ABSTRACT

Aim: Turbidity is one of the integral parameters used to ascertain beer quality and clarity. This has been achieved through a combination of filtration processes and utilization of kieselghur as filter aid. Kieselghur on the other hand is expensive and not readily available; therefore there is need to find potential filter aids from locally available materials. The aim of this research is to determine the effectiveness of egg shell powder and snail shell powder in sorghum beer clarification.

Study Design: This study was made to fit into using a combination of T-test and one way Analysis of Variance.

Place and Duration of Study: The research was carried out at Department of Food Science and Technology Laboratory, Federal University of Technology, Owerri, Nigeria, between May 2018 and November 2018.

Methodology: Sorghum grains were malted by adoption of barley malting protocols while snail shells and egg shells obtained from the market were processed into powders. Temperature programmed infusion mashing method was used to produce wort from sorghum malt that was...
germinated for 2 days and 3 days. The resulting worts were fermented using Saccharomyces carlsbergensis to obtain sorghum beer and the beer was clarified with kieselghur, egg shell powder and snail shell powder. Turbidity and other quality analyses were carried out on the sorghum wort, sorghum beer and clarified sorghum beer.

**Results:** The results showed that beers clarified with Kieselghur had better clarity than egg shell powder and snail shell powder clarified sorghum beers. Egg shell powder showed better clarification potentials than snail shell powder. There is a correlation between total solids and turbidity. Germination period significantly influenced the turbidity of sorghum beer.

**Conclusion:** This study showed that egg shell powder can serve as potential filter aid in beverage clarification.

**Keywords:** Beer quality; egg shell; filter aid; kieselghur; sorghum beer; snail shell; turbidity.

1. **INTRODUCTION**

Beer is an alcoholic beverage made from cereal grains especially malted barley [1]. Beer may be produced from the principal ingredients such as: water, barley, hops and fermenting yeasts. Among several malts, barley malt is the most widely used starch source owing to its high enzyme content (which facilitates the breakdown of starch into sugars), less problem in filtration being a covered caryopsis as well as its composition which contributes to the distinct beer characteristic quality. In Nigeria, the frequent use of barley malts in brewing has diverted a greater percentage of the country’s foreign earnings on importation of malted barley since it is not grown in the tropics. An alternative towards solving this can be by the use of other cereal grains especially sorghum malt in place of barley malt in brewing since it is one of the prominent crops grown in the tropics.

Sorghum is a staple crop grown in Nigeria and in many African countries like Benin, Ghana, Zimbabwe, South Africa, etc [2]. It is cheap, locally available and can grow in the tropics. Sorghum has an endosperm structure similar to that of maize, though it gives nearly same qualities in beer but it has a problem in filtration due to its naked caryopsis, its single aleurone layer and its pericarp containing more polyphenols (about 413 mg/100 g) than that of barley [3].

However, it is worthy to note that the first visual impression of beer quality is determined by its turbidity. Beer quality and clarity on the other hand, can be improved through clarification which can be achieved by the use of certain filter aids or clarifying agents. These agents help to trap impurities and/or suspended particle to form clogs or sediment [4]. Hence, such clarifying agents among these categories include Isinglass, Kieselghur, Irish moss, Kappa carrageenan, gelatins, etc. In addition, due to more demand for these commercial filter aids, there is more burden on foreign exchange to import these raw materials especially kieselghur as they are relatively expensive and are not readily available. That being the case, there is need to find alternative filter aid that is cheap and locally available so as to save foreign exchange for importation of kieselghur. This can be actualized by finding substitutes for these commercial filter aids from some of the sources that are locally available such as egg shell powder and snail shell powder.

Egg shell and snail shell have different components aside the mineral contents which composed mainly of calcium compounds especially CaCO$_3$ [5] unlike kieselghur which is diatomaceous earth that contains mainly silica from the remains of diatoms. Calcium compounds also help to precipitate the suspended particles in the solution making them to form lumps and acquire more density which enable them to settle under gravity, thus reducing turbidity. Consequently, the use of some of these locally available products as filter aids in brewing may yield products of desired qualities that can be comparable to those obtained from the use of commercial filter aids, while reducing the overall cost of importation of other commercial filter aids. Therefore the objective of this research is to evaluate the potentials of utilizing egg shell powder and snail shell powder in sorghum beer clarification.

2. **MATERIALS AND METHODS**

2.1 Source of Materials

Grains of white sorghum variety (Sorghum bicolor L. Moench) of less than one year old and snail shells were both purchased at Nkwo Nnewi main market Nnewi, Anambra state,
2.2 Sample Preparation

2.2.1 Production of sorghum malt

The sorghum malt was produced using the method of Kunze [6] which has similar protocols for malting of barley. The weighed and sorted sorghum grains (2 kg) were suspended in 10 litres of water and steeped for 18 hours at a temperature of 25-30°C with 6 hours wet steep period and 45 minutes of air rest. The steeped grains were drained and heaped on a moistened jute bags previously sterilized with steam and allowed to germinate at 25-30°C for 2 and 3 days. Water was being sprinkled on the grains throughout the germination period to avoid local overheating during germination. Kilning of the 2 days and 3 days germinated grains were done in a hot air oven at temperatures between 70°C for about 3 hours. The sorghum malt was continuously stirred to aerate and achieve uniform heat distribution. The rootless were removed and winnowed (cleaned) to remove dust and other particles. The malted sorghum was milled (crushed) into different particle sizes – coarse, medium and fine grist using attrition mill and then packaged in an air tight container.

2.2.2 Production of egg shell powder

Egg shells from restaurant were cleaned and washed followed by the removal of the shell-membrane using hot water in order to prevent or minimize the interference of protein molecules with the final product. The shells were later oven dried at 105°C for 3 hours followed by aeration at room temperature for proper cooling. The dried egg shells were milled into powder using attrition mill and then packaged in an air tight container.

2.2.3 Production of snail shell powder

Snail shells obtained from the market were cleaned to remove sand and thoroughly washed. The resulting clean shells were oven dried at 105°C for 3 hours followed by aeration at room temperature for proper cooling. The resultant material was crushed into powder and packaged in an air tight container.

2.3 Mashing of Sorghum Malt

Temperature programmed infusion mashing was used to mash the sorghum malt as described by the method of Ofodu [7] as well as Osuji and Anih [8] with slight modification. Six hundred (600) gram of malted grains comprising of 75% of malted sorghum and 25% of malted barley were dissolved in 6,600 ml of portable water that was previously adjusted to a pH of 11.0 using Ca(OH)₂ solution. This step was repeated twice for different mash water for day 2 and day 3 sorghum malts. The mixture was stirred continuously while the temperature was being raised to 45°C (for protein rest) and maintained at this temperature for 20 minutes after the addition of bacterial α-amylase (0.4 ml). With constant stirring but not vigorous, the temperature of the sample mixture was raised to 55°C and maintained at this temperature for 10 minutes. The temperature of the content of the mashing vessel was raised to 65°C and maintained at this temperature for 1 hour with the addition of fungal α-amylase (0.4 ml). Conversion (hydrolysis) of starch in the medium was tested by pipetting 2 drops of iodine solution on a white ceramic tile. The mash temperature was raised to 75°C and more fungal α-amylase (0.4 ml) was added to the mixture as the temperature of the mash was maintained at 75°C until complete saccharification is achieved. This was confirmed with another iodine test.

The converted medium was filtered across a triple layer muslin cloth and the spent grains were sparged with 1000 ml of hot water. The spent grains which also include husk, seedlings and other insoluble materials were separated from the wort to recover as much extract as possible from the spent grains.
2.4 Sorghum Beer production

The procedure used for beer production was the method described by Kunze [9]. The recovered wort was boiled using classical method involving atmospheric boiling at 100°C for 45 minutes. Hop extracts were added to the boiling wort so as to impart unique characteristic flavor and herbal aroma to the product. The boiled wort was cooled and the hot trubs and other undissolved particles (debris) were removed. The filtered wort was transferred into a fermenting vessel with each vessel containing wort from 2nd day and 3rd day malted grains. An already activated yeast (200ml) was pitched into each of the vessel at a temperature of 20°C and the wort was allowed to ferment for 8 days at 10°- 20°C.

2.5 Beer Filtration and Clarification

After fermentation, the beer was filtered and transferred into another vessel for maturation and clarification. During the lagering process, the beer was made in triplicate for each of the brew from the different germination periods (day 2 and day 3). Kieselghur in the ratio of 2:1 (Male: Female), egg shell powder and snail shell powder were added to each of the three separate vessels containing beer from day 2 and day 3 germination period for clarification and gently stirred. The mixture was allowed to stand undisturbed for 2 weeks followed by bottling of the clarified beer in a clean sterilized glass bottle and sealed with manual crowning machine.

2.6 Quality Evaluation of Sorghum Malt, Wort and Beer

2.6.1 pH determination

The wort and beer pH were determined using a method of A.O.A.C. [10] with using hand-held pH meter.
2.6.2 Apparent brix determination

The apparent brix (°B) of the wort and beer samples was determined according to the method described by Montanez-Soto et al. [11] using a Milwaukee Digital refractometer.

2.6.3 Determination of specific gravity

Specific gravities (original and final gravity) of the wort and the beer were determined using specific gravity bottle as described by A.O.A.C [10].

2.6.4 Determination of alcohol

The percentage alcohol-by-volume of beer was determined by the method of A.O.A.C. [10] through distillation technique.

2.6.5 Determination of total solids

Total solids were determined using the method of Osuji and Anih [8].

2.6.6 Determination of turbidity

Turbidity of the beer samples was determined by the photometric method using HACH DR/21010 spectrophotometer at a wavelength of 860nm and programme number 750 according to A.O.A.C [12].

2.6.7 Determination of total dissolved solids

The total dissolved solid of the beer sample was determined using a method of A.O.A.C. [10].

2.7 Statistical Analysis

Data obtained from these analyses were subjected to T-test and one-way analysis of variance. Means obtained were separated using Fisher’s Least Significant Difference at P<0.05.

3. RESULTS AND DISCUSSION

Quality Evaluation of Sorghum wort and Sorghum beer: The sorghum wort and beer quality before clarification are shown in Table 1.

3.1 pH

The pH of the sorghum wort from day 2 and day 3 sorghum malt were 5.60 and 5.50 respectively while the pH of sorghum beer from day 2 and day 3 sorghum malt were 4.10 and 3.90. There was no significant difference (P>0.05) between the pH of day 2 & day 3 sorghum wort and the pH of day 2 & day 3 sorghum beer. pH is used to denote the degree of the alkalinity or acidity of a substance. The pH of the sorghum wort obtained in this study was in agreement with the range of 5.40 – 5.70 reported by Kunze [6]. The sorghum beer pH was significantly lower than its corresponding sorghum wort. However, previous research works have reported a pH range of 3.70 – 4.50 for lager beer and the pH of sorghum beer obtained in this study falls within this range. This indicates that beer pH tends to be more acidic after fermentation. The fall in pH of sorghum wort after fermentation must be the action of yeast activity. It was reported by Coote and Kirsop [13] that yeast may be used to reduce the pH of wort by altering the wort buffering capacity, absorbing bases (amino acids) and excreting organic acids such as pyruvic, malic, lactic, succinic acids, etc; thereby giving beer its unique pH in acidic region.

3.2 Apparent Brix (°B)

The apparent brix of the sorghum wort from day 2 and day 3 sorghum malt were 15.4°B and 16.0°B respectively while the apparent brix of sorghum beer recorded were4.60°B for day 2 malt and 4.70°B for day 3 malt. There was no significant difference (P>0.05) between the apparent brix of day 2 & day 3 sorghum wort and the apparent brix of day 2 & day 3 sorghum beer. The major solids present in wort were mainly hydrolyzed sugars which include monosaccharide, disaccharides, dextrins as well as amino acids, polypeptides, vitamins, minerals, etc [11]. Brix refractometer measures the degree to which a solution refracts or bends light as it is normally used to measure the amount of sucrose in a solution [14]. The apparent brix of sorghum beer was significantly lower than its corresponding sorghum wort probably because of fermentation. During fermentation, soluble extracts in form of hydrolyzed sugars and soluble proteins were absorbed by the yeast, thus reducing the final brix of the beer after soluble extracts utilization is complete.

3.3 Specific Gravity of the Wort

The original gravity of the sorghum wort from day 2 and day 3 sorghum malt were 1.076 and 1.076 respectively while the final gravity of sorghum beer recorded 1.020 for day 2 malt and 1.020 for day 3 malt. The original gravity obtained in this work is in line with the range of 1.000-1.130 as
Table 1. Quality evaluation of sorghum wort and beer before clarification

<table>
<thead>
<tr>
<th>Samples</th>
<th>pH</th>
<th>Apparent brix (°B)</th>
<th>Specific gravity</th>
<th>% Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wort</td>
<td>Beer</td>
<td>Wort (Original gravity)</td>
<td>Beer (Final gravity)</td>
</tr>
<tr>
<td>Day 2 Malt</td>
<td>5.60±0.141</td>
<td>4.10±0.141</td>
<td>15.4±0.282</td>
<td>4.60±0.071</td>
</tr>
<tr>
<td>Day 3 Malt</td>
<td>5.50±0.007</td>
<td>3.90±0.0212</td>
<td>16.0±0.141</td>
<td>4.70±0.141</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are the means of duplicate determinations

a,b….means with the same superscript along a column for each treatment are not significantly different (P>0.05)

*….means with asterisk (*) within a row and within pH, Apparent Brix and Specific gravity is significantly different (P>0.05)

Key: Day 2 Malt = Sorghum grains germinated for 2 days; Day 3 Malt = Sorghum grains germinated for 3 days; NS = Not Significant

Table 2. Physicochemical properties of clarified beer samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specific gravity</th>
<th>Turbidity (NTU)</th>
<th>Total solids (g/ml)</th>
<th>Total dissolved solids (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.001±0.001</td>
<td>8.280±0.280</td>
<td>0.016±0.001</td>
<td>0.008±0.003</td>
</tr>
<tr>
<td>B</td>
<td>1.001±0.001</td>
<td>21.880±0.140</td>
<td>0.017±0.001</td>
<td>0.012±0.000</td>
</tr>
<tr>
<td>C</td>
<td>1.002±0.000</td>
<td>23.240±0.140</td>
<td>0.024±0.000</td>
<td>0.011±0.003</td>
</tr>
<tr>
<td>D</td>
<td>1.004±0.001</td>
<td>8.720±0.280</td>
<td>0.016±0.001</td>
<td>0.009±0.001</td>
</tr>
<tr>
<td>E</td>
<td>1.003±0.003</td>
<td>23.420±0.140</td>
<td>0.022±0.003</td>
<td>0.010±0.001</td>
</tr>
<tr>
<td>F</td>
<td>1.004±0.001</td>
<td>26.560±0.000</td>
<td>0.026±0.000</td>
<td>0.011±0.001</td>
</tr>
<tr>
<td>LSD</td>
<td>NS</td>
<td>0.47</td>
<td>0.004</td>
<td>NS</td>
</tr>
</tbody>
</table>

a,b….means with the same superscript along a column for each treatment are not significantly different (P>0.05)

Keys: A = Day 2 beer clarified with Kieselghur; B = Day 2 beer clarified with egg shell powder; C = Day 2 beer clarified with snail shell powder; D = Day 3 beer clarified with Kieselghur; E = Day 3 beer clarified with Egg shell powder; F = Day 3 beer clarified with snail shell Powder.
3.4 Alcohol Content

The alcohol content of sorghum beer from day 2 malt and day 3 malt were 6.00\% and 4.60\% respectively. There was significant difference (p<0.05) on alcohol content between sorghum beer from day 2 malt and sorghum beer from day 3 malt. The alcohol content in the sorghum beer was predominantly ethanol. The higher alcohol content in sorghum beer from day 2 malt when compared to the beer from day 3 malt could be as a result of varying concentrations of sugars produced during malting and mashing operation. However, it could be stated that a correlation exists between duration of germination and hydrolysable sugar concentration as well as alcohol content. In other words, it could be that the alcohol yield decrease with increase in malting loss or decrease in malt yield [6].

Quality evaluation of the clarified sorghum beer samples: The quality of sorghum beer clarified with kieselghur, egg shell powder and snail shell powder are shown in Table 2.

3.5 Total Solids

The total solids of the clarified beer samples ranged from 0.016g/ml to 0.024g/ml. For Day 2 clarified beer, there was no significant difference (P>0.05) between sample A (Day 2 beer clarified with Kieselghur) and sample B (Day 2 beer clarified with egg shell powder) but both showed significant difference (P<0.05) with sample C (Day 2 beer clarified with snail shell powder). Also, for Day 3 clarified beer, there was no significant difference (P>0.05) between sample D (Day 3 beer clarified with Kieselghur) and sample E (Day 3 beer clarified with Egg shell powder) but both showed significant difference (P<0.05) with sample F (Day 3 beer clarified with snail shell Powder). The variations in the total solids of the clarified beer samples may be attributed to the nature and/or the chemical composition of the filter aids. It was observed that the germination period of malt used in producing these clarified sorghum beers had no significant influence on the amount of total solids in the beer sample. However, the filter aids influenced the quantity of solids present in the sorghum beer samples. For both Day 2 and Day 3 clarified sorghum beer samples, beer clarified with kieselghur had the least total solids, followed by egg shell powder clarified beer, while snail shell powder clarified beer recorded a higher total solid content. According to Anger et al., [15] and Ofoedu [7] total solids are largely composed of soluble and insoluble constituents of a solution such as sugars, organic acids, amino acids (nitrogenous compounds) and minerals. Comparing the egg shell and snail shell powders used as filter aids in this research work, it could be that the chemical composition of the egg shell powder tends to have more affinity for insoluble or suspended solids by trapping insoluble and/or suspended solids and precipitating them out of solution in form of flocs, thereby resulting to a lesser solid content compared to snail shell powder clarified beers.

3.6 Total Dissolved Solids (TDS)

The total dissolved solids of the clarified beer samples ranged from 0.008g/ml to 0.012g/ml. There was no significant difference (P>0.05) in the total dissolved solids of the clarified beer samples from Day 2 and Day 3. The total dissolved solids are the soluble solutes that were not precipitated by the filter aids. The differences in the total dissolved solids of the clarified beer samples could be interpreted as a result of variations of germination periods and some chemical reaction that occurred during mashing operation, thereby yielding different concentrations of soluble solutes. According to Ofoedu [7], total dissolved solid is the measure of the combined content of all organic and inorganic substances contained in a liquid in molecular and/or ionized form.

3.7 Specific Gravity

The specific gravity (SG) of the clarified beer samples ranged from 1.001 to 1.004 and there was no significant difference (P>0.05) in the SG of the beer samples. Specific gravity of beer is a function of the density of beer and
density of water at equal volumes [7]. It could be observed that the SG of the clarified beer samples from Day 2 and Day 3 were lower than the final gravity of the beer after fermentation in Table 1. The decrease in SG observed in the clarified beer samples is because of the combined action of beer maturation and clarification with the aid of filter aids. Maturation is the transformation between the end of primary fermentation and final filtration of beer [16]. During this stage of brewing process, complex biochemical, chemical and physical reactions that lead to beer clarity as well as flavour development occur.

3.8 Turbidity

The turbidity of the clarified beer samples ranged from 8.28 to 26.56NTU (Nephelometric Turbidity Unit). There were significant differences (P<0.05) in the turbidity of the clarified beer samples. Sample F (Day 3 beer clarified with snail shell Powder) recorded the highest turbidity of 26.56NTU while sample A (Day 2 beer clarified with Kieselghur) had the least turbidity of 8.28NTU followed by sample D (Day 3 beer clarified with Kieselghur) with a turbidity of 8.72NTU and both showed no significant difference (P>0.05). The differences in the clarified beer turbidity could be as a result of variations in the concentration of soluble solutes and suspended particles in the beer samples as well as the extent of grain modification during germination/malting [6] and also due to the degree of hydrolysis during mashing. Sorghum grain was without husk and this tends to cause filtration problem due to their naked caryopsis.

In addition, the type of filter aids used, might have partly contributed to the variations in the turbidity of the beer samples. It could be observed that for Day 2 and Day 3 clarified beer, beer samples clarified with kieselghur had a lower turbidity, followed by egg shell powder clarified beer while snail shell powder clarified beer recorded the highest turbidity and this also corresponds to the total solid content of the individual beer samples. In other words, the concentration of total solids in a beer sample or solution can be correlated to turbidity. It is important to note that Day 3 clarified beer samples had a higher turbidity than its corresponding Day 2 clarified beer samples, probably due to production of more proteins (amino acids) and other compounds that can cause haze in beer. Turbidity is simply the cloudiness or haziness of a beer. Haze in beer can be as a result of polyphenols and proteins. These compounds are always present in beer and can form haze when they cross-link to form insoluble particles [17]. Unlike insoluble particles and/or suspended solids, coloured compounds such as melainoidins that are produced during kilning and mashing operation and forms part of soluble solutes can also contribute to beer’s turbidity. Turbidity is measured when incident light is scattered at right angles from the sample by the concentration of suspended solids. However, though melainoind pigments may be present in low concentrations in beer, they also contribute significantly to the turbidity of the beer.

Four beer samples analyzed for turbidity by Thermoscientific [18] reported that lager beer had 16.3NTU, light pilsner had 7.2NTU, seasonal ale had 13.5NTU and stout had 5.8NTU; however, the sorghum lager beer produced in this study had 8.28-8.72NTU for kieselghur clarified beer, 21.88-23.42NTU for egg shell powder clarified beer and 23.24-26.56NTU for snail shell powder clarified beer. According to literature, 1EBC = 4NTU = 69ASBC [19], and the turbidity of beer samples are graded based on their degree of haziness (Brilliant for 0.0 to 0.5EBC, Almost Brilliant for 0.5 to 1.0EBC, Very Slightly hazy for 1.0 to 2.0EBC, Slightly hazy for 2.0 to 4.0EBC, hazy for 4.0 to 8.0 EBC and Very hazy for >8.0EBC). This implies that the turbidity of kieselghur clarified sorghum may be classified as slightly hazy while egg shell powder and snail shell powder clarified sorghum beer may be classified as hazy. This is in agreement with the work of Olu et al. [20] who reported 5.25EBC lager beer from 100% barley malt and a range of 5.25EBC to 7.00EBC lager beer from blends of barley malt and sorghum adjunct.

4. CONCLUSION

The effectiveness of egg shell powder and snail shell powder as potential filter aids for brewing exhibited some clarification properties. Kieselghur clarified sorghum beer had better clarity than egg shell powder and snail shell powder clarified sorghum beer; but egg shell powder showed better clarification potentials than snail shell powder. Sorghum beer clarified with kieselghur was classified as slightly hazy while egg shell powder and snail shell powder clarified sorghum beer was classified as hazy in terms of its turbidity. Malting of sorghum improves the hydrolysis and
modification of starchy endosperm. The concentration of total solids in the beer samples influenced the beer quality and clarity; consequently, there was a direct correlation between total solid content and turbidity. Germination period significantly influenced the turbidity of the beer samples. It is therefore recommended that further research be carried out on the treatment of egg shell, snail shell and other crustacean shells so as to improve their effectiveness in beer/beverage clarification for a better product quality and acceptability.

COMPETING INTEREST

Authors have declared that no competing interest exists.

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