Evaluation of malting properties and activities of three enzymes from sorghum (*Sorghum bicolor*) during malting

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ABSTRACT

Sorghum (*Sorghum bicolor* (L) Moench), is used as human food such as bread, malt drinks and beers; as livestock feed, renewable energy source such as bioethanol. The experimental monitoring of enzyme activity during malting permits the detection of germination-specific genes. The products of α- amylase (simple sugars), β-amylase (maltose), protease (free amino nitrogen) and polyphenol oxidase (quinone), combined during kilning and mashing, with the development of characteristics flavor and coloured malt. In this study, we investigate malting properties of white and yellow varieties of sorghum while the variation in the activity of the three enzymes namely polyphenol oxidases, amylases and proteases, were monitored as the malting progresses. The result obtained were 95% and 86% germinative energy, 1.49 g and 1.09 g water absorption, 0.751 mg/ml and 0.639 mg/ml crude protein for yellow and white sorghum respectively. The activity of the three enzymes was higher in dormant yellow sorghum than dormant white sorghum. For both varieties, polyphenol oxidase activities were lowest during steeping than in dormant grain, while the highest activities were obtained during germination. Amylases activity did not changed significantly from dormant to germination. The proteases activities were highest during steeping than in dormant while gradually decreased to zero level during germination.

Keywords: Sorghum, *Sorghum bicolor* (L) Moench, Malting properties, Polyphenol oxidases, Amylases, Proteases.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench), indigenous to Africa, is a member of the grass family Poaceae. Sorghum is a grain that grows primarily around the equator in semi-arid climates (Owuama 1997). Sorghum is ranked as the fifth most important grain in terms of production, preceded by wheat, rice, maize, and barley (Veith, 2007). The top sorghum producers are Mexico, Nigeria, U.S.A and India. Sorghum, locally called guinea-corn, is the most extensively grown cereal grain in the Nigeria (Aba *et al.,* 2004). Two of the best known species are *Sorghum vulgare* and *Sorghum bicolor* (L.) Moench. Nigeria is the largest sorghum producer in West Africa, accounting for about 71% of the total regional sorghum output, and the breweries have continued to use sorghum and corn as the key raw materials despite the barley malt importation in Nigeria (USDA, 2011).

The grain is composed of three main parts: seed coat (pericarp-testa), germ (embryo) and endosperm (storage tissue). The seed coat contains copious amount of polyphenolic compounds which combine with other flavonoids (anthocyanins, anthocyanidins, etc.,) to give it varying colours (Okrah, 2008). The germ fraction of sorghum is rich in minerals (ash), protein and lipids as well as B-group vitamins: thiamine, niacin, and riboflavin which also occur in the aleurone layer (Pomeranz, 1987; Palmer *et al*., 1989) while the endosperm consists mainly of starch granules, storage proteins and cell-wall materials (Ogbonna, 2011). Sorghum is a source of

nutrients and bioactive compounds, especially 3-deoxyanthocyanidins, tannins, and polycosanols, which beneficially modulate, in vitro and in animals, parameters related to noncommunicable diseases such as obesity, diabetes, dyslipidemia, cardiovascular disease, cancer, and hypertension (Awika, *et al*., 2009; Farrar, *et al*., 2008; Kamath, *et al*., 2007; Kim and Park, 2012; Moraes *et al.,* 2012; Muriu *et al.,* 2002; Shih *et al*., 2007; Woo, *et al*., 2012; Yang *et al*., 2009). It does not contain gluten, which make it suitable for the celiac disease patient.

Sorghum is used as human food such as bread, malt drinks and beer; as livestock feed, renewable energy source such as bioethanol (Dahlberg *et al*, 2011). Previous studies demonstrated that sorghum plants were able to accumulate large quantities of heavy metal in the shoots, as well as highly tolerant to metal pollution (Roy *et al.,* 2016). The availability of the sorghum full genome sequence (Paterson *et al.,* 2009), makes it a reasonable C_4 model plant, which could be combined with the C_3 plant models Arabidopsis and rice, to study the gene products involved in adaptation to heavy metal stress. Traditionally, sorghum malting occurs outdoors by placing the steeped sorghum grain in thin layers on covered or uncovered floors. Following a 4-6 day germination period, the green malt is dried in thin layers in the sun (Daiber and Taylor, 1995). The traditional brewing process utilizes malted grain sorghum and two different fermentations. The first fermentation is by lactic acid bacteria to produce lactic acid, which provides the characteristic flavor and lowers the pH, thus reducing microbial growth. The second fermentation is by yeast, *Saccharomyces cerevisiae*, to produce alcohol (Watson and Novellie 1975).

Malting is the controlled germination of the grain followed by the controlled drying of a seed. The goal of malting is to produce high enzyme activity, endosperm modification, and a characteristic flavor with a minimum loss of dry weight (Hoseney, 1994). Thus, experimental monitoring of enzyme activity during malting will permit the detection of germination-specific genes. Physically sorghum does not contain a husk like barley does, has a higher starch gelatinization temperature, and has less diastatic, β-amylase, and glucanase activities. Therefore the traditional brewing procedures for barley have to be altered to account for the differences between the grains (Pozo-Insfran *et al*., 2004). Why malting? Sorghum components, especially its protein is less digestible than other cereals for human and monogastric animals. The removal of these undesirable components during malting improves the nutritional quality of sorghum and effectively utilizes its potential as human food or animal feed. The report of Narsih *et al.* (2012) showed that, soaking for 24 hours and germination for 36 hours produced sorghum with higher nutritional values.

However, slow digestibility of sorghum starch and protein makes it foods useful in diabetic treatments, in that its polyphenol content mostly tannin, serves as antioxidants.

Alpha-amylase, the liquefying amylase, is an endoenzyme hydrolyzing α1→4 bonds within amylose and amylopectin that develops during the germination phase of malting (Bamforth 2006; Owuama 1999). Proteases are vital components in the malting process as not only do they provide yeast with a source of free nitrogen, sulphur and amino acids, but they are also important contributors to the clarity, flavour and filterability of both whisky and beer, and are also involved beer foam stability and haze formation (Bamforth, 2006). Enzymatic browning occurs as a result of the oxidation by polyphenol oxidase, of phenolic compounds to quinones and their eventual (non-enzyme-catalyzed) polymerization to melanin pigments (Whitaker, 1996). Polyphenol Oxidase proved to be advantageous in the malting process, involving in the development of flavor and characteristic brown coloration resulted by Strecker degradation, a typical maillard reaction (Yoruk and Marshall, 2003). Thus, the products of amylase (simple sugars), protease (free amino nitrogen) and polyphenol oxidase (quinone) combine during kilning and mashing, leading to the development of characteristics flavor and coloured malt. In this study, we evaluated the malting properties of two variety of sorghum and the behavior of three malting enzymes, namely polyphenol oxidase, amylase and protease.

MATERIALS AND METHODS

MATERIALS

Sample collection

Dried white and yellow Sorghum was purchased from a seller at Oja-oba market in Akure, Nigeria. It was classified as *Sorghum bicolor* at the Department of Crop and Pest Science of Federal University of Technology Akure, Nigeria.

Apparatus and chemicals

Top loading balance (OHAUS Inc.), Homogenizer, Measuring cylinder, Micropipette, pH meter, Beakers, Laboratory oven, Refrigerator, Heating baths, Eppendorf Cold Centrifuge, Spectrophotometer, Catechol , Bovine serum albumin (BSA), Bradford reagent, 3,5-dinitrosalisylate reagent (DNS), Potato starch, Casein. All other reagents were all of analytical grade.

METHODS

Physical analysis

The weight and volume of 1000 sorghum grain were

determined in duplicates as described by Makeri et al. (2013). Density was calculated as a ratio of mass of the kernel to its volume in g/ml.

Determination of the Germinative energy (GE)

The germinative energy was determined following the method of the Institute of Brewery method (Makeri *et al*, 2013) and the European Brewery Convention (EBC) method (Frančáková *et al*, 2012). The 100 grains were germinated in 90 mm Petri dishes with two layers of filter paper (Whatman No. 1) wetted with 5 ml H_2O. Petri dish was then covered and incubated at an average temperature of 30ºC for 24-72 hours. The kernels that shoot (sprouted) at the end of the incubation were counted and expressed as germinative energy.
GE (%) = 100-N; where; GE=Germinative Energy and N = number of ungerminated grains. OR
GE (%) = $(n_{24} + n_{48} + n_{72})$
where: n_{24}, n_{48}, n_{72} – numbers of germinated kernels at 24, 48, and 72 h

Determination of the germination index (GI)

The germination index (GI) was calculated from the results of the germination energy determination according to EBC method (Frančáková *et al*, 2012):
$GI = 10 \times (n_{24} + n_{48} + n_{72})/(n_{24} + 2n_{48} + 3n_{72})$
where: n_{24}, n_{48}, n_{72} – numbers of germinated kernels at 24, 48, and 72 h

Germinative capacity

One hundred sorghum grains were placed in a 100ml glass beaker containing distilled water and steeped at 30ºC for 48 hours. The steep water was strained off and the sprouted grains separated from the un-sprouted ones and counted. The un-sprouted grains were then transferred onto moist filter papers (Whatman No.1) in petri dishes covered with another moist filter paper and the lid replaced. The dishes were then wrapped in jute bag and allowed to germinate at ambient temperature (32ºC ± 2) for about 24 hours while water was sprinkled at intervals. Newly germinated grains were counted and the result added to the first (Makeri *et al*, 2013).
The germinative capacity (GC) was calculated as follows:
GC % (dH_2O) = (200-N)/2
where; N =Number of ingeminated grains.

Water sensitivity

Two lots of 100 grains each were grown on filter papers (Whatman No.1) in petri dishes (9cm diameter); the two

Water absorption

Hundred (100) sorghum kernels of each cultivar were soaked in a 100 ml beaker containing 50 ml of distilled water at ambient temperature (32oC ± 2). Steeping was done continuously until constant weights were attained and recorded. Soak waters were drained off the grains by the use of sieves, while soaked grains were weighted at 3hours interval; from 0 hr to 24 h. The water absorbed over the 24 h soaking period was calculated as the difference between the weights of the original and soaked grains (Makeri et al, 2013).

Malting

Malting consists of steeping, germination and drying or kilning of sorghum grains (Ogbonna et al, 2012; Makeri et al, 2013).

Steeping: The sorghum grains were steeped at 32oC (± 2) for 36 hours as follows: 7 hours steeping: 2 hours air-rest in four cycles. Air-rests were done by draining off the steep water completely.

Germination: After the last 4 hours air-rest, the grains were placed on a rectangular wooden frame that has net, to enable bottom and top air-flow circulation. The grain germinated at room temperature (32ºC ± 2) with water sprinkled at intervals. The green malts were harvested continuously at 9 hours interval for 72 hours of continuous germination.

Preparation enzyme crude extract

Twenty-five grams (25g) of each sorghum grains were weighed and homogenized with 50ml of chilled phosphate buffer (0.1M, pH6.8) for 3mins and filtered. The filtrate was centrifuged at 10000rpm at 4ºC for 15mins. The supernatant was stored and used as crude extract for enzymes assay.

Assay for enzyme activity

Determination of α-amylase

Saccharolytic activity of α-amylase was measured with the DNSA method (Bernfeld, 1955). The reaction mixture consisted 0.7 ml of 1% substrate (potato starch) solution dissolved in 0.1M phosphate buffer (pH 6.8), and 0.3 ml of enzyme solution. After incubation at 30ºC for 5 min in a thermo stable water bath, the reaction was stopped by the addition of 1 ml of DNSA reagents. Samples were then one moistened with 5 ml and the other with 10 ml water. The difference in the number of grains that germinated in Petri dishes was noted as the water sensitivity value (Makeri et al, 2013).

Table 1. Malting Properties of Sorghum

PARAMETERS	SORGHUM SAMPLES	
	WHITE	YELLOW
Weight per 1000 grains	31.505g	37.645g
Density	1.125g/cm^3	1.176g/cm^3
Germination Energy	86%	95%
Germination Index	6.98	8.12
Germination Capacity	88.5	96.0
Water Sensitivity	2	11
Water Absorption	1.09g	1.49g

placed in a boiling water bath for 15 min and subsequently cooled down to room temperature. Absorbance was measured using a spectrophotometer at 540 nm against a blank prepared using the identical method, except the enzyme solution was added to the mixture after the addition of the DNSA solution (Dutta *et al.,* 2006).

Determination of Protease Activity

0.3mL of enzyme extract and 0.3mL of 1% casein prepared in 0.1M phosphate buffer pH 6.8 were incubated for 1 h at 37 °C. The reaction was stoppe d by addition of 2 mL of 10 % (v/v) trichloracetic acid (TCA). For the control, the substrate was precipitated with 2 mL of 10% TCA before adding the enzyme solution and then treated as described above. After 10 min on ice, the samples were filtered using Whatman No1 filter paper (Shimogaki *et al.,* 1991). 0.5mL supernatant was then added to 1 mL of 1 M NaOH, and the absorbance was taken at 440 nm (Karmous *et al.,* 2012). One unit (U) of protease activity was defined as the amount of the enzyme that increased the absorbance by 0.001 minute^{-1} under the conditions of the assay.

Determination of polyphenol oxidase activities

The activity of PPO was determined by reaction mixture which contained of 0.1ml freshly prepared enzyme extract and 0.7ml of 30mM catechol in 0.1M phosphate buffer pH 6.8 incubated for 10 minutes and the absorbance was read at 420nm. One unit (U) of PPO activity was defined as the amount of the enzyme that increased the absorbance by 0.001 minute^{-1} under the conditions of the assay (Deepaa and Wong, 2012).

Protein determination

Protein concentration was measured according to the method of Bradford (1976) using bovine serum albumin (BSA).

RESULT AND DISCUSSION

The white, yellow and cream seed of sorghum have been reported useful for malting and brewing (Abu *et al.,* 2004). Dewar *et al*. (1997) reported that steeping conditions applied to sorghum brewing indicates malt quality. Measured parameters of diastatic power, FAN content, and extract increased when steeping time was increased from 16 to 40 h and optimum temperature was between 25° and 30°C. The study showed that aeratio n during steeping was necessary to maximize malt quality. Steeping is a very important aspect of sorghum malting, and dilute alkaline steeping improves the overall quality of the malt (Obeta, 1999). A study by Lefydei and Taylor (2006) indicated that steeping in 0.2% sodium hydroxide solution reduced fungal and bacterial contamination without causing cytotoxicity, along with increasing the diastatic power by increased water absorption in the grains.

The overall performances of white and yellow sorghum follow the same paradigm. The protein concentrations of the crude extract were 0.639 mg/ml and 0.751 mg/ml for white and yellow sorghum respectively. This is slightly higher than the amount that was reported by Adefila *et al* (2012), where 0.59 mg/ml was obtained for crude enzyme from green malt of *Sorghum bicolor*. The malting properties obtained in this study (Table 1), showed that white sorghum has lower germinative energy and germinative capacity than the yellow sorghum. Ogbonna (2011) reported a range of 96-97% and 90.5-96.5% for germinative energy and germinative capacity respectively for some sorghum varieties. Makeri *et al* (2013) reported germinative capacities greater than 90% for some improved Nigeria barley cultivars. Beta *et al*. (1995) found the germinative energy of 16 sorghum cultivars ranged from 43-99% while Demuyakor and Ohta (1992) found the germinative energy of sorghum varieties grown in Ghana ranged from 72-90%.

The result of this study showed that polyphenol oxidase activities were lowest during steeping than at dormant seed, while the highest activities were obtained during germination. This indicated the development of brown coloration which is essential for malt production, and this can be optimized and control based on the type

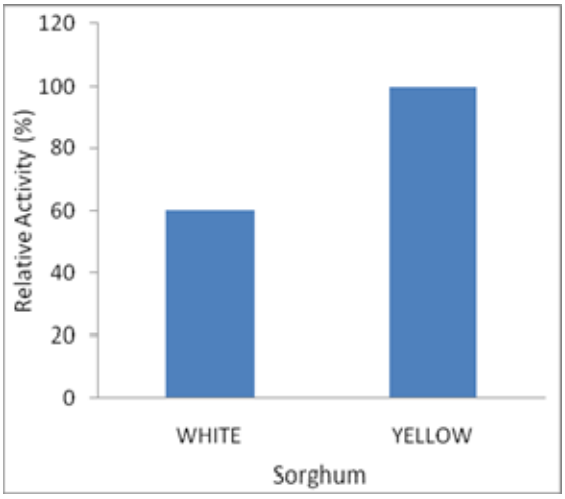

Figure 1. Polyphenol Oxidases Activity in dormant grains (0 Hour).

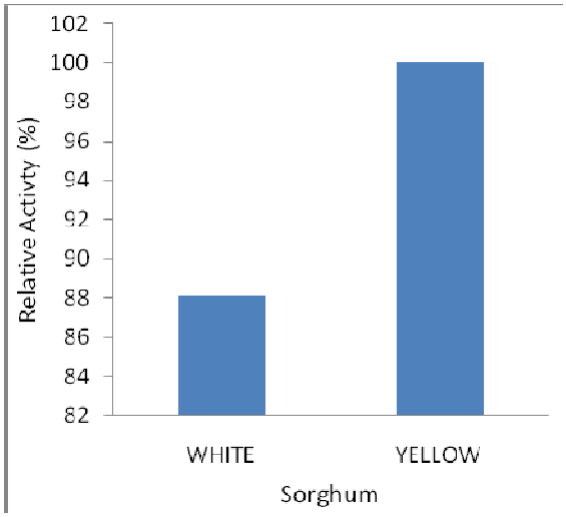

Figure 2. Amylases Activity in dormant grains (0 Hour).

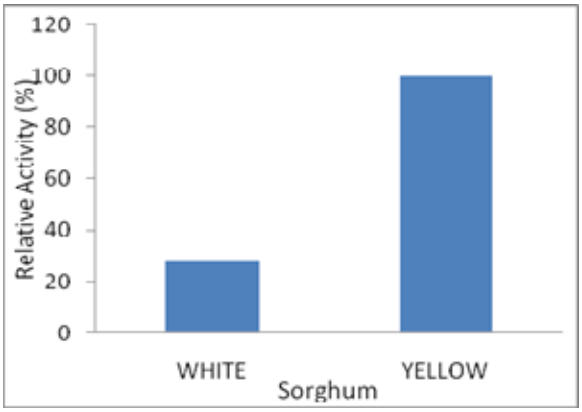

Figure 3. Proteases Activity in dormant grains (0 Hour).

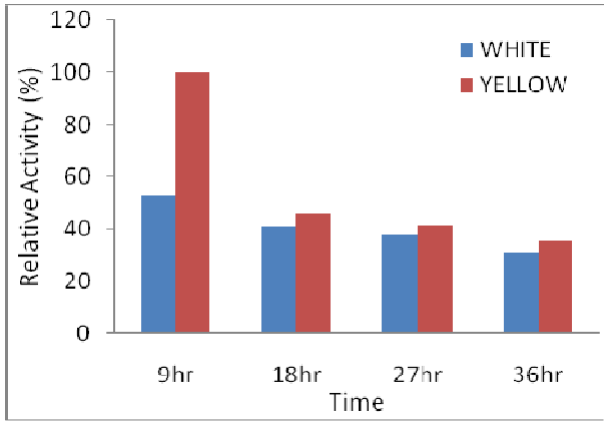

Figure 4. Polyphenol Oxidase Activity at 9 hour intervals during Steeping.

of malt in view. The activities of PPO are possible on the adequate presence of phenolics such as 3-deoxyanthocyanidins, tannins, and polycosanols, as in figure 1, 4, 7, and 10. Huynh and Jerumanis (1977) found that polyphenol oxidase activity increased by three fold after steeping.

The result of this study showed that an amylases activity did not change greatly from dormant to germination. This could have negative impact in the malting process in that the amounts of simple sugar or maltose released were unchanged as in figure 2, 5, 8 and 11. Sorghum malting yields high proportions of hydrolytic enzymes such as α-glucosidase, and α- and β-amylases (Owuama 1999). Dufour *et al*. (1992) evaluated 49 different sorghum cultivars grown in 10 different Asian and Africa nations and found that eighty percent of the sorghum malts exhibited α-amylase activities similar to or higher than industrial lager barley malts, and that the white sorghums in the study performed better in malting and brewing than red sorghum. Taylor (1994) found that sorghum has a lower ratio of β- to α- amylase (0.2), which limits the conversion of starch to simple sugars. Aisien and Ghosh (1978) report that α-amylase rather than β-amylase is the primary amylase that degrades

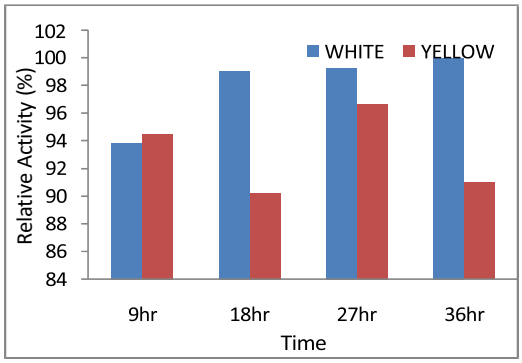

Figure 5. Amylases Activity at 9 hour intervals during Steeping.

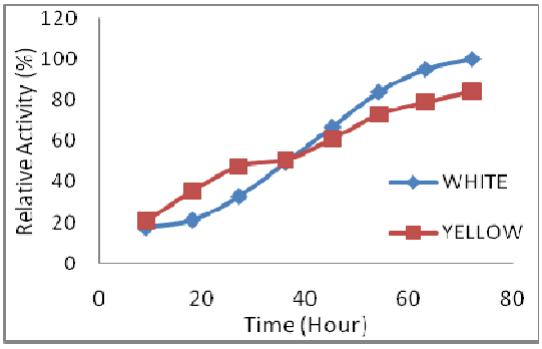

Figure 7. Polyphenol Oxidase Activity at 9 hour intervals during Germination.

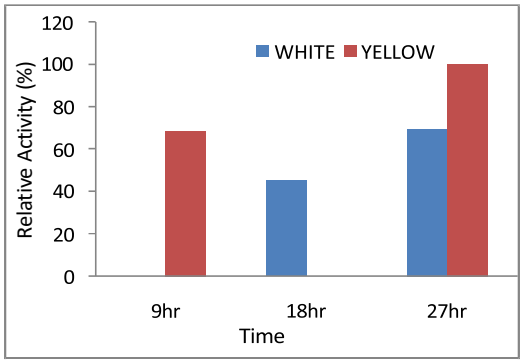

Figure 6. Proteases Activity at 9 hour intervals during Steeping.

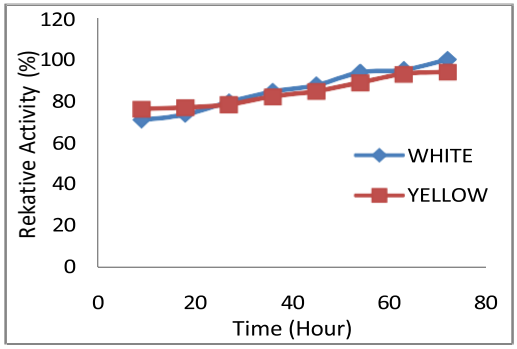

Figure 8. Amylases Activity at 9 hour intervals during Germination.

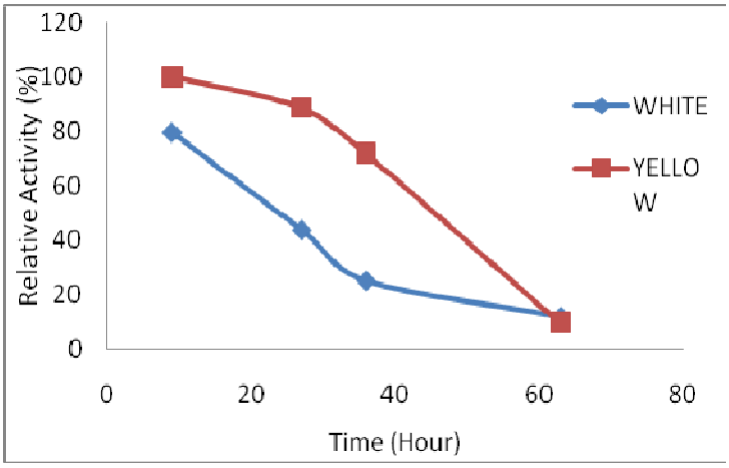

Figure 9. Proteases Activity at 9 hour intervals during Germination.

starch during malting. β-amylase, the saccharifying amylase, catalyzes the hydrolysis of α(1→4) glucosidic bond at a non-reducing end of polysaccharides, causing the release of maltose. It has been reported that un-germinated sorghum grain exhibits virtually no β-amylase activity (Owuama 1999).

The result of this study showed that the proteases activities were highest during steeping than in dormant while gradually decreased to zero level during germination. This indicated the production of free α-

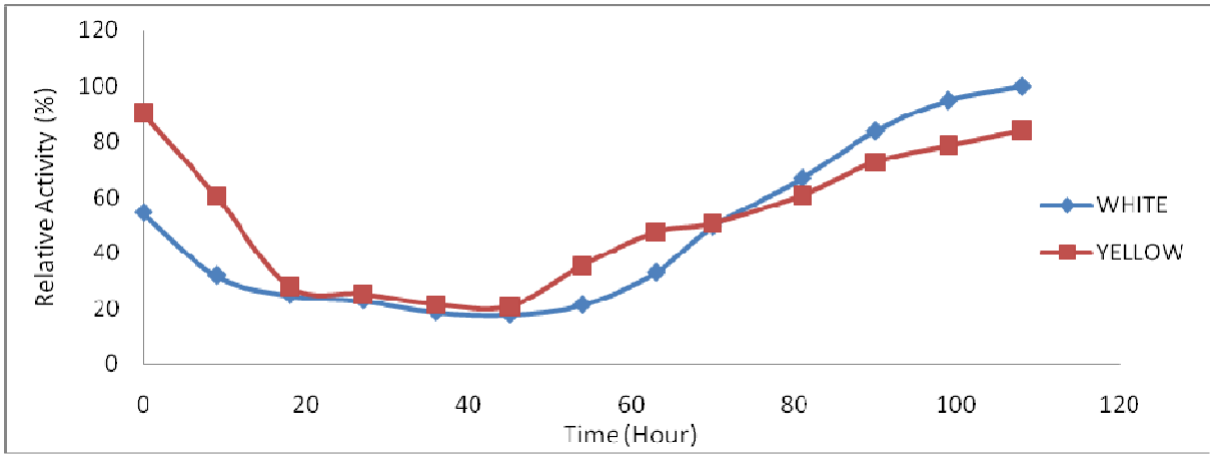

Figure 10. Polyphenol Oxidase Activities during Sorghum Malting. Dormant = 0 hour. Steeping = 0 - 36 hours. Germination = 36-108 hours.

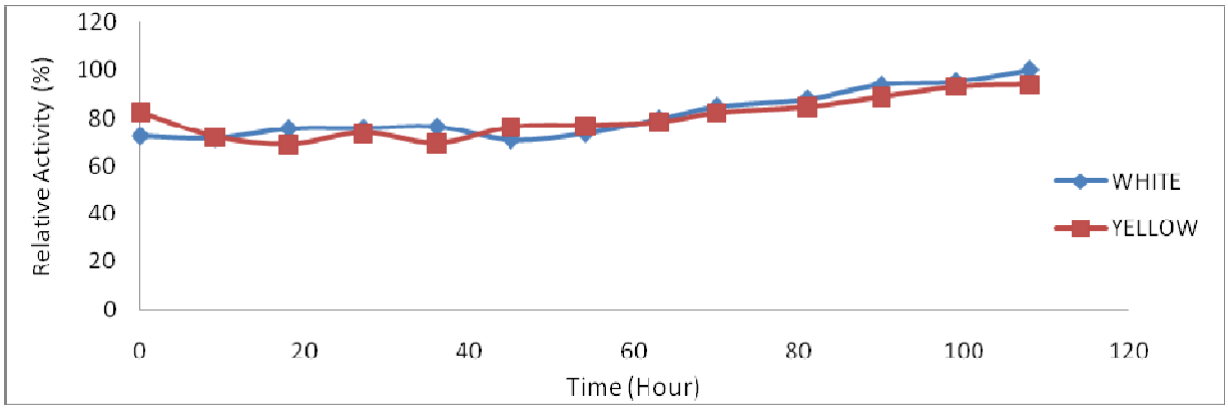

Figure 11. Alpha-amylase Activities during Sorghum Malting. Dormant = 0 hour. Steeping = 0 - 36 hours. Germination = 36-108 hours.

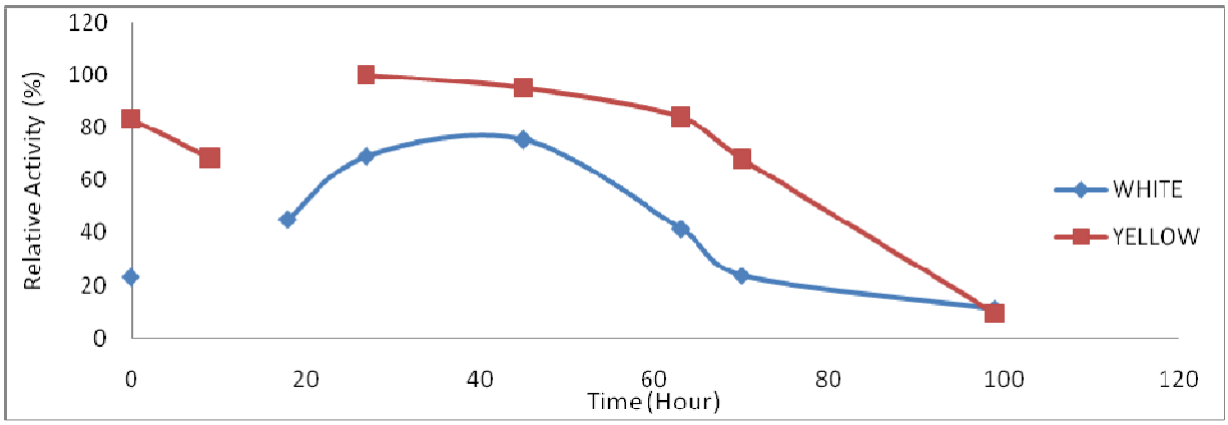

Figure 12. Protease Activities during Sorghum Malting. Dormant = 0 hour. Steeping = 0 - 36 hours. Germination = 36-108 hours.

amino nitrogen (FAN), as in figure 3, 6, 9 and 12. The carboxypeptidase and proteinase enzyme activities in three sorghum malts has been reported (Ogbonna *et al.,* 2003). Endopeptidases including carboxypeptidases and proteinases are important in grain germination to hydrolyze proteins into FAN, which is necessary for yeast

metabolism (Owuama 1999). Afify *et al.,* (2012), observed that after germination, crude protein was decreased and free amino acids were increased.

CONCLUSION

We have been able to provide data for industrial production of sorghum malt. Based on the result of this study, polyphenol oxidase like the other well known malting enzymes such as amylases and proteases, proved to be greatly expressed during germination stage of malting, and it is important in the colour development of malt before kilning. Thus, to obtain the highest levels of sugars, free amino nitrogen, and quinone, the amylases, proteases and polyphenol oxidases activities and other malting conditions need to be controlled and optimized.

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