



MICROBIAL CHANGES DURING THE FERMENTATION OF BAOBAB (*Adansoniadigitata*) FRUIT PULP YOGHURT

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ABSTRACT

This study was conducted to assess the microbial changes during the fermentation of Baobab (*Adansoniadigitata*) fruit pulp yoghurt. The Baobab fruit pulp yoghurt was prepared in the Laboratory using the conventional method. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were used as starter cultures while a control was produced without the starter cultures. de Man Rogosa Sharpe (MRS) agar was used to culture lactic acid bacteria. The microbial load, succession and percentage occurrences were determined using standard methods. The total aerobic bacterial count was found to be within the range of 1.9×10^3 – 1.4×10^5 cfu/ml. The Lactic acid bacteria and fungal count ranges were 4.5×10^3 – 7.5×10^3 cfu/ml and 8.0×10^1 – 2.8×10^4 cfu/ml respectively. At the end of fermentation time, there was significant difference between the test and control Baobab yoghurt at $P < 0.05$. Lactic acid bacteria recorded the highest count of 6.2×10^4 and 7.5×10^3 cfu/ml in the test and control respectively. *Bacillus* species, *Staphylococcus aureus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Micrococcus* species were the bacteria isolated while the fungal isolates were *Saccharomyces cerevisiae* and *Hansenula* species. *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Bacillus* species and *Saccharomyces cerevisiae* were the only microorganisms found at the end of fermentation time. The study obtained low microbial count and isolated less number and type of microorganisms from Baobab fruit pulp yoghurt because of the antimicrobial effect of baobab pulp and pasteurization treatment. Based on the results of this study, Baobab fruit pulp yoghurt can be said to be of good microbiological quality for human consumption. The industrial use of Baobab fruit pulp in the production of yoghurt is recommended.

Keywords: Baobab, Fermentation, Fruit pulp, Microbial changes and Yoghurt

INTRODUCTION

Yoghurt is one of the most popular fermented dairy products widely consumed all over the world. It is obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus* (Helmenstine, 2016). It can also be made by using a portion from the former fermentation – backslipping. The role of these two genera in yoghurt manufacture can be summarized as milk acidification and synthesis of aromatic compounds (Adams and Moss, 2009). In Nigeria, it is a popular drink due to its nutritional, probiotic and organoleptic

characteristics (De *et al.*, 2014). The high pH and low acidity may be due to the fact that there is no proper system of culture dosage in unbranded yogurt, which largely affects the acidity of the final yogurt. Milk derived from animal such as cow milk, is high in nutrients such as protein, fat, carbohydrates, vitamins and minerals necessary food required by microorganisms (Efiosa *et al.*, 2017).

Baobab (*Adansoniadigitata*) fruit pulp yoghurt is made by combining commonly available commercial animal milk with milk obtained from Baobab fruit pulp.

The pulp, seeds and the fibre are separated by sieving to produce a fine powdery pulp (Baobab Fruit Company Senegal, BFCS, 2011). The Baobab Pulp is 100% natural and organic, no chemicals or preservatives added. It has a unique pleasant flavor reminiscent of pear with a mild slightly acidic after taste due to its high percentage of organic acids such as citric acid, tartaric acid, malic acid, succinic acid and ascorbic, with pH 3.3 (Abdalla *et al.*, 2010; BFCS, 2011).

Yoghurt has milk as a raw material for its production. Milk even in powdered form has been reported to support the growth of microorganisms for instance *Staphylococcus aureus* (Efiosa *et al.*, 2017).

There have been a number of foods borne illnesses resulting from the ingestion of raw milk, or dairy products made with milk that was not properly pasteurized or was poorly handled causing post-processing contamination (Eze *et al.*, 2014). The following bacterial pathogens are still of concern today in raw milk and other dairy products: *Bacillus cereus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella* spp, *Escherichia coli* O157:H7 and *Campylobacter jejuni* (De *et al.*, 2014). It should also be noted that moulds, mainly of species of *Aspergillus*, *Fusarium*, and *Penicillium* can grow in milk and dairy products. If the conditions permit, these moulds may produce mycotoxins which can be a health hazard (Bhattacharjee, 2013).

Dirisu *et al.* (2015) reported that related researches have shown that most yoghurt sold in Nigerian markets are contaminated with pathogenic bacteria such as *E. coli*, *Staphylococcus aureus*, *Bacillus* spp and moulds, such as *Rhizopus* spp., *Aspergillus* spp. etc

Yoghurt also serves as a medium for the growth of microorganisms due to its high nutritional content hence it is liable to contamination. Moulds and yeast are the primary contaminants in yoghurt.

Fungi growing in yoghurt utilize some of the acid, which will invariably reduce the acidity and hence favour the growth of putrefactive bacteria (Dirisu *et al.*, 2015) or other pathogenic organisms such as *Staphylococcus aureus*. Caplice and Fitzgerald (1999) noted that at the beginning of the fermentation step, the food is vulnerable to

contamination since it is not acidic. It is known that pathogenic microorganisms normally found in food will not be able to grow in an acid environment, which is at pH below four. This acidity is normally found in lactic acid fermented food (Adams and Moss, 2009).

Several authors have reported the effect of fermentation on the microbial and / or biochemical changes of food products: Parkouda *et al.* (2015) reported Biochemical changes associated with the fermentation of baobab seeds in Maari. Eze *et al.* (2014) investigated the Biochemical and Microbiological Changes associated with Fermenting African Oil Bean (*Pentaclethra macrophylla* Benth) Seeds; Achi *et al.* (2007) worked on Microbiological and Chemical Changes during Fermentation of Crabs for *ogirinsiko* Production.

It is postulated that the organic acids in Baobab pulp milk and lactic acids produced during fermentation of Baobab fruit pulp Yoghurt could be effective against the growth of spoilage Microorganisms. This study was conducted to assess microbiological changes during the fermentation of this new food (Baobab Fruit Pulp Yoghurt).

MATERIALS AND METHODS

Laboratory Preparation of baobab fruit pulp yoghurt

The traditional method of preparing baobab fruit pulp yoghurt was employed in the laboratory (Eke *et al.*, 2013).

The large lumps of the pulp containing the seeds and fibre were carefully pounded without breaking the seeds using pestle and mortar to loosen the content. The pulp, seeds and the fibre were separated by sieving to produce a fine powdery pulp (BFCS, 2011).

Powdered milk (300g) was dissolved in one and half litres (1.5L) of water and 100g of baobab pulp was dissolved in one litre (1L) of water. The two solutions were pasteurized separately at 80-85°C for 15 minutes after which they were then mixed together. The sample was inoculated with culture of *actobacillus bulgaricus* and *Streptococcus thermophilus* and incubated at room temperature for 15 hours. A control was made in the same way without the use of starter cultures.

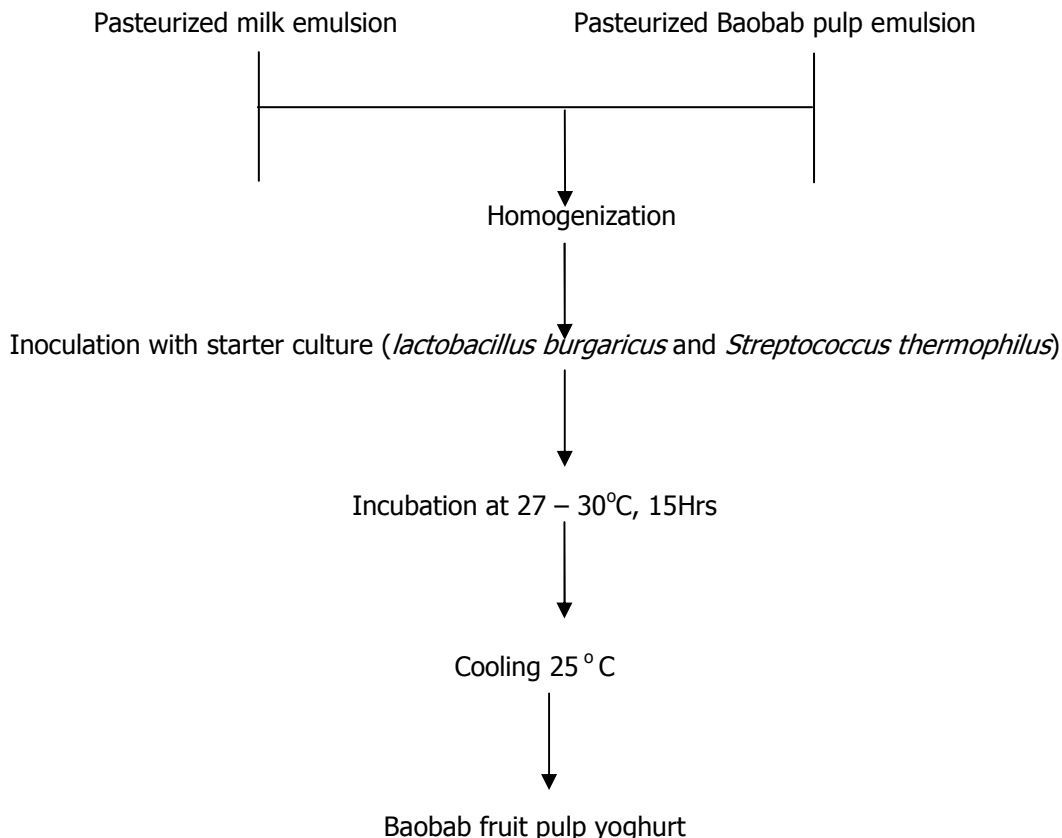


Fig.1: Flow chart for production of Baobab fruit pulp Yoghurt (Eke *et al.*, 2013)

Microbiological analysis

Serial dilution

One ml of the sample was thoroughly mixed with 9 ml of normal saline water as diluents in a McCartney bottle and the content was thoroughly shaken. Subsequent serial dilutions (10^{-2} , 10^{-3} , 10^{-4} and 10^{-5}) were made from this solution by adding serially 1 ml of solution from preceding concentration to 9 ml of diluents, using sterile syringe.

Isolation of Bacteria

A 0.1 ml of various dilutions were transferred separately to agar plates using streak method in triplicates of Nutrient Agar (for total aerobic bacteria), de Man Rogosa Sharpe (MRS) agar (for lactic acid bacteria), *Salmonella Shigella* agar (SSA) for isolation of *Salmonella* and *Shigella* species (Ezeet *al.*, 2014). Bacteria counts were made on nutrient agar plates incubated at 37°C for 24 hours. The total number of colonies developed were counted and expressed as cfu/ml of the original sample. Colonies were differentiated on the basis of morphology and

counts of different colonial types. For specific counts of de Man Rogosa Sharpe (MRS) Agar, plates were incubated at 37°C for 3 – 4 days (Ezeet *al.*, 2014).

Identification of Bacteria

Colonies obtained after incubation were aseptically sub-cultured on Nutrient agar and were incubated for 24 hours at 37°C. The cultural characteristics of isolates on the agar plates were observed. The isolated colonies were then gram stained to differentiate bacteria into gram positive and negative. Different biochemical tests were carried out using standard methods described by Vaughan (1994) and pure cultures of the different organisms identified were sub-cultured and preserved on agar slants at refrigeration temperature (4°C) (Ezeet *al.*, 2014).

Isolation and Identification of Fungi

A 0.1 ml of various dilutions were transferred separately to Potato Dextrose agar (PDA) using streak method in triplicates (Ezeet *al.*, 2014). The plates were incubated at room temperature (25°C) for 5 to 14 days.

The total number of colonies developed were counted and expressed as cfu/ml of the original sample. The method described by Ibrahim and Rahma (2009) was adopted for identification of the fungi using Lactophenol cotton blue. The species encountered were identified in accordance with Cheesbrough (2000) and Ellis, *et al.* (2007).

Monitoring microbial changes

The microbial changes were monitored by Periodical pH measurement to show pH decrease or increase at three hourly, Periodical Temperature measurements to note increase or decrease at three hours interval, Determination of total acidity (lactic acid) at three hours interval and Microbial analysis at three hours interval.

Statistical Analysis

The results were presented in tables and graphs. Analysis of Variance (ANOVA) was used to obtain the effect of fermentation time on the product (Baobab fruit pulp Yoghurt) as well as compare the difference between the test and control group at 5% significance level. The statistical calculations were done using SPSS software version 22.0.

RESULTS AND DISCUSSION

Table 1 shows the microbial count obtained from the test and control samples of Baobab fruit pulp yoghurt which were taken at the interval of every three hours for the period of fifteen hours. There was no growth of *Salmonella* and *Shigella* in both the test and control group. Highest count of total aerobic bacteria was recorded at the onset (0 hour) that is 1.4×10^5 , 2.4×10^4 cfu/ml in the test and control group respectively. The lactic acid bacterial count recorded the highest in the fifteenth hour of 6.2×10^4 and 7.5×10^3 cfu/ml in the test and control respectively. Highest fungal count was observed in the third hour of fermentation (2.8×10^4) and at the onset of fermentation (1.8×10^4) in the test and control respectively. There was significant difference between the test yoghurt and the control yoghurt at $P < 0.05$ toward the end of fermentation.

Table 2 below shows the succession of microorganisms isolated from Baobab Fruit Pulp Yoghurt. The organisms isolated included *Bacillus* species, *Staphylococcus aureus*, *Lactobacillus bulgaricus*, *Saccharomyces cerevisiae*, *Streptococcus thermophilus*, *Hansenula* species and *Micrococcus* species. *Bacillus* species, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Saccharomyces cerevisiae* were found from the beginning to the end of fermentation. *Micrococcus* species appeared in the third hour of

fermentation and disappeared after nine hours. *Staphylococcus aureus* was encountered at the onset and disappeared after six hours of fermentation. *Hansenula* species endured till after twelfth hours when it got weeded out.

The occurrence of microbial isolates which are shown in Table 3 revealed that *Lactobacillus* species were the most predominant organism encountered while *Bacillus* species occurred less in the Baobab fruit pulp yoghurt sample during the investigation period. The table gave the percentage occurrences of microbial isolates obtained from Baobab Fruit Pulp Yoghurt in which *Lactobacillus* species had the highest frequency of occurrence (31%) while *Bacillus* species had the lowest frequency of occurrence (9.2%).

Table 1: Microbial count of Baobab fruit Pulp Yoghurt

Microbial Count (cfu/ml)	GROUP	Time (hrs) of fermentation					
		0	3	6	9	12	15
TBC	Test	$1.4 \times 10^5 \pm 1.0 \times 10^3$	$1.1 \times 10^4 \pm 1.0 \times 10^2$	$1.9 \times 10^4 \pm 1.0 \times 10^3$	$6.8 \times 10^3 \pm 1.0 \times 10^{2a}$	$4.2 \times 10^3 \pm 1.0 \times 10^{2a}$	$2.6 \times 10^3 \pm 1 \times 10^2$
	Control	$1.3 \times 10^4 \pm 1.0 \times 10^{2bcde}$	$2.2 \times 10^4 \pm 1.0 \times 10^{3bc}$	$2.4 \times 10^4 \pm 1.0 \times 10^{3b}$	$1.7 \times 10^4 \pm 1.0 \times 10^{3c}$	$2.7 \times 10^3 \pm 1.0 \times 10^{2d}$	$1.9 \times 10^3 \pm 1 \times 10^{2e}$
SSC	Test	NG	NG	NG	NG	NG	NG
	Control	NG	NG	NG	NG	NG	NG
LBC	Test	$4.5 \times 10^3 \pm 1.0 \times 10^2$	$5.3 \times 10^4 \pm 1.0 \times 10^2$	$7.5 \times 10^3 \pm 1.0 \times 10^{2a}$	$8.0 \times 10^3 \pm 1.0 \times 10^{2a}$	$3.6 \times 10^4 \pm 1.0 \times 10^3$	$6.2 \times 10^3 \pm 1 \times 10^3$
	Control	0.0 ± 0.0^b	0.0 ± 0.0^b	$6.1 \times 10^3 \pm 1.0 \times 10^{2cd}$	$7.1 \times 10^3 \pm 1.0 \times 10^{2d}$	$7.2 \times 10^3 \pm 1.0 \times 10^{2cd}$	$7.5 \times 10^3 \pm 1 \times 10^{cd}$
FC	Test	$1.7 \times 10^4 \pm 1.0 \times 10^{3ae}$	$2.8 \times 10^4 \pm 1.0 \times 10^{3a}$	$1.9 \times 10^3 \pm 1.0 \times 10^{2bc}$	$1.8 \times 10^3 \pm 1.0 \times 10^{2b}$	$1.6 \times 10^2 \pm 1.0 \times 10^{bc}$	$8.0 \times 10 \pm 1 \times 10^{bc}$
	Control	$1.8 \times 10^4 \pm 1.0 \times 10^{3de}$	$1.7 \times 10^4 \pm 1.0 \times 10^{3d}$	$1.1 \times 10^4 \pm 1.0 \times 10^3$	$9.5 \times 10^3 \pm 1.0 \times 10^2$	$7.0 \times 10^3 \pm 1.0 \times 10^{2c}$	$8.5 \times 10^3 \pm 1 \times 10^2$

"Means sharing the same superscript are not significantly different from each other within the same row (Bonferroni's, $P < 0.05$)"

Note: TBC = Total Bacterial Count, SSC = *Salmonella*, *Shigella* Count, LBC = Lactic Acid Bacteria Count, FC = Fungal Count, NG = No Growth

Table 2: Microbial succession during Baobab fruit pulp yoghurt fermentation

Time (Hrs)	Bacteria	Microbial Isolates		pH value
		Lactic acid bacteria	Fungi	
0	<i>Bacillus</i> sp. <i>Staphylococcus aureus</i>	<i>Lactobacillus bulgaricus</i> , <i>S. thermophilus</i>	<i>Saccharomyces cerevisiae</i> <i>Hansenula</i> sp.	6.1
3	<i>Micrococcus</i> sp. <i>Bacillus</i> sp. <i>Staphylococcus aureus</i>	<i>Lactobacillus bulgaricus</i> , <i>S. thermophilus</i>	<i>Saccharomyces cerevisiae</i> <i>Hansenula</i> sp.	6.0
6	<i>Staph. aureus</i> <i>Lactobacillus</i> <i>Micrococcus</i> sp. <i>Bacillus</i> sp.	<i>S. thermophilus</i> <i>Lactobacillus bulgaricus</i> ,	<i>Hansenula</i> sp. <i>Saccharomyces cerevisiae</i>	5.9
9	<i>Micrococcus</i> sp. <i>Bacillus</i> sp. <i>Lactobacillus</i> sp.	<i>S. thermophilus</i> <i>Lactobacillus bulgaricus</i>	<i>Hansenula</i> sp. <i>Saccharomyces cerevisiae</i>	4.9
12	<i>Bacillus</i> sp. <i>Lactobacillus</i>	<i>S. thermophilus</i> <i>Lactobacillus bulgaricus</i>	<i>Hansenula</i> sp. <i>Saccharomyces cerevisiae</i>	4.8
15	<i>Bacillus</i> sp. <i>Lactobacillus</i>	<i>S. thermophilus</i> <i>Lactobacillus bulgaricus</i>	<i>Saccharomyces cerevisiae</i>	4.6

Table 3: Percentage Occurrence of Microbial Isolates of test and control Baobab fruit pulp Yoghurt

Isolate	Test No. (%)	Control No. (%)	Total No. (%)
<i>Bacillus species</i>	03 (6.5)	05 (12.2)	08 (9.2)
<i>Staphylococcus aureus</i>	04 (8.7)	06 (14.6)	10 (11.5)
<i>Micrococcus species</i>	06 (13.1)	08 (19.5)	14 (16.1)
Lactic acid bacteria	18 (39.1)	09 (22.0)	27 (31.0)
<i>Saccharomyces cerevisiae</i>	08 (17.4)	08 (19.5)	16 (18.4)
<i>Hansenula species</i>	07 (15.2)	05 (12.2)	12 (13.8)
Total	46 (100)	41 (100)	87 (100)

No growth of *Salmonella* and *Shigella* was observed because of Pasteurization treatment and probably because of antimicrobial effect of constituents of the Baobab fruit pulp. This disagrees with the finding of Eze *et al.* (2014) who isolated diverse microorganisms including pathogenic organisms like *Pseudomonas aeruginosa*, *Salmonella* Spp, *Aspergillus* Spp etc from fermenting oil bean seeds.

The low total bacterial count obtained in this study as against higher count reported by De *et al.* (2014); Igbabul (2014) and Dirisu *et al.* (2015) whose reports showed high microbiological load in yoghurt sold in Central Market, Kaduna State, Makurdi metropolis, Benue State and Omoku Schools, Rivers State Nigeria respectively shows the microbial quality of this product.

Toward the end of fermentation of the product, there was decrease in all the microbial count except LBC which was observed to increase, this might be linked to increase in Lactic acid production and other inhibitory factors. This finding is in conformity with the report of Adams and Moss (2009) who reported the following as factors contributing to microbial inhibition by Lactic acid bacteria: Low pH, Organic acids, Bacteriocins, Hydrogen Peroxides, Ethanol, Nutrient depletion and low redox potential.

At the end of the fermentation period (15hrs), the TBC and FC of the test group were found to have decreased while LBC was found to increase. There was more fungal count in the control than the test group probably because the test group recorded

high count of Lactic Acid Bacteria which may produce large amount of lactic acids and bacteriocin that might have inhibitory effect on the growth of Yeasts like *Saccharomyces cerevisiae* and *Hansenula* Species (Thomas *et al.*, 2001). The authors reported that yeast growth and fermentation rates could be adversely affected by the presence of high numbers of lactobacilli in incoming mash or in transfer lines.

Groups of microorganisms were isolated from the Baobab fruit pulp yoghurt. These included *Bacillus* species, *Staphylococcus aureus*, *Lactococcus bulgaricus*, *Saccharomyces cerevisiae*, *Streptococcus thermophilus*, *Hansenula* species and *Micrococcus* species. The isolation of microorganisms like *Bacillus* species, *Staphylococcus aureus* and *Micrococcus* species could be due to post pasteurization contamination or their ability to survive pasteurization treatment. This result agrees with the submission of Adams and Moss (2009) who reported that members of the genera *Microbacterium*, *Enterococcus*, *Micrococcus* and *Lactococcus* can survive mild pasteurization treatment. Ng *et al.* (2010) also found out that pasteurized yoghurt had no effect on some bacterial strains. *Saccharomyces cerevisiae* was frequently encountered in the course of the fermentation and this position has since been observed by Adams and Moss (2009) that it is the most frequently encountered yeast in fermented beverages and foods based on fruits and vegetables. That all strains ferment glucose and many ferment other plant associated

carbohydrates such as sucrose, maltose and raffinose.

The persistence of *Bacillus* species, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Saccharomyces cerevisiae* in the yoghurt up to the end of fermentation period could be due to their ability to produce bacteriocins and organic acids such as lactic acid, acetic acid etc which led to the decrease in pH (Jay, 2005). The organic acids, bacteriocin and low pH must have greatly contributed to the inhibition of the growth of other microorganisms especially pathogenic ones as opined by Caplice and Fitzgerald (1999) who reported that fermentation makes foods less hospitable to pathogenic microorganisms. *Bacillus* species though pathogenic, has been reported to produce bacteriocin which inhibit growth of other organisms but favours its growth.

The absence of *Micrococcus* species in the 12th and 15th hours of fermentation may not be

unconnected to the continued presence of *Bacillus* species which has been reported to produce bacteriocin that *Micrococcus* species cannot withstand (Konkur *et al.*, 2006).

CONCLUSION

This study reported low Microbial count and isolated less number and type of Microorganisms from Baobab Fruit Pulp Yoghurt because of the antimicrobial effect of baobab fruit pulp and Pasteurization treatment. The microbes isolated were all fermentative organisms except *Bacillus* species and *Staphylococcus aureus* which might be contaminants of the product.

Baobab Fruit Pulp Yoghurt can be said to be of good Microbiological quality for human consumption. The product if standardized can be marketed as a cheap source of income to the teeming Nigerian population.

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