Original Research Article

Extract development of sorghum grains during Malting

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ABSTRACT

Aims: The aim of this research is to malt sorghum grains, extract amylase enzymes, and produce sorghum beer.
Study design:
Place and Duration of Study: sample obtained from a local market around University of Port Harcourt in Choba, study conducted at Pre-lite research laboratories, Alakahia, between June 2021 and September 2021.
Methodology: The grains were bought at a local market, steeped for 24h, germinated for 5days at 30°c and kilned at 45°c for 24h. Alpha and beta amylases were extracted from the malted sorghum and their activities were determined by measuring the maltose produced. Mashing was done using infusion method, bitter leaf extract were used in place of hops, and 500ml of the wort was pitched with 50ml inoculum of *Saccharomyces cerevisiae* isolated from fresh palm wine. Fermentation lasted for 14 days.

Results: Beta amylase activities were higher with a peak of 1.5mg/ml maltose as against 0.9mg/ml maltose for alpha amylase. Wort properties such as diastatic power and hot water extract were measured as 40°l and 415°l/kg respectively. The resulting beer gave alcohol content (%) of 3.6 and 3.4, bitterness was 100.38 IBU (International Bitterness Units) and 101.82 IBU while colour was 19.15 EBC (European Brewery Convention) and 21.45 EBC for sorghum wort having 5ml and 10ml of bitter leaf extract respectively.
Conclusion: The results obtained in this study depicts sorghum malt as a source of enzymes and further reveals the brewing potentials of sorghum grains in beer production, however bitter leaf offered a good degree of bitterness to the beer but lacked the characteristic aroma of hops.
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Keywords: Sorghum, malting, amylase, fermentation, beer

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal that ranks fifth after rice, wheat, maize, and barley. In sub-Saharan Africa (SSA), it ranks second in importance after maize [22]. Sorghum comprises the main food source from which over half a billion people in developing countries, derive their energy requirements [20]. Sorghum belongs to the genus Sorghum, tribe Andropogoneae, Poaceae family which is made up three main species namely *S. bicolor, S. halepense* and *S.propinquum* (Kunth) Hitchc. [25]. Sorghum bicolor includes all the cultivated sorghums which are Bicolor, Kafir, Caudatum, Durra and Guinea races. These races further have various sorghum use types that include; grain, forage, sweet/syrup, biomass and broom sorghum. These differ in their morphological features of the stem, inflorescence, grain and glumes [6].

Malting of cereal grains is the process of changing the biochemical modifications that improve its nutritional and bioactive quality under controlled germination [8]. During the malting process, the hydrolytic enzymes amylases, proteases and

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fiber-degrading enzymes are activated by the germinated grain to breakdown the starchy endosperm into simpler forms. Malting consists of three stages: steeping (soaking the grain in water), germination, and kilning. During steeping, the sorghum is submerged in water with some periods of aeration, to increase the moisture and activate important enzymes. During this stage, grain moisture content reaches around 45% [26][7]. The germination stage usually takes between 4 and 5 days, depending on temperature, moisture, light conditions, and grain. It consists of rotating the sorghum at some periods maintaining moisture and temperature. Synthesis of all malting enzymes in sorghum takes place during germination. In this stage, the metabolism is very active, and enzymes such as hemicellulases, amylases, proteases, oxidases, among others, are involved. The process is stopped by kilning when the root of the grain has the same size of the grain [7]. During kilning, there is an initial drying where the grain is exposed to 50˚C, and enzymatic activity decreases. At this point, the moisture of malt is reduced by up to 12%. A second drying is performed at 85–90 ˚C to achieve desirable moisture of 4–5%. This stage is key to stopping the metabolic activity and maintaining the quality characteristic of malt, such as the enhancing flavor, aroma, and obtaining malt with a long shelf life [26].

During the malting process, the hydrolytic enzymes amylases, proteases and fiber-degrading enzymes are activated by the germinated grain to breakdown the starchy endosperm into simpler forms. Generally, grains are known to contain a varying quantity of amylases (both alpha and beta-amylases). Sorghum has been reported to be a good source of alpha amylase in comparison to other available grains such as rice, maize or millet owing to the relatively high heat stability of the alpha-amylase in the intact malt [1][10] while unmalted sorghum has no β -amylase and there is very little when the grain is malted.

Diastatic power is a measure of how effective the malt is at converting starch to sugar [12], and is an important quality trait for malt used in brewing and distilling [14]. Sorghum has been recognized for its potential to substitute barley malt in lager beer production and it is now widely used in European-type lager beer brewing in many developing countries of the tropics [15]. The main problems when brewing with sorghum are the lower diastatic power of its malt, especially deficient in beta-amylase activity, and the higher gelatinization temperature of sorghum starch [23]. Sorghum malt worts generally contain lower amounts of fermentable sugars and high amounts of dextrins that increase viscosity [23]. Thus, optimization of conditions for mashing and fermentation are necessary for the production of acceptable sorghum lager beer [15]. This study was aimed at developing and extracting enzyme from sorghum grains during malting and using the sorghum malt for beer production.

2.1 Sample Collection

The sorghum grains were purchased at a University of Port-Harcourt Junction market in Choba community, Obi-Akpor LGA in River state, Nigeria. The grains were carefully sorted to remove damaged and broken seeds. All reagents used were of analytical grade.

2.2 Proximate analysis of sorghum

All Proximate analysis was carried out according to the method of [3]. The moisture content, ash content, fats and oil, crude fibre, protein and carbohydrate compoisition were determined respectively.

2.3 Malting of sorghum

The grains were surface-sterilized by immersion for at least 40 min in a sodium hypochlorite (NaOCl) solution to discourage microbial activity. Thereafter, the grains were drained of the sterilant, washed vigorously three times in tap water. 200g of the sterile sorghum grains were steeped in 400 mL of distilled water in a clean container for 24h at room temperature (28°C) with steep water changed at 6h intervals to minimize microbial contamination. Germination was done according to the method of [4]. It was carried out on a sterile clothe at 30°C for 5 days in dark germination chambers with 12 hourly spray of 10ml water using a clean perforated bottle to prevent drying out. After the grains had germinated, kilning was done in an oven at 45°C for 24h.

2.4 Milling of sorghum malt

Dried malts were rubbed in between palms to remove the rootlets and shoots from the kernels. The grains were then milled for two 30secs period in a cooled blender at high speed.

2.5 Extraction of α -amylase and β –amylase

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The method of [21] was used with slight modification. 5g of the milled sorghum malt was homogenized in 10ml of 0.1 M acetate buffer, pH 5.5 for α-amylase while same volume of 0.1 M phosphate buffer, pH 6.0 for β-amylase extraction. They were poured in separate 250ml conical flask and labelled accordingly. They were kept in the refrigerator for 1 h with intermittent stirring every 10 min. This was followed by centrifugation at 6000 rpm for 20 min to remove the debris. They were then filtered with filter paper and the supernatants were taken as the crude enzymes. Assay for the enzyme activities were then carried out.

2.6 Enzyme assay

2.6.1 Assay of α-Amylase

This procedure was used for the determination of α-Amylase activity. Four test tubes were labelled tube S1, S2, S3 and S4 (blank) and 1ml of starch solution was added in all and properly mixed. The following volumes: 0.50ml, 0.70ml, 1ml and 0ml of buffer solution and extracted crude α-amylase were added in tube S1, S2, S3 and S4 respectively, properly mixed and all tubes were incubated for 3mins at room temperature. 1ml each of colour reagent was added to all tubes excluding the blank and allowed to boil for 15mins in water bath. The tubes were cooled on ice and 9ml of distilled water was added to each tube. The tubes were mixed by inversion and spectrophotometric reading at 540nm was taken. The absorbance values were compared to the standard and used to determine the sugar concentration produced.

2.6.2 Standard Curve Preparation

8 test tubes were labelled standard (std) 1, 2, 3, 4, 5, 6, 7 and blank respectively. The following volumes: 0.05ml, 0.20ml, 0.40ml, 0.60ml, 0.80ml, 1ml, 2ml and 0ml of 0.2 % maltose standard were added to each tube as labelled respectively. The following volumes: 1.95ml, 1.80ml, 1.60ml, 1.40ml, 1.20ml, 1ml, 0ml and 2ml of distilled water were added to each tube as labelled respectively. To the mixture of each tube, 1ml of colour reagent was added to all tubes then boiled for 15mins in a water bath. The tubes were removed, cooled on ice and 9ml of distilled water was added to each tube. The tubes were mixed by inversion and spectrophotometric reading at 540nm was taken. Absorbance values were used to plot the enzyme standard curve.

2.6.3 Assay of β-AMYLASE

This procedure was used for the determination of β-Amylase activity. Four test tubes were labelled tube S1, S2, S3 and S4 (blank) and 1ml of starch solution was added in all and properly mixed. The following volumes: 0.50ml, 0.70ml, 1ml and 0ml of Sodium Acetate Buffer and extracted crude β-amylase were added in tube S1, S2, S3 and S4 respectively, properly mixed and all tubes were incubated for 3mins at room temperature. 1ml each of colour reagent was added to all tubes excluding the blank and then placed in boiling water bath to boil for 15mins in water bath. The tubes were removed from the water bath, cooled all ice and 9ml of distilled water was added to each tube. Due to the short enzymatic incubation time of 3mins, each test lot was run one at a time. The tubes were mixed by inversion and spectrophotometric reading at 540nm was taken. The absorbance values were compared to the standard and used to determine the sugar concentration produced.

2.6.4 Standard Curve Preparation

8 test tubes were labelled standard (Std) 1, 2, 3, 4, 5, 6, 7 and blank respectively. The following volumes: 0.05ml, 0.20ml, 0.40ml, 0.60ml, 0.80ml, 1ml, 2ml and 0ml of 0.2 % maltose standard were added to each tube as labelled respectively. The following volumes: 1.95ml, 1.80ml, 1.60ml, 1.40ml, 1.20ml, 1ml, 0ml and 2ml of distilled water were added to each tube as labelled respectively. To the mixture of each tube, 1ml of colour reagent was added to all tubes then boiled for 15mins in a water bath. The tubes were removed, cooled on ice and 9ml of distilled water was added to each tube. The tubes were mixed by inversion and spectrophotometric reading at 540nm was taken. Absorbance values were used to plot the enzyme standard curve.

2.7 Mashing using infusion method

The recommended method of analysis by the Institute of Brewing (1997) and as applied by Eneje et.al. [11] was used in this determination. Fifty grams (50g) of the ground sample was weighed into the mashing beaker and placed in a hot water bath for 15min and 360ml of equilibrated water (65°C) was added, stirred at 30min interval for 1h to eliminate all lumps. The mash was later cooled and transferred to a 515ml measuring flask. The beaker was rinsed inside into the flask and made up to 515ml and mixed by inversion. Thereafter, the mash was filtered off and the specific gravity was obtained. The extract yield was obtained from the relation:

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Extract (as is) = Excess gravity x 10.31°/kg

Extract (d\Dry) = Extract (as is) x 100 / (1000 – m)

where, m = moisture content of grain.

2.8 Diastatic Power (DP)

Diastatic Power (DP) is the total activity of malt enzymes that hydrolyze starch to fermentable sugars. Determination of Diastatic Power was done according to the method of [17]. Infusion extract of the sorghum malts prepared from hot water extract was used without filtering and 3ml aliquots of the extracts was separately pipetted into 100ml of 2% 0.1M citrate phosphate buffer starch solution in 200ml flasks. The mixtures was shaken and maintained at room temperature for 1h from the time the aliquots was added. At the end 30ml of 0.1M NaOH was then added to stop the reaction and total volume raised to 200ml with distilled water. The diastatic power of the maize malts was determined by titrating the starch digests against 5ml of Fehling's solution (that is equal volumes of solution A and B mixed together) contained in a 150ml conical boiling flask. Solution A was prepared by mixing 7g of Copper Sulphate and 2 drops of dilute Sulphoric acid in 100ml of water and Solution B by mixing 35g of Potassium tartarate and 12g of sodium hydroxide in 100ml of water. The flask contents were boiled as titration continues until the blue colour of Fehling's solution is discharged. 3 drops of 1% (w/v) aqueous solution of methylene blue was added and the titration and boiling continued to the end point when the methylene blue became decolourized and the reaction mixture became bright red.

The diastatic power (D.P) in degrees Linter (°L) was calculated from the relation:

D.P = 2000 – 200 / Xy -Xs

Where X = number of ml of malt extract used in digesting starch

y = number of ml of starch digest used in titration

s = Titre for starch blank.

200 and 2000 are constants

2.9 Beer Production

2.9.1 Isolation of Yeast (Saccharomyces cerevisiae) from fresh palm wine

This was carried out according to the method of [2]. 1 ml of palm wine sample was diluted serially in 9ml of sterile distilled water. 1ml of the diluted sample was dispensed into 2 petri dishes each that contain yeast peptone dextrose agar that was supplemented with 0.1mg/ml of chloramphenicol to inhibit the growth of bacteria. The plates were incubated at 25°C for 48 hours. After 48hours, the plates were examined for the development of colonies. The colonies were further streaked on PDA agar to obtain pure colonies.

2.9.2 Preparation of Sorghum wort

Infusion mashing was carried out according to [16]. Malt samples from the maize were ground in a hand mill to produce grists with 2 mm particle size. Ten grams of each grist sample was added to 72 mL of distilled water contained in a 250 mL Erlenmeyer flask placed in a water bath at a temperature of 67 °C. The contents of the flasks were stirred using a glass rod until the temperature fell to 65 °C. The flasks were allowed to remain at this temperature for 60 min, and the contents stirred at intervals of 10 min. Thereafter, the contents of the flasks (mash) were cooled with tap water and allowed to stand at room temperature (28 °C) for 25min. The mash samples were transferred through wide-necked funnels into 100 mL Erlenmeyer flasks. Precipitates from the mash samples left behind in the 250 mL flask were rinsed out with some water. The contents of the flasks were filtered through a 16 cm diameter filter paper placed in a funnel. The first 50 mL of each filtrate collected was returned to the funnel. Filtration was allowed to continue for 30 min. The resulting final filtrate was used for specific gravity determinations, which were then used to calculate the hot water extract values and then used for fermentation.

2.9.3 Preparation of bitter leaf extract

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Bitter leaf extract was used in the place of hops. Fresh bitter leafs were obtained, washed and rubbed vigorously between palms to get the extract. 15ml of this was used in the fermentation process.

2.9.4 Fermentation of sorghum wort

500ml of the maize wort was separated into two 250ml conical flasks. The bitter leaf extract added was varied with flask 1 containing 5ml and flask 2 containing 10ml respectively. The flasks were boiled for 30mins, cooled to 20°C and pitched with 25ml of liquid Saccharomyces cerevisiae each. The inoculum had an optical density of 0.015 at 625nm wavelength. The inoculated wort were corked with cotton wool and incubated at room temperature for 3days. The cotton plug was removed and fermentation lock was used so that CO2 produced may be evolved and then allowed to ferment until 14days is completed. Test for completion was carried out by measuring specific gravity at day 10 and day 12. On day 15, the fermented liquid was centrifuged at 4000-5000rpm for 15mins to remove all yeast cells and the supernatant (beer) was stored in the refrigerator at low temperature.

2.9.5 Measurement of bitterness

This was measured using EBC/MEBAK method. The bitter substances in beer and wort, in particular iso-α acids are extracted from the acidified sample with isooctane and the concentration in the extract is measured by spectrophotometry. The wort was clarified by centrifuging at 3000 rpm for 15 min and no filtration was done. CO2 was expelled from the sample (beer) without loss of any foam. 10 ml of the sample (tempered to 20 °C) was pipetted into a centrifuge glass and 0.5 ml of 6M HCl was added followed by the addition of 20 ml of isooctane, and 3 glass beads. The glass was close centrifuged and shaken mechanically at 20°C and at optimum mixing intensity for 15min. Then, Centrifugation at 3000 rpm for 3 min to separate the phases and break the emulsion was done. The clear supernatant was immediately transferred to curvette and measured at 275nm.The bitterness (IBU) was calculated using the formula below:

Bitterness (IBU) = Absorbance reading 275nm × 50

2.9.6 Measurement of colour

This was measured using EBC/MEBAK method. The absorbance is measured by spectrophotometry in a 10-mm rectangular OS cell. The color, expressed in EBC units, is calculated by conversion with a predefined factor. CO2 was expelled from the beer sample and the sample was filtered using a membrane filter. The sample was optionally clarified by adding 0.1% kieselguhr and filtered prior to the membrane filtration step. Filtration can be dispensed with in the event that the turbidity of the diluted sample is lower than 1 EBC turbidity units. In the event of EBC units > 60, dilute the sample so that its color is within the measurement range; use the corresponding dilution factor when subsequently calculating the result (measurement result x dilution factor). Transfer the filtrate to the curvette and read at 430nm. The colour in EBC units was calculated using the formula below:

EBC = 25 × D × Absorbance reading 430nm

Where D = dilution factor;

D = 1 for undiluted sample

D = 2 for 1:1 dilution

2.9.7 Determination of alcohol content

Alcohol content (%) was determined by measuring the amount of fermentable sugars. Fermentable sugars were measured using hydrometer and it was calculated by the formula below:

Fermented sugars (%) = [(O.G – F.G) × 100] / O.G

Where, O.G = Original gravity

F.G = Final gravity

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3. RESULTS AND DISCUSSION

3.1 PROXIMATE ANALYSIS

The result in table 1 shows the proximate composition of the analyzed sorghum grain with protein content 10.75g, carbohydrate content 68.41g, fat 3.11g, ash content 0.72g, crude fiber 2.40g and moisture 11.59%.The result obtained correlated with the study of [13] on proximate analysis of selected sorghum cultivars where they reported that protein content ranged from 6.23 - 13.81%, carbohydrate 65.57 - 76.28%, lipid 3.60 - 10.54, fibre 1.65 - 7.94%, ash 1.12 - 1.68%, and moisture 9.75 -16.32%.

Table 1. Proximate analysis of sorghum

Parameters	Sorghum sample
Protein(g)	10.75
Carbohydrates (g)	68.41
Fats (g)	3.11
Ash content (g)	0.72
Crude fiber (g)	2.40
Moisture content (%)	11.59

3.2 Alpha-Amylase Activity

The outcome of the amylase activity was calculated from the α-amylase standard curve where various maltose concentrations liberated were as follows: S1 with 0.9mg/ml, S2 produced 0.7mg/ml and S3 with 0.6mg/ml. This result reveals low sugar concentration which is an indicator of the relatively low α –Amylase produced during malting. This might probably be as a result of the sorghum cultivar used, and poor malting conditions.

Table 2. α-Amylase assay readings

Samples	Reading (540nm)
S1	0.575
S2	0.553
S3	0.464
S4	0.754

Table 3. α -Amylase standard curve reading

Standard (STD)	Reading (Abs $_{540nm}$)

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1%	0.995
2%	1.070
3%	1.170
4%	1.264
5%	1.390
6%	1.430
7%	1.967
(Blank)	0.500

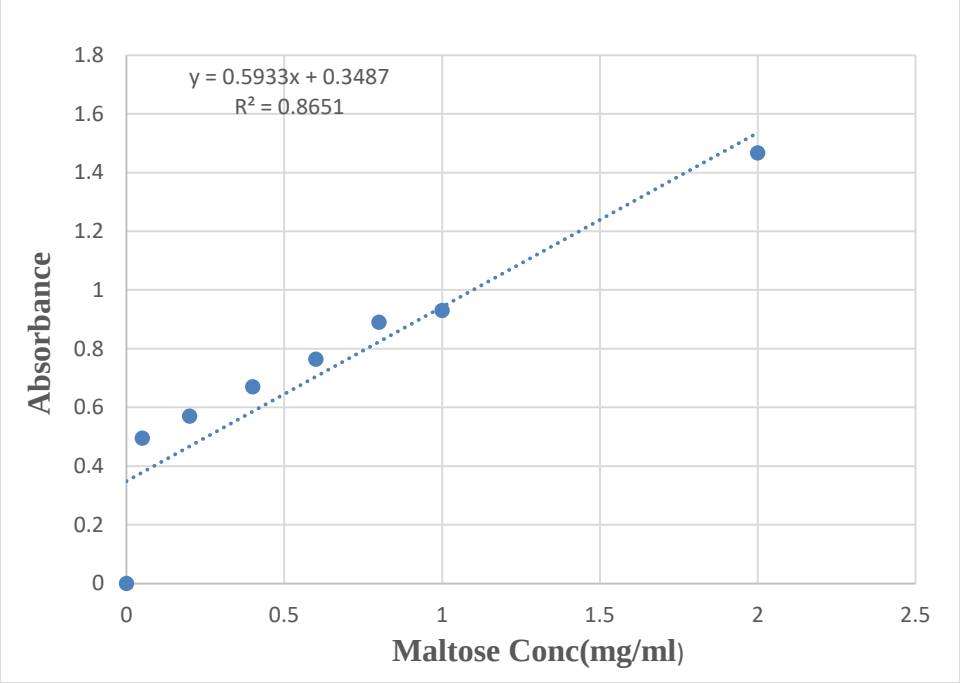

Fig 1: Alpha Amylase Standard Curve

3.3 Beta-Amylase Activity

β-amylase activity was recorded with the following maltose liberated, S1 with 1.5mg/ml, S2 produced 1.4mg/ml and S3 with 1.2mg/ml. From this result, the low sugar concentration is an indicator of the relatively low β –Amylase produced during malting. This might probably be as a result of the sorghum cultivar used, and/or poor malting conditions.

Table 4. β –Amylase Standard curve reading

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Standard (STD)	Reading (Abs $_{540nm}$)
1%	0.890
2%	1.070
3%	1.170
4%	1.264
5%	1.390
6%	1.430
7%	1.967
Blank	0.410

Table 5: β –Amylase assay readings

Samples	Reading (540nm)
S1	0.311
S2	0.158
S3	0.014
S4	0.441

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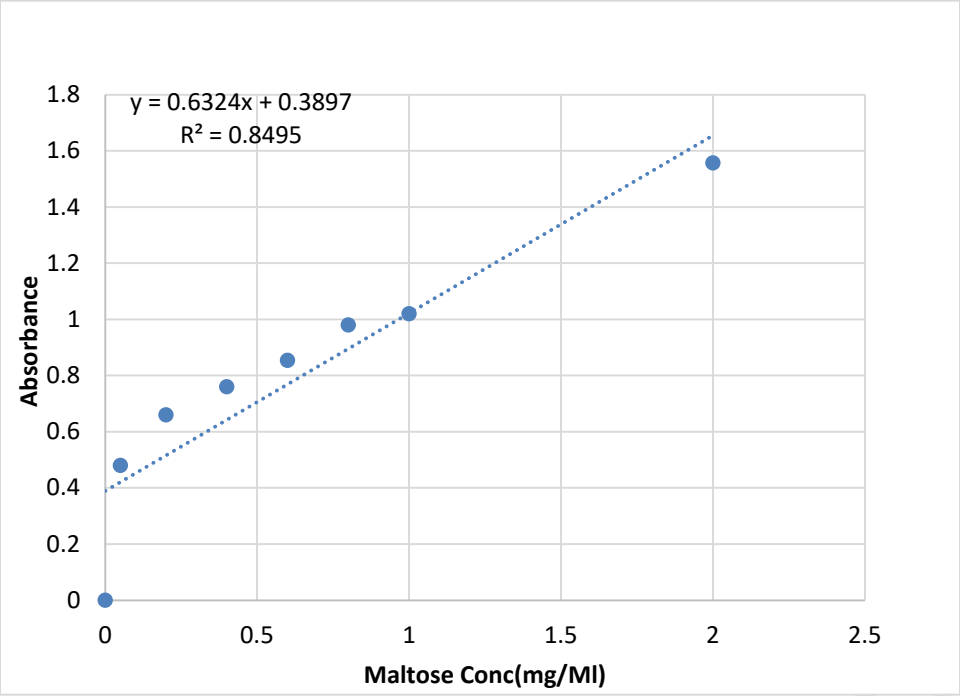

Fig 2. Beta Amylase Standard Curve

3.4 Wort Properties

The result obtained in table 6 revealed the HWE value as 415°/kg. This result is within the acceptable range for HWE performed with infusion method because high values are always recorded. The study conducted on the Grain and malt quality properties of some improved Nigerian sorghum varieties showed HWE values 0f 180-250°/kg [16]. The result obtained in this study is higher as a result of the sorghum variety used. The HWE value is in agreement with the work of [18] who obtained value as high as 315°/kg in his study on development of amylolytic potentials of sorghum. The diastatic power recorded as 40°L as showed in table 6. This is a bit low which is as a result of poor enzyme activities during conversion of starch to fermentable sugars. Diastatic power is also expressed in sorghum diastatic units (SDU), where one SDU equals approximately 2° Linter. The total diastatic activity of sorghum malt is less than half of barley (53°L) which is probably because of the apparently very low β-amylase activity of sorghum malt [24]. As reported in a study about the production of local sorghum beer, diastatic power ranged between 32 and 42.5 SDU/g across malting conditions [9].

Table 6. Hot water extract and diastatic power

Wort Properties	Values
Hot water extract HWE	415°/kg
Diastatic power DP	40°L

3.5 Beer Quality

The alcohol content (%) of the beer for both 5ml and 10ml bitter leaf extract were obtained in table 7 as 6 and 3.4% respectively. The alcohol content of both 3.6% for 5ml and 3.4% for 10ml were within the range of alcohol content of 100% brewed sorghum beer. The result was in agreement with [19] who reported that the desired alcohol content of beer is generally 3.92 ± 0.08%. The bitterness of the beer for both 5ml and 10ml bitter leaf extract were shown in table 7 as 100.38 IBU and 101.82 IBU respectively. These values are within the acceptable range both for 5ml and 10ml respectively

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but comparatively higher as expected in 10ml bitter leaf extract. The bitter leaf offered a good degree of bitterness to the beer but lacked the characteristic aroma of hops when used in beer production. The colour when physically observed was brown and the corresponding EBC value were high. Table 7 shows the colour of the beer for both 5ml and 10ml bitter leaf extract and the values were 19.15 EBC and 21.45 EBC respectively. This could be attributed to the color of raw sorghum used due to the presence and oxidation of polyphenols and melanoidin in the sorghum, though not toxic, but inimical to producing good beer [5].

Table 7. Quality parameters of sorghum beer

Parameters	5ml bitter leaf extract	10ml bitter leaf extract
Alcohol content (%)	3.6	3.4
Bitterness (IBU)	100.38	101.82
Colour (EBC)	19.15 EBC	21.45 EBC

4. CONCLUSION

The outcome of this study revealed that sorghum malt contains important enzymes such as alpha and beta amylases even though their activities were reportedly low as shown in this study. Sorghum malt has always had the potentials of replacing barley malt in beer production but some properties such as malt loss during malting process, low enzyme activities; high gelatinization temperature and low production of fermentable sugars are all key hindrances. However, the appropriate malting conditions and the introduction of external enzyme to further gelatinized sorghum malt during mashing can further improve the beer qualities produced from sorghum malt. This study also revealed that bitter leaf offered a good degree of bitterness to the beer but lacked the characteristic aroma of hops.

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