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Bacterial flora associated with some stages of Burukutu production

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ABSTRACT: 'Burukutu' which is an indigenous alcoholic drink has been shown to be rich in bacterial diversity, with a total of thirteen (11) bacterial species isolated from the three stages of production (namely: steeping, mashing and maturation) sampled from two different selling points in Ilorin, metropolis. The bacterial species isolated are *Streptococcus* sp; *Lactobacillus* sp; *Bacillus* sp; *Pseudomonas* sp; *Klebsiella* sp; *Staphylococcus* sp; *Enterococcus* sp; *Escherichia coli*; *Proteus* sp; *Leuconostoc* sp and *Micrococcus* sp. Samples from the steeping stage had the highest bacterial flora followed by the mashing stage. The maturation stage of production had the least bacterial diversity of all the stages tested. Samples collected from the steeping stage, also had the highest total heterotropic count which ranged from 1.5 X 10^4 to 2.2 X 10^4 cfu/ml. This was followed by samples from the mashing stage with counts ranging from 8.9 X 10^3 to 1.1 X 10^4 cfu/ml. Samples from the maturation stage had the least heterotrophic count of between 6.9 X 10^2 and 8.4 X 10^2 cfu/ml. The public health implication of these bacterial flora and their distribution among the various stages of 'burukutu' production was discussed.

Introduction

'Burukutu' is a popular alcoholic beverage of a vinegar-like flavour, brewed from guinea corn (*sorghum bicolor*), consumed in the Northern Guinea savanna region of Nigeria, in the Republic of Benin and in Ghana. (Ekundayo, 1969).

As reported by (Faparusi, 1970) and as established during the course of this study, the preparation of 'burukutu' involves steeping sorghum grains in water overnight, following which excess water is drained. The grains are then spread out on to a mat or tray, covered with banana leaves and allowed to germinate. During the germination process, the grains are watered on alternate days and turned over at intervals. Germination continues for 4-5 days until the plumule attains a certain length. The malted grains are spread out in the sun to dry for 1-2 days, following which the dried malt is ground into a powder. 'Gari', (a farinaceous fermented cassava product) is added to a mixture of the ground malt and water in a ratio of one part 'gari' to two parts malt and six parts water. The resulting mixture is seeded and allowed to ferment for 2 days, following which it is boiled for approximately 4 hrs and allowed to mature for a further 2 days. The resulting product is a cloudy alcoholic beverage.

As with other fermentative processes, the main objective of these processing stages is to improve the bioavailability of the nutrients and to partially or completely remove antinutrients or toxic compounds from the substrates (Emechebe, 1998).

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Fermentation processes play important roles in food technology in developing countries. In traditional fermentation processes, natural microorganisms are employed in the preparation and preservation of different types of food. These processes add to the nutritive value of foods as well as enhancing flavour and other desirable qualities associated with digestibility and edibility. The fermentation techniques are often characterized by the use of simple, non-sterile equipment, chance or natural inoculum, unregulated conditions, sensory fluctuations, poor durability, and unattractive packaging of the processed products (Nout, 1985).

As would be expected, while some of these bacteria are normal flora associated with the production processes, and play positive roles in ensuring a better quality of the end product, some others are actually mere contaminants which can constitute a threat to the quality and shelf-life of the end product (Nakazato *et al*. 1990).

As such, the main thrust of this work is to isolate and identify bacteria associated with some stages of 'burukutu' production and subsequently determine the bacteriological quality of 'burukutu' produced and sold in local places.

Materials and Methods

Sample Collection.

'Burukutu' samples were collected from two notable production centres in Ilorin metropolis namely: Post Office area (C1) and Maraba area (C2). Samples were collected from three (3) different stages of production (Steeping; Mashing and Maturation). Using sterile 250ml sampling bottles 100ml sample was collected from each stage of production from each production center and transported to the laboratory in an ice bucket immediately after collection for microbiological analysis.

Microbiological Analysis

Total heterotrophic count

For each sample, 10ml was aseptically transferred to sterile 90ml of 0.1% peptone water and the mixture shaken vigorously. Serial 10-fold dilutions were prepared by transferring 1ml of these solutions to 9ml tubes of 0.1% peptone water. The level of total heterotrophic count was established by dispensing 1ml of 10^{-2} dilutions into duplicate sterile plates and 20ml of molten nutrient agar (Oxoid) added. The dilution and molten agar were mixed and allowed to solidify. Inoculated plates were incubated for 48hr at $37^{o}C$ before colonies were counted and reported as colony forming units/ml (cfu/ml).

Identification of Bacterial isolates

Representative bacterial isolates obtained from each sample was identified based on colonial morphology and biochemical characteristics. The biochemical tests carried out are: Gram staining; Spore staining; Capsule staining; Motility; Catalase; Coagulase; Indole; Citrate utilisation; MR-VP; Oxidase; Gelatin hydrolysis; Oxidase and Sugar fermentation.

Results

The total heterotrophic counts varied for samples collected at the different stages of production. Samples from the steeping stage had the highest bacterial count of $2.2 \text{ X } 10^4$ cfu/ml and $1.5 \text{ X } 10^4$ cfu/ml for the post office production centre and maraba production centre respectively. The heterotrophic count for samples collected from the mashing stage ranged from $8.9 \text{ X } 10^3$ to $1.1 \text{ X } 10^4$ cfu/ml while samples from the maturation stage had the least heterotrophic count of $6.5 \text{ X } 10^2$ and $8.4 \text{ X } 10^2$ cfu/ml for the post office and maraba production centres respectively (Table 1).

The distribution of the bacterial species isolated from the different production stages shows that *Lactobacillus* species, *Bacillus subtilis*, *E. coli* and *Leuconostoc* species were isolated from all the

stages of production from both production centres (Tables 2 & 3). However, *Klebsiella* species, *Enterococcus* species and *Micrococcus* species, that were isolated from the steeping stage in samples collected from post office area were not isolated from any of the stages in maraba area
(Table 3).

Table 1: Total heterotrophic count of samples collected from some stages of 'burukutu' production.

Production Centres	Bacterial Count (cfu/ml)		
	Steeping stage	Mashing stage	Maturation stage
Post Office Area (C1)	2.2×10^4	1.1×10^4	8.4×10^2
Maraba Area (C2)	1.5×10^4	8.9×10^3	6.5×10^2

Data represent the mean of duplicate determinations.

Table 2. Distribution of bacterial species associated with the three stages of 'burukutu' production sampled from post office area (C1)

Bacterial isolates	Production stages sampled		
	Steeping stage	Mashing stage	Maturation stage
Streptococcus sp.	+	+	–
Lactobacillus sp.	+	+	+
Bacillus subtilis	+	+	+
Pseudomonas sp.	+	–	+
Klebsiella sp.	+	–	–
Staphylococcus aureus	+	–	–
Enterococcus sp.	+	–	–
Escherichia coli	+	+	+
Proteus sp.	+	–	–
Leuconostoc sp.	–	+	+
Micrococcus sp.	+	–	–

Data represent the mean of duplicate determinations.

Table 3. Distribution of bacterial species associated with the three stages of 'burukutu' production sampled from Maraba area (C2)

Bacterial isolates	Production stages sampled		
	Steeping stage	Mashing stage	Maturation stage
Streptococcus sp.	–	–	+
Lactobacillus sp.	+	+	+
Bacillus subtilis	+	+	+
Pseudomonas sp.	–	–	+
Klebsiella sp.	–	–	–
Staphylococcus aureus	+	–	–
Enterococcus sp.	–	–	–
Escherichia coli	+	+	+
Proteus sp.	+	–	–
Leuconostoc sp.	+	+	+
Micrococcus sp.	–	–	–

Data represent the mean of duplicate determinations.

Discussion

The bacterial species isolated from the different stages of 'burukutu' production sampled, was quite diverse, with the steeping stage having the highest diversity of bacterial isolates (Tables 2 & 3). Of the eleven (11) bacterial species identified from the three stages sampled, ten (10) species were isolated from the steeping stage from post office area (C1) (Table 2) and eight (8) species were isolated from the steeping stage from maraba area (C2) (Table 3). However, some species such as, *Streptococcus, Pseudomonas, Klebsiella, Enterococcus* and *Micrococcus* that were isolated from the steeping stage in C1 were not isolated from samples collected from the steeping stage in C2. The isolation of some of these organisms is in conformity with the report of Faparusi, (1970), who reported the isolation of *Streptococcus lactis*, *Bacillus* species, *Lactobacillus* species, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* species and *Saccharomyces* species from 'burukutu'.

However, the isolation of *Bacillus subtilis*, in all the stages of production sampled, and its lack of particular role in the fermentative process, shows that it is possibly a contaminant that has been carried from the raw material (sorghum) to the last stage of production. *Bacillus* species are quite common in the agricultural environment and may contaminate farm produce from various sources both during the production, storage and processing (Janstova and Lukasova, 2001). It may also be owing its resilience in this circumstance to some of its exceptional properties such as the spores it produces (Nicholson *et al*. 2000). Aerobic and facultative anaerobic spore-forming bacteria of the genus *Bacillus* have also been reported to cause serious problem in milk industry. Because of the heat resistance of spores and ability of vegetative cells to produce extra-cellular enzymes they may cause food deterioration (Christiansson *et al*. 1999). It is known that the spores of bacilli survive pasteurisation and can have suitable conditions for germination created in the pasteurised product (Meer *et al*. 1991).

Since heat resistance is an important character of *Bacillus* species spores, appropriate temperature selection and duration of heating are crucial for the required microbial quality of the product. As with a linear temperature increase, the time needed to achieve nearly complete abiosis becomes shorter exponentially (Janstova and Lukasova, 2001).

The isolation of *E. Coli* in all the stages of production sampled is of major public health implication. This is because *E. Coli* has been reported to be causative agent for some diseases such as diarrhoea and urinary tract infection (NIAID fact sheet, 2002). *E. Coli* is of enteric origin, and their isolation from these stages is an indication of contamination from unclean water or unhygienic practices by handlers.

A reduction in the bacterial count and diversity was observed from samples collected from the maturation stages. This is not unexpected as the boiling of the fermented wort prior to the maturation stage is expected to have reduced the bacterial load and also concentrated the wort, inactivated the enzyme and coagulated the proteins (Haard *et al.*, 1999). The fermentative process also could have contributed to the reduction of the bacterial load and diversity (Campbell-Platt, 1994).

However, the isolation of *Pseudomonas* species and *E. Coli* at the maturation stage is of major public health implication considering the pathogenicity of these organisms to man. *Pseudomonas cocovenenans* has been reported to be responsible for bongrek acid and toxoflavin formation in fermented cereals (Ko, 1985).

It was observed that product from maraba production centre generally had lower bacterial count and fewer bacterial isolates compared to the product from post office centre.

Therefore, with safer hygienic practices by the workers and the use of high quality raw materials, 'burukutu' with much higher bacteriological quality can be produced. . It is also recommended that the final product be pasteurised to eliminate postproduction contaminants.

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