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Effect of steeping conditions on the amylolytic development of some Nigerian improved sorghum cultivars

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Abstract Nigerian improved sorghum cultivars 'L538', 'YG5760', 'L1499', 'SSH1' and 'SSH3' were investigated for the effect of steeping conditions on their amylolytic development. The grains were steeped using 3 steep regimes SR 1, SR 2 and SR 3 which involved steeping and re-steeping in cold distilled and de-ionized water for 36, 45 and 54 h respectively. Grains in each SR were divided into 4 portions and further steeped for 6 h using final warm steep temperatures (FWST) of 30, 35, 40 and 45°C. α-and β-Amylase as well as diastatic activity were determined at different FWST. SR and FWST were correlated with enzyme development. Steeping conditions significantly ($p<0.05$) affected amylolytic development of the sorghum malts. Optimum moisture content (48%) was obtained at FWST of 35°C. α-Amylase was the predominant enzyme. All enzyme activities were at a peak at FWST of 30 and 35°C and at SR 2. The highest enzyme activity was recorded by cultivar 'YG5760' malt-(α-amylase 272, β-Amylase 169 μg equivalent glucose). High relationship existed between α-amylase and moderate relationship between β-Amylase and SR. Similar relationship existed between enzyme development and FWST.

Keywords Sorghum cultivars · α-Amylase activity · β-Amylase activity · Diastatic activity

Introduction

The ability of malted sorghum to generate complex system of enzyme associated with starch hydrolysis is a key to its use as brewing raw material. It is therefore necessary to understand the basic physiological process underlying the development of good quality sorghum malt (Etokakpan and Palmer 1990b, Etokakpan 1992). The conditions of steeping are believed to decide what may be optimal for sorghum germination and subsequent enzyme development (Igyor et al. 1989). Proper enzyme development ultimately leads to complete saccharification and high extract yield. Grain re-steeping is one of the several methods which have been used for the development of good quality barley malt. Advantages that accrue from the application of re-steeped include a reduced malting loss as well as fast modification of steeped grains. Studying condition of re-steeping which may be favourable for malt quality development in barley, Lubert and Pool (1964) found that the induction of water sensitivity in barley by the initial steep is influenced by factors such as temperature of steeping water and length of steeping rather than moisture uptake rate. Hence, re-steeping process is proposed for sorghum malting. As part of the on-going attempts to optimize sorghum steeping condition, Ezeogu and Okolo (1994) evaluated effect of final warm water steep and air rest cycles on malt properties of 3 improved Nigeria sorghum cultivars. They found that air rest periods incorporated into steep cycles enhance water uptake by sorghum. Sorghum moisture content is an underlying factor for sorghum modification. Furthermore, the effect of air rest periods on malting response to final warm water steep was determined (Ezeogu and Okolo 1995). They found that sorghum quality parameters such as root length, malting loss, diastatic activity, α and β-Amylolytic activity and extract activity were significantly affected by the air rest duration, cultivar type and their pair-wise interactions.

To find optimum conditions for sorghum malt development work is still continuing. In spite of several improved sorghum cultivars developed in Nigeria, efforts have been concentrated only on few cultivars notably 'KSV8', 'ICSV400' and 'SK5912'. The present work therefore was aimed to study the effect of steeping conditions on the amylolytic activity of some Nigerian improved sorghum cultivars.

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Materials and methods

Sorghum (*Sorghum vulgare*) cultivars 'L538', 'YG5760', 'L1499', 'SSH1', 'SSH3', were obtained from the Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The grains were sorted manually and 500 g of each cultivar were surface sterilized by immersion in sodium hypochlorite solution for 20 min. The grains were drained and washed thrice with distilled and de-ionized water. The grains were steeped in cold distilled and de-ionized water using 3 different steep regimes (SR) 1 to 3. In SR1, grains were steeped for 6 h, then brought out of steep (air rest) for 3 h, re-steeped (6 h) and out of steep (3 h) continuously alternating 6 h re-steeping with 3 h air rest for a total of 36 h. After this the grains were divided into 4 portions and re-steeped at different temperatures of 30, 35, 40 and 45^{0}C, respectively each for 6 h. SR2 and SR3 involved similar re-steeping and air rest as in SR1 except that re-steeping and air rest was carried out for 45 and 54 h, respectively before increasing the temperature of steeping.

At the end of the steep cycles, grains were germinated for 4 days in shallow germination trays with fine mesh bottom and kept in dark humidified wooden germination boxes at 25 ± 3°C. Grains were turned every 12 h and sprayed with 10 ml of distilled and de-ionized water using atomizer spray. The grains were kilned in an oven (Gallenkamp, Britain) at 50°C for 24 h and rootlets were removed manually. The malted grains were milled in a cooled blender and stored in refrigerator.

Analysis: The grains were analysed for moisture according to recommended methods (IOB 1982). α- and β-Amylase activity as well as diastatic activity (total reducing activity) were determined by the diamylase procedure of Etokakpan and Palmer (1990a). In this procedure, β-amylase was inhibited and then α-amylase activity was the residual saccharifying power. A unit of enzyme activity was the amount of enzyme that could release 1 µg glucose equivalent per min. Relationship between enzyme development and SR, final warm steep temperatures (FWST) was demonstrated by correlation analysis. Data were subjected to analysis of variance and read at 0.05 confidence level. Means that differed significantly were shown by the least significant difference (Steele and Torrie 1980).

Results and discussion

Steep-out moisture content of various sorghum cultivars at different FWST was in the range of 35–48% (Table 1). Rate of water uptake by sorghum cultivars was significantly affected ($p<0.05$) by FWST and SR. There was an increase in moisture with increase in steep temperature from 30 to 35^{0}C FWST after which a progressive decrease was observed in FWST of 40 and 45°C. This made the FWST of 30 and 35°C the most active in terms of grain moisture pick up. During steeping of grains, respiration of grains rises rapidly as absorbed oxygen is removed from the steep liquor by the grain. These metabolic changes during steeping increase the temperature of steeping water. Some workers have advocated the use of flush steeping in which the grain is alternatively immersed in water and exposed to the air a number of times (Ezeogu and Okolo 1995). This process requires control since it can lead to increase in temperature and ex-

Table 1 Effect of steeping conditions on the moisture content (%) of some Nigerian improved sorghum cultivars at different final warm steep temperatures (FWST)

		FWST, °C			
Steep regime	Sorghum cultivar	30	35	40	45
SR 1	'L538'	44 ± 0.3^a	45 ± 0.4^a	43 ± 0.2^a	40 ± 0.2^b
	'YG5760'	43 ± 0.2^b	45 ± 0.4^b	40 ± 0.2^c	38 ± 0.1^c
	'L1499'	41 ± 0.1^c	42 ± 0.2^c	39 ± 0.1^d	37 ± 0.1^d
	'SSH1'	43 ± 0.2^a	44 ± 0.3^a	42 ± 0.2^b	41 ± 0.2^b
	'SSH3'	42 ± 0.2^a	45 ± 0.4^b	39 ± 0.1^c	36 ± 0.1^d
SR 2	'L538'	46 ± 0.5^b	48 ± 0.5^b	41 ± 0.2^c	39 ± 0.1^c
	'YG5760'	45 ± 0.4^c	47 ± 0.5^c	41 ± 0.2^e	40 ± 0.2^e
	'L1499'	44 ± 0.3^a	46 ± 0.5^b	43 ± 0.2^a	41 ± 0.2^c
	'SSH1'	45 ± 0.4^b	48 ± 0.5^d	44 ± 0.3^b	42 ± 0.2^a
	'SSH3'	47 ± 0.5^f	48 ± 0.5^f	42 ± 0.2^c	40 ± 0.1^c
SR 3	'L538'	40 ± 0.2^b	43 ± 0.2^b	37 ± 0.1^f	35 ± 0.1^f
	'YG5760'	42 ± 0.2^c	44 ± 0.3^c	36 ± 0.1^d	35 ± 0.1^d
	'L1499'	43 ± 0.2^a	45 ± 0.4^a	40 ± 0.2^b	37 ± 0.1^b
	'SSH1'	41 ± 0.2^b	42 ± 0.2^b	38 ± 0.1^c	37 ± 0.1^c
	'SSH3'	45 ± 0.4^f	46 ± 0.4^f	42 ± 0.2^c	40 ± 0.2^c

Means ± SD with different superscripts in the same row are significantly different ($p<0.05$) (n=3) SR1, SR2, SR3: As in text

cessive rootlet growth during germination and consequently high malting losses. If properly controlled, Brookes et al. (1976) said that it is a much more rapid process which has economic advantages. Although high moisture levels were obtained in all SR1, SR2 and SR3 at FWST 30 and 35°C, sorghum grains steeped under SR2 had the highest moisture content (Table 1).

In the experiments where sorghum grains were steeped at 25°C for 15 h, followed by 4 h air rest and 25 h re-steeping, the out-of-steep moisture (after 4 h draining of the grain) was about 40% (w/w) (Palmer et al. 1989). Similar steeping of barley gave 45% moisture (Hough et al. 1971). Manipulation of steeping conditions in combination with use of improved sorghum cultivars have increased sorghum moisture content up to 48%. However, such high moisture content may not necessarily cause optimal hydration of starchy endosperm which usually results in rapid and uniform enzymatic breakdown of the sorghum endosperm food reserves.

Grain water uptake may be influenced by many factors which include grain size, nitrogen content and initial moisture content of the grains (Brookes et al. 1976).

α-*Amylase activity:* α-Amylase is involved in carbohydrate breakdown (Palmer 1989, 1991, Sanwo and Demazon 1992). They are endo-enzymes which hydrolyse α – 1–4 glucosidic linkages randomly in the chain after which the terminal sugar is left in α-configuration. In the present study, α-amylase was the predominant enzyme in sorghum. FWST significantly ($p<0.05$) affected α-amylase activity (Table 2). With respect to FWST, there were highly significant variations in malt α-amylase activity. Increase in FWST resulted in decreased mean values of α-amylase activity. Invariably, FWST of 30°C was therefore optimum for α-amylase activity of all the sorghum malt under all the SR conditions. Malt α-amylase in malt differed significantly from each other with respect to SR. SR 2 supported the highest α-amylase activity. A maximum activity of 272 µg equivalent glucose was recorded by sorghum variety 'YG5760 malt and 193 µg equivalent was the lowest activity exhibited by 'L538' malt under the same SR. Higher α-amylase activity at 30°C FWST compared to other FWST could be due to heat on enzymes which results in some loss of activity and enzymes can even be completely inactivated with intense heating. The grain variety was a significant determinant for malt α-amylase activity. 'YG 5760' malt consistently produced highly significantly more α-amylase activity than other cultivars studied under all the conditions of steeping. The pattern of α-amylase activity in the sorghum malts as a response to FWST was similar as observed in each sorghum malt. High α-amylase activity can appear during the germination of grains due to *de novo* synthesis of enzymes which is triggered by gibberellins. They can also be influenced by steeping condition. Ezeogu and Okolo (1995) found that incorporating air rest periods during steeping were optimum for α- and β-amylase activity for 'KSV8' and 'SK5912' sorghum cultivars. This means that among other factors that influence amylase production by sorghum during malting is the grain genetic property which was evident in the mean α-amylase activity values obtained for all the sorghum cultivars (Table 2).

β-*Amylase activity:* β-Amylase, the sarccharifying malt-enzyme is an exo-enzyme and attack the amylose chain from the non-reducing end liberating β-maltose

Table 2 Effect of steeping conditions on the α-Amylase activity (µg equivalent glucose) of some Nigerian improved sorghum cultivar malts at different final warm steep temperatures (FWST)

		FWST, °C			
Steep regime	Sorghum cultivar malt	30	35	40	45
SR 1	'L538'	160 ± 0.7^{a}	158 ± 0.7^{a}	70 ± 0.4^{b}	63 ± 0.4^{b}
	'YG5760'	120 ± 0.6^{b}	108 ± 0.6^{b}	70 ± 0.4^{c}	46 ± 0.3^{d}
	'L1499'	113 ± 0.6^{c}	104 ± 0.6^{c}	63 ± 0.4^{d}	59 ± 0.4^{d}
	'SSH1'	115 ± 0.6^{d}	106 ± 0.6^{d}	69 ± 0.4^{g}	53 ± 0.4^{f}
	'SSH3'	117 ± 0.6^{a}	110 ± 0.6^{a}	65 ± 0.4^{b}	56 ± 0.4^{b}
SR 2	'L538'	193 ± 0.8^{b}	184 ± 0.8^{b}	137 ± 0.6^{c}	35 ± 0.3^{d}
	'YG5760'	272 ± 1.0^{a}	242 ± 1.0^{b}	206 ± 0.8^{c}	185 ± 0.8^{c}
	'1499'	265 ± 1.0^{b}	232 ± 0.9^{c}	162 ± 0.7^{d}	146 ± 0.7^{d}
	'SSH1'	270 ± 1.0^{c}	225 ± 0.9^{d}	187 ± 0.8^{a}	135 ± 0.6^{f}
	'SSH3'	271 ± 1.0^{b}	228 ± 0.9^{c}	194 ± 0.8^{d}	143 ± 0.7^{e}
SR 3	'L538'	168 ± 0.7^{a}	150 ± 0.7^{a}	105 ± 0.6^{b}	68 ± 0.4^{c}
	'YG5760'	130 ± 0.6^{d}	127 ± 0.6^{d}	94 ± 0.5^{c}	54 ± 0.3^{f}
	'L1499'	125 ± 0.6^{g}	114 ± 0.6^{g}	70 ± 0.4^{a}	43 ± 0.3^{b}
	'SSH'	132 ± 0.6^{a}	118 ± 0.6^{a}	93 ± 0.5^{b}	64 ± 0.4^{b}
	'SSH3'	146 ± 0.7^{b}	132 ± 3.6^{c}	101 ± 0.6^{d}	75 ± 0.5^{c}

Means ± SD with different superscripts in the same row were significantly different ($p<0.05$) (n=3). SR1, SR2, SR3: As in text

which has the free hydroxyl group in β-configuration (Enari and Sopanen 1986). The low level of β-amylase in sorghum is an important physiological difference between sorghum malt and barley malt. Combinations of sorghum cultivar selection and malting manipulation have been reported to have a positive impact in the development of β-amylase during malting (Dufour and Melotte 1992, Taylor and Robbins 1993, Ezeogu and Okolo 1994, 1995, Okolo and Ezeogu 1996). Table 3 indicates one of such steeping manipulations using FWST on sorghum cultvars .Although the values of β-amylase activity obtained were still far lower than their corresponding α-amylase values, β-amylase activity was significantly ($p<0.05$) affected by steeping conditions and cultivars. β-Amylase activity followed a

Table 3 Effect of steeping conditions on the β-Amylase activity (μg equivalent glucose) of some Nigerian improved sorghum cultivar malts at different final warm steep temperatures (FWST)

		FWST, °C			
Steep regime	Sorghum cultivar malt	30	35	40	45
SR 1	'L538'	74 ± 0.5[a]	78 ± 0.5[a]	56 ± 0.4[b]	54 ± 0.4[b]
	'YG5760'	93 ± 0.5[b]	90 ± 0.5[b]	59 ± 0.4[c]	58 ± 0.4[c]
	'L1499'	85 ± 0.5[d]	86 ± 0.5[d]	65 ± 0.4[e]	63 ± 0.4[e]
	'SSH1'	72 ± 0.5[a]	89 ± 0.5[a]	49 ± 0.3[b]	49 ± 0.3[b]
	'SSH3'	93 ± 0.5[b]	86 ± 0.5[b]	56 ± 0.4[c]	52 ± 0.4[c]
SR 2	'L538'	148 ± 0.6[c]	166 ± 0.7[c]	75 ± 0.5[d]	56 ± 0.4[d]
	'YG5760'	156 ± 0.7[d]	169 ± 0.7[d]	83 ± 0.5[f]	62 ± 0.4[f]
	'L1499'	126 ± 0.6[a]	139 ± 0.6[a]	75 ± 0.5[b]	70 ± 04[b]
	'SSH1'	108 ± 0.6[a]	125 ± 0.6[a]	53 ± 0.4[b]	50 ± 0.3[b]
	'SSH3'	102 ± 0.6[b]	110 ± 0.6[b]	60 ± 0.4[c]	57 ± 0.4[c]
SR 3	'L538'	83 ± 05[a]	88 ± 0.5[a]	40 ± 0.3[b]	40 ± 0.3[b]
	'YG5760'	85 ± 0.5[a]	91 ± 0.5[a]	47 ± 0.3[d]	43 ± 0.3[d]
	'L1499'	73 ± 0.5[c]	80 ± 0.5[c]	36 ± 0.3[a]	18 ± 0.2[b]
	'SSH1'	55 ± 0.4[a]	62 ± 04[a]		24 ± 03[c]
	'SSH3'	50 ± 0.3[b]	87 ± 0.5[c]	35 ± 03[d]	24 ± 0.3[d]

Means ± SD with different superscripts in the same row were significantly different ($p<0.05$) (n=3) SR1, SR2, SR3: As in text

Table 4 Effect of steeping conditions on the diastatic activity (μg equivalent glucose) of some Nigerian improved sorghum cultivar malts at different final warm steep temperatures (FWST)

		FWST, °C			
Steep regime	Sorghum cultivar malt	30	35	40	45
SR 1	'L538'		234 ± 0.9[a]	236 ± 0.9[a]	126 ± 0.6[b]
	'YG5760'	213 ± 0.9[b]	190 ± 08[b]	129 ± 06[c]	104 ± 06[d]
	'L1499'	198 ± 0.8[c]	190 ± 08[c]	128 ± 0.6[d]	122 ± 0.6[d]
	'SSH1'	187 ± 0.8[a]	195 ± 08[a]	118 ± 6[b]	
	'SSH3'	196 ± 0.8[b]	203 ± 0.8[b]	121 ± 0.6[c]	108 ± 0.6[c]
SR 2	'L538'	341 ± 1.2[a]	350 ± 1.2[a]	212 ± 0.9[b]	91 ± 0.5[c]
	'YG5760'	428 ± 1.5[a]	411 ± 1.4[a]	289 ± 1.0[b]	247 ± 1.0[c]
	'L1499'	391 ± 1.4[c]	361 ± 1.3[c]	237 ± 09[d]	216 ± 0.9[d]
	'SSH1'	378 ± 1.3[c]	350 ± 1.2[c]	240 ± 0.9[d]	185 ± 0.8[f]
	'SSH3'	373 ± 1.3a	338 ± 1.1[a]	254 ± 1.0[b]	200 ± 08[c]
SR 3	'L538'	251 ± 1.0[a]	238 ± 0.9[a]	145 ± 07[b]	208 ± 0.8[c]
	'YG5760'	215 ± 0.9[c]	218 ± 0.9[c]	140 ± 0.6[d]	97 ± 05[c]
	'L1499'	198 ± 0.8[a]	195 ± 08[a]	106 ± 0.6[b]	61 ± 0.4[c]
	'SSH1'	187 ± 08[a]	120 ± 0.6[b]	117 ± 0.6[b]	81 ± 0.5[c]
	'SSH3'	196 ± 0.7[b]	219 ± 09[b]	136 ± 0.6[c]	99 ± 0.5[d]

Means ± SD with different superscripts in the same row were significantly different $p<0.05$) (n=3) SR1, SR2, SR3: As in text

Table 5 Relationship between steep regime and enzyme development of some Nigerian sorghum cultivar malts

	Correlation co-efficient, r		
Sorghum cultivar malt	SR1	SR2	SR3
	α-Amylase		
'L538'	0.63	0.65	0.68
'YG5760'	0.68	0.98	0.74
'L1499'	0.65	0.91	0.70
'SSH1'	0.69	0.90	0.75
'SSH3'	0.78	0.92	0.76
	β-Amylase		
'L538'	0.46	0.50	0.57
'YG5760'	0.54	0.75	0.56
'L1499'	0.46	0.67	0.40
'SSH1'	0.35	0.47	0.38
'SSH3'	0.43	0.51	0.42

SR1, SR2, SR3: As in text

Table 6 Relationship between final warm steep temperatures (FWST) and enzyme development of some Nigerian sorghum cultivar malts

	Correlation co-efficient, r			
Sorghum cultivar	30°C	35°C	40°C	45°C
		α-Amylase		
'L538'	0.68	0.23	0.18	0.32
'YG5760'	0.94	0.92	0.88	0.80
'L1499'	0.93	0.90	0.87	0.78
'SSH1'	0.93	0.89	0.80	0.77
'SSH3'	0.93	0.89	0.82	0.78
		β-Amylase		
'L538'	0.34	0.62	0.32	0.25
'YG5760'	0.45	0.68	0.33	0.26
'L1499'	0.47	0.48	0.43	0.42
'SSH1'	0.44	0.49	0.34	0.31
'SSH3'	0.50	0.53	0.42	0.41

30 to 45°C: FWST

different trend of development from the α-amylase activity. There was an increase in β-amylase as the FWST was raised from 30 to 35°C after which a progressive decrease was observed in subsequent FWST (Table 3). Based on FWST, 35°C was optimum for β-amylase development in sorghum cultivars.

β-Amylase development was highest under SR2. This confirms the fact that steeping manipulations can affect the amylolytic development of sorghum cultivars. The β-Amylase values obtained also indicated cultivar dependence as "YG5760" and "L538" sorghum cultivars showed the highest activity values (169 and 166 μg equivalent glucose, respectively) under SR 2.

Diastatic activity: The diastatic activity of the sorghum cultivars investigated showed that FWST and steep regime significantly ($p<0.05$) affected their activity as illustrated in Table 4. Diastatic activity was higher in sorghum cultivars under SR2 when compared to the other steep regimes. Most of the sorghum cultivars diastatic activity increased when the FWST was raised from 30 to 35°C but dropped thereafter with further increase in FWST. Sorghum cultivar 'L1499' witnessed a progressive decrease in diastatic activity with increase in FWST while 'SSH' had similar decrease in SR2 and SR3. Since the diastatic activities values at 30 and 35°C were not significantly different ($p>0.05$), it implies that FWST of 30 and 35°C were optimum for diastatic activity of these sorghum cultivars. Morrall et al. (1986) observed increase in diastatic power with germination time. A rapid increase occurred up to day 4 of germination when diastatic power reached a peak but declined slowly there-

after. Although malting manipulation employed in this study has significantly affected the diastatic activity, α-amylase was still the principal contributor of the sorghum diastatic activity rather than β-amylase.

Enzyme activity correlated highly positively with SR (Table 5). In SR2 sorghum malt 'YG5760' had r = 0.98 for α-amylase and r = 0.75 for β-amylase. β-Amylase activity however showed lower r-values than α-amylase activity. A similar trend was observed when the relationship between FWST and enzyme development was investigated (Table 6). Enzyme development correlated positively with FWST. Low correlation levels were noted with β-amylase activity. In some sorghum cultivars such as 'L538' and 'YG5760', α- and β-amylase activities correlated poorly with FWST of 40 and 45°C (Table 6).

Conclusion

Steeping conditions were highly determinant factors for amylolytic development of the Nigerian sorghum cultivars investigated. Sorghum water uptake under the various steep regimes was directly related to enzyme development of malts. All the improved sorghum cultivars investigated showed cultivar dependent characteristics in their rate of water uptake and the pattern of their enzyme development. α-Amylase contributed more than β-amylase to the diastatic activity of the sorghum malts. Steeping for 6 h wet, 3 h air rest for a total of 45 h followed by 6 h final warm water steep at 30–35°C resulted in optimum water uptake and amylolytic development of the sorghum cultivars. Sorghum grain is genetically different from barley grain. Hence, research results in sorghum malt enzyme activity will be more appreciated if a procedure is standardized for their determination.

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