $MgSO_4$, 1 g/l. mixed and autoclaved at 121^{o}C for 20min. The media was inoculated with 1ml of spore suspension with cell count of (7×10^6) and incubated at 30^{o}C.

Enzyme Extraction and assay

The incubation ended at the 8th day and the fermented solid was mixed 30 ml of phosphate buffer (pH 6.5, 0.1 M) was added to the culture flasks and mixed well in a rotary shaker (200 rpm) at room temperature for 30 min. The mixture was filtered through muslin cloth and centrifuged at 10,000rpm for 10 min at 4ºC with Zigmarefrigated centrifuge. Collected supernatant, after centrifugation, were used for determination of amylase activity. Estimation of α-amylase activity was carried out according to the dinitro salicylic acid (DNS) method of Miller (1959). Each of the enzyme samples was assayed by adding 0.5 ml of the enzyme solution to 0.5 ml of phosphate buffer (pH 6.5) into which 1% starch substrate was added. The solution incubated at 37°C for 10 min and the reaction mixture was stopped by adding 5 ml of DNS reagent. It was heated at 100ºC for 15 min and cooled 2.5 ml of distilled water was added and allowed to cool down to room temperature for 10 min. The absorbance was read at 540 nm with the help of a colorimeter against glucose as the standard. One unit of enzyme activity is defined as the amount of amylase which releases 1µmole of reducing sugar as glucose per minute (U/ml/min), under the assay conditions. The experiments were carried out in triplicates and standard error was calculated.

Protein estimation and enzyme purification

The amount of Protein content in the enzyme extracts were estimated by the method of Lowry et al. (1951), using bovine serum albumin as the standard. Enzyme activity is expressed as specific activity which is equivalent to U/mg protein. All the experiments were carried out in triplicates and the standard error was calculated. Partial purification of crude enzyme sample was achieved by fractionation using ammonium sulphate $(NH_4)_2SO_4$, in 70% saturation, the supernatant did not contain any measurable amount of the enzyme, indicating that it had precipitated. The precipitates were dissolved in a minimum volume of 0.1M phosphate buffer (pH 6.5), and dialyzed overnight against 0.05M phosphate buffer (Gomori, 1955).

Effect of temperature on enzyme activity

This was carried out by adding 0.1ml of the enzyme to 0.2ml phosphate buffer (6.5) followed by the addition of 0.5ml of starch, 0.1ml NaCl and shaken. Then, incubated for 20mins at various temperatures,(40°C, 50°C, 60°C, 65°C, 70°C, 80°C) with the addition of 0.1ml NaOH. The reaction is stopped by the addition of Dinitrosalicylic acid (DNS) and heated in boiling water for 10mins, then the absorbance checked at 540 nm.

Effect of pH on enzyme activity

This was carried out by incubating the enzyme in 0.2ml phosphate buffer at various pH (4.0, 5.0, 6.0, 6.5, 7.0, 8.0) followed by the addition of 0.5ml of starch and shaken. Also 0.1ml NaCl was added and the enzyme mixture incubated for 20mins, followed by the addition of 0.1ml NaOH and the reaction stopped by the addition of Dinitrosalicylic acid (DNS). This is heated in boiling water for 10 mins and allowed to cool, the absorbance was taken at 540 nm.

Effect of metal ions on enzyme activity

The substrate was prepared using 0.1g in 10ml (1%) phosphate buffer and heated to gelatinize, 5mM of each metal salt was prepared dissolving (molar mass of salt/1000 X 5)g in 1000ml of water at different concentration (2g/l, 5g/l and 7g/l) were prepared. The effect of salts ($CaCl_2$, $CuSO_4$, $MgSO_4$, $FeCl_2$ and $MnCl_2$) on enzyme activity was determined. A control was set up with 0.2ml of substrate plus 0.2ml of enzyme. 0.2ml of

enzyme was added to 0.2ml of substrate plus 0.2ml of 5mM of salt. This was incubated at 55°C for 10 mins; reaction was stopped with 0.4 DNS, boiled for 10min, cool and 2ml of water added. Absorbance was taken at 540nm.

Estimation of glucose, maltose and maltotriose using Merck silica G60 TLC

The wort was estimated for approximate soluble sugar content by measuring the refractive index using a refractometer. This estimate was used to determine the amount of carbohydrate to be spotted on the TLC plates for quantitative purposes which is in range of 1- 25 µg. De-fatting was done by mixing 50µl with 100l of chloroform in a micro-tube and capped. It was placed on a vortex shaker for one minute and centrifuged at 6,500rpm for 5min to separate the chloroform from the wort. The lower chloroform layer was discarded. Dilution or concentration of wort was carried out to acceptable spotting concentrations of sugars within the volume of 0.5 to 1.0 µl. Spotting points on Merck G60 silica TLC plates were designated at intervals 0f 1.5cm and 2cm from plate edge. Appropriate standard sugar solutions of expected sugars in the range 1 to 25 µlg were spotted to give at least four concentration variables which will be used to prepare a standard calibration curve. The dried spotted sugars were developed and used to estimate concentration of unknown sugars.

Wort physical properties determination: Wort viscosity was determined using Zigma Viscometer and wort colour was determined using The Lovibond comparator (Model – Lovibond 2000)

Data analysis: Each set of data in the experiments was collected in three replicates and the result was the mean of three data sets. The standard deviations (error bars) and statistical differences (5% level of significance) were analyzed by using GraphPad Prism 6® software (trial version) (GraphPad Software, CA, USA) and SPSS version 21(Nwankwegu *et al.,* 2016).

RESULTS

Characteristics of unmalted sorghum grains varities (SSV98001, SSV98002 and SK5912)

SSV98001 has the highest moisture content while SSV98002 had the least (Table 1). The grains had high germinative capacity, energy and good water sensitivity showing that the grains were viable and suitable for malting. SSV98002 had the highest tannin content. 1000 corn weight, cold and hot while SSV98001 had the lowest. While SK5912 also had the highest diastatic power and protein while SSV98001 had the least values. Statistical analysis of the data obtained showed no significant difference among the varieties at P-value <0.05.

Table 1. [a]Characteristics of unmalted Sorghum grain varieties

		Parameter			
Sample	Volume (ml)	Protein (mg)	Activity (U)	Specific Activity (U/mg)	Fold
Crude enzyme	250.0±1.30	62.2±1.13	432.4±2.12	8.4±0.34	-
70 % saturation	5.0±0.10	53.4±0.57	235.2±2.53	51.1±0.64	8.5±0.39

[a]Values are mean of replicate analyses ±SD

Table 2. [a]Malt characteristics of Sorghum grain varities for five days period

Parameter	Days of germination				
	1	2	3	4	5
Moisture content (%)	35.2±0.31	26.4±0.13	18.8±0.21	17.8±0.44	16.2±0.41
	*32.8±0.31	24.6±0.13	18.1±0.13	16.8±0.34	16.4±0.61
	[d]34.4±0.13	25.8±0.14	18.6±0.21	18.2±0.02	16.4±0.25
Protein content (%)	7.2±0.11	5.4±0.26	4.1±0.35	3.2±0.51	3.0±0.07
	*7.8±0.17	6.7±0.27	4.4±0.31	4.1±0.41	3.6±0.20
	[d]7.9.4±0.11	7.0±0.14	4.8±0.31	4.4±0.12	4.0±0.20
Cold Water Extract (Kg/°L)	15.7±0.14	16.2±0.36	18.9±0.24	26.8±0.08	30.5±0.06
	*16.3±0.15	18.2±0.36	27.8±0.23	30.1±0.18	32.4±0.33
	[d]19.4±0.15	24.6±0.34	30.7±0.41	38.5±0.12	41.4±0.23
Hot Water Extract (Kg/°L)	25.6±0.32	28.9±0.36	48.6±0.42	46.9±0.44	104.6±0.56
	*25.7±0.22	29.2±0.27	50.5±0.32	90.1±0.19	112.1±0.47
	[d]29.8±0.16	36.2±0.34	58.4±0.21	98.6±0.02	120.2±0.45
Diastatic Power (°L)	2.9±0.13	3.1±0.15	4.6±0.23	9.3±0.66	11.2±0.35
	*3.4±0.13	3.9±0.15	5.8±0.21	10.2±0.06	12.1±0.28
	[d]4.1±0.33	4.6±0.14	6.8±0.24	11.4±0.32	14.2±0.05

[a]Values are mean of replicate analyses ±SD; *SSV98002 sorghum variety; [d]SK5912 sorghum variety

Table 3. [a]Partial purification of α-amylase by Ammonium sulphate fractionation

		Sorghum varities	
Parameter	SSV98001	SSV98002	SK5912
Moisture content (%)	14.4±0.11	12.8±0.33	13.4±0.55
Germinaive capacity (%)	99.0±0.12	97.0±0.15	69.0±0.07
Germinative energy (%)	99.0±0.22	99.0±0.23	77.0±0.11
Water sensitivity (%)	98.0±0.00	3.0±0.12	86.0±0.44
Tanins (%)	5.0±0.13	8.0±0.11	6.0±0.01
Percentage corn weight (g)	34.1±0.32	42.7±0.44	35.5±0.34
Cold Water Extract (Kg/°L)	15.3±0.04	16.1±0.06	15.9±0.25
Hot Water Extract (Kg/°L)	25.2±0.02	25.5±0.18	25.2±0.43
DiastaticPower (°L)	2.9±0.13	3.1±0.19	3.3±0.34

[a]Values are mean of replicate analyses ±SD

Malt Characteristics of studied varieties

The cold water extract, hot water extract and diastatic power of the three varities (SSV98001, SSV98002 and SK5912) increased from the first day of germination to the fifth day, while the moisture and protein content decreased accordingly (Table 2). Though, it had the least values of the above mentioned parameters compared to SSV98002 and SK5912 (Table 1 and 2). The data obtained from the above tested parameters showed no significant difference at P-value <0.05.A result of partial purification of the alpha amylase is presented in Table 3.

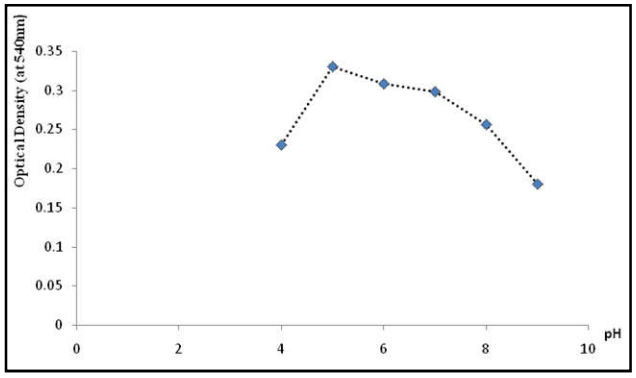

Fig. 1. Effects of pH on partially purified enzyme activity

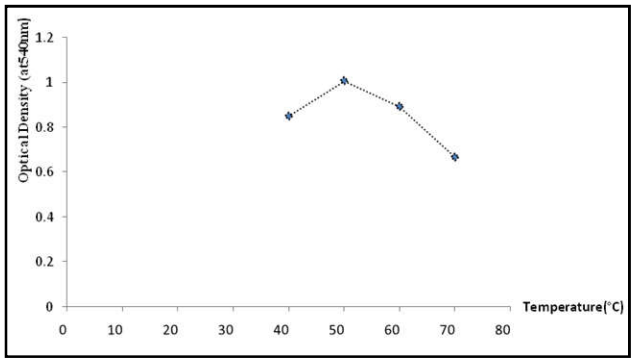

Fig. 2a. Effect of temperature on partially purified enzyme activity

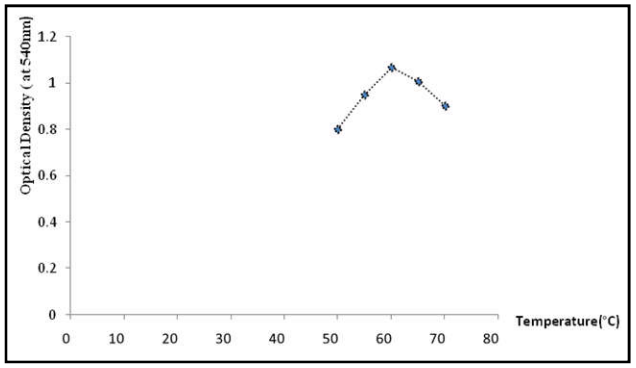

Fig.2b. Effect of Ca2+ on alpha -amylase stability

Effects of pH and temperature on partially purified enzyme activity

The partially purified enzyme from *Aspergillus oryzae* exhibited minimal activityat pH. 4.0, the optimum activity was at 5.0 and gradually decreased with increase in pH (Fig 1).At 50°C,an optimum activity was observed and the crude enzyme activity gradually decreased with increase in temperature (Fig 2).

Effects of Ca^{2+} on alpha -amylase stabilityand partially purified enzyme

Eeffects of Ca^{2+} on temperature of enzyme. From 50°C the crude enzyme showed an increasing enzyme activity till an optimum activity was attained at 60°C (Fig 2b). The effects of metals ions on the enzymes varied; Mn^{2+} had the highest activity, while the rest (Hg^{2+}, Cu^{2+}, Ag^{2+}, Na^{+}, Mg^{2+} , K^{+}, Fe^{2+}, Mn^{2+}) showed an increasing difference in their other of activity (Fig.3). The three parameters evaluated above (Effects of pH. Temperature and Metal ion of the crude enzyme) was used to design a suitable mashing regime to be used to mash the improved varieties.

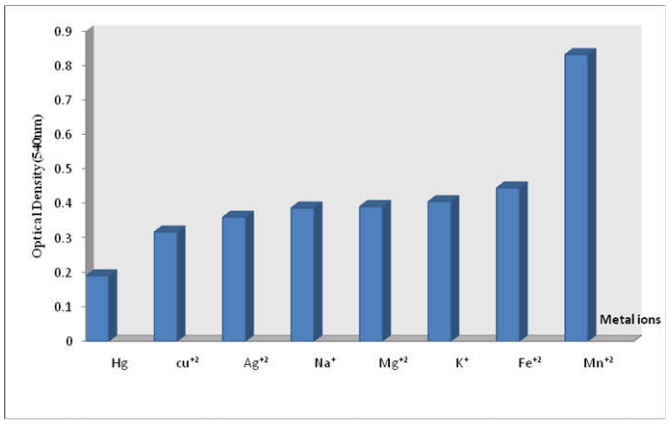

Fig. 3. Effects of metal ions on partailly purified enzyme

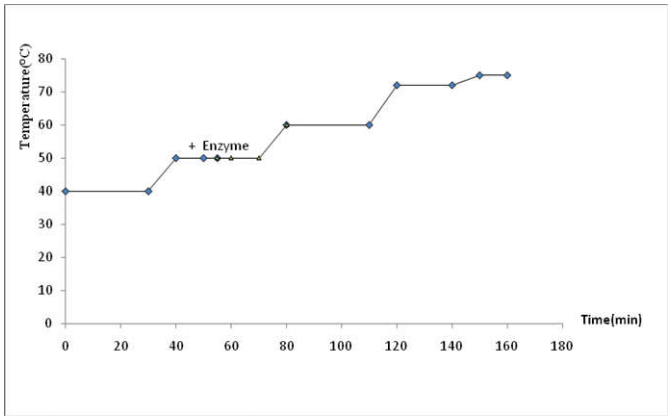

Fig.4.First tempreture programed mashing regime for sorghum malt varieties

First and second Temperature programmed mashing regime

The malt was mashed in at 40°C, and rested for 30mins, then raised to 50°C for 10mins, and rested for 30mins with the addition of enzyme, later raised to 60°C and rested for 30mins, with and without enzyme addition and later raised to 70°C and rested for 20mins, and mashed off (Fig 4). In the second temperature programmed mashing regime (Fig 5), the malt was mashed in at 50°C, and rested for 50mins, then raised to 60°C for 10mins, and rested for 70mins with the addition of

enzyme, later raised to 65°C and rested for 20mins, with and without enzyme addition plus Ca^{2+} and later raised to 70°C and rested for 20mins, and mashed off.

Single decoction mashing regime

The malt was mashed in at 50°C, and rested for 30mins, then 1/3 of the mash was decocted and heated to 80°C for 10mins and returned to the main mash, the temperature rose to 60°C, it was rested for 60mins, with and without enzyme addition, later raised to 70°C for 20mins and mashed off (Fig 6).

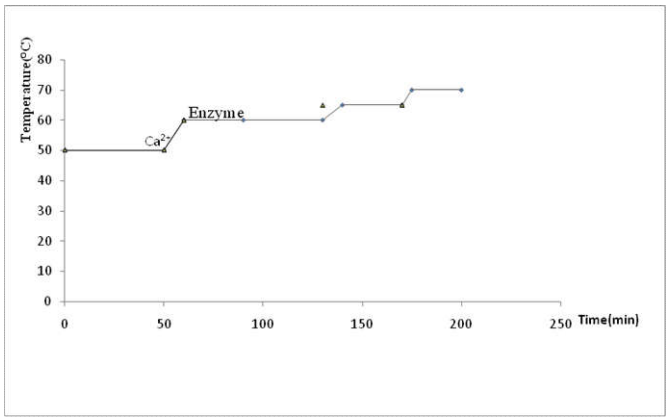

Fig.5. Second Temperature programmed mashing regime for sorghum malts

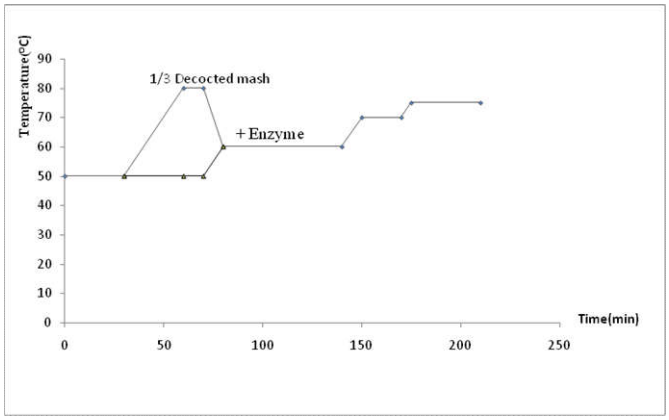

Fig.6.Single decoction mashing regime for sorghum malts

DISCUSSION

The results obtained showed that the sorghum varieties were suitable for malting because they had high germination capacities, germinative energy and good water sensitivity. Differences in moisture content are also presented, the data obtained showed no significant difference at *P*.value<0.05 and was similar with the reports of (Eneje *et al.,* 2012). Their moisture and protein contents decreased with increased days of germination for the five cultivars, while diastatic power, cold and hot water extract increased with increased germination time, and the highest values were attained by SK5912 while SSV98002 had lowest values. The decline in the value of percentage protein was indicative of protease activity during malting (Ezeogu and Duodu 2005; Wong and Lau, 2009). The increase in malt amylolytic activity reflects the wide varying difference in the ability of sorghum grain cultivars to release bond enzyme forms and this has been reported by Etokakpan and Palmer (1990b). The α-amylase isolated from the brewing spent grains was precipitated using ammonium sulphate at different saturation according to the works Archibong et al (2015). The properties of the enzyme were carefully studied, like the effect of metal ions, temperature, pH and Ca^{2+} stability. Amylase as a metalloenzyme contains at least one activating Ca^{2+} ion. The affinity of Ca^{2+} to amylase is much stronger than other ions (Gupta *et al.,* 2003). Enhancement of amylase activity such as Mn^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Ba^{2+}, could be based on its ability to interact with negatively charged amino acids residue such as aspartic and glutamic acid (Linden *et al.,* 2003). The influence of temperature on the produced alpha amylase shows that the enzyme activity increased progressively with increase in temperature from 50 to 60°C optimum as a result of the Ca^{2+} ion addition (Fig2b). Above 50°C as optimum temperature in the absence of Ca^{2+} ion there was a marked decline in the enzyme activity and this agreed with the report of patel *et al* (2005). They found that the optimum temperature for alpha amylase activity isolated from brewing spent grain, produced by *Aspergillus oryzea* was 50°C. The effect of pH on the purified enzyme showed that the enzyme attained it optimum activity at the pH of 5.0 (Fig 1). These results tally with that obtained by Egwin and Oloyede (2006). At the same time, the obtained data agreed with the findings of Nirmala and Muralikrishna (2003) who found that the optimum pH of alpha amylase from finger millet was the range of 5.0-5.5. Mohamed *et al.,*(2009) reported that the optimal pH for different alpha amylase of wheat ranged from 5.0 to 7.0. The adoption of different mashing regimes yielded varying reducing sugars (Fig 4 and 5). This is similar to the reports of Archibonget al (2015), Veith, (2009), and Arendt *et al.,* (2004). Such difference in reducing sugars during malting and mashing can be attributed to different diastatic activities of exogenous enzymes and the amount of starch available for hydrolysis (Okafor and Aniche, 1980). Results obtained from the second temperature programmed mashing regime with partially purified enzyme from *Aspergillus oryzea*and Calcium ion addition produced more reducing sugars compared with that of first single decoction and first temperature programmed mashing regime enzyme without Calcium ion addition. This indicates the complete breakdown of the starch molecules was as a result of the activities of Calcium ion on the partially purified enzyme.

It has generally been reported that partially purified alpha-amylase particularly those of fungal origin, lose activity rapidly above 50°C but the activity could be retained in the presence of calcium. Alpha-Amylase does not contain co-enzyme but they are calcium metallo-enzyme with at least one atom of this metal per molecule of the enzyme. Calcium free amylases are very susceptible to denaturation by acid, heat, and urea and readily degraded by protease (patel *et al.,* 2005). At 50°C, the alpha-amylase specific activity was 0.52 U/mg proteins. On the other hand, when the reaction was carried out at 60°C in the presence of 10mM $CaCl_2$ (0.25 mL) in the reaction mixture, the activity was much better than that at 50°C (Patel *et al.,* 2005). Some important relations have been shown to exist between amylolytic enzymes (α-amylase and β-amylase) and the ratio of glucose to maltose in malted sorghum or malted barley and this agrees with the work of Agu (2005). In this study, it has been shown that while α-amylase was directly linked to extract recovery, the ratio of glucose to maltose was directly linked to the ratio of α-

amylase to β-amylase enzymes of the malt. This also corroborated the results obtained by Agu and Palmer 1997b) which showed that different sorghum varieties malted and mashed under similar conditions presented wide variations in their sugar profiles due to seasonal and processing differences. Results obtained from this research shows that brewing spent grains served as a good sources of hydrolysing enzyme, of which the presence of Ca^{2+}ultimately stabilize and enhance its activityduring mashing to produce improved results during brewing operations.

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Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors

Conflict of interest

All the authors declare that there are no conflicts of interest

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