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Effects of Cultivar and Germination Time on Amylolytic Potential, Extract Yield and Wort Fermenting Properties of Malting Sorghum

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Abstract: Malts from three improved Nigerian sorghum cultivars (*Sorghum bicolor* L.; ICSV 400, ICSH 89009 and ICSH 89002) were evaluated for amylolytic enzyme development, extract yield, free α-amino nitrogen (FAN) and wort fermentability. In general, diastatic activity, α-and β-amylolytic activities were significantly affected at $p \leq 0.001$, $p \leq 0.001$ and $p = 0.05$ respectively, by cultivars and germination time. Peptone extraction showed that high portions of total malt amylase activity occurred in the bound form for ICSH 89002 (60-73%), relative to ICSV 400 (26-48%) and ICSH 89009 (21-25%) cultivars. Moreover, levels of malt amylases occurring in the free and bound forms for ICSH 89002 and ICSV 400 were influenced by duration of germination at $p = 0.05$. However, cold-water extract development was only affected by grain cultivars, while hot water extracts of malts were influenced by germination periods ($p = 0.1$). Maltose constituted the major wort fermentable sugar (74-80% of total fermentable sugar), followed by maltotriose (9.6-15.6%) and glucose (7.6-8.8%). ICSV 400 recorded the highest levels of wort FAN (162-176 mg FAN/L), relative to ICSH 89009 (106-152 mg FAN/L and ICSH 89002 (85-115mg FAN/L). Similarly, worts from ICSV 400 recorded better fermentability values (80%), compared to ICSH 89009 (78 and 76%) and ICSH 89002 (76 and 77%), for the 4 and 6-day malt worts, respectively. The high disparity between malt β-amylolytic activities and wort maltose levels, suggests that malt amylolytic activities alone, are inadequate for assessing mash performance and extract development of sorghum particularly with bound enzyme forms. Moreover, there exists an enzymic/substrate critical level for the mashes of ICSH 89009 and ICSV 400 cultivars, within which sugar production increases with substrate concentration.

Key words: Sorghum, diastatic power, β-amylase, extract, maltose, wort fermentable sugars, free α-amino nitrogen, cultivars, germination

INTRODUCTION

Malt is the major raw material used in the brewing industry. Barley is traditionally the cereal chosen for malting in order to develop enzymes (Kuntz and Bamforth, 2007). In Nigeria, where attempts to cultivate barley have met with little success, the high cost of importing barley malt, in conjunction with the rising demand for European-type lager, has forced the use of local cereals particularly sorghum, as a malting and brewing grain. Sorghum

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(*Sorghum bicolor* L. Moench) is a cultivated tropical grass which originated in Africa about 3000 to 5000 years ago (Taylor and Belton, 2002). Sorghum like other cereal grains such as barley, maize, rice and wheat belong to the grass family-the Gramineae and it is widely grown in other parts of the countries. It is an important cereal crop to humankind ranking fifth in terms of overall cereal production after wheat, rice, maize and barley (Taylor and Belton, 2002). Among other applications, sorghum is used in the production of traditional opaque beer, non-alcoholic beverages in developing countries and until recently production of lager-type clear bear (Taylor and Dewar, 2001; Palmer *et al*., 1989). In Nigeria sorghum is malted commercially on a large scale for production of lager-beer, stout and for non-alcoholic malt-based beverages (Taylor and Belton, 2002). The primary quality criterion for their use is their potential to produce malt with diastatic power (amylase activity) which are needed to hydrolyze starch molecules and produce fermentable sugars. Hence, the fundamental role of the malting process is the mobilization of these endogenous hydrolytic enzymes (amylases) of the grain that will digest starch (Kanauchi and Bamforth, 2008). Sorghum malting involves three steps: steeping, germination and drying/kilning. Germination of grains is an essential part of the malting process because when grains do not germinate, or germinate poorly, they do not contribute to the enzyme development of the malt and uneven modification of the malt occurs (Agu and Palmer, 1998).

Etokakpan (1992) opined that sorghum malt is inferior to barley in the development of key hydrolytic enzymes and extract potential. This could however, be attributed to malting conditions and the variety of sorghum used as differences have been reported in the composition and quality of sorghum malted by the regime used for barley (Etokakpan, 1992) and that developed for sorghum (Ezeogu and Okolo, 1994, 1995, 1999; Okolo and Ezeogu, 1996). This observation, besides demonstrating differences in malting behaviour between sorghum cultivars, also shows the possibility of optimizing sorghum malting and mashing performance. Recent agronomy research on sorghum has therefore centered mainly on its improvement as a malting and brewing grain. Intensified research on the maltability of sorghum would therefore, be useful for the selection of progeny with high mating potential from a breeding programme. As part of research geared towards the optimization of sorghum brewing potential through improvements in sorghum malt enzymic character and other malt quality parameters, the influence of varietal differences and duration of germination of diastatic enzyme development, extract yield and wort fermentable properties of three improved Nigerian sorghum cultivars are hereby reported.

MATERIALS AND METHODS

Sorghum Varieties

Three improved Nigerian sorghum varieties (ICSV 400, ICSH 89009 and ICSH 89002) were obtained from the National Seed Service, Zaria Nigeria. The three cultivars had good germinative energies and were not water sensitive.

Sorghum Malting

The Sorghum grains were cleaned by winnowing and sorting to removed dust, broken kernels and foreign materials. Thereafter, samples (250 g) were surface sterilized by immersion for 20 min in sodium hypochlorite (NaOCl) solution containing 1% available chlorine, then drained and washed thrice in tap water (Ezeogu and Okolo, 1995; Kuntz and Bamforth, 2007). A steep cycle of 6 h wet; 3 h dry for 45 h was generally applied followed by a final warm water (40°C) steep period of 3 h. At the end of the steep cycle, grains were again subjected

to surface sterilization. Germination was carried out for 6 days in shallow wooden trays with fine mesh bottoms, in an atmosphere of near water saturation at 30°C. At 12-hourly intervals, the sprouted grains were turned and sprayed with 5 mL-distilled water using an atomizer spray (Ezeogu and Okolo, 1995). Samples of each cultivar were collected at 24 h intervals and kilned for 24 h in a forced drought oven maintained at 50°C. The dried roots and shoots (culms) were rubbed off by hand in a sieve. The grains were milled for two 30 sec periods in a cooled warning blender at high speed and used for analyses.

Malt Characteristics Methods

Germinative activity was determined using triplicate samples (50 seeds each) in open petri dishes lined with Whatman No. 4 filter paper. Water (4 mL) was added and the dishes were placed in a germinator at 28°C, 95% rh. Seeds that developed roots and shoots were counted after 72 h and the percentage was recorded (Beta *et al*., 1995).

Diastatic Power and β-Amylase Activity Assays

Diastatic power (total reducing activity) and β-amylase activity were determined by the diamylase procedure of Etokakpan and Palmer (1990), in which β-amylase activity is selectively inhibited by $HgCl_2$ and calculated as the difference between diastatic power and β-amylase activity. One unit of enzyme activity is defined as any amount of enzyme capable of releasing lug glucose equivalent per minute.

Bound Amylase Activity Assay

Bound amylase was determined by the diamylase procedure of Etokakpan and Palmer (1990) with minor modification. Two percent (2%; w/v) Bactopeptone was added to the extractant (sodium acetate butter pH 5.7) prior to extraction as described by Novellier (1960).

Cold and Hot Water Extract

Cold-water extracts were determined by Molmes (1991) modification of the Institute of Brewing method #2.5. Hot water extracts were determined using Etokakpan's (1992) decantation procedure, in which enzymic wort is separated and then re-added to the gelatinized and cooled sorghum starch.

Free α-Amino Nitrogen

Wort Free α-amino nitrogen was determined by the Ninhydrin method of the Institute of Brewing.

Total Reducing Sugars and Wort Fermentability

Total reducing sugars were analysed by the method of Jayarayamau (1981). The reducing sugar profiles of wort were determined by the paper chromatographic method of Dubois *et al*. (1956). Wort fermentability was determined using the standard yeast fermentation procedure #3.9.8 of the Institute of Brewing.

Statistical Analysis

The effects of cultivar and duration of germination on diastatic power, α-and β-amylase activity, free α-amino nitrogen and extract development, as well as fermentable sugar profiles and wort germentability, were resolved by Analysis of Variance (ANOVA) and correlation studies. Means that differed significantly were identified by students' t-test and Least Significant Difference (LSD) tests. Results are presented as means of duplicate experiments.

RESULTS

The three sorghum varieties (ICSV 400, ICSH 89009 and ICSH 89002) investigated had good germinative properties as revealed in their high viabilities with no tendency of dormancy (Table 1). Figure 1 showed the time course development of diastatic activity in the three sorghum cultivars. The result indicated that the diastatic enzyme development generally increased from the first day of steeping for the three cultivars. Maximum values were observed on the second day of germination for ICSH 89002 and on the third day of germination for both ICSH 89009 and ICSV 400. Thereafter, a second Diastatic Power (DP) high point for both ICSV 400 and ICSH 89002 was obtained on the fifth day of germination. Analyses of variance data indicate that DP development for these cultivars was highly significantly ($p = 0.001$) dependent on cultivar and malting time. ICSV 400, generally showed better DP activity, compared to both ICSH 89009 and ICSH 89002. At maximum activity, DP development in ICSH 89002 represented 2.4 and 2.7-fold lower DP values compared to corresponding malts from ICSH 89009 and ICSV 400, respectively.

The time course development of α-amylolytic activity in three sorghum cultivars is depicted in Fig. 2. The data obtained showed that malt α-amylase activity increased from day 1 and peaked on the second day of germination for both ICSH 89002 and ICSH 89009 but on the fifth day of germination for ICSV 400. Whereas α-amylolytic activity for cultivars ICSH 89009 and ICSH 89002 increased with time until peak values, ICSV 400 showed two high points of activity on the third and fifth days. Two-way analysis of variance showed that the development of α-amylolytic activity was highly significantly ($p \leq 0.001$) influenced by cultivars and malting period.

Table 1: Germinative properties of the three sorghum varieties

	Sorghum varieties		
Characteristics	ICSH 89002	ICSH 89009	ICSV 400
Moisture content (%)	9.65	9.76	10.86
Germinative capacity	95.5	95.5	97.5
Germinative energy (%)	96.5	96.5	98.0
Water sensitivity (%)	1.0	1.0	2.0

Data are presented as means of triplicate

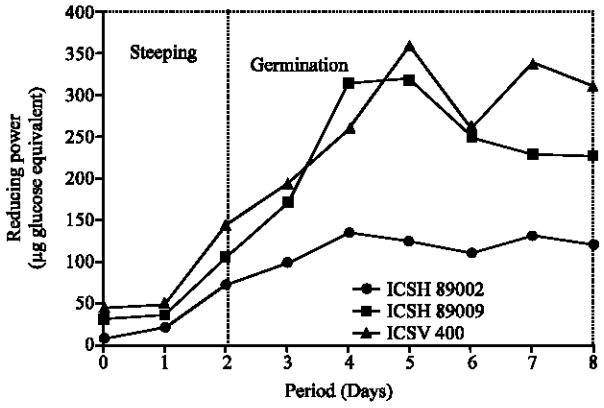

Fig. 1: Time course development of diastatic activity (Total reducing power) three sorghum cultivars

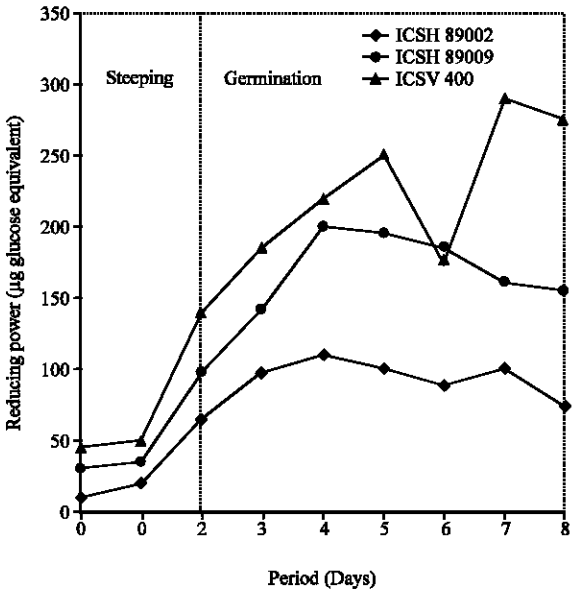

Fig. 2: Time course development of alpha-amylolytic activity in the three sorghum cultives

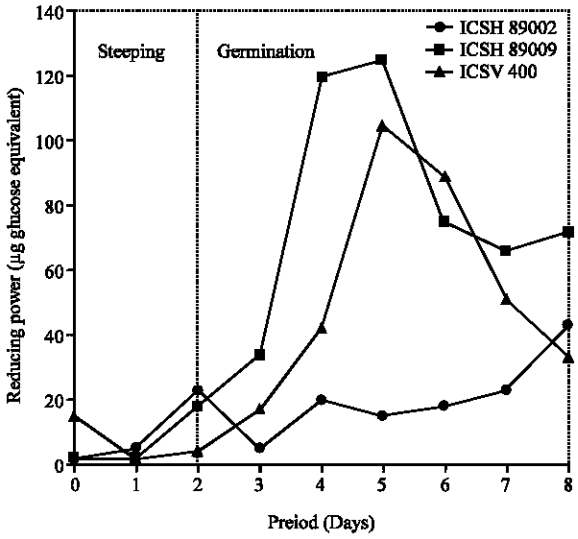

Fig. 3: Time course development of beta-amylolytic activity in three sorghum cultivars

Figure 3 showed the time course development of β-amylolytic activity in the three sorghum cultivars. The data obtained indicated that β-amylase activity was optimal on the sixth day of germination for ICSH 89002 and on the third day of germination for both ICSH 89009 and ICSV 400. At peak diastatic activity (DP high points), β-amylolytic activity represented 36.7, 28.6 and 15.6% of total malt saccharifying amylase activity for ICSH 89009, ICSV 400 and ICSH 89002 cultivars, respectively. The extraction of bound amylases (Table 2) indicate that only a small portion of malt amylases occurred in the bound form for

ICSH 89009 (12-25%) compared to ICSV 400 (26-48%) and ICSH 89002 (60-73%), for the fourth and sixth day malts, respectively. Table 3 shows the Cold Water Extracts (CWE) of the sorghums for the 4 and 6-day malts profile of the cultivars. The result obtained for the Cold Water Extract (CWE) development of the four and six-day sorghum malts revealed that the four-day malts had more cold-water soluble materials than 6-day malts, with ICSV 400 recording the highest values (25.0 and 22.3, respectively) of CWE at both duration of germination.

The effects of cultivar and germination time on the development of malt Hot Water Extracts (HWE) are shown in Table 4. Patterns of HWE development were similar to the CWE, except that ICSH 89009 recorded a higher value of HWE from the 6-day malt. It could be observed that generally the HWE levels from the 4 and 6 day malts of ICSH 89002 respectively, were significantly ($p \leq 0.05$) lower relative to ICSV 400 at both duration of germination.

Table 5 showed the effects of cultivar and germination time on the development of wort free α-amino nitrogen (FAN). Results obtained revealed that all the cultivars had higher FAN presented in Table 6. Paper chromatogram showed that maltose, maltotriose and glucose, were the principal sugars of the worts. The effects of exogenous starch (soluble starch) concentration on total soluble sugar production in the 4 and 6-day malt worts are shown in

Table 2: Development of amylolytic enzyme activity in the 4 and 6 day sorghum malts

		*DEP[1]			*D-PEP[2]			*PSA[3]		
Cultivars	Germination time (days)	DP[4]	α -A[5]	β-A[6]	DP[4]	α -A[5]	β-A[6]	DP[4]	α -A[5]	β-A[6]
ICSH 89002	4	111	93	18	407	246	161	296	153	143
	6	113	74	40	285	221	64	172	147	24
ICSH 89009	4	269	196	73	359	230	129	90	34	56
	6	236	164	72	300	209	91	64	45	19
ICSV 400	4	270	182	88	522	344	178	252	162	90
	6	315	279	36	426	289	137	111	10	101

*DEP[1]: Diamylase extraction procedure, *D-PEP[2]: Diamylase-peptone extraction procedure, *PSA[3]: Peptone soluble amylase, DP[4]: Diastatic power activity, α -A[5]: α-amylase activity, β-A[6]: β-amylase activity. *Activity expressed as μg glucose equivalent

Table 3: Cold Water Extracts (CWE) of the sorghums for the 4 and 6 day malts (%)

	Germination time (days)	
Cultivars	4	6
ICSH 89002	20.7±1.05	19.2±0.9
ICSH 89009	19.2±0.95	18.5±0.9
ICSV 400	25.0±1.25	22.3±1.1

Table 4: Hot Water Extracts (HWE) of 4 and 6 day the sorghums malts (L° kg^{-1})

	Germination time (days)	
Cultivar	4	6
ICSH 89002	282.4±14.1	245.7±12.3
ICSH 89009	258.4±12.9	274.3±13.7
ICSV 400	312.8±15.7	296.1±14.8

Table 5: Free α-amino nitrogen (FAN) of the sorghum worts from the 4 and 6 day malts (mg FAN/L)

	Germination time (days)	
Cultivars	4	6
ICSH 89002	115±5.8	85±4.3
ICSH 89009	152±7.6	106±5.3
ICSV 400	176±8.8	162±8.1

Table 6: Sugar profiles of the 4 and 6 day malt worts

Cultivars	Germination time (days) worts	Total sugars (%)	Maltose	Glucose	Maltotriose
			----------(mg mL^{-1})----------		
ICSH 89002	4	2.5	18.5±0.45	1.9±0.25	3.9±0.35
	6	2.5	19.3±0.75	2.1±0.30	2.8±0.40
ICSH 89009	4	2.5	20.0±0.00	2.2±0.10	2.4±0.20
	6	2.5	19.0±0.75	2.0±0.05	3.2±0.40
ICSV 400	4	2.5	20.0±1.50	1.9±0.45	2.7±0.25
	6	2.5	20.0±0.50	1.9±0.05	2.7±0.20

Table 7: Effects of exogenous starch (soluble starch) concentration on total soluble sugar production in the 4 and 6 day malt worts

Cultivars	Germination time (days) worts	Starch	Concentration	Presentage
ICSH 89002	4	2.6	3.6	3.2
	6	3.8	3.3	3.0
ICSH 89009	4	2.9	3.1	3.6
	6	3.9	3.5	3.2
ICSV 400	4	2.8	3.8	3.5
	6	2.6	3.5	3.7

Table 8: Percentage fermentability of the 4 and 6 day sorghum malt worts (%)

	Germination time (days)	
Cultivars	4	6
ICSH 89002	76.0±1.6	77.0±1.9
ICSH 89009	78.0±2.1	76.0±2.6
ICSV 400	80.0 ±1.8	80.0±1.4

Table 7. The data obtained revealed that the supplementation of sorghum malt worts with exogenous (soluble) starch, elicited an improvement in wort TFS values for most of the sorghum malt worts. At 1% starch supplementation, increase in wort TFS values of between 0.1 to 1.4 (4-56%) was observed. Worts from ICSH 89009 recorded the highest increases (16-56%), followed by ICSH 89002 (4-52%) for the 4 and 6 – day malt worts, respectively. ICSV 400 however, showed little increase in wort TFS levels when supplemented with 1% soluble starch. Table 8 depicted the effect of cultivar and germination time on wort fermentability. Data obtained from the percentage fermentabilities of the worts indicated that relatively high maltose (representing a 10.5-fold increase over glucose) and FAN levels in the ICSV 400 malt worts are reflected in their higher percentage fermentabilities. Except for the worts of ICSV 400, the percentage fermentabilities of all the worts differed significantly ($p \leq 0.05$).

DISCUSSION

The three sorghum varieties (ICSV 400, ICSH 89009 and ICSH 89002) investigated had good germinative properties as revealed in their high viabilities with no tendency of dormancy. Data obtained on the properties of the three sorghum varieties are within the range for malting sorghum grains and are consistent with other reports (Aisien, 1988; Palmer, 1980). Changes in amylolytic enzyme development during malting were investigated using standard methods. As illustrated in the result, the diastatic enzyme development generally increased from the first day of steeping (day 1) for the three cultivars. Maximum values were attained on the second day of germination for ICSH 89002 and on the third day of germination for both ICSH 89009 and ICSV 400. Thereafter, a second Diastatic Power (DP) high point for both ICSV 400 and ICSH 89002 was observed on the fifth day of germination. Analyses of variance data indicate that DP development for these cultivars was highly significantly ($p = 0.001$) dependent on cultivar and malting time. ICSV 400, generally showed

better DP activity, compared to both ICSH 89009 and ICSH 89002. At maximum activity, DP development in ICSH 89002 represented 2.4 and 2.7-fold lower DP values compared to corresponding malts from ICSH 89009 and ICSV 400, respectively.

Malt α-amylase activity also increased from day 1 and peaked on the second day of germination for both ICSH 89002 and ICSH 89009 but on the fifth day of germination for ICSV 400. Whereas α-amylolytic activity for cultivars ICSH 89009 and ICSH 89002 increased with time until peak values, ICSV 400 showed two high points of activity on the third and fifth days. Two-way analysis of variance showed that the development of α-amylolytic activity was highly significantly ($p \leq 0.001$) influenced by cultivars and malting period.

The β-amylase activity was optimal on the sixth day of germination for ICSH 89002 and on the third day of germination for both ICSH 89009 and ICSV 400. At peak diastatic activity (DP high points), β-amylolytic activity represented 36.7, 28.6 and 15.6% of total malt saccharifying amylase activity for ICSH 89009, ICSV 400 and ICSH 89002 cultivars, respectively. This supports the earlier observation by Novellier (1960), that sorghum malts contain β-amylase in considerably more than traces. Aniche and Palmer (1990) had postulated that screening of more sorghum cultivars might reveal some with significant β-amylolytic activity. This activity has recently been shown to be dependent on cultivars, steep regime and their pair-wise interactions (Ezeogu and Okolo, 1994).

Unlike the sorghum cultivars used by some earlier workers (Novellier, 1960; Dyer and Novellie, 1966), the cultivars used in this study showed β-amylolytic activity in the ungerminated (raw) state and in this sense resembled the barley (Palmer, 1989). In their ungerminated state, ICSV 400 showed significantly high β-amylase activity ($p = 0.05$) compared to grains of both ICSH 89009 and ICSH 89002. However, there was no significant difference in β-amylolytic activity in the latter two grains. Considering the high level of β-amylase activity observed for ICSH 89009 (at day 3), compared to malts of the other two grains, it is possible that as in sorghum á-glucosidase (Taylor and Dewar, 1994) and barley β-amylase, sorghum β-amylase may additionally occur in the bound form. Where this is so, time course differential behaviour of β-amylolytic activity for ICSH 89009, would be due to either of two factors; a higher capacity to solubilize bound amylases possibly owing to higher levels of appropriate processing enzymes or s-s reducing environment or a high capacity to synthesize β-amylase enzymes de novo. However, results presented in this study indicate the former to be true not only for β-amylase activity, but also for the total reducing power of ICSH 89009 (i.e., ICSH 89009 has the lowest degree of $*PSA^3$).

The extraction of bound amylases indicate that only a small portion of malt amylases occurred in the bound form for ICSH 89009 (12-25%) compared to ICSV 400 (26-48%) and ICSH 89002 (60-73%), for the fourth and sixth day malts, respectively. These differences in malt amylolytic activity, as observed by the diamylase procedure of Etokakpan and Palmer (1990), may thus also reflect widely varying differences in the ability of sorghum grain cultivars to release bound enzyme forms. Generally, data obtained for the Cold Water Extract (CWE) development of the four and six-day sorghum malts revealed that the four-day malts had more cold-water soluble materials than 6-day malts, with ICSV 400 recording the highest values of CWE at both duration of germination. Grain cultivar was the only significant factor ($p = 0.05$) influencing malt cold-water extract potential.

The patterns of HWE development were similar to the CWE, except that ICSH 89009 recorded a higher value of HWE from the 6-day malt. Generally, the HWE levels from the 4 and 6-day malts of ICSH 89002 respectively, were significantly ($p = 0.05$) lower relative to ICSV 400 at both duration of germination. The hypothesis that the HWE obtained from sorghum malt is greater at 6 days germination than 4 days (Moral *et al*., 1986) is not entirely

supported by this evidence. Since only ICSH 89009 followed this trend, additional environmental factors distinct from cultivar and germination time, like steep regime (Ezeogu and Okolo, 1995), steep liquor and their pair-wise interactions (Ezeogu and Okolo, 1999), could possibly have had some contributory effects, to this variation of HWE development.

However, results obtained on the effects of cultivar and germination time on the development of wort free á-amino nitrogen (FAN).revealed that all the cultivars had higher FAN levels in their corresponding 4 day worts. This observation further showed grain cultivar and germination time to play, albeit marginal significant ($p = 0.1$) roles in influencing FAN development. The lower FAN levels obtained for the 6-day malt worts, suggest that the increased development of roots and shoots in the 6-day sprouted grains (as observed in our laboratory), favours increased anabolic processes, thereby leading to lower levels of kernel of kernel FAN in the 6-day malts. ICSH 89002 and ICSV 400 recorded the lowest and highest levels of wort FAN respectively, at each duration of germination. A value of 100mg FAN/L of wort, with a lower limit of 8.5% sugar, has been recommended as the target figure for sorghum beer fermentations (Pickerel, 1986). The FAN content of all the worts in this study, were well above this target figure, with about a three-fold (ICSH 89002) and a nine-fold (ICSV 400) increase, in the lower and upper FAN regions respectively, relative to wort total fermentable sugar values.

Paper chromatogram showed that maltose, maltotriose and glucose, were the principal sugars of the worts. Taylor and Dewar (1994) have demonstrated that pre-cooking the starch-rich mash residue prior to mashing, resulted in worts with a much lower proportion of glucose and a higher proportion of maltose. This is consistent with the findings in this study and that of some earlier workers (Etokakpan, 1992; Etim and Etokakpan, 1992; Aniche and Palmer, 1990). However, unlike previous studies of mashing sorghum malt (Etokakpan, 1992; Etim and Etokakpan, 1992; Taylor and Dewar, 1994), it is especially remarkable that in the present investigation, maltose recorded a 9-10.5-fold increase over glucose.

In previous study (Etokakpan, 1992), maltose comprised 75% (50 mg mL^{-1}) and 42% (17 mg mL^{-1}) of total fermentable sugar (TFS) for barley and sorghum worts respectively. Similarly, maltose represented 41% (13 mg mL^{-1}) of TFS in an all-sorghum wort (Etim and Etokakpan, 1992). In the present investigation, maltose comprised 74-80% of wort TFS. These results are viewed with keen interest against the background of relative maltose contribution, where sorghum wort TFS comprised 4% (Etokakpan, 1992) and 3.2% (Etim and Etokakpan, 1992), as against 2.5% obtained in the present study.

The maltose levels obtained in this study would therefore be expected to increase with mash substrate concentration. These results strongly indicate that a change in the relative amounts of maltose over glucose was brought about by increased mash maltogenic amylase activity. Since these results differ considerably from the malt saccharifying activity, where maltose-producing β-amylase comprised 11.4-35.4% of the total reducing power activity, net increase in wort sugar would therefore be due to water-insoluble bound fraction of amylases, which remained bound during malting, but are active during the mashing operation (Novellier, 1960). These amylases also possess distinct properties from that of barley (Novellier, 1960). Novellier (1959) had earlier stated that the failure of the DP method to give an adequate indication of sugar production is probably due to; (1) Higher concentration of materials, particularly proteins, in the mash (2) A different ratio of amylase to starch and (3) Proteolysis occurring to a greater degree over the period of reaction. Time and temperature thus, favour mash production of protein degradation products resembling peptone. From the pint of view of malting conditions, it is conceivable that malt amylolytic activities are

somewhat partial indicators of wort TFS profiles, especially where wort substrate concentration is the rate determinant step. The concept that the low β-amylase activity in sorghum malt is responsible for the low levels of maltose in sorghum malt worts (Palmer, 1989), is not in agreement with the findings in this study and that of some other workers (Taylor and Dewar, 1994). Considering the implications of steep treatment on the development of amylolytic enzymes and soluble extracts (Ezeogu and Okolo, 1994-1995), desirable improvement of β-amylolytic activity in the mashing process, would thus be thus be dependent on the twin factors of modified malting regimes and appropriate cultivar selection. The somewhat similar TFS values obtained for the worts could be due to substrate limitation. Novellie (1966) had observed that when an enzyme reacts with its substrate, the rate of product formation is proportional to the enzyme concentration if an excess of substrate is present. But substrate concentration becomes rate determining, where enzyme concentration is in excess. It was thus found necessary to supplement the malt mashes with gelatinized starch (Difco) at 1, 2.5 and 4% concentrations, respectively. The supplementation of sorghum malt worts with exogenous (soluble) starch, led to an improvement in wort TFS values for most of the sorghum malt worts. At 1% starch supplementation, increase in wort TFS values of between 0.1 to 1.4 (4-56%) was observed. Worts from ICSH 89009 recorded the highest increases (16-56%), followed by ICSH 89002 (4-52%) for the 4 and 6-day malt worts, respectively. ICSV 400 however, showed little increase in wort TFS levels when supplemented with 1% soluble starch.

Novellier (1966) had opined that there exists a low critical value, within which sugar production is wort is very dependent on the actual diastatic activity employed. Thus, it appears that the DP activity of the 6-day malts of ICSH 89002 and ICSH 89009, are well within this range (D-PEP[2]), while the DP of ICSV 400 exceeded this range, entering a less critical range, where amylase concentration is more sufficient for sugar production. Amylase must therefore, be present in excess and starch concentration; the controlling factor in the case of the malt with highest DP. Generally, the total yield of sugar declined somewhat with increasing sugar concentration, except for the 4 and 6-day worts of ICSH 89009 and ICSV 400, respectively, where sugar yield increased with starch concentration. This phenomenon suggests that there exists an optimum value of sugar yield at a particular malt/starch ratio. This ratio has been shown (Novellie, 1966) to be independent of the DP. It is also noteworthy, that for the 4 and 6-day malts of ICSH 89009 and ICSV 400 respectively, bound, malt (Table 2) β-amylolytic activities (PSA), were more than the corresponding β-amylolytic activity. At the various starch supplementation levels, only the TFS mean values observed at the 1% exogenous starch concentration for the 4 and 6 day malt worts differed significantly ($p = 0.001$).

Data obtained from the percentage fermentabilities of the worts indicate that relatively high maltose (representing a 10.5-fold increase over glucose) and FAN levels in the ICSV 400 malt worts are reflected in their higher percentage fermentabilities. Except for the worts of ICSV 400, the percentage fermentabilities of all the worts differed significantly ($p = 0.05$). Malt provides amino acids, small peptides and larger polypeptides (Baxter, 1981), but yeasts are only capable of assimilating simple amino acids (Jones and Pierce, 1964; Palmqvist and Ayrapaa, 1969) and peptides (Mac William and Clapperton, 1969), but not proteins. In wort, the amino acids are preferentially used to provide nitrogen to the yeast cells for growth (Rose and Keenan, 1981). Pickerel (1986) working with sorghum, observed that the FAN levels required by yeast to fully utilize the available wort carbohydrates in 48h is dependent on the initial wort sugar content. The higher maltose level in the 6-day malt wort of ICSH 89002, with subsequently proportionate interactions with FAN, would thus be responsible

for the significantly ($p = 0.001$) higher percentage fermentability (relative to the 4 day malt wort). Similarly, ICSH 89009 with a higher maltose level in the 4-day wort, as well as a higher FAN content, recorded a higher percentage fermentability (relative to the 6-day malt wort).

The parity observed in the fermentable sugar levels of the worts from ICSV 400 is worthy of consideration. This is further compounded by the fact that β-amylolytic activity in the 6 day malt, accounts for only 40.9% of its 4 day maltogenic activity. Moreover, β-amylolytic activity for the 6 day malt of ICSH 89009, recorded a 2-fold increase over that of ICSV 400. As shown earlier (Table 2), more β-amylase activity occurred in the bound form for the 6 – day malts of ICSV 400, than in the 4-day germinated grains. Furthermore, while β-amylolytic activity represented 90.9% of total (bound) amylolytic activity (*PSA^3) for the former, it only accounted for 35.7% in the latter. It thus appears that mashing among other things, served to compensate for the bound β-amylase fraction in the 6 day malt of ICSV 400. This confirms the earlier observation that malt amylolytic activities would not adequately typify wort TFS profiles. Pickerel (1986) had earlier suggested that an optimum level of wort FAN ($= 113 < 170$ mg L^{-1} wort) may exist for sorghum beer fermentations, above which slight improvements are gained for large increases in FAN.

It appears that both worts from ICSV 400 supported extensive yeast growth equally well. Therefore, similar interactions of FAN-sugar levels would be responsible for the similar fermentability values recorded for ICSV 400. It thus follows that the differences observed in the fermentability values of the 4-day malt worts of ICSV 400 and ICSH 89009, relative to the similitude of their maltose levels is attributable to differences in sugar-FAN interactions during fermentation. At the same sugar level, the high FAN worts promote more rapid fermentations of the available sugars than do low FAN worts, presumably because they support more extensive yeast growth and supply the wort with more yeast cells in an active state of fermentation (Kirsop and Brown, 1972). Jones and Pierce (1964) investigating yeast uptake of free amino acids in barley wort during fermentation, reported that variations in wort composition as regards differences in a-amino nitrogen, would ensure preferential absorption of groups A and B amino acids, leaving groups C and D. It would appear therefore, that from the point of view of yeast nutrition, worts from ICSV 400 are superior to those from ICSH 89009 because they probably contain more standard values of readily assimilable nitrogen. When brewer's wort is fermented by *Saccharomyces* species, the sugar present are utilized in the order; sucrose, glucose, fructose, maltose and maltotriose, with dextrins remaining unfermented (Stewart *et al*., 1985; Palmer, 1989). However, maltose and maltotiose uptake by the yeast cells is repressed by the presence of glucose and only when half of the wort glucose has been fermented, do the yeast cells take up maltose (Stewart *et al*., 1985). Under the same conditions, glucose levels in the wort of ICSV 400 would be expected to decrease to 50% of their original value in a shorter time than that of ICSH 89009. Exhaustion of fermentable materials in 48 h would also follow the same trend. A more critical repression of maltose by the higher glucose levels of ICSH 89009 (relative to ICSV 400) would thus be responsible, at least in part, for its lower fermentability value.

CONCLUSIONS

The study reported in this paper showed that diastatic activity and β-amylolytic activity were significantly ($p \leq 0.001$) influenced by sorghum cultivar and germination time. Thus, confirming previous reports that sorghum grains can develop diastatic power during malting and that some cultivars may possess significant levels of β-amylolytic enzymes. However, results of this study showed that Cold Water Extracts (CWE) development was only affected

by grain cultivar while Hot Water Extracts (HWE) of malts were affected by germination time. Among the three sorghum cultivars, ICSV 400 had the highest levels of free α-amino nitrogen (FAN) and worts from ICSV 400 also showed better fermentability potentials when compared with ICSH 89009 and ICSH 89002. Data from this study also revealed that total malt amylase activity occurred in the bound form for the three cultivars. Maltose was the major fermentable sugar followed by maltotriose and glucose. The high disparity between malt β-amylolytic activities and wort maltose levels suggests that amylolytic alone is insufficient in assessing mash performance and development of sorghum particulars with bound enzyme forms. Considering the implications of steep water treatment on the development of amylolytic enzymes and soluble extracts (Ezeogu and Okolo, 1994, 1995), desirable improvement on β-amylolytic activity during the mashing process, would thus be dependent on the twin factors of modified malting regimes and appropriate cultivar selection.

In conclusion, the result of this study showed that the three improved Nigerian sorghum cultivars (*Sorghum bicolor L*; ICSV 400, ICSH 89009 and ICSH 89002) used in this study have great potential in brewing industries. Thus, will reduce the cost of importing barley malts for beer and malt brewing.

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