

Lipase Activities During Malting and Fermentation of Sorghum for Burukutu Production

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ABSTRACT

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Local red and white, and SK₅₉₁₂ varieties of sorghum grains were malted, and assayed for amylase, diastatic and lipase activities. The 5-day malts from the red and white varieties were fermented for 48 h to produce burukutu, during which, lipase activities were monitored. Malt values peaked on day 5, and the red malts had the highest diastatic activities while the white variety had the lowest. Lipase activity in the malts peaked on day 4 with the red having the highest activity. The pH of the fermenting gruels decreased from 5.33 and 5.35 and levelled off at 3.88 and 3.85 for the red and white malts respectively. During fermentation, lipase activity peaks were detected at 0 h, 21 h and 45 h for the red malt with the highest peak at 45 h. The peak fermentation times for lipase activity in the white malt was at 0 h, 21 h and 48 h with the highest peak at 48 h. Lipase activity within the first 39 h was higher in the white gruel. Sorghum malt lipase from the red and white varieties may consist of three isoforms, two of which may have activity optima in the acidic pH range.

Key words: Burukutu, fermentation, lipase, malting, sorghum.

INTRODUCTION

Burukutu is an alcoholic beverage produced by the germination of sorghum grains, drying under the sun, and without removing the sprouts, milling and slurring in water for simultaneous saccharification and fermentation. After 1-2 days of fermentation, the broth is sieved, boiled, and matured for 2 days and pasteurisation at 60-65°C for 30 min^{19,20,43}. *Burukutu* is produced at the cottage level, and has a short shelf life of 1-8 days. The short shelf life may be due to the very low lactic acid content (0.3-0.6%), the low titratable acidity (0.4-0.6%, as acetic acid), the low alcohol content (2-4% v/v), the high concentration of vitamins and fermentable sugars^{1,5,15,19,20,31} and the presence of lipoxidation products.

The presence of lipoxidation products could be particularly contributory since sorghum contains a high concentration of fatty acids^{13,25} relative to millet⁴⁸, another cereal which may also be used in burukutu production and bar-

ley^{39,47,48,51}. Lipoxidation is the product of two enzyme activities – lipases that hydrolyse triacylglycerols (TAGs) to produce free fatty acids, the unsaturated ones of which are oxidized by lipoxygenases. Lipoxidation^{6,38} results in flavour instability in beer^{6,40}, which has been controlled by the use of antioxidants⁹ and reduction of oxygen concentration in packaged beer⁸.

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3.) are enzymes that hydrolyse esters of long chain aliphatic fatty acids from their glycerol backbone only at aqueous-lipid interfaces, and differ from esterases by their ability to hydrolyse insoluble fatty acyl esters⁶⁰. An exception to this is guinea pig pancreatic lipase⁵⁵ and *Pseudomonas aeruginosa* lipase⁴¹, which are not interfacially activated. The fatty acids released during TAG hydrolysis are linear with increasing concentration of enzyme⁴⁶ and are used in the provision of metabolic energy, in glucose synthesis³¹ when aleurone and scutellar soluble sugars are depleted or as precursors for synthesis of phospholipid necessary for the proliferation of cellular endomembrane systems^{56,61}.

Lipase activity increases during malting of sorghum grains⁴² and germination of oleaginous seeds⁴⁶. In sorghum seedlings, about 68% of the lipase is found in the plumule, 29% in the endosperm, and 3 percent in the radicle. During germination, the pH of the starchy endosperm of cereals such as barley is actively maintained in the range 5.0-5.2²³. Barley lipase has an optimum pH of 6.8⁴², while lipase from sorghum has a pH optimum of 7.0²⁵. The latter enzyme is active between pH 6.0 and 9.0 indicating that it is active during steeping, malting and mashing. In contrast, *Curcumeropsis edulis* lipase has an optimum pH of 5.0⁴⁶ due to the presence of phosphatidyl choline (cephalin) which has alkali-binding properties.

In seeds, triacylglycerols (TAGs) which are the storage form of fats/oils in plants are partitioned into small sub-cellular droplets called lipid bodies²⁸ and account for up to 85 percent of the nonpolar fraction of sorghum lipids⁴⁷. Lipid bodies decrease in direct proportion to the lipase content during germination²⁸ due to the activation of synthesised lipase. This has been observed in the aleurone^{25,57} and scutellum²². Free fatty acids (FFAs) resulting from its hydrolysis during germination will therefore contribute significantly to the pool of FFAs available for peroxidative reactions^{9,48}.

In lager beer production, much of the approximately 2% lipid of barley malt is retained in the spent grains³², while some are present in wort and contribute to the occa-

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sional occurrence of foam problems resulting from the foam-negative properties of the lipids. In barley, linoleic acid is the most abundant fatty acid in malt, wort and beer derived from it³⁵. In the case of burukutu production, all sorghum lipids would be part of the “wort” during fermentation since the grain malt is milled and fermented without removing the sprouts. The potential for the appearance of peroxidative products is therefore very high. The low pH resulting during fermentation of cereals¹¹, the presence of peroxidative products, the decrease in substrate concentration and increase in alcohol content may affect lipase activity during burukutu production. This provided the motivation for this investigation on lipase activities during malting and fermentation of sorghum for burukutu production to elucidate the basis for the short shelf life of the product.

MATERIALS AND METHODS

Grains

Native sorghum grains of the red and white varieties were purchased from a market in Nsukka, Nigeria, while SK₅₉₁₂, an improved sorghum variety was obtained from the National Seed Service, Zaria, Nigeria.

Malting

The sorghum grains were germinated by the two-step wet steep process of EtokAkpan¹⁸ and dried. Some of the germinated grains were dried to constant weight in 24 h at 50°C using a convection oven, while other samples were dried under the sun (26 ± 2°C) for 2 weeks. The dried malts were milled in a disc attrition mill (Bentall Plate Mill, Model 200 L 090) without removing the sprouts and assayed for amylase, diastatic and lipase activities on the basis of which the further use of SK₅₉₁₂ was discontinued.

Fermentation

Burukutu was prepared by a modification of the methods of Ekundayo¹⁵ and Faparusi¹⁹. A 1:4 (w/v) slurry of flours from each of the 5 day red and white sorghum malts in tap water (pH 6.20) was fermented in lightly covered plastic containers using the natural flora from the malt,

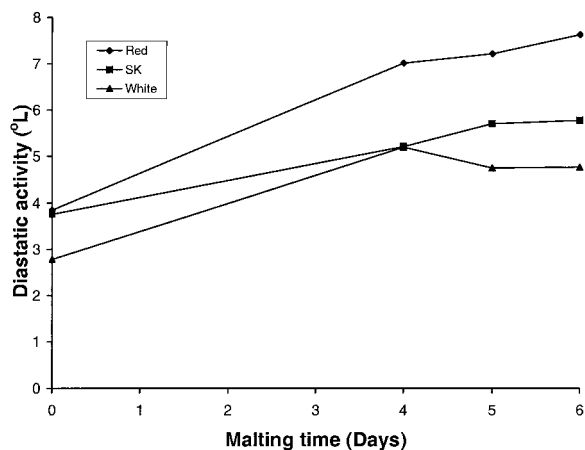


FIG. 1. Diastatic activity of sorghum malts (°L). Diastatic activities are the means of three determinations.

water and the container as inoculum as practiced traditionally in Nigeria. Samples were collected after 0, 3, and subsequently 6 h intervals and tested for lipase activity.

Determination of enzyme activities

In the malts, α - and β -amylase activities were determined by the diamylase procedure of EtokAkpan and Palmer¹⁶, diastatic activity by the method of Hulse et al²⁹, while lipase activity was monitored by a modification of the method of Nwanguma et al⁴². The modifications consisted of using 18 mL of 0.1M phosphate buffer instead of 3 mL; during fermentation, 4.5 mL of the fermenting liquor was used in place of 0.5 g of the acetone powder. The controls were prepared by adding 0.5 g of the acetone powder or 4.5 mL of the fermenting slurry to the reaction mixture after incubation as applicable. Lipase activities were derived by calculation based on the 1:1 molar relationship between a fatty acid and NaOH during a neutralisation reaction and expressed as micromoles free fatty acids released per minute.

RESULTS AND DISCUSSION

Amylase and diastatic activities during malting

The results for diastatic and amylase activities of the sorghum malts are presented in Figs. 1 and 2 respectively. The diastatic power of the sorghum malts increased with malting time and showed that the red malt had the highest values followed by SK₅₉₁₂ and then the white malt (Fig. 1). SK₅₉₁₂ however had the highest α - and β -amylase activities (Fig. 2) which suggests that the diastatic activity of SK₅₉₁₂ malt which recommended its use in the Nigerian brewing industry may be mainly due to its high α - and β -amylase activities.

The highest diastatic activity recorded for the red malts could be due to a low protein content and thermostable cell wall degrading enzymes¹⁷. High protein contents and their structural organisation in the cell wall may limit cell wall degradation. Bamforth⁴ had reported that barley β -

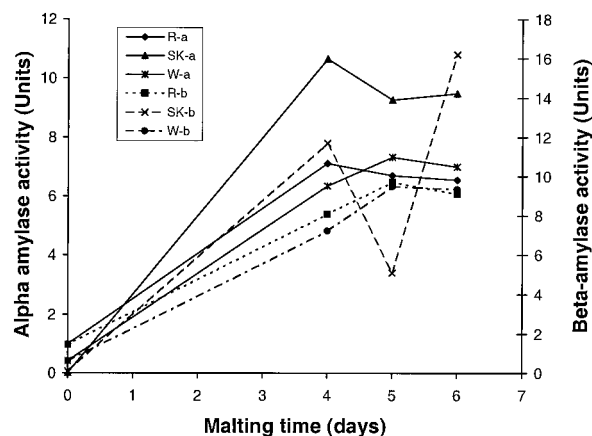


FIG. 2. Amylase activities of sorghum malts. Ra, SKa and Wa - α -amylase activities of the red, SK₅₉₁₂ and white malts; Rb, SKb and Wb - β -amylase activities of the red, SK₅₉₁₂ and white malts. Amylase activities are the means of three replicate determinations.

glucan is crosslinked by proteins or peptides to form a complex rigid matrix. That all of the β -glucan is not always released is attributed to inaccessibility of enzyme to substrate or to the likelihood that different barley varieties display different degrees of cross-linking in their walls. The higher diastatic activity of the red over the white sorghum malts may therefore be due to differences in the degree of crosslinking in their walls with the red possibly having less cross-linking that allows proteolytic and amylolytic enzymes pass through portals in the cell wall⁴⁹. It may also be due to unhydrolysed proteins attached to the starch granules^{48,49,50,52}. The presence of polyphenols and tannins in the red variety may therefore not inhibit diastatic activity as reported by Daiber¹² and could be due to the loss of most of the tannins during germination^{10,24}.

Peaks of diastatic activities were recorded on days 4 (white) and 6 (red and SK₅₉₁₂) and may be due to structural and varietal differences. The white variety had the lowest activity which may be due to the effect of kilning⁴⁴ or it may be due to inhibition by tannin compounds which increase during malting²⁴. The highest diastatic activity recorded for the red malt tends to corroborate the results of Glennie²⁴ that bird-resistant sorghums have high diastatic power.

The results (Fig. 2) indicate that SK₅₉₁₂ had the highest α -amylase activities with the peak on day 6. The red and white malts had peak α -amylase activities on days 4 and 5 respectively. Difference in peak days of α -amylase activity may be attributed to the grain structure or to the differential rate of synthesis of the enzyme by the embryo^{3,48}. The higher α -amylase activity of the white malts over the red may therefore be due to a lower malting loss value.

β -Amylase activities as shown in Fig. 2 indicate that it occurs in unmalted sorghum grains but this is not in agreement with the results of Dyer and Novellie¹⁴ and Okon and

Uwaifo⁴⁴. In the malts, SK₅₉₁₂ had the highest activity with the peak on day 6. The red and white malts had β -amylase peaks on day 5 as was reported by Okon and Uwaifo⁴⁵ for some improved varieties of sorghum. The difference in peak days may be due to the differential rate of synthesis of cysteine proteases responsible for their activation^{23,26,27,33} or due to differences in malting loss⁴⁵. The higher β -amylase activity in the red compared to white malts may be the result of a more heat-stable cysteine protease or the presence of a higher content of heat-stable β -amylase in the inactive state in the unmalted grain. The lower levels of β -amylase activity towards the end of the malting period may be due to increasing malting loss or the presence of more heat-labile isoforms. β -amylase is quite heat-labile and some activity is lost during kilning³⁴. Malt β -amylase enzymes are more heat-labile than barley enzymes. The "body" of burukutu, which is preferably produced from red sorghum malt may therefore be the result of inadequate starch conversion, which is sub-optimal below 80°C³⁴.

Changes in lipase activity during sorghum malting and fermentation for burukutu production

Lipase activity decreased during steeping and subsequently increased during germination (Fig. 3). The decrease during steeping may be due to leaching and indicates that sorghum contains lipase in the mature seed⁴² as was reported for castor oil bean²⁸ where it is present in an inert form and in barley aleurone layers as the "storage" form in association with protein bodies³⁶. The decrease in activity is consistent with the results of Macleod and White³⁸ but differ from that of Nwanguma et al⁴² who reported a slight increase in activity. This difference in the

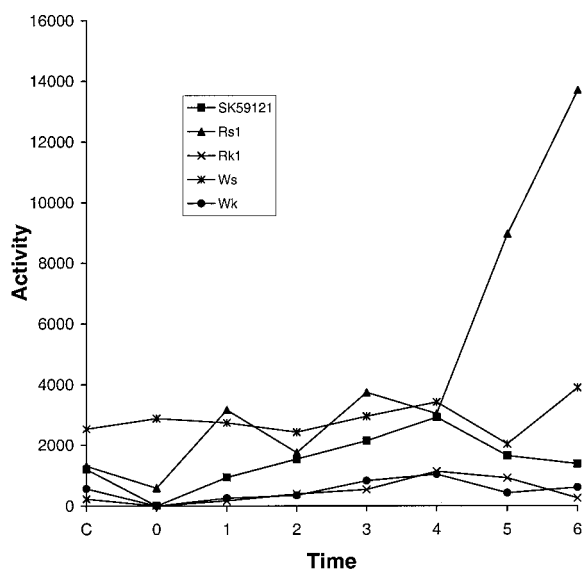


FIG. 3. Changes in lipase activities during malting. SK₅₉₁₂, Rs, Rk, Ws and Wk – lipase activities of SK₅₉₁₂, sun-dried red, kilned (50°C) red, sun-dried white and kilned (50°C) white malts. Lipase activities are the means of three replicate determinations.

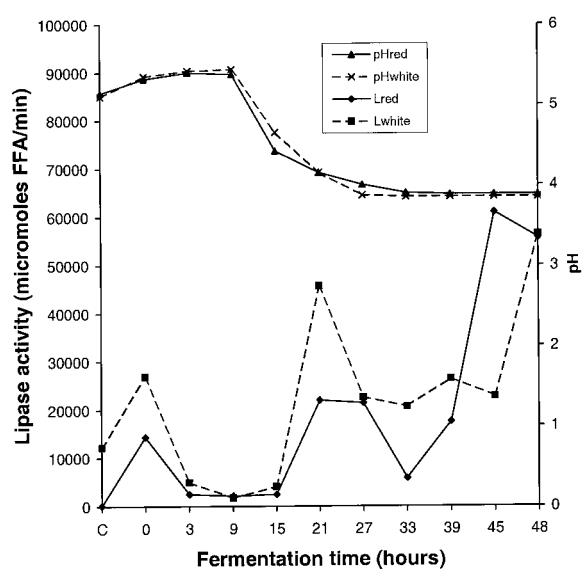


FIG. 4. Changes in lipase activities during fermentation of 5-day sorghum malts. pH_{red}, pH_{white} – pH of the kilned (50°C) red and white malts; L_{red}, L_{white} – lipase activities of the kilned (50°C) red and white malts. Lipase activities and pH are the means of three replicate determinations.

results may be due to differences in variety, malting methods, and the presence of different levels of endogenous regulators of lipase activity.

The increase in lipase activity during germination is probably due to *de novo* synthesis in the aleurone⁵⁸, scutellum⁷ or to oleosin activation²⁸. The increased lipase activity is consistent with the result of Nwanguma et al⁴² as has also been observed in barley^{21,38}, corn³⁷ and wheat⁵⁴. With the exception of red malts dried under the sun in which the lipase activity peak was on day 6, maximum lipase activity was observed on day 4. Sun-dried malts had higher activities than 50°C dried malts, which may be due to less inactivation at the lower drying temperatures under the sun (26 ± 2°C). In the 50°C dried malts, the higher activity in SK₅₉₁₂ may be due to induction resulting possibly from a higher oil content since enzymes are synthesised in proportion to the available substrate or anchorage sites on the oil body membrane^{57,59}.

The red malts had higher lipase activity than the corresponding white malts. This suggests that the red malts have a higher oil content^{57,59}, or it may be due to oleosin activation²⁸ and the presence of different levels of endogenous regulators of lipase activity. Drying at 50°C had a far greater effect on the red lipase activity especially toward the end of the malting period and may be due to a higher free space potential in the white malts. Malt enzymes are known to be more heat-labile than those from the grain. There is also the possibility of the presence of lipase activities having different stabilities to heat⁴², with the red malts having more heat labile isoforms towards the end of malting.

During fermentation for burukutu production (Fig. 4), the pH increased slightly (from 5.35 to 5.50) during the first 9 h and the increase may be due to the leaching of materials including tannins into the fermentation medium^{10,24}. Thereafter, the pH decreased sharply between 9 h and 27 h and more gradually between 27 h and 48 h. The pH decreases could be due to the production of organic acids and changes in the basic constituents of the medium¹¹. The pH decreased from 5.33 and 5.35 and stabilized at 3.88 and 3.85 for the red and white malts respectively as from 39 h. Coote and Kirsop¹¹ attributed the decreasing rate of pH change during active fermentation to the conversion of [H⁺] to its negative logarithm (pH), the consequence of which is that progressively greater quantities of hydrogen ions are required to decrease pH as the pH value of fermenting wort falls. The apparent small differences between the white and red fermenting liquors especially towards the end of fermentation may therefore be due to the use of pH instead of [H⁺].

Lipase activity was detected throughout the 48 h of fermentation. This suggests that malt lipase from native sorghum grains is active within the acidic range, in contrast to the activity in the improved varieties which lie in the alkaline and near alkaline range²⁵. Three main activity peaks were detected in the red liquor at the 0, 21 and 45 h at which the pH values were 5.32, 4.15 and 3.88, and at 0, 21 and 48 h at which the pH values were 5.35, 4.15 and 3.85 in the white liquor. Lipase activity at 0 h and 21 h was higher in the white and suggests the presence of the less inhibitory polyphenolic compounds or a faster rate of metabolism of the tannin/polyphenol concentration. To-

wards the end of fermentation, the rate of metabolism may have slowed down possibly as a result of the unfavourable environment, which may have exerted a more inhibitory effect on the activity of lipase from white sorghum malts. The higher peak of lipase activity some 3 h earlier (45 h vs 48 h) suggests some modification of tannins in the red. Low pH is known to precipitate wort tannins and they may have been inactivated earlier in the red compared to the white gruel.

In contrast, the red malt had a higher peak of activity at the 45 h which tends to support the fact that the inhibitory tannins may have been lost or precipitated during fermentation and that the red lipase is a more acidic isoform. This may explain the short shelf life of an otherwise acidic product which is primarily made from red malts in Nigeria. The peak of activity towards the end of fermentation is at variance with the results of Nwanguma et al⁴², which indicated that sorghum malt lipase activities decreased at the low pH conditions prevalent towards the end of fermentation of barley malt used for beer production. This difference may be attributed to differences in raw materials and methods of fermentation for the production of lager beer and "burukutu beer".

Lipase activity during fermentation was higher than during malting suggesting that dispersion into water before the assay improved the activity. This suggests that the larger the water-oil interface for lipase activity, the higher the number of molecules of TAGs transformed and that the tannins in the malt do not significantly inhibit lipase activity. This seems to explain the higher lipase activities obtained when the volume of the extracting medium (0.1M phosphoric acid) was increased from 3 mL to 18 mL.

CONCLUSIONS

Burukutu fermentation is of the acidic type. The low pH achieved and the tannins and tannin precursors did not inhibit malt lipase activities under the conditions of fermentation for *burukutu* production. Sorghum malt lipase may be made up of three acidic isoforms, the activities of which contribute to the short shelf life of the product.

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APPENDIX 1

Lipase activity was derived by calculation using the formulae:

$$\text{For the acetone powder preparation: } \frac{\text{vol. of NaOH used in titration} \times 10^5 \text{ micromoles} \times 0.5 \times 18}{180 \times 3}$$

$$\text{For the fermenting gruel: } \frac{\text{vol. of NaOH used in titration} \times 10^5 \text{ micromoles} \times 1.125 \times 18}{180 \times 3}$$