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THE IMPACT OF INDUSTRIAL ENZYMES ON MALT AND WORT USING NIGERIAN SORGHUM VARIETIES

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ABSTRACT

Studies on the impact of industrialised enzymes on malt and worts using Nigerian sorghum (red and white) varieties were carried out. The red and white sorghum grains were subjected to grain analysis, malted, milled and analysed (malt analysis). Each of the red and white sorghum malts were mashed (infusion technique) with and without exogenous enzymes. The exogenous enzymes used were (α-amylase, β-amylase, β-glucanase and proteinase). Worts were produced and analysed for their physicochemical properties before boiling with hops for one hour and thirty minutes. The worts were cooled and pitched with yeast strain *Saccharomyces cerevisiae* to begin primary fermentation which lasted seven days followed by secondary fermentation for fourteen days. The physicochemical properties of resulting beer were analysed. The results of physicochemical properties of the worts in this study showed samples with enzymes having original gravity (1.032 and 1.031°), sugar content (8.04 and 7.8°Brix), pH (5.31 and 5.40), flow rate (18.61 and 18.60 second) and viscosity (1.05cp) while samples without enzymes have original gravity (1.021 and 1.020°), sugar content (5.33 and 5.08°Brix), pH (5.03 and 5.11), flow rate (18.53 and 18.45 second) and viscosity (1.03cp). The results of beer analysis showed specific gravity range of 1.001–1.002°ρ, sugar 0.2 –0.52°Brix, pH 3.89–3.99, alcohol content 2.45–4.0 and apparent ferment ability 90–96.88%. The result of sensory evaluation showed that there was no significant difference among the samples based on parameters tested at P≤0.05 level of significance. The use of industrial exogenous enzymes have been found to be more effective in the preparation of beer worts as observed from the result obtained with red and white sorghum malt in this study.

Keywords: Industrial enzymes, sorghum, malt, grains, sensory

INTRODUCTION

Enzymes are catalysts, which speed up chemical reactions without change their nature or structure (Malomo *et al.* 2012). Some of them broke down the starch, while others broke down distinct parts into smaller molecules and other new substances made from different starting materials. One of the main applications of enzymes in food industry is brewing (Agu and Palmer, 2007). Brewing is defined as the process of beer production in which the sugars in starch are fermented to ethyl alcohol through the action of yeast (Schemedding *et al.* 2002). It is among the earliest methods of producing food, dating back to before the time of Christ (Gomaa, 2018).

The traditional source of enzymes used for the conversion of cereals into beer is malt (Archibong *et al.* 2015). Enzymes derived from sources other than malt may be used at various stages during brewing, provided that this is allowed by local regulations (Bamforth, 2008). Complex proteins of grain embryo or aleurone layer contain several enzymes, however, most of them are formed during malting process. For example, α-amylase, does not exist in grain however it exists in malt.

Endo-β-1,3:1,4glucanases, α-amylase, β-amylase, and other enzymes (e.g. proteinase, carboxypeptidases, lipoxyiganase) make up the most widely utilized class of enzymes in brewing for the hydrolysis of the wort. These enzymes catalyze the conversion of polymeric substances, including starch, proteins, and starch cell walls, into low molecular weight materials, which mainly influence the fermentation of wort sugars and the ultimate product's quality (Guerra *et al.* 2015). Each enzyme catalyzes only a specific chemical reaction (Agu *et al.* 2005).

Starch hydrolysis by enzymes is the process that leads to the breakdown of starch into small

molecules such as sugars (Bajomo and Young, 2012). Both crystalline and amorphous parts of starch granules can be dissolved by α-amylase, which targets the (α-1-4)-linkages of oligosaccharides that produce starch. β-amylase attacks also (α-1-4)-linkages from the non-reducing ends of amylose and amylopectin molecules (Lalor and Goode, 2009). During the mashing process, these enzymes operate on starch to produce a variety of fermentable sugars, including glucose, sucrose, fructose, and mostly maltose, as well as some low molecular weight dextrins. However, because amorphous starch portions are easier for enzymes to attack and hydrolyze than crystalline ones, starch hydrolysis is highly dependent on the physical state of the starch. Consequently, because it increases the starch's vulnerability to enzyme hydrolysis, starch gelatinization is crucial to starch conversion (Gomaa, 2018).

Traditionally, sorghum grains have been in use for the production of local alcoholic drinks such as 'Kaffir beer' in South Africa and 'Burukutu' in Nigeria (Aastrup and Olsen, 2008). For some time now and until recently the grain has been used in large amounts as unmalted adjunct and as malted cereal to brew beer in Nigeria (Malomo *et al.* 2012). It is, however, low in beta-amylase, an important diastatic power enzyme, which necessitates the use of extraneous enzymes during mashing (Okoro *et al.* 2021).

Because malt is the traditional source of the enzymes required to ferment cereals into beer, if there is insufficient enzyme activity in the mash, there will be a number of negative consequences, including a low extract yield, a lengthy wort separation process, a sluggish fermentation process, insufficient alcohol production, a decreased beer filtration rate, and poorer beer flavor and stability (Malomo *et al.* 2012). Brewers may acquire under-modified malts or raise the adjunct ratio if they want to employ local raw materials or reduce the cost of raw ingredients. The limiting factor is to ensure an adequate complex of enzymatic activities for high-quality wort (Schemedding *et al.* 2002). To avoid these issues, the malt's natural enzymes are supplemented with exogenous enzymes. Additionally, industrial enzymes are employed to generate low-carb beer, or "light beer," improve adjunct liquefaction, speed up the beer maturing process, and make beer from less expensive raw materials. The substitution of a varying proportion of malt with sorghum introduces new dimensions to the grist composition (Archibong and Onuorah, 2010). However, it is unclear to what extent adding enzyme supplements to sorghum cultivar mashes could lower the amount of fermentable sugars in the worts (Okoro *et al.* 2021).

Sorghum has a significant role as a malt substitute, particularly in Africa. Due to its high starch content and lack of gluten, sorghum has an intriguing potential as a raw material for brewing in addition to its economic benefits. However, in order to properly compete with barley in the production of European-style beer, a few fundamental issues with sorghum need to be resolved. A major disadvantage for the use of malted sorghum in brewing is the usual low enzymatic composition, low diastatic power and amylolytic activity during mashing. To avoid these issues, the sorghum malt's natural enzymes are supplemented with exogenous enzymes. Hence, this study.

The aim of this study is to investigate the impact of industrialised enzymes on malt and worts using Nigerian sorghum varieties. The specific objectives of this study are to:

i. To determine the grain and malt properties of the sorghum grain used.
ii. To produce malts from two Nigerian Sorghum Varieties
iii. To evaluate the effect of enzymes on mash using infusion mashing technique.
iv. The determine the physicochemical properties of the worts produced.
v. To determine the physicochemical and organoleptic properties of the beer produced.

MATERIALS AND METHOD

Sources of Materials

The four basic raw materials used for these research were water, malts (red and white sorghum varieties), hops and yeast. The red and white sorghum varieties were purchased from Eke-Agbani Market in Nkanu-West LGA of Enugu state. The hops, yeast, chemicals and reagents used were supplied by the Laboratory of the Department of Applied Microbiology and Brewing, ESUT.

Methods used

The methods used in this work were according to that of Institute of Brewing (IOB), America Society of Brewing Chemists (ASBC) and European Brewing Convention (EBC) methods.

Grain analysis

Methods Used

The methods used in this work were according to that of Institute of Brewing (IOB), America Society of Brewing Chemists (ASBC) and European Brewing Convention (EBC) methods.

Malting process

Malting was done by selecting the grain first, followed by steeping (the steep liquor was changed at 6 hr interval after which the grains were allowed to have a 2 hr air rest) for 40 hr, casting (the grains were drained off water and heaped on a jute bag) for 24 hr, germination (the grains were spread out on the jute bag very well for uniform aeration and germination) for 3 days and kilning (the malt is dried to reduce the moisture content). After kilning, the rootlets were removed by using fiction (abrasion).

Malt Analysis

The malted grains were analysed for hot water extract, cold water extract and diastatic power.

Milling Process

The malts were coarsely milled in the form that ensures that the husks are substantially left intact in order to aid filtration after mashing.

Mashing

The red and white sorghum malt grist (50g of each sample) were weighed into 4 different 500ml conical flask labelled A, B, C and D. A total of 360ml of water was added into each of the conical flask containing the grists. Two milliliters (2ml) of α–amylase, β–amylase, α-1,6 amyloglucosidase, proteinase and β–glucanase were added into two conical flask containing grist from red and white sorghum malt and shake properly. No enzyme was added to the remaining two flasks containing grist from red and white sorghum malt. The samples were as follows:

Sample A contained 50g of red sorghum with enzymes.
Sample B contained 50g of red sorghum without enzymes.
Sample C contained 50g of white sorghum with enzymes.
Sample D contained 50g of white sorghum without enzymes.

The labelled conical flask containing the samples were covered with aluminium foil and placed in a water bath where the temperature was raised to 35^0C and maintained for 30mins. The temperature was raised again to 45^0C and maintained for 30mins for Beta-glucanase activities. The temperature was raised again to 50^0C and maintained for 30°C for proteolysis. The temperature was further raised to 63^0C and maintained for 1 hour for Beta-amylase activities. Finally, the temperature was raised to 72^0C and maintained for 30mins. These temperatures activate alpha-amylase to act on work converting starch to glucose. One (1) drop of iodine solution was added to 2ml of the mash in a test tube to check for saccharification. After a complete saccharification, which was got through yellowish colour, the worts were vigorously boiled to boiling point for extra 10mins to mash off. The sample (mash) was allowed to cool and filtered using filter cloth to obtain a clear solution known as worts.

Wort Analysis

The parameters determined were original gravity (O.G) (°), sugar (°Brix), pH, flow rate (sec), viscosity (cp), temperature (°C) and reducing sugar (glucose and maltose). This was done using the method of the Methods of Analysis of the Institute of Brewing.

Wort Boiling

This was carried out before fermentation to sterilize the worts, inactivate the enzymes and extract the hop constituent. The worts were poured into 500ml conical flasks and arranged in water bath, hops (pellets) were added and boiled for $1^{1/}_{2}$ hrs.

Wort cooling and filtration

By setting the conical flasks in a large basin filled with cold water, the hopped boiling worts were allowed to cool to room temperature using the heat exchanger technique. Using a filter and sterile muslin cloth, hop debris and the coagulant protein (trub) were separated from the wort.

Wort Fermentation

The wort was now prepared for yeast fermentation after cooling and aerating. Strain of *Saccharomyces cerevisiae* was employed for the fermentation process. The yeast was first reconstituted by mixing 20g of yeast, 10g of glucose with water in a container. It was shake vigorously for 10mins and checked for pressure which signified that the yeast was back to life. Ten (10ml) of the yeast inoculum was added to the worts (pitching) and the container was stocked with cotton wool. The primary fermentation took 7days at 25^0C. At the end, the yeasts were skimmed off and the product filtered to obtain green beer which was further allowed for 7days for secondary fermentation at 10^0C. After the secondary fermentation, the beer was filtered with filter paper to obtain a bright clear beer.

Beer Analysis

After fermentation, the pH, gravity and percentage alcohol were determined after filtration.

Organoleptic Analysis

Organoleptic test was carried out on the beer produced. A group of ten panel lists tested the products and recorded their inferences and insights about the product. The evaluation varied from "dislike extremely" to "liked extremely" for parameters (colour, taste, mouth feel and general acceptability). The judgements were further analysed statistically.

Data Analysis

Analysis off variance (ANOVA) were used to evaluate the parameters. The results of the analysis were tested at $P \leq 0.05$ level of significance.

RESULTS

Table 1 showed the result of grain analysis indicating that the red and white sorghum varieties used for this study had good grain quality required for brewing. Table 2 presented results of malt analysis indicating that the malts from red and white sorghum grains are suitable for the production of beer. Infusion mashing technique was used to obtain worts of varying properties and concentrations from red and white sorghum malts with and without enzymes. The physicochemical properties of the worts were determined and recorded in Table 3. The high gravity obtained for samples with enzymes showed the effect of enzymes on the mash. The physicochemical properties of the beer samples after three days, primary and secondary fermentation were recorded in Tables 4, 5 and 6, respectively. While table 7 was the sensory evaluation table.

Table 1: Grain Analysis

Samples	Germinative Energy (%)	Germinative Capacity (%)	Moisture content (%)	Thousand corn weight (g)
Red Sorghum	98	96	9.6	24
White Sorghum	96	96.5	9.5	29

Table 2: Malt Analysis

Samples	HWE (%/kg)	CWE (%)	Diastatic power (%)
Red Sorghum	295	24	27.3
White Sorghum	264	22	25.1

Table 3: Wort Analysis

Samples	O.G (^{0}p)	Sugar level (^{0}Brix)	pH	Temp (^{0}C)	Flow rate (Sec)	Viscosity (cp)	Reducing sugars (mg/l)	
							Glucose	Maltose
A	1.032	8.04	5.31	25	18.61	1.05	54.72	88.80
B	1.021	5.33	5.03	25	18.53	1.03	60.15	98.1
C	1.031	7.8	5.40	25	18.60	1.05	50.21	81.17
D	1.020	5.08	5.11	25	18.45	1.03	50.21	81.17

Key:

Sample A contained 50g of red sorghum with enzymes.

Sample B contained 50g of red sorghum without enzymes.

Sample C contained 50g of white sorghum with enzymes.

Sample D contained 50g of white sorghum without enzymes

Table 4: Physicochemical Properties of the Liquor after 3 days of Fermentation

Samples	Specific Gravity (^{0}ρ)	Sugar level (^{0}Brix)	Temp. (^{0}C)	pH	% Alcohol	Apparent Fermentability (%)
A	1.009	2.31	25	4.11	2.97	71.88
B	1.006	1.54	25	4.10	1.94	71.43
C	1.010	2.56	25	4.16	2.71	67.74
D	1.007	1.8	25	4.10	1.67	65

Table 5: Physicochemical Properties of the Green Beers after Primary Fermentation

Samples	Specific Gravity (^{0}ρ)	Sugar level (^{0}Brix)	Temp. (^{0}C)	pH	% Alcohol	Apparent Fermentability (%)
A	**1.003**	**0.77**	**25**	**4.03**	**3.74**	**00.63**
D	**1.003**	**0.77**	**25**	**4.01**	**3.32**	**85.71**
C	**1.003**	**0.77**	**25**	**4.02**	**3.61**	**90.32**
D	**1.003**	**0.77**	**25**	**4.01**	**2.19**	**85**

Table 6: Physicochemical Properties of the Beers after Secondary Fermentation

Samples	Specific Gravity (^{0}ρ)	Sugar level (^{0}Brix)	Temp. (^{0}C)	pH	% Alcohol	Apparent Fermentability (%)
A	1.001	0.26	25	3.89	4.0	96.88
B	1.001	0.26	25	3.95	2.58	95.23
C	1.002	0.52	25	3.99	3.87	93.55
D	1.002	0.52	25	3.94	2.45	90

Table 7: The Sensory Evaluation Table

	Colour	Taste	Mouth Feel	General Acceptability
F – Cal. Values	3.08	2.26	1.76	2.28
F – Tables values	3.354	3.354	3.354	3.354
LSD	0.484	0.482	0.554	0.443

Key:
F- Cal Value = F – calculated value
F-Tabulated= F – Table values.
LSD = Leas Significant Difference

The result of sensory evaluation showed that there was no significant difference among the samples based on parameters tested at $P \leq 0.05$ level of significance. The beer samples are therefore accepted and can be sold to the public.

DISCUSSION

The traditional source of enzymes used for the conversion of cereals into beer is malt. Enzymes derived from sources other than malt may be used at various stages during brewing. Sorghum offers an interesting potential as raw material for brewing due to its high starch content and the absence of gluten but they have limited enzymes composition required for mashing. This study investigated the impact of commercial exogenous enzymes on worts produced from local Nigerian sorghum varieties.

The germinative energy (98% and 96%) and germinative capacity (96% and 96.5%) obtained from the red and white sorghum grain variety, respectively showed that the red and white sorghum grain variety were relatively good enough for malting. Jayatisa *et al.* (2013) reported that germinative capacity of 98% and germinative energy of 97% for sorghum are above average. The results obtained in this study corresponds with the range of values recommended by Ogu *et al.* (2006) for barley grain. Thousand corn weight of 24 and 29g obtained from the red and white sorghum grain variety was slightly below the values obtained from different varieties of barley which ranges from (31.4 – 38.2)g (Dabija *et al.* 2021). High quality grain is indicated by high thousand corn weight and low moisture content. Moisture content of (13 – 15%) is suitable without fungal attack, therefore, the moisture of 9.6 and 9.5% obtained from the red and white sorghum grain variety is of advantage to the brewer. Since grain with low moisture content are required for brewing.

Under modified malts are malts with cold water extract less than 18%, between (18 – 21)% are modified, while 22% and above are well modified (Dabija *et al.* 2021). Therefore, the cold-water extract of 24 and 22% obtained from the red and white sorghum malts showed the obtained from the red and white sorghum malts to be well modified. The 295 and 264^{0}L/kg obtained from the red and white sorghum malts, respectively for hot water extract falls within the range of lager and ale malt (250 – 300)^{o}L/kg as specified by I.O.B., 1986. The value for the diastatic power 27.3 and 25.1^{0}L obtained from the red and white sorghum malts, respectively is lower than the values for lager and ale malt which are 70^{o}L and 45^{o}L respectively as specified as specified by Jayatisa*et al.* (2013). The value of diastatic power produced by red and white sorghum malts is lower because sorghum produced very little hydrolytic enzyme during malting and this is why commercial hydrolytic enzymes were added during mashing which help in the hydrolysis of the sorghum malt extracts.

The results of physicochemical properties of the worts in this study showed samples with enzymes having original gravity (1.032 and 1.031^o), sugar content (8.04 and 7.8^{o}Brix), pH (5.31 and 5.40), flow rate (18.61 and 18.60 second) and viscosity (1.05cp) while samples without enzymes have original gravity (1.021 and 1.020^o), sugar content (5.33 and 5.08^{o}Brix), pH (5.03 and 5.11), flow rate (18.53 and 18.45 second) and viscosity (1.03cp). This findings showed red and white sorghum varieties to be a good source of substrate for brewing. These showed that the worts are of good quality and the starch in red and white sorghum varieties were completely broken down by the commercial hydrolytic enzymes. Consequently, addition of exogenous enzymes helps improve the extract yield as seen in the result above indicating the amylolytic effects of enzyme activities during mashing. These results are in consonance with the results of Malomo*et al.* (2012). Notably, treatments with exogenous enzymes mainly affected the extraction of sorghum malts as well as sugar composition of sorghum worts. Moreover, enzyme treatment can significantly increase the real extract of sorghum mash especially those used in this study. Also, the exogenous addition of enzyme-assisted mashing facilitated the hydrolysis of starch and

polysaccharides and improved sugar composition of the sorghum worts; thus bolstering its fermentation performance.

Looking at the results, one can see that there is a general trend for both original gravity and pH to decrease from day 1 to the final day after secondary fermentation when maximum fermentation is attained. Both observations are consistent with the fact that during the fermentation stage in beer production, sugars (glucose, fructose, maltose) in the wort are converted to ethanol and carbon dioxide through the action of the yeast with consequent decrease in pH and specific gravity of the product. The explanation for this change may be due to the degradation of sugar by the action of yeast during primary fermentation and the production of other products like ethanol and CO_2 during secondary fermentation.

There is also noticeable increase of alcoholic content with increased time of fermentation. This alcoholic content increases gradually after primary fermentation and to a higher level after secondary fermentation due to continues action of yeast until there is no more extract to utilize. Noticeably, samples that are produced from worts mashed with enzymes have higher alcohol because of the initial high extracts obtained during mashing. Moreover, enzyme treatment significantly improved extract yield which in turn produced higher alcohol content.

The use of industrial exogenous enzymes have been found to be relatively more effective in the preparation of beer wort as observed for the result obtained with red and white sorghum malt in this study. The use of red and white sorghum as brewing main raw material with commercial enzymes are found to be useful in production of high quality wort with low cost and profitability.

CONCLUSION

The overall experiment recorded significant changes in pH, flow rate, viscosity, extract yield, improved with addition of industrial exogenous enzymes. The use of industrial exogenous enzymes have been found to be more effective in the preparation of beer worts as observed for the result obtained with red and white sorghum malt in this study. The high performance of the enzymes may be as a result of the environmental changes such as temperature, ion strength, the types and proportion of enzymes used. Therefore, presence of commercial enzyme will enhance the quality characteristics of the wort, increase yield and profitability due to high efficiency, specificity and ability to control their activities in the brewing process. Therefore, the use of local Nigerian sorghum (red and white varieties) as brewing raw materials will be a welcome development and further enhance better utilization of sorghum and reduces importation of barley with more profit.

Based on the results of the research carried out, it is therefore recommended that local Nigerian sorghum (red and white varieties) should be given serious attention in Nigeria, or indeed in the rest of Africa, as a substrate for brewing because it can be used to produce beer of acceptable quality. Commercially available enzyme (exogenous enzymes) should be incorporated to sorghum mash to ensure complete conversion starch present in the sorghum to fermentable sugar as endogenous enzymes are lacking in sorghum. Using industrial (exogenous) enzyme in brew house is more efficient because in the same time it is increase extract yield, improve wort filter ability, protein coagulation and it needs less energy for wort boiling. Government should invest on sorghum farming to ensure its supply for home use as well as for beer making. Further studies should be carried out in the same subject matter with other local cereals.

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