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Microbiological Analysis of Baobab Yoghurt Produced Using *Lactobacillus bulgaricus*

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Abstract: This research was carried out to determine the microbial loads on Baobab Yoghurt produced using *Lactobacilus bulgaricus*. *Lactobacilus bulgaricus* was isolated from fermented cow milk and was identified using Analytical Profile Index 50 CH kit. Four fifty grams of powdered Baobab was formulated with 1L of sterile water and 1L of milk emulsion was added after which the *Lactobacilus bulgaricus* was inoculated using 0.5 macfarlane standard, it was allowed to ferment for 9h. The pH and TTA of the finished product was recorded as 3.34 and 1.089 respectively. The microbial was observed using MRS media, Nutrient agar and PDA and the following results were observed; 2.52 x 10^2, 2.06 x 10^2, <10 respectively. The identified microorganisms are Lactic acid bacteria and Saccharomyces species. All the results were within the acceptable limits set by NAFDAC. The reason for the low fungal count is owed to the lower pH of the yoghurt.

Keywords: Baobab, *Lactobacillus bulgaricus,* Yoghurt, Fermentation

1. Introduction

Baobab (*Adansonia digitata*) which belongs to the family "*Bombacaceae"* is indigenous to arid regions [1]. It is a well-adapted deciduous tree native to the arid parts of Central Africa and widely spread in the savannah regions in Nigeria [2]. It is a massive, majestic tree up to 25m high and spends only (4) months of the year in leaf and (8) months leafless. This is possible because some photosynthesis takes place in the trunk and branches during the eight months of leafless periods using water stored in the trunk [3]. Baobab fruit pulp is a powder produced from the fruit of the Baobab tree (*Adansonia digitata*) which grows predominantly in Southern Africa. The Baobab tree produces large pendulous white flowers from October to December. The fruit is usually green or brownish and covered in pale yellow-brown hairs. Inside the hard outer shell of the Baobab fruit is a dry, white fruit pulp with red fibers and black, kidney-shaped seeds randomly distributed inside the fruit pulp [4]. All parts of the baobab tree are absolutely useful and can either be used as food, beverages or ingredient [3]. The leaves, for instance, are used in the preparation of soup. Seeds are used as a thickening agent in soups, and they can be fermented and used as a flavouring agent, or roasted and eaten as snacks [5]. The pulp is either sucked or made into a drink while the bark is used in making ropes [5]. During acute seasonal food supply fluctuations or famine periods, the leaves and fruit of baobab are of particular importance as supplementary and emergency food [2].

Yoghurt is a Turkish name for a fermented milk product. It is originated by early nomadic herdsman, especially in Asia, Southern and Eastern Europe. Yoghurt is made by adding a culture of acid forming bacteria to milk that is usually homogenized, pasteurized and fermented. Yoghurt is defined as a fermented milk product that evolved empirically some centuries ago by allowing naturally contaminated milk to sour at a warm temperature, in the range of 40-50°C [3]. The micro-organisms which are used conventionally in this process are referred to as "Starter Culture". They include *Lactobacillus delbrueckii* subsp. *bulgaricus* and

Streptococcus thermophiles. During the fermentation, hydrolysis of the milk proteins occurs, the pH drops, the viscosity increases and bacterial metabolites are produced that contribute to the taste and possibly to the health promoting properties of yoghurt. Not only is yoghurt a wonderful quick, easy and nutritious snack, but also research evidence point to the fact that milk and yoghurt may actually add years to life as found in some countries where fermented dairy products are a dietary staple [6].

Traditional fermentation is a form of food processing, where microbes, for example, lactic acid bacteria (LAB) are utilized. The bacteria use food as a substrate for their propagation. This is a form of food preservation technology, used from ancient times. The rural folks have come to prefer fermented over the unfermented foods because of their pleasant taste, texture and colour. Using LAB fermentation for detoxification is more advantageous in that it is a milder method which preserves the nutritive value and flavour of decontaminated food. In addition to this, LAB fermentation irreversibly degrades mycotoxins without leaving any toxic residues. The detoxifying effect is believed to be through toxin binding effect [7]. Moreover, LAB are also known to produce protein antimicrobial agents such as bacteriocins. LAB also synthesises other anti-microbial compounds such as, hydrogen peroxide, reuterin, and reutericyclin. Other applications of LAB include their use as probiotics that restore the gut flora in patients suffering from diarrhoea, following usage of antibiotics that destroy the normal flora. In addition, the consumption of food products and beverages rich in LAB helps to alleviate constipation and abdominal cramps [7].

Lactic Acid Bacteria (LAB) are Gram positive non-spore forming cocci, cocco-bacilli or rods. They ferment glucose primarily to lactic acid, CO_2 and ethanol. They grow anaerobically in the presence of oxygen as aerotolerant anaerobes [8]. They lack catalase but possess superoxide dismutase and have alternative means to detoxify peroxide radicals, generally through peroxidase enzymes [8]. To a lesser extent, L. A. B is beneficial components of the human normal flora and probiotics. They are among the most important groups of microorganisms in food fermentations contributing to the taste and texture of fermented food as well as inhibiting food spoilage microorganism by producing a growth inhibiting substances and lactic acid [8].

The growing incidences of malnutrition especially in a developing country like Nigeria are quite alarming. Thus, the need for protein, energy and micronutrients to support the growing world population. Baobab fruit is grossly underutilized and thus this research aims at increasing the utilization of the fruit. Fermentation could help to remove anti-nutrients, natural toxicants and mycotoxins. Hence, both methods may therefore help to improve the nutritional quality and increase in consumption of Baobab fruit which will translate into increased production. Thus an improvement in house holds' income. The objective of this research is to produce Baobab Yoghurt using *Lactobacillus bulgaricus.*

2. Methodology

2.1. Sample Preparation

The Baobab (*Adanisonia digitata*) fruit was bought from Institute of Agricultural Research (IAR) Zaria. The fruit pods were opened by knocking them against hard materials to open the shell. The fruit pulp was packaged and stored. Four hundred and fifty grams (450g) of the baobab sample was used for the production of Baobab yoghurt [3].

2.2. Preparation of Baobab Yoghurt by Fermentation

The baobab yoghurt was prepared by the method described by [3]. Thirty (30) grams of sugar was added to 1 L of reconstituted powdered milk emulsion and 50 g of sugar was added to 1 L of baobab fruit pulp solution and were pasteurized separately at 80-85°C for 3-5 minutes, it was then formulated and homogenized. Submerged fermentation method was employed. The sample was inoculated with 3% pure culture of *Lactobacllus bulgaricus* using 0.5 Mac farlane standards and was incubated at room temperature (37°C) for 9 hours.

2.3. Analysis of the Baobab Yoghurt

2.3.1. Physico-Chemical Analysis of the Baobab Yoghurt

The baobab sample was analyzed for physico-chemical analysis in accordance with the standard procedures of association of official analytical chemist [9].

i. pH Determination

The mixture was allowed to stand for 15mins, shaken at 5min interval and filtered with whatman No. 4 filter paper, the pH of the filtrate was measured using the pin electrode of pH meter [9].

ii. Total Titrable Acidity Determination

Aliquots (10 ml) in triplicates were pipette from the filtrate obtained for pH above, into an Erlenmeyer flask, and then 2 drops of phenolphthalein was added. This was titrated using 0.1 N NaOH until a faint pink color appeared. The titre volume was noted and used to calculate Total Titrable Acidity (TTA) which was expressed as Percentage Lactic Acid [9]. TTA was determined and expressed as follows:

% Lactic acid = A × 0.009 × 100/V;

Where A = mL of 0.1NaOH required for the titration;

And V = mL of sample taken for the test.

The acidity was calculated as lactic acid using the relationship:

$$\frac{\text{Volume of base used x Normality of NaOH (N) x 9}}{\text{Volume of Sample used (average titre)}}$$

iii. Determination of Viscosity

The viscosity of water at 27°C and 28°C were noted. The water was poured into a

Flow-cup viscometer to the brim, while the discharge outlet was blocked with the index finger. This was so done to ascertain the time taken for the water to discharge on releasing.

The average time taken for the sample was recorded. By using the method of linear interpolation, the viscosity of the sample was determined by comparing with standard viscosity of water at 27°C and 28°C respectively [10].

iv. Determination of Vitamin C Content

Thirty grams of the sample was blended with about 100 ml of 04% oxalic acid for two minutes in a blender. The blended mixture was made up to 500ml in volumetric flask with 04% oxalic acid and filtered. The ascorbic acid in the filtrate was titrated against standard 2-6 Dichlorophenol Indophenol.

$$\text{Ascorbic acid } \left(\frac{\text{mg}}{100\text{g}}\right) = \frac{\text{Titre value X strength dye X 100}}{\text{Factor}}$$

$$\text{Factor} = \frac{\text{Sample weight X titration for volume Sample}}{\text{Total volume of sample}}$$

2.3.2. Proximate Analysis the Baobab Yoghurt

i. Determination of % Protein Content

The sample was digested with concentrated H_2SO_4, concentrated NaOH (40%), K_2SO_4 and $CuSo_4$. Five (5) ml of the digested sample was placed into a micro-kjeldahl distillation apparatus and excess concentration NaOH was added to make the solution strongly alkaline. Ammonia was distilled into 5ml of boric acid indicator in a titrating flask. Above 45ml of the distillates was collected. Titration was done with 0.01m HCL. The end point of the titration was light green [9].

% protein = % N × F

Where F = conversion factor 100 (% in food protein)

$$\text{Where \% N} = \frac{V_S\, V_B \times \text{Nacid} \times 0.01401 \times 100}{\text{W}}$$

V_S = vol. (ml) of acid required to titrate sample

V_B = vol. (ml) of acid required to titrate blank

N_{acid} = normally of acid (0.1)

W = weighed of sample in grams

Each food type has it percentage nitrogen. The common factor for most food and food mixture is 6.25.

ii. Determination of % Fat Content

The percentage fat content of the sample was determined by direct soxhlet extraction using petroleum ether (bp 40-60°C) as solvent. Five (5) g of the sample was measured and transferred into filter paper and was placed in the extractor and the set up was placed on a heating mantle. The heat source was adjusted so that the solvent boiled gently and refluxed for 6 hours until the ether had siphoned over and the barrel of the extractor was empty. On removal, the filter paper was placed in an oven at 50°C and dried to constant weight. The percentage in fat was calculated [9].

$$\text{\% Fat} = \frac{\text{Weight of fat}}{\text{weight of sample}} \times 100$$

iii. Determination of % Carbohydrate Content

The total percentage of carbohydrate content of the sample was calculated by subtracting the total protein, fat, crude fibre from the organic matter.

2.3.3. Anti-nutrients Determination of the Baobab Yoghurt

i. Phytate Content

This was determined using [9] method. Four grams (4g) of sample was soaked in 100 ml of 2% hydrochloric acid for 3 h and then filtered. Five millilitre (5 ml) of 0.3% ammonium thiocyanate solution was added to 25 ml of the filtrate. Then, 53.5 ml of distilled water was also added to the mixture. This was then titrated against a standard iron (III) chloride solution until a brownish yellow colour persisted for 5 min. The phytate content was calculated from the iron determinations, using a 4:1 iron-to-phytate molecular ratio.

ii. Tannin Content

Tannin content was determined by the vanillin- HCl method as described by [9]. Samples (0.6 g) was extracted for 60s at room temperature (28°C±2°C) with 3 ml of methanol. Extract will be reacted with 3 ml of 0.1M FeCl in 0.1N HCl and 33ml of 0.008Mv KFe (CN). Absorbance of the colour 36 developed was read at 720 nm. Catechin was used as standard.

2.3.4. Microbiological Analyses of the Baobab Yoghurt

The microbial analysis of the Baobab fruit and its products was done according to [11].

i. Serial Dilution of the Baobab Yoghurt

A sterile syringe was used to take 1ml of the sample and dispensed into one of the test tubes containing peptone water (10), and the test tube was shook. Another 1ml was drawn from test tube (10) and was dispensed into test tube (100). The same was repeated for test tube (1000).

ii. Enumeration and Isolation of Bacteria

Two millilitres was drawn from test tube (1000) and 1ml each was dispensed into 2 Petri dishes. Nutrient agar was poured onto the inoculated petri dish. The Petri dishes was incubated for 24 hours. Following incubation, the colonies obtained were counted and the average bacterial counts was enumerated. Pure colonies of the colonial growth was finally transferred onto de Man, Rogosa and Sharpe agar (MRS agar) plates for further isolation of the bacteria present.

iii. Enumeration and Isolation of Fungi and Yeast

Two millilitres was drawn from test tube (1000) and 1ml each was dispensed into 2 Petri dishes. Sabouraud's dextrose agar was poured into the two agar plates respectively. The Petri dishes was kept in the incubator for 48 hours. Following incubation, the colony obtained was counted and the average fungal counts were enumerated. Pure colonies of the colonial growth were finally transferred onto Potato Dextrose Agwar (PDA) and Sabouraud's dextrose agar (SDA) for further isolation of yeast and fungi respectively.

2.3.5. Sensory Evaluation of the Baobab Yoghurt

A ten panellist were used to evaluate the products on a 9-point hedonic scale for appearance, flavour, taste, texture and

overall acceptability.

9 – LIKE EXTEREMELY
8 – LIKE VERY MUCH
7 – LIKE MODERATELY
6 – LIKE SLIGHTLY
5 – NEITHER LIKE NOR DISLIKE
4 – DISLIKE SLIGHTLY
3 – DISLIKELY MODERATELY
2 – DISLIKE VERY MUCH
1 – DISLIKE EXTEREMELY [12]

2.3.6. Statistical Analysis

Data generated in the research was subjected to analysis of variance (ANOVA) and student t-test to evaluate the difference [13].

3. Results

Table 1: Results for Physico-chemical Analysis of Baobab Yoghurt

Table 1 below shows results for the Physico-chemical analysis of both Baobab yoghurt. This was done using student t-test. Titrable acidity and vitamin content have a significant difference of 0.00. The pH values of both samples has no significant difference of 0.1 where Baobab yoghurt is having a pH value of 3.34. Also, the results for viscosity of the both samples has no significance difference of 0.14 where Baobab yoghurt is having a viscosity of 26.80rpm.

Table 1. *Results for Physico-chemical Analysis of Baobab Yoghurt.*

Parameter	Baobab yoghurt	P-value
Titrable acidity	1.089	0.00s
pH	3.34	0.10ns
Viscosity (rpm)	26.80	0.14ns
Vitamin C (mg/l)	219.59	0.00s
Total	250.82	

Key: S = Significant; Ns = Not significant

Table 2: Results for Proximate Analysis of Baobab Yoghurt

The results for the proximate analysis of the Baobab yoghurt is shown in table 2 below. This was done using student t-test. The percentage total solids has the highest significant difference of 0.03. Baobab yoghurt has a percentage total solids of 21.17% which is high. The percentage ash content do not differ significantly as Baobab yoghurt is having 1.14%. Percentage moisture content has the lowest significant difference of 0.00 with Baobab yoghurt having 78.83%. It is closely followed by percentage protein, percentage total carbohydrates and energy contents with significant differences of 0.01 each. Then lastly followed by the percentage fat content with significant difference of 0.02 with Baobab yoghurt having 0.642%.

Table 2. *Results for Proximate Analysis of Baobab Yoghurt.*

Parameter	Baobab yoghurt (%)	P-value
Total solid (%)	21.17	0.03s
Ash (%)	1.14	0.47ns
Moisture content (%)	78.83	0.00s
Protein (%)	4.025	0.01s
Fat (%)	0.642	0.02s
Total carbohydrate (%)	15.37	0.01s
Energy (Kcal/g)	83.33	0.01s
Total	189.137	

Key: S = Significant; Ns = Not significant

Table 3. Results for Anti-Nutritional Analysis of Baobab Yoghurt.

Table 3 below shows the results for the Anti nutritional analysis for both Baobab yoghurt. ANOVA was used for the statistical analysis. There is no significant difference in the tannin content of baobab yoghurt and little significant difference for the raw baobab pulp. For the Phytate content, no significant difference exist within all the two samples.

Table 3. *Results for Anti-Nutritional Analysis of Baobab Yoghurt.*

Parameter	Baobab yoghurt	Raw Baobab
Tannin (%)	2.92^{b}	7.05^{a}
Phytate (%)	0.09^{a}	0.07^{a}

Table 4: Results for Microbiological Analysis of Baobab Yoghurt

Table 4 below shows the results for the Microbial analysis for both Baobab yoghurt. This was done using student t-test. There is no significant difference for all the three results. Lactic acid bacteria has a non-significant difference of 0.35 where Baobab yoghurt has a count of 2.52×10^2. Fungi has a non-significant difference of 0.84 where both Baobab yoghurt. For the bacteria, there is a non-significant difference of 0.48 where Baobab yoghurt has a count of 1.64×10^2.

Table 4. *Results for Microbiological Analysis of Baobab Yoghurt.*

Strains of microorganisms	Baobab	P-value
MRS (lactic acid bacteria) agar	2.52×10^2	0.35ns
Potato dextrose agar (fungi)	<10	0.84ns
Nutrient agar (Bacteria)	1.64×10^2	0.48ns

Key: S = Significant; Ns = Not significant

Figure 1: Sensory Evaluation of Baobab Yoghurt

Figure 1 below reveals the sensory evaluation of Baobab yoghurt using 10 panellists based on 9-points hedonic scale.

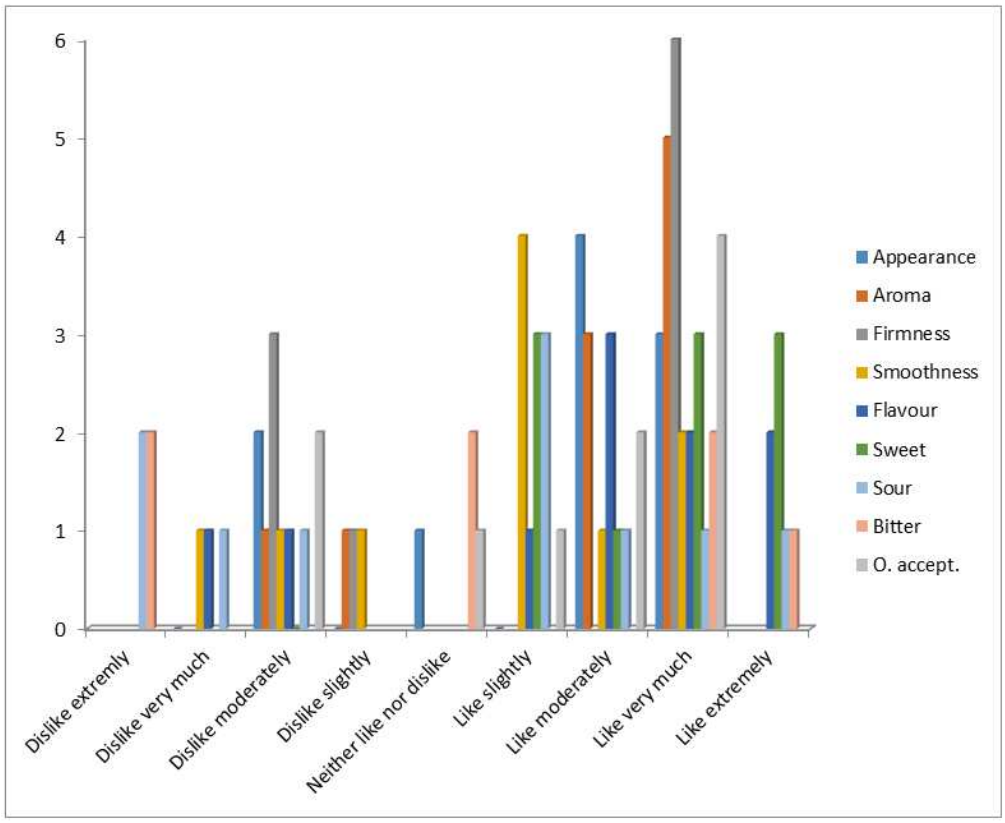

Figure 1. *Sensory Evaluation of Baobab Yoghurt.*

Figure 2: Sensory Evaluation of Powdered Milk Yoghurt

Figure 2 below reveals the sensory evaluation of powdered milk yoghurt using 10 panellists based on 9-points hedonic scale.

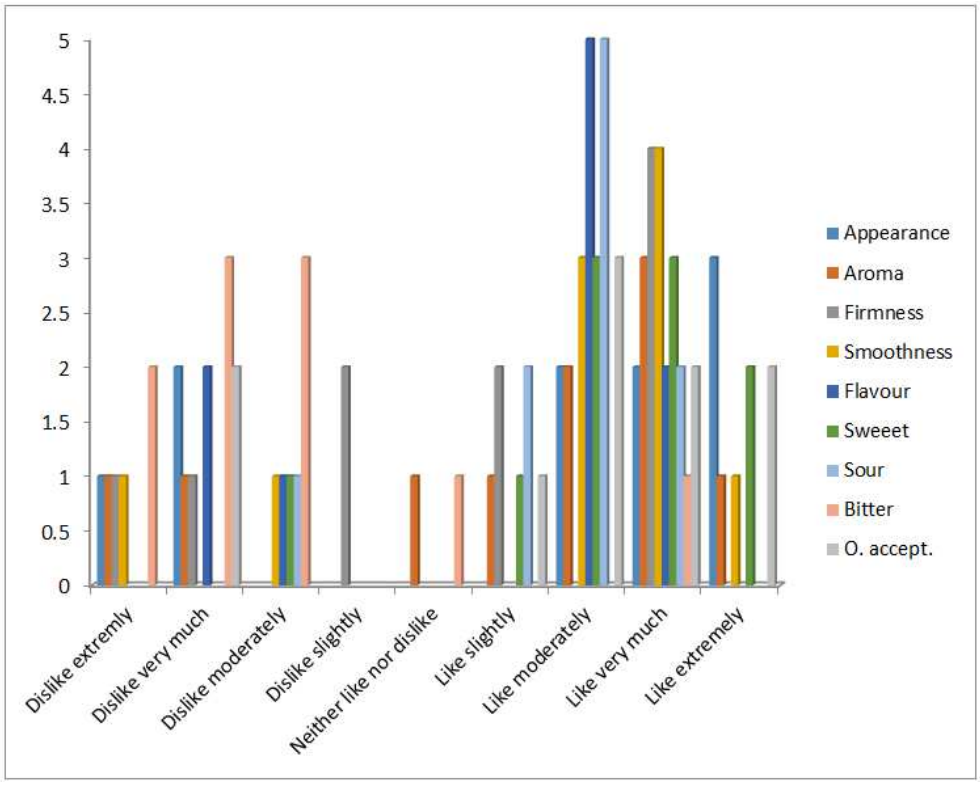

Figure 2. *Sensory Evaluation of Powdered Milk Yoghurt.*

Table 5: Results for Storage Stability for Baobab Yoghurt

Table 5 below shows the storage stability of Baobab yoghurt within 21 days. The readings were taken at two days interval. ANOVA was used. The results revealed little to no significant difference at 0.05 between day-1 and day-7. The pH value increases from day-1 to day-21 resulting in a decrease in the acidity of the yoghurt with little significant difference within the treatments from day-9 to day-21. The Titrable acidity also show slight significant difference from day-7 to day-21 with no any significant difference at 0.05 between day-1 and day-7. However, the value for the Titrable acidity decreases with increase in the number of storage days, this can be attributed to the increase in the pH values from day-1 to day-21. The fungal counts increases from day-1 to day-21 due to decrease in acidity of the yoghurt sample. The bacterial counts increased within the storage period due to the decrease in the acidity of the yoghurt sample during the storage period. The lactic acid bacterial counts as well as the variability decreased within the storage period due to the decrease in the acidity of the yoghurt sample as well as increase in the fungal and bacterial counts.

Table 5. *Results for Storage Stability for Baobab Yoghurt.*

DAYS	pH	TITREABLE ACIDITY	FUNGAL COUNTS	BACTERIAL COUNTS	LAB COUNTS
1	3.34 ± 0.05^{a}	1.089 ± 0.01^{b}	$<10\pm0.01^{f}$	$1.06X10^{1}\pm3.23^{d}$	$2.52\ X\ 10^{2}\pm1.16^{a}$
3	3.31 ± 0.32^{a}	1.096 ± 0.00^{b}	$<10\pm0.00^{f}$	$1.17X\ 10^{1}\pm2.15^{d}$	$2.08\ X\ 10^{2}\pm1.53^{a}$
5	3.09 ± 0.07^{a}	1.163 ± 0.02^{b}	$1.12X10^{1}\pm0.04^{e}$	$1.3\ X\ 10^{1}\pm3.45^{d}$	$2.01\ X\ 10^{2}\pm4.06^{a}$
7	3.005 ± 0.65^{a}	1.24 ± 0.01^{ab}	$1.28X1O^{1}\pm0.34^{d}$	$1.63X\ 10^{1}\pm2.35^{c}$	$1.97X10^{2}\pm1.48^{a}$
9	2.86 ± 0.32^{ab}	1.56 ± 0.03^{b}	$1.53X10^{1}\pm2.54^{d}$	$1.83X\ 10^{1}\pm0.98^{b}$	$1.2X\ 0^{2}\pm3.65^{b}$
11	2.63 ± 0.18^{b}	$1.781\pm0.00\pm^{ab}$	$1.92X\ 10^{1}\pm1.25^{c}$	$1.867X10^{1}\pm0.33^{b}$	$1.0X10^{2}\pm5.13^{b}$
13	2.31 ± 0.09^{b}	1.94 ± 0.01^{ab}	$1.94X\ 10^{1}\pm3.15^{c}$	$1.98\ X\ 10^{1}\pm5.43^{b}$	$1.87X10^{1}\pm3.21^{c}$
15	2.07 ± 0.21^{cb}	1.986 ± 0.06^{ab}	$1.98X\ 10^{1}\pm1.18^{c}$	$2.10\ X\ 10^{1}\pm4.23^{b}$	$1.67X10^{1}\pm3.25^{c}$
17	1.96 ± 05^{c}	2.02 ± 0.05^{a}	$1.08X10^{2}\pm3.45^{b}$	$1.5\ X\ 10^{2}\pm5.32^{a}$	$1.53X10^{1}\pm0.46^{cd}$
19	$1.52\ \pm0.08^{c}$	2.163 ± 0.03^{a}	$1.15X10^{2}\pm4.17^{b}$	$1.62\ X\ 10^{2}\pm3.26^{a}$	$1.32X10^{1}\pm3.25^{cd}$
21	$1.240.02^{d}$	2.238 ± 0.03^{a}	$1.12X\ 10^{3}\pm3.15^{a}$	$1.7\ X\ 10^{2}\pm1.25^{a}$	$1.02X\ 10^{1}\pm3.67^{d}$

Means with the same superscript letters per column did not differ significantly at 0.05

4. Discussion

Physico-chemical Analysis of the Yoghurt Sample

The Titrable acidity of the yoghurt samples is 1.089. This according to [3] complies with the minimum of 0.6 Titrable acidity in commercial yoghurt. The pH is a determining factor in the decrease or increase in the Titrable acidity of the yoghurt samples. The pH ranged between 3.34 indicating acidity of the samples. The pH value is lower than the recommended pH of (3.9-4.5) yoghurt, according [3], the presence of organic acid is responsible for low pH in both samples. The viscosity of the samples was between 26.8rpm. However, the viscosity of both samples did not comply with the favourable limit for viscosity which according to [3] is 80-170rpm. The vitamin C content is 219.5885mg/l. The reason for high baobab content is due to the fact that baobab pulp is particularly rich in vitamin C.

Proximate Analysis of the Yoghurt Sample

The total solids are 20.12% as compared to 9.24-16.27% as reported by [3]. There was an increase in the percentage ash content production, the ash content for the raw Baobab was 5.14% whereas for the yoghurt is 1.14% which according to [3] did not comply with the standard percentage ash content for non-fat skimmed milk. The increased in ash content of the baobab fruit could be due to high content of minerals in the Baobab [14]. The moisture content is 78.83% a significant increase in the moisture content was observed as the raw Baobab was having a moisture content of 13.90%. However, the moisture content of both samples was below the standard limit of moisture content for most commercial yoghurt which according to [3] is between 80-85%. There was an increase in the percentage protein content after production with the raw baobab having a percentage protein content of 3.075%. 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 according to [15] is due to possible increase of microflora that uses protein for their metabolism. The fat content ranged between 0.642%. The fat content fall within the limit for low fat yoghurt (<3.5%) There was an increased in the percentage fat content with the raw baobab having a fat content 0.25%. According to [5], the increase in fat content after fermentation maybe due to the activities of the lipases which hydrolyse fat to glycerol and fatty acids. The total carbohydrate content is 15.37%. The initial total carbohydrate content for the raw baobab was 56.37%, this indicate a significate decrease in the total carbohydrate content after fermentation which according to [3] could probably be due to the fact that carbohydrate moiety was fermented by the lactic acid bacteria. According to [5], the decrease in the total carbohydrate content was likely due to the use of the nutrient especially the metabolite, the simple sugar as a source of energy and also the use of carbohydrate to provide carbon skeleton foe the synthesis of other absorbable compounds which in turn increase the availability of the nutrients in the fermentation samples. The energy is 83.33Kcal/g.

Anti-nutritional Content of the Sample

The Phytate content is 0.09mg, according to Abdoulaye *et al.,* (2011) Phytate level in food should ideally be not more than 25mg or 0.035% of the food. The initial Phytate content for the raw baobab pulp before fermentation was 0.17mg,

hence a decrease in Phytate level of both yoghurt samples was observed after the lactic acid bacteria fermentation. According to [16], lactic acid fermentation provides an optimum pH condition for enzymatic degradation of Phytate in food. According to [5] decrease of Phytate after microbial fermentation could be due to presence of microbial phytatse present during microbial fermentation. The tannin level is 22.85mg. The raw baobab sample was found to be having a tannin level of 7.05mg. According to [5], the decrease in tannin level after fermentation may likely be due to the breakdown of tannin complexes to release free nutrients and also as a result of the leaching of the tannin in the fermentation medium which in turn improve the availability of the nutrients. Tannin is responsible for the astringent taste of baobab [16].

Microbiological Composition of the Sample

The fungal counts was found to be <10 which is the acceptable limits of fungi in yoghurt. Limits higher than 10 are capable of producing toxic metabolites leading to food poisoning and cancer of the liver [17]. According to [18], the presence of lactic acid bacteria in yoghurt prevent the proliferation of fungi in yoghurt. The bacterial counts was observed to be 1.06×10^1 which an acceptable limits for bacteria in yoghurt is. According to [18], the reason for the lower bacterial was also as a result of the presence of lactic acid bacteria in the yoghurt. The lactic acid bacterial count is 2.52×10^2. The morphological characteristic of the microorganisms was observed to be cocci, bacilli, circular, creamy, flat and smooth isolates. They appeared to be gram positive, catalase negative, non-motile, some scanty, some clustered and some appeared in chain.

Sensory Evaluation of the Yoghurt Samples

A consumer acceptance panel was conducted using the experimental yoghurt. Consumer panellists were chosen from Department of Microbiology Kaduna State University. Panellists scored on a 9 point hedonic scale on how they either liked or disliked the appearance, texture, colour, smell and aftertaste of the baobab flavoured yoghurt where 9 is like extremely and 1 is dislike extremely [12].

During the sensory evaluation of the yoghurt samples, the two yoghurt samples i.e. Baobab yoghurt was compared against powered milk yoghurt. The powdered milk yoghurt has higher appearance and aroma score than both baobab yoghurt, this can be as a result of the baobab having an off-white colour which is not consistent with commercial yoghurt which the panellist are familiar with. Powdered milk yoghurt also has higher aroma score than baobab yoghurt. The intense acid taste masks the other flavour and odour notes of the yoghurt [19]. Baobab yoghurt has higher firmness score than powdered milk yoghurt. The reason for higher firmness in the baobab yoghurt might be due to higher viscosity in the yoghurt samples than in the powdered milk yoghurt. Powdered milk yoghurt has higher smoothness score than the baobab yoghurt. Baobab yoghurt has higher flavour score than powdered milk yoghurt. The high flavour score in baobab yoghurt indicates that the panellists like the yoghurt despite the astringent aftertaste. This also conforms to the work of [19]. Powdered milk yoghurt has higher sweetness score than baobab-milk and baobab-soy yoghurt, this can be attributed to the intense acid taste in baobab which may tend to mask the sweet taste of the yoghurt as earlier cited by [19]. The sourness score of baobab yoghurt is higher than powdered milk yoghurt. This could be due to the presence of organic acid in baobab which makes it highly acidic. Baobab has higher overall acceptability than powdered milk yoghurt despite the low appearance and aroma scores.

Storage Stability of the Yoghurt Sample

Baobab yoghurt was stored for 21 days at 5^0c during which they were observed for changes in pH, Titrable acidity, microbial counts, odour colour and taste. There was a continuous increase in pH during storage of the yoghurt sample with little to no significant differences at 0.05. Low pH in the yoghurt samples could be attributed to the presence of organic acids in baobab and also continuous fermentation caused by oxidation of organic compounds present in the yoghurt samples. Decrease in pH results in increase in Titrable acidity, causing lower flavour score with increase in storage time. Citric acid was used in moderation as a preservative, according to USP, (2012), citric acid serves as a buffer to control the pH of a food sample. There was an increase in Titrable acidity during the storage period which as earlier stated was as a result of decrease in pH. High Titrable acidity syneresis in yoghurt which is not acceptable in yoghurt [20]. The fungal loads was within the acceptable limits of <10 within the first 3 days, it was then followed by continuous increase in fungal load during the storage period. This resulted in the production of off-flavour and gassy appearance towards the end of storage period. The increase in fungal load maybe due to the decrease in pH which provide a selective environment for the proliferation of fungi. Yeast are major causes of spoilage in yoghurt [18]. Another reason for the increase in the fungal loads of the yoghurt sample was due to the decrease in Lactic acid bacterial load, lactic acid bacteria are synergistic to the growth of microorganisms. However, the presence of citric acid interfere with fungal load of the yoghurt sample during storage period by acting as a buffer in regulating the acidity of the yoghurt hence making the environment less favourable for their propagation. There was also an increase in the bacterial load of the yoghurt samples with increase in storage period. However, the bacterial load was within the acceptable limit between day-1 and day-11. The increase in bacterial counts indicated a continuous deterioration of the yoghurt quality [18]. 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 samples to increased microbial growth during storage. Lactic acid bacterial counts decreases gradually with increase in storage time. This could be due to accumulation of by-product of their metabolism i.e. lactic acid as well as decrease in available nutrients during the storage period. As earlier stated, decrease in lactic acid bacteria results in increase in microbial loads. The sensory score remained the

same between day-1 and day-11, after which sensory scores decreases with increase in storage period. This can be attributed to decrease in pH and increase in Titrable acidity leading to subsequent increase in microbial load [19]. Colour change was observed from day-11 to day-21 from off-white colour to brownish-white colour, there was change in odour also from day-11 to day-21 ranging from pleasant to unpleasant odour, and gassy odour. The taste of the yoghurt samples was also affected from day-11 ranging from pleasant-sour to unpleasant-sour taste. According to [18], low pH provide selective environment for fungal growth leading to fermentation off-flavour and gassy appearance. According to [19] decrease in sensory score of yoghurt during storage period maybe due to the production of diacetyl and acetyl aldehyde compounds in the yoghurt during storage.

5. Conclusion

It p can be conclude that baobab yoghurt was having the most merit in Physico-chemical, proximate, microbial and anti-nutrient, and Sensory properties despite the astringent after taste and its acidic flavour. The fermentation of yoghurt especially on shelf storage samples was due to the oxidation of organic compounds present in the yoghurt by microorganisms. The preservative (citric acid) concentration was within the threshold values specified by the Standard Organization of Nigeria (SON) and National Agency for Food and Drug Administration and Control (NAFDAC).

Recommendations

It is recommended that baobab should be encourage in the production of yoghurt as an economical means to solve the problem of protein-calorie malnutrition in Africa.

It is recommended that yoghurt produce with Baobab fruit pulp should be promoted and commercialize in order to improve household income.

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