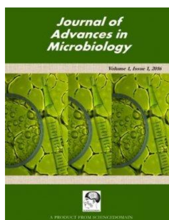

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Characteristic Properties of Derived Wort from Lactic Acid Bacteria (LAB) Challenged Sorghum Samples

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Authors' contributions

This work was carried out in collaboration between both authors. Author AAO designed the study. Author FTA performed the experiments, the statistical analyses, wrote the first draft of the manuscript and managed the literature searches. Authors AAO and FTA managed the analyses of the study, read and approved the final manuscript.

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ABSTRACT

Aim: This study aimed at investigating lactic acid bacteria (LAB) as starter cultures for the improvement of alcoholic beverages.

Methodology: Sorghum was obtained from Bodija market and also from the Institute of Agricultural Research and Training, Ibadan. LAB were isolated from spontaneously-fermenting sorghum. The abilities of the LAB strains to produce antimicrobials and their antagonistic activity against known cereal pathogens were used to select the best three strains for further work. The selected strains were applied singly and in combination at inoculum concentration of 2.3 x 10^4 cells/mL for five days to challenge sorghum seeds prior to malting and wort production. Sorghum wort was fermented for five days with *Saccharomyces carlsbergensis.* Physiological and nutritional characteristics of the unchallenged and challenged sorghum, and fermentative characteristics of the wort were determined.

Results: One hundred and twenty seven strains of LAB were isolated and identified as *L. plantarum* (32), *L. brevis* (31), *L. fermentum* (25), *L. delbrueckii* (8), *L. casei* (12) and *L. acidophilus* (19). The

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pH reduced from 6.50±0.03 to 2.93±0.03. *L. plantarum* (WS) and *L. casei* (WS) also had the same total residual sugar content with value of 0.97±0.03% at day 5. *L. plantarum* (WS) and *L. casei* (WS) produced the highest Total titratable acidity (TTA) with values of 4.77±0.03% while control (RS) had the least TTA with value of 3.97±0.09%. Control (WS) had the highest protein content of 1.17±0.03%, *L. casei* (RS) had the least protein content with value of 0.93±0.03%. *L. plantarum* (WS) had the highest FAN content of 29.97±0.19mg/L while the least Free amino nitrogen (FAN) was produced by control (RS) with value of 19.37±0.07mg/L. Fermentation of the unchallenged wort with pH of 6.2 yielded ethanol content of 2.2 %. The subsequent fermentation produced 4.8 % ethanol.

Conclusion: This study demonstrated the use of biological control methods, involving the use of LAB as starter cultures. It improved the quality of the end products in brewing. The use of LAB as starter cultures is an alternative treatment to chemical treatment which can be used to control microbial contamination during sorghum malting.

Keywords: Wort; lactic acid bacteria; sorghum; starter cultures; pathogens.

1. INTRODUCTION

The lactic acid bacteria (LAB) are a group of Gram-positive bacteria, non-motile, non-spore forming, cocci or rods, which produce lactic acid as the major end product of fermentation of carbohydrates. They are the most important bacteria in food fermentations, being responsible for the fermentation of sour bread, sorghum beer, all fermented milks, cassava (to produce *gaari* and *fufu*) and most “pickled” (fermented vegetables) [1]. *Lactobacillus* is the main genus of lactic acid bacteria (LAB) and plays an important role in balancing microflora in the gut ecosystem of animals [2].The genera include *Carnobacterium, Enterococcus, Lactobacillus, Oenococcus, Pediococcus, Streptococcus* and *Tetragenococcus*, [3].

Microbial proliferation has been documented as an indigenous component of the malting and brewing environment [4] with the resultant microflora having both beneficial and detrimental effects on malt quality [4]. LAB omnipresent on the surface of malt barley may positively influence the quality and safety of the malt and derived products; this property has been exploited for the biological improvement of the malting and beer process.

Biological control methods, involving inoculation with LAB and yeast (*Geotrichum candidum*) starter cultures [5]; have shown promise for the control of unwanted bacteria and fungi during malting. Bacterial cultures have also been added to the grain during malting to inhibit the growth of fungi [6]. The various factors contributing to the antimicrobial activity of LAB are low pH due to the production of organic acids (lactic acid and acetic acid), carbon dioxide, hydrogen peroxide, ethanol, diacetyl, bacteriocins, depletion of nutrients and microbial competition [7]. The use of “starter”culture in malting is a relatively new process that controls indigenous microbial growth and is technically and economically feasible [7].

Sorghum is a major food crop and is ranked fifth in terms of world cereal grain production after wheat, rice, maize and barley [8]. Much sorghum is malted to brew opaque beer in most parts of Africa, including South Africa and European type beer (e.g. larger) and non- alcoholic malt beverages for example ‘pito’ ‘togwa’ ‘obiolor’ and ‘kunu-zaki’ in several African countries [9]. Sorghum malting yields high proportions of hydrolytic enzymes such as α-glucosidase, and α- and β-amylases [10]. Malting has been defined as a traditional processing technology that could possibly be used to improve the nutritional quality of the protein [11].

The conditions of transportation and storage of sorghum make it be susceptible to microbial attack and this affects the quality of the end products of malting and brewing. The germination conditions, especially not turning the grain, encourages entangling of the roots and shoots growing from the grains. This then leads to the formation of matted clumps, which encourages the growth of bacteria and fungi [12]. Heavy microbial populations may negatively impact on the quality of the malt by causing discolouration of the malt and development of unacceptable off flavours [13]. The sorghum malt with coliforms and moulds is of more concern because they are associated with diarrhoeal diseases [14] and moulds have the potential of producing mycotoxins, which are toxic to animals and humans [15].

In order to avoid potential hazards associated with the high microbial load and the possible presence of mycotoxins in sorghum malts, a method that can prevent or inhibit the growth of potentially harmful microorganisms during the sorghum malting process should be implemented. One of such methods is the use of biological methods including lactic acid bacteria as biocontrol agents to control pathogens of the malting process.

Selection of LAB as starters will help in reducing the problems encountered in brewing because it will improve malt processing, quality and aids utilisation of products made from sorghum. LAB as starters alleviate the variations that occur in natural fermentation and thereby also enhance the shelf -life and quality of the end products. To enhance and maintain the quality and shelf- life of alcoholic beverages, there is the need to undertake basic studies on product processing aimed at selecting the best starters for the improvement of the alcoholic beverages. This work therefore aimed at using lactic acid bacteria (LAB) as starter cultures for the improvement of alcoholic beverages.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Different varieties of maize (*Zea mays*), sorghum (*Sorghum bicolor* and *Sorghum vulgaris*) and millet (*Eleusine coracana*) were purchased locally from Bodija market in Oyo State, Nigeria. The defective seeds were removed and the viable or healthy seeds were stored in sterile polythene bags.

2.2 Preparation of 'Ogi' from the Cereal Samples

This was carried out using the method of Odunfa and Adeyele [16].

2.3 Isolation and Identification of Lactic Acid Bacteria (LAB)

The medium for the isolation of Lactic acid bacteria was de Mann Rogosa and Sharpe agar (MRS Agar, Oxoid) [17]. The medium was prepared according to the manufacturer's direction and sterilised by autoclaving at 121ºC for 15 minutes.

LAB strains were isolated from the different fermented cereal gruels using the serial dilution method [18]. Purification of the isolates was done by subculturing into fresh medium (MRS) to obtain pure cultures [19]. Identification of the isolates was carried out based on their microscopic, macroscopic, cultural, physiological and biochemical characteristics with reference to Bergey's Manual of Systematic Bacteriology [20] and an Approach to the classification of Lactobacilli [21].

2.4 Brewery Flocs

Brewery floc was obtained from Nigerian Breweries factory in Ibadan and the yeast was isolated and identified using conventional methods. The yeast was then used for the fermentation of the wort.

2.5 Screening of LAB for the Production of Lactic Acid, Hydrogen Peroxide and Diacetyl Production

For these measurements, the test organisms were grown anaerobically in MRS broth for 48hrs at 37°C and centrifuged using 80-2 Bench centrifuge at 3000xg for 15mins. The supernatant fluid was used for all the determinations [22].

2.6 Application of Inoculum of LAB Isolates to the Sorghum Samples

The LAB used as starter cultures were: *L. plantarum, L. fermentum* and *L. casei*. The organisms were used singly and also in combination. An inoculum size of 2.3 X 10^{4} cells/mL was used to treat the steeped sorghum grains [23].

2.7 Malting of Sorghum and Production of Wort from the Malted Sorgum

The red sorghum and white sorghum varieties (800g) were germinated on trays after steeping with the LAB isolates. After 5days, the germination process was terminated. The malted sorghum were then dried using the oven at 50°C for 24hrs. The wort was produced using the single infusion method [24]. 600g of sorghum malt was used to produce the wort and 200g of maize grits was used as an adjunct and it was dissolved in 3000mls of water.

2.8 Analyses of the Produced Wort

2.8.1 pH of the wort

The pH value of the wort was determined at intervals. It was determined at the 3 stages of the

fermentation process. The pH was determined using a previously calibrated pH meter (Hanna Instrument HI 8521) that was inserted into the wort and gently stirred until a stable pH reading was displayed.

2.8.2 Total Residual Sugar of the wort

Distilled water (9mls) was added to the supernatant and vortexed. A standard curve was made from 0.01g of 1ml of glucose [25].

2.8.3 Total Titratable Acidity (TTA) of the wort

Twenty (20) mls of the wort was pipetted into a clean flask and titrated against 0.1M NaOH using two drops of phenolphthalein indicator. The titer volume was multiplied by 0.09 to give percentage total titratable acidity [26]. This was done at intervals during the fermentation process.

2.8.4 Total Solids (TS) of the wort

The total solid was determined by using a hand refractometer. The results was expressed as (%).

2.8.5 Protein Content of the wort

Kjedahl method of nitrogen/protein determination was used [27]. Two milliliters aliquot of each solution was then read at 540nm.

2.8.6 Free Amino Nitrogen (FAN) content of the wort

This was determined using Ninhydrin assay method of the Institute of brewing. The absorbance of the sample was measured at 570nm. A blank (distilled water) and a glycine standard solution (10.72mg/L) were also analysed following the same procedure. A colour correction was included by taking into account the absorbance caused by coloured compounds [28].

3. RESULTS

The organisms that were used as starter culture were selected based on their ability to produce antimicrobials and high antagonistic activity against pathogenic organisms. The organisms selected as starter cultures were *L. plantarum, L. casei* and *L. fermentum.* They were used singly and also in combination.

The pH of all the worts produced during fermentation were all in the acidic range (Table 1) The pH reduced considerably from 6.50±0.03 to 2.93±0.03. At day 0, control (RS) had the highest pH value of 6.50±0.03 while LP (WS) had the lowest pH value of 5.16±0.03. At day 5, control (WS) had the highest pH value of 3.80±0.03 while *L. plantarum* (WS) had the least pH with value of 2.93+0.03. The pH of the control worts i.e control (WS) and control (RS) had the highest pH value after the fermentation process with values of 3.80±0.03 and 3.75±0.03 respectively when compared with the wort challenged with Lactic acid bacterial isolates.

The total residual sugar content of the wort samples reduced significantly from day 0 day 5 of the fermentation period (Table 2). On day 0, *L. casei* (WS) had the highest total residual sugar content of 3.63±0.03% while *L. casei* (RS) and control (RS) had the least values of 3.27±0.03% and 3.27±0.07%. Also on day 0, *L. fermentum* (WS) and *L. plantarum*+ *L. fermentum* (WS) had the same total residual sugar content with value of 3.60±0.06%, *L. fermentum* (RS) and *L. plantarum* + *L. fermentum* (RS) had the same total residual sugar content with value of 3.37±0.03%. On day 5, the control worts i.e control (WS) and control (RS) residual had higher total sugar content of 1.23±0.03% while *L. plantarum*+ *L. fermentum* (WS) had the least with value of 0.93±0.03%. *L. plantarum* (WS) and *L. casei* (WS) also had the same total residual sugar content with value of 0.97±0.03% at day 5. There were significant (P<0.05) differences in the control worts and the wort derived from the malted sorghum challenged with the Lactic acid bacterial isolates.

The total titratable acidity (TTA) of the wort increased from day 0 to day 5 during the fermentation process. At day 0, control (RS) had the least total titratable acidity of 0.63±0.03% while LF (WS) had the highest value of total titratable acidity of 0.83±0.03%. There was no significant difference (p<0.05) between *L. plantarum* (RS), *L. casei*(WS) and *L. fermentum* (RS), they had the same TTA with value of 0.73±0.03%. At day 5, *L. plantarum* (WS) and *L. casei* (WS) had the highest TTA with values of 4.77±0.03% while control (RS) had the least TTA with value of 3.97±0.09%. Overall, the control worts had lowest TTA values when compared with the wort derived from malted sorghum subjected to challenge by LAB isolates as shown in Table 3.

There was a reduction in the total solids of the wort produced from the malted sorghum challenged with the LAB isolates during

fermentation. On day 0, LC (WS) had the highest total solids with the value of 13.87±0.03% while *L. plantarum* (RS) had the least total solids of 13.03±0.03. Also on day 0, *L. casei* (RS) and *L. fermentum* (RS) had the same total solids with value of 13.23±0.03%. On day 5, control (WS) had the highest total solid with value of 13.10±0.06% while *L. plantarum*+ *L. fermentum* (RS) gave the least total solid with value of 12.10±0.00% (Table 4).

The protein content of the wort reduced during the fermentation period as shown in Table 5. On day 0, *L. casei* (WS) had the highest protein content with value of 1.77±0.03% while *L. plantarum*+ *L. fermentum* (RS) had the least value of 1.53±0.03% and the control worts i.e control (WS) had protein contents of 1.63±0.03, control (RS) also had the same value. Also at day 0, *L. plantarum*(RS) and *L. casei* (RS) had protein content of 1.63±0.03% which was the same as that of the control worts. At day 5, control (WS) had the highest protein content of 1.17±0.03% while *L. casei* (RS) had the least protein content with value of 0.93±0.03%. *L. plantarum* (RS), *L. fermentum* (WS), *L. fermentum* (RS) and *L. plantarum*+ *L. fermentum* (RS) all had the same protein content with value of 0.97±0.03%. Also, *L. plantarum* (WS) and *L. plantarum*+ *L. fermentum* (RS) had the same protein content with value of 1.03±0.03% at day 5.

Free amino nitrogen content (FAN) of the sorghum wort increased throughout the fermentation period as shown in Table 6. On day 0, *L. plantarum*(WS) had the highest FAN content of 3.83±0.03mg/L while *L. fermentum* (RS) produced the least FAN content of 3.40±0.00mg/L. *L. plantarum* (RS), Control WS, and Control (RS) all had the similar values of 3.47±0.03, 3.47±0.07 and 3.47±0.03mg/L respectively. Also at day 0, *Lactobacillus casei* (WS) and *L. fermentum* (WS) had the same FAN content of 3.77±0.03mg/L and 3.77±0.07mg/L. *L. casei*(RS) and *L. plantarum*+ *L. fermentum* (RS) also had the same value of 3.43±0.03mg/L. At day 5*, L. plantarum* (WS) had the highest FAN content of 29.97±0.19mg/L while the least FAN was produced by control (RS) with value of 19.37±0.07mg/L. Overall, the treated worts had higher FAN content when compared with the control worts.

4. DISCUSSION

The organisms selected as starter cultures were; *L. plantarum, L. casei* and *L. fermentum.* They were used singly and also in combination. The LAB isolates were screened for the rate of production of antimicrobial compound since LAB are reported to be important in the biopreservation of food and feed [29]. In this study, the isolated LAB, *L. plantarum, L. casei, L. fermentum* were observed to produce more of lactic acid, hydrogen peroxide and diacetyl an observation in line with the findings of Borch and Molin [30] which reported an increase in the production of lactic acid with time due to low pH. Low pH (4.0-6.0) permits the growth of lactic acid bacteria. The organisms were also able to inhibit pathogenic organisms selected as indicator organisms when compared with other LAB isolates. The rate of production of these antimicrobials and inhibitory activity formed an important factor for the selection of the LAB strains for further screening by subjecting them to various physiological tests in the search for suitable starter cultures for malting of sorghum. Furthermore, the results observed in this study were in accordance with the work of Ogunbanwo et al. [31] who reported that production of the primary metabolite, lactic acid and the resulting pH decrease is the main preserving factor in food fermentation.

The pH of all the worts including the control reduced considerably during fermentation. The pH started dropping after 24 hrs. A rapid drop in pH can be correlated to the yeast viability and yeast growth. Wort amino acid stimulates yeast growth, which in turn promotes pH decline. A low pH is desirable for the the final flavour of the beer [32].

It was also observed that the total residual sugar of the wort reduced during fermentation. The decrease of the total residual sugar with prolonged fermentation was attributed to utilisation by the fermenting yeast i.e. *Saccharomyces carlsbergensis*. The sugars are being utilised to produce ethanol, carbon dioxide and other metabolites according to the work of Mensah [33]. Residual sugar are prime component of fermentation, after consumption of which alcohol is produced. The total residual sugar decreased with the increase in fermentation days. Michodjehoun-Mestres et al. [34] also reported a decrease in the reducing sugar of ‘gowe’. A reduction in total sugars was conversely correlated with the total titratable acidity of wort. The amount of total residual sugar in beer influences the organoleptic properties of beer and indicates the completion of fermentation.

Table 1. pH at different time intervals of wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates

LAB isolates (Sorghum Variety)	pH value / Treatment period (days)					
	0	1	2	3	4	5
L. plantarum (WS)	5.2±0.03^{d}	4.2±0.03^{de}	3.7±0.03^{de}	3.3±0.03^{c}	3.0±0.03^{de}	2.3±0.03^{d}
L. plantarum (RS)	5.4±0.03^{bc}	4.3±0.00^{cd}	3.9±0.03^{ab}	3.5±0.03^{ab}	3.2±0.03^{bcd}	3.0±0.03^{bcd}
L. casei(WS)	5.37±0.03^{cd}	4.3±0.06^{cd}	3.7±0.03^{ef}	3.2±0.03^{c}	3.1±0.03^{cde}	3.0±0.00^{cd}
L. casei(RS)	5.3±0.03^{bc}	4.4±0.03^{bc}	3.8±00^{cd}	3.4±0.03^{b}	3.1±0.06^{bcde}	3.0±0.03^{bcd}
L. fermentum(WS)	5.2±0.03^{cd}	4.2±0.03^{de}	3.6±0.03^{efg}	3.3±0.03^{c}	3.1±0.03^{cde}	3.0±0.03^{bcd}
L. fermentum(RS)	5.3±0.03^{bc}	4.3±0.03^{cde}	3.9±0.07^{bc}	3.5±0.07^{ab}	3.2±0.06^{abc}	3.1±0.07^{abc}
L. plantarum + *L. fermentum*(WS)	5.5±0.03^{a}	4.2±0.00^{de}	3.4±0.03^{hi}	3.2±0.00^{c}	2.9±0.03^{e}	2.9±0.07^{d}
L. plantarum + *L. fermentum* (RS)	5.5±0.07^{a}	4.2±0.03^{e}	3.6±0.00^{fg}	3.2±0.03^{c}	3.1±0.07^{cde}	3.0±0.03^{bcd}
Control (WS)	6.1±0.07^{de}	6.0±0.03^{de}	5.3±0.03^{cd}	4.6±0.03^{bc}	3.9±0.03^{ab}	3.8±0.03^{cd}
Control (RS)	6.5±0.03^{de}	5.8±0.03^{de}	5.1±0.03^{cd}	4.2±0.03^{e}	3.9±0.03^{bc}	3.8±0.03^{de}

**Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.*
KEY: WS-White sorghum variety; RS-Red sorghum variety

Table 2. Total residual sugar content at different time intervals of wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates

LAB isolates (Sorghum Variety)	Residual sugar content (%) / Treatment period (days)					
	0	1	2	3	4	5
L. plantarum (WS)	3.43±0.07^{bcd}	3.07±0.03^{b}	1.93±0.03^{bc}	1.30±0.06^{c}	1.13±0.03^{def}	0.97±0.03^{c}
L. plantarum (RS)	3.30±0.06^{d}	3.00±0.00^{bc}	2.06±0.03^{b}	1.53±0.03^{b}	1.20±0.06^{cde}	1.03±0.03^{bc}
L. casei(WS)	3.63±0.03^{a}	2.97±0.03^{bc}	1.83±0.03^{c}	1.27±0.03^{cd}	1.10±0.06^{efg}	0.97±0.03^{c}
L. casei(RS)	3.27±0.03^{d}	2.93±0.07^{bc}	2.00±0.00^{b}	1.47±0.03^{b}	1.23±0.03^{cd}	1.07±0.03^{bc}
L. fermentum(WS)	3.60±0.06^{ab}	2.97±0.03^{bc}	2.03±0.07^{b}	1.57±0.09^{b}	1.30±0.06^{c}	1.13±0.07^{ab}
L. fermentum(RS)	3.37±0.03^{cd}	3.03±0.03^{bc}	2.30±0.06^{a}	1.73±0.03^{a}	1.43±0.03^{b}	1.20±0.06^{a}
L. plantarum + *L. fermentum* (WS)	3.60±0.06^{ab}	2.93±0.07^{bc}	1.63±0.03^{d}	1.13±0.03^{d}	1.00±0.00^{g}	0.93±0.03^{c}
L. plantarum + *L. fermentum* (RS)	3.37±0.03^{cd}	2.97±0.03^{bc}	1.53±0.03^{d}	1.17±0.03^{cd}	1.03±0.03^{fg}	1.00±0.00^{c}
Control (WS)	3.53±0.03^{abc}	3.33±0.03^{a}	2.33±0.03^{a}	1.87±0.07^{a}	1.57±0.03^{a}	1.23±0.03^{a}
Control (RS)	3.27±0.07^{d}	3.37±0.03^{a}	2.23±0.03^{a}	1.77±0.03^{a}	1.43±0.03^{b}	1.23±0.03^{a}

**Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.*
KEY: WS-White sorghum variety; RS-Red sorghum variety

Table 3. Total Titratable Acidity (TTA) at different time intervals of wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates

LAB isolates (Sorghum Variety)	Total titratable acidity (%) / Treatment period (days)					
	0	1	2	3	4	5
L. plantarum (WS)	0.77±0.03^{ab}	1.97±0.03^{def}	2.93±0.03^{de}	4.17±0.03^{ab}	4.57±0.03^{ab}	4.77±0.03^{a}
L. plantarum (RS)	0.73±0.03^{abc}	1.80±0.06^{h}	2.77±0.03^{f}	3.87±0.03^{d}	4.43±0.07^{bc}	4.70±0.06^{ab}
L. casei(WS)	0.73±0.03^{abc}	2.03±0.03^{cde}	2.93±0.07^{de}	4.10±0.06^{ab}	4.57±0.03^{ab}	4.77±0.03^{a}
L. casei(RS)	0.66±0.03^{bc}	1.87±0.03^{fgh}	2.70±0.00^{f}	3.87±0.03^{d}	4.27±0.03^{d}	4.57±0.03^{bcd}
L. fermentum(WS)	0.83±0.03^{a}	1.93±0.03^{efg}	2.97±0.07^{d}	4.17±0.07^{ab}	4.50±0.00^{b}	4.67±0.03^{abc}
L. fermentum(RS)	0.73±0.03^{abc}	1.83±0.07^{gh}	2.80±0.06^{ef}	3.87±0.03^{d}	4.23±0.03^{d}	4.50±0.06^{cd}
L. plantarum + L. fermentum(WS)	0.77±0.03^{ab}	2.23±0.03^{a}	3.17±0.09^{abc}	4.06±0.12^{bc}	4.47±0.03^{bc}	4.57±0.03^{bcd}
L. plantarum + L. fermentum (RS)	0.67±0.03^{bc}	2.17±0.03^{ab}	3.03±0.03^{cd}	3.80±0.00^{d}	4.23±0.03^{d}	4.47±0.03^{d}
Control (WS)	0.70±0.06^{bc}	1.47±0.03^{i}	2.33±0.03^{g}	3.43±0.03^{e}	3.67±0.09^{e}	4.03±0.07^{e}
Control (RS)	0.63±0.03^{c}	1.33±0.03^{j}	2.27±0.03^{g}	3.50±0.06^{e}	3.67±0.09^{e}	3.97±0.09^{e}

**Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.*
KEY: WS-White sorghum variety; RS-Red sorghum variety

Table 4. Total solids at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates

LAB isolates (Sorghum Variety)	Total solids (%) / Treatment period (days)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	13.77±0.03^{a}	13.20±0.06^{cd}	13.03±0.03^{ef}	12.90±0.00^{d}	12.83±0.03^{de}	12.67±0.03^{cd}
Lactobacillus plantarum (RS)	13.03±0.03^{d}	12.67±0.03^{e}	12.53±0.03^{g}	12.40±0.06^{ef}	12.30±0.06^{f}	12.13±0.03^{e}
Lactobacillus casei(WS)	13.87±0.03^{a}	13.10±0.00^{d}	12.90±0.06^{f}	12.80±0.06^{d}	12.70±0.06^{e}	12.63±0.03^{d}
Lactobacillus casei(RS)	13.23±0.03^{c}	12.80±0.06^{e}	12.47±0.03^{gh}	12.40±0.06^{ef}	12.30±0.06^{f}	12.23±0.03^{e}
Lactobacillus fermentum(WS)	13.80±0.06^{a}	13.47±0.03^{b}	13.20±0.00^{cd}	13.07±0.03^{c}	12.93±0.03^{cd}	12.77±0.03^{bcd}
Lactobacillus fermentum(RS)	13.23±0.03^{c}	12.73±0.07^{e}	12.53±0.03^{g}	12.47±0.03^{e}	12.30±0.06^{f}	12.20±0.06^{e}
Lactobacillus plantarum + Lactobacillus fermentum (WS)	13.60±0.06^{b}	13.23±0.03^{cd}	13.07±0.03^{de}	13.03±0.03^{c}	12.93±0.03^{cd}	12.80±0.06^{bc}
Lactobacillus plantarum + Lactobacillus fermentum (RS)	13.17±0.03^{cd}	12.47±0.03^{f}	12.30±0.06^{i}	12.20±0.06^{g}	12.13±0.03^{g}	12.10±0.00^{e}
Control (WS)	13.53±0.03^{b}	13.70±0.06^{a}	13.57±0.03^{a}	13.40±0.00^{a}	13.23±0.03^{a}	13.10±0.06^{a}
Control (RS)	13.57±0.09^{b}	13.27±0.09^{c}	13.13±0.07^{de}	13.07±0.00^{c}	13.00±0.00^{bc}	12.83±0.03^{b}

**Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.*
KEY: WS-White sorghum variety; RS-Red sorghum variety

Table 5. Protein content at different time intervals of the wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates

LAB isolate (Sorghum Variety)	Protein content (%) / Treatment period (days)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	1.77±0.03[a]	1.47±0.03[bc]	1.23±0.03[abc]	1.13±0.03[bcd]	1.07±0.03[bcd]	1.03±0.03[bcd]
Lactobacillus plantarum (RS)	1.63±0.03[bcd]	1.27±0.03[d]	1.10±0.06[cde]	1.00±0.00[ef]	0.97±0.03[ef]	0.97±0.03[cd]
Lactobacillus casei(WS)	1.73±0.03[ab]	1.40±0.06[c]	1.20±0.06[bcd]	1.13±0.03[bcd]	1.07±0.03[bcd]	0.96±0.03[cd]
Lactobacillus casei (RS)	1.63±0.03[bcd]	1.17±0.03[d]	1.03±0.03[e]	0.97±0.03[f]	0.93±0.03[f]	0.93±0.03[d]
Lactobacillus fermentum(WS)	1.70±0.00[ab]	1.43±0.09[bc]	1.27±0.03[ab]	1.10±0.06[cde]	1.03±0.03[cde]	0.97±0.03[cd]
Lactobacillus fermentum (RS)	1.56±0.03[cde]	1.17±0.03[d]	1.06±0.03[de]	1.03±0.03[def]	0.97±0.03[ef]	0.97±0.03[cd]
Lactobacillus plantarum + *Lactobacillus fermentum* (WS)	1.73±0.03[ab]	1.50±0.06[bc]	1.27±0.03[ab]	1.17±0.03[abc]	1.07±0.03[bcd]	1.03±0.03[bcd]
Lactobacillus plantarum + *Lactobacillus fermentum* (RS)	1.53±0.03[def]	1.20±0.00[d]	1.10±0.00[cde]	1.03±0.03[def]	0.97±0.03[ef]	0.97±0.03[cd]
Control (WS)	1.63±0.03[bcd]	1.70±0.06[a]	1.37±0.00[a]	1.26±0.03[a]	1.20±0.00[a]	1.17±0.03[a]
Control (RS)	1.63±0.03[bcd]	1.47±0.03[bc]	1.23±0.03[abc]	1.16±0.03[abc]	1.10±0.00[bc]	1.13±0.03[ab]

**Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.*
KEY: WS-White sorghum variety; RS-Red sorghum variety

Table 6. Free Amino Nitrogen (FAN) at different time intervals of sorghum wort derived from malted sorghum subjected to challenge by lactic acid bacteria isolates

LAB isolates (Sorghum Variety)	FAN (mg/L) / Treatment period (days)					
	0	1	2	3	4	5
Lactobacillus plantarum (WS)	3.83±0.03[a]	11.60±0.06[cde]	17.33±0.03[d]	21.73±0.18[d]	27.40±0.06[c]	29.97±0.19[ab]
Lactobacillus plantarum (RS)	3.47±0.03[c]	11.43±0.03[efg]	16.60±0.06[f]	19.90±0.06[g]	25.30±0.11[gh]	27.53±0.12[e]
Lactobacillus casei(WS)	3.77±0.03[a]	11.70±0.00[bcd]	17.43±0.09[d]	21.73±0.03[d]	27.37±0.07[c]	29.73±0.09[b]
Lactobacillus casei(RS)	3.43±0.03[c]	11.23±0.03[h]	16.30±0.06[g]	19.73±0.03[g]	25.50±0.06[f]	27.37±0.03[ef]
Lactobacillus fermentum(WS)	3.77±0.07[a]	11.73±0.03[bc]	17.63±0.03[c]	21.27±0.09[e]	21.17±0.07[d]	29.30±0.06[c]
Lactobacillus fermentum(RS)	3.40±0.00[c]	11.33±0.07[gh]	16.33±0.09[g]	19.83±0.03[g]	25.27±0.03[h]	27.20±0.06[f]
Lactobacillus plantarum+ *Lactobacillus fermentum* (WS)	3.80±0.06[a]	11.93±0.03[a]	17.93±0.03[b]	22.87±0.03[b]	27.50±0.06[c]	29.90±0.06[ab]
Lactobacillus plantarum+ *Lactobacillus fermentum* (RS)	3.43±0.03[c]	11.53±0.03[def]	16.77±0.03[e]	21.33±0.03[e]	25.60±0.00[f]	27.56±0.03[e]
Control (WS)	3.47±0.07[c]	7.433±0.12[i]	9.03±0.03[h]	13.33±0.09[h]	17.47±0.03[i]	19.87±0.07[g]
Control (RS)	3.47±0.03[c]	6.63±0.12[j]	8.20±0.06[i]	11.90±0.06[i]	17.03±0.03[j]	19.37±0.07[h]

**Within rows, values with the same superscript are not significantly different from one another by Duncan's Multiple Range Test.*
KEY: WS-White sorghum variety; RS-Red sorghum variety

Total titratable acidity (TTA) of the wort derived from malted sorghum subjected to challenge by LAB increased from day 0 to day 5 during the fermentation of the wort. The increase in TTA is one of the common features in the fermentation of fermented food and beverages [35]. During fermentation, the metabolic activities of yeasts lead to production of carbondioxide and ethanol from the breakdown of hexoses and pentoses [36] and this explains the increase in titratable acidity of the wort. Similar observations have also been made by Hounhouigan et al. [37] and Mugula et al. [38] who observed an increase in titratable acidity of 'mawe' and 'togwa' respectively while using LAB as starter culture in the preparation of these products. The accelerated acidification observed constituted an asset that can contribute to the improvement of the final product quality as observed in other studies [39,40]. The growth of pathogens can also be avoided with the rapid acidification obtained [34].

The total solids reduced during fermentation. Such decrease in the total solids was attributed to the alcoholic fermentation carried out by yeast [35]. During fermentation, the metabolic activities of yeasts lead to the production of ethanol and carbondioxide from the breakdown of hexoses and pentoses. This explains the decrease in the total solids during fermentation.

The protein content of the wort derived from malted sorghum subjected to challenge by LAB isolates reduced significantly during fermentation compared with the control. Although this was contrary to the report of Elyaas et al. [41] who reported that an increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles. The reduction in protein could also result from nutrient depletion by microorganisms during fermentation.

The Free Amino Nitrogen (FAN) of the wort derived from malted sorghum challenged with LAB increased considerably when compared with the control worts. The free amino nitrogen gives estimate of the amount of amino acids, ammonia, and in addition, the terminal α- amino nitrogen groups of peptides and proteins in the wort. The work by Taylor et al. [42] provided evidence to support the Ninhydrin assay as a good indicator of yeast fermentation performance. Evaluation of FAN content in wort indicates how well yeast can grow and reproduce. Owuama [10] reported that a high level of FAN in wort is necessary to support rapid and proper fermentation. FAN is important because it is an essential component of yeast nutrition in brewing as it promotes proper yeast growth and fermentation efficiency [43]. It also plays a role in the maintenance of foam stability of beer.

5. CONCLUSION

This study demonstrated the use of biological control methods, involving the use of LAB as starter cultures. It improved the quality of the end products in brewing. The use of LAB as starter cultures is an alternative treatment to chemical treatment which can be used to control microbial contamination during sorghum malting

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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