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Assessment of the physicochemical and sensory properties of probiotic malt beverage produced from sorghum and barley using probiotic strains (*Lactobacillus acidophilus* and *saccharomyces cerevisiae*)

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Abstract

Proper nutrition is characteristically essential as it promotes gut balance and enhances protection from diseases and overall human survival. Functional foods inoculated with lactic acid bacteria (LAB) are a multifaceted group of microorganisms with a wide range of applications in industry and humans due to their health benefits. This study involved the evaluation of the physicochemical and sensory properties of probiotic malt beverages produced from sorghum and barley using *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*. The malt beverages were divided into three groups, inoculated with *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* and the mixture of both *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*, and were coded 177, 277 and 377, respectively. The proximate and sensory properties of the probiotic malt beverage were evaluated. The data generated were subjected to statistical analysis using a significant difference of 5%. There was variation in the evaluated sensory properties of the probiotic malt beverage with the control (normal malt) standing out. After the control, 277 had the highest score for all the sensory parameters evaluated, followed by 377. No significant difference exists ($p > 0.05$) in their appearance, smell and general acceptability. However, there was a significant difference in their taste. Results from this research work suggest that the production of probiotic malt beverage can be achieved using *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*. The production and consumption of probiotic beverages should, therefore, be encouraged because of its immense health benefits, especially in the balance of human gut flora level.

Keywords: Lactic Acid Bacteria; Probiotic malt; Cereals; Mixed cultures; Fermentation; Physicochemical composition; Sensory evaluation

1. Introduction

Proper nutrition is characteristically essential as it promotes gut balance and enhances protection from diseases and overall survival. Gut bacteria are important components of the microbiota ecosystem in the guts of humans and the balance or imbalance of bacteria in the digestive system is linked to one's overall health and disease [1]. An imbalance in the composition of gut bacteria has been associated with intestinal symptoms such as bloating, abdominal pain, and diarrhoea [2]. Other diseases that have been linked to gut bacteria include inflammatory bowel diseases, carcinoma, obesity, diabetes, HIV, chronic heart diseases, liver diseases, autism, and cancers [3]. Recent studies also relate gut bacteria with several other diseases and malaise, such as bad sleep, rheumatic diseases, and kidney diseases [4].

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Modern consumer trends are gravitating towards functional foods as a cost-effective alternative to prophylaxis for disease prevention. Studies have shown that these foods contain a mix of ingredients, in addition to their basic nutritive value, that aid in disease prevention and treatment [5]. Prominently, probiotics or products containing lactic acid bacteria have gained increased relevance within the functional food category. Lactic Acid Bacteria (LAB) are gram-positive, diverse groups of bacteria which produce lactic acid as their major product, and are often non-sporulating or coccus-shaped organisms [6, 7]. They are described as fermentative agents and produce a variety of end products (lactic acid, carbon dioxide, ethanol or acetic acid) that help to define their state as either homo-fermentative or hetero-fermentative [8]. LAB has vast array of applications and benefits in humans, industries, agriculture, and health. They exist in the gastrointestinal tracts of humans and are reported to produce myriad beneficial effects such as alleviation of lactose intolerance, diarrhea, stimulation of the immune system and anti-allergic effects [7].

Ample scientific information is available on the health and industrial benefits of LAB [9, 6]. Industrially, LAB is added to dietary products for shelf-life benefits, nutrient and taste improvement, and for packaging purposes. The addition of LAB into dietary products such as beverages, milk, yoghurts, and malts, etcetera makes them probiotics and also contributes to the taste, flavor and texture, particularly for milk and yoghurts [10] (Tamime, 2012).

Although research shows that there are over 60 genera of LAB used in food fermentation [11], here, we focus on two common strains: *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*. *Lactobacillus acidophilus* is an obligate homofermentative microorganism that produces lactic acid from the fermentation of carbohydrates. It is microaerophilic and among the least oxygen-tolerant *Lactobacilli* [12]. It exhibits important technical properties such as thermostability, retaining activity at a wide range of pH and inhibitory actions against food spoilage and pathogenic microorganisms [13]. These properties explain its extensive application in the food industry as an important class of biopreservatives. It is one of the most important strains with anticarcinogenic properties [14]. Following the report of Wagner *et al.* (2000) [15], *L. acidophilus* possesses good antifungal properties and the ability to protect immunocompromised individuals from opportunistic infections by *Candida albicans*. Research has shown that decreased incidences of lactose intolerance in cattle-raising cultures are due to increased consumption of dairy products containing *L. acidophilus* [7]. Thus, the need to produce malted beverages and other dietary products using *L. acidophilus* becomes more apparent.

1.1. *Saccharomyces cerevisiae*

Non-alcoholic malt beverage is one of the most highly consumed beverages in Sub-Saharan Africa, particularly Nigeria, and it originated from Albani Brewery of Denmark [16]. Malt is a non-fermented cereal-based carbonated beverage that is highly fortified with various vitamins and rich in minerals, starch, protein and trace elements [17]. Typically, the production process of malt-based beverages (malting) is similar to beer production but with full fermentation. Malting involves three processes, namely steeping, germination and drying [5], and detailed information on the production of malt-based beverages from mashing a mixture of sorghum and barley are well documented [17, 18, 5]. Malt beverages are considered healthy foods, and in addition, LaMarco (2011) [19] compiled some health benefits which include but are not limited to good sources of B vitamins and amino acids, promoting good vision, colon cancer and kidney stones.

1.2. Sorghum and Barley

Sorghum (*Sorghum bicolor* (L.) Moench) and barley (*Hordeum vulgare* L.) are cereals from the family Poaceae and are traditionally mixed for malt production. Though sorghum is gluten-free while barley contains gluten, both grains contain high phenolic compounds like flavonoids [20, 21], and are rich in protein, starch, fibre, vitamins, and minerals [22]. Diverse industrial applications of Sorghum and barley are well documented [23, 24, 25]. These include: flour, flakes, malted foods, beverages, and beer. This portrays their high consumption rate (either raw or processed) in addition to their nutritional value.

Research has shown that the balance or imbalance of bacteria in the digestive system is linked to the overall health and disease of an individual. Gut bacteria are important elements in the microbiota ecosystem of humans [1]. Contrary to the healthy conditions associated with gut bacteria balance in humans, an imbalance in the composition of gut bacteria is associated with multiple symptoms and diseases [3, 2, 4]. This clearly shows that gut-imbalance diseases are public health problems at a global scale, and an attempt to balance the gut bacteria flora in humans through healthy food could mitigate the prevalence of such diseases. It, therefore, follows the suggestion for the addition of probiotics to frequently consumed foods and beverages as a way of maintaining the right gut flora balance.

Based on the popular adage "health is wealth", it is believed that good health can only be achieved through good nutrition, and good nutrition starts with maintaining the right balance of gut flora. Here, we introduce *L. acidophilus* and *S. cerevisiae* in the production of malt beverages as an important and inexpensive way of maintaining gut flora balance.

It is expected that the probiotic malt beverages produced will be of great health benefit and provide the right gut flora balance.

2. Material and Methods

2.1. Sampling

The raw materials used in this study include: CSR-02 variety of white sorghum grain sourced from the Institute for Agricultural Research, Kaduna State, Nigeria; germinated and kilned barley sourced from Kawahara Flour Mill, Japan; *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were both sourced from Applied Biotech International Nigeria Limited.

2.2. Sample Preparation and Inoculation

The sample preparation and production methods of Pradhanang (2013) [26] were adapted for this study but with slight modification by the inoculation of the LAB strains. For the inoculation, the microorganisms were introduced into thirty-six (36) bottles filled with the malt mixture. They were separated into 3 groups of 12 bottles each. The bottles in the first group were inoculated with only *Lactobacillus acidophilus* (at 37 °C). The bottles in the second group were inoculated with *Saccharomyces cerevisiae* (at 32.3 °C) while the bottles in the third group were inoculated with a mixture of both *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* (at 37 °C). *Saccharomyces cerevisiae* was diluted in 10ml of distilled water and 1ml of the dilution was introduced into 100ml of the sample.

2.3. Incubation

The bottles and their contents were incubated for 24 hours. The *Lactobacillus acidophilus* bottles were incubated at 37 °C, *Saccharomyces cerevisiae* was incubated at 32.3 °C while the mixture of *L. acidophilus* and *S. cerevisiae* bottles were incubated at 37 °C.

2.4. Microbiological Analysis

Probiotic malt beverages were analyzed for microbial load using the method described by Sanjee and Karim (2016) [27].

2.5. Total Viable Count (TVC)

The total viable count of probiotic malt samples was enumerated by the standard plate count (SPC) method as described by Maturin and Peeler (2001) [28]. Serial dilution of each sample was prepared up to a 10⁻⁵ dilution with 9 mL sterilized 0.1% peptone water. A 1 mL aliquot from each dilution was aseptically transferred into duplicate sterile Petri plates. Sterile molten Plate Count Agar (around 40–45 °C) was poured into each plate, gently swirled for uniform mixing, allowed to solidify, and finally incubated in an inverted position at 37 °C for 24–48 hours. After incubation, the plates with well-spaced colonies (30–300) were counted using a colony counter (Stuart Scientific, UK). The total viable count per mL of sample was calculated by multiplying the average number of colony per plate by the reciprocal of the dilution and expressed as colony-forming units (CFU/mL).

2.6. Enumeration of Total Coliforms

Total coliforms were enumerated using the Most Probable Number (MPN) method as described by Feng et al. (2002) [29]. Serial dilution of the samples was prepared as described above, and 1 mL of each dilution was inoculated into triplicate test tubes of 9 mL Lauryl Tryptose Broth (LTB) containing inverted Durham tubes. The inoculated tubes were incubated at 37 °C for 48 hours. Gas-positive test tubes were recorded as presumptive positive for coliforms, and results were interpreted using an MPN chart for coliform counts (MPN/mL) determination.

2.7. Enumeration of Fecal Coliforms

The fecal coliform count was determined using the method described by Oblinger and Koburger (1975) [30]. About one loopful from each gas-positive LTB was inoculated into a test tube of sterilized BGLBB and a test tube of sterilized 10 mL Tryptone Broth and then incubated at 44.5 ±0.5 °C for 48 hours. After incubation, gas production was recorded, and 2-3 drops of Kovac's reagent were added to each of the Tryptone Broth tubes. A cherry red color indicated a positive indole reaction and confirmed the presence of *E. coli*. Results were interpreted using an MPN chart to determine the total fecal coliform count (MPN/mL).

2.8. Determination of Physicochemical Analysis

Moisture content was determined gravimetrically using the method described by the Association of Official Analytical Chemists AOAC (2000) [31]. The pH was determined by the method described by AOAC (2010), which was read off a digital Hanner (pHS-25) pH meter calibrated with pH 7 buffer. Acidity was determined using the method described by AOAC (1990) [32], where 5ml aliquot of the sample solution was titrated with 0.1N NaOH using phenolphthalein solutions as an indicator and the titratable acidity was calculated as a percentage of lactic acid. The TSS of the samples was determined by use of a hand refractometer and the values expressed in °Brix. Total reducing sugar was determined using the Lane-Eynon method as described by Pal *et al.* (2019) [33], and the results were expressed as maltose. The protein was determined using a micro-Kjeldahl method as described by (AOAC, 2005) [34]. Crude Fat content was determined by the continuous solvent extraction and gravimetric using a Soxhlet apparatus, using the method described by AOAC, (2000). The color of the sample was determined as per AOAC official method 976.08 (2005) [34] with slight modification. Briefly, the sample was filtered through Whatman No. 1 filter paper and its absorbance was read at 430nm using a 10 mm cuvette in a spectrophotometer. Lastly, the color intensity was calculated by multiplying its Absorbance at 430nm by 10. The calorific value was computed by summing up the values obtained by multiplying the values with Atwater constants for carbohydrates, crude fat and crude protein with the factors; 4, 9, and 4, respectively. The ash content was determined using the furnace incineration and gravimetric method as recommended by AOAC (1990) [32]. The density of the samples was determined using a pycnometer and the results were recorded.

2.9. Sensory Analysis

Probiotic malt beverages were analyzed for sensory quality. The purpose of the sensory evaluation was to compare the probiotic malts against other brand malt beverages with respect to color, aroma, taste, and overall acceptability. As suggested by Pal *et al.* (2019) [33], a nine-point hedonic rating test was adopted as the method of evaluation. A sensory panel consisting of lecturers, technical/laboratory staff and students of the *Anonymous department, Anonymous University*, were invited. A total of 20 panelists were selected and trained for the sensory evaluation. Each panelist was provided with coded samples and a sheet of the sensory evaluation form. The panelists were asked to score for the quality attributes of color, smell, taste, and overall acceptability in order of their individual preferences.

2.10. Statistical Analysis and Experimental Design

The data generated were analyzed in triplicates and expressed as mean±standard deviation. The statistical data analysis was by Analysis of Variance (ANOVA) using Genstat programming (Genstat Discovery ed. 3, 2010). The Least Significant Difference (LSD) method was used to compare means of the data at 5% level of significance.

The experimental design adopted for this study was a completely randomized design (CRD) following the method described by Hinkelmann and Kempthorne (2008) [35].

3. Results and Discussion

3.1. Microbial Composition of Probiotic Malt Beverage

From the microbial composition result shown in Table 1, *L. acidophilus* and *S. cerevisiae* were fermented at 37 °C and 32.3 °C for 24 hours. The maximum number of *L. acidophilus* is 5.0×10^7 CFU/ml while *S. cerevisiae* is 1.5×10^7 CFU/ml and there was no significant difference ($p < 0.05$) between them.

Table 1 Result Showing the Microbial Composition of Probiotic Malt

Sample	Total Viable Count (CFU/ml)	Total Coliforms (MPN)	Fecal coliforms (MPN)
177	$5.00 \times 10^{7a} \pm 0.00$	$1.00 \times 10^{1a} \pm 0.00$	$0.00 \times 10^{1a} \pm 0.00$
277	$3.00 \times 10^{7a} \pm 0.00$	$1.30 \times 10^{1a} \pm 0.00$	$0.00 \times 10^{1a} \pm 0.00$
377	$1.50 \times 10^{7a} \pm 0.00$	$0.90 \times 10^{1a} \pm 0.00$	$0.00 \times 10^{1a} \pm 0.00$

a-e: Means with the same superscript within the same column are not significantly different at ($P > 0.05$).

Where;

177 = Malt sample inoculated with *Lactobacillus acidophilus*

277 = Malt sample inoculated with *Saccharomyces cerevisiae*

377 = Malt sample inoculated with *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*

The microbial composition of *L. acidophilus* and *S. cerevisiae* did not show any significant differences in their final viable counts despite their wide microbial growth levels. However, high microbial growth was recorded in *L. acidophilus* as compared to *S. cerevisiae*. The high microbial growth of *L. acidophilus* aligned with the findings of Gao *et al.* (2019) [41] but in a probiotic beverage using breadfruit as substrate. However, the low microbial growth in *S. cerevisiae* could be attributed to its slower growth rate during the short fermentation period. Our findings demonstrated that all tested probiotic strains, including the single and mixed cultures, achieved viable cell counts exceeding 10^7 CFU/ml in the final products. This confirms the suitability of sorghum and barley as good substrates for probiotic fermentation. It also showed that the single culture of LAB grew faster compared to the mixed culture of *L. acidophilus* and *S. cerevisiae*, likely due to competition for nutrients in optimal growth conditions. The total coliforms and fecal coliform counts were undetectable for all samples, which confirms proper handling and production practices. These results are within the recommended limit specified by the International Commission of Microbiological Specification for Food (ICMSF).

3.2. Physicochemical Composition

The result of the physicochemical composition of the probiotic malt is shown in Table 1. This shows a significant difference ($p < 0.05$) in color, pH, ash, total solids, and specific gravity. However, there were no significant differences in the acidity, conductivity, soluble solids and reducing sugars among the samples.

Table 2 Physicochemical Composition of Probiotic Malt

SAMPLE	COLOR (g/ml)	pH at 25°C	Acidity (g/g)	conductivity	TSS	TS	Ash content	Protein	Fat	Density	Specific density	Reducing Sugar
177	18900.00 ^a ±0.00	3.50 ^{a+} _{0.007}	0.21 ^{a+} _{0.00}	1871.00 ^a ±0.0	17.85 ^{b+} _{0.78}	0.47 ^{b+} _{0.08}	3.30 ^{c+} _{0.01}	23.58 ^{b+} _{0.65}	0.73 ^{a+} _{0.61}	1.11 ^{c+} _{0.00}	1.08 ^{c+} _{0.01}	5.51 ^{a+} _{1.19}
277	19300.00 ^a ±0.00	3.48 ^{b+} _{0.007}	0.22 ^{a+} _{0.01}	1726.50 ^a ±0.0	18.60 ^{a+} _{0.57}	0.44 ^{b+} _{0.57}	4.58 ^{a+} _{0.11}	25.74 ^{a+} _{0.41}	1.65 ^{a+} _{1.91}	1.14 ^{b+} _{0.01}	1.12 ^{b+} _{0.01}	5.31 ^{a+} _{1.52}
377	20500.00 ^a ±0.00	3.45 ^{c+} _{0.000}	0.23 ^{a+} _{0.00}	1205.00 ^a ±0.0	16.90 ^{a+} _{0.99}	0.69 ^{a+} _{0.01}	4.09 ^{b+} _{0.07}	25.38 ^{a+} _{0.54}	1.33 ^{a+} _{1.03}	1.17 ^{a+} _{0.01}	1.16 ^{a+} _{0.01}	5.19 ^{a+} _{1.29}

a-e: Means with the same superscript within the same column are not significantly different at ($P > 0.05$).

Key

177 = Malt sample inoculated with *Lactobacillus acidophilus*

277 = Malt sample inoculated with *Saccharomyces cerevisiae*

377 = Malt sample inoculated with *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*

TSS = Total Soluble Solid

TS = Total Solid

The difference in moisture content when compared to previous research could be attributed to the degree of exposure to humidity during the storage of sorghum seeds. However, a lower percentage of moisture content in sorghum indicates that the grains can be stored for a longer period of time since higher moisture content affects the shelf life. The sorghum protein content is in line with the proposed range (8-11%) of Palmer (1989) [36] as the acceptable range for effective proteolysis during malting. Meanwhile, our protein content value is lower than that of Ape *et al.* (2016) [37] and Kulamarva *et al.* (2009) [38]. On the other hand, the protein content of germinated and kilned barley is lower compared to Farooqui *et al.* (2018) [39] for only germinated barley (14.87%). This difference could be because the barley was malt kilned before analysis and thus, lost some portions of protein during drying. The sorghum fat content is below the range (13.50-16.00%) obtained by Raihanatu *et al.* (2011) [40] for five local varieties, but higher than the value (3.1%) reported by Kulamarva *et al.* (2009) [38]. Nevertheless, the barley fat content aligned with the value (2.1%) of Farooqui *et al.* (2018) [39] for germinated barley. Thus, drying had no significant effect on the fat content of barley as compared to its protein content.

The physicochemical properties of the probiotic malt beverages were significantly different in color, pH, ash, total solids, and specific gravity except for the acidity, conductivity, soluble solids and reducing sugars. The high color reading observed in the probiotic malt may be attributed to the reaction between the probiotic strain and the malt beverage, given that the same factors (formulas) and ingredients were maintained for all products. According to Gao *et al.* (2019)

[41] and confirmed in our result, the fermentation pH readings of probiotic beverages moved from high to low and usually between 4.6 and 3.49. This observed phenomenal decrease could be due to the lactic acid produced by the LAB. Study indicates that differences in metabolism and production of organic acids by the different strains are key drivers of the difference in acidification of probiotic beverages [41]. Malting and fermentation processes increased the protein content of the probiotic malt beverage compared to the raw seeds, and this correlated with the findings of Elkhilil *et al.* (2001) [42] that protein content and digestibility can be improved by malting. On the other hand, malting and fermentation led to a decrease in the fat content of the probiotic malt compared to the raw seeds which is beneficial for lower cholesterol levels.

3.3. Sensory Evaluation of Probiotic Malt Beverage

The result of the sensory evaluation of the various probiotic malt beverages is shown in Table 2. It revealed that the control (Dubic malt) was the most preferred in terms of taste, appearance, smell and general acceptability. The three probiotic malt beverages showed no significant difference ($p>0.05$) in terms of appearance, smell, and general acceptability. However, there was a significant difference ($p<0.05$) in the taste.

Table 3 Table of Results Showing the Sensory Properties of the Probiotic Malt

Sample	Appearance	Taste	Odor	General Acceptability
177	6.25 ^a ±1.12	6.25 ^c ±1.29	5.65 ^a ±1.59	6.55 ^b ±0.94
277	5.80 ^b ±1.58	7.00 ^b ±1.34	6.35 ^b ±1.39	9.95 ^b ±1.15
377	5.65 ^b ±1.35	6.50 ^{bc} ±0.95	5.95 ^b ±1.19	6.65 ^b ±0.99
477	8.10 ^a ±1.12	8.05 ^a ±0.69	7.90 ^a ±0.91	8.30 ^a ±0.80

a-e: Means with the same superscript within the same column are not significantly different at ($p>0.05$)

Where;

177 = Malt sample inoculated with *Lactobacillus acidophilus*

277 = Malt sample inoculated with *Saccharomyces cerevisiae*

377 = Malt sample inoculated with *Lactobacillus acidophilus* and *Saccharomyces cerevisiae*

477 = Control (Dubic malt)

Based on the sensory evaluation, we mention that the control (Dubic malt) was the most preferred in terms of taste, appearance, smell and general acceptability. The sensory evaluation of the probiotic malt beverages had no significant difference ($p>0.05$) in terms of appearance, smell, and general acceptability. However, there was a significant difference ($p<0.05$) in the taste. Here, we noticed that the control (Dubic malt) was most preferred and followed in this order: probiotic malt beverage with *S. cerevisiae*, mixtures of *L. acidophilus* and *S. cerevisiae* and lastly probiotic malt beverage with *L. acidophilus*. The low preference for *L. acidophilus* fermented beverage may be due to its high acidic content when compared to other products. Similarly, such a condition was reported by Gao *et al.* (2019) [41] as a factor responsible for decrease in acceptability.

4. Conclusion

This study was borne out of the need to produce healthy food (probiotic malt beverage) from cereals using probiotic strains (*Lactobacillus acidophilus* and *Saccharomyces cerevisiae*). The formulated beverage had good cell viability and acceptable sensory characteristics which are favorable sensory properties for consumer acceptability. The market demand for probiotic beverages is driven by the increasing number of health-conscious consumers demanding for food products with added value and improved functionality. It is expected that increased awareness of the health benefits associated with the consumption of probiotic beverages would increase the market of these products globally. The nutritional values of the probiotic malt beverage are higher compared to the raw cereal grains and a normal malt beverage. Furthermore, this study has demonstrated the following: first, production of probiotic malt beverages from sorghum and barley can be achieved using *L. acidophilus* and *S. cerevisiae*. Second, probiotic malt beverages are nutritious and healthy for consumption. Third, probiotic beverages produced using *L. acidophilus* and *S. cerevisiae* were acceptable to consumers. To this end, the production of probiotic malt beverages needs further study to facilitate its commercialization in the food industry. Future research may study probiotic products formulated with other LAB strains in comparison with the strains used in this research to develop a novel probiotic malt beverage that is acceptable to consumers

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no competing interests.

Statement of ethical approval

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Authors' contributions

This work was carried out in collaboration among all authors. All authors investigated the study, did literature searches and did data validation and visualization. All the authors reviewed and approved the final draft, and are responsible for all aspects of the work.

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