



Microbial Characterization of Maize Fermentation Water during Ogi Production

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Abstract

This study characterized the microorganism found in 0 – 96 hours maize fermentation medium during ogi production (a cereal based porridge). Dried yellow maize were purchased from three maize sellers at Rumuomasi market, Port Harcourt, Nigeria. The samples were fermented using sterile water for 0 – 96 hours. About 2ml of the fermentation water was aseptically collected. Standard microbiological characterization of the isolates was carried out. Results revealed that *Saccharomyces cerevisiae*, *Corynebacteria* and *Lactobacillus* species were the main microbes found in the fermentation medium within 96 hours of fermentation. Other microbes found in the medium included *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter*, *Pseudomonas*, *Bacillus*, *Micrococcus* species (bacteria), *Aspergillus flavus*, *Aspergillus niger*, *Penicillium*, *Rhizopus*, *Mucor*, *Fusarium*, *Geotrichum* species (Fungi). There was decline in the TCFU ml⁻¹ followed by increasing pH as the fermentation progressed. This would suggest that the fermentation medium gradually became unfavorable to the microbial population. The implication of the effect of pH variability with the preponderance of the isolates over the period of fermentation was discussed.

Keywords: Cereal, Fermentation, Maize, Microorganisms, Ogi

1 Introduction

Like rice and wheat, maize is an important cereal used globally for the production of several products. Typically, maize belongs to the *Poaceae* family; maize thrives in both tropical and temperate regions of the world. Maize is an annual plant that produces grains that are edible and as such have economic importance. Maize is produced in several countries of the world notably, United States, China, Brazil, Mexico, India, Argentina, Spain, Italy, Nigeria Australia etc.

Maize is used for the production of several foods including fermented foods. Abdulrahman and Kolawole [1] listed 28 different food dishes that could be produced from maize. Among the notable uses of maize is *Ogi* production (a fermented maize slurry), which is used especially as weaning food for infants [2-5] as well as a dietary staple for adults in West Africa especially for the elderly and sick. Maize is also used for the production of *Masa* (a sorghum-maize blend) [6]; *Kokoro* (a Nigerian indigenous fermented maize snack) [7]; *sekete* (a fermented maize beverage) [8]. In addition, *Agidi* can also be produced from fermented maize slurry. Like *ogi*, *Agidi* is an essential traditional cereal based food consumed in Africa that is processed by natural fermentation [9, 10] of cereal mainly maize. Like *Ogi*, *Agidi* is also consumed with soup, stew, akara, moimoi as light meal for the sick [11, 12].

Maize typically has health benefits. For instance, Abdulrahman and Kolawole [1] reported that maize has 6 medicinal values. Some of the major health benefits of maize include as a rich source of vitamins and minerals; controls of diabetes and hypertension; reduction in cholesterol absorption in the body and risk of various cardiovascular diseases;

anemia prevention; boosting of immune system; maintenance of vision and healthy skin; lowering risk of colon cancer and hemorrhoids and enhancement of bone strength [13]. Similarly, maize is rich in nutrients (i.e. dietary fiber, carbohydrate, calories and low in protein), minerals (magnesium, phosphorus, potassium and manganese) and vitamins (thiamin, vitamin C, folate and niacin) [13]. However, during processing of maize for ogi production, notably during sieving of milled fermented maize some nutrients are lost. For instance Farinde [14] reported reduction in protein, ash, fiber, carbohydrate, iron, zinc, magnesium, sodium and calcium content in sieved ogi than in the unsieved type.

During fermentation of maize for ogi production water is typically used to ferment sieved maize. Authors have variously reported that initial fermentation period of 1 – 3 days is ideal [3, 14-20]. On appearance, the maize gets swollen and the water becomes turbid. On microbial presence, authors have variously reported that microbial consortia of maize fermentation medium increases with density [2, 15, 18, 21, 22] and reduces in diversity as fermentation progresses. Similarly, authors have reported that most of the microbial diversity that participate in fermentation is mainly of the genera *Lactobacillus*, *Leuconostoc*, *Saccharomyces*. Oyewole and Isah [23] listed genera of microbes that play essential role in fermentation of traditional food to include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Streptococcus*, *Penicillium* and *Saccharomyces*. In a recent review by Izah et al. [12], the authors reported that *Saccharomyces cerevisiae*, *Lactobacillus* species and *Aspergillus niger* were the predominant microbial isolates found in maize ogi fermentation medium.

Several studies have been carried out with regards to microbial diversity of maize fermentation water [15, 18, 21, 22, 24, 25]. Therefore, this study focuses on the microbial

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characterization of four days maize fermentation medium in Port Harcourt Metropolis.

2 Materials and Methods

2.1 Field Sampling

Dried yellow maize samples were purchased from three maize sellers at Rumuomasi market, Port Harcourt, Nigeria. They were packaged in sterile Ziploc bags and taken to the laboratory for fermentation.

2.2 Sample preparation

In a sterile 1000 ml conical flask, the maize samples were separately soaked using 950ml sterile water which was aseptically added. The control was set up (using sterile water without maize added). The cap of the conical flask was loosely covered. 2ml of the ferment water samples from both media viz. the flask containing maize and the one containing only sterile water was collected after shaking for microbiological examination.

2.3 Identification of microbial isolates

Four media i.e. Nutrient agar (for total heterotrophic bacteria counts), MacConkey Agar (for the enumeration of Enterobacteriaceae family), Potato dextrose agar (for mould and Yeast), DeMan, Rogosa and Sharpe Agar i.e. MRS Agar (for Lactobacillus). The media were prepared according to the manufacturer's instruction. The Pour plate method was used for TCFU m^{-1} determination employed. For bacteria (Enterobacteriaceae family) 0.1 ml of the 10-fold serially diluted ferment water samples were plated in the various media. For bacterial total viable counts, incubation was for 24 – 48 hours at 37°C; for Mould and Yeast, incubation was for 3-4 days at 30°C; For Lactic acid bacteria incubation was at 37°C for 2-3 days under anaerobic condition using MRS Agar containing 10mg/ml cycloheximide.

The bacteria isolates were subjected to biochemical tests (viz: Gram's reaction, citrate, catalase, oxidase, Indole, coagulase, motility, methyl red) using the guides of Cheesbrough [26] and Benson [27]. Thereafter, the resultant characteristics were compared with those of known taxa using scheme of Cheesbrough [26] and Bergey's Manual of Determinative Bacteriology by Holt et al. [28]. Based on gram reaction, the gram positive organisms were streaked in Mannitol Salt Agar plate and incubated inverted at 37°C for 24 hours. The presence of yellowish pigments in Mannitol Salt Agar indicates *Staphylococcus aureus*. Also, the pure cultures from MacConkey agar were streaked in Levine's eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hours. The presence of small nucleated colonies with greenish metallic sheen indicates *E. coli* [27, 29]. Similarly the lactic acid bacteria were further streaked in MRS agar from where the colonial morphology and cellular characteristics for the various colonies obtained were studied. Identification method for the lactic acid bacteria include macroscopic, microscopic, and biochemical tests (such as gram reaction, catalase and sugar fermentation i.e. lactose, sucrose, salicin, mannitol, sorbose, xylose and growth on 4% sodium chloride following the method described by Oyarekua [30]. The resultant characteristics were compared with characteristics presented by Oyarekua [30].

Both microscopic and macroscopic techniques were employed for the identification of the moulds. The observation of the mould isolates were compared with guide provided by Benson [27], while the microscopic morphology was determined using Lactophenol cotton blue stain as described by Pepper and Gerba [29] and Benson [27]. The resultant microscopic

characteristics were compared with the scheme of Pepper and Gerba [29], Ellis et al. [31] and Benson [27].

The yeast isolates were streaked onto glucose yeast agar and yeast malt agar under aerobic condition at 28°C [32] from where morphological and physiological tests were carried out. The result characteristics were compared according to Barnett et al. [33], Kregger Van-Rij [34], Ellis et al. [31] and Benson [27].

2.4 Statistical Analysis

The frequency of occurrence of microbial isolates was computed and their type determined (i.e. as total heterotrophic bacteria count/lactic acid bacteria counts and bacterial of the Enterobacteriaceae family) and fungi (mould/yeasts). The charts were plotted using Microsoft excel.

3 Results and Discussion

The microbial isolates of maize fermentation water for ogi production is presented in Table 1. The bacterial isolates include *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter*, *Lactobacillus*, *Pseudomonas*, *Bacillus*, *Micrococcus* and *Corynebacteria* species. While the mould and yeast diversity included *Aspergillus flavus*, *Aspergillus niger*, *Penicillium*, *Rhizopus*, *Mucor*, *Fusarium*, *Geotrichum* species and *Saccharomyces cerevisiae*. The distribution of microbes during the different fermentation of this study showed similarity to the work of Adegbehingbe [2], who reported that microbes such *Corynebacteria*, *Lactobacillus*, *Saccharomyces cerevisiae* were found in the fermentation medium from the second day upward. Similarly, the authors also reported that *Staphylococcus aureus*, *Rhizopus nigricans* and *Candida crusei* were found in the fermentation medium on the first day and absent from the second day upward, while *Micrococcus lutens*, *Aspergillus niger* occurred from day 1 to day 2 but absent from day 3 upwards. The bacterial and yeast/mould frequency of occurrence isolates are presented in Figures 2 and 3 respectively. Based on the frequency of occurrence the bacterial isolates indicated; *Staphylococcus aureus* (34.9%) and *Enterobacter* sp (3.3%) were highest and least at 0 hour. *Lactobacillus* sp (48.3%) and *Enterobacter* sp (2.3%) after 24 hours, *Lactobacillus* species (63.1%) and *Pseudomonas*, *Bacillus* and *Micrococcus* species (0.0%) occurred at 48 hours, *Lactobacillus* species (82.3 - 86.8%) and *E.coli*, *Pseudomonas*, *Bacillus* and *Micrococcus* species (0 %) occurred at 72 to 96 hours of fermentation respectively (Figure 2). The frequency of occurrence of *Lactobacillus* species indicated that they were increasing in number as fermentation progresses. Similarly, and *Corynebacteria* species progressed up to 48 hours before there was decline in the population. But other aerobic mesophilic bacteria including *E.coli*, *Pseudomonas*, *Bacillus*, *Enterobacter* and *Micrococcus* species population generally were in a decreasing trend. For yeast and mould isolates, the frequency of occurrence were highest but least for *Aspergillus niger* (28.2%) and *Saccharomyces cerevisiae*, *Aspergillus flavus* and *Geotrichum* species (0.0%) at 0 hour, *Saccharomyces cerevisiae* (29.1%) and *Fusarium* sp (5.2%) at 24 hours, *Saccharomyces cerevisiae* (47.4%) and *Fusarium*, *Rhizopus* species and *Aspergillus flavus* (0.0%) at 48 hours, *Saccharomyces cerevisiae* (80.8%) and *Geotrichum*, *Penicillium*, *Rhizopus*, *Fusarium*, *Mucor* species and *Aspergillus flavus* (0.0 %) at 72 hours respectively (Figure 3). While only *Saccharomyces cerevisiae* were isolated at 96 hours of fermentation. The trend in the frequency of occurrence in this study has some similarity with the work of Akinleye et al. [22]. *Enterobacter* species and *E. coli* are indicator organisms, thus their presence could stem from

contamination of maize by fecal material probably from materials used for storage or even handling process.

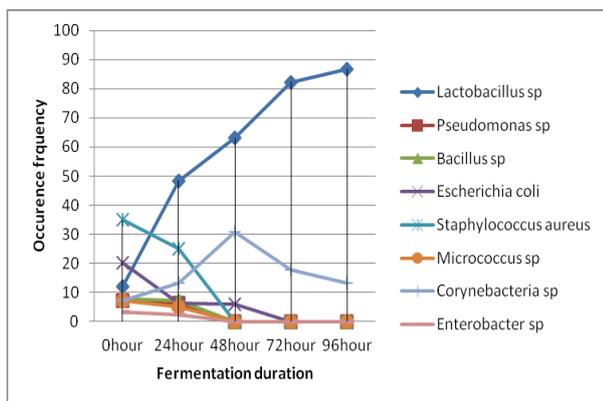


Figure 1: Occurrence frequency of the bacteria isolates

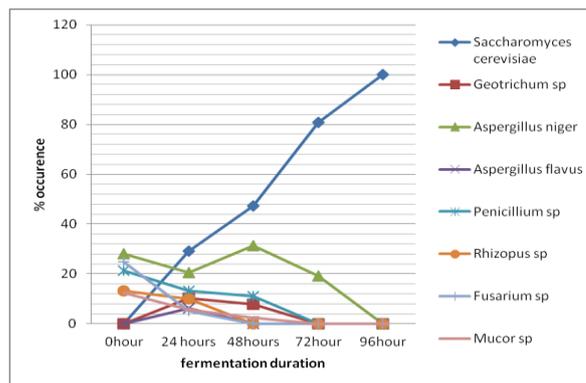


Figure 2: Occurrence frequency of the yeasts/mould isolates

Table 1: Microbial isolates of maize fermentation water for ogi production

Microbes		Hours				
		0	24	48	72	96
Bacteria	<i>Lactobacillus sp</i>	+	+	+	+	+
	<i>Pseudomonas sp</i>	+	+	-	-	-
	<i>Bacillus sp</i>	+	+	-	-	-
	<i>Escherichia coli</i>	+	+	+	-	-
	<i>Staphylococcus aureus</i>	+	+	-	-	-
	<i>Micrococcus sp</i>	+	+	-	-	-
	<i>Corynebacteria sp</i>	-	-	+	+	+
	<i>Enterobacter sp</i>	+	+	-	-	-
Yeast and mould	<i>Saccharomyces cerevisiae</i>	-	+	+	+	+
	<i>Geotrichum sp</i>	-	+	+	-	-
	<i>Aspergillus niger</i>	+	+	+	+	-
	<i>Aspergillus flavus</i>	-	+	-	-	-
	<i>Penicillium sp</i>	+	+	+	-	-
	<i>Rhizopus sp</i>	+	+	-	-	-
	<i>Fusarium sp</i>	+	+	-	-	-
	<i>Mucor sp</i>	+	+	+	-	-

Note: Some microbes only occurred in one set of the triplicate samples; + = Present; - = Absent

The occurrence of some microbes from day 24hours of fermentation in this study could be attributed to changes in the acidity of the fermentation medium. For instance, authors have variously reported that pH of fermentation of maize products increases decreases as fermentation period increased [2, 35 -38]. For instance, as pH decreases it encourage the growth of microbes such as *Lactobacillus*, *Corynebacteria* species and *Saccharomyces cerevisiae* at 72 to 96 hours. This suggested that these are the main microbial isolates that play significant role in fermentation of maize medium. This microbes typically aid in acid fermentation. Generally, the various group of microorganisms isolated in this study have been reported by Nwokoro and Chukwu [21], Akinleye et al. [22], Wakil and Daodu [15], Omemu [24], Oyedeji et al. [25], Adegbehingbe [2], Ijabadeniyi [18].

Specifically, the occurrence of microbes such as *Pseudomonas aeruginosa* and *Aspergillus niger* could be due to laboratory contamination. In a study by Akinrotoye [39] and Kigigha et al. [40] on palm wine, the authors have variously reported that microorganisms such as *Pseudomonas aeruginosa* and *Proteus vulgaris*, *Aspergillus niger* in palm wine was probably due to unhygienic mode of handling and or laboratory contamination. Also, Oyelana and Coker [41] have reported that *Aspergillus flavus*, *Aspergillus niger*, *Penicillin oxalicum*, *Fusarium oxysprium* and *Rhizopus stolonifer* were frequently found in water used in the fermentation of ogi

production. The occurrence of *Staphylococcus aureus* in the medium could be due to contamination. Typically, *Staphylococcus aureus* is a normal flora on the skin of humans, which could have been possible source of their presence in the maize fermentation sample. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Enterobacter*, *Penicillium*, *Rhizopus*, *Fusarium*, *Mucor* species and *Aspergillus niger* found in the maize medium in 0 hours suggesting that the maize could be contaminated by them through handling and marketing processes prior to laboratory analysis.

4 Conclusion

Maize is an important cereal cultivated in several parts of the world. Maize is important in the compounding of a number of foods especially infant winning foods such as Ogi. In Nigeria, ogi (a cereal porridge) is produced from maize and to a lesser extent from sorghum and millet). Ogi production is a typical cereal fermentation process. This study characterized the diversity of microbes found in fermentation medium between 0 – 96 hours. The study showed that *Saccharomyces cerevisiae*, *Corynebacteria* and *Lactobacillus* species were the predominant isolates which during the 96 hours of fermentation in the medium. The study also indicated the presence of several aerobic microbes in the fermentation

medium which were probably introduced as contaminants prior to purchasing.

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