

Quality Assessment of Different Sorghum Varieties for Their Brewing Potential

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ABSTRACT

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Four sorghum varieties (SK 5912, KSV 4, KSV 8, ICSV 400) were malted and extracted under similar conditions to assess their quality for brewing. The results showed that, in general, the sorghum varieties had high malting loss which was attributed to the high germination temperature used. The sorghum varieties also developed low levels of amylolytic activity (α -amylase and β -amylase), and with similar ratios. When the sorghum malts were mashed at different temperatures with the aid of commercial enzyme preparations, it was observed that mashing temperatures were more important in sugar release than additions of commercial enzymes. This was because at the lower mashing temperature, sorghum starch was not adequately gelatinised. However, when commercial enzyme preparations were added, low levels of enzymes were very effective in reducing wort viscosity and producing free amino nitrogen (FAN). Although, both commercial enzyme preparation and mashing temperature influenced sugar production, the malts produced glucose and maltose at similar ratios. Therefore good quality malts can be produced from sorghum, however mashing will employ commercial enzymes and mashing regimes are not yet optimised.

Key words: Commercial enzymes, mashing temperature, sorghum, sugar profile.

INTRODUCTION

Research studies into sorghum are progressing rapidly, and making a great impact in brewing despite the earlier misunderstanding that malted sorghum developed insufficient hydrolytic enzymes^{3–10,12–20,29–32,39–41}. Differences in malting and mashing temperatures employed in studies of sorghum in the past were a major contributory factor and complicated our understanding of the physiological behavior of sorghum during malting^{3,14,27,28,41}. In recent times, the large body of work carried out on sorghum to understand the physiological behavior of sorghum during malting has led to the suggestion that a malting temperature of 30°C would produce commercially acceptable sorghum malt^{6,13,21–23,26,30,36,37}. This malting temperature

(30°C) was attributed to the tropical nature of sorghum^{3,6,9}. However, this high malting temperature for sorghum has been reported to facilitate the growth of aflatoxin producing *Aspergillus flavus*¹¹. Nevertheless, earlier studies¹⁰ showed that microbial growth during the malting of sorghum could be controlled. Also, like in barley, the development of sorghum varieties suitable for malting and brewing purposes has been a major area of research and development. This paper presents the results of investigations on the performance of different sorghum cultivars (SK 5912, KSV 4, KSV 8, ICSV 400) malted under similar conditions, with a view to assessing their brewing potentials in the absence and presence of commercial enzymes.

MATERIALS AND METHODS

The sorghum samples used in this study were grown in the northern part of Nigeria where high temperature conditions favour the growth of sorghum. Samples of sorghum (SK 5912, KSV 4, KSV 8, ICSV 400) were obtained from Institute of Agricultural Research, Ahmadu Bello University, Samaru, Zaria. They were screened by hand to remove broken or damaged kernels and foreign material since damaged grains cause microbial infection during germination^{10,18}. Quality assessments such as thousand-corn weight, germinative energy, germinative capacity, total nitrogen and moisture content were also carried out³⁸.

Total nitrogen of sorghum samples

Total nitrogen was determined by the Kjeldahl method as described in the Recommended Methods of the Institute of Brewing³⁸. The Tecator digester block (System 2020 digester) and Tecator distillation unit (System 1002) were used.

Steeping and malting of sorghum

A steeping procedure (steeping temperature 20°C) – 20 h water steep, 4 h air-rest, 20 h water steep was used, and germinated as described previously³. An out of steep moisture of 33–35% was achieved^{3,9,16}. Formaldehyde (0.1%) was added to the steep water to reduce microbial load on the sorghum grains¹⁰. Germination was carried out at 30°C for a period of 5 days. Germinated grains were kilned at 50°C for 16 h.

Malt analyses

Malting loss. This was determined by the Recommended Method of the Institute of Brewing³⁸.

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α -Amylase (Dextrin Units – DU). The method described by Palmer³³ was used for this determination as follows. The crude substrate was prepared by mixing 1% starch with excess β -amylase dissolved in 20 mM sodium acetate buffer containing 10 mM CaCl₂ (pH = 5.7).

Five grams of milled malt of each sample was prepared by extracting in 80 mL of extraction buffer (20 mM sodium acetate buffer pH = 5.7). The mixture was shaken for 30 min, and then centrifuged at 2,000 rpm for 10 min at 4°C (Sorvall RC24 Superspeed Refrigerated DU Pont Company, Delaware, U.S.A.). The supernatant was removed and diluted appropriately ($\times 20$), before 0.5 mL of the enzyme extract was used in duplicate for assay.

Substrate (0.5 mL) was mixed with 0.5 mL of the diluted enzyme and the mixture was allowed to digest for 5 min at 25°C. Dilute iodine solution (10 mL) (0.254 g/L iodine in 4 g/L potassium iodide) was added to the digest to stop the enzyme reaction. The colour of the iodine-dextrin complex was determined using a spectrophotometer (Philips PU 8730 UV/VIS Scanning Spectrophotometer and Philips colour plotter) at 565 nm. Digests containing no substrate or no enzyme were also examined with iodine at 565 nm.

The alpha-amylase in Dextrin Units was calculated using the formula:

$$(DU) = A_{565 \text{ nm}} (\text{absorbance}) \text{ units} \times 2 \times \text{Dilution}$$

where A_{565} (absorbance) units = A_{565} substance control – A_{565} Assay value.

β -Amylase (Diastatic Power – DP). This was determined by the Fehling's solution procedure following the IoB Methods of Analysis³⁸. The starch used was Fisher Chemical Starch, Lintner's grade. Equal volumes of commercially prepared Fehling's solution A and B (BDH chemical) were mixed, and prepared fresh before use according to Recommended Methods of the Institute of Brewing³⁸.

Mashing of sorghum

In the first part of the experiment, sorghum malt grist (0.2 mm Buhler Miag mill) was mashed at 65°C (water/grist ratio; 4:1) without the addition of commercial enzyme preparations. In the second part of the experiment, sorghum malt was mashed at 65°C with equal volumes (20 μ L) of commercial enzyme preparation combinations (Hitempase, Bioglucanase ME 250 and Bioprotease N100L). The commercial enzymes whose properties are shown in Table I were kindly supplied by Quest International, Ireland.

In the third part of the experiment, equal volumes (20 μ L) of commercial enzyme combinations (Hitempase, Bioglucanase ME 250 and Bioprotease N100L) were used during the mashing process⁷, but different mashing temperatures of 75°C, 85°C and 95°C were employed. The choice of enzymes was based on a previous study⁷. Hitempase enzyme was used because of the temperature tolerance of this enzyme and our mashing was extended to 95°C. Preliminary study showed that when the different enzymes were used individually during the mashing of sorghum at 65°C they did not influence extract recovery of sorghum malt. Sorghum malt, rather than raw sorghum, was used in this study because sorghum malt provides a buffering effect in the wort; also when commercial enzymes were used to mash raw sorghum, they destroyed not only the foam, but also foam stabilizing proteins⁷.

α -Amino nitrogen – ninhydrin assay. This was determined by the ninhydrin colorimetric method as described in the IoB Methods of Analysis³⁸.

Analyses of sugars in sorghum malt extracts

Hot water extract was determined by placing wort samples into a density meter (Calculating Digital Density meter, Stanton Redcroft PAAR DMA 46), and after conversion to specific gravity, hot water extract was calculated³⁸. The reducing sugars present in the extract were determined by the Lane and Eynon Fehling's solution method³⁸ as described elsewhere²⁵.

Wort colour

The Lovibond comparator (Model – Lovibond 2000) was used to determine wort colour as previously described²⁴.

Wort viscosity

A modified Oswald Viscometer was used to determine the wort viscosities of water and wort. The viscosity of the wort was obtained using the formula:

$$\frac{n_1}{n_2} = \frac{t_1}{t_2} - \frac{P_1}{P_2}$$

n_1 = Coefficient of viscosity of water = 1.00 cP

n_2 = Coefficient of viscosity of wort

t_1 = time of flow of water

t_2 = time of flow of wort

p_1 = density/specific gravity of water (1.000)

p_2 = density/specific gravity of wort

Variations in the duplicate results presented in this study did not exceed $\pm 5\%$.

TABLE I. Properties of the commercial enzyme preparations used in this study.

Name	Type	Microbial source	pH range	Optimum temp. range	Activities
Hitempase	Heat-stable α -amylase	<i>Bacillus licheniformis</i>	5.0–8.0	75–92°C	Hydrolyses α -1,4-glycosidic linkages in amylose and amylopectin
Bioferm L	α -Amylase	<i>Aspergillus oryzae</i>	4.0–7.0	55–60°C	Hydrolyses amylose and amylopectin at α -1,4-glucosidic linkage
Bioprotease N100L	Proteolytic enzyme	<i>Bacillus subtilis</i>	5.5–6.0	45–53°C	Produces high level of FAN
Bioglucanase ME 250	Endo-beta glucanase	<i>Penicillium emersonni</i> and <i>Bacillus subtilis</i>	5.0–6.5	50–65°C	Hydrolyses both 1,4 and 1,3 glucosidic linkage found in beta glucan
Bioglucanase HS	Beta-glucanase, cellulase and hemicellulase	<i>Trichoderma reesei</i> and <i>Penicillium emersonni</i>	4.5–6.2	60–75°C	Degrades cell wall and carbohydrates in plant cells

RESULTS AND DISCUSSION

Some properties of the four sorghum varieties studied are shown in Table II. The sorghum varieties were suitable for malting because they have high germination capacities. Apart from ICSV 400, which has lowest value for thousand corn weight (23 g) and the highest total nitrogen content (1.75%), the other sorghum varieties have suitable corn weights and nitrogen contents (Table II). Differences in moisture content are also presented. The malt analyses of the malted samples of sorghum are shown in Table III. In general, all the sorghum varieties have high malting loss. The high malting loss obtained for malted sorghum was caused by the high malting temperature used during the germination of sorghum^{3,9} because of the tropical nature of sorghum⁹. Sorghum malt gave high cold water extract (CWE), above 30% at a germination temperature of 30°C. In contrast, modified barley malt is reported to give values of 17–20% when malted at 17°C³². The relationship between cold water extract and germination temperature of sorghum is not known and further investigation is required. A maximum out of steep moisture obtained in this study (33–35%) has been reported for sorghum^{3,9,16}. However, the high CWE obtained for sorghum malt seems to be a general characteristic of sorghum malt studied. It is not clear at present if the low out of steep moisture (33–35%) obtained for sorghum would cause localised endosperm modification in sorghum. Another interesting observation is that the sorghum varieties studied produced α -amylase (measured as dextrin unit – DU) and β -amylase (measured as diastatic power – DP) at similar levels and ratios (Table III).

In Table IV the results of sorghum malts mashed at 65°C, with no added commercial enzyme preparations, are presented. At the mashing temperature of 65°C, the iodine starch test carried out on the mash was positive, because the starch of sorghum malt was not adequately gelatinised at the 65°C mashing temperature and therefore not optimally hydrolysed during mashing. It is also clear from the results in Table IV that the wort viscosity was high. It has

been reported that β -glucanase enzymes in malted barley are not active during mashing³⁴. The β -glucanase enzymes of sorghum may also be susceptible to high mashing temperatures. Most important, sugar release and free amino nitrogen (FAN) production were very low and variable. As regards the action of amylases during mashing, it was observed that although the sorghum varieties released glucose and maltose at different rates, they produced glucose and maltose at similar ratios. This observation may reflect the pattern of development of amylolytic enzymes in the sorghum varieties during malting. Some important relationships have been shown to exist between amylolytic enzymes (α -amylase and β -amylase) and the ratio of glucose to maltose in malted sorghum¹ or malted barley². In these studies, it was shown that while α -amylase was directly linked to extract recovery, the ratio of glucose to maltose was directly linked to the ratio of α -amylase to β -amylase enzymes of the malt.

Sorghum malts were mashed at 65°C with added commercial enzyme preparations (second part of the experiment) and the results obtained are shown in Table V. Again, the iodine starch test of the mash was positive because this mashing temperature was not high enough to gelatinise the sorghum starch, even though commercial enzymes were present. However, the addition of commercial enzymes was significant in reducing the wort viscosity (see Tables II and III), suggesting that, like in barley, β -glucanase enzymes of malted sorghum are not active during mashing³⁴. Added commercial enzymes also increased FAN production almost 2-fold and the sugars released into wort were increased dramatically. Despite the dramatic increase in sugars, the glucose to maltose ratio was again, similar to those obtained when no commercial enzymes were added to the mash (see Table IV).

Sorghum malt was mashed at a higher temperature of 75°C plus added commercial enzyme preparations (20 μ L) and the results obtained are shown in Table VI. The iodine starch test showed that of the four sorghum varie-

TABLE II. Some properties of sorghum varieties studied.

	SK5912	KSV 4	KSV 8	ICSV 400
Moisture (%)	9.5	10.5	9.6	8.5
1000 corn weight (g)	30	32	29	23
G. E. (4 mL test) %	96	96	96	94
G. C. (%)	98	97	99	98
TN (%) as is	1.65	1.54	1.55	1.75
Crude protein (N \times 6.25) %	10.3	9.6	9.7	10.9

TABLE IV. Some properties of sorghum malt mash (65°C) no added commercial enzymes.

	SK5912	KSV 4	KSV 8	ICSV 400
Wort original gravity 20°C	1025	1030	1025	1025
HWE (L°/Kg)	235	262	209	221
Iodine starch test	+ve	+ve	+ve	+ve
Wort viscosity (cP)	1.20	1.18	1.22	1.27
FAN (mg/L) ninhydrin	42	51	80	55
Reducing sugars				
Glucose (g/L)	7.1	7.5	6.7	6.7
Maltose (g/L)	11.6	12.3	10.9	10.9
Glucose : maltose ratio	1:1.6	1:1.6	1:1.6	1:1.6

TABLE III. Some properties of the malt of the sorghum varieties studied.

	SK5912	KSV 4	KSV 8	ICSV 400
Moisture (%)	5.5	5.2	5.7	5.5
Malting loss (%)	25.5	24.6	21.5	22.1
Cold water extract %	36	36	31	36
Hot water extract (L°/kg)	235	262	209	221
Wort colour (°Lovibond)	10.0	10.5	9.0	9.5
Dextrinizing units (DU)	25	26	23	24
Diastatic power (DP) °L	29	30	27	28
DU:DP ratio	1:1.2	1:1.2	1:1.2	1:1.2

TABLE V. Some properties of sorghum malt mash (65°C) plus commercial enzymes (20 μ L).

	SK5912	KSV 4	KSV 8	ICSV 400
Wort original gravity 20°C	1025	1028	1027	1025
HWE (L°/Kg)	242	268	215	228
Iodine starch test	+ve	+ve	+ve	+ve
Wort viscosity (cP)	1.05	1.11	1.08	1.03
FAN (mg/L) ninhydrin	84	101	152	97
Reducing sugars				
Glucose (g/L)	40.3	46.4	44.7	54.7
Maltose (g/L)	64.3	74.7	71.8	88.9
Glucose : maltose ratio	1:1.6	1:1.6	1:1.6	1:1.6

ties studied, only SK 5912 had a negative iodine starch test, whilst KSV 4, KSV 8 and ICSV 400 showed a positive iodine starch test. It is therefore likely that the starches present in SK 5912 are more susceptible to enzyme hydrolysis during mashing at 75°C. This may explain why SK 5912 was one of the pioneer sorghum varieties selected and recommended for brewing in the early 1980's²⁶. The iodine starch test results therefore suggest that SK 5912 may have physiological differences from KSV 4, KSV 8 and ICSV 400, which is reflected in starch structure. The increase in mashing temperature showed a marginal increase in FAN production and sugars released into the wort, but the effect on wort viscosity was not significant. Although the sugar released into the wort increased marginally at the 75°C mashing temperature, the glucose to maltose ratio did not change, and were similar to those shown in Tables IV and V.

Some interesting results were obtained when the sorghum malts were mashed at 85°C with similar concentrations of commercial enzyme preparations (20 µL). The results obtained are shown in Table VII. Here, all the sorghum varieties studied showed a negative iodine starch test because the starches of the sorghum samples were effectively gelatinised followed by hydrolysis. This observation confirms, though indirectly, that sorghum starch will gelatinize above 80°C³². The negative iodine starch test confirmed the release of higher levels of reducing sugars into the wort. Again, as was the case with the previously reported data in Tables IV, V and VI, the glucose to maltose ratio did not change (see Tables IV to VII). However, the higher mashing temperature of 85°C resulted in an increase in FAN production, probably because of the high temperature tolerance of the commercial neutral protease enzyme used in this study. It is interesting to note that the wort viscosity did not change, and suggests that the low level of β-glucanase enzyme used in

this study was sufficient to produce a low viscosity in the wort of sorghum malt by hydrolysing the β-glucans and releasing glucose into the wort (see Tables III to VII). This is important because these enzymes are expensive to purchase.

When the mashing temperature was further increased to 95°C, plus added commercial enzyme at the 20 µL level, some important changes in the wort properties were observed (Table VIII). At this 95°C mashing temperature, a drop in FAN production was seen for the sorghum malts made from SK 5912, KSV 4 and KSV 8, but not ICSV 400. This highlights more physiological differences between the sorghum varieties studied. The most probable reason for the drop in FAN production is because this mashing temperature was very high for the commercial protease enzymes used in this study, or because the proteins of the sorghum samples had been denatured⁷. Again, whilst the reducing sugars released into the wort increased further at this mashing temperature for SK 5912, KSV 4 and KSV 8, no further increase in reducing sugar production was observed for ICSV 400. As was the case with the glucose to maltose ratio reported in Tables III–VI, the sorghum malts mashed at 95°C also produced glucose and maltose at a similar ratio (Table VIII). These results showed that ICSV 400 and SK 5912 were physiologically different from KSV 4 and KSV 8, both of which behaved in a similar way.

CONCLUSIONS

This work showed that the mashing temperature employed in mashing sorghum is very important in assessing the performance of sorghum as a brewing material. At the mashing temperature of 65°C, generally used in mashing barley malt, sorghum starch was not adequately gelatinised and sugar release was sub-optimal even when commercial enzymes were added during mashing at this temperature. However, at the mashing temperature of 85°C and above, sorghum starch was gelatinised effectively and sugar release into the wort was higher than at 65°C; and even higher when commercial enzymes were included at a very low rate. Although higher temperatures and added commercial enzyme preparations used in mashing sorghum malt dramatically increased the sugars released into the wort of sorghum mash, the ratio of glucose to maltose did not change. This might relate to the structure of sorghum starch, as well as to the pattern of development of α-amylase and β-amylase enzymes during the malting of sorghum^{1,2}. The influence of commercial enzyme addition

TABLE VI. Some properties of sorghum malt mash (75°C) plus commercial enzymes (20 µL).

	SK5912	KSV 4	KSV 8	ICSV 400
Wort original gravity 20°C	1030	1025	1032	1030
HWE (L°/Kg)	258	276	238	236
Iodine starch test	-ve	+ve	+ve	+ve
Wort viscosity (cP)	1.06	1.12	1.12	1.09
FAN (mg/L) ninhydrin	92	118	163	127
Reducing sugars				
Glucose (g/L)	44.7	60.2	52.4	48.2
Maltose (g/L)	71.8	98.1	84.9	77.8
Glucose : maltose ratio	1:1.6	1:1.6	1:1.6	1:1.6

TABLE VII. Some properties of sorghum malt mash (85°C) plus commercial enzymes (20 µL).

	SK5912	KSV 4	KSV 8	ICSV 400
Wort original gravity 20°C	1030	1026	1034	1038
HWE (L°/Kg)	282	296	268	292
Iodine starch test	-ve	-ve	-ve	-ve
Wort viscosity (cP)	1.07	1.15	1.10	1.04
FAN (mg/L) ninhydrin	275	358	167	147
Reducing sugars				
Glucose (g/L)	54.7	63.3	50.2	54.7
Maltose (g/L)	88.9	103.4	81.2	88.9
Glucose : maltose ratio	1:1.6	1:1.6	1:1.6	1:1.6

TABLE VIII. Some properties of sorghum malt mash (95°C) plus commercial enzymes (20 µL).

	SK5912	KSV 4	KSV 8	ICSV 400
Wort original gravity 20°C	1032	1034	1034	1035
HWE (L°/Kg)	298	302	306	298
Iodine starch test	-ve	-ve	-ve	-ve
Wort viscosity (cP)	1.06	1.16	1.10	1.07
FAN (mg/L) ninhydrin	160	126	114	147
Reducing sugars				
Glucose (g/L)	57.3	66.8	72.4	54.7
Maltose (g/L)	93.3	109.3	117.3	88.9
Glucose : maltose ratio	1:1.6	1:1.6	1:1.6	1:1.6

was also apparent on other substrates such as β -glucan and proteins, because wort viscosity was reduced and FAN production increased, when commercial enzymes were used at the lowest dose rate. The different behaviour of the sorghum varieties studied under similar conditions further highlight physiological differences in sorghum varieties. Therefore good quality sorghum malts can be produced. However, mashing will employ commercial enzymes and mashing regimes are not yet optimised.

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