*Journal of Scientific Research & Reports*

*25(6): 1-8, 2019; Article no.JSRR.53750*
*ISSN: 2320-0227*

# Changes in the Activities of Alpha and Gluco-amylases during Malting of Some Improved Nigerian Sorghum Varieties

**Chukwudi I. Nnamchi[1*], Chukwunweike O. Okoye[1] and Arinze L. Ezugwu[2]**

*[1]Department of Microbiology, University of Nigeria, Nsukka, Nigeria.*
*[2]Department of Biochemistry, University of Nigeria, Nsukka, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors CIN and ALE designed the study. Author COO carried out most of the laboratory work and together with author ALE wrote the first draft. Author CIN wrote the final manuscript. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JSRR/2019/v25i630201
Editor(s):
(1) Assoc. Prof. Dr. Suleyman Korkut, Department of Forest Industry Engineering, Duzce University, Division of Wood Mechanic and Technology, 81620 Beciyorukler Campus Duzce, Turkey.
Reviewers:
(1) Mohamed A. Hassan, Egypt.
(2) Marco Benvenga, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53750

***Original Research Article***

***Received 25 October 2019***
***Accepted 30 December 2019***
***Published 06 January 2020***

## ABSTRACT

In this study changes in the alpha and glucoamylase activities of four different improved sorghum varieties were monitored over a 96-hour malting time to determine how the expression of the enzymes were affected by malting. Preliminary tests showed that SK5912 had the highest weight of 40 g/1000 grains and lowest malting loss of about 13%, while KSV 8 followed a weight of 36 g/1000 grains and the most malting loss of 22.6%. Enzyme results showed that the different sorghum varieties differed in their expression of the two of them across different malting regimes. However, all the varieties showed much higher expressions of glucoamylase than α-amylase at all the malting regime. Glucoamylase consistently showed its highest activities of over 71 U/ml across the four sorghum varieties after the first day of germination with variety SRNA giving the highest value slightly above 72 U/ml. The least glucoamylase activities were also given consistently across the four varieties by the unmalted raw grain. The highest α-amylase activities were generally shown across the four varieties after the third day of germination, with variety KSV 8 showing the highest value of about 14 U/ml. The control also gave the least α-amylase activities across all varieties with

**Corresponding author: E-mail: chukwudi.nnamchi@unn.edu.ng;*

KSV8 giving the least. Considering the many important and multi-faceted roles that amylases are nowadays known to perform, the study of their expression dynamics in different plants and processes, one of which we report here, could help further the understanding of their characteristics and thus facilitate their maximal utilization.

*Keywords: Alpha amylase; beta amylase; sorghum; grains; cereals; malting.*

## 1. INTRODUCTION

Cereal malting is a process involving the germination of grains under controlled conditions [1,2]. Ultimately, the malting process leads to the development and activation of enzymes that will attack the endosperm matrix and breaks down the underlying carbohydrates [3]. Amylases are the enzymes responsible for breaking down amylose (starch). Three types of amylases exist, namely: α-amylase, b-amylase and glucoamylase also called α-glucosidase. α-amylase (endo-1, 4-α-D-glucan glucohydrolase, 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 [4]. Because it is an endozyme, it splits the substrate in the interior of the molecules. β-amylase (β-1, 4-glucan maltohydrolase, EC 3.2.1.2) is an exoacting enzyme that cleaves non-reducing chain ends of amylase, amylopectin and glycogen molecules. It hydrolyses alternate glycosidic linkages yielding glycoside linkages maltose [4]. Glucoamylase (exo-1, 4-α-D-glucan glucano-hydrolase, EC 3.2.1.3) hydrolyses single glucose units from the non-reducing ends of amylase and amylopectin in a stepwise manner [4]. Amylases extracted from plants and plant sources because of their immense biological activity and other useful properties, some of which may not be obtained from other sources, are of great significance in the biotechnological industries and therefore highly sought after. One major area where their immense usefulness finds domiciliation is in the pharmaceutical and fine chemicals. As a matter of fact, the first industrially produced enzyme was an amylase obtained from a fungal source in 1894 and used as a pharmaceutical remedy for the treatment of some disorders of the digestive system [5]. Incidentally, with the increasingly new numbers of cutting edge areas in biotechnology, the range and rich varieties of amylase application has extended to many other fields including clinical, medicinal and analytical chemistry and also their many and broadly spread traditional applications in starch saccharification as well as in the textile, food, brewing and distilling industries [6].

Quantitatively and in production terms, sorghum is the world's fifth largest and most important cereal, after wheat, maize, rice and barley [7]. In most parts of Africa, sorghum is still largely a subsistence food crop helping to feed her huge and ever increasing population; however, increasingly, it is becoming the foundation for many successful food and beverage industries [8]. One of the reasons for this is the immense improvement that have been made in terms of unleashing innate enzymes such as the numerous types of amylases towards mobilizing its stored carbohydrates and thereby making them available for many biotechnological purposes [9]. For instance, one of the important uses of sorghum grains presently is with respect to brewing. Although barley has historically been the oldest and most used brewing grain [10] it is essentially a temperate crop and therefore the high importation cost incurred in many tropical countries including Nigeria and many other African countries caused governments in the affected countries to compel industries to seek alternative grains as brewing raw material [7,11]. Those policy measures has resulted in the fact that today many beer brewing industries in most parts of the world are able to produce continental type beers using sorghum as the major brewing material. The immense improvements in the developing the innate enzymes of the different sorghum varieties has played a large role in these developments. There is at present many deliberate but concerted efforts towards developing new and improved varieties of sorghum so much so that now over 10,000 varieties of it exists [7]. Because of the immense role their contents of amylases whether in their raw states or during malting process as is often required during brewing, we aimed in the present work to assess the content of α-amylase, b-amylase and glucoamylase of four improved Nigerian sorghum varieties during a 4-day malting period.

## 2. MATERIALS AND METHODS

### 2.1 Grain Sourcing

Four different varieties of sorghum grains (*Sorghum bicolor* L. Moench var. SK5912, KSV8,

SRN39 and SSV98001 all grown in 2015 and purchased from the Institute for Agricultural Research of the Ahmadu Bello University Zaria, Nigeria were used for this work.

## 2.2 Cleaning and Sorting of Grains

The sorghum grains were carefully sorted manually one after the other, to remove broken kernels and foreign materials before being surface sterilized by immersion in 1% (v/v) hypochlorite solution [12] and allowed to stand for a few minutes. After thoroughly rinsing with tap and distilled water, the grains were spread on a clean surface layered with soft absorbent papers and allowed to dry at room temperature overnight.

## 2.3 Preliminary Tests

Certain preliminary tests including thousand kernel weight, germinative energy, germinative capacity, malting loss etc. were carried out first on the grains before the full malting process. All the listed parameters were assessed using the Recommended Methods of the Institute of Brewing, London [13]. Triplicate or duplicate analyses of the parameters were done in all cases to ensure appropriate statistical considerations.

## 2.4 Grain Steeping

The steeping of the sorghum grains was done by weighing out exactly 200 grams of each sorghum variety in triplicates, and immersing them in 400 ml of distilled water such that the grain/water mixture was in a ratio of 1:2. Thereafter, steeping was carried out for 24 hours at room temperature (28ºC) and the steep water changed at 6 hourly intervals to remove any untoward microbial contaminant.

## 2.5 Germination

At the end of steeping, the sorghum grains were germinated on wet beds, in the dark, for a period lasting 4 days. Before usage, the wet beds and cupboards were thoroughly cleaned and sterilized of microbial contaminants using the hypochlorite solution. During germination, the sorghum grains were regularly sprinkled with water, and also mixed and turned so as to achieve uniform temperature and moisture levels.

## 2.6 Kilning

Germination was terminated by kilning (drying) the germinated seedlings in a hot-air (thermostat-controlled) oven, previously set at 48ºC. The kilning lasted for 24 hours. Afterwards, the radicle and plumule of each dry now seedling was manually removed.

## 2.7 Enzyme Extraction and Analysis

After the malting of the sorghum grains enzymes were extracted using a modification of the method described by Oyewole and Agboola [4]. Exactly 10 g of the seeds or malts were weighed out and ground using a mortar and pestle with 6.0 ml of appropriate buffer depending on the enzyme to be extracted (0.1 M acetate buffer, pH 5.5 for a-amylase; 0.5 M acetate buffer, pH 4.5 for glucoamylase). The mixture was sieved with muslin cloth and centrifuged at 4500 rpm for 10 mins. The supernatant was decanted and used as crude enzymes. Amylase activities were assayed from day zero to the end of malting.

## 2.8 Glucoamylase Activity Assay

Glucose standard curve was prepared by dissolving 0.009 g of glucose and made up to 100 ml using distilled water and used as stock solution. 0.1 ml to 1 ml of the stock solution was added to 10 different test tubes and distilled water was used to make up to 1 ml. 1 ml of DNSA was added to each test tube and it was boiled for 10 mins and 1 ml of sodium tartarate was added and allowed to cool and absorbance was taken. To determine enzyme activity, starch solution was prepared by dissolving 1 g of starch in 40 ml of distilled water and adding the whole content to 50 ml of boiling water while stirring. The volume was made up to 100 ml after allowing it to cool. Glucoamylase activity was assayed in triplicates. Each test tube contained 0.5 ml of starch solution, 2 ml of sodium acetate buffer and 0.5 ml of the crude enzyme and was incubated for 10 mins at 50ºC. The released glucose was measured by adding 1 ml of DNSA and it was immersed in a boiling water bath for 10 mins and 1 ml of sodium potassium tartarate was added to stabilize the colour. The test tubes were allowed to cool at room temperature and the absorbance taken against the substrate blank at 540 nm using Spectrumlab 722s spectrophotometer. Amylase activity was estimated by the analysis of reducing sugar released during hydrolysis of starch. The amount of glucose produced per unit time was estimated

from a standard glucose curve using 5 mM glucose solution. Glucoamylase activity unit was expressed as the amount of enzyme releasing 1 µmole of glucose equivalent per minute per ml.

### 2.9 Alpha Amylase Activity Assay

Maltose standard curve was prepared by dissolving 0.068 g of maltose standard in 100 ml of distilled water and used as stock solution. 0.1 ml to 1 ml of the stock solution was added to 10 different test tubes and distilled water was used to make up to 1 ml. 1 ml of DNSA was added to each test tube and it was boiled for 10 mins and 1 ml of sodium tartarate was added and allowed to cool and absorbance was taken. To determine enzyme activity, starch solution was prepared by dissolving 1 g of starch in 40 ml of distilled water and adding to 50 ml of boiling water while stiring. The volume was made up to 100 ml after allowing it to cool. Alpha amylase activity was assayed in triplicates. Each test tube contained 0.5 ml of starch solution, 2 ml of phosphate buffer and 0.5 ml of the crude enzyme and was incubated at room temperature for 1 h. 1 ml of DNSA was added and it was immersed in a boiling water bath for 10 mins and 1 ml of sodium potassium tartarate was added to stabilize the colour. The test tubes were allowed to cool at room temperature and the absorbance taken against the substrate blank at 600 nm using Spectrumlab 722s spectrophotometer. Amylase activity was estimated by the analysis of reducing sugar released during hydrolysis of starch.

## 3. RESULTS AND DISCUSSION

Fig. 1 shows the thousand kernel weight (TKW) of the four sorghum varieties used in this work. The TKW which measures the pooled weight of a thousand quantities of each grain gives an appropriate measure of the grain size. As seen in the figure, the variety with the most weight was Sk5912 with a TKW of about 40 g, followed by KSV8 with 36.2 g, while the least was SRNA with nearly 27 g. Grain weight is often considered an important measure of grain quality. This is because higher weighted grains are believed to confer some advantages such as having less husks in addition to having higher starch content when compared to their smaller-sized counterparts [7]. Table 1 shows some preliminary malting properties of the four sorghum grain varieties used in this work. The result showed that they all have high germinative energies, which is an indication of the grain's ability to germinate, with variety SSV98001 having the highest value of 99% followed closely by SRNA with 98% while the least value of 95% was given by variety SK5912. A similar trend was also observed with the germinative capacity, a measure of the degree of dormancy, of the different sorghum varieties. As observed with germinative energy, variety SSV98001 again gave the highest value of 98% followed closely by both varieties SRNA and KSV8 with 97% while the least value of 96% was given by variety SK5912. The import of this result is that all the sorghum grain varieties used in this work showed high germinative energies and capacities of close to 100, which is a positive trait usually considered by grain buyers as it indicates that they are all alive and possess positive malting abilities [7]. The results are in agreement with previous reports for sorghum [14] but higher than those previously observed for barley [15,16]. A very important fact is that these values are higher than the recommended values acceptable for malting grains [17]. The highest value of 22% for malting loss was observed for variety KSV8, followed distantly by varieties SRNA (15.77) and SSV98001 (14.61) while the least value of 12.98% was given by variety SK5912. The takeaway from the malting loss values is that they are comparatively low compared with the high malting losses typically observed with sorghum which are often above 30% [18,19]. Typically malting losses in sorghum is a measure of the losses incurred because of the metabolic process associated with the germination of grains and manifests in rootlet and plumule growth and development [20,21].

Results obtained in respect of the enzymes showed that the four sorghum varieties differed in how they expressed the two enzymes across the different regimes. In all cases, we found that the least amount of enzymes was obtained with the control which is the raw unmalted grains. This is expected, considering that the grains in that state had not been subjected to any form of treatment. Malting is known to improve and increase enzyme development and expression in grains [1]. Among the various controls, variety KSV8 gave the least amount of α-amylase, 2.88 U/ml, followed by variety SSV98001 with 3.14 U/ml while the highest expression of the enzyme was given by SK5912 with 5.8 U/ml. As shown in Fig. 2, α-amylase activities increased with the commencement of malting starting with the end of steeping when the activities of all the four sorghum varieties witnessed over 100% increase relative to their controls. Thus, variety KSV8

increased to 6.45 U/ml compared to the control (2.88 U/ml), SSV98001 increased from 3.14 U/ml to 8.05 while variety SK5912 more than doubled to 10.04 U/ml. The highest α-amylase value after steeping was however given by variety SRNA which increased from 4.57 U/ml given by the control to 11.07 U/ml. It has been observed that steeping which involves the soaking of grains in water, is a key factor in the malting of cereals [22] and that it is the point during germination when the activities of many endosperm modifying enzymes such as amylases, proteases, and similarly working enzymes are induced [23,24]. That no doubt explains the increased incidence of α-amylase after the grains had been steeped. Similar progressive increase in α-amylase activities continued during the other stages of the malting process, with most of the sorghum grain varieties increasing their α-amylase activities beyond the values attained after steeping starting from the first day of germination. However as also shown in Fig. 2, most of the grains experienced their highest activities at the end of the third day of germination, with variety SRNA achieving the highest value of 14.06 U/ml, followed in second and third places by varieties KSV8 and SSV98001 while the least value at that point (third day) was given by SK5912, whose highest value occurred on the fourth day of germination (12.57 U/ml). We had previously noted in our work with peroxidases, that the third day of germination seemed to be the point during which the highest amount of enzyme activities is observed [25]. The present work seems to also confirm that fact. Fig. 2 also showed that all the enzyme activities decreased from the highs observed during germination after they had been kilned. This is probably because subjecting the germinated grains to heat treatment which is essentially what is done at this point to halt further enzyme activities resulted in the lowering of further α-amylase activities.

Fig. 3 showed the changes in the glucoamylase activities of the different sorghum grain varieties after malting. What is immediately apparent from the figure is that all the grains have more glucoamylase content than alpha amylase. For example, in the control alone, the ratio between alpha amylase to glucoamylase is 4.57 to 22.37 for variety SRN39; 3.14 to 45.6 for SSV98001, 2.88 to 32.48 for KSV8 and 5.8 to 40.48 for SK5912. This means that the glucoamylase content of all grains is more than their

**Table 1. Preliminary malt quality properties of sorghum grain varieties**

| Sorghum variety | Germinative energy (%) | Germinative capacity (%) | Malting loss (%) |
|---|---|---|---|
| KSV8 | 97 | 97 | 22.58 |
| SRN39 | 98 | 97 | 15.77 |
| SK5912 | 95 | 96 | 12.98 |
| SSV98001 | 99 | 98 | 14.61 |

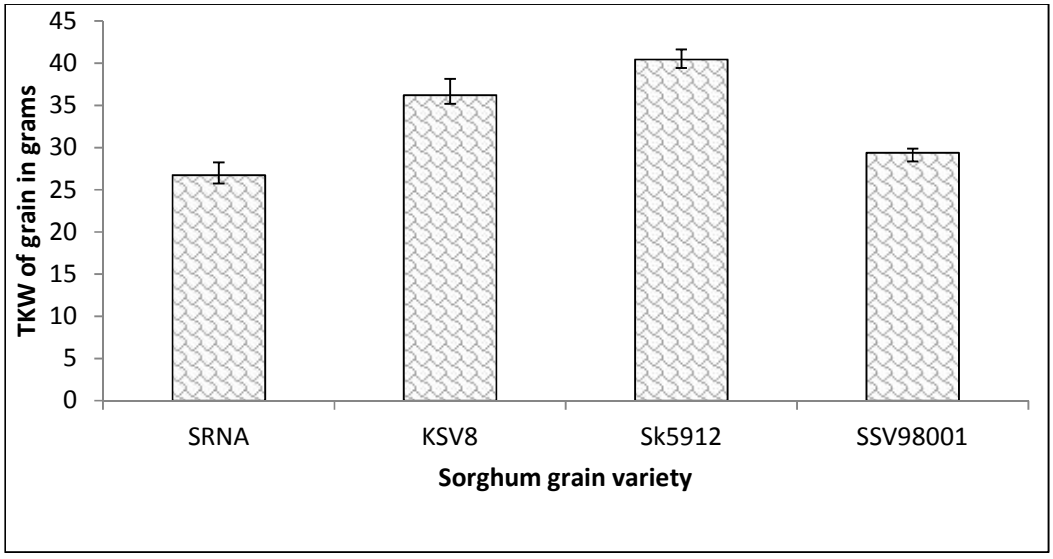


**Fig. 1. Thousand kernel weight (TKW) of different sorghum grain varieties used**

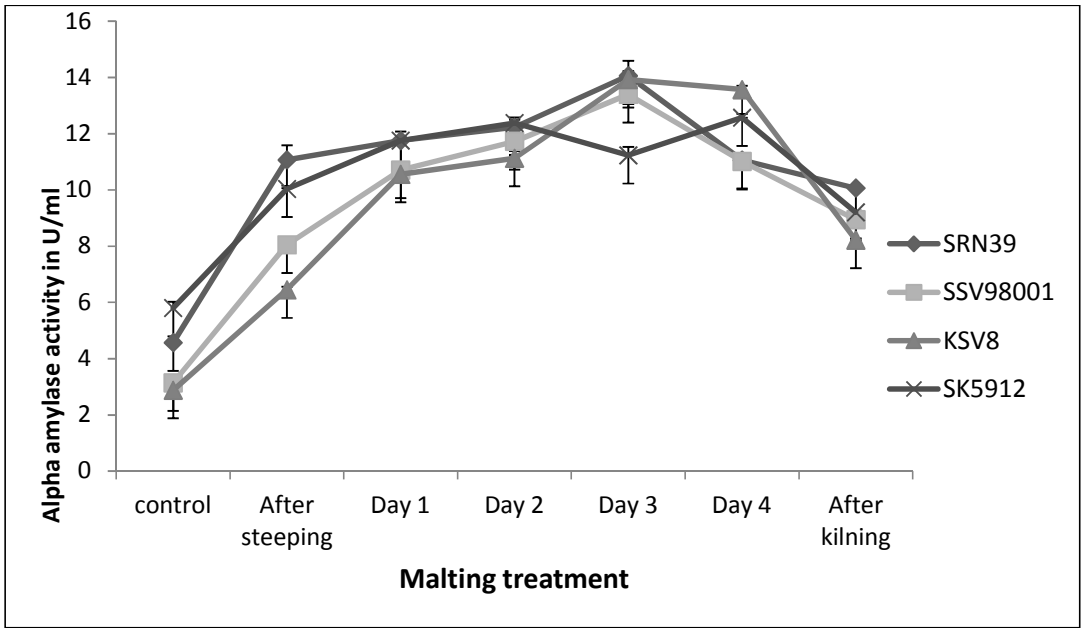


**Fig. 2. Changes in the alpha amylase composition of some improved sorghum grains after different malting treatments**

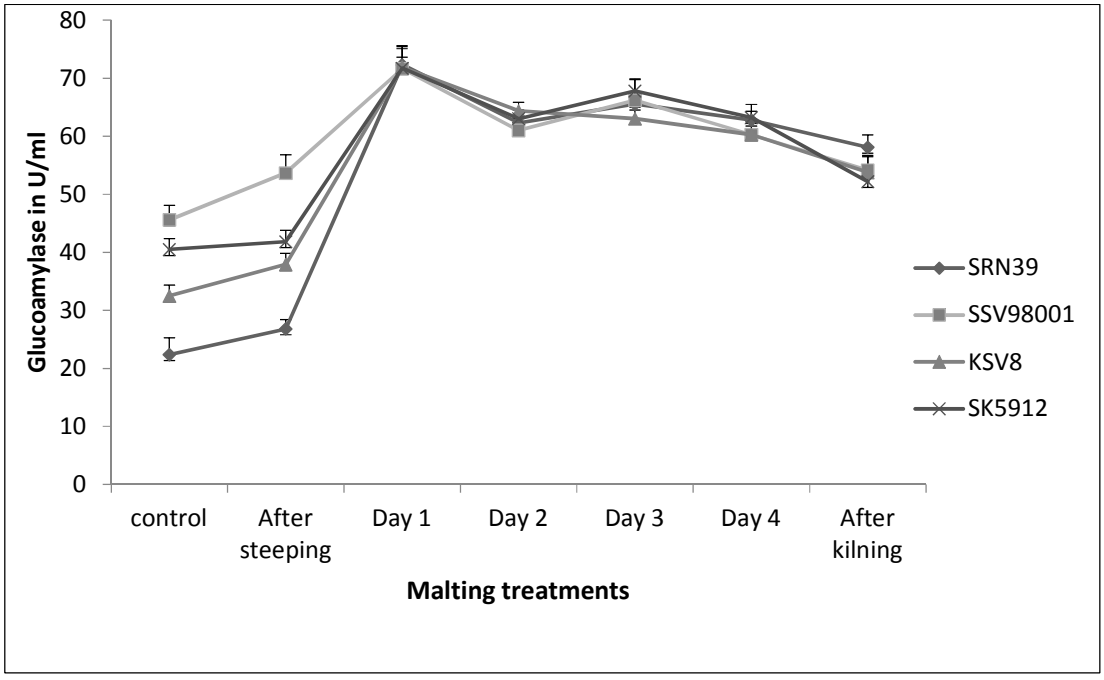


**Fig. 3. Changes in the glucoamylase composition of some improved sorghum grains after different malting treatments**

corresponding alpha amylase counterparts by 4.9, 14.5, 11.3 and 6.98 times respectively for the different sorghum varieties as listed above. Considering that the two enzymes are essentially amylases, this result is significant and could mean that sorghum grains even when in their raw, untreated state, that is, when not malted nor subjected to further treatment, already contain

more intrinsic glucoamylases than α-amylases. Part of the reason for this could be the fact which had been suggested by some persons, glucoamylases and β-amylases are of plant origin and therefore, that plant sources typically contain more glucoamylases and β-amylases than α-amylases, while in microorganisms and microbial sources the reverse is the case [4,26]. Essentially the same trend of the incidence of more glucoamylases than α-amylases was observed across the rest of the malting regime although the ratios were smaller considering that the onset of malting seemed to have increased the activities of both enzymes alike. Further on the progress of glucoamylase activities across the malting spectrum, we observed that after steeping the activities of glucoamylases did not increase as was the case with α-amylases after steeping. More significant increases in activities were only observed when germination commenced. Incidentally, the highest glucoamylase activities occurred after the first day of germination with variety SRN39 having the most activity (72.31 U/ml). The other three varieties also had their most glucoamylase activities after the first day of germination while the least activity during the germination period occurred on the fourth day of germination. This is another reinforcement of the fact that we had observed above with α-amylases and also when we assayed for peroxidase previously [25], that the best enzyme activity results during the malting of sorghum grains may well occur at earlier periods of malting than the fourth day when it most researchers typically conclude it. This could result in the saving of the extra time and cost of resources.

## 4. CONCLUSION

In this work, we had followed the course of two amylase enzymes during a 4-day malting schedule and found that malting improved the activities of the enzymes. We also found that glucoamylases occurred more than α-amylases both before malting, and after malting. These results are significant considering that the activities of theses enzymes together with that of β-amylases, are largely responsible for the modification of cereal endosperms during malting.

## ACKNOWLEDGEMENT

The authors are grateful to TETFUND for their 2015-2016 IBR grant given in 2017 which to a large extent helped to fund this project.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Briggs DE. Malt and Malting, First Ed. Blackie Academic and Professional, London; 1998.
2. Ayenor GS, Ocloo FCK. Physicochemical changes and diastase activity associated with germinating paddy rice (PSB. Rc 34). African Journal of food Science. 2007;1: 037-041.
3. Palmer GH. Cereals in malting and brewing, in Cereal Science and Technology (Palmer, G. H. Ed.), Aberdeen University Press, Aberdeen. 1989;61–242.
4. Oyewole OI, Agboola FK. Comparative studies on properties of amylases extracted from kilned and unkilned malted sorghum and corn. International Journal for Biotechnology and Molecular Biology Research. 2011;2(9):46-149.
5. Manners DJ, Marshall JJ. Studies on carbohydrate metabolizing enzymes. Part XXIII. The ß-glucanase system of malted barley. J. Instit. Brewing. 1969;75:550-561.
6. Alli AL, Ogbonna CL, Rahman AT. Hydrolysis of certain Nigerian cereal starches using crude fungal amylase. Nig. J. Biotech. 1998;9:2436.
7. Nnamchi CI, Okolo BN, Moneke AN. Grain and malt quality properties of some improved Nigerian sorghum varieties. J Inst Brew. 2014;120:353–359.
8. Agu RC, Palmer GH. A reassessment of sorghum for larger beer brewing. Bioresource Technology. 1998;66:253-261.
9. Adejumo AL, Agboola FK, Layokun SK. Hydrolysis of maize starch using amylolytic enzymes extracted from sorghum malt. Int. J. Biol. Chem. Sci. 2009;3(5):1030-1041.
10. Bamforth CW. New brewing technologies: Setting the scene In: Brewing Bamforth CW ed. Cambridge, Woodhead Publishing Limited. 2006;3.
11. Taylor JRN, Dlamini BC, Kruger J. 125th Anniversary review: The science of the tropical cereals sorghum, maize and rice in relation to lager beer brewing. 2013;119:1–14.
12. Ogbonna AC, Obi SKC, Okolo BN. Protein modification in malting sorghum. World

Journal of Microbiology and Biotechnology. 2003;19:495-503.
13. Institute of Brewing. Recommended methods of analysis. London; 1989.
14. Palmer GH. Cereals in malting and brewing. In: Cereal Science and Technology, ed. Palmer GH. Aberdeen: Aberdeen University Press. 1989;61–242.
15. Novellie L. Kaffir corn malting and brewing studies XI. Effect of malting conditions on the diastatic power of kaffir corn malts. Journal of the Science of Food and Agriculture. 1962;13:115–120.
16. Okon EU, Uwaifo AO. Evaluation of malting sorghum I: The malting potentials of Nigerian varieties of sorghum. Brewer's Digest. 1985;60:24-29.
17. Briggs DE, Hough JS, Stevens R, Young TW. Malting and Brewing Science (Malt and sweet wort). Vol 1. Chapman and Hall, Ltd, USA; 1981.
18. Ogu EO, Odibo FJC, Agu RC, Palmer GH. Quality assessment of different sorghum varieties for their brewing potential. Journal of the Institute of Brewing. 2006;112:117–121.
19. Bekele A, Bultosa G, Belete K. The effect of germination time on malt quality of six sorghum (Sorghum bicolor) varieties grown at Melkassa Ethiopia. Journal of the Institute of Brewing. 2012;118:76–81.
20. Aisien AO, Muts GCJ. Micro-scale malting and brewing studies of some sorghum varieties. Journal of the Institute of Brewing. 1987;93:328–331.
21. Daiber KH, Taylor JRN. Opaque beers. In: Sorghum and the Millets: Chemistry and Technology. (D.A.V. Dendy ed.), American Association of Cereal Chemists, St. Paul. 1995;299-323.
22. French BJ, McRuer GR. Malt quality as affected by various steep aeration regimes. MBAA Technical Quarterly. 1990;27:10–14.
23. Dewar J, Taylor JRN, Berjak P. Determination of improved steeping conditions for sorghum malting. Journal of Cereal Science. 1997;26:129–136.
24. Nnamchi CI, Okolo BN, Moneke AN, Nwanguma BC. Effect of different steeping conditions on the peroxidase activities of some improved sorghum varieties. Bio Research. 2012;11:819–824.
25. Nnamchi CI, Okolo BN, Moneke AN, Nwanguma BC. Changes in the activities of peroxidases during different stages of sorghum malting. Int. J. Adv. Res. 2013; 1(7):44-58.
26. EtokAkpan OU, Palmer GH. Comparative studies of the development of endosperm-degrading enzymes in malting sorghum and barley. World J. Microbiol. Biotechnol. 1990;6:408-417.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sdiarticle4.com/review-history/53750*