Sorghum β-Amylase Production: Relationship With Grain Cultivar, Steep Regime, Steep Liquor Composition and Kilning Temperature

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ABSTRACT

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Steep regime, nature of alkaline liquor, and kilning conditions were studied for their effects on sorghum malt β-amylase development in four Nigerian sorghum cultivars. Malt β-amylase activity was markedly ($p < .001$) influenced by all the four factors as well as their various interactions. Overall, malts from KSV 8 variety were the most β-amylolytic followed in sequence by those from Local Red (LR), SK 5912, and Local white (LW) respectively. The presence or absence of air rests in steep regimes was a significant ($p < .001$) determinant of sorghum β-amylase response to final warm steeping, steep liquor and kilning condition. The nature of the alkaline steep liquor was also a major determinant of the pattern of malt β-amylase response to the kilning condition. Steeping in $Ca(OH)_2$ enhanced malt β-amylase activity at the higher temperature of kilning, while KOH produced the opposite effect. $Ca(OH)_2$ enhancement of β-amylase development, at a kilning temperature of 50°C, was variety-dependent suggesting that different sorghum cultivars may employ different biosynthesis models for β-amylase synthesis. The regime-dependence of β-amylase response to kiln temperature suggests that this was an important modulator of sorghum germination physiology.

Key words: Alkaline liquor, β-amylase, kilning temperature, sorghum malt, steeping regime, cultivars.

INTRODUCTION

β-Amylase is the key saccharifying enzyme of the brewers' malt[22]. While the low β-amylase activity of sorghum malts has been defined as one of the most serious obstacles to its use as replacement for barley malt[2], results of recent studies using some improved varieties of the grain suggest that the production of high β-amylase-containing sorghum malts may be possible with the proper choice of variety and conditions of malting[9,10,25-27].

Recent research has shown that sorghum malt α-amylase level is critically correlated with environmental conditions of steeping and kilning[24]. While some work has been conducted on the effects of environmental conditions of steeping on sorghum malt β-amylase levels, very few data are available with respect to the effects of kilning conditions on the development of the sorghum enzyme[29].

In this communication, we present the effects of kilning conditions on the β-amylase activity of sorghum malts prepared from different cultivars, and under varying conditions of steeping including dissimilar steep liquors as well as different steeping regimes.

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MATERIALS AND METHODS

Sources of sorghum cultivars

Cultivars SK 5912 and KSV 8 were improved varieties obtained from the National Seeds Service, Zaria, Nigeria. Grains of the local white (LW) and the local red (LR) varieties were purchased from the open market at Nsukka, Nigeria. Germinative energies were above 95% for all the grains and none was water-sensitive.

Sorghum malting

The grains were sorted manually to remove broken kernels and debris. Alkaline steeping was carried out using glass-distilled water containing 0.1% of the alkali (NaOH, KOH, or $Ca(OH)_2$). Double glass-distilled water served as the control liquor. Steeping was by the four different regimes presented below:

Steep regime I. Grains were steeped at 30°C for 51 hours using the schedule; 6 h wet: 3 h dry (×5) followed by a final wet period of 6 h at 30°C.

Steep regime II. Same as regime I, except that the final wet period of 6 h was maintained at 40°C.

Steep regime III. Grains were steeped with liquor changes at the 9th, 18th, 27th, 36th, and 45th hours. Like regime I, the final wet period was maintained at 30°C.

Steep regime IV. As regime III. The final wet period of 6 h was however maintained at 40°C.

Before and after steeping, grains were sterilized by immersion for 20 min in NaOCl solution with 1% available chlorine. These were subsequently rinsed with sterile distilled water as described elsewhere[9]. Germination was carried out in the dark, in shallow trays with fine mesh bottoms in an atmosphere of near water saturation at 30°C for four days. Thereafter, grains were processed for analysis as described previously[9].

Kilning conditions

The effects of kilning conditions were studied by drying the green malts at either 40°C or 50°C for 24 h. Kiln controls were prepared by drying milled green malts in anhydrous acetone.

Assay of β-amylase activity

β-Amylase extraction and assay were as outlined in Sun and Henson[32], except that the assay medium contained 10 mM ethylene di-amine tetra-acetic acid (EDTA) and the maltopentaose substrate (1% final concentration) was reduced[1,13] prior to use. All other conditions were as in Sun and Henson[32]. Reducing sugar was quantitated by the Nelson and Somogyi method[21]. Enzyme activities are expressed in μg glucose equivalents per minute per milliliter of dialysed enzyme extract.

Statistical analyses

All data were subjected to the four-way analysis of variance tests as described by Cohen[5]. Means that differed significantly were identified by the least significant difference (Lsd) method[5].

RESULTS AND DISCUSSION

Sorghum variety and β-amylase activity

Malting conditions and grain variety were studied in relation to their effects on the β-amylase activity of malts from the four sorghum cultivars. All the factors under study, in all their possible combinations, significantly ($p < .001$) influenced sorghum malt β-amylase activity development (Table I). As has earlier been shown for sorghum α-amylase[24], β-amylase activity development was markedly ($p < .001$) affected by grain cultivar. Mean average sorghum malt β-amylase activities for KSV 8, LR, SK 5912 and LW were 526.6, 469.52, 458.1, and 433.0 units respectively indicating KSV 8 malts as being by far ($p < .001$) the most β-amylolytic. Similarly, malts of the LW variety gave the poorest overall performance while mean average β-amylase activity values were statistically ($p > .1$) the same for SK 5912 and LR.

Varietal factors are important determinants of the patterns of enzyme development during cereal grain malting[3,4,6,9,10,11]. Earlier reports[7,15,23] on sorghum malt β-amylase activity development have also clearly suggested the existence of a possible relationship between the genetic make-up of the sorghum grain and its β-amylase activity. Data obtained in this work agree with those earlier views.

Kilning temperature and sorghum β-amylase activity

β-Amylase activity was significantly influenced ($p < .001$) by the conditions of kilning, irrespective of grain variety. With a mean amylase activity of 560.5 units, kilning at 40°C supported markedly ($p < .001$) more β-amylase activity development compared to drying with either acetone (459.0 units) or at 50°C (448.3 units) (Table I). Unlike earlier observations[24] in similar studies for sorghum α-amylase activity, mean average β-amylase activity was statistically similar ($p > .1$) for both acetone and 50°C drying.

β-Amylase response to kilning conditions was also influenced ($p < .001$) by sorghum variety. All the malts, except those made from LW, were significantly ($p < .005$) more β-amylolytic when kilned at 40°C than their kiln control (acetone-dried) counterparts. All 40°C-treated malts were also better ($p < .001$) than the 50°C-dried products except those made from KSV 8 for which similar ($p > .1$) enzyme values were obtained suggesting identical β-amylase potentials. The highest β-amylase activity of all the cultivars at 50 and 40°C was given by KSV 8. Malts made

TABLE I. Effect of variety, steeping regime, steep liquor chemical composition and conditions of kilning on sorghum malt β-amylase activity.

		Sorghum malt β-amylase activity (μg glucose equivalents)											
		Cultivar											
		SK 5912			KSV 8			LW			LR		
	Steep liquor	Ac	40°C	50°C	Ac	40°C	50°C	Ac	40°C	50°C	Ac	40°C	50°C
Reg I	NaOH	345.4	465.0	411.9	624.4	531.4	930.0	531.4	385.3	451.7	504.9	611.4	597.9
	KOH	425.1	106.3	425.1	465.0	93.0	425.1	385.3	612.4	149.6	531.4	465.0	504.9
	$Ca(OH)_2$	438.4	651.0	899.4	504.9	225.9	89.1	518.4	518.1	611.1	511.3	584.6	624.4
	H_2O	239.1	318.9	465.0	225.9	544.7	577.9	513.6	149.6	116.9	305.6	438.3	455.9
Reg II	NaOH	114.3	518.1	690.4	544.7	385.3	690.9	1391.9	757.3	106.3	611.1	677.6	49.6
	KOH	110.6	1182.4	252.4	491.6	1594.3	826.4	418.5	372.0	232.5	542.8	833.7	397.1
	$Ca(OH)_2$	338.8	896.8	677.6	531.4	225.9	677.6	451.7	358.7	520.1	372.0	504.9	584.6
	H_2O	790.5	644.3	451.1	524.8	1926.4	797.1	458.4	372.0	156.7	524.8	504.9	126.2
Reg III	NaOH	551.6	332.1	332.1	551.6	491.6	491.5	551.6	178.9	179.8	551.6	166.4	332.1
	KOH	511.5	318.9	104.4	279.0	319.9	332.1	465.0	451.7	318.9	465.8	465.0	212.6
	$Ca(OH)_2$	465.0	717.4	358.7	462.0	358.7	649.6	438.4	305.2	292.3	438.4	345.4	531.4
	H_2O	385.3	358.7	332.1	451.1	518.1	385.3	478.3	785.3	565.7	478.3	584.6	565.7
Reg IV	NaOH	504.9	491.5	398.6	345.4	345.4	478.3	594.9	724.1	398.9	491.6	491.6	478.3
	KOH	343.4	518.1	308.6	664.3	345.4	872.9	239.6	724.1	149.6	132.9	533.4	504.9
	$Ca(OH)_2$	478.3	358.7	584.6	597.9	54.7	345.4	318.9	544.7	607.2	358.7	358.7	318.9
	H_2O	398.6	135.1	843.6	597.9	318.9	100.3	531.4	531.2	564.6	451.7	850.3	564.6

Results are presented as means of triplicate experiments.
Ac = Acetone drying.
40°C, 50°C = kilning temperatures.

from LW were the most amylolytic with acetone drying followed by those of KSV 8, LR and SK 5912 in that sequence.

Mean average β-amylase values for SK 5912 and KSV 8 malts were significantly ($p < .001$) higher at 40 and 50°C kilning temperatures than with acetone drying. On the other hand, mean enzyme activities with acetone were far ($p < .001$) in excess of values at 50°C, and 40 and 50°C for LR and LW malts respectively. The above differences in mean malt β-amylase response to kilning conditions probably reflect differences in the physiological behaviours of the varieties. This agrees well with earlier observations on sorghum malt β-amylase response to conditions of malting[9,34]. Apparently, conditions of kilning are important determinants of the β-amylase potentials of sorghum grains irrespective of the grain variety as strongly suggested by the large within variety differences in sorghum malt β-amylase activity.

Steep liquor chemical composition and malt β-amylase activity

Mean sorghum malt β-amylase activity varied markedly ($p < .001$) between steep liquors (Table II). With alkaline steeping, the chemical nature of the steeping agent was an important determinant of the pattern of β-amylase development. Markedly ($p < .001$) less β-amylase was given by steeping in KOH compared to either of the two other alkaline liquors, NaOH or $Ca(OH)_2$. Although the mean malt β-amylase levels for NaOH steeps were higher than those for $Ca(OH)_2$, the lack of statistically significant differences at $p = 0.1$ suggests sorghum malt performance was similar with the two steep liquors. Compared to the distilled water controls, only KOH, out of all the alkaline liquors, showed any overall suppression of β-amylase development. In contrast, mean β-amylase activity was significantly improved by steeping in NaOH, while figures for $Ca(OH)_2$ steeping were statistically similar to those given by steep liquor (distilled water) controls. The present enhancements in β-amylase activity by steeping in NaOH, when compared to the earlier reported[24] suppression of α-amylase production by the same liquor and under similar conditions of malting, suggest that the two enzymes are produced/secreted by entirely different physiological processes in sorghum. In contrast marked suppression, in this study, of β-amylase production by KOH agrees quite well with earlier findings[11,24].

Grain cultivar significantly ($p < .001$) modulated the patterns of sorghum β-amylase response to steep liquor. In three of the varieties (SK 5912, KSV 8, and LW), significant ($p < .01$ to $p < .001$) differences in β-amylase potential were engendered by steeping in liquors that varied in their chemical composition. In contrast, β-amylase activity in LR seemed unaffected by steep liquor chemical composition. Further variety-dependent differences in malt β-amylase response to steep liquor composition were also noted. For example, steeping in $Ca(OH)_2$, distilled water and NaOH were optimal for β-amylase development in SK 5912, KSV 8, and LW respectively. Similarly alkaline steeping was, notwithstanding the steeping agent, beneficial to β-amylase development in LW contrasting with KSV 8 in which, mean average malt β-amylase activity for alkaline-steeped grains (508.4 units) was significantly ($p < .001$) less than the mean values for steep liquor controls (573.3 units). This suggests a possible suppression of β-amylase production in KSV 8 by alkaline steeping. A look at the mean KSV 8 β-amylase values for each alkaline steep liquor however shows this to be true only for the $Ca(OH)_2$ liquor (Table II), levels of β-amylase engendered by NaOH and KOH steeps being statistically similar ($p > .1$) to those for steep liquor controls in KSV 8. NaOH and KOH were also significantly ($p < .001$) better steeping agents for β-amylase development in KSV 8 than $Ca(OH)_2$. In contrast to KSV 8, the use of $Ca(OH)_2$ steeps was highly ($p < .001$) beneficial to β-amylase development in SK 5912, giving 28.0, 33.1 and 49.1% more β-amylase than distilled water, NaOH and KOH steeps respectively. Overall, steeping in alkaline liquor caused improvements in malt β-amylase potential only in SK 5912 ($Ca(OH)_2$) and LW ($Ca(OH)_2$ and NaOH). Significant suppression of β-amylase activity occurred with KOH (SK 5912) and $Ca(OH)_2$ (KSV 8). Apparently, β-amylase development in LR was insensitive to steep liquor chemical composition.

β-Amylase response to steep liquor was markedly ($p < .001$) influenced by the conditions of sorghum malt drying (Table II). The reverse was also true for the effects of drying conditions on malt β-amylase levels. However, no single kiln treatment was found to be generally most favourable for β-amylase production with all the steep liquors. This finding contrasts clearly with earlier observations made in our laboratory[24] on α-amylase development in sorghum grains malted under similar conditions, and for which, kilning at 40°C was highly optimal irrespective of the chemical composition of the steep liquor. Kilning at 40°C was nevertheless most favourable for β-amylase development with distilled water and KOH as steep liquors (Table II). On the other hand, drying at 50°C best suited β-amylase development in $Ca(OH)_2$-treated grains while acetone drying was optimal with NaOH steeping. Drying at either 40 or 50°C was significantly ($P < .001$)

TABLE II. Mean malt β-amylase activity as influenced by steep liquor, grain variety and kilning temperature and their interactions.

Grain cultivar	Steep liquor	Malt β-amylase activity (µg glucose equivalent) by kilning condition		
		Ac	50°C	40°C
SK 5912	NaOH	379.0	458.3	451.7
	KOH	347.7	271.9	531.5
	$Ca(OH)_2$	430.1	630.1	656.0
	Dist. water	453.4	523.0	364.3
KSV 8	NaOH	516.5	647.7	430.9
	KOH	475.0	614.1	587.9
	$Ca(OH)_2$	524.0	440.4	338.8
	Dist. water	425.1	467.7	827.0
LW	NaOH	767.5	333.8	504.9
	KOH	377.2	212.7	540.2
	$Ca(OH)_2$	431.9	510.2	431.7
	Dist. water	401	351.0	334.5
LR	NaOH	540.0	364.6	486.8
	KOH	418.1	404.9	574.3
	$Ca(OH)_2$	420.1	514.8	448.4
	Dist. water	440.1	428.1	594.5

Results are presented as means of triplicate experiments.
Ac = Acetone drying, 40°C, 50°C = kilning temperatures.

suppressive of β-amylase development in NaOH-steeped grains of sorghum, giving respectively 14.9 and 18.0% less β-amylase activity than the corresponding acetone-dried controls. With all the other steep liquors, drying at 40°C gave between 4 and 45% more β-amylase activity than was obtained with the corresponding green malts. Thus, the chemical composition of the steep liquor is an important determinant of the mode in which sorghum malt β-amylase activity responds to the conditions of drying. Similar steep liquor-dependent differences in sorghum malt β-amylase response to kilning at 50°C were also noted. However, only the $Ca(OH)_2$- and distilled water-steeped malts displayed any enhancement in mean malt β-amylase activity at that kilning temperature when compared to the corresponding green (acetone-dried) malts.

We have, in an earlier report[24], argued that profound differences in the nature of the physiological stress engendered in sorghum grains by varying steep liquors could be responsible for the wide-ranging differences in enzyme potentials observed when they are malted using conditions similar to the ones described in the present article. While confirming those views, data from the present work further highlight the possibility of increasing sorghum malt β-amylase potentials through proper choice of steeping liquor and kilning regimes. Overall, the mean β-amylase activity for acetone-dried malts was higher ($P < .001$) with alkaline steeping than with distilled water. This was also true for 50°C-dried malts but markedly different at 40°C kiln temperature where significantly ($P < .001$) more β-amylase was given by distilled water liquor. Generally, the highest malt β-amylase development, for both alkaline steeping and the distilled water controls, was given by kilning at 40°C. However, the malts were least β-amylolytic with acetone drying for distilled water steep controls, while the lowest overall β-amylase activity of alkaline-steeped grains was obtained at 50°C for all the kilning conditions investigated in the present work. This suggests that the chemical nature of steep liquors was a significant influence on the extents to which β-amylase development in germinating sorghum grains responded to the conditions of drying.

Some increases in enzyme activity have been reported to occur during the kilning phase of cereal malt production, its extent being controlled by (a) the length of the kilning process, and (b) the initial air-on temperature[18]. The significantly ($p < .001$) higher β-amylase activity given at 40°C kilning temperature, if compared to values at 50°C, suggests the possible development of markedly more enzyme during the kilning process at the lower temperature, as well as a possibly higher loss of enzyme activity at the higher temperature of drying, most likely due to faster kilning phase transition to the enzyme-denaturing phase[18]. Higher air-on temperatures during kilning have been linked to faster synthetic–to–curing phase transitions[18,24]. It is thus likely that a faster onset of the malt curing phase and a subsequent lengthening of that phase at the higher drying temperature may have been additionally responsible for the lowered β-amylase activity at 50°C. Acetone drying is expected to cause an abrupt termination of all enzyme development in treated grains. This shortening in the phase of enzyme development may explain the markedly ($p < .001$) lower β-amylase values associated with the green (acetone-dried) malts in comparison to values for corresponding malts dried at 40°C. On the other hand, post-translational activation by proteases has been associated with improved β-amylase activity during cereal grain malting[12,13,16,31,35]. Thus in addition to probable *de novo* β-amylase synthesis[17], possible proteolytic activation of β-amylase zymogens during the enzymic phase of malt kilning[17] may in part account for the higher β-amylase levels observed in 40°C-dried grains as compared to values for the green malts. β-Amylase activity of the acetone-dried malts was however markedly higher than mean values at 40°C for NaOH-steeped grains. This suggests that steeping in NaOH caused reductions in rates of β-amylase development during kilning to values lower than those for enzyme inactivation. It is additionally likely that β-amylase engendered by NaOH steeping was more sensitive to heat inactivation than the enzyme whose production was stimulated by steeping in the other liquors. Alternatively there is the likelihood that steeping in NaOH caused reductions in rates of sorghum β-amylase zymogen activation by endo-proteases. This in turn could have led to significant reductions in malt β-amylase activity during kilning especially if proteolytic activation was a key contributor to the overall enzyme activity of the grain. Irrespective of the reasons for this fall in β-amylase activity in 40°C-dried grains, the relationship between the chemical composition of the steep liquor and the quality and amounts of β-amylase produced by sorghum grains remains quite evident.

Cereal malt β-amylases are known to be neither thermostable nor to require Ca^{2+} ions for optimal activity or temperature stability[14]. $Ca(OH)_2$-steeped grains, gave higher β-amylase activity after kilning at 50°C than they did at 40°C suggesting the possibility that significantly ($p < .025$) more enzyme could have developed or been activated during kilning at the higher temperature, or that malt β-amylases produced during the synthetic phase of kilning at 50°C in $Ca(OH)_2$-treated malts were of a more temperature-resistant variety. While earlier works have reported heterogeneity in sorghum β-amylases[28,34] there is not, to the best of our knowledge, any mention of thermo-stable cereal β-amylases in the literature. A third explanation for the above phenomenon would have been that β-amylase extracts were possibly contaminated with α-amylase. However that possibility is abolished by the specificity of the β-amylase assay procedure employed in this work which, in addition to the use of the β-amylase-specific α-limit dextrin substrate also employs EDTA[13,30] to inhibit all possible α-amylase activity in the assay mixture. Rather the probability that sorghum βamylase exist in a zymogen form possibly activated by Ca^{2+}-requiring malt endoproteases[20], which are probably stabilized by the cation and ostensibly more active at the higher kilning temperature of 50°C, seems possible. While the activation of β-amylase in barley has been associated with cysteine proteinases, it is however possible that similar enzyme activation in sorghum be due to the mediation of metallo-enzymes. Earlier works have shown that cereal grains could differ significantly in their modes of β-amylase development[17,19]. In that sense the fact, that the presence of Ca^{2+} has been associated with significantly improved β-amylase synthesis in maize kernels[8,17], becomes quite instructive. It is known that proteolysis occurs optimally at 45 to 50°C in cereal malts[33]. Higher rates of β-amylase zymogen activation by processing proteases during kilning would be expected to have the same effect as that of increased β-amylase synthesis. If the overall rate of malt β-amylase development is higher than that of its inactivation, a net increase in the enzyme levels will be established at the end of the kilning process.

Malt β-amylase response to the interactions of steep liquor and kilning temperature was strongly ($p < .001$) influenced by sorghum grain variety (Table II). With NaOH liquor, enzyme activities at 40 and 50°C kilning temperatures were higher than activities in corresponding green (acetone-dried) malts only when the sorghum grains were of the SK 5912 variety. Conversely, mean average enzyme activity levels at 40 and 50°C, for the three other varieties, were significantly ($p < .001$) lower than values for their corresponding green malts signifying that rates of loss in β-amylase activity during kilning were probably higher than the rates of its development under those kilning conditions. NaOH-steeped malts of LW and LR varieties prepared by kilning at 50°C were also significantly ($p < .001$) less β-amylolytic than their counterparts dried at 40°C. In contrast, the mean average β-amylase activity for 50°C-dried, NaOH-steeped grains of KSV 8 was higher than values obtained at 40°C and with acetone drying. Variety-dependent differences in malt β-amylase response to steep liquor and kilning conditions were also noted with KOH. With that agent, patterns of enzyme response contrasted with the trends earlier observed with NaOH. Mean average malt β-amylase activity at 40°C kilning temperature was, irrespective of grain cultivar significantly higher than values for corresponding green malts. Also, in comparison to their corresponding green malts, significantly ($p < .001$) more β-amylase was engendered by drying KOH-steeped KSV 8 grains at 50°C therefore contrasting with results from SK 5912 and LW in which, the combination of KOH steeping and drying at 50°C resulted in respectively 21.6 and 43.6% less enzyme compared to the corresponding green malts. Mean average β-amylase activity for KOH-steeped LR malts was statistically ($p > .1$) similar to that for matching green malts. Compared to acetone drying, kilning at 40°C, for $Ca(OH)_2$-steeped grains, was significantly ($p < .001$) inhibitory to β-amylase development. This was found also to be true for KSV 8 and the local red (LR) varieties. The reverse however occurred with SK 5912 (Table II) in which malts, a substantial proportion of total β-amylase activity appeared to develop during the kilning phase. The most dramatic effect of $Ca(OH)_2$ steeping on the response of malt β-amylase activity to drying temperature was however observed at 50°C. At that temperature of drying, the β-amylase activities of all $Ca(OH)_2$-steeped malts, except those from SK 5912, were significantly ($p < .001$) higher than values at 40°C. This could probably be due to enhancements in rates of zymogen activation at the higher kiln temperature, the possible development of more thermostable forms of the enzyme during the kilning process or to both. The fact that malt β-amylase activity at 40°C, for those varieties, was generally less than values for kiln controls strongly supports the argument of zymogen as possible reason for improved β-amylase activity after kilning at 50°C for $Ca(OH)_2$-steeped malts. The possibility that sorghum malt β-amylase may exist in both processed and unprocessed forms agrees quite well with earlier reports of multiple β-amylase isoforms in sorghum malts[28,34]. The $Ca(OH)_2$ malts of SK 5912 dried at 50°C were, like their 40°C-dried counterparts, significantly ($p < .001$) more β-amylolytic than their kiln controls, even though their mean enzyme activity was also significantly ($p < .001$) lower than that for the former.

With distilled water as steep liquor, mean β-amylase activity for KSV 8, LW and LR were highest at 40°C, sharply contrasting with the trend in SK 5912 where kilning at the above temperature engendered the lowest enzyme activity value for distilled water steeps. These results suggest that the heightening of malt β-amylase levels after kilning is probably more due to enhanced development of the enzyme during the drying process for grains steeped with NaOH, KOH or distilled water while possible proteolytic activation may be responsible for the enhanced post-kilning phase β-amylase activity of $Ca(OH)_2$-steeped grains. The fact that the patterns of β-amylase development differed significantly with each combination of the three factors of cultivar, liquor and kiln temperature suggests that each affected grain physiology in a peculiar manner.

Steep regime and sorghum malt β-amylase activity

Mean average malt β-amylase levels were significantly ($p < .001$) influenced by the steep regime. Differences between the mean malt β-amylase activities for individual steep regimes were found to be statistically very high even at levels as high as $p = .005$ and $p = .001$. By far ($p < .001$) the highest mean malt β-amylase activity was engendered by steep regime II (air rest with final warm steeping). This was followed in performance by malts made with regimes IV (continuous with final warm steep), I and III (air rest and continuous steeping respectively) in that order. Attempts were also made to compare the mean malt β-amylase performance of air rested grains to that for the continuously steeped malts. At a value of 437.3 units, the mean malt β-amylase of the latter was significantly ($p < .001$) lower than the value of 501.9 units given by the air rested grains. This suggests that steep aeration was beneficial to the development of β-amylase in sorghum during malting, thus agreeing with earlier reports associating air resting with improved sorghum malt β-amylase development[9,10].

The pattern of sorghum malt β-amylase development, with each regime, was investigated as regards the influence of each of the other factors (Table III). Regime-to-regime, mean malt β-amylase activity varied strikingly ($p < .001$) in relation to the chemical nature of the steep liquor. For example, the mean malt β-amylase activity with regime I at 539.3, 382.4, 514.7 and 312.8 units respectively for NaOH, KOH, $Ca(OH)_2$ and distilled water indicates how the nature of the steep liquor defined sorghum malt β-amylase productivity for each of the steep regimes. While similar steep liquor-dependent differences were also noted for each of the other regimes, steeping sorghum with a combination of air rests and a final warm steep (Steep Regime II) appeared optimal for β-amylase production irrespective of steep liquor and notwithstanding the existence of large ($p < .001$) liquor-to-liquor variations in enzyme activity values. Similarly, steeping continuously without final warm steeping (i.e. steep regime III) caused the poorest malt β-amylase performance for all the steep liquors except distilled water, for which, the lowest β-amylase activity was given by regime I (air rest without warm steeping). Like regimes II- and III-malted grains, the β-amylase activity of regimes IV- and I-steeped grains varied strikingly ($p < .005$ to $p < .001$) in relation to steep liquor composition. Interestingly, final warm steeping was generally beneficial to β-amylase development irrespective of steep liquor and regardless of whether the grain was steeped continuously or discontinuously. For instance, the mean β-amylase value by continuous steeping improved by over 5, 25 and 26% of its value after the final warm steeping of distilled water-, NaOH- and KOH-treated grains respectively. With air resting, an even more dramatic improvement in amylase values of over 58 and 90% was noted respectively for KOH- and distilled water-steeped malts. In comparison with results given at regime IV by NaOH-steeped grains, the application of final warm steeping to the air rested grains provided only a marginal increase of less than 1% in β-amylase activity. Both air rested and continuously steeped, $Ca(OH)_2$-malted grains were similar ($p > .1$) in their β-amylase activity values notwithstanding final warm steep treatment. This suggests that β-amylase production, in $Ca(OH)_2$-steeped grains, was insensitive to final warm steeping. Steeping in NaOH was optimal for β-amylase development with regimes I and IV. With regimes II and III, maximum enzyme activity was obtained with distilled water. Steeping in KOH provided the least β-amylase values with most steep regimes (except regime II). Differences such as the ones mentioned above confirm our earlier observations[24] on the modulation of sorghum malt enzymes' responses to steeping regime by the chemical composition of steep liquors.

Regime II provided the highest β-amylase activity for most of the steep liquors. Nevertheless, very distinct ($p < .001$) within-liquor differences in enzyme activity were still noted in relation to steep regime. For instance mean β-amylase values with KOH were 382.4, 604.5, 353.6 and 444.6 units respectively for regimes I, II, III, and IV. The order of β-amylase development with the other liquors were as follows; Regime II > Regime IV > Regime III > Regime I (distilled water liquor); Regime II > Regime IV > Regime I > Regime III (NaOH); and Regime I = Regime II > Regime IV = Regime III ($Ca(OH)_2$). Wide-ranging variations in enzyme values occurred in relation to steep regime, thus confirming that steep regime significantly influenced malt β-amylase response to steep liquor composition.

Compared to the corresponding steep liquor controls, all the alkaline liquors (except KOH) were generally more beneficial to β-amylase development in regime I-malted sorghum. This trend was however not replicated with the other steep regimes. For example, at steep regime III, malts made with the alkaline liquors, with the exception of $Ca(OH)_2$, were significantly ($p < .001$) less β-amylolytic than their corresponding distilled water controls. Similar results were also noted with steep regimes II and IV with the possible exception that, with regime II, enzyme activity was similar ($p > .1$) between control malts and KOH malts. Malts made from NaOH steeps were also more β-amylolytic than distilled water controls at regime IV. Overall, the lowest mean β-amylase performance of all the alkaline steeps, for each of the regimes (except regime II) was associated with the KOH liquor. This strongly reinforces the views that differences in alkaline liquor composition may have wide-ranging effects on the physiological behaviour of germinating sorghum seeds.

We also evaluated the effects of steeping regime on malt β-amylase response to drying condition (Table III). β-Amylase response to the conditions of drying was markedly ($p < .001$) influenced by the nature of the steep regime employed, very often, leading to significant ($p < .01$ to $p < .001$) within-kiln temperature differences in the malt enzyme levels. Combining air rests and final warm steeps was beneficial to β-amylase development in acetone-dried malts for which, markedly ($p < .001$) more activity was observed compared to matching malts from steep regimes III, IV and I. Statistically, differences in amylase potentials were established at $p = .025$ for regimes III and IV, and regimes III and I malts but not for those from regimes IV and I ($p > .1$). Steep regime-determined within-kilning temperature variations in malt β-amylase values were significantly more pronounced at 40°C where the mean enzyme activities of most malts varied markedly ($p < .001$). Nevertheless, the highest β-amylase activity of the 40°C-dried grains was, as has earlier been noted with acetone drying, achieved with regime II (i.e. air rest + final warm steeping), while mean activities for regimes IV, I and III followed in that order. Regardless of whether the steep regime incorporated an air rest cycle or not, β-amylase development in 40°C-dried malts was markedly ($p < .05$ to $p < .001$) improved by final warm steeping. At 50°C drying temperature, regimes IV and I malts showed similar ($p > .1$) activity while enzyme levels with either was significantly higher than the mean β-amylase values of malts from regimes II ($p < .05$) and III ($p <$

TABLE III. Mean malt β-amylase activity as influenced by steep regime, steep liquor and kilning temperature and their interactions.

Steep liquor	Steeping regime	Malt β-amylase activity (μg glucose equivalent) by kilning condition		
		Ac	50°C	40°C
NaOH	I	501.5	597.9	491.5
	II	665.5	384.3	577.1
	III	551.6	333.8	292.5
	IV	484.2	488.3	513.2
KOH	I	451.8	376.2	319.2
	II	390.9	427.1	995.6
	III	430.1	242.0	388.6
	IV	345.0	458.2	530.4
$Ca(OH)_2$	I	493.3	556.0	494.9
	II	423.5	617.5	496.6
	III	451.0	458.0	431.7
	IV	438.4	464.0	451.7
H_2O	I	196.5	403.9	337.9
	II	574.6	382.8	861.9
	III	448.4	462.2	461.7
	IV	465.4	520.8	458.9

Results are presented as means of triplicate experiments.
Ac = Acetone drying, 40°C, 50°C = kilning temperatures.

.001). Final warm steeping highly ($p < .001$) improved mean β-amylase productivity at 50°C drying temperature when combined with continuous steeping (i.e. regime IV) but slightly inhibited (6.3% reduction) enzyme development when used with air resting. In acetone-dried malts, final warm steep promoted β-amylase development only when used with air rests.

Drying conditions influenced malt β-amylase response to steeping regime. Drying at 40°C always supported more ($p < .001$) β-amylase activity than acetone drying, except at steep regimes I and III. We have in an earlier communication attributed the higher α-amylase activity of 40°C-dried malts (compared to acetone drying) to the probable extension of the periods of enzyme synthesis during kilning[24]. This may, in part, explain the higher mean β-amylase levels of 40°C-dried regimes II and IV malts vis-à-vis their corresponding green malt counterparts. On the other hand, β-amylase values were higher for acetone drying at steep regimes I and III compared to corresponding 40°C figures. This suggests that enzyme loss during kilning (at 40°C) occurred at probably much higher rates than did enzyme development in grains steeped by those steep regimes, perhaps owing to reduced processing ability or due to higher β-amylase thermolability. The latter would of course suggest heterogeneity in sorghum malt β-amylase enzyme – a fact that is quite consistent with the findings of Taylor and Robbins[34]. Together, drying at 40°C or with acetone were significantly ($p < .025$ to $p < .001$) more salutary to β-amylase production than 50°C drying at steep regimes II and III. This was however untrue with the two other steep regimes where malts dried at the latter temperature were markedly ($p < .001$) more β-amylolytic than drying with acetone (steep regimes I and IV) and at 40°C (steep regime I only). Higher mean malt β-amylase activity at 50°C than at 40°C could probably be due to the activity of processing proteases which were probably more active during the kilning phase at the higher drying temperature probably resulting in faster rates of enzyme activation in regime I-steeped grains. Net enzyme production during kilning represents the difference between the amounts of enzyme synthesized and lost during kilning. It is thus possible that the net enzyme synthesis be higher at 50°C for regime I malted grains than at 40°C if the rates of enzyme activation are significantly higher than corresponding values at the lower temperature. On the other hand, enzyme activity at 50°C drying temperature could be higher than that for green malts if more enzyme development/activation took place during the kilning phase at the higher temperature, and if the cumulative rates of enzyme development/activation were higher than those for their inactivation as has earlier been proposed by Lloyd[18].

Steep regime greatly influenced ($p < .001$) the manner in which malt β-amylase activity was affected by the interaction of steep liquor chemical composition with kilning condition (Table III). Regardless of steep liquor and kilning conditions, the highest mean malt β-amylase activity was at all times achieved with steep regime II (air rest with final warm steeping) – a result which was replicated at the 40°C drying temperature but not with the other drying conditions. With acetone and 50°C, the steep regime responsible for the highest β-amylase activity varied markedly ($p < .001$) in relation to the nature of the steep liquor. For example, at 50°C the highest β-amylase activities for each of the steep liquors were offered by regimes I, IV, II, and IV respectively for NaOH, KOH, $Ca(OH)_2$ and distilled water. Results for the acetone-dried green malts showed that steep regime I supported maximum β-amylase productivity with KOH and $Ca(OH)_2$, while the highest enzyme activity with NaOH- and distilled water was given by regime II. Of special interest in this work is the observation that all $Ca(OH)_2$-steeped, 50°C-dried sorghum malts had, irrespective of their steep regime, significantly ($p < .001$ to $p < .1$) more β-amylase activity than all their acetone- or 40°C-dried counterparts (Table III). While no immediate reasons can be given for this, it nevertheless remains possible that (as we have earlier asserted in this communication); (i) sorghum β-amylase occurs in a zymogen form requiring activation during germination for optimum activity; (ii) that the major activating proteolytic enzymes act optimally at or about 50°C; (iii) that these major activating proteases are metalloenzymes probably requiring Ca^{2+} for their maximum synthesis, activity and stability; (iv) that steeping in $Ca(OH)_2$ leads to a possible increase in the levels and activity of these proteases enabling them act longer during the drying process at 50°C thus possibly converting very high levels of zymogen sorghum β-amylase to the active forms and; (v) that the rates at which active β-amylase is released by the activity of processing proteases at the 50°C kilning temperature, in $Ca(OH)_2$-steeped grains, is high enough to supersede rates of sorghum β-amylase activity loss during the denaturing phase of kilning at that temperature, thus ensuring a net increase in enzyme activity after drying in comparison with levels of activity in the corresponding green malts. In this sense, the higher β-amylase activity of the 50°C-dried, $Ca(OH)_2$-steeped malts, when compared to the lower values for the corresponding 40°C-dried grains, may be attributed to possibly faster rates of β-amylase activation at the higher drying temperature where the activities of the processing enzymes would be expected to be closer their optimum. Still within the $Ca(OH)_2$-steeped grains, the extents to which the β-amylase activity of malts dried using the three conditions of kilning varied was significantly ($p < .001$) higher with steep regimes I and II. If our arguments on the processing proteases are to be accepted, then the above observation would suggest that the combination of air resting and $Ca(OH)_2$ steeping was most salutary to the development those enzymes in sorghum malts dried at 50°C. A few 50°C-dried malts generated with NaOH (regime I) or with distilled water steeps (regimes I and IV) were also associated with more β-amylase activity than their corresponding acetone- and 40°C-dried counterparts. While the explanations given above for the $Ca(OH)_2$ steeps may apply here, the fact that the above phenomenon occurred more frequently with the latter reinforces the opinion earlier expressed by us in this communication that one or more of the major processes involved in the activation of zymogen sorghum β-amylase may primarily require Ca^{2+} or a related ion and that sorghum β-amylase activation may occur optimally at or close to 50°C. With NaOH, KOH and the distilled water liquors, instances existed where malt β-amylase activity at 50°C was higher than the value for the corresponding green malt but less than enzyme activity given by the matching 40°C-dried malts. In such cases, it is suggested that the higher β-amylase activity at 50°C (vis-à-vis green malts) may have been essentially due to kilning phase-associated de novo enzyme synthesis[18,24] rather than to zymogen activation. It is nevertheless possible that zymogen activation may have been additionally responsible for this post-germination phase increase in sorghum malt β-amylase activity.

The within- and between-variety β-amylase productivities of the grains were markedly ($p < .100$) influenced by steep regime (Table IV). With each of the varieties, the mean malt β-amylase activity varied significantly ($p < .001$) with respect to steep regime. Except for LR malts, the highest mean β-amylase activity of each of the sorghum grain cultivars was achieved with either regime II or regime IV thus suggesting that the application of final warm steeping (irrespective of whether grains were steeped continuously or with air resting) was beneficial to sorghum malt β-amylase development. This enhancement in malt β-amylase activity by the application of final warm steeping was particularly pronounced with the air rested malts of the KSV 8 variety for which the application of final warm steeping resulted in 75.4% improvement in the malt enzyme's activity. Percentage increases in malt β-amylase activity as a result of the application of final warm steeping were 28.4 and 25.8% for air rested malts of SK 5912 and the LW varieties respectively. Similar values for continuously steeped grains were 3.3, 20.1, 32.5 and 9.0% respectively for malts of the varieties KSV 8, SK 5912, LW and LR. The above findings agree quite well with a similar observation on α-amylase development earlier made with sorghum grains malted using conditions identical to the ones reported in the present study[24]. Obviously, final warm steep-engendered enhancements in β-amylase activity of LR malts occurred only in continuously steeped grains, as a similar treatment was significantly ($p < .001$) deleterious to enzyme development in air rested grains of that cultivar (Table IV). Overall, mean malt β-amylase activity of the sorghum grains were higher ($p < .005$) with air resting, except for the LW variety. The highest malt β-amylase activity was however given by steep regime II-malted KSV 8 malt.

As can also be seen, the combined effects of grain cultivar and steep regime on sorghum malt β-amylase activity were significantly influenced ($p < .001$) by the nature of the steep liquor (Table V). Similarly malt β-amylase response to any combination of two of the above factors was influenced by the third. For example steep regimes IV, II, I and II supported the highest malt β-amylase activity for SK 5912 treated with NaOH, KOH, $Ca(OH)_2$ and distilled water respectively. Similar differences with the three other cultivar were as follows regimes I, II, IV and II (KSV 8), regimes II, III, I and IV (LW), and regimes I, II, I, and IV (LR) respectively for the above liquors. Of particular interest here was the observation that the highest β-amylase activity, for SK 5912 grains, was, irrespective of steep regime, given by $Ca(OH)_2$. This suggests that, like in maize[17], Ca^{2+} may be required for optimum β-amylase synthesis in sorghum varieties. With the other varieties, no single steep liquor was noted to enhance β-amylase development across all the steep regimes. The incorporation of air rests was also, notwithstanding steep liquor composition, generally beneficial to β-amylase development in SK 5912. This was also replicated in alkaline-steeped malts of LR, while the two other varieties showed a more varied pattern of response to air resting, even among alkaline-steeped grains. Similarly no general pattern was established with respect to the application of final warm steeping.

TABLE IV. Mean malt β-amylase activity as influenced by steep regime, grain variety and kilning temperature and their interactions.

Grain cultivar	Steeping regime	Malt β-amylase activity (µg glucose equivalent) by kilning condition		
		Ac	50°C	40°C
SK 5912	I	362.0	550.4	385.3
	II	338.5	517.9	810.4
	III	477.6	206.9	431.8
	IV	431.3	533.1	375.9
KSV 8	I	455.0	505.5	348.8
	II	523.1	748.0	1025.5
	III	436.1	464.6	421.8
	IV	526.4	451.7	388.6
LW	I	397.3	332.4	384.6
	II	680.1	356.4	465
	III	483.3	339.1	330.5
	IV	416.7	479.8	631.2
LR	I	463.3	545.7	524.8
	II	512.7	289.4	630.3
	III	483.3	410.5	390.4
	IV	358.7	466.7	558.5

Results are presented as means of triplicate experiments.
Ac = Acetone drying, 40°C, 50°C = kilning temperatures.

TABLE V. Mean malt β-amylase activity as influenced by steep regime, grain variety and steep liquor composition.

Cultivar	Steep liquor	Malt β-amylase activity (µg glucose equivalent) by steep regime			
		I	II	III	IV
SK 5912	NaOH	407.4	440.9	405.3	465.0
	KOH	318.9	515.1	311.6	389.1
	$Ca(OH)_2$	663.0	637.7	513.7	473.9
	Dist. water	341.0	628.6	358.7	459.1
KSV 8	NaOH	695.3	530.3	511.6	389.7
	KOH	327.7	970.7	310.0	627.5
	$Ca(OH)_2$	273.3	478.3	490.1	496.0
	Dist. water	449.5	1082.9	451.7	309.0
LW	NaOH	447.1	751.8	303.6	638.9
	KOH	382.5	341.0	411.9	371.3
	$Ca(OH)_2$	549.2	446.8	345.3	490.2
	Dist. water	106.7	329.0	476.4	536.4
LR	NaOH	571.4	446.1	350.1	487.2
	KOH	500.4	591.2	380.9	390.4
	$Ca(OH)_2$	573.4	487.1	438.4	345.4
	Dist. water	400.0	385.3	542.9	622.2

CONCLUSIONS

In this communication, we have presented the effects of steeping and germination conditions on β-amylase development in sorghum. The wide-ranging effects, which the conditions of steeping had on malt β-amylase response to kilning condition, in this work, suggest that the period of steeping is an important step in sorghum malting. Results also showed that the choice of kilning regimes is critical to the development of β-amylase in sorghum, although this must be predicated on the choice of the appropriate steeping conditions as well as the right variety of sorghum if the product will be used successfully in the brewing industry. The differences in the varietal responses of sorghum grains to conditions of steeping and kilning suggest the possibility that grains from different cultivars may employ varying biosynthesis models in their β-amylase development. This further highlights the need to study the physiology and molecular biology of β-amylase development in every sorghum cultivar being considered as possible candidate for commercial malting. Proper understanding of the molecular control mechanisms involved in β-amylase evolution and how they respond to the environmental conditions of germination may be necessary if malt from sorghum is to become a viable alternative to barley malt in lager beer brewing.

REFERENCES

1. Abdel-Akher, M., Hamilton, J. K., Montgomery, R. and Smith, F., *Journal of American Chemical Society*, 1952, **74**, 4970.
2. Aisien, A. O. and Muts, G. C. T., *Journal of the Institute of Brewing*, 1987, **93**, 328.
3. Bush, D. S., Biswas, A. K. and Jones, R. L., *Planta,* 1989, **178**, 411.
4. Bush, D. S., Cornejo, M-J, Huang, C. N., and Jones, R. L., *Plant Physiology*, 1989, **82**, 566.
5. Cohen, S. S., *Practical Statistics*. Edward Arnold: London, 1988.
6. Cole, N., MacLeod, L. and Mitchell, K., *Technical Quarterly Master Brewers Association of the Americas*, 1998, **35**, 104.
7. Daussant, J. and Lauriere, C., *Planta,* 1990, **181**, 505.
8. Demuyakor, B. and Ohta, Y., *Journal of the Institute of Brewing*, 1993, **99**, 227.
9. Ezeogu, L. I. and Okolo, B. N., *Journal of the Institute of Brewing*, 1994, **100**, 335.
10. Ezeogu, L. I. and Okolo, B. N., *Journal of the Institute of Brewing*, 1995, **101**, 31.
11. Ezeogu, L. I. and Okolo, B. N., *Journal of the Institute of Brewing*, 1999, **105**, 49.
12. Fincher, G. B., *Annual Review of Plant Physiology and Molecular Biology*, 1989, **40**, 305.
13. Grime, K. H. and Briggs, D. E., *Journal of the Institute of Brewing*, 1996, **102**, 261.
14. Jacobsen, J. V. and Higgins, T. J. V., *Plant Physiology*, 1983, **70**, 1647.
15. Jayatissa, P. M., Pathirana, R. A. and Sunderan, S. K., *Journal of the Institute of Brewing*, 1980, **88**, 18.
16. Laberge, D. E. and Marchylo, B. A., *Journal of the American Society of Brewing Chemists*, 1983, **41**, 120.
17. Lauriere, C., Doyen, C., Thevenot, C. and Daussant, J., *Plant Physiology,* 1992, **100**, 877.
18. Lloyd, W. J. W., *Journal of the American Society of Brewing Chemists*, 1988, **46**, 8.
19. MacGregor, A. W., *Louvain Brewing Letters*, 1991, **4**, 21.
20. Mitsuhashi, W. and Oaks, A., *Plant Physiology*, 1994, **104**, 401.
21. Nelson, N., *Journal of Biological Chemistry*, 1944, **153**, 375.
22. Norris, K. and Lewis, M. I., *Technical Quarterly Master Brewers Association of the Americas*, 1965, **22**, 154.
23. Novellie, L., *Journal of the Science of Food and Agriculture*, 1962, **13**, 121.
24. Obeta, J. A. N., Okungbowa, J. and Ezeogu, L. I., *Journal of the Institute of Brewing*, 2000, **106**, 295.
25. Okolo, B. N. and Ezeogu, L. I., *Journal of the Institute of Brewing*, 1995, **101**, 463.
26. Okolo, B. N. and Ezeogu, L. I., *Journal of the Institute of Brewing*, 1996, **102**, 79.
27. Okolo, B. N. and Ezeogu, L. I., Journal *of the Institute of Brewing*, 1996, **102**, 277.
28. Owuama, C. I., *Journal of the Institute of Brewing*, 1999, **105**, 23.
29. Palmer, G. H., *World Journal of Microbiology and Biotechnology*, 1989, **7**, 17.
30. Smith, O. B. and Gill, A. A., *Journal of the Institute of Brewing,* 1986, **92**, 360.
31. Sopanen, T. and Lauriere, C., *Plant Physiology*, 1989, **89**, 244.
32. Sun, Z. and Henson, C. A., *Archives of Biochemistry and Biophysics*, 1991, **284**, 298.
33. Taylor, J. R. N. and Boyd, H. K., *Journal of the Science of Food and Agriculture,* 1986, **39**, 1109.
34. Taylor, J. R. N. and Robbins, D. J., *Journal of the Institute of Brewing*, 1993, **99**, 413.
35. Ziegler, P., Daussant, J. and Loos, K., *Journal of Experimental Botany*, 1994, **45**, 1147.