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Effects of steeping condition and germination time on the alpha-amylase activity, phenolics content and malting loss of Nigerian local red and hybrid short Kaura sorghum malts

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The effects of steeping condition (STC) and germination time (GMT) on the phenolics content (PC), alpha-amylase activity (DP) and malting loss (ML) of Nigerian local red (LRS) and hybrid (HSK) grain sorghum were investigated in 6-day micro-malting trials. Air-rested steeping (ARM) effected the highest increase in DP of both LRS (51.7%) and HSK (30.2%), which occurred on the fifth day of germination, while the lowest increase was from the low-temperature steeping treatment (LTM) in LRS (12.6%) and HSK (5.32%), based on the control (CSM). Reduction in PC was proportional to GMT. The greatest reduction, which occurred on the sixth day, in LRS (71.4%), was caused by formalin re-steeping of ARM germinates (FRM), while the least reduction was caused by ARM (28.3%) in HSK. Malting loss (ML) increased with GMT while ARM caused the greatest loss (46.2%) in the 6-day LRS malt. On average, ML of LTM was not significantly different from that of CSM in HSK, but it was in LRS ($P \leqslant 0.05$). Results of second-order ANOVA showed that the main factors—STC (A), GMT (B) and sorghum variety, SGV (C)—have significant influences ($P \leqslant 0.025$) on DP and ML, while SGV had none on PC. The interaction A×B affected DP ($P \leqslant 0.10$) and ML ($P \leqslant 0.005$); A×C exerted no statistically significant effects on any of the three quality attributes, while B×C influenced only DP ($P \leqslant 0.05$). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Steeping condition (Axcell *et al.*, 1983) and duration of germination (Novellie, 1962*a,b*; Morral *et al.*, 1986; Iwuoha, 1988; Ilori & Akingbala, 1991) have been identified as crucial quality factors of malted grains.

Sorghum is fast becoming a substitute for barley (the traditional cereal of choice in conventional brewing) in the brewing sector of the Nigerian economy as a result of recent deliberate policy. Consequently, scientists, technologists, research institutes and companies have attempted to contribute in various ways to realizing the objective of this decision (Skinner, 1976; Okafor & Anichie, 1980, 1987; Aina & Olapade, 1981; Federal Institute for Industrial Research Oshodi, 1983; Onwuzulike, 1984; Chukwura, 1985; Apata, 1986; Malomo, 1987; Toyon, 1987; Iwuoha, 1988; Ilori & Akingbala, 1991).

Two main problems have been reported to be responsible for low amylolytic activity in sorghum malt: the presence of phenolics, which have the capacity to inhibit amylase activity in sorghum (Miller & Kneen, 1947; Strumeyer & Malin, 1969; Daiber, 1975; Davis & Hoseney, 1979; Chukwura & Muller, 1982; McGrath *et al.*, 1982); and inherent inappropriate structuring and functions of the grain in enzyme release during malting and mashing relative to barley (Aisien, 1982; Aisien *et al.*, 1983; Palmer *et al.*, 1989; Etokakpan & Palmer, 1990, 1994; Palmer, 1991; Etokakpan, 1992). In response to this problem, several workers initiated researches to control the inhibitory activity of phenolics and to simultaneously reduce malting loss; chemicals were used in steep liquor and/or sprayed onto seeds during germination (Daiber & Novellie, 1968; Toyon, 1987; Ilori & Akingbala, 1991).

Studies on steeping with higher frequency of air-resting prior to steep-out are relatively few. Information

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on the use of chemical re-steeping on grain sorghum germinates, as had been done for barley (Withey & Briggs, 1966; Briggs *et al.*, 1981; Axcell *et al.*, 1983), is scarce. There is a need to assess the effects of steeping Nigerian sorghums at reduced (sub-ambient) temperature.

The present study was designed to appraise the influence of steeping conditions and duration of germination on some quality attributes of Nigerian local red and hybrid sorghum malts.

MATERIALS AND METHODS

Chemicals

Glacial acetic acid GPR (99.5%, 1.048–1.051 g ml^{-1}), anhydrous ferric chloride GPR (96%) and Lintner's starch were obtained from BDH Chemicals (Poole, UK). Formaldehyde solution USP (37%, w/w, 1.09 g ml^{-1}), hydrochloric acid RG (37%, 1.1 g ml^{-1}), potassium ferricyanide reagent AGS (99%), potassium iodide reagent, ISO (99.5%) and anhydrous sodium thiosulphate (99%) were acquired from Hoechst Celanese Corp., Somerville, NJ, USA.

Plant materials

The local, short, red guinea corn (*Sorghum vulgare*) was procured from a retail outlet, while the short hybrid (SK 5912) strain (*Sorghum bicolor* Moench) was obtained from the Federal Institute for Industrial Research, Oshodi (FIIRO), Lagos, Nigeria.

Malt samples preparation

Each of the two grain types was prepared in batches of $\geq$1000 healthy kernels for each steeping condition, during which the steep liquor was changed six times within 24 h, giving rise to a steep-out moisture range of 37–43%.

Four steeping treatments were carried out:

- Steeping treatment 1. Control (CSM): grains were steeped for 24 h at 28°C without air-resting.
- Steeping treatment 2. ARM: grains were steeped as for treatment 1 but with four equal-duration, intermittent drainings of the steep liquor and half-hourly ventilation of the damped grains.
- Steeping treatment 3. FRM: a batch of 48-h germinates of ARM was re-steeped in 750 mg l^{-1} formaldehyde solution for 24 h (NB: formaldehyde is prohibited in certain countries as a food additive).
- Steeping treatment 4. LTM: grains were steeped at 18°C for 24 h without air-resting.

All the steeping treatments were duplicated.

After steeping, each batch of imbibed grains was germinated in a well-ventilated room that excluded photosynthesis (i.e. with simulated darkness) at 28°C and 68 ± 2% relative humidity. The grains were watered twice a day to prevent dehydration and the seeds were germinated for up to 6 days.

One-sixth of the total germinates was withdrawn at 24-h intervals, dried at 50°C (Okon & Uwaifo, 1984) and cleaned for further analyses.

Grain and malt analyses

The thousand kernel masses of both the grains and malts were measured according to the Institute of Brewing (1977) method. Moisture content was determined following the Association of Official Analytical Chemists (1984) method. Malting loss was measured using the 1000 kernel weight approach (Novellie, 1962*b*). Alpha-amylase activity (also referred to as diastatic power, DP) was determined using the aqueous extraction method as modified by Novellie (1959). Five millilitres of 5% (w/v) cold water extract was allowed to act on 100 ml of 2% buffered Lintner's starch solution. The reducing sugar produced was determined on a 5-ml aliquot of the filtrate using the alkaline ferricyanide titration method (American Society of Brewing Chemists, 1976). The titre was converted to sorghum diastatic units per gram (SDU g^{-1}), adopting the Novellie (1959) procedure for *Sorghum kaffircorn* (SDU is the amount of enzyme activity which, when acting under the conditions of the method, gives a thiosulphate titre of 0.5 ml of 0.05 N solution). The amylase activity was then calculated as diastatic power (DP, expressed as SDU g^{-1}, dry matter basis) as follows:

$$DP = \frac{200 \times V_t \times 100}{V_{EA} \times E(100 - MC)}$$

where V_t is titre (ml), i.e. blank less determination, V_{EA} is aliquot (ml) of extract used for diastasis (ml), E is cold water extract (%, w/v), and MC is moisture content (%, fresh weight basis) of the sample analysed.

The total phenolics contents of the grain and malt samples (10% (w/v) extracts) were evaluated following the method of Price & Butler (1977), which involves measuring the optical densities (OD) in 1-cm glass cells after a 10-min exposure at 720 nm on a Zeiss PMQ II spectrophotometer which has been zeroed with water. The OD values were then used to quantify the phenolics as absorbance units at 720 nm per gram dry matter (A_{720} g^{-1}).

Statistical analyses of data

The means of the quality attributes of the malts, as functions of the steeping conditions, were statistically assessed using the two-way analysis of variance (ANOVA) technique (Miller & Freund, 1977) for each of the two grain sorghum types. Multiple comparison tests were carried out using Tukey's procedure (Romano, 1977) to establish the extents of significant differences (where they occurred) for each attribute. Furthermore, integration of the steeping conditions, STC (4), germination time, GMT (6 days), and sorghum

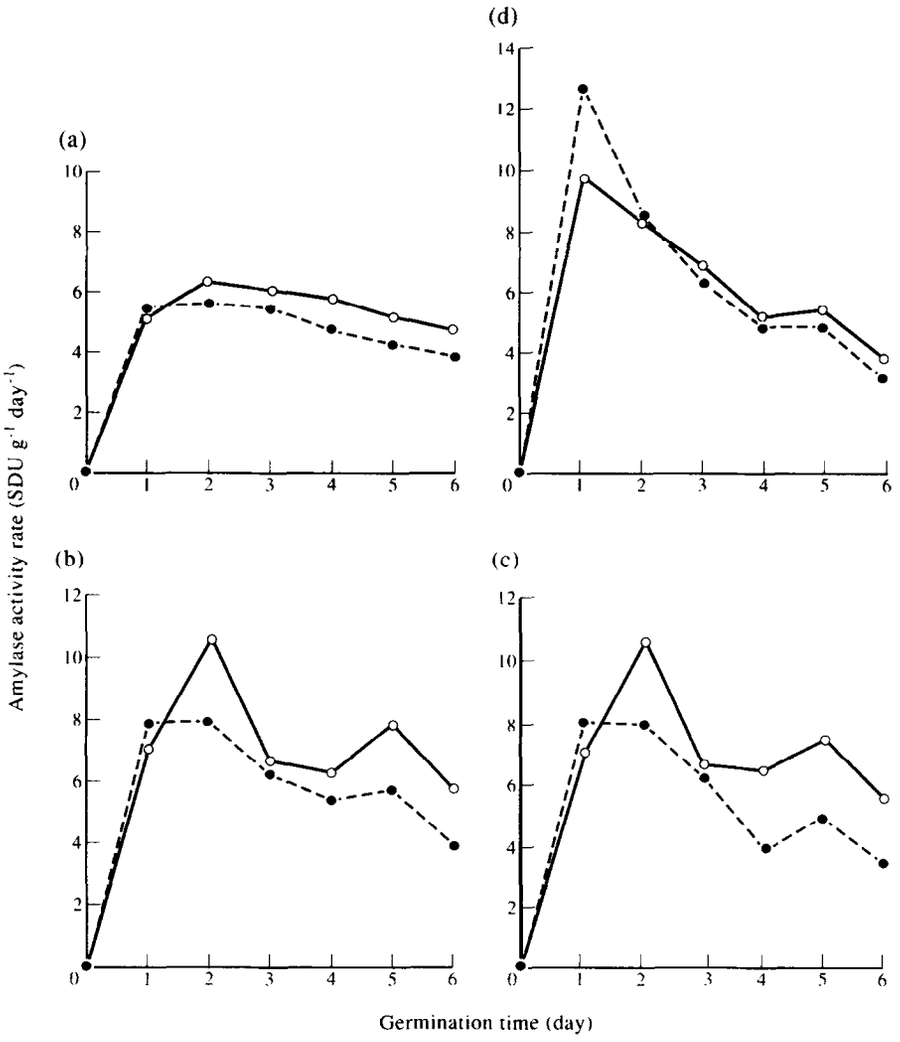

Fig. 1. Change in amylase activity of sorghum malts as a function of steeping condition and germination time: LRS (○—○), HSK (●- -●), A (control, CSM), B (ARM), C (FRM), D (LTM).

varieties, SGV (2), were fitted into a 4×6×2 factorial experimental design. The original test scores were then subjected to three-way (factor) ANOVA as described by Steel & Torrie (1980).

RESULTS AND DISCUSSION

Amylase activity

The results for alpha-amylase activity (DP) of Nigerian sorghum malts (NSM) as a function of steeping conditions (STC) and germination time (GMT) are illustrated in Fig. 1 (as rate quantities, AAR), while the means and standard deviations of the DP points are shown in Table 1 (local red sorghum, LRS) and Table 2 (hybrid short Kaura, HSK). The three-factor (STC (A), GMT (B) and sorghum variety, SGV (C)) ANOVA for statistical analyses of the quality parameters of NSM are reported in Table 3.

Amylase activity (DP) was directly proportional to GMT, reaching a maximum at the fifth day, while the rate of change of amylase activity (AAR) peaked within the first 48 h of germination and then decreased. The steepest decrease was in the low-temperature steeped (LTM) samples (Fig. 1d). This indicates some degree of chilling injury in the Nigerian (tropical) grain SGV (Iwuoha, 1988) as opposed to its temperate counterpart, barley (Brookes *et al.*, 1976; Aisien & Ghosh, 1978). The effects of STC (A) and GMT (B) on DP were found to result in different levels of significance (Tables 2 and 3), while their joint effects (interaction A×B) were not significant (Table 3). This underscores the point that steeping is the most critical stage in the malting process (Axcell *et al.*, 1983). In all, ARM caused the highest increase in DP of both LRS (51.7%) and HSK (30.2%) while the least increase was by LTM treatment in LRS (12.6%) and HSK (5.32%). These figures show that LRS, a pure (basic) strain, exhibited relatively higher DP than HSK, a hybrid, which agrees with the report by Daiber & Novellie (1968).

Phenolics content

The residual phenolics contents (PC) of NSM are shown in Table 1 (LRS) and Table 2 (HSK), and the rate of

Table 1. Effects of steeping conditions and germination time on the quality parameters of Nigerian local red (LRS) sorghum malt

Treatment	Parameter[a]		
	Amylase activity (DP, SDU g^{-1})	Phenolics content[b] (PC, A_{720} g^{-1})	Malting loss (ML, %)
CSM	19.2 ± 8.3^{a}	23.5 ± 2.5^{ab}	15.7 ± 8.1^{a}
ARM	24.8 ± 10.7^{b}	28.8 ± 6.6^{b}	37.2 ± 10.1^{a}
FRM	24.5 ± 10.2^{b}	26.5 ± 8.6^{ab}	32.6 ± 7.5^{c}
LTM	19.8 ± 5.5^{a}	21.3 ± 4.6^{a}	22.7 ± 6.6^{d}

[a]Values are mean ± standard deviation of duplicate determinations for 24 h steeped grains, germinated at 28°C for up to 6 days.
[b]The value for untreated LRS grain was 54.5 A_{720} g^{-1}.
[a–d]Means with common superscripts in the same column do not differ significantly according to Tukey's test ($P = 0.05$).
DP, diastatic power; SDU, sorghum diastatic unit; CSM, malt from control (no air-rest) steeping (28°C, 24 h); ARM, malt from air-rested steeping (28°C, 24 h); FRM, malt from formalin re-steeped ARM germinates (28°C, 24 h); LTM, malt from low-temperature steeping (18°C, 24 h).

Table 2. Effects of steeping conditions and germination time on the quality parameters of Nigerian hybrid short Kaura (SK 5912) (HSK) sorghum malt

Treatment	Parameter[a]		
	Amylase activity (DP, SDU g^{-1})	Phenolics content[b] (PC, A_{720} g^{-1})	Malting loss (ML, %)
CSM	16.6 ± 6.4^{a}	23.8 ± 5.4^{ab}	18.4 ± 8.8^{a}
ARM	19.6 ± 6.9^{a}	28.3 ± 2.5^{b}	24.0 ± 3.3^{a}
FRM	17.5 ± 6.6^{a}	25.0 ± 7.0^{ab}	23.3 ± 3.0^{a}
LTM	18.9 ± 3.7^{a}	21.7 ± 4.9^{a}	21.5 ± 9.5^{a}

[a]Values are mean ± standard deviation of duplicate determinations for 24 h steeped grains germinated at 28°C for up to 6 days.
[b]The value for untreated HSK grain was 33.9 A_{720} g^{-1}.
[ab]Means with common superscripts in the same column do not differ significantly according to Tukey's test ($P = 0.05$).
DP, diastatic power; SDU, sorghum diastatic unit; CSM, malt from control (no air-rest) steeping (28°C, 24 h); ARM, malt from air-rested steeping (28°C, 24 h); FRM, malt from formalin re-steeped ARM germinates (28°C, 24 h); LTM, malt from low-temperature steeping (18°C, 24 h).

Table 3. Three-factor (second order) ANOVA of steeping conditions (STC), germination time (GMT) and grain variety (SGV) on the quality parameters of sorghum malts

Parameter	Variance ratios (F value)						Mean square error
	A (STC)	B (GMT)	C (SGV)	Interactions			
				A×B	A×C	B×C	
Amylase activity (DP)	5.0^{***}	53.8^{****}	21.6^{****}	1.5^{NS}	2.6^{*}	3.2^{**}	8.5
Phenolics content (PC)	13.2^{****}	29.5^{****}	0.1^{NS}	1.2^{NS}	0.3^{NS}	0.4^{NS}	8.2
Malting loss (ML)	23.2^{****}	23.3^{****}	17.3^{****}	1.8^{NS}	8.4^{****}	0.6^{NS}	19.0

Analyses were done for two grain varieties, four different steeping conditions and each grain sample malted for up to 6 days.
Significant at $^{*}P < 0.01$, $^{**}P < 0.05$, $^{***}P < 0.02$ and $^{****}P < 0.005$ levels; ^{NS}not significant ($P > 0.005$, $P > 0.10$).

change of phenolics reduction (PRR) as a function of STC and GMT is depicted in Fig. 2.

The PC decreased progressively with an increase in GMT. The reason for the latter could be in line with the reports that, during steeping and subsequent germination of sorghum grains, phenolics, which are primarily located in the pericarp and testa (Blessin *et al.*, 1963; Leucere, 1982; Kock *et al.*, 1985), are leached (Chavan *et al.*, 1981), resulting in lowering of the residual PC values. The greatest reduction in PC was caused by FRM treatment in LRS (from 54.5 to 15.6 A_{720} g^{-1}, 71.4%) and in HSK (from 33.9 to 11.1 A_{720} g^{-1}, 67.3%), while the lowest reduction was by ARM steep-treatment in HSK (28.3%). From the stand-point of PRR, LTM gave the greatest (Fig. 2d) while ARM was least (Fig. 2b).

The effects of STC (A) and GMT (B) on PC were statistically significant (Tables 1–3) but SGV (C) was not. Their joint effects (interactions A×B, A×C, B×C) were not (Table 3). These observations indicate the importance of each of these factors (optimum STC and GMT) in reducing PC in sorghum grains during malting.

Malting loss

Malting loss (ML) increased with GMT, more for LRS (37.2%) caused by ARM treatment (compare Tables 1

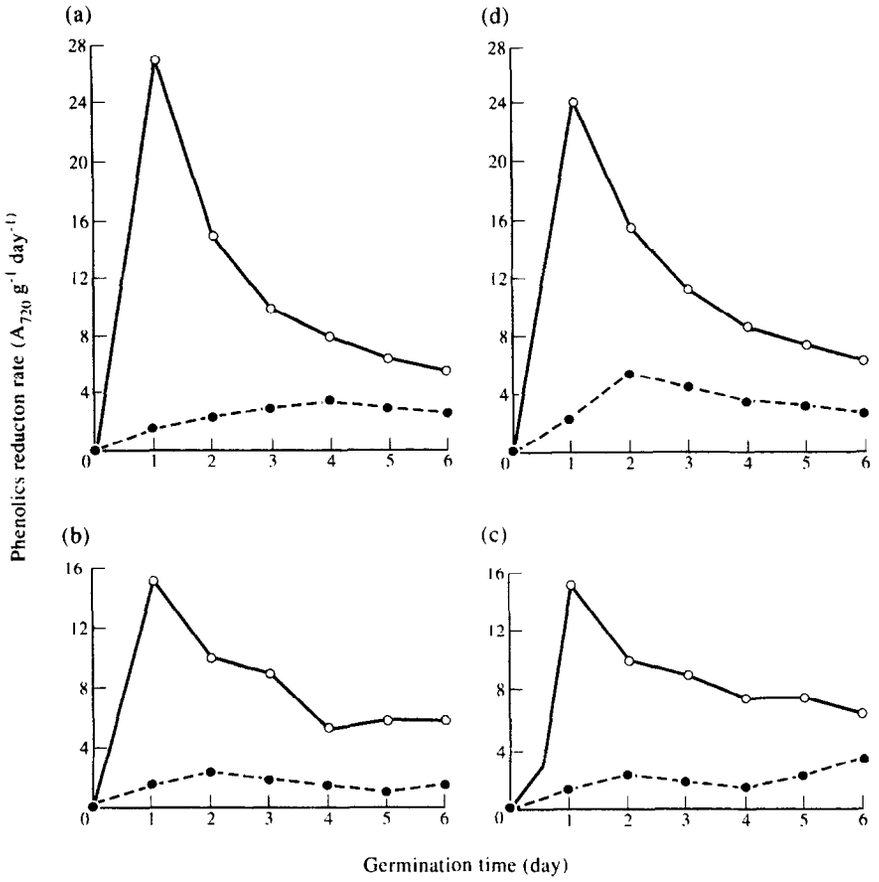

Fig. 2. Rate of reduction in phenolics content of sorghum malts as a function of steeping condition and germination time: LRS (○—○), HSK (●- -●), A (CSM), B (ARM), C (FRM), D (LTM).

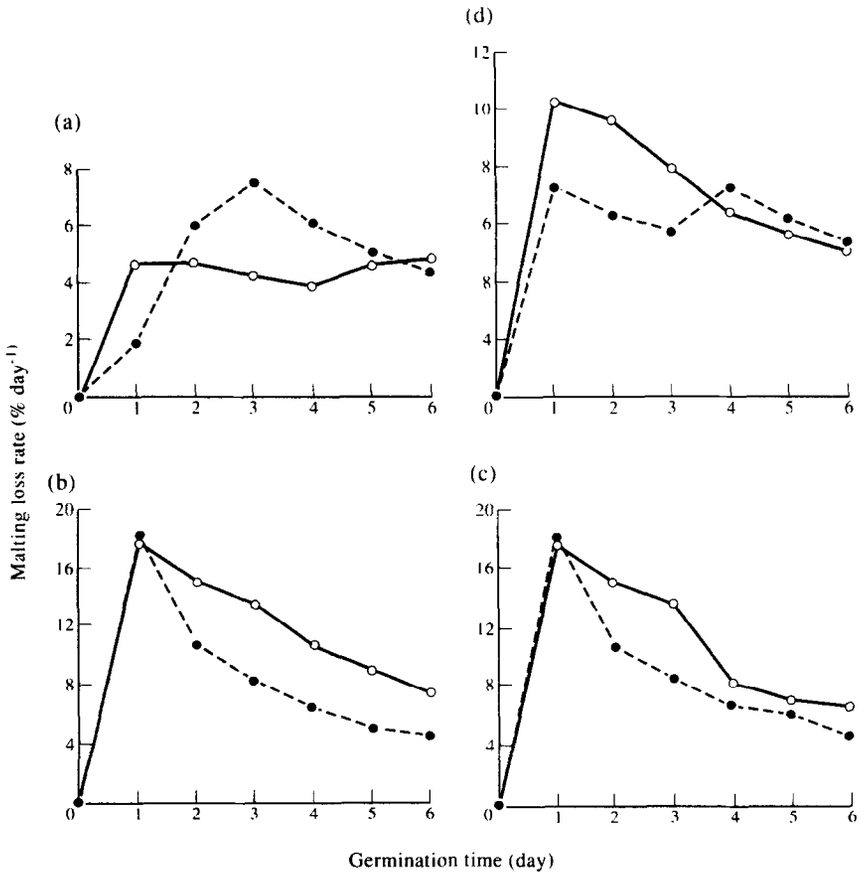

Fig. 3. Change in malting loss during sorghum malting as a function of steeping condition and germination time: LRS (○—○), HSK (●- -●), A (CSM), B (ARM), C (FRM), D (LTM).

and 2). This outcome has been reported as being not in any way supplementary to the simultaneous improvement in DP (Novellie, 1962*b*); rather, the increased ML is a characteristic feature of seedling growth and malting (Aisien, 1982; Aisien *et al.*, 1983; Iwuoha, 1988). The rate of change of malting loss (MLR) in NSM was also highest with ARS (Fig. 3(b)) while the least was caused by CSM treatment for HSK (Fig. 3(a)).

The effects of STC (A) on ML was significant for LRS (Table 1) but not for HSK (Table 2), while GMT (B) and SGV (C) also affected ML significantly ($P \leqslant 0.005$, Table 3). Only the interaction A×C affected ML statistically, which seems to suggest that adequate control of STC for any SGV will influence the magnitude and extent of ML.

CONCLUSION

Steeping treatments (STC) and germination time (GMT) effected various significant changes in the test quality parameters of Nigerian sorghum malts (NSM). The maximum diastatic power obtained in this study for SGV (LRS), which is equivalent to 20°L, fell short of that for barley (lager) malt which is up to 80°L. The LRS (pure strain) exhibited relatively better malting characteristics than HSK (hybrid) as functions of STC and GMT.

With the poor-quality attributes of sorghum, the alternatives open to Nigeria are:

1. to use the indigenous SGV and produce national beer brands, as done in South Africa with Kaffir Beer,
2. to aggressively employ genetic engineering to improve on the DP of the SGV,
3. to go on using barley malt, solely or predominantly, or
4. to introduce extraneous enzyme systems to the NSM during mashing.

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