

Biochemical Changes during the Fermentation of Baobab (*Adansonia digitata*) Fruit Pulp Yoghurt

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Abstract: This study was conducted to assess biochemical changes during the fermentation of *Adansonia digitata* 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. The chemical composition, nutritional content and enzyme activity of the Baobab fruit pulp Yoghurt were determined during its fermentation period at every three hour interval using standard procedures. The proximate composition of the test Baobab fruit pulp yoghurt was 75.72 – 77.52% Moisture, 5.43 – 7.16% Protein, 4.86 – 5.85% Fat, 0.72 – 0.99 Ash, 0.67 – 0.95% Fibre and 8.98 – 10.78% carbohydrate. At the end of fermentation time, there was significant difference between the test and control Baobab fruit pulp yoghurt at 5% level of significance. The levels of Calcium (Ca), Iron (Fe), Sodium (Na), Magnesium (Mg) and Potassium (K) were found to be in the range of 4.8 – 16.3 mg/ml, 0.4 – 1.2mg/ml, 3.0 – 5.9mg/ml, 19.9 – 25.3mg/ml and 3.0 – 4.0mg/ml respectively. The activity of the enzyme Amylase, Protease and Lipase measured in unit/ml were found to be in the range of 1.30 – 5.33, 0.00 – 5.69 and 0.89 – 2.31 respectively. The results of proximate analysis and mineral determination showed that the product is chemically and nutritionally rich. The addition of Baobab fruit pulp improved the quality of the Yoghurt. Baobab fruit pulp Yoghurt is therefore recommended for human consumption based on its good chemical and nutritional quality.

Keywords: Baobab, Biochemical changes, Fermentation, Fruit pulp, Yoghurt

INTRODUCTION

Fermentation is one of the methods used to preserve food and alter its quality. Yeast, especially *Saccharomyces cerevisiae*, is used to leaven bread, brew beer and make wine. Certain bacteria, including lactic acid bacteria, are used to make yoghurt, cheese, hot sauce, pickles, fermented sausages and dishes such as kimchi (Helmenstine, 2016). A common effect of these fermentations is that the food product is less hospitable to other microorganisms, including pathogens and spoilage-causing microorganisms, thus extending the food's shelf-life. Some cheese varieties also require molds to ripen and develop their characteristic flavors (Caplice and Fitzgerald, 1999).

Eze *et al.* (2014) said food fermentation involves the use of microorganisms and enzymes for the production of foods with distinct quality attributes that are quite different from the original agricultural raw materials. They maintained that many of the

food fermentations are natural or controlled fermentation consisting of different species and genera of yeast, fungi and bacteria.

Baobab fruit pulp yoghurt is made from baobab pulp and cow or goat milk using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as starter cultures (Eke *et al.*, 2013). Similar product made in Zimbabwe is called 'mutandabota' (Mpofu *et al.*, 2014).

Abdalla *et al.* (2010) reported that Baobab fruit pulp, due to the combination of health claims (such as probiotic and anti-oxidation properties, high calcium content, and anti-inflammatory effect) and food technological functions (due to its high pectin and fiber content, baobab fruit pulp gives beverages a thicker consistency and can be also used as filler), is a very interesting candidate for a new generation of functional foods and drinks. According to Eke *et al.* (2013), the growing incidences of malnutrition especially in a developing country like Nigeria are quite alarming.

Researchers are now being directed to explore new and nonconventional sources of food such as baobab that is grown in the arid and semiarid regions of the world.

The development of Baobab fruit pulp Yoghurt may reduce the problem of malnutrition reported by Eke *et al.* (2013) hence the need to investigate the effect of fermentation on the quality of this new food product.

It was against this background that this study was undertaken to assess the biochemical changes during the fermentation of Baobab fruit pulp Yoghurt. The study investigated the chemical composition, nutritional content and the enzyme activity of the product.

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).

Three hundred grammes (300g) of powdered milk was dissolved in One and half litres (1.5L) of water to give One and half litres (1.5L) of powdered cow milk emulsion and 100g of baobab pulp was dissolved in one litre (1L) of water to form one litre (1L) of baobab fruit pulp solution. (Mpofu *et al.*, 2014) modified. The two solutions were pasteurized separately at 85°C for 15 minutes after which they were then mixed together. The sample was inoculated with culture of *Lactobacillus 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 (Eke *et al.*, 2013).

Proximate Analysis of the Samples

The parameters that were determined included crude protein, carbohydrates, lipid, moisture content, ash and crude fibre.

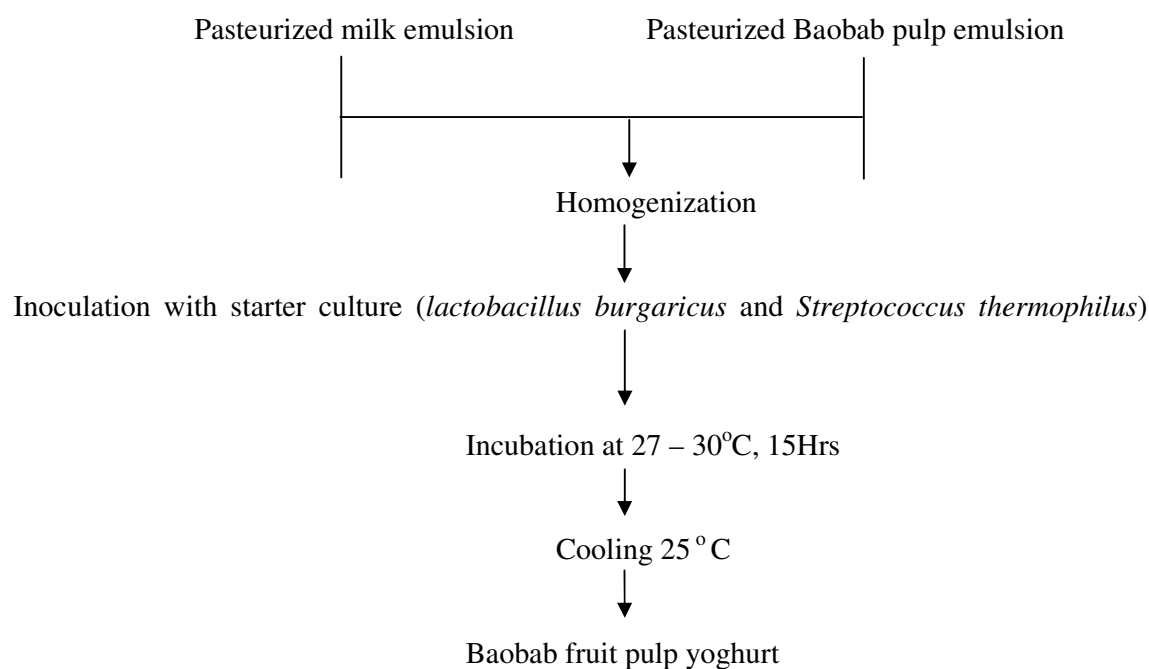


Fig.1: Flow Chart for Production of Baobab Fruit Pulp Yoghurt (Eke *et al.*, 2013)

These parameters were determined using standard procedures described by Association of Official Analytical Chemist (AOAC, 2000) and Dublec (2011).

Determination of Mineral Composition

The baobab fruit pulp yoghurt samples were analyzed for mineral composition such as Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg) and Iron (Fe) with Atomic Absorption Spectrophotometer (AAS) model 210 VGP (Hernandez 2005; Akinola *et al.*, 2008).

Crude Enzyme Extraction from Baobab Fruit Pulp Yoghurt

Standard procedures described by Titilayo and Musliu (2016) were followed in the extraction of enzymes for protease, amylase and lipase activities.

Determination of Protease Activity in Baobab Fruit Pulp Yoghurt

Protease activity was determined according to Titilayo and Musliu (2016). This was done by adding 5ml of enzyme extract to 10ml of 2% light soluble casein solution and incubated at 35°C for 30min. The reaction was terminated by adding 10ml of 10% trichloroacetic acid. Undigested protein was removed by centrifugation at 4000rpm for 15 min. The trichloroacetic acid soluble peptides in the supernatant were determined by using tyrosine as standard solution. One unit of protease activity was defined as the amount that produced 1.0μmol of tyrosine in 1.0ml of the trichloroacetic acid-soluble peptides under the assay conditions.

Determination of Amylase Activity in Fermenting Baobab Fruit Pulp Yoghurt

Amylase activity was determined by mixing 2ml of the enzyme extract with 1ml of 1% starch solution and incubated for 1 hour at 40°C. The reaction was stopped by adding 3 ml of dinitrosalicylic acid reagent, and heated for 5 minutes. After cooling, it was diluted with 18 ml distilled water and the optical density measured at 550 nm in a spectrophotometer. In a blank determination, the dinitrosalicylic acid reagent was added before the starch solution (Titilayo and Musliu, 2016).

Determination of Lipase Activity in Fermenting Baobab Fruit Pulp Yoghurt

Lipase activity was determined according to Titilayo and Musliu (2016). Five millilitre (5ml) of the extract was added to 1ml of Olive oil, 0.4g of sodium fluorochlorate, 1ml of 0.1M CaCl and 6ml of 0.1M sodium acetate buffer, pH 5.5. After incubation of the mixture at 35°C for 1 h for the enzyme to liberate fatty acids from the oleic acid, the reaction was terminated by adding 40 ml of absolute alcohol and titrated against 0.02M potassium hydroxide using phenolphthalein as an indicator. A blank determination was carried out by using 5ml of distilled water in place of the enzyme extract. The titre value, i.e. the amount of alkali required to neutralize the liberated fatty acids, was expressed as oleic acid with the unit of enzyme being the amount of enzyme required to liberate 1.0mg of oleic acid per minute (Titilayo and Musliu, 2016).

Monitoring Biochemical Changes and Enzyme Activities during Fermentation Process

Proximate analysis, Mineral determination and Measurement of enzyme activity were carried out during the fermentation of the baobab fruit pulp yoghurt every three hours (AOAC, 2000; Dublec, 2011; Eke *et al.*, 2013; Eze *et al.*, 2014 and Titilayo and Musliu, 2016).

Statistical Analysis

The results were presented in tables. 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 a significance level of 0.05. The statistical calculations were done using SPSS software version 22.0.

RESULTS

Proximate Composition of Baobab Fruit Pulp Yoghurt

At the end of fermentation time (15hrs), there was significant difference between the test and control Baobab fruit pulp yoghurt at $P > 0.05$ of the moisture, protein, fat, ash, fibre and carbohydrate as shown in Table 1.

Table 1: Proximate Composition of Baobab Fruit Pulp Yoghurt (Mean % with S.E)

Chemical Composition (%)	GROUP	Time (hrs) of fermentation					
		0	3	6	9	12	15
Moisture	Test	77.52±0.01	76.53±0.01 ^a	76.85±0.01	75.72±0.01	76.08±0.01	76.60±0.01 ^a
	Control	76.94±0.01	75.72±0.01 ^b	74.9±0.01	74.85±0.01	75.72±0.01 ^b	75.64±0.01
Protein	Test	5.43±0.01	6.32±0.01	6.67±0.01	6.85±0.01 ^b	6.87±0.01 ^b	7.16±0.01
	Control	5.37±0.10	5.83±0.01	6.48±0.01	6.57±0.01 ^a	6.62±0.01 ^a	6.84±0.01
Fat	Test	4.86±0.01	5.00±0.01	5.04±0.01	5.62±0.01	5.84±0.01 ^{ac}	5.85±0.01 ^a
	Control	5.45±0.01	5.56±0.01	5.73±0.01	5.78±0.01 ^b	5.85±0.01 ^{bc}	5.82±0.01 ^b
Ash	Test	0.73±0.01 ^b	0.98±0.01 ^a	0.81±0.01	0.99±0.01 ^a	0.74±0.01 ^b	0.72±0.01 ^b
	Control	0.92±0.01 ^c	0.87±0.01 ^d	1.15±0.01	1.03±0.01	0.9±0.01 ^d	0.88±0.01 ^{cd}
Fibre	Test	0.68±0.01 ^a	0.79±0.01	0.76±0.01	0.95±0.01	0.67±0.01 ^b	0.69±0.01 ^{ab}
	Control	0.79±0.01 ^c	0.76±0.01 ^c	0.61±0.01 ^e	0.70±0.01	0.82±0.01	0.75±0.01 ^c
Carbohydrates	test	10.78±0.01	10.38±0.01	9.87±0.01 ^a	9.85±0.01 ^a	9.82±0.01 ^a	8.98±0.01
	Control	10.53±0.01	11.26±0.01	11.13±0.01 ^b	11.10±0.01 ^b	10.09±0.01 ^c	10.07±0.01 ^c

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

The Mineral Composition of Baobab Fruit Pulp Yoghurt

Table 2 revealed the nutritional elements contained in the yoghurt sample. The

Calcium concentration of the Baobab fruit pulp yoghurt sample increases with increase in the fermentation time

Table 2: Mineral Composition of Baobab Fruit Pulp Yoghurt (mean concentration with S.E)

Elements (mg/ml)	GROUP	Time (hrs) of fermentation					
		0	3	6	9	12	15
Ca	Test	4.83±0.15	10.77±0.50	12.7±0.33	15.33±0.06 ^a	16.13±0.11 ^b	16.27±0.40 ^{abf}
	Control	11.97±0.15 ^c	12.83±0.37 ^{cd}	17.43±0.23 ^c	14.2±0.17 ^d	20.23±0.15	16.47±0.15 ^{ef}
Fe	Test	1.17±0.58	0.6±0.00 ^a	0.60±0.00 ^a	0.63±0.06 ^{ab}	0.37±0.06 ^c	0.37±0.06 ^{bcg}
	Control	0.60±0.00 ^e	1.0±0.00 ^d	1.30±0.06	0.40±0.00 ^f	0.90±0.00 ^d	0.40±0.00 ^{efg}
Na	Test	5.93±0.31 ^a	4.86±0.11 ^h	3.00±0.0 ^b	4.0±0.0 ⁱ	5.0±0.0 ^{ak}	3.0±0.17 ^b
	Control	7.13±0.11 ^e	5.00±0.00 ^{gh}	4.00±0.0 ^{dh}	4.0±0.0 ^{dj}	5.0±0.0 ^{djfk}	9.0±0.0 ^{ef}
Mg	Test	25.27±0.15	21.70±0.0 ^a	21.5±0.10 ^{ab}	21.13±0.06 ^{bch}	20.77±0.15 ^{cd}	19.9±0.17 ^d
	Control	22.00±0.10	24.27±0.06 ^e	24.10±0.10 ^{ef}	21.2±0.10 ^{gh}	23.73±0.06 ^f	20.63±0.06 ^g
K	Test	3.33±0.58 ^a	4.00±0.00 ^a	4.00±0.00 ^a	4.00±0.00 ^a	3.00±0.00 ^a	3.00±0.00 ^a
	Control	4.00±0.00 ^a	4.00±0.00 ^a	4.00±0.00 ^a	4.00±0.00 ^a	4.00±0.00 ^a	3.00±0.00 ^a

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

Measurement of Enzyme Activity of the Fermenting Baobab Fruit Pulp Yoghurt

The enzyme activity of the fermenting Baobab fruit pulp yoghurt was measured

after crude enzyme extraction. Table 3 indicated that there was increase in the microbial enzyme activity as the fermentation progresses with time.

Table 3: Enzyme Activity of Baobab Fruit Pulp Yoghurt (Mean activity with S.E)

Enzyme Activity (unit/ml)	GROUP	Time (hrs) of fermentation					
		0	3	6	9	12	15
Amylase	Test	1.3±0.1 ^a	1.32±0.01 ^a	4.12±0.01	4.55±0.01	4.63±0.01	5.33±0.1
	Control	1.14±0.01 ^b	1.27±0.01 ^d	3.08±0.01	3.26±0.01	3.59±0.01	3.76±0.01 ^f
Protease	Test	0.0±0.0 ^a	1.39±0.01	3.95±0.01	5.30±0.01	5.40±0.01	5.69±0.01
	Control	0.00±0.00 ^a	1.23±0.01	3.7±0.01	4.20±0.01	4.63±0.01	3.48±0.01
Lipase	Test	0.89±0.01	1.12±0.01 ^a	1.28±0.01	1.52±0.01	1.71±0.01	2.31±0.01
	Control	0.65±0.01	1.11±0.01 ^a	1.17±0.01	1.24±0.01	1.38±0.01	1.89±0.01

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

DISCUSSION

Proximate Analysis of Baobab Fruit Pulp Yoghurt

There was remarkable increase in protein and fat with increase in the fermentation time of both the test and control groups (Table 1). The increase could be attributed to release of protein by some microorganisms and enzymes which are proteinous in nature (Adams and Moss, 2009). There was decrease in percentage moisture and carbohydrate of both the test and control samples of the yoghurt. The decrease in the percentage of Carbohydrate might be because of its conversion to lactic acid by Lactic acid bacteria and other fermentative microorganisms. This result agrees with the report of Omafuvbe *et al.*, (2004) who found that Fermentation led to increase in Lipid, Crude Protein, Moisture content and reduction in Ash, Fibre and Carbohydrate. The decrease in the moisture level at time 3 and 15 hours of the test samples and between time 3 and 12 of the control samples were not statistically significant at $P < 0.05$. There was slight increase in the percentage ash and fibre in both the groups. The results of the proximate analysis of Baobab fruit pulp yoghurt had higher percentage protein (7.16) compared to the values obtained by Ndife *et al.* (2014) who reported 3.05 as the percentage protein in plain yoghurt. The values of the protein and carbohydrate revealed in this study were lower than the values obtained by Ihemeje *et al.* (2015) who reported percentage

composition of plain yoghurt as 1.70, 9.97, 1.8, 0.44, 0.32 and 84.67 for carbohydrate, protein, fat, ash, fibre and moisture respectively.

The proximate composition of Baobab Fruit Pulp Yoghurt produced in the Laboratory was similar to the values obtained with plain Yoghurt by Igbabul *et al.* (2014) who reported 5.26, 3.25, 0.21, 1.02, 78.62 and 11.69 as the percentage composition of Protein, Fat, Crude Fibre, Ash, Moisture and Carbohydrate respectively.

The Mineral Composition of Baobab Fruit Pulp Yoghurt

From table 2, Calcium content increases significantly at 5 % significant level with fermentation time in both test and control group. The increase in Calcium content agrees with the finding of Nnam and Obiakor (2015) who observed that there was increase in Calcium, Potassium and Zinc levels during the fermentation of baobab seeds and rice grains.

Magnesium decreases significantly with time in the test group but fluctuates with time in the control group. Iron, Sodium and Potassium did not show remarkable changes with time in both the test and control group. Potassium did not differ with fermentation time and between test and control group.

The levels of Calcium, Sodium, Potassium, Magnesium and Iron determined in this study showed to have fallen within the recommended values of U.S. Food and Drug Administration (FDA, 2013).

The organization reported that recommended intake of nutrients vary by age and gender setting the following for adults per day: Mg = 400mg, Ca = 1,000mg, Fe = 18g, Na = 2,400mg and K = 3,500mg.

Enzyme Activity in Baobab Fruit Pulp Yoghurt

Significant increase in amylase enzyme activity in both test and control samples except at the early hours of fermentation (from 0 – 3 hours) as indicated in Table 3. Amylase activity differed significantly at 5% level of significance between the test and the control group. Protease activity differed significantly at 5% level of significance with increase in time of fermentation and between the test and control group except at the onset (0 time) when the test and control group recorded similar protease activity. Lipase activity was observed to differ within fermentation time and between groups except at the third hour when the control and test groups were the same. The increase in enzymatic activity might be related to the increase in number of micro-organisms especially lactic acid bacteria and yeasts whose increase may be the reason for the concomitant production and release of enzyme. The enzymes released in the Baobab fruit pulp yoghurt led to the decrease in carbohydrate content. This finding is in agreement with the report of Eze *et al*, (2014) who found out that

enzymes are involved in fermentation of raw materials leading to products production with distinct quality. The amounts of amylase and protease enzymes measured were higher than that of Lipase and hence this might be the reason why no decrease in fat was noticed.

The nutritional elements determined were Calcium, Sodium, Potassium, Magnesium and Iron. Their level fall within the recommended values set by U.S. food and drug administration (FDA, 2013).

CONCLUSION

The proximate composition of Baobab Fruit Pulp Yoghurt was found to change with fermentation time. The finding revealed that the product is rich in nutritional elements such as Calcium, Iron, Sodium, Magnesium and Potassium. Baobab Fruit Pulp Yoghurt showed protease, amylase and lipase enzymes activities during its fermentation process.

RECOMMENDATIONS

1. Baobab Fruit Pulp Yoghurt is recommended for human consumption based on its good chemical and nutritional quality.
2. Studies should be carried out on enzyme extraction and purification during the fermentation of Baobab Fruit Pulp Yoghurt.

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