Chemical Composition and Microbiological Quality of Baobab (*Adansonia digitata*) Fruit Fortified Yoghurt

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Abstract: The effect of fortification of reconstituted skimmed cow milk with baobab fruit milk on the physicochemical, microbial and sensory characteristics of yoghurt was studied. Pasteurized milk was distributed and substituted as follows: 100% cow skimmed milk (control-sample A), 100% baobab milk (sample B), 90% cow milk: 10% baobab milk (sample C), 80% cow milk: 20% baobab milk (sample D) and 70% cow milk: 30% baobab milk (sample E) milk: baobab milk juice . The five (5) samples were inoculated with 5% concentration of starter culture and incubated at 45^{o}C for 18hours. The coagulation obtained after incubation were broken to obtain smooth texture yoghurt. The yoghurts were stored at 6 °C for 21 days (3 weeks) and their quality monitored. Results showed that moisture, fat, protein, viscosity and pH decreased with increase in fortification levels. Moisture content ranged from 80.21% in sample A to 77.15% in sample E, same trend was observed in fat and protein contents. However, the carbohydrate and ash content increased with fortification level. pH ranged from 4.84 in sample A to 4.01 in sample E. Same trend was observed in ascorbic acid content and Titratable acidity. Viscosity decreased from 351.01% in sample A to 227.00% in sample E. Calcium and Sodium decreased with increase in fortification level while Phosphorous and Potassium increased. The sensory evaluation results showed that sample C and sample E were most preferred in terms of appearance (6.4). Sample A (control) had the highest overall acceptability score followed by Sample C. It can be concluded that yoghurt made from milk fortified with baobab fruit milk, is nutritious and safe for consumption.

Keywords: Baobab fruit, Chemical, Microbial quality, Yoghurt

INTRODUCTION

The Baobab fruits pulp is an important raw material that can be sucked chewed or made into drinks when mixed with water or milk either with or without sugar. The fruit pulp of baobab is about 90% dry matter, the extract reverse of the expectation is a fruit. The dry matter is nutritionally unlike that of cereals or root crops such as potatoes. The baobab fruit pulp comprises of about 80% Carbohydrate, 10% Fiber, 5% Crude protein, and 0.2% Fats (De Caluwé *et al.,* 2010). The food energy level has been found to range from 200 to 350 calories per 100g of dry weight. It has low fat content; the high energy content must have come from carbohydrate, mainly pectin, glucose, and other sugars and miscellaneous matter (Blasko, 2011).

Yoghurt production have principally employed milk as its raw material and the use of baobab fruit milk that has proximate composition similar to the natural cow milk, has not actually been fully exploited; this has led to the under-utilization of the indigenous fruit (Baobab fruits milk) as well as limited the scope of yoghurt production to conventional raw materials. However, yoghurt produced from skimmed milk is found to be too expensive and many people may not be able to afford it. The baobab fruit pulp has been reported by various authors to have a particularly high antioxidant capability mainly because of its high natural vitamin C content (Eke et al, 2013), which is equivalent to 6 oranges per 100g. Anti-oxidants protect the cells of organisms from damage by free radicals. A deficiency of vitamin C weakens the immune system and promotes the susceptibility to disease. Deficiency of vitamin C also results in scurvy. Additionally, vitamin C aids the bodily uptake of iron and calcium, of which the baobab fruit pulp contains more than double than the same amount of milk (Adams and Moss, 1999). The use of baobab fruit pulp in the production of yoghurt could be nutritious and reduce the cost of the production, thus making it more affordable.

This research work was carried out to assess the nutritional composition, microbial quality and sensory qualities of stored yoghurt fortified with baobab milk.

MATERIALS AND METHODS

Good quality Baobab fruits were purchased from Rimi Market in Kano State.

The skimmed milk was also obtained in a supermarket in Ota.

Method of producing conventional Yoghurt

Four (4) liters of Reconstituted skimmed cow milk powder were heated to 90°C for 15 minutes to pasteurize the milk; it was then cooled to 45°C. The pasteurized milk was distributed into four (4) portion (1 liter per sample), one sample is hundred percent skimmed milk which served as control, and the other three (3) samples were substituted with baobab milk at various percentages (90: 10%, 80: 20%, 70: 30% and 100% baobab milk) respectively (Table 1).

Table 1: Formulation of milk for yoghurt production

S/N	Samples	Powdered milk (%)	Baobab milk (%)
1	Sample A	100	0
2	Sample B	0	100
3	Sample C	90	10
4	Sample D	80	20
5	Sample E	70	30

The five (5) samples were inoculated with 5% concentration of starter culture (50ml of branded yoghurt with live organism was used as starter culture). Incubation was done 45°C for 18hours. The coagulation obtained after incubation were broken using blender to obtain smooth texture yoghurt.

Reconstituted Skimmed cow milk powder
⇩
Heated
⇩
(90°C for 15mins)
⇩
Cooling (45°C)
⇩
Inoculation
⇩
(Starter culture, 50ml/1Liter)
⇩
Incubation
⇩
(45°C for 18hours)
⇩
Homogenization
⇩
Cooling to 5°C
⇩
Yoghurt

Figure 1: Flow chart for the production of conventional yoghurt

Method of Producing Baobab Fruit Milk

Baobab pulp (500g) was placed in a 1liter of water and allowed to soak; it was then squeezed and filtered using a 2mm pore size muslin cloth to obtain the milky juice. The seed and other residues were removed.

Baobab fruit
⇓
Sorting
⇓
Removal of shell
⇓
Cleaning
⇓
Soaking
⇓
Squeezing (To remove the seed)
⇓
Filtration (Using muslin cloth)
⇓
Incubation (at room temperature for 24hours
⇓
Homogenization
⇓
Baobab Milk

Figure 2: Flow chart for extraction of juice from Baobab fruit

Proximate analyses of the Yoghurt samples

The ash, moisture and fat content of the yoghurt samples were determined by standard method as described by Association of Official Analytical Chemists (AOAC, 2010). The crude protein content was determined by Kjeldah method as described by AOAC (2005) and Carbohydrate content was determined by difference as described by AOAC (2010).

Physicochemical analyses of the Yoghurt samples

The pH was measured using pH meter (BDH England). The Titratable acidity and total solids were determined by method described by Association of Official Analytical Chemists (AOAC, 2010).

Determination of Anti-nutritional composition of Yoghurt samples

Phytate content in the samples was determined according to AOAC (2010) and Oxalate content was determined according to the method described by Munro (2000).

Mineral Content determination of Yoghurt samples

Calcium, Sodium Potassium and Phosphorous of the yoghurt samples were carried out according to the standard method described by AOAC (2010).

Microbiological Analysis of Yoghurt samples

Aerobic plate count

The total plate count was carried out on the fortified yoghurt samples using the method of Fawole and Oso (1988). Tenfold serial dilution was made by transferring aseptically, one milliliter of yoghurt to a tube containing 9ml of sterile distilled water. This was labelled 10^{-1} dilution. From the 10^{-1} dilution, 1ml was further transferred to another tube containing 9ml of the diluent and this second tube was labelled as 10^{-2}. The procedure was repeated up to 10^{-9} dilutions. From the dilution tubes, inoculation was made into appropriately labelled duplicate Petri dishes. This was followed by pouring aseptically, molten nutrient agar. The plates were mixed well, allowed to solidify and incubated at 37^{o}C for 24 hrs. Following incubation, plates with between 30 and 300 hundred colonies were selected for counting. The average number of colonies was multiplied by the inverse of the dilution factor to get the cfu/ml.

Total Fungal Counts (TFC) (Yeasts and moulds)

Potato Dextrose Agar (PDA) was used for the enumeration of yeasts and moulds and the plates were incubated at 28˚C for 72 hours. Visible colonies were counted and expressed in log cfu/g per sample homogenate.

Storage Stability

Microbial analysis and sensory evaluation of the yoghurt samples were determined immediately after processing and during storage at one week intervals for three weeks under ambient temperature, refrigerating temperature and freezing temperature.

Sensory Evaluation of Yoghurt samples

The sensory evaluation was carried out using the multiple comparison tests as described by (Hashim *et al.,* 2009). The five (5) samples of yoghurt were served to 30 semi-trained panelists made up of staff and students of Bells University of Technology, Ota, Ogun state, who are familiar with the sensory attributes- taste, aroma, flavor, color and mouth feel of yoghurt. A 9-point hedonic scale was designed to measure the degree of preference of the samples; samples were presented in identical containers, coded with 3-digit random numbers served simultaneously to ease the possibility of the panelists to re-evaluate a sample. The categories were converted to numerical scores and the results were subjected to analysis of variance. Precautions were taken to prevent carry-over flavor during the tasting by ensuring that panelists ate tiny piece of cracker biscuit or rinse their mouth with water after evaluating each sample.

Statistical Analysis

The data obtained from the analyses were statistically analysed using analysis of variance (ANOVA) and means were separated using Duncan multiple range test; in the Statistical Package for the Social Sciences (SPSS 23, 2018). The statistical significance was accepted at $p< 0.05$.

RESULTS AND DISCUSSION

Effect of Baobab milk fortification on the proximate analysis of the Yoghurt sample

The effect of baobab fruit on the proximate analysis of yoghurt sample is shown in Table 2. There was an increase in the percentage ash content production, the ash content for the baobab yoghurt was 1.14% whereas for the yoghurt is 0.18% which according Eke *et al*., (2013) comply with the standard percentage ash content for non-fat skimmed milk. The ash content increased as the proportion of baobab fruit pulp was increased in the product. This could be due to the fact that baobab fruit pulp has high ash content (Conceptual, 2008). The high ash value in the produced samples agreed with the ash content (0.89-1.13%) of non-fat skimmed yoghurt (Mistry and Hassan, 1992). This result is similar to earlier reports of Obadina *et al.* (2013), with ash content values of 0.23- 0.74%, during soymilk fermentation. There was an increase in the percentage of protein content after substituting with the baobab fruit. This is as a result of the addition of protein sources (milk) during the formulation of the yoghurt as baobab has low protein content and fermentation also decrease the level of proteins which is due to possible increase of microflora that uses protein for their metabolism. This is in line with the findings of Obadina *et al.* (2013). The fat content ranged between 0.64 - 4.65%. The fat content fell within the limit for low fat yoghurt (<4.7%) there was an increase in percentage fat content with the baobab milk having a fat content 0.64%. The increase in fat content after fermentation may be due to the activities of the lipases which hydrolyse fat to glycerol and fatty acids. The total carbohydrate content of the yoghurt is 10.43%. The total carbohydrate content for the baobab milk was 19.36%, this indicate a significant increase in the total carbohydrate content after fermentation which could probably be due to the addition of carbohydrate sources (baobab milk) during the production of yoghurt.

Table 2: Effect of Baobab milk fortification on the nutritional value of the yoghurt samples

Sample	Protein (%)	Ash (%)	Fat (%)	Carbohydrate (%)	Dry matter (%)	Moisture content (%)
A	4.46[e]	0.18[a]	4.65[d]	10.42[a]	19.67[a]	80.21[d]
B	2.03[a]	1.14[c]	0.64[a]	19.35[e]	23.17[e]	76.82[a]
C	4.14[d]	0.62[ab]	3.90[c]	12.51[b]	21.17[b]	78.84[c]
D	3.51[c]	0.99[ab]	3.21[c]	14.39[c]	22.20[c]	77.90[b]
E	2.32[b]	1.13[c]	2.21[b]	17.14[d]	22.90[d]	77.15[ab]

Means with different superscripts on the same row are significantly different (P<0.05).

Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Effect of Baobab fruit fortification on the Physicochemical properties and ascorbic acid content of Yoghurt

The effect of Baobab fruit enrichment on the physicochemical properties of yoghurt is shown in Table 3. The Titratable acidity of the yoghurt samples ranged between 0.38-0.42. This complies with the minimum standard (0.6) of Titratable acidity in commercial yoghurt. The pH is a determining factor in the decrease or increase in the Titratable acidity of the yoghurt samples. The pH ranged from 3.09 - 4.84 with sample A having the highest pH. The results were similar to that of Oluwabamiwa and Kolapo (2007) that reported pH values of 4.81 in soy yoghurt.

The viscosity of the samples was between 29.80-351.01rpm Yoghurt viscosity is directly related to the protein content (Sodini *et al*., 2005).

The vitamin C content of 100% baobab milk is 209.6mg/100g. The reason for high vitamin C content is due to the fact that baobab pulp is particularly rich in vitamin C and it increase with increase in fortification level with 70% milk + 30% baobab having the highest value of 14.82mg/100g.

Effect of Baobab milk fortification on the Minerals composition of Yoghurt

The mineral content of the yoghurt is shown on Table 4. The 100% yoghurt has the lowest phosphorus content of 3.77mg/kg. The 90%, 80% and 70% milk had 0.99mg/kg, 0.95mg/kg and 0.86mg/kg respectively, which increased with the addition of Baobab milk. The high phosphorus content is desirable as it increases bone health among humans (Lee *et al*., 2014). Same trend was observed in Potassium contents which ranged from 594.42– 740.38mg/kg with Sample E having the highest value. Potassium helps the nerves to function and muscles to contract as well as helping with normal heartbeat, increasing iron utilization (Elinge *et al*., 2012) and is beneficial to people taking diuretics to control hypertension who suffer from excessive excretion of potassium through the body fluid (Gemede *et al*., 2016). Sample A (100% yoghurt) had the highest calcium content of 1.33mg/kg. Calcium plays key roles in bone formation and mineralization. The calcium requirements during growth, pregnancy, and lactation are increased (Oscar *et al*., 2004). This, therefore, means that the Baobab enriched yoghurt would be a beneficial drink for the children, pregnant and lactating mothers as well as the elderly whose calcium requirement is high. The 100% baobab fruit had the lowest amount in calcium and sodium, 0.68mg/kg and 86.73mg/kg respectively while the 100% yoghurt had the highest amount of sodium (298.94mg/kg).

Effect of Baobab milk fortification on Anti-nutritional content of Yoghurt samples

The phytate content of the baobab fruit is 2.103mg (Table 5), according to Abdoulaye *et al.,* (2011) Phytate level in food should not be more than 25mg. Hence a decrease in phatate level in yoghurt samples was observed after the lactic acid bacteria fermentation. Lactic acid fermentation provide an optimum pH condition for enzymatic degradation of phytate in food. The oxalate level is 444.27mg the decrease in oxalate level after fermentation may likely be due to the breakdown of oxalase complexes to release free nutrients which improve the availability of the nutrients. Oxalate is responsible for the astringent taste of baobab fruit (Abdoulaye *et al.,* 2011).

Table 3: Effect of Baobab fortification on the physico chemical properties and ascorbic acid content of yoghurt.

Samples	pH	Titratable acidity	Viscocity	Ascorbic acid mg/100g
A	4.84[c]	0.38[a]	351.01[e]	0.8[a]
B	3.09[a]	0.51[c]	29.80[a]	209.6[e]
C	4.69[bc]	0.40[a]	332.10[d]	9.3[b]
D	4.41[b]	0.41[b]	267.00[c]	10.57[c]
E	4.01[b]	0.42[b]	227.00[b]	14.82[d]

Means with different superscripts on the same row are significantly different (P<0.05).
Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Table 4: Effect of baobab milk fortification on the minerals composition of yoghurt samples.

Sample	Calcium (mg/kg)	Sodium (mg/kg)	Potassium (mg/kg)	Phosphorous (mg/kg)
A	1.33[e]	298.94[e]	894.33[d]	3.77[d]
B	0.68[a]	86.73[a]	1001.36[e]	5.16[e]
C	0.99[d]	229.61[d]	594.42[a]	2.16[a]
D	0.95[c]	199.22[c]	636.41[b]	2.41[b]
E	0.86[b]	138.44[b]	740.38[c]	2.96[c]

Means with different superscripts on the same row are significantly different (P<0.05).
Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Table 5: Effect of Baobab milk fortification on anti-nutritional content of yoghurt samples.

Samples	% Phytate	% Oxalate
A	1.931[c]	563.20[e]
B	2.103[c]	444.27[a]
C	1.570[a]	427.45[a]
D	1.671[b]	460.75[c]
E	1.912[c]	487.74[d]

Means with different superscripts on the same row are significantly different (P<0.05).
Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Effect of Baobab milk fortification on the storage stability of Yoghurt samples

Baobab yoghurt was stored for 21 days at refrigeration temperature during which they were observed for changes in pH, Titratable acidity and microbial counts (Table 6-9). There was a continuous increase in pH during storage of the yoghurt sample with little or no significant differences. Low pH in the yoghurt samples could be attributed to the presence of organic acids in baobab fruit and also continuous fermentation caused by oxidation of organic compounds present in the yoghurt samples. An increase in titratable acidity supports earlier reports of some researchers (Gesinde *et al.*, 2008;

Almeida *et al*., 2007), and this could be due to the accumulation of some organic acids such as lactic acid and acetic acid resulting from the activities of the Lactic acid bacteria in the fermenting foods.

The increase in the bacterial counts (Table 6) indicated a continuous deterioration of the yoghurt quality. This could be due to increase in acid levels resulting from continuous fermentation caused by the oxidation of organic compounds present in yoghurt sample and since baobab is rich in organic acid, this attribute further expose the yoghurt sample to increased microbial growth during storage.

The fungal load (Table 7) was within the acceptable limits of <10 within the first 14 days, it was then followed by continuous increase in fungal load during the storage period. The increase in fungal load may be due to the decrease in pH which provides a selective environment for the proliferation of fungi. Yeast is the major causes of spoilage in yoghurt.

Effect of Baobab milk fortification on the sensory characteristics of Yoghurt samples

The results of the sensory analysis are shown on Table 10. The powdered milk yoghurt (control) has higher score in appearance and aroma than baobab yoghurt; this can be as a result of the baobab having an off-white colour which the panelists are not familiar with. Powdered milk yoghurt also has higher aroma score than baobab yoghurt. However, the 100% yoghurt has highest overall acceptability score; followed by the 90% milk + 10% baobab, then 80% milk + 20% baobab and 70% milk + 30% baobab, the 100% baobab yoghurt had the least overall acceptability, appearance and aroma scores respectively.

The sensory score remained the same between day-1 and day- 14, after which sensory scores decreases with increase in storage period. This can be attributed to decrease in pH and increased in Titratable acidity leading to subsequent increase in microbial load. Decrease in sensory score of yoghurt during storage period may be due to production of diacetyl and acetyl aldehyde compounds in the yoghurt during storage

Table 6: Total bacterial count of the Baobab yoghurt samples during storage

Samples	A (x 10^3 cfu/ml)	B (x 10^3 cfu/ml)	C (x 10^3 cfu/ml)	D (x 10^3 cfu/ml)	E (x 10^3 cfu/ml)
DAY 1	1.50	0.00	0.50	0.00	0.00
DAY 7	5.50	2.50	4.50	4.00	3.00
DAY 14	21.50	4.50	17.50	14.50	12.50
DAY 21	29.50	7.50	18.50	18.00	15.50

Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Table 7: Total fungal count of baobab yoghurt samples during storage

Samples	A (x 10^3 cfu/ml)	B (x 10^3 cfu/ml)	C (x 10^3 cfu/ml)	D (x 10^3 cfu/ml)	E (x 10^3 cfu/ml)
DAY 1	0.00	1.00	0.00	0.50	1.00
DAY 7	3.00	5.00	3.50	4.50	4.50
DAY 14	5.50	17.50	7.50	12.00	14.00
DAY 21	8.50	14.50	10.50	11.50	13.50

Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Table 8: pH value of Baobab yoghurt samples

Samples	A	B	C	D	E
DAY 1	4.48	3.09	4.69	4.41	4.01
DAY 7	4.65	2.84	4.34	4.03	3.77
DAY 14	4.60	2.32	4.44	4.32	4.16
DAY 21	4.45	2.21	4.03	3.84	3.59

Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Table 9: Titratable acidity of Baobab yoghurt samples

Samples	A	B	C	D	E
DAY 1	0.38	0.51	0.38	0.41	0.42
DAY 7	0.57	0.89	0.59	0.62	0.63
DAY 14	0.59	0.89	0.60	0.62	0.66
DAY 21	0.59	0.89	0.61	0.62	0.66

Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

Table 10: Effect of Baobab fruit fortification on the sensory properties of the yoghurt samples.

Sample	Appearance	Taste	Mouth feel	Aroma	Colour	Overall Acceptability
A	7.9^{c}	7.4^{b}	6.7^{b}	6.9^{b}	6.8^{b}	7.5^{c}
B	5.2^{a}	5.8^{a}	4.7^{a}	4.8^{a}	4.8^{a}	5.3^{a}
C	6.4^{b}	7.0^{b}	6.5^{b}	6.5^{b}	6.2^{b}	6.2^{b}
D	6.4^{b}	5.9^{a}	5.6^{ab}	5.2^{a}	5.9^{b}	5.8^{ab}
E	6.0^{b}	5.8^{a}	5.5^{ab}	4.7^{a}	4.6^{a}	5.3^{a}

Means with different superscripts on the same row are significantly different (P<0.05).

Key: A: 100% yoghurt B: 100% baobab C: 90% milk + 10% baobab, D: 80% milk + 20% baobab E: 70% milk + 30% baobab

CONCLUSION

Fortification of skimmed cow's milk with baobab milk at different levels resulted in increase in the ash, fat and carbohydrate content of yoghurt as level of addition increases. The fat content ranged between 0.64 - 4.65 and increased as fortification level increases. The protein content ranged between 4.14 -2.32 and it decreases as fortification level increases. The vitamin C content of 100% baobab milk is 209.6mg/100g and it increased with increase in fortification level with 70% milk + 30% baobab having the highest value of 14.82mg/100g. The sensory evaluation results showed that 90% milk + 10% baobab most preferred in terms of appearance and overall acceptability. The total bacteria and fungi counts increase significantly with increase in days of storage. Results from this study shows that Baobab fortified yoghurt produced was nutritious as well as safe for consumption.

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