Effects of Lactic Cultures on Fermented Drink Produced from Sorghum (*Sorghum bicolor*).

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Abstract: A total of thirty four lactic acid bacteria (LAB) isolates were isolated from fermented cereal gruels and processed yoghurt and they were identified as *Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus fermentum, Lactobacillus bulgaricus*. Three of the LAB that produced the highest antimicrobials i.e lactic acid, diacetyl and hydrogen peroxide when the lactic acid bacteria were screened for the production of antimicrobials were selected as starter organisms to treat the germinating sorghum that was used for the drink production. Production of drink was done after the sorghum samples were malted. Proximate analysis which include specific gravity, total protein, viscousity, total sugar, pH, sugar level and colour were also carried out on the sorghum drink. The sample treated with *L. plantarum* and *L. acidophilus* had the highest specific gravity of 1.03 and the least was *L. brevis* 1.02; *L.brevis* and *L. plantarum* used for treating sorghum the drink sample produced the highest protein value of 0.62% and *L. brevis* produced drink with highest viscousity with value of 13.25. The highest sugar was produced by *L. acidophilus* and *L. plantarum* treated sample with values of 3.98mg/ml. The pH of the drink were ranged between 3.85 to 4.08. The sugar level was highest with value of 0.25mg/ml in the sample treated with *L. brevis. L. acidophilus* had the highest effect on the colour of the drink with value of 5.92 with *L.brevis* being the least with value of 4.82. Analysis of the sensory evaluation results revealed consumer acceptance of the lactic acid bacteria (LAB) treated sorghum drink than the sample not treated with Lactic acid bacteria.

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1. Introduction

Lactic acid bacteria (LAB) form a phylogenetically diverse group and are defined as Gram – positive, non- sporing rods and cocci, non-motile, catalase-negative, fastidious, acid tolerant and strictly fermentative bacteria that secrete lactic acid as the major end product of sugar fermentation (Salminen and Wright, 1998). The genera *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* are recognized as LAB (Ercolini *et al*., 2001). Historically, lactic – acid producing bacterial starter cultures are used in the production of fermented dairy, meat and plant products, fermented cereals and legumes and the fermentation results in products with improved shelf life, flavour, aroma and texture (Steinkraus *et al*., 1983).

Sorghum is the fifth most important cereal crop after wheat, rice, maize and barley in term of production (FAO, 2005). Total world annual sorghum production is about 60 million tons from cultivated area of 46 millions hectare. African sorghum beer is brewed using grain sorghum and undergoes lactic acid fermentation as well as alcoholic fermentation. The steps in brewing African sorghum beer are; malting, mashing, souring and alcoholic fermentation.

Biological preservation aims to improve the microbiological safety of foods and beverages through the use of competitive or antagonistic microorganisms or their metabolic products, to prevent or inhibit the growth of undesired microorganisms in foods or beverages (Schillinger *et al*., 1996). Certain lactic acid bacteria (LAB) are widely exploited for biopreservation. The biopreservative actions of LAB are due to their production of antimicrobial compounds which inhibit the growth of other bacteria or fungi. LAB play a positive role in the wort production process by eliminating undesirable microorganisms and contributing to wort bioacidification. The common occurrence of LAB in foods and feeds coupled with their long- lived use contribute to their natural acceptance as GRAS (Generally Regarded As Safe) product for human consumption (Aguirre and Collins, 1993).

This present work aimed at studying the effects of using lactic acid bacteria (LAB) as starter cultures for the malting of sorghum and the production of fermented drink from sorghum samples and to study the effects of the LAB on the nutritional composition of the wort treated samples.

2. Materials and Methods

Collection of Samples

Samples of maize (*Zea mays*), Sorghum (*Sorghum bicolor*) and Millet (*Eleusine coracana*) were purchased locally at the Bodija Market in Ibadan Metropolis, South Western Nigeria. Yoghurt samples were also collected from different retail outlets in Ibadan. The samples were collected in clean sterile polythene bags and transported to the laboratory. The grains were manually cleaned to remove stones, sticks, damaged and broken kernels and other foreign materials.

Traditional preparation of Ogi

The cereal grains (*Zea mays, Sorghum bicolor and Eleusine coracana*) were cleaned and steeped in water separately for 2 days in sterile containers. The water was decanted and the grains wet-milled before sieving with muslin cloth. The pomace was discarded and the starch suspension was allowed to sediment after which fermentation was carried out for 2-3 days by the natural flora of the grains (Odunfa and Adeyele, 1985).

Isolation of Bacterial Strains

Lactic acid bacterial strains were isolated from Ogi and Yoghurt. 1g of the samples were added to 9ml of sterile diluent containing 0.1% peptone water and was then homogenized. Isolation of bacteria was carried out on MRS agar using pour plate method (Harrigan and Mc Cance, 1976) and incubated anaerobically at 30^{o}C for 48hrs. After incubation, the culture was observed for growth. Single and isolated colonies were picked and subcultured on MRS agar to obtain pure culture of the isolates. Simultaneously, the smears were prepared and stained with Gram's stain and examined under microscope for the staining characteristics and morphology of the isolates.

Culture preservation

The isolated Lactobacilli were subcultured onto maintenance medium consisting of MRS broth with 12% (w/v) glycerol end incubated at 30°C for 48hours until growth becomes visible. The stock cultures were stored at – 4^{o}C until required for use.

Production of Antimicrobial agents by the test isolates

For these measurements, 0.1ml of a suspension of the test organisms were grown anaerobically on MRS broth for 48hours at 37^{o}C. After incubation, known volume of the supernatant fluid was used for all the determinations. Quantitative estimation of lactic acid, hydrogen peroxide and diacetyl was done according to the method of A.O.A.C. (1980). The LAB isolates that produced the highest antimicrobials were selected as starter cultures for the purpose of this work.

Malting of Sorghum

The cleaned sorghum grains were then steeped with the lactic acid bacteria inoculum. The lactic acid bacteria that was used as starter cultures are; *Lactobacillus acidophilus, Lactobacillus brevis and Lactobacillus plantarum*. These strains were chosen based on their ability to produce antimicrobial best. An inoculum size of 7.0 x 10^3 cells/ml was used to treat the steeped sorghum grains. The sorghum grains were then malted using the method of (Demuyakor and Ohta, 1993).

Production of Wort from the Sorghum Samples

Worts were produced with the 200g each of the malted sorghum, 204g of the adjunct and 1 litre of water (Demuyakor and Ohta, 1993). The mash was then prepared using the 3 – decoction method (Osorio *et al*., 2001). The malted sorghum was ground, mixed with warm water (35^{0}C) and a third of the obtained mash was boiled and mixed properly with the other two thirds to obtain a mash at 50°C. The mixture was allowed to stand for a period of 30 minutes after which a third of the mash was boiled again and returned to the remaining mash to increase the temperature of the mixture to 65°C. The process was repeated the third time thus, the temperature of the mash was increased to 75^{o}C. At this time, all the enzymic activities had ceased.

The mash was then boiled quickly, cooled and filtered using a 250 mesh sieve, muslin cloth and through a Whatman No. 1 filter paper. The spent grain was sparged with water to remove the remaining wort. When the wort was prepared, the sugar level of the wort was determined and the adjunct was added to it (Osorio *et al*., 2001).

Fermentation and Analyses of the Sorghum drink

Worts for analysis were obtained from the malted sorghum samples. The wort was fermented in lightly covered plastic containers. The yeast strain that was used for the fermentation was *Saccharomyces uvarum (Carlsbergensis)*, which is a bottom – fermenting yeast. 1250ml of the wort was inoculated with 6.0 X 10^6 yeasts cells/ml. The fermentation was then carried out at 30^{o}C under static conditions. After fermentation, the yeast was separated from beer by filtration.

Specific gravity of the drink was determined using the method of Agu *et al*., (2002). The sugar content and total sugar of the drink were determined using the phenol-sulphuric acid method of Dubois *et al.,* (1956). Total protein content and total nitrogen were determined using the soluble nitrogen content method of the Association of Agricultural chemist (A.O. A.C, 1980). Colour of the drink was estimated using Spectrophotometer with glass cells of 2mm path length to obtain the absorption spectra from which L* a* b* values were calculated using illuminant D65 and a 10^{t} observer according to the CIELAB 76

convention (McLaren, 1980). The viscousity was determined using a Viscometer (Agu *et al*., 2002). pH of the drink was determined using a previously calibrated pH meter (Demuyakor and Ohta, 1992b).

Organoleptic assessment of the fermented sorghum drink was carried out by a panel of ten judges. The panelists were asked to score each characteristics based on appearance, taste, flavour or aroma, colour and overall acceptability on a nine – point Hedonic scale (Larmond, 1977).

3. Results

The frequency of occurrence of lactic acid bacteria (LAB) isolated from ogi and processed yoghurt is shown in Table 1. The lactic acid bacteria that were used as starter cultures were; *Lactobacillus acidophilus, Lactobacillus brevis and Lactobacillus plantarum* and these strains were chosen based on their ability to produce antimicrobial best and the antimicrobials produced by the isolates are shown on Table 2.

Specific gravity, Total protein and Viscousity

Specific gravity obtained was all in the same range. The value ranged from 1.02 to 1.04. *L. plantarum and L. acidophilus* treated drink gave the highest specific gravity of 1.03 while *L. brevis* treated drink produced the least specific gravity of 1.02. The specific gravity of the experimental drink was higher than the control drink. Total protein was produced most in the drink treated with *L. brevis and L. plantarum* with value of 0.62% (Table 3). *L. brevis* treated drink gave the highest viscousity with a value of 13.25 (cm^2/secs) while L. *plantarum* treated wort gave the least viscousity with a value of 11.00(cm^2/secs).

Total sugar, pH and sugar Level

The drink treated with *L. plantarum* and *L. acidophilus* produced the highest total sugar with values of 3.98 mg/ml while the drink treated with *L. brevis* gave the least total sugar with value of 3.93 mg/ml. The drink treated with *L. brevis* had the highest sugar with value of 0.25mg/ml while the drink treated with *Lb.acidophilus* had the least sugar with value of 0.20 mg/ml (Table 4).

The drink treated with *L. brevis* produced the highest pH value of 4.12 while *L. plantarum* produced the least pH value of 3.85. The pH of the drink was within acidic range.

Colour of the sorghum drink

L. acidophilus produced the greatest effect on the colour of the drink with value of 5.92 while *L. brevis* produced the least effect on the colour of the drink with value of 4.82. Sensory analysis indicated that the sorghum drink treated with the starter cultures had a good taste, aroma, colour and the appearance was also acceptable when compared with the untreated sorghum drink used as control (Table 5).

4. Discussions

The lactic acid bacteria isolated were identified as *Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus fermentum* and *Lactobacillus bulgaricus*. These organisms have been reported by Halm *et al.* (1993) to be responsible for various fermentation processes involved in food production. Dike and Sanni (2010) also isolated lactic acid bacteria from various fermented cereal gruels.

The specific gravity of the experimental drink was higher than the control drink. This is as a result of the lactic acid bacteria suspension on the drink. The specific gravity obtained were in the same range with the specific gravity of beer (Demuyakor and Ohta, 1993). Total protein was produced most in the drink treated with *L. brevis and L. plantarum* with value of 0.62% (Table 3).

The protein content of the drink treated with the suspension of the lactic acid bacteria cultures was lower than that of the control drink. This may be due to the application of the starter cultures because sorghum seeds with high protein content are slow to modify during malting and are not desirable for sorghum drink production (Briggs *et al.,* 1981). High protein contents and their structural organization in the cell wall may limit cell wall degradation and this may not give a quality drink according to Etok Akpan (1990).

The viscosity of the drink was low because of the application of starter cultures. Low viscosity ensures high quality of beer in the brewing process (Haikara and Latila, 1995).

The sugar level and the total sugar of the drink was high; possibly due to the fact that lactic acid bacteria utilize starch and convert it to sugar. The lactic acid bacteria break down starch to produce more sugars (Novellie, 1961). The pH of the drink was within acidic range and this could be as a result of the Lactobacillus used as the starter culture and as a result of the lactic acid produced by the organisms. The starter culture converted the sugar into acid during malting process and this lowered the pH of the drink produced from the malted sorghum. The Lactobacillus could only grow and survive in the acidic range. The pH of the wort obtained in this study is beneficial at these values and enzyme activity is optimized while beer flavour is improved and this is in agreement with the work of Oliver (1988).

The colour of the drink could be attributed to the higher content of reducing sugars and alpha amino nitrogen that favoured the heat treatments used for mashing (Urias, 2001). According to EBC (1998), the colour of the drink ranged from 5.33 to 6.76. The

colour of the drink produced also falls within this range suggesting good quality.

Sensory analysis indicated that the sorghum drink treated with the starter cultures had a good taste, aroma, colour and the appearance was also acceptable when compared with the untreated sorghum drink used as control. In all, the sensory results were quite favourable towards the sorghum treated drink.

In conclusion, Lactic acid bacteria (LAB) starter cultures are applied in the brewing for improving beer characteristics. LAB produces antimicrobial substance, which restricts the growth of harmful Gram- negative and positive bacteria. Lactobacillus increased the sugar content of the wort. The use of LAB improved taste and flavour stability of starter beers. The results of proximate analysis and the pH were within the acceptable limits expected for a fermented drink. The sorghum drink that was treated with the LAB starter cultures were acceptable when compared with the untreated drink. However, there should be large- scale production of Lactobacillus species that can be used for fermentation of sorghum in the brewing industry so as to improve the quality of the finished product and also to reduce contamination.

Table 1. Frequency of occurrence of lactic acid bacteria Isolated From "Ogi And Processed Yoghurt.

Lab Isolates	Number Of Isolates	Frequency Of Occurrence (%)
Lb. plantarum	14	41
Lb. acidophilus	3	9
Lb. brevis	4	12
Lb. casei	2	5
Lb. delbrueckii	3	9
Lb. fermentum	4	12
Lb. bulgaricus	4	12
Total	34	100

Key: Lb-Lactobacillus

Table 2. Antimicrobial Production By The Lactic Acid Bacteria Isolates Yield Of Antimicrobials (G/L)

Isolates	Code	Lactic Acid	Hydrogen Peroxide	Diacetyl
Lb. brevis	**WO_1**	**0.93± 0.02^b**	**0.007± 0.01**	0.46±0.03
Lb. acidophilus	**YO_6**	**0.81± 0.03^a**	**0.010± 0.03**	0.40±0.01
Lb. plantarum	SO_6	0.88± 0.02^b	0.009± 0.03	0.62±0.03

*Values with the same letter are not significantly different.

Key: Lb-Lactobacillus

Table 3. Effect of lactic acid bacteria suspension on the specific gravity, total protein content and the viscousity of drink from sorghum samples.

Lab Isolates	*Specific Gravity*	*Total Protein (%)*	*Viscousity (Cm^2/Secs)*
Lb. Plantarum	1.03 ±0.02^a	0.62±0.04^a	11.00 ±0.03^b
Lb. acidophilus	1.03±0.02^a	0.60 ±0.03^a	11.25±0.03^b
Lb. brevis	1.02±0.03^a	0.62±0.01^a	13.25 ±0.02^b

*Means having the same superscripts are not significantly different from one another.

Key: Lb-Lactobacillus

Table 4. Effect of lactic acid bacteria suspension on the total sugar, p^H, sugar level and colour of drink from sorghum samples.

LAB Isolates	*Total sugar (mg/ml)*	*P^H*	*Sugar level (mg/ml)*	*Colour*
Lb. plantarum	3.98± 0.03^a	3.89± 0.04^b	0.24±0.02^a	5.20±0.03^a
Lb. acidophilus	3.98± 0.03^a	4.12±0.03^a	0.20±0.03^a	5.92±0.04^b
Lb. brevis	3.93±0.02^a	3.85±0.05^b	0.25±0.02^a	4.82±0.01^b

*Means having the same superscripts are not significantly different from one another.

Key: Lb-Lactobacillus

Table 5. Sensory analysis of sorghum drink

Samples	Appearance	Taste	Flavour/Aroma	Colour
SO6red	6.6	7.1	6.2	6.3
WO1 red	6.7	7.1	7.6	6.4
YO6red	7.6	7.1	7.3	6.2
SO6 white	6.8	5.8	6.9	7.6
WO1 white	6.3	5.4	5.9	5.9
YO6 white	6.6	6.4	6.5	6.1
SO6 brown	6.4	6.7	7.0	6.0
WO1 brown	6.5	6.8	6.8	6.4
YO6 brown	6.2	6.4	6.2	6.5
Red control	5.4	4.6	4.9	6.0
White control	5.2	4.4	4.1	5.7
Brown control	5.5	4.6	4.3	5.7

Key: SO6-White sorghum treated with *Lactobacillus plantarum*
WO1-Red sorghum treated with *Lactobacillus brevis*
YO6-Red sorghum treated with *Lactobacillus acidophilus*

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