Blend of Sorghum and Yellow Cassava as a Substrate for Beer production using *Saccharomyces cerevisiae*

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Abstract: The search for local alternatives to barley for brewing has been a major concern to stakeholders in the beer industry in Nigeria and Africa. One of such alternative and which can be sustained is the use of Sorghum and cassava as raw materials. This research work was aimed at investigating the possibility of producing lager beer using a blend of sorghum and hybrid yellow cassava (*IBA 070593* and *IBA 070539)*. The two yellow cassava varieties were blended with the sorghum malt at ratio 0:100 (control), 20:80, 30:70, 40:60 and 50:50. Fermentation was carried out for duration of 10 days and samples analyzed every 2 days interval. Parameters analyzed were yeast count, pH, total soluble sugars, alcohol content and sensory evaluation using standard procedures. The results showed that the formulation ratio of 20:80 had the highest yeast count and alcohol content of 286.7 ± 2.60 × 10^{12}cfu/ml and 6.78 ± 0.41 % respectively, while the least values of 247.3 ± 1.76 × 10^{12}cfu/ml and 3.63 ± 0.49 % were from 50:50 ration.Sensory evaluation showed that overall acceptability of 8.00 ± 0.05 was from 20:80 ration while the least of 7.30 ± 0.13 was from 40:60. The study revealed that the hybrid of yellow cassava blended with sorghum can be a favourable raw material for beer production.

Keywords: Beer, Cassava, Sorghum, *Saccharomyces cerevisiae*, Alcohol content

INTRODUCTION

Lager beers use barley as their major raw material (Stewart, 2016). The tropical climate prevalent in Nigeria does not favour the cultivation of barley as it is a temperate cereal (Taylor *et al*., 2013). To attend to this problem, importation of barley must be carried out which requires the loss of hard earned foreign exchange and consequent rise in beer price which could be well above the pockets of a majority of Nigerians. Another drawback of importation is that it denies local farmers potential markets. In 1988, the Federal Government of Nigeria placed a ban on the importation of barley and other grains like wheat with a view to reduce foreign exchange losses through importation and encouraging indigenous production of possible substitutes for brewing (Taylor *et al*., 2012; Akinyoade *et al*., 2016; Chavan *et al*., 2016). Even though the ban has been lifted a long time ago following inconsistent government policies, grains like sorghum have been in consistent use due to their low cost and affordability (International Sorghum and Millet Collaborative Research, 2008; Adegbola *et al*., 2013). This evolution has provided lots of economic advantages (Adegbola *et al*., 2013).

Over the years, giant strides have been made to locally source for alternative carbohydrate sources (sorghum, maize, millet, cocoyam, cassava) and enzymes for brewing (Akinyoade *et al*., 2016; Gomaa, 2018). This has become imperative as the demand of the beer industry and activities have far outweighed the requisite supply of raw materials for brewing in Nigeria (Antia-Obong, 2019). It has been reported that lager beer has been brewed using other carbohydrate sources in place of barley such as maize, rice, plain sorghum, sprouted sorghum and maize grits, sorghum malt and cassava (Bailly *et al*., 2014; Ore *et al*., 2018; Ceccaroni *et al*., 2019). Other sources employed tentatively include sweet potato, whey, millet, starch syrup and cocoyam (Onwuka and Eneh, 2005; Usai *et al*., 2013; Bano *et al*., 2015; Panda *et al*., 2015; Macwan *et al*., 2016).

Sorghum is a cereal crop of the grass family Graminae, with more than 10,000 known varieties (Owuama, 2019). Sorghum ranks fifth when it comes to global cereal production and is an important food crop in several

continents including Africa, America and Asia Some particular sorghum varieties have brewing potential as they contain several brewing enzymes (such as α-amylase, β-amylase and proteinases) and good malting properties (such as high diastatic power and extract yield) (Ogbonna, 2011; Owuama, 2019). Several improved varieties of sorghum with relatively better brewing potentials have been developed and is still being developed by research institutes (Ogu *et al*., 2006).

Nigeria is the world's largest producer of cassava (Ashaye*et al*., 2018). Cassava is the third largest source of human food in the world (Karri and Nalluri, 2016). Yellow cassava varieties have been bred genetically, developed and grown in Nigeria through collaborative efforts of International Institute for Tropical Agriculture (IITA), National Root Crops Research Institute (NRCRI) and other research institutes, scientific institutions as well as Government agencies (Ilona *et al*., 2017; Onyeneke *et al*., 2019). Yellow cassava is biofortified with beta-carotenes and is a very rich source of vitamin A (Talsma *et al*., 2016). It is therefore imperative that carbohydrate source from yellow cassava (so far an underutilized crop) would not only be more economical but could help address some of the impending nutritional deficiency issues (especially that of vitamin A) prevalent in Nigeria (De Moura *et al*., 2015; Ilona *et al*., 2017). Production of beer using yellow cassava could help create a ground for competitive demand and awareness of the crop as well as employment opportunities on a broad spectrum basis and generate more revenue for the government with a view to raising the economy. The use of yellow cassava in brewing on the long run could also create an avenue for training Nigerian Food Scientists and Microbiologists with high level skills in cassava brewing and technology (Kolawole and Agbetoye, 2007). The technologies developed in Nigeria could help countries like the USA and Australia to (Abiodun, 2002; Hailu and Assefa, 2018). produce cassava lager beers to help individuals who are gluten intolerant due to celiac disease (Cohen *et al*., 2019; Cela *et al*., 2020). Thus, this study focused on the use of sorghum blended with yellow cassava as a substrate for beer production using *Saccharomyces cerevisiae*

MATERIALS AND METHODS

Collection of Samples and Preparation

Yellow cassava varieties *IBA 070539* and *IBA 070593* (5 kg of each variety) was obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria and transported to the Biotechnology laboratory of the Federal Institute for Industrial Research Oshodi (FIIRO), Lagos State, Nigeria. Sorghum hybrid variety, CSR-03H was obtained from the National Cereals Research Institute (NCRI), Zaria, Kaduna State, Nigeria through Food and Agro Allied Ltd, Sango-Otta, Ogun State, Nigeria; and transported to the laboratory. Yeast (*Saccharomyces cerevisiae* Strain *B05*) and hops extract was supplied by Nigerian Breweries Plc, Iganmu, Lagos State, Nigeria.

Measured 5 kg of each variety of fresh cassava roots were prepared separately. The roots was thoroughly washed, peeled and milled using a Quadrumat Jr. Laboratory mill (Brabender, Duisburg, Germany) into fine slurry. The slurry was pressed and squeezed to reduce the hydrocyanic acid content. Thereafter, it was dried and stored in a plastic bag at 28 ± 2^{o}C until further analyses or use.

Measured 5 kg of sorghum grains were cleaned by first manually sorting to remove shapeless, broken and immature kernels, dust, stones and other extraneous materials. The kernels were washed in tap water contained in a 40 L bucket. Thereafter, the grains were stirred in the water and sieved out. The sieved grains were sun dried and stored in clean plastic bags.

Preparation of Sorghum for beer production

Germinative energy (GE), Germinative capacity (GC), Water sensitivity, Diastatic power (DP), Cold water extract (CWE) and Hot water extract (HWE) tests were performed on the sorghum variety to find out whether it was suitable for saccharification.

Germinative energy and Germinative capacity

Germinative energy (GE) test was performed by addition of 4 ml of water to a petri dish containing 100 seeds and 2 pieces of filter paper. Germinated grains was removed every 24h. Acceptability of grains was set at 95% germination after 72 h as recommended by the American Society of Brewing Chemists' official method of analysis (Francakova *et al*., 2012; ASBC, 2009).

Germinative capacity (GC) or dormancy test was carried out by placing 100 grains in a petri dish containing 100 ml 0.75% hydrogen peroxide for 48 h. The dish was drained; germinated grains were removed and counted with 95% germination set at acceptability level as recommended by the American Society of Brewing Chemists' official method of analysis (Hailu and Assefa, 2018; ASBC, 2009).

Water sensitivity

Water sensitivity was determined using the method of Sanni and Fatoki (2017). Measured 100 grains of sample each in petri dishes lined with filter papers (Whatmann No. 1) were wetted with 5 ml and 10 ml of distilled water and allowed to germinate for 72 h. The water sensitivity value was measured as the difference in the number of grains that germinated in the two petri dishes.

Malting and Kilning of sorghum seeds

Malting was carried out by weighing 5 kg of sorghum grain into 50 L plastic bucket containing 40 L of water and steeped for 10 h at temperature of 30^{o}C. The steeped water was drained and replaced with fresh water every 6 h for 48 h. After steeping, the grains were blotted with towels to remove surface water and placed on aluminium tray. The tray was covered with foil papers and left to germinate for another 72 h. During the 72 h germination process, the foil papers were partially removed and the surface of the kernels was sprayed with tap water and turned periodically at 6 h intervals. The resulting seedlings were kilned in a hot air oven at 60^{o}C for 48 h. The kernels were allowed to cool and the rootlets of the resulting seedlings were removed by rubbing vigorously in a sieve of mesh size 1.30 mm.

Milling the malt

Measured 3kg sorghum malt was dry-milled using a Quadrumat Jr. Laboratory mill (Brabender, Duisburg, Germany) into fine malt flour.

Diastatic Power

The Diastatic power of the malt was obtained using Fehling's solution as described by the American Society of Brewing Chemists recommended method of analysis (ASBC, 2009). Measured 3 ml aliquot of malt infusion extract was pipetted into 100 ml of 2% buffered starch solution in a 200 ml Erlenmeyer flask. The mixture was shaken thoroughly and allowed to stand at 28 ± 2^{o}C for a period of 1 h.

After this period, measured 15 ml of 0.1 M NaOH was added to halt the reaction and the mixture was made up to 200 ml with distilled water. This mixture was transferred to a burette and titrated against 5 ml of mixed Fehling's solutions A and B in a 150 ml boiling flask using 3 drops of methylene blue as indicator. The contents of the flask were thoroughly mixed and boiled for 2 min and the end point was reached when the indicator turned reddish brown. The blank was also prepared by titrating undiluted 2% starch solution against 1 ml of mixed Fehling's solution A and 2 ml Fehling's solution B using methylene blue as indicator. The diastatic power was obtained by calculation in Linter (^{o}L) units using the formula:

$$DP = \frac{2000 - 200}{xy - xs}$$

Where,

x = Number of ml of malt extract

y = Number of ml of converted starch to 5 ml of the Fehling's solution

s = Titre for starch blank

Cold water extract

For determination of Cold water extract (C.W.E), the Institute of Brewing recommended method of analysis was used (IOB, 1989). Measured 3 g of malt was extracted with 30 mL of ammonium hydroxide solution (27.5 ml distilled water + 2.5 ml 0.1 M NH_4OH) at 25°C. The extract was filtered using Whatman filter paper and the percentage of dissolved sugar read at 20°C using a refractometer. The cold water extract was calculated using the formula:

$$\% \text{ C.W.E} = \frac{P\ (m + 1000)}{100 - P}$$

$$\% \text{ C.W.E (dry basis)} = 100 \times (100 - m)$$

Where,

P = Percentage sugar by refractometer reading,

M = Malt moisture content

Hot water extract

The Institute of Brewing recommended analytical method was used for the determination of the Hot water extract (H.W.E) (IOB, 1989). Measured 50 g of malt grist in a 100 ml Erlenmeyer flask was extracted with 360 ml distilled water in a water bath at 45°C for 30 min. The wort was separated from the mash by decantation and the temperature of the mash was raised to 100°C in a water bath. The mash was maintained at this temperature for 2 min and then lowered to 65°C. The wort was again added to the mash and further extraction was carried out at 65°C, stirred at 30 min interval for a period of 1 h to prevent seed formation after which the solution was cooled and the volume made up to 515 ml with distilled water. This mixture was allowed to stand for 20 min followed by shaking. The mixture was decanted into a Whatman filter paper and the percentage sugar content of the filtered wort was read using a handheld Bellingham refractometer. The percentage hot water extract was calculated using the relation:

Extract = Excess gravity × 10.31°/kg

$$\% \text{ H.W.E (dry basis)} = \frac{Extract \times 100}{1000 - m}$$

Where, m = moisture content of grain

Mashing, Iodine test and Wort boiling

Measured 200 g of sorghum malt suspended in 400 ml mash liquor was gelatinized at 80°C for 20 min. The sample was subjected to a three-mash decoction mashing as described by Rajagopal (1976). One-third of the mash was withdrawn and boiled separately in a boiling flask for 3 min while stirring continuously to prevent seed or lump formation and then returned to the main mash so that the temperature rose to 55°C. The pH of the mash was adjusted to 5.5 and the mash was maintained at this temperature for 15 min in a water bath. Again, one-third of the mash was once again withdrawn and boiled separately for 3 min while stirring before being added back to the main mash. This temperature was raised to 65°C and the pH of the mash was again adjusted to 5.5. The mash was maintained at this temperature for 15 min in a water bath. A last one-third of the mash was removed, boiled separately for 3 min while stirring and returned to the main mash raising the temperature of the mash to 69°C. The pH was adjusted to 5.5 and the mash was allowed to be maintained at this temperature for 15 min. Thereafter, the iodine test was performed to determine the extent of saccharification followed by filtration of the mash using muslin cloth to obtain the wort. To 2 ml of wort sample, two (2) drops of iodine solution was added.

The result was colourless. A blue black result would have indicated partial saccharification while a colourless result indicates complete saccharification.

The wort was boiled for 1h in a 2 L beaker followed by addition of yeast nutrients (0.5 g/L $(NH_4)_2H_2PO_4$, 1.5 g/L KH_2PO_4, 10 g/L yeast extract, 0.5 g/L $MgSO_4.7H_2O$), 0.05 w/v of ammonium sulphate and 2 g of hops. After boiling, the wort was filtered, autoclaved and kept cold at 10^{o}C. The pH of the wort was adjusted to 5.5 prior to fermentation. Iodine test was carried out to check for the presence of starch (Onwuka and Eneh, 2005).

Yeast propagation

The yeast was cultured and sub-cultured on Yeast Extract Peptone Dextrose (YEPD) medium. Three pure colonies were inoculated into 20 ml autoclaved liquid wort at 20^{o}C in a 100 ml Erlenmeyer flask and shaken at 110 rpm for 40 h. The 20 ml wort was transferred to 200 ml autoclaved liquid wort at 18^{o}C in a 1.5 L flask and shaken at 110 rpm for 40 h. The 200 ml wort was transferred to 800 ml autoclaved liquid wort at 16^{o}C in a 2 L flask and shaken at 100 rpm for 40 h. The collected cells were counted using a hemocytometer and used for fermentation (Fawole and Oso, 1988).

Cassava/Sorghum substrate formulation for beer production

Measured 40 g weight of cassava grits suspended in 400 ml mash liquor was gelatinized at 80^{o}C for 20 min. After cooling, measured 160 g weight of malt powder was added to give 50 % mash. In this case, the mixing ratio of cassava grits to sorghum malt was in the ratio 20:80. Other wort mixing ratios for cassava grits to sorghum malt of 30:70, 40:60, and 50:50 were prepared. A control experiment was also prepared and consisted of 200 g sorghum malt only (without cassava grits) to give a ratio of 100:0. This procedure was carried out using the two cassava varieties separately to give a total of nine (9) wort samples as shown in table 1. These samples were subjected to three-mash decoction mashing as previously described. Iodine test was performed on the worts obtained followed by wort boiling as previously described.

Table 1: Blending proportion of samples.

Sample varieties	Sample Code	Sorghum (%)	Yellow cassava (%)
Sorghum + Yellow Cassava (*IBA 070593*)	SC593 80:20	80	20
	SC593 70:30	70	30
	SC593 60:40	60	40
	SC593 50:50	50	50
Sorghum + Yellow Cassava (*IBA 070539*)	SC539 80:20	80	20
	SC539 70:30	70	30
	SC539 60:40	60	40
	SC539 50:50	50	50
Sorghum	SC 100:0	100	0

Key: C – Yellow Cassava
S – Sorghum.

Fermentation process

The fermentation of the different cassava/sorghum blended portions and 100% sorghum wort was carried out using 200 ml of wort in 1.0 L Erlenmeyer flask at pH 5.50, inoculated with 1.24×10^8 cells/ml of yeast and incubated at 28 ± 2 ^oC for 6 days. At every 2 days intervals samples were analyzed for microbiological, physicochemical, proximate analyses and sensory evaluation. After 6 days, the resulting 'green beer' was transferred aseptically into another 1.0 L Erlenmeyer flask for secondary fermentation to take place for another 4 days.

Beer maturation, sterilization and packaging

The resulting beer was centrifuged and filtered using cellulose acetate filter. Further clarification was performed by addition of 2 g of bentonite and the product was again filtered using cellulose acetate filter. The product was maturated for a period of 3 months at a temperature of 4^oC to cause "cold break" and enhance flavour development. Thereafter, the beer was filtered and pasteurized at 60^oC for 15 min in a water bath prior to bottling (Hailu and Assefa, 2018).

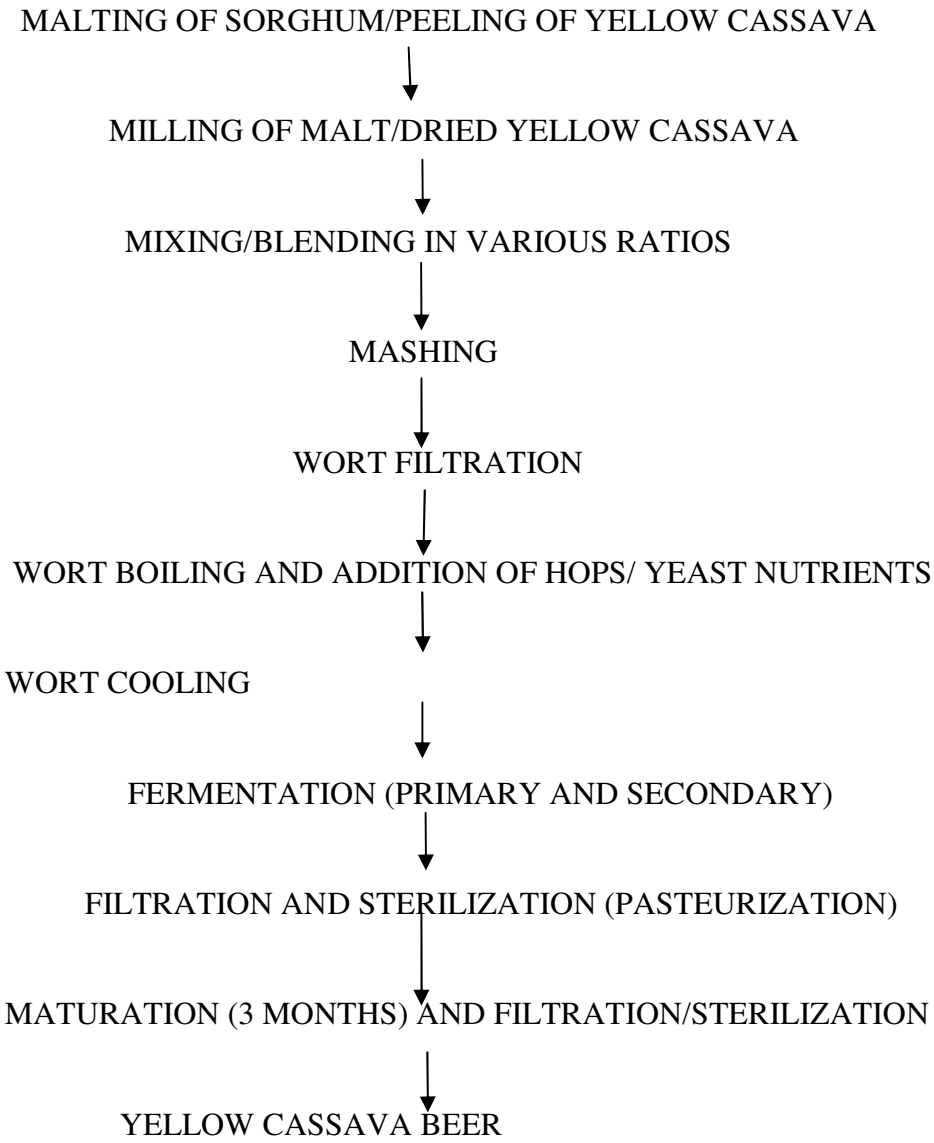

Figure 1: Production of Yellow Cassava Beer

Microbiological analysis of Yellow Cassava beer

Serial dilution was carried out serially up to 10^{-10} dilution and used for enumeration of microorganisms. Microbiological analysis involved total viable count, coliform count, fungal count and lactic acid bacteria identification using plate count technique.

Total viable count

Measured 1 ml of the 10^{-1} to 10^{-3} dilutions of each sample were aseptically drawn with the aid of sterile Pasteur pipette and dispensed into sterile labeled petri dishes. Measured 15 ml of sterilized nutrient agar (cooled to 45^{o}C) was poured into each petri dish and carefully swirled to homogenize. The plates were allowed to cool and set, thereafter incubated at inverted position at 37^{o}C for 48 h for bacterial counts. The colonies on the plates were counted using a haemocytometer to obtain the colony forming unit and recorded in cfu/ml (Fawole and Oso, 1988).

Total coliform count

Total coliform count was performed using most probable number technique (MPN) (Fawole and Oso, 1988).

Fungal count

Measured 1 ml of each dilution of sample was introduced into sterile petri dishes and 20 ml of sterile molten Potato Dextrose Agar (PDA) supplemented with 0.5 g Chloramphenicol to inhibit the growth of bacteria was introduced into the inoculum aseptically. The petri dishes were swirled to homogenize and then allowed to cool and set. The PDA plates were incubated for 72 h at 28 ± 2^{o}C and the fungi colonies were counted using a haemocytometer and recorded in cfu/ml (Fawole and Oso, 1988).

Lactic acid bacteria count

Measured 1 ml sample was inoculated into selective media De Mann Rogosa Sharpe (MRS) agar supplemented with 0.5% cycloheximide for total Lactic acid bacteria (LAB) enumeration as well as *Lactobacilli* isolation while Yeast Glucose Lamco Agar (YGLA) was used for the isolation of *Streptococci* of LAB origin. Inoculated plates were incubated at 37^{o}C for 48 h (Fawole and Oso, 1988).

Physico-chemical and proximate analyses of Yellow Cassava beer

pH

Measured 10 ml of sample was transferred into 100 ml beaker. The pH was determined using a pH meter (Model Hanna P 211). The pH meter was calibrated using pH 4.0 and 7.0 buffers.

Total acidity

The total acidity was determined using the method of AOAC (2016).

Total soluble sugar

This was determined using a handheld Bellingham and Stanley refractometer at 28^{o}C.

Specific gravity

The specific gravities of the samples were determined using a handheld Stanley and Bellingham refractometer at 28^{o}C.

Alcohol content

The percentage ethanol of the fermented products was determined using the specific gravity (bottle) method (AOAC, 2016).

Free amino nitrogen (FAN)

The EBC-Ninhydrin method for determination of free alpha amino nitrogen was used (European Brewing Convention, 1998). Measured 1 ml of reagent (100 g $Na_2HPO_4.12H_2O$, 60 g of KH_2PO_4, 5 g of ninhydrin and 3 g of fructose dissolved in 1 litre of distilled water at pH 6.7) was added to 2 ml of the samples (diluted with water 50-fold) and heated for 16 min in a water bath.

This was followed by cooling for 20 min in a water bath at 20^{o}C. Measured 5 ml of diluted solution (2 g $KClO_3$ in 600 ml distilled water and 400 ml of 96% ethanol) was added to each tube and mixed thoroughly. Absorption at 570 nm was read using a spectrophotometer within 30 min after addition of diluted solution. The blank was also prepared using 2.0 ml of water in place of the sample following exactly the same procedures. Free amino nitrogen (FAN) was obtained by calculation using the relation:

$$FAN = \frac{(Ap - Ab - Af) \times 2 \times d}{As - Ab}$$

Where,
FAN = The amount of free amino nitrogen in the sample in mg/l,
Ap = Average absorbance of the test solution,
Ab = Average absorbance for the blank,
Af = Average absorbance for the correction for dark worts and beers,
2 = Amount of free amino nitrogen (mg/l) in the glycine standard solution,
As = Absorbance of the glycine standard solution.

Bitterness

Measured 10 ml of samples were transferred into 100 ml test tubes containing 20 ml 2,2,4-trimethylpentane and placed in a water bath at 20^{o}C for 30 min. After this period, the samples were acidified with 1 ml of 0.1 N HCl and shaken vigorously for 1 min. The samples were then placed in a water bath at 20^{o}C for another 30 min. The supernatant organic phase was carefully decanted into cuvette and the optical densities (OD) read at 275 nm wavelength and European Brewing Convention (EBC) bitterness units calculated by multiplying the OD by a factor of 100 (EBC, 1998).

EBC bitterness units = OD × 100

Colour

Measured 10 ml of samples were filled into cuvettes and the optical densities (OD) were read at 430 nm wavelength using a UV-Visible spectrometer. The optical density (OD) was expressed in terms of European Brewing Convention (EBC) colour units as follows,

EBC colour units = OD × 25

Foam stability

This was determined using the Nibem method as described by ASBC (2009).

Haze characteristics

Turbidity of the samples was measured using a hazemeter VOS ROTA 90/25 model and results were expressed in EBC (Formaline Nephelometric Unit).

CO_2 content

The method of Popescu*et al.* (2013) was adopted. Measured 50 ml solution of sodium trioxocarbonate (IV) was added to 25 ml of beer at 4^{o}C in a 1000 ml glass cylinder. The contents were thoroughly mixed using a glass rod. Measured 400 ml of cold water (at 4^{o}C) which have been previously boiled was added to the contents in the cylinder and titrated against 0.2 M HCl solution using phenolphthalein as indicator. The end point was reached when the solution turned colourless. Again, measured 100 ml of distilled water was added to 25 ml of beer (at 4^{o}C) in a 500 ml Erlenmeyer flask and boiled for 3 min. The solution was cooled to 20^{o}C and titrated against 0.2 M sodium trioxocarbonate (IV) solution using phenolphthalein as indicator until the solution turned colourless. The amount of CO_2 in grams per 100 ml of beer was calculated using the relation:

CO_2 content (g/100 ml) = 4 [(50 - 2V_1) – V_2] × 0.0044

Where V_1 = Volume of 0.2 M HCl in ml used in the first titration

V_2 = Volume of 0.2 M Na_2CO_3 in ml used in the second titration and

0.0044 is the amount of CO_2 in grams which corresponds to 1 ml 0.2 M Na_2CO_3.

Sensory evaluation of Yellow Cassava beer

Sensorial characteristics of the final beer product were assessed by twenty (20) trained panelists of the Department of Biotechnology, Federal Institute for Industrial Research Oshodi (FIIRO), Lagos State, Nigeria. A nine (9) point hedonic rating scale was employed and two known commercial lager brands ('Star' and 'Life') were used as the standard set at nine (9) point. Each of the panelists was provided with a score card and coded samples.

The panelists were asked to allot scores for the quality attributes of colour, flavor, taste, mouth feel and overall acceptability based on their individual preferences. The data obtained was subjected to statistical analysis to locate significant differences between means of samples.

Data analysis

An average of triplicate readings for each parameter was used for all the determinations. Analysis of variance (ANOVA) was performed using SPSS statistical package (version 20) to locate significant differences between means of triplicates.

RESULTS

Analyses of Sorghum seeds and Cassava

Analysis of the sorghum grains before malting is presented in table 2. Analysis of the malted sorghum before mashing is presented in table 3.

Microbiological analysis of Yellow Cassava beer

The total viable count for bacteria of Sorghum amended with cassava during fermentation of beer is shown in table 4. The total lactic acid bacteria count on MRS (De Mann Rogosa Sharpe) agar is shown in table 5. The yeast count is presented in table 6. The values increased for all samples.

Physico-chemical analysis of Yellow Cassava beer

The pH of the beer during fermentation is shown in table 7. The pH decreased for all the samples throughout the period of fermentation. Table 8 shows the titratable acidity of the beer during fermentation. The values increased for the samples during fermentation. The total soluble sugar (TSS) of the beer during fermentation is revealed in table 9. table 10 shows the specific gravity values of the beer during fermentation. The values decreased for all the samples during fermentation.

The alcoholic content of the beer increased during the fermentation period as shown in table 11. The free amino nitrogen (FAN) values of the beer during fermentation are shown in table 12. FAN values for all the samples decreased during fermentation. The bitterness of the beer after fermentation is presented in table 12. The colour of the beer is shown in table 12. The foam stability of the beer is shown in table 12. The haze values of the beer are shown in table 12. The CO_2 content of the beer is shown in table 12.

Sensory evaluation of Yellow Cassava beer

The sensory analysis of the beer after 240 h fermentation period is shown in table 13.

For colour, sample SC593 50:50 had the highest value (6.06 ± 0.13) while SC539 80:20 had the least (4.32 ± 0.12). For taste, the highest value (7.77 ± 0.13) was from sample SC593 80:20 while the least (7.08 ± 0.09) was from sample SC539 50:50. The highest mean sensory score for taste was recorded for SC593 80:20. Similarly, flavour was highest (8.16 ± 0.18) in sample SC593 50:50 and least (7.86 ± 0.16) in sample SC593 60:40. The mouth feel was highest (7.10 ± 0.14) in sample SC593 80:20 and least (6.43 ± 0.19) in sample SC539 80:20. Overall acceptability was highest (8.00 ± 0.05) for sample SC593 80:20 and least (7.30 ± 0.13) for sample SC593 60:40. There was significant difference between the beer products in terms of overall acceptability.

Table 2: Analysis of sorghum grains before malting

Time (hr)	Germinative capacity (%)	Germinative energy (%)	Water sensitivity (%)
24	79.0 ± 0.00	76.0 ± 0.00	4.00 ± 0.00
48	89.0 ± 0.00	95.0 ± 0.00	2.00 ± 0.00
72	95.0 ± 0.00	96.0 ± 0.00	1.00 ± 0.00

Values are expressed as mean ± standard deviation of triplicates

Table 3: Analysis of sorghum malt before mashing

Parameter	Value
Diastatic power (^{o}L)	142.100 ± 0.924^{b}
Cold Water Extract (%)	19.367 ± 0.555^{d}
Hot Water Extract (%)	68.267 ± 0.555^{c}

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column shows mean that are not significantly different (P > 0.05)

Table 4: Total viable count (× 10^{3} CFU/ml) during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0	48	96	144	192	240
SC593 80:20	21.57 ± 0.49^{e}	19.67 ± 0.88^{d}	10.00 ± 0.58^{c}	ND	ND	ND
SC593 70:30	25.22 ± 0.66^{e}	23.00 ± 0.58^{c}	12.67 ± 0.88^{c}	ND	ND	ND
SC593 60:40	34.50 ± 0.72^{d}	32.67 ± 0.88^{b}	17.67 ± 0.88^{b}	2.00 ± 0.58	ND	ND
SC593 50:50	29.75 ± 0.57^{e}	27.67 ± 0.88^{c}	15.00 ± 0.58^{b}	ND	ND	ND
SC539 80:20	31.25 ± 0.49^{d}	28.00 ± 1.15^{c}	9.00 ± 0.58^{c}	ND	ND	ND
SC539 70:30	39.67 ± 0.61^{c}	37.67 ± 0.88^{b}	13.00 ± 0.58^{b}	ND	ND	ND
SC539 60:40	48.30 ± 1.18^{a}	44.00 ± 1.15^{a}	14.67 ± 0.88^{b}	1.67 ± 0.33	ND	ND
SC539 50:50	55.33 ± 1.13^{a}	49.00 ± 0.58^{a}	20.00 ± 1.15^{a}	3.00 ± 0.58	ND	ND
SC 100:0	16.63 ± 0.55^{f}	14.0 ± 0.58^{e}	5.67 ± 0.88^{d}	ND	ND	ND

KEY: ND – Not Detected

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

Table 5: Total Lactic acid bacteria (LAB) count (× 10^3 CFU/ml) during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0	48	96	144	192	240
SC593 80:20	14.00 ± 0.58^{d}	11.67 ± 0.88^{d}	3.00 ± 0.58^{d}	ND	ND	ND
SC593 70:30	20.67 ± 1.20^{c}	18.00 ± 1.16^{c}	7.67 ± 0.88^{b}	ND	ND	ND
SC593 60:40	26.00 ± 0.58^{b}	23.33 ± 1.76^{b}	4.00 ± 0.58^{d}	ND	ND	ND
SC593 50:50	21.00 ± 0.58^{c}	17.33 ± 0.88^{c}	2.67 ± 0.33^{d}	ND	ND	ND
SC539 80:20	27.33 ± 0.88^{b}	24.00 ± 0.58^{b}	5.00 ± 0.58^{c}	ND	ND	ND
SC539 70:30	31.00 ± 0.58^{a}	28.00 ± 0.58^{b}	8.00 ± 0.58^{b}	ND	ND	ND
SC539 60:40	35.67 ± 0.67^{a}	31.33 ± 0.88^{a}	12.33 ± 1.45^{a}	ND	ND	ND
SC539 50:50	37.67 ± 0.67^{a}	33.33 ± 1.20^{a}	10.67 ± 1.20^{a}	ND	ND	ND
SC 100:0	26.00 ± 1.73^{b}	24.00 ± 2.31^{b}	11.0 ± 0.58^{a}	ND	ND	ND

KEY: ND – Not Detected

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

Table 6: Total yeast count during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0 (cfu/ ml × 10^8)	48 (cfu/ ml × 10^9)	96 (cfu/ ml × 10^9)	144 (cfu/ ml × 10^{10})	192 (cfu/ ml × 10^{11})	240 (cfu/ ml × 10^{12})
SC593 80:20	1.28 ± 0.01^{b}	78.33 ± 1.20^{b}	245.33 ± 2.33^{b}	193.67 ± 2.03^{b}	245.33 ± 0.88^{b}	286.67 ± 2.60^{a}
SC593 70:30	1.36 ± 0.03^{b}	75.33 ± 2.03^{b}	230.67 ± 2.91^{c}	191.67 ± 3.18^{b}	238.67 ± 2.03^{b}	274.33 ± 2.60^{b}
SC593 60:40	1.31 ± 0.05^{b}	74.33 ± 0.88^{b}	214.00 ± 3.46^{e}	175.00 ± 1.73^{c}	238.67 ± 2.03^{b}	259.33 ± 2.03^{b}
SC593 50:50	1.29 ± 0.03^{b}	64.67 ± 1.45^{c}	203.33 ± 2.60^{e}	165.00 ± 2.65^{d}	231.67 ± 5.36^{b}	248.33 ± 1.76^{c}
SC539 80:20	1.25 ± 0.03^{b}	69.00 ± 1.73^{c}	248.33 ± 1.45^{b}	191.67 ± 3.18^{b}	218.00 ± 1.53^{c}	267.00 ± 2.08^{b}
SC539 70:30	1.31 ± 0.12^{b}	75.33 ± 2.03^{b}	237.67 ± 2.60^{c}	179.00 ± 2.31^{c}	207.33 ± 2.33^{d}	263.33 ± 2.33^{b}
SC539 60:40	1.34 ± 0.08^{b}	64.67 ± 1.45^{c}	226.67 ± 1.76^{d}	165.00 ± 2.65^{d}	194.67 ± 1.76^{e}	260.00 ± 2.31^{b}
SC539 50:50	1.37 ± 0.09^{b}	78.33 ± 1.20^{b}	222.67 ± 2.33^{d}	160.67 ± 2.91^{d}	175.00 ± 1.53^{e}	248.33 ± 1.76^{c}
SC 100:0	1.38 ± 0.08^{b}	88.67 ± 2.60^{a}	296.00 ± 2.31^{a}	219.00 ± 2.89^{a}	269.00 ± 1.73^{a}	290.00 ± 2.31^{a}

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

Table 7: pH changes during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0	48	96	144	192	240
SC593 80:20	5.50 ± 0.00	5.27 ± 0.01^{c}	4.60 ± 0.01^{c}	3.92 ± 0.01^{c}	3.66 ± 0.01^{b}	3.65 ± 0.01^{b}
SC593 70:30	5.50 ± 0.00	5.30 ± 0.00^{b}	4.75 ± 0.01^{a}	4.36 ± 0.01^{b}	3.70 ± 0.01^{b}	3.70 ± 0.01^{b}
SC593 60:40	5.50 ± 0.00	5.31 ± 0.01^{b}	4.76 ± 0.01^{b}	4.61 ± 0.01^{a}	3.85 ± 0.01^{b}	3.71 ± 0.01^{b}
SC593 50:50	5.50 ± 0.00	5.36 ± 0.01^{b}	4.81 ± 0.01^{a}	4.66 ± 0.01^{a}	3.89 ± 0.01^{b}	3.74 ± 0.01^{b}
SC539 80:20	5.50 ± 0.00	5.12 ± 0.01^{c}	4.75 ± 0.01^{b}	4.37 ± 0.01^{b}	3.74 ± 0.01^{b}	3.65 ± 0.01^{b}
SC539 70:30	5.50 ± 0.00	5.37 ± 0.01^{b}	4.81 ± 0.01^{a}	4.38 ± 0.02^{b}	3.85 ± 0.01^{b}	3.72 ± 0.01^{b}
SC539 60:40	5.50 ± 0.00	5.27 ± 0.01^{c}	4.83 ± 0.00^{a}	4.43 ± 0.01^{b}	4.05 ± 0.03^{a}	4.04 ± 0.01^{a}
SC539 50:50	5.50 ± 0.00	5.34 ± 0.01^{a}	4.84 ± 0.01^{a}	4.45 ± 0.02^{b}	4.15 ± 0.02^{a}	4.10 ± 0.01^{a}
SC 100:0	5.50 ± 0.00	5.34 ± 0.01^{a}	4.75 ± 0.01^{b}	4.20 ± 0.12^{b}	3.87 ± 0.01^{b}	3.50 ± 0.01

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

Table 8: Total titratable acidity (ml) during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0	48	96	144	192	240
SC593 80:20	1.36 ± 0.01^{d}	1.68 ± 0.01^{c}	2.51 ± 0.01^{d}	3.44 ± 0.01^{a}	3.69 ± 0.03^{b}	3.91 ± 0.01^{a}
SC593 70:30	1.34 ± 0.01^{d}	1.50 ± 0.01^{d}	2.48 ± 0.01^{d}	2.88 ± 0.01^{b}	3.69 ± 0.03^{b}	3.84 ± 0.01^{a}
SC593 60:40	1.30 ± 0.01^{d}	1.41 ± 0.01^{d}	2.48 ± 0.01^{d}	2.73 ± 0.01^{b}	3.55 ± 0.03^{b}	3.80 ± 0.01^{a}
SC593 50:50	1.35 ± 0.01^{d}	1.29 ± 0.01^{e}	2.44 ± 0.01^{d}	2.62 ± 0.12^{b}	3.51 ± 0.01^{b}	3.75 ± 0.01^{a}
SC539 80:20	1.31 ± 0.01^{d}	1.75 ± 0.01^{b}	2.48 ± 0.01^{b}	2.90 ± 0.01^{b}	3.63 ± 0.01^{b}	3.87 ± 0.01^{a}
SC539 70:30	1.40 ± 0.01^{d}	1.62 ± 0.01^{c}	2.43 ± 0.01^{b}	2.85 ± 0.01^{b}	3.38 ± 0.01^{b}	3.76 ± 0.01^{a}
SC539 60:40	1.38 ± 0.01^{d}	1.54 ± 0.01^{c}	2.37 ± 0.01^{b}	2.78 ± 0.01^{b}	2.87 ± 0.01^{b}	3.09 ± 0.01^{b}
SC539 50:50	1.27 ± 0.01^{d}	1.38 ± 0.01^{d}	2.34 ± 0.01^{b}	2.73 ± 0.01^{b}	2.84 ± 0.01^{b}	2.97 ± 0.01^{b}
SC 100:0	1.95 ± 0.01^{a}	2.85 ± 0.01^{a}	3.24 ± 0.01^{a}	3.58 ± 0.01^{a}	3.80 ± 0.01^{a}	3.93 ± 0.01^{a}

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

Table 9: Total soluble sugar (^{o}Brix) during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0	48	96	144	192	240
SC593 80:20	17.00 ± 0.00	12.83 ± 0.09^{c}	8.63 ± 0.05^{c}	6.70 ± 0.05^{b}	5.81 ± 0.06^{b}	4.69 ± 0.05^{b}
SC593 70:30	17.20 ± 0.00	13.33 ± 0.04^{c}	9.00 ± 0.15^{c}	6.90 ± 0.03^{b}	6.30 ± 0.05^{a}	5.42 ± 0.06^{b}
SC593 60:40	17.60 ± 0.00	14.19 ± 0.11^{b}	9.21 ± 0.04^{c}	7.10 ± 0.04^{a}	6.50 ± 0.03^{a}	6.10 ± 0.04^{a}
SC593 50:50	17.80 ± 0.00	15.10 ± 0.52^{a}	11.30 ± 0.03^{a}	7.42 ± 0.07^{a}	6.81 ± 0.07^{a}	6.31 ± 0.05^{a}
SC539 80:20	16.80 ± 0.00	12.46 ± 0.28^{c}	9.07 ± 0.08^{c}	5.31 ± 0.06^{c}	5.21 ± 0.06^{b}	4.90 ± 0.05^{b}
SC539 70:30	17.10 ± 0.00	13.90 ± 0.46	9.50 ± 0.03^{c}	5.91 ± 0.06	5.50 ± 0.25^{b}	5.50 ± 0.05^{b}
SC539 60:40	17.40 ± 0.00	14.00 ± 0.46^{b}	10.20 ± 0.05^{b}	6.61 ± 0.06^{b}	6.30 ± 0.06^{a}	6.20 ± 0.06^{a}
SC539 50:50	17.60 ± 0.00	15.60 ± 0.06^{a}	10.50 ± 0.05^{b}	6.90 ± 0.05^{b}	6.80 ± 0.06^{a}	6.50 ± 0.06^{a}
SC 100:0	16.00 ± 0.00	11.00 ± 0.00^{d}	9.00 ± 0.00^{c}	7.00 ± 0.00^{a}	5.10 ± 0.00	4.00 ± 0.00^{c}

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

Table 10: Specific gravity during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0	48	96	144	192	240
SC593 80:20	1.069 ± 0.000	1.052 ± 0.001^{b}	1.035 ± 0.001^{b}	1.026 ± 0.001^{b}	1.023 ± 0.001	1.019 ± 0.001^{b}
SC593 70:30	1.071 ± 0.000	1.055 ± 0.001^{b}	1.037 ± 0.001^{b}	1.028 ± 0.001^{a}	1.025 ± 0.001	1.021 ± 0.001^{a}
SC593 60:40	1.072 ± 0.000	1.059 ± 0.001^{b}	1.038 ± 0.001^{b}	1.029 ± 0.001^{b}	1.026 ± 0.001	1.024 ± 0.000^{a}
SC593 50:50	1.073 ± 0.000	1.062 ± 0.001^{a}	1.046 ± 0.001^{a}	1.031 ± 0.001^{a}	1.027 ± 0.000	1.026 ± 0.001^{a}
SC539 80:20	1.069 ± 0.000	1.052 ± 0.001^{b}	1.037 ± 0.001^{b}	1.021 ± 0.000^{b}	1.021 ± 0.001^{b}	1.020 ± 0.001^{a}
SC539 70:30	1.070 ± 0.000	1.057 ± 0.001^{b}	1.038 ± 0.001^{b}	1.025 ± 0.001^{b}	1.023 ± 0.001^{a}	1.022 ± 0.000^{a}
SC539 60:40	1.071 ± 0.000	1.063 ± 0.002^{a}	1.042 ± 0.001^{a}	1.026 ± 0.001^{b}	1.025 ± 0.001^{a}	1.024 ± 0.001^{a}
SC539 50:50	1.072 ± 0.000	1.066 ± 0.001^{a}	1.042 ± 0.000^{a}	1.027 ± 0.001^{b}	1.027 ± 0.001^{a}	1.026 ± 0.001^{a}
SC 100:0	1.066 ± 0.000	1.046 ± 0.001^{b}	1.036 ± 0.001^{b}	1.028 ± 0.001^{a}	1.020 ± 0.001^{b}	1.016 ± 0.001^{b}

Table 11: Alcohol content (%) during beer fermentation of sorghum supplemented with different concentration of cassava

Sample code	Fermentation period (h)					
	0	48	96	144	192	240
SC593 80:20	0.00 ± 0.00	2.57 ± 0.56	4.34 ± 0.35^{a}	5.33 ± 0.35^{a}	6.04 ± 0.36^{a}	6.74 ± 0.41^{a}
SC593 70:30	0.00 ± 0.00	1.79 ± 0.67	3.29 ± 0.28^{b}	4.13 ± 0.23^{b}	4.91 ± 0.52^{b}	5.53 ± 0.27^{b}
SC593 60:40	0.00 ± 0.00	1.58 ± 0.22	2.81 ± 0.35^{c}	3.31 ± 0.29^{c}	4.01 ± 0.24^{b}	4.86 ± 0.39^{b}
SC593 50:50	0.00 ± 0.00	1.05 ± 0.56	1.83 ± 0.13^{d}	2.25 ± 0.07^{d}	2.93 ± 0.34^{c}	3.65 ± 0.39^{c}
SC539 80:20	0.00 ± 0.00	2.42 ± 0.67	4.25 ± 0.36^{a}	5.15 ± 0.29^{a}	5.82 ± 0.51^{b}	6.43 ± 0.26^{a}
SC539 70:30	0.00 ± 0.00	1.67 ± 0.67	3.06 ± 0.38^{b}	3.79 ± 0.27^{c}	4.71 ± 0.31^{b}	5.15 ± 0.43^{b}
SC539 60:40	0.00 ± 0.00	1.45 ± 2.89	2.58 ± 0.13^{c}	3.02 ± 0.38^{c}	3.80 ± 0.44^{c}	4.62 ± 0.34^{b}
SC539 50:50	0.00 ± 0.00	1.41 ± 0.56	2.34 ± 0.36^{c}	2.82 ± 0.50^{d}	3.04 ± 0.27^{c}	3.63 ± 0.49^{c}
SC 100:0	0.00 ± 0.00	2.94 ± 0.67	4.86 ± 0.37^{a}	5.95 ± 0.48^{a}	6.48 ± 0.79^{a}	7.55 ± 0.20^{a}

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

Table 12: Physico-chemical analysis of beer from sorghum supplemented with different concentration of cassava

Sample Code	Bitterness (IBU)	Colour (EBC) at 0 h	Colour (EBC) at 240 h	FAN(mg/l) at 0 h FP	FAN (mg/l) at 240 h FP	Foam Stability (Seconds)	Haze Value (EBC)	CO_2 Content (g/100 ml)
SC593 80:20	9.27 ± 0.18^{c}	11.56 ± 0.05^{b}	4.58 ± 0.01^{b}	204.91 ± 1.09^{b}	109.13 ± 0.58^{c}	181.7 ± 0.17^{b}	1.25 ± 0.01^{b}	0.50 ± 0.03^{a}
SC593 70:30	9.50 ± 0.06^{c}	10.85 ± 0.03^{c}	4.62 ± 0.02^{b}	193.85 ± 1.34^{c}	113.02 ± 0.46^{c}	182.35 ± 0.37^{b}	1.47 ± 0.26^{a}	0.49 ± 0.03^{a}
SC593 60:40	8.83 ± 0.12^{d}	10.71 ± 0.02^{c}	4.69 ± 0.02^{b}	186.56 ± 0.48^{c}	117.84 ± 0.69^{c}	183.01 ± 0.51^{b}	1.15 ± 0.01^{c}	0.48 ± 0.03^{a}
SC593 50:50	7.80 ± 0.06^{d}	10.21 ± 0.03^{c}	4.54 ± 0.02^{b}	180.12 ± 0.37^{c}	125.08 ± 0.46^{a}	183.63 ± 0.24^{b}	1.15 ± 0.00^{c}	0.46 ± 0.05^{b}
SC539 80:20	10.27 ± 0.09^{b}	12.03 ± 0.04^{a}	4.59 ± 0.02^{b}	208.13 ± 0.74^{b}	115.29 ± 0.48^{c}	180.34 ± 0.49^{b}	1.47 ± 0.26^{a}	0.48 ± 0.05^{a}
SC539 70:30	9.23 ± 0.09^{c}	11.74 ± 0.02^{b}	4.63 ± 0.01^{b}	200.43 ± 0.48^{b}	118.78 ± 0.71^{c}	180.62 ± 0.35^{b}	1.20 ± 0.01^{b}	0.47 ± 0.03^{a}
SC539 60:40	9.77 ± 0.09^{c}	11.17 ± 0.04^{b}	4.69 ± 0.02^{b}	173.34 ± 0.46^{d}	129.37 ± 0.81^{a}	181.00 ± 0.27^{b}	1.15 ± 0.01^{c}	0.46 ± 0.05^{b}
SC539 50:50	9.00 ± 0.12^{c}	10.77 ± 0.02^{c}	4.94 ± 0.02^{a}	164.63 ± 0.58^{d}	136.34 ± 0.69^{a}	184.00 ± 0.27^{b}	1.20 ± 0.01^{b}	0.44 ± 0.05^{b}
SC 100:0	12.40 ± 0.06^{a}	12.47 ± 0.06^{a}	4.44 ± 0.02^{c}	226.88 ± 0.50^{a}	101.89 ± 0.71^{d}	187.32 ± 0.24^{a}	1.10 ± 0.01^{c}	0.51 ± 0.05^{b}

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column show means that are not significantly different (P > 0.05)

KEY: FAN – Free Amino Nitrogen; FP – Fermentation Period

Table 13: Sensory evaluation of beer from sorghum supplemented with different concentration of cassava

Sample Code	Colour	Taste	Flavour	Mouthfeel	Overall Acceptability
SC593 80:20	4.39 ± 0.16^{c}	7.77 ± 0.13^{a}	8.15 ± 0.17^{a}	7.10 ± 0.14^{a}	8.00 ± 0.05^{a}
SC593 70:30	5.05 ± 0.11^{b}	7.41 ± 0.11^{a}	8.14 ± 0.17^{a}	6.77 ± 0.12^{b}	7.67 ± 0.07^{b}
SC593 60:40	5.71 ± 0.16^{b}	7.26 ± 0.16^{a}	7.86 ± 0.16^{b}	6.47 ± 0.18^{b}	7.30 ± 0.13^{b}
SC593 50:50	6.06 ± 0.13^{a}	7.51 ± 0.06^{a}	8.16 ± 0.18^{a}	6.84 ± 0.22^{b}	7.80 ± 0.05^{b}
SC539 80:20	4.32 ± 0.12^{c}	7.13 ± 0.12^{a}	7.87 ± 0.07^{b}	6.43 ± 0.19^{b}	7.70 ± 0.12^{b}
SC539 70:30	4.94 ± 0.09^{c}	7.27 ± 0.12^{a}	8.02 ± 0.08^{a}	6.57 ± 0.23^{b}	7.33 ± 0.03^{b}
SC539 60:40	5.67 ± 0.19^{b}	7.52 ± 0.13^{a}	8.07 ± 0.09^{a}	7.00 ± 0.05^{a}	7.77 ± 0.09^{b}
SC539 50:50	6.00 ± 0.06^{a}	7.08 ± 0.09^{a}	8.03 ± 0.27^{a}	6.80 ± 0.03^{b}	7.53 ± 0.07^{b}
SC 100:0	5.90 ± 0.17^{b}	6.87 ± 0.15^{b}	7.86 ± 0.08^{b}	6.40 ± 0.03^{b}	7.08 ± 0.13^{b}

Values are expressed as mean ± standard deviation of triplicates

Note: All similar alphabets within a column shows means that are not significantly different ($P > 0.05$)

DISCUSSION

Analyses of Sorghum seeds and Cassava

The germinative energy (G.E) and germinative capacity (G.C) values of 96% and 95% obtained respectively for the sorghum grains used for this research work was well within the acceptable viability limit set by the European Brewing Convention (EBC) which recommended a minimum GE and GC value greater than or equal to 95% for barley (Bekele *et al*., 2012). Also, a G.E and G.C minimum value of 90% has been recommended by Dewar *et al.* (1995) as required for sorghum to be suitable for malting purposes. The values obtained were similar to the work of Agu and Palmer (1998), Sanni and Fatoki (2017) and Nnamchi *et al.* (2014). The water sensitivity values obtained were similar to that reported by Nnamchi *et al*., 2014; Owuama, 2019 and Sanni and Fatoki, 2017.

The diastatic power value was higher than the standard of > 65 ^{o}L set by the Institute of Brewing (IOB) (O'Rourke, 2002). It is also comparable to the values previously quoted for barley malts (Muoria *et al*., 1998; Agu *et al*., 2007; Makeri *et al*., 2013; Bera *et al*., 2018). The cold water extract (C.W.E) value was in line with the minimum limit of 18-19% set by the Institute of Brewing (IOB) and the limit of 2.0-2.2% for modified lager malt specified by the European Brewing Convention (EBC) (O'Rourke, 2002). The values reported are similar to those reported for other sorghum varieties by Ogbonna *et al.* (2012) and Ogu *et al.* (2006). The H.W.E value is comparable to that of Eneje *et al.* (2012) and higher than that of Nnamchi *et al* (2014). The hot water extract (H.W.E) value obtained for the sorghum malt was higher than the values of 63.1, 10.87 and 15.40% per 50 g of sample quoted for sorghum, colocassia and barley malts respectively (Onwuka and Eneh, 1998; Dicko *et al*., 2006; Hailu and Assefa, 2018). The H.W.E value reported for the sorghum hybrid used in this research work was higher than the value reported for two well-known established sorghum varieties *SK5912* and *KSV8* (12.02 and 11.78% per 50 g respectively) (Nnamchi *et al*., 2014).

Microbiological analysis of Yellow Cassava beer

The values recorded at 0 h fermentation time for total bacteria count could be due to contamination during milling and malting as well as from the flora present at the surface of the grains prior to collection (Agu and Palmer, 1998; Hassani *et al*., 2013).

The values steadily decreased as fermentation proceeded and disappeared during secondary fermentation after 192 h fermentation period so that no bacteria count was detected in the final products. This may be due to the accumulation of organic acids in the products as fermentation progressed causing a rise in the total titratable acidity for the samples and a consequent fall in pH readings (Okhonlaye and Foluke, 2016; Nemo and Bacha, 2021). pH below 4.5 destroys the kinetics of bacteria (Laetitia *et al*., 2005). It has been reported that bacteria are unable to survive under low pH or high acidic conditions due to the high osmotic stress their cell walls undergo (Yanez *et al*., 2008; Guan and Liu, 2020).

The decrease in LAB count from 0 h to 144 h fermentation period could be due to the inability of the species of LAB present to withstand the stress conditions in the wort (such as minimal oxygen, low pH and inavailable nutrients which could have been 'swallowed up' by yeasts) for prolonged periods of fermentation (Hayek and Ibrahim, 2013; Pittet*et al*., 2018). It could be that the inability of the LAB strains to resist the stresses in the wort showed that they lacked the glucosyltransferase (gtf) gene and could not produce exopolysaccharide (EPS) (Pittet et al., 2018). It has been reported that LAB strains such as *Lactobacillus plantarum* and *Lactobacillus buchneri* showed no growth under multi-stress conditions created by wort (Dysvik*et al*., 2020). It could be that the strains of LAB present in the wort were unable to survive in the presence of antimicrobial compounds generated by hops (Dysvik*et al*., 2020; Suzuki *et al.*, 2007). The decrease in Lactic acid bacteria count is supported by Nwachukwu *et al* (2010) and Wakil and Ajayi (2013).

The increase in yeast count during fermentation for all the samples may be attributed to the presence of adequate fermentable sugars and nutrients for yeast growth and metabolism. Also, the disappearance of bacteria and lactic acid bacteria as well as absence of faecal coliforms could have created a monopolistic ground for utilization of sugars and nutrients by yeasts. The low pH or acidic conditions created a suitable environment for yeast to thrive giving them a competitive advantage over other microorganisms which could have been present in the substrate medium (Reddy *et al*., 2010). The fact that sample coded SC593 80:20 had the highest yeast count and sample coded SC539 50:50 had the least yeast count after 240 h fermentation period could indicate that yeast proliferation was relatively more enhanced when the formulated substrates had lower amounts of cassava than when the concentration of cassava was higher. This means that the higher the ration of sorghum in the formulation, the higher the yeast counts. This trend could be as a result of increase in the cyanide content which inhibited yeast growth (Tetchi *et al*., 2012). Also, the decrease in yeast count with increasing proportion of cassava in the ration could be caused by increase in the formation and concentration of medium-chained fatty acids (MCFAs) which inhibited the growth of the yeasts. The MCFA formation could have been facilitated by the low pH and ethanol concentration of the wort (Baron *et al*., 2017). It could be that during cultivation and harvesting of the cassava roots, herbicides were used. Concentrations of these herbicides must have been retained by the roots which could have inhibited the growth of the yeasts (Singh and Wright, 2002). The presence of anti-nutrients like tannin and phytate could inhibit the growth of the yeast due to lowered availability of available nutrients causing lowered proliferation of the yeasts on increasing the ration of cassava (Igbua *et al*., 2020). Therefore, sample containing 80% sorghum and 20% cassava gave the highest yeast count compared to the other rations. Also, the trend in yeast count showed that yeast growth was relatively more enhanced when the substrate contained yellow cassava variety *IBA 00593* than when it contained

variety *IBA 00539*; as the yeast counts were higher for the former than for the latter irrespective of the blending ratio used for the formulation. The increase in yeast count observed in this research work is supported by Wakil and Ajayi (2013), Erten *et al.* (2007) and Wilson *et al.* (2012). There were no coliforms and mould growth in all the samples. The absence of coliform growth in the beer products could be tied to the strict sanitary and hygienic standards maintained throughout the production process.

Physico-chemical analysis of Yellow Cassava beer

The decrease in the pH of the different formulated products from 0 h to 240 h fermentation period could be attributed to the consumption of the sugars and nutrients by yeast with consequent release of organic acids thereby increasing acidity in the samples as fermentation progressed. This could be as a result of the uptake of ammonium ions, potassium ions and amino acids by yeast and the resultant discharge of hydrogen ions and organic acids into solution by the yeast (Lewis and Young, 1995). This may also be due to the increase in titratable acidity as pH is inversely proportional to titratable acidity (Akpoghelie and Omonigho, 2018). The least pH after 240 h fermentation period recorded for 80:20 ration revealed that fermentation of the wort by yeasts was more effective when the concentration of cassava in the blend was lower. The pH range recorded for the blended products after 240 h fermentation period fall within the acceptable limit of 3.50-4.50 recommended by NAFDAC for beer products manufactured in Nigeria (Ogu and Ogunbodede, 2017). The pH values obtained were close to that obtained for *Kodome* Sorghum beer by Hailu and Assefa (2018) who reported a pH of 4.53 in the final beer product. This result obtained for pH is similar to the work of Asante (2008) who reported a pH range of 3.95 to 4.13. The pH range is in consonance with the value of 3.90-5.40 reported by Lyumugabe *et al* (2014) for "Ikigage" which is a traditional sorghum beer.

The increase in titratable acidity of the beer products from 0 h to 240 h fermentation period could be tied to the production of organic acids by the fermenting yeasts which led to a corresponding decrease in pH (Onwuka and Eneh, 1998). The titratable acidity values after 240 h fermentation period for the formulated beer products is similar to the commercial recommendation of acidity of 2.5-3.5 for beer by NAFDAC (Ogu and Ogunbodede, 2017). This was supported by Odibo *et al.* (2002) and Rajagopal (1976). The decrease in pH and increase in titratable acidity observed in this research work is supported by Obi and Ugwu (2019). This is in conformity with the works of Lyumugabe *et al.* (2010), Bhuyan *et al.* (2014) and Ekberg *et al.* (2015).

The decrease in values of TSS revealed progressive increase in the chemical reactions that consumed the sugars or reduced the concentration of sugars (Braide and Nwaoguikpe, 2011; Ocloo and Ayernor, 2008). The TSS values for the wort is much higher than the wort value of 13^{o}Plato recorded by Rajagopal (1976) for cassava beer and the value of 14.51 ^{o}Plato and 14.04 ^{o}Plato quoted by Onwuka and Eneh (1998) for colocasia stout beer. The TSS values for the final products were much higher than the value of 0.99^{o}Brix obtained by Hailu and Assefa (2018) for kodome sorghum beer. However, the values were much lower than that recorded for "Kapsiki" beer (7.0-7.46 ^{o}Brix) by Ronald and Roger (2017) and "Ikigage" beer (11.6 ± 1.53 ^{o}Brix) by Lyumugabe *et al.* (2010).

The decrease in specific gravity values during fermentation for all the samples may be attributed to the type of brewing yeast used in the production process and the consequential decrease in total soluble sugars during fermentation (Ajibola *et al.*, 2012).The low values obtained for specific gravity after 240 h fermentation period suggests efficient yeast

performance and adequate wort components (Ocloo and Ayernor, 2008; Braide and Nwaoguikpe, 2011). The results obtained for specific gravity of the products at 0 h fermentation time is a little higher than the standard limit of 1.040-1.060 recommended by NAFDAC (Ogu and Ogunbodede, 2017). The specific gravity of the products at 0 h fermentation time is higher than the value of 1.056 reported by Segura *et al.* (2011). The specific gravity values for the products after 240 h fermentation period were a little higher than the value of 1.013 reported for sorghum beer by Segura *et al.* (2011). The specific gravity of the products at 0 h and 240 h fermentation times are in conformity with the values reported by Onwuka and Eneh (1998).

The increase in alcohol content could be as a result of the continuous fermentation and conversion of the soluble sugars in the wort into ethanol and other metabolites by the yeasts (Stewart, 2016; Bokulich and Bamforth, 2013). The results obtained for the products were much lower than the range of 12.50-12.55% found by Adenuga*et al* (2010) for sorghum beer but similar to the findings of Olsovska and Sterba (2015) and Tan *et al.* (2015) who reported values of 3.8% v/v and 2.5-4.3% v/v respectively for lager type beers. They were also similar to 4.78% v/v found by Hailu and Assefa (2018) and fell within the standard values of 4.71-4.89% v/v quoted for standard beers according to Hailu and Assefa (2018). The alcoholic content is in line with the range of 1.49-4.56% v/v found by Lyumugabe*et al.* (2014) as well as the range of 4.91-6.87% v/v reported by Veljovic*et al.* (2015).

The decrease in FAN could be due to the consumption of the FAN by the yeasts for their growth and proliferation as well as the decrease in pH during fermentation (Lekkas*et al*., 2005; Hill and Stewart, 2019). The decrease in FAN during fermentation is supported by Lekkas*et al.* (2005). A minimum acceptable limit of 120 mg/L for FAN of wort is required for optimal yeast growth and fermentation efficacy as reported by Lekkas*et al.* (2005).

This suggests that the brewing was a high gravity brewing.The specific gravity values obtained for the products at 0 h fermentation time is much similar to the wort value of 1.064 obtained by Villicana and Saldivar (2004) for 100% sorghum malt.

The fall in bitterness values from 0 h to 240 h fermentation period could be attributed to the loss in iso-α-acids and decrease in pH during fermentation (Pospescu*et al*., 2013). Also, the decrease in bitterness could be due to the absorption of the bittering compounds by the yeast cells (Pospescu*et al*., 2013). The decrease in bitterness values is supported by Popescu*et al.* (2013) and Haseleu*et al.* (2010). The result of bitterness is similar to the work of Hailu and Assefa (2018) who reported a bitterness value of 9.854 EBU but much lower than that for maize beer reported by Diakabana*et al* (2013). The bitterness values obtained for the finished beer products fall below the standard of 15-30 EBU suggested by NAFDAC. The bitterness values of the formulated products were found to be low which could be as a result of the condition and quantity of hops added during the wort boil. However, the low bitterness of the products could be amended by increasing the amount of hops added during wort boiling.

The low colour values recorded at 0 h fermentation time for all the products could be attributed to process parameters such as the temperature of wort during boiling, wort pH, concentration of free amino nitrogen (FAN) and original gravity of wort (Shellhammer and Bamforth, 2008). The low colour values obtained for the products at 0 h fermentation time could be due to low product formation via maillard reaction during malting which is a function of the extent of kilning of the malt (Daniels, 2000). The low colour values of the beer products at 0 h fermentation time may also be due to the complex precipitation of proteins that occurred during the wort boiling

process (Onwuka and Eneh, 1998; Ndukwu and Udofia, 2016). The colour values obtained for this research work was much lower than that for kodome sorghum beer (7.5EBC) by Hailu and Assefa (2018). However, the colour range for the finished beer products were in line with the standard range of 3.5-4.5 EBC recommended by the Nigerian Industrial Standard (NIS) for pale lager beers.

The values obtained for foam stability in this research work is similar to the findings of Chen *et al* (2015). However, the values were lower than that reported by Kordialik-Bogacka and Antczak (2011). The relatively lower foam retention times of the beer products compared to literature values quoted for barley and other cereals beer like sorghum could be traced to the amounts of fats and proteins in the beer products (Evans and Bamforth, 2009; Devolli*et al*., 2018).

The haze values recorded were higher than that of 0.165 EBC found by Hailu and Assefa (2018). The values for haze obtained in this research work did not fall within the value range of 0.175-0.180 EBC quoted for standard beers (Hailu and Assefa, 2018). However, the values reported in this research work fall well within the acceptability limit of 5.00 EBC after six months set by NAFDAC and were also very close to the average value of 1.00 EBC recorded for commercial lager beers in Nigeria (Ogu and Ogunbodede, 2017). The haze values of the formulated products reveals that the malt used for this study was of high quality and also the absence of microbial contamination coupled with standard brewing process protocols.

The values obtained for CO_2 are higher than that reported by Hailu and Assefa (2018) for sorghum beer but falls in line with the quoted range of 0.501-0.506 g/100 ml for standard beers by the same researchers. The values of CO_2 content of the beer products complies with the recommended standard of 0.45-0.62 g/100 ml by NAFDAC for light lager beers. This indicates that the CO_2 content of the product coded SC593 80:20 is well within the acceptable standard limit and would therefore be acceptable to consumers.

Sensory evaluation of Yellow Cassava beer

The values reported for colour could be due to higher maillard reaction in the blended products than in the plain sample as a result of the addition of yellow cassava substrate to the blended products. There was significant difference between the beer products for colour.The trend observed for taste could be attributed to the addition of equal amount of hops during wort boiling. The mean sensory scores for flavour were higher for the rations containing cassava than for the ration containing 100% sorghum. This could be as a result of the generation of higher amount of flavor compounds and esters in the blended products than in the plain product thereby leading to higher maillard reactions in the former than in the latter (Van Boekel, 2006).

The results of overall acceptability showed that product SC593 80:20 showed significant difference between the other products which were not significantly different from each other. The main determinants to the overall acceptability of the products by the panelists are flavor and taste followed by mouthfeel and lastly colour. Based on this, the product coded SC593 80:20 was considered to be the most acceptable by the panelists and could therefore be the most acceptable to consumers.

CONCLUSION

This research work has revealed that hybrid yellow cassava and hybrid sorghum were good substrates for beer production. The microbiological and physico-chemical properties of the beer product met statutory standards and are acceptable to consumers. The sensory evaluation results also reveal that the product is acceptable and could enjoy robust acceptability by consumers. Formulated sample SC593 80:20 containing fermented blend of 80% hybrid sorghum and 20% hybrid yellow cassava was chosen as the best treatment based on microbiological, physico-chemical and sensory evaluation results. Also,

of the yellow cassava varieties used, *IBA 070593* gave better yield.Therefore, lager beer can be produced using hybrid yellow cassava blended with hybrid sorghum and the product could serve as a means to cut the excesses recorded in overhead production cost incurred in barley importation as well as improve the variety of available brands in the beer market. However, further research is still needed to optimize the potentials of the raw materials relinquished in this study for beer production.

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