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Microorganisms Isolated during Fermentation of Sorghum for Production of *Akamu* (A Nigerian Fermented Gruel)

O. H. Ekwem[1*] and B. N. Okolo[1]

[1]Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

Authors' contributions

This work was carried out in collaboration between the authors. Both authors designed the experiment. Author OHE carried out the experiment and author BNO supervised the work. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aim: The aim of this study is to isolate microorganisms present during fermentation of sorghum for production of *akamu*.
Place and Duration: The study was done at Nsukka, Enugu State for a period of ten weeks.
Methodology: The production processes of two local producers were monitored. They were designated as P1 and P2. Samples were taken at each stage of the production process and inoculated on different microbial culture media. Counts were taken after incubation to ascertain the microbial load. Biochemical tests were done to identify the isolated organisms.
Results: From the grains no coliform was isolated, but the presence of coliform was noticed after 24 h steeping and was detected throughout the production. Lactic acid bacteria were also isolated and they significantly increased throughout the production process confirming them as major players in the fermentation of these cereals. The lactic acid bacteria were identified as *Lactobacillus delbrueckii, Lactobacillus lactis, Lactobaciluus plantarum Lactobacillus fermentum*

**Corresponding author: E-mail: ogechi.ekwem@unn.edu.ng;*

and *L. casei*. The yeasts isolated were identified as *Saccharomyces* spp and *Candida* spp. The fungi were identified as *Aspergillus niger, Aspergillus flavus, Penicillium* spp *and Fusarium* spp. *Eschericia coli, Stapylococcus, Klebsiella and Pseudomonas* were also isolated.

Keywords: Fermentation; Lactobacillus; akamu sorghum; yeast.

1. INTRODUCTION

The community of microorganisms found in fermented foods is rather complex with notable diversity observed among different samples. This could be attributed to the fact that these products are produced under uncontrolled fermentation and has no particular organisms as starter culture. In the fermentation of sorghum, different microorganisms are involved though a few are usually dominant and determine the quality of the end products. Results have shown that during fermentation of sorghum, the lactic acid bacterial count increases as yeast counts also increases, and pH decreases [1,2]. The pH of this fermentation process is usually reduced and this is sufficient to inhibit the growth of most pathogenic organisms. Although the extent to which pathogens are reduced or inhibited by low pH depends on the organisms involved, quantity of acids produced by the predominant organisms, the buffering capacity of the food etc. The acids produced permeate into the bacterial cell and slows down metabolic activity. A large quantity of lactic acid bacteria must be present in order for the inhibition of pathogens to be effective.

Generally, spontaneous cereal-based fermentations are performed by cooperation between lactic acid bacteria and yeasts [3]. The predominance of lactic acid bacteria in fermented food is commonly due to their ability to tolerate low pH [4]. This tolerance along with the propensity of lactic acid bacteria to produce antimicrobial substances such as organic acids (lactic acid, acetic acid, propionic acid, etc.), bacteriocin and hydrogen peroxide create an unfavorable condition for growth of pathogens, as well as spoilage organisms [4]. The hydrogen peroxide is also known as a strong oxidizing agent and it is a bacteriocide. The lactic acid bacteria also produce vitamins, amino acids and serves as a single cell protein after cooking.

According to a report by [5,6] and [7] the number of lactic acid bacteria and yeasts differ in household samples and increasing lactobacilli create an acidic environment conducive for yeast proliferation while the yeasts produce vitamins with other growth factors for lactobacilli; and also contribute to the flavor improvement. The simultaneous increase in the number of lactic acid bacteria and yeasts has been attributed to their symbiotic association [8].

According to [2], throughout the fermentation of maize, the predominant organisms are the lactic acid bacteria and yeasts, increasing in number as fermentation continued. This was followed by the acidification of the product. Besides their involvement in enhancement of flavor of the fermentation products, they also show, amylolytic, protease and phytase activities; and these enzymatic abilities contribute to breakdown of maize and sorghum starch and allow access to nutritionally essential minerals. The spectrum of yeasts found in fermentation of maize for *akamu* production has been identified as *Saccharomyces cerevisiae* and *Candida spp* [2]. It is important to note that simple fermentation can result in products that are sometimes microbiologically not safe [9,10]. The reason is that the final quality and safety of the fermented food depend on the quality of raw material, contamination level, fermentation condition, and the degree of acidity.

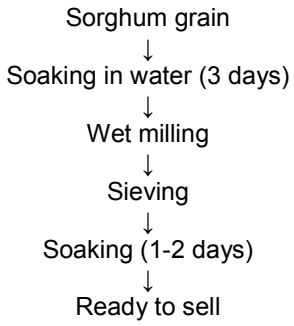

Fig. 1. Process of sorghum fermentation

2. METHODOLOGY

2.1 Materials and Methods

Samples from each step of sorghum fermentation for production of *akamu* were collected from two local producers at Zik's flats and Beach Junction, Obukpa, Nsukka. The samples were taken to the Microbiology

Laboratory, University of Nigeria, Nsukka for immediate analysis.

2.2 Enumeration of Microorganisms

Microbial counts were determined on selective media after serial dilutions and inoculation of the samples using the spread plate method.

2.3 Total Plate Count

These were done on Nutrient agar. Plates were incubated for 24 h at 37°C. The colonies which appeared after incubation were counted as colony forming unit per ml (cfu/ml).

2.4 Lactic Acid Bacteria Count

These were done on de Mann Rogosa Sharpe (MRS) agar. Plates were incubated in a candle jar for 48 h at 30°C. The colonies which appeared after incubation were counted as colony forming unit per ml (cfu/ml).

2.5 Coliform Counts

These were done on MacConkey agar and incubated for 24 h at 37°C. Confirmation of coliform was done on eosin methylene blue agar and incubation at 37°C for 24 h. The growth of green metallic sheen colonies confirmed Coliforms.

2.6 Staphylococcus Count

Staphylococcal count was done using Mannitol salt agar, followed by incubation at 37°C for 24 h. [11].

2.7 Yeast and Mold Count

Enumeration of yeasts and molds were done on Sabouraud Dextrose Agar (SDA) substituted with chloramphenicol. A 0.1 ml of each sample was dropped in sterile agar plates and evenly spread using a glass spreader. The inoculated plates were incubated at room temperature (28 ± 2°C) for 5 days. The isolates were identified using conventional microbiology methods, i.e. morphology.

2.8 Identification and Characterization of Isolates

All the isolated organisms were identified using conventional microbiological methods. For bacteria, the standard tests used for characterization of the isolates were microscopic examination, biochemical tests and sugar fermentation. Identification was also based on comparison of characteristics of the isolates with those in Bergey's Manual of Determinative Bacteriology, 8th edition, 1974. Fungi were identified using their morphology.

3. RESULTS

The raw material (sorghum grains) had a mean total plate count of $4.10x10^9$ cfu and $4.13x10^8$ cfu for P1 and P2. There was a significant decrease in the mold count from $6.63x10^9$ to $1.3x10^8$cfu and $6.96x10^9$ to $1.2x10^8$cfu for P1 and P2 respectively. No mold was isolated from both producers beyond 24hrs of steeping. The lactobacilli count increase from $3.03x10^8$ to $9.03x10^9$ and $4.33x10^8$ to $8.87x10^9$ for P1 and P2 respectively.

Table 1. Microorganism isolated from the sorghum fermentation

Isolates	Sorghum grains	After 24 hrs steeping	After washing	Wet-milling and sieving	Ready to sell
E. coli	NP	P	P	P	P
S. aureus	P	P	P	P	P
Staphylococcus spp	P	P	P	P	P
Pseudomonas	P	P	P	P	P
Klebsiella	P	P	P	P	P
Lactobacillus spp	P	P	P	P	P
Candida spp	P	P	P	P	P
Sacharomyces spp	P	P	P	P	P
Aspergillus flavus	P	P	NP	NP	NP
Aspergillus niger	P	P	NP	NP	NP
Fusarium	P	P	NP	NP	NP
Penicillium	P	P	NP	NP	NP

P= Present, NP = Not present

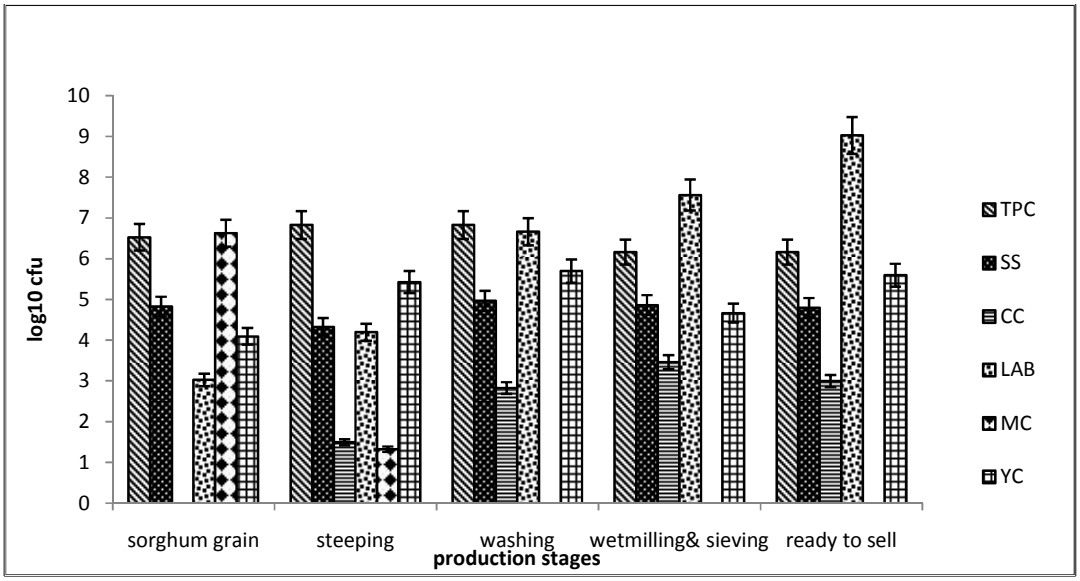

Fig. 2. Microbial count of producer 1

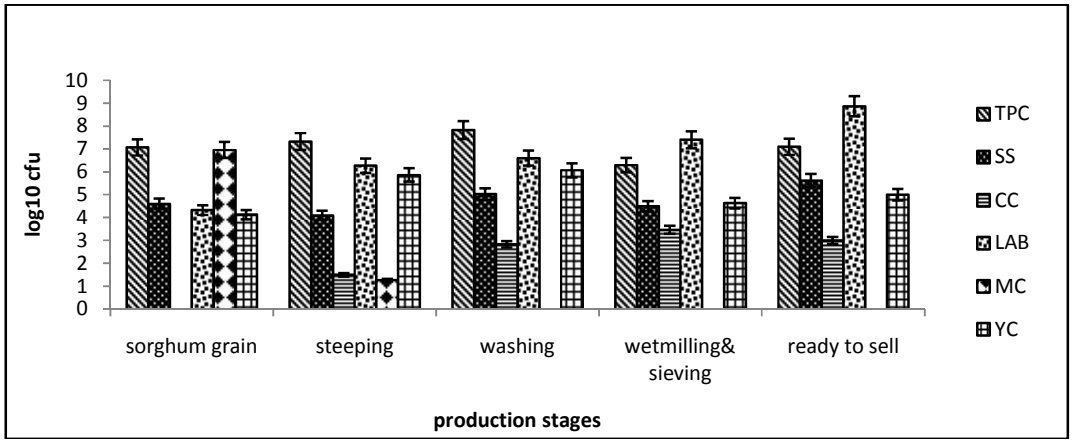

Fig. 3. Microbial count of producer 2

Key: TPC-Total plate count; SS - Staphylococcus count; CC -Coliform count; LAB -Lactobacillus count; MC - Mold count; YC -Yeast count

4. DISCUSSION

Different microorganisms were isolated during the course of this research, and were subjected to biochemical test to determine them. Above Figs. 2 and 3 show the trend of microbial growth. The lactobacilli show continuous and significant increase throughout the experiment. This shows a similar observation with the work of [12]. A report according to [13] also described the lactic acid bacteria as the dominant organisms in cereal fermentation. The microorganisms isolated from this study have been reported in the research of [1,14] and [15]. More so, a lot of microorganisms are involved during sorghum fermentation though some could be externally introduced by producers or from the utensils. From the grains, no coliform was isolated, but the presence of coliform was noticed after 24hr steeping and was detected throughout the production. This shows that the coliform would have been introduced through water. Lactic acid bacteria significantly increased throughout the production process confirming them as major players in the fermentation of these cereals.

5. CONCLUSION

Akamu generally has a safety record. However, the incidence of pathogens in it as highlighted by

this research work suggests that measures that minimize the risk of foodborne illness should be taken. The isolation of *Penicillium*, and *Aspegillus* from the sorghum grains shows the grains support mold growth. The lactic acid bacteria occur in high frequency in the product and confer beneficial effects on it, which include improved texture, flavor and increased nutritional value Regardless of the place of production of *akamu*, continuous good hygienic practice is important to ensure safe products. In as much as fermentation is beneficial, loss of control during production could result to negative effects, example is diarrhea.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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