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Evaluation of the Wort Properties of Four Improved Sorghum Varieties Mashed with and Without Commercial Enzyme

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ABSTRACT

To determine the wort properties of recently improved sorghum properties, the malt of four sorghum varieties namely Samsorg 46, Samsorg 47, Samsorg 48 and Samsorg 49 were analyzed. The grains were malted and mashed with and without commercial enzyme, using the single decoction mashing technique. The wort derived from the mashing was filtered and boiled, then analyzed. The wort properties looked out for include, specific gravity, Protein estimation using Micro Kjeldahl method, reducing sugar using the DNS method and free α -amino nitrogen (FAN) using method described by Association of Official Analytical Chemists, pH, filterability rate. Generally, there were significant differences across the different varieties of sorghum for, FAN, reducing sugars, cold water extract and hot water extract at both $p<0.05$ and $p<0.01$. There was a general increase in specific gravity, reducing sugar, FAN, filterability rate and pH, as the days of germination increased across the varieties, while there was a noticeable decrease in protein content as the days of germination increased in single decoction method of mashing. The varieties gave good results using single decoction method as there were increases in the specific gravity with Samsorg 47 having the highest with 100% malt wort (1.035) and malt enzyme wort (1.036), reducing sugar with Samsorg 46 having the highest with 100% malt wort (130mg/ml) and malt enzyme wort (130mg/ml), FAN with Samsorg 48 having the highest with 100% malt wort (284.69mg/L) and Samsorg 49 for malt enzyme wort (351.38mg/L) and filterability rate with Samsorg 47 having the highest with 100% malt wort (3.00ml /min) and malt enzyme wort (3.80ml/min). The four recently improved sorghum varieties had good wort properties but more works need to be done on then to realize their full potential.

KEYWORDS: *sorghum, mashing, single decoction, wort*

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INTRODUCTION

In Nigeria, the Institute for Agricultural Research, Samaru established in 1922 has produced over 40 improved sorghum malt varieties. These varieties have improved nutrients and high resistance to disease and pest. Using some of these improved varieties; it is noteworthy and indeed salutary to report that more than 80% of the biochemical problems of sorghum malts have been resolved through research efforts [1]. Among these new sorghum varieties were some whose malts possess beneficial qualities for beer brewing, such as good diastatic power, α- and β-amylase activities and extract recovery [2]. These qualities of sorghum malts are further enhanced by the incorporation of starch hydrolyzing enzymes such as amylases during mashing [3]. Among starch degrading enzymes are endoamylases, exoamylases, glucoamylases, debranching enzymes and glycosyl transferases. Depending on the type of amylase, starch is degraded to simple sugars such as glucose, maltose, maltotriose or to oligosaccharide malto-oligosaccharides or dextrins [3].

Sorghum is used in lager beer brewing as malt and/or raw sorghum [4]. Much interest has been generated in the use of sorghum in brewing. The use of malted sorghum in the production of kaffir beer, a traditional beverage has been well documented [5]. There have

also been reports on the use of sorghum in the production of Western-type beer [6]. The successful research that led to the development of commercial sorghum lager beer brewing focused on enzymes in sorghum malting, sorghum malting technology and sorghum brewing technology [7] However, identification of sorghum types with specific grain characteristics suitable for lager brewing remains a major area of concern [8]. Currently, sorghum types that differ substantially in chemical composition are used for lager beer brewing in Africa, including white pericarp type II tannin sorghums in Nigeria [9;10] and white pericarp, type I non-tannin, tan-plant sorghums in Uganda [11].

Mashing with malted sorghum in lager beer brewing yields a high level of free amino nitrogen (FAN) needed to ensure efficient buffering capacity and optimum yeast performance during fermentation [12]. However, a low level of fermentable sugars is produced in sorghum malt mashing, which has been attributed to the high starch gelatinization temperature and low β-amylase activity in sorghum compared with barley [8]. The traditional source of enzymes used for the conversion of cereals into beer is barley malt. If too little enzyme activity is present in the mash, there will be several undesirable consequences: the extract yield will be too low; wort separation will take too long; the fermentation process will be too slow; too little alcohol will be produced; the beer filtration rate will be reduced; and the flavour and stability of the beer will be inferior [7]. In practice, sorghum malt mashing requires addition of exogenous enzymes in order to produce fermentable sugars [14]. Industrial enzymes are used to ensure better adjunct liquefaction, to produce low carbohydrate beer ("light beer"), to shorten the beer maturation time, and to produce beer from cheaper raw materials. It is not however clear how far mashing enzyme supplements in sorghum cultivar mashes could help alleviate the levels of fermentable sugars in its worts [19].

In barley malt brewing, cultivar differences are a major cause of variability in wort quality [18]. Thus, wort quality in sorghum grain brewing is presumably a very important criterion for determining which types of sorghum are most suitable. Therefore, the objective of this study was therefore to determine the wort properties of recently improved sorghum varieties Samsorg 46, Samsorg 47, Samsorg 48 and Samsorg 49.

MATERIALS AND METHODS

Sample collection and preparation: The improved sorghum varieties (Samsurg 46, Samsurg 47, Samsurg 48 and Samsurg 49), were obtained from the Institute of Agricultural Research, Ahmadu Bello University Samaru, Zaria, Kaduna State, Nigeria. The grains were sorted and screened by hand to remove broken or damaged grains and foreign materials before being used for analysis.

Malting of sorghum grains: The method described by Agu and Okeke [13] was used to malt the recently improved sorghum varieties.

Mashing: The malt of the sorghum varieties were mashed with and wihtout commercial enzyme using single decoction mashing regime. After mashing, the mash was filtered using Whatman No. 1 filter paper to produce worts which were boiled for 10 minutes and cooled in a refrigerator at 4^{o}C before analysis.

Protein estimation: Protein content of the worts was determined using the Micro Kjeldahl method for determination of nitrogen according to Association of Official Analytical Chemists AOAC, [16].

Determination of reducing sugar: The total reducing sugars (as glucose) present in the wort samples was determined using the 3, 5-dinitrosalicyclic acid (DNS) method of Miller [27]

Determination of free *α* -amino nitrogen (FAN): was carried out using the Ninhydrin method of A.O.A.C [16].

Determination of *pH:* The *pH* was determined using a pH meter.

Staistical Analysis: Each analytical determination was carried out in triplicates with average mean recorded. Data were subjected to One way Analysis of Variance (ANOVA) using SPSS (Statistical Package for Social Sciences).

Results

The four improved sorghum varieties under study (Samsorg 46, Samsorg 47, Samsorg 48 and Samsorg 49) that were mashed without external commercial enzyme (100% malt) and with commercial enzyme alphalase sorghum (malt + alphalase sorghum), using the single decoction method indicated that the temperature increased after each decoction in all varieties and this was seen in figures 1.The saccharification test was positive for all mashes.

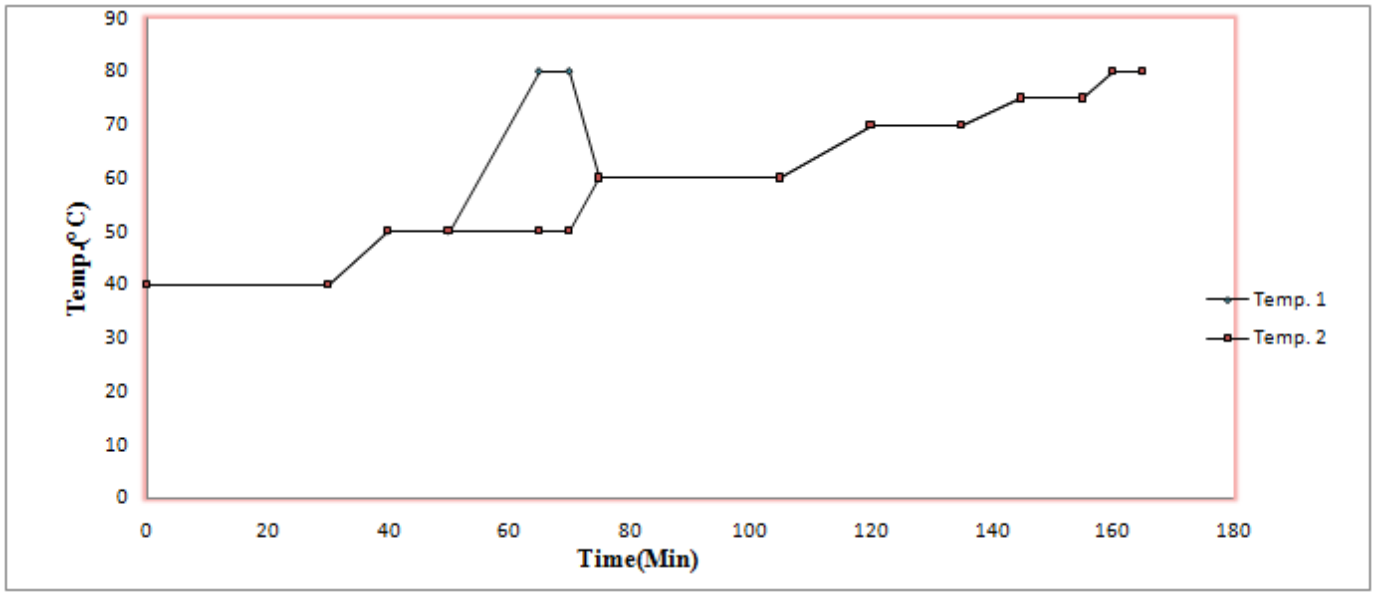

Fig 1: Single Decoction Mashing Regime

The analysis of all malt wort (100% malt wort) without enzyme and analysis of wort with alphalase-sorghum enzyme for day one germination is shown in table 1. The table showed that the 100% wort produced from Samsorg 47 and 48 varieties had the highest value in Specific Gravity (1.010), Samsorg 46 Reducing Sugar (40mg/ml), pH (5.60), Samsorg 48 and 49 filterability rates (3.00ml/min), Samsorg 46 Protein content (9.98%). The wort produced from Samsorg 48 variety gave highest value in all parameters analysed except for protein content which was highest in Samsorg 46 variety and FAN in which Samsorg 47 had the highest. Also analysis of day 2 malt wort (100% malt wort) and that of malt plus enzyme was as seen in table 2 showed that the 100% wort produced from Samsorg 46 gave the highest value in all parameters analysed except for FAN content and filterability which gave the highest value in Samsorg 49 variety. The malt that was added exogenous(100% malt + alphalase sorghum), produced wort with SAMSORG 46 and 48 varieties having the highest value in Specific Gravity (1.012), Samsorg 46 highest Reducing Sugar (85mg/ml) and protein content (8.93%), SAMSORG 48 had the highest filterability rate (6.91ml/min) in the analysis of Day 2 enzyme malt wort. The wort produced from day 3 all malt mashing and enzyme malt wort (malt + alphalase sorghum) (table 3) indicated that Samsorg 49 variety had the highest specific gravity, FAN content and filterability rate. Samsorg 46 had the highest protein content for 100% malt wort. The enzyme malt wort (malt + alphalase sorghum) as shown in table 3 indicated that the wort produce from Samsorg 49 variety gave highest value in all parameters analysed except for protein content and pH which was highest in SAMSORG 46 variety.

The day 4 malt wort (100% malt wort) analysis indicated that the wort produced from Samsorg 48 gave the highest value in *pH*, FAN and protein content while SAMSORG 46 had the highest reducing sugar and filterability rate. The enzyme malt wort analysis of Day 4 showed that the wort (100% malt + alphalase sorghum) produced from Samsorg 49 varieties have the highest value of Specific Gravity (1.030), SAMSORG 46 highest Reducing Sugar (85mg/ml) and FAN (325.38), while SAMSORG 48 had the highest filterability rate (6.98ml/min) and protein content (3.73%), (table 4). The analysis of day 5 malt wort (100% malt) as seen in table 5 indicated that, the wort produced from Samsorg 47 variety had the highest specific gravity (1.035), FAN content was seen to be highest in Samsorg 49. Samsorg 46 had the highest protein content while Samsorg 46 gave the highest in Filterability rate and reducing sugar and having the lowest in FAN content. Also the analysis of Day 5 enzyme malt wort (100% malt + alphalase sorghum), as seen in table 5 too indicated that, Samsorg 47 varieties had the highest value of Specific Gravity (1.036), Samsorg 46 highest Reducing Sugar (130mg/ml) and filterability rate (3.85ml/min), FAN content (351.38) was highest in Samsorg 49 while Samsorg 48 had the highest protein content (1.48%).

Table 1: Analysis of Day 1 Wort

Parameters	Samsorg 46		Samsorg 47		Samsorg 48		Samsorg 49	
	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt
Specific Gravity	1.005± 0.001	1.005±0. 001	1.010± 0.001	1.009±0 .001	1.010± 0.003	1.011±0 .001	1.007± 0.001	1.009±0 .002
Reducing Sugar (mg/ml)	40±5	70±10	30±5	70±10	20±5	80±10	30±5	60±10
Protein Content (%)	9.98± 0.02	9.98±0.1	9.56± 0.02	9.56± 0.04	8.50± 0.02	8.52± 0.1	9.20±0 .02	9.21± 0.3
FAN (mg/L) ninhydrin	120.37 ±0.02	156.64±1	132.51 ±0.03	167.84 ±1	144.54 ±0.03	156.86± 2	102.39 ±0.02	121.39± 1
pH	5.60± 0.02	5.60±0.1	5.54±0 .02	5.54± 0.2	5.54± 0.02	5.54± 0.2	5.54±0 .02	5.54± 0.2
Filterability Rate (ml/min)	2.98± 0.02	6.42±0.1	2.00±0 .02	4.53± 0.1	3.00± 0.02	6.91± 0.1	3.00± 0.02	6.80± 0.1

Table 2: Analysis of Day 2 Wort

Parameters	Samsorg 46		Samsorg 47		Samsorg 48		Samsorg 49	
	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt
Specific Gravity	1.008± 0.002	1.008± 0.002	1.012± 0.002	1.012± 0.002	1.012± 0.002	1.012± 0.002	1.009± 0.002	1.009± 0.002
Reducing Sugar (mg/ml)	60±10	85±10	30±5	75±0.5	20±5	80±10	50±5	80±10
Protein Content (%)	8.93± 0.04	8.93± 0.03	8.52± 0.03	8.52± 0.02	7.30± 0.3	7.30± 0.02	7.51± 0.01	7.52±0.1
FAN (mg/L) ninhydrin	121.34 ±1	160.87 ±3	244.25 ±1	278.50 ±4	154.85 ±6	195.84 ±3	266.50 ±8	279.10 ±6
pH	5.69± 0.01	5.60± 0.1	5.64± 0.01	5.54± 0.1	5.67± 0.02	5.54±0.1	5.64± 0.01	5.54±0.1
Filterability Rate (ml/min)	2.98± 0.02	6.42± 0.1	1.99± 0.02	4.53± 0.1	3.00± 0.02	6.91±0.1	3.00± 0.02	6.80±0.1

Table 3: Analysis of Day 3 Wort

Parameters	Samsorg 46		Samsorg 47		Samsorg 48		Samsorg 49	
	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt
Specific Gravity	1.015± 0.003	1.015± 0.002	1.019± 0.002	1.018± 0.003	1.020± 0.005	1.021± 0.002	1.022± 0.003	1.023± 0.00
Reducing Sugar (mg/ml)	70±9	100±5	30±9	80±5	40±5	95±13	50±9	100±5
Protein Content (%)	5.57± 0.03	5.23± 0.3	5.82± 0.05	4.56± 0.1	35± 0.07	4.20± 0.2	4.97± 0.2	4.50± 0.2
FAN (mg/L) ninhydrin	130.17 ±0.8	165.82 ±4	256.26 ±1	285.30 ±7	205.04 ±2	228.70 ±1	269.79 ±4	293.00 ±9
pH	5.78± 0.1	5.68± 0.02	5.75± 0.1	5.67± 0.03	5.82± 0.05	5.67± 0.04	5.74± 0.1	5.65± 0.02
Filterability Rate (ml/min)	3.01± 0.1	6.74± 0.2	2.23± 0.2	4.78± 0.2	3.20± 0.3	7.02± 0.04	3.30± 0.3	7.10± 0.1

Table 4: Analysis of Day 4 Wort

Parameters	Samsorg 46		Samsorg 47		Samsorg 48		Samsorg 49	
	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt
Specific Gravity	1.020± 0.003	1.021± 0.003	1.025± 0.005	1.027± 0.002	1.027± 0.003	1.026± 0.003	1.029± 0.002	1.030± 0.003
Reducing Sugar (mg/ml)	80±5	125±5	40±0	85±5	57±7	98±5	70±5	105±5
Protein Content (%)	3.43± 0.1	3.36± 0.02	3.95± 0.2	3.50± 0.03	4.28±0.3	3.73± 0.04	3.31± 0.1	3.20±0.2
FAN (mg/L) ninhydrin	265.49 ±3	273.60±2	265.30 ±2	295.72±3	283.19±3	302.00±3	275.80 ±2	325.38± 4
pH	5.92± 0.05	5.92± 0.04	5.86± 0.2	5.86±0.1	5.95± 0.05	5.95± 0.02	5.86± 0.04	5.86± 0.04
Filterability Rate (ml/min)	3.95± 0.05	6.20± 0.2	2.50±0	4.51±0.1	3.61± 0.2	6.98± 0.02	3.58± 0.2	6.81± 0.02

Table 5: Analysis of Day 5 Wort

Parameters	Samsorg 46		Samsorg 47		Samsorg 48		Samsorg 49	
	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt	100% malt	Enzyme malt
Specific Gravity	1.024± 0.002	1.025± 0.002	1.035± 0.003	1.036± 0.004	1.030± 0.002	1.029± 0.003	1.007± 0.001	1.034± 0.003
Reducing Sugar (mg/ml)	130±5	130±5	100±10	100±10	105±5	105±9	30±5	125±9
Protein Content (%)	1.84± 0.04	1.32± 0.01	1.81± 0.01	1.41± 0.02	2.12± 0.02	1.48± 0.02	9.20± 0.02	1.24± 0.02
FAN (mg/L) ninhydrin	262.62± 2	313±4	293.46 ±3	318±2	294.69 ±2	345.87±4	102.39 ± 0.02	351.38± 5
pH	6.01± 0.01	6.01± 0.01	6.02±0	5.54± 0.2	6.01± 0.01	6.01± 0.01	5.54± 0.02	6.01± 0.01
Filterability Rate (ml/min)	7.48± 0.2	3.86± 0.04	4.96± 0.1	4.53± 0.1	7.42± 0.2	3.80± 0.1	3.00± 0.02	3.80± 0.1

Discussion

The mashing methods used in this work are similar to that carried out by Ogu, [30]. The test for saccharification showed positive result, this indicates that the hydrolytic enzymes were largely produced and released in the 5th day of germination [30].

The enzyme alphalase sorghum was added same time with the grist. The enzyme alphalase sorghum has α-amylase as its component whose optimum temperature range of activity is 50-60°C to enable the breakdown of α-1,4-glucosidc linkages in sorghum starches [26]. During the mashing processes, the time at which it takes to rise to specific temperatures were seen to be the same. The wort produced at different days of germination showed continuous increase in the specific gravity of wort from the one-day germinated malts to the five-day germinated malts. The free amino nitrogen (FAN) values increased as the days increased. The values produced by 100% malts were relatively low when compared to worts produced with alphalase sorghum, the reducing sugar produced from malts increased with increasing days. The increase indicated in FAN, specific gravity and reducing sugar level are indications that during the malting periods, hydrolyzing enzymes were produced which aided in the breakdown of the macromolecules to smaller molecules during mashing [33]. The test for saccharification showed negative in the worts derived from the first three days of germination but positive on the 4th and 5th days of germination.

Wort is a product of filtration of mashes, the residue of the filtration is called the spent grains [21]. During filtration of the various mashes, spent grains were removed in order to ensure clarity of the wort. In this work, the addition of exogenous enzyme alphalase sorghum aided the rate of filtration. This agrees with the result obtained by the EBC [21]. The specific gravity of the worts produced is quite lower than that recorded as standard for laboratory analysis of barley. Literature has recorded specific gravity of 1.040 as

the standard for laboratory analysis of barley [34]. The specific gravity of the worts produced both from the hundred percent malts and those added exogenous enzymes might have a shorter period of fermentation process but when given the right fermentation conditions and adequate cellular operations the wort should give acceptable high values [23].

A commonly accepted optimal range for wort pH is 5.2-5.7 with 5.5 being optimal for starch conversion activity but many authors report wort quality benefits if the pH is lowered into the 5.2-5.4 range [25, 28].The pH of the wort has a great influence in the enzyme's activity. A mash pH below 4.3 will reduce amylase activity while high alkaline mash pH can cause the extraction of phenols which will impart a stringent character to the finished beer [34]. There was no significant different in the pH of the wort when exogenous enzyme was added to the malted grain.

Reducing sugars play an important role in establishing the quality and identity of beer. The values obtained in this study were within the range of values obtained in the study conducted by [31]. This is an indication of hydrolyzing enzymes produced during the malting period, which were then involved in the breakdown processes of macro molecules to their lower units during mashing [17].

The protein concentration in all malt wort reduced with increase in germination days. These values were higher than that obtained with the addition of alphalase sorghum, but fell within the range of values obtained by [32].The protein content of brewing grains must first be converted to amino acids and small peptides (denoted as assimilable nitrogen) before it can be utilized by fermenting yeasts for growth, therefore, FAN determination is a very important index of brewing grain quality [35]. The FAN levels obtained in the current work for the different improved sorghum varieties were all at levels of >100, showing that they have moderately high FAN levels. Their levels fell within the recommended FAN level necessary for satisfactory yeast growth and fermentation, which has been put at between 100 and 150 mg/L [29]. The FAN levels obtained in this work, differed significantly from those reported previously without added commercial enzymes, but showed some level of agreement with those obtained with added commercial enzymes [31]. However, the FAN values were mostly lower than those previously obtained by [2; 20]. Seasonal variations in raw materials owing to a multiplicity of reasons– environmental, varietal changes, pest infestation and even factors that can be ascribed to humans, and also differences in grist composition – could be responsible for some of these differences [15, 24]. It has been reported that FAN levels generally increase with increasing germination time [22].

Conclusion

The objective of this study was to investigate the different wort properties of four different malts of recently improved sorghum varieties (Samsorg 46, Samsorg 47, Samsorg 47, Samsorg 48 and Samsorg 49) for different germination days produced with single decoction mashing. The malts of the improved sorghum varieties shows that they can be used to achieve desired results in brewing in terms of extract yield, FAN level, proper saccharification and total reducing sugar levels at the end of 5th day of germination. From the analysis of the malts produced by the improved grains it can be said that the malts from Samsorg 48 and 49 produced better malts when assessed using their extracts. The success of saccharification makes decoction type of mashing (single decoction) suitable for the production of the malt wort produced from these improved sorghum varieties. Addition of commercial enzyme preparations improved the extract yield with single decoction mashing of sorghum malt with Samsorg 48 and 49 showing to have the best quality when assessed with the extract yield, FAN level, proper saccharification and total reducing sugar levels after the 5th day of germination. Malting mashing studies have revealed that the nutritional quality of grain sorghum can be significantly improved by malting and mashing treatment, therefore, it is necessary that more work be done on these sorghum varieties to improve their utility as a brewing material.

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