

Assessment of Physicochemical Composition and Enzymes Activity on the Effect of Fermentation Techniques During Production of Baobab Pulp Yoghurt

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ABSTRACT

The goal of the study was to evaluate the effect of fermentation time on some physicochemical properties and enzyme activity in yoghurt supplemented with baobab fruit pulp. Fresh baobab fruits were collected, processed, and then incorporated at 10% (w/v) into milk prior to fermentation. Fermentation of yoghurt samples was carried out for 0–15 hours. Changes in pH, TTA, sugar content (Brix), temperature, viscosity, and enzyme activities (amylase, protease, and lipase) were determined using the standard analytical method. The results indicated that the fermentation time significantly ($p < 0.05$) influenced TTA values that ranged from 0.12% to 0.89%, but the pH decreased from 4.67 to 3.83, within the acceptable range of yoghurt acidity. Sugar content increased slightly from 11.43% to 12.00%, and the apparent viscosity varied between 257.30 and 876.20 mPa.s at different fermentation times. Temperature slightly declined from 32.50 to 31.80°C. Enzyme activities were constant through the fermentation time of amylase (2.72 mg/mL), protease (31.65 mg/mL), and lipase (0.37 mg/mL) since no significant differences existed in the analyzed samples, which was obtained at $p > 0.05$. This means that supplementation of baobab pulp affects the physicochemical properties and did not affect changes in enzyme activities during fermentation. Baobab-enriched yoghurt is presented as a functional dairy product because enzyme activity did not change with increasing fermentation times.

Keywords: Physicochemical composition, enzymes, fermentation, yoghurt

Introduction

Adansonia digitata L. *Malvaceae* is a multipurpose tree termed African baobab by both the English and the French (Kaboré *et al.*, 2011). The tree belongs to the *Malvaceae* family with great ecological tolerance which makes it valuable in both hot and dry cultivating conditions (Buchmann *et al.*, 2010). This beneficial tolerance is attributed to the thick fire-resistant bark, shedding of leaves, as well as possession of a trunk that can absorb water in the rainy season and contracts in the dry season. The genre *Adansonia* was coined from Adanson Micheal, (1727– 1806) a French botanist name, who discovered a specimen on the islands of Sor, Senegal, while the term digitate (digitals of the hand or hand-like) was about the shape of the leaves of the African baobab tree (Kamatou *et al.*, 2011) with a usual range of 5–7 leaflets. However, concerning physiological attributes, several other names have been ascribed to the African baobab, including “magic tree”, “chemist tree”, “symbol of the earth”, “monkey bread of Africa” (Vermaak *et al.*, 2011) “dead rat tree”, “cream of tartar”, etc. (Rahul *et al.*, 2015).

African baobab is indigenous to the Savannah regions of Africa (16° N and 26° S), whilst several others have been identified in other tropical regions of the world (Darr *et al.*, 2020). African baobab is an angiosperm - having flowers and producing seeds covered by a carpel, they are pachycauls, with thick stems. Records have shown that the African baobab tree is the oldest and largest surviving angiosperm (Patrut *et al.*, 2018) where some of them including the Panke of Zimbabwe (a sacred tree in Matabeleland North), Chapman of Botswana (a historic African baobab tree), are now dead. Initial growth of the African baobab tree is characterized by single stems which consequently develops to several other stems as a result of its characteristic ability to produce stems periodically.

Importantly, a recent study (Patrut *et al.*, 2018) has highlighted the wood volume of the African baobab to range between 300 and 500 m³, aside the Plat land tree (501 m³), previously known to be the most advocated African baobab found the Limpopo province of South Africa (Patrut *et al.*, 2018). Seed production has been recorded to begin between the ages of 8– 23, while the mature tree (> 60 years) produces about 160–250 fruits annually (UNCTAD, 2005). Physiological examination of *A. digitata* reveals that it is a massive deciduous tree growing up to about 20–30 m tall with a gigantic girth ranging between 20 and 35.10 m, with a circumference varying from 14.3 to 32.0 m, having a lifespan of up to 450 years (Patrut *et al.*, 2018). The smooth, reddish-brown or gray bark, possesses longitudinal fibers. The tree is substantially branched, producing a lateral system of about 50m from the trunk (Rahul *et al.*, 2015).

Material and Methods

Sample Collection and Preparation

Baobab fruits were collected from mature baobab trees located in the arid regions of northern Nigeria. The fruits were carefully selected based on their size, color, and absence of physical damage. Once collected, the fruits were transported to the laboratory for further processing. In the laboratory, the fruits were washed thoroughly with clean water to remove any dirt or contaminants. The hard outer shells of the baobab fruits were cracked open using a mallet, and the pulp was extracted. The pulp was then separated from the seeds and fibers using a clean, sterile sieve. The sieved pulp was collected in sterile containers and stored at -20°C until further use as previously described by Mounjouenpou *et al.* (2018).



Figure 1. African Baobab Tree (Darr *et al.*, 2020)

Yoghurt Production

The baobab pulp was used as an ingredient in the yoghurt production process as previously described by Adelekan *et al.* (2020) and Stadlmayr *et al.* (2020) with little modification. Fresh cow milk was obtained from a local dairy farm and was pasteurized by heating it to 85°C for 15 minutes to eliminate any pathogenic microorganisms. After pasteurization, the milk was cooled to 45°C, the optimal temperature for the growth of yoghurt starter cultures. Starter cultures containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were added to the cooled milk. The inoculated milk was then divided into two batches. In the first batch, 10% (w/v) of baobab pulp was added to the milk, while the second batch served as the control without any baobab pulp. Both batches were incubated at 45°C for 6 hours to allow fermentation to occur.

Physicochemical Analysis

The physicochemical properties of the baobab pulp yoghurt were evaluated to assess its quality and stability. The analyses included pH, temperature, and titratable acidity

Determination of pH

The pH value of the sample was determined using a pH meter. The initial pH value displayed by the pH meter before taking the actual reading was noted, and the meniscus of the pH meter was allowed to stabilize. The reading was taken immediately after the sample was prepared in the laboratory and at 3-hour intervals for up to 15hours (Eze *et al.*, 2014).

Determination of temperature

The temperature determination was conducted using a calibrated mercury-in-glass thermometer. The temperature readings were taken at 3-hour intervals by dipping the thermometer into the sample for up to 15hours (Eze *et al.*, 2014).

Measurement of titratable acidity

The titratable acidity, expressed as a percentage of acid produced, was determined by titrating 10 ml of the fermenting sample with 0.1N NaOH, using phenolphthalein as an indicator until the endpoint (pink color) was achieved. The percentage of Total Titratable Acidity (%TTA), representing the percentage of lactic acid content, was calculated according to the method previously described by Igbabul *et al.* (2014) as follows:

$$\% \text{ Total Titratable Acid} = \frac{\text{Titre volume} \times 0.1 \text{ NaOH} \times 0.09}{\text{weight of sample}} \times \frac{100}{1}$$

Determination of Enzyme Activity

Enzyme activities of amylase, protease, and lipase in yoghurt samples supplemented with baobab pulp were determined using standard spectrophotometric and titrimetric procedures according to established analytical methods. Enzyme activity was monitored at various fermentation times: 0, 3, 6, 9, 12, and 15 hours.

Determination of Amylase Activity

The activity of amylase was determined by the starch-iodine colorimetric assay. A 1-mL yoghurt sample was mixed with 1 mL of 1% soluble starch solution, incubated at 37°C for 10 minutes. The reaction was stopped after incubation by the addition of 1 mL of iodine reagent. Absorbance was measured at 620 nm using a spectrophotometer. Amylase activity was calculated based on the reduction in the intensity of the blue colour, reflecting starch hydrolysis, and expressed in mg/mL (Alkan, 2007).

Assay of Protease Activity

Protease activity was assayed by a casein digestion method. One milliliter of yoghurt sample was incubated with 2 mL of 1% casein solution at 37°C for 30 minutes. The reaction was stopped by adding 3 mL of 5% trichloroacetic acid (TCA). The mixture was filtered and the absorbance of the clear filtrate was measured at 280 nm. Protease activity was expressed in mg/mL in terms of tyrosine equivalents released during hydrolysis (Abada, 2009).

Determination of Lipase Activity

The lipase activity was assayed by a titrimetric technique based on hydrolysis of an olive oil emulsion. Then, 1 mL of yoghurt sample was added to 5 mL of olive oil emulsion followed by incubation at 37°C for 30 minutes. After incubation, 10 mL of ethanol–acetone mixture (1:1) was added to stop the reaction. The liberated free fatty acids were titrated with 0.05 N NaOH, using phenolphthalein as indicator. Lipase activity was calculated based on the volume of NaOH used and was expressed in mg/mL (Wang *et al.*, 2024).

RESULTS

Physicochemical Properties

The physicochemical properties and effect of fermentation of the yoghurt supplemented with baobab fruit pulp is shown in Table 1. Result revealed that titratable acidity (TTA) decrease significantly ($p < 0.05$) during fermentation except at 3 hour fermentation with value ranged from 0.12 to 0.89%, while the pH decreased significantly from 4.67 to 3.83. The sugar content (Brix) increased significantly from 11.43 to 12.00%. Viscosity shows significant decrease except at 15 hour fermentation, it varied from 649.97 mPas to 876.20 mPas,. Temperature significantly ($p < 0.05$) decreases from 32.50 to 31.80°C.

Table 1. Effect of fermentation time on physicochemical properties of yoghurt supplemented with baobab pulp

Fermentation time (hours)	TTA (%)	Ph	Brix (%)	Temperature (°C)	Viscosity (mPa.s)
0	0.71±0.01 ^b	4.67±0.01 ^a	11.43±0.01 ^d	32.50±0.01 ^a	649.97±0.01 ^b
3	0.89±0.01 ^a	4.10±0.01 ^c	11.60±0.07 ^{cd}	32.40±0.01 ^{ab}	257.30±0.07 ^f
6	0.12±0.01 ^e	3.83±0.01 ^d	11.47±0.01 ^{cd}	32.10±0.01 ^b	413.73±0.01 ^e
9	0.40±0.01 ^d	3.83±0.01 ^d	11.63±0.01 ^c	31.80±0.01 ^c	430.90±0.07 ^d
12	0.69±0.01 ^c	4.17±0.01 ^c	11.83±0.01 ^b	31.90±0.01 ^c	496.47±0.01 ^c
15	0.66±0.01 ^c	4.40±0.07 ^b	12.00±0.07 ^a	31.80±0.01 ^c	876.20±0.07 ^a

Values are means ± SD. Means having similar superscript letter in a column are not significantly different ($p < 0.05$). Key words: TTA – titratable acidity, Brix – Sugar content, (%) – percentage, (°C) – degree centigrade, (mPa.s) – millipascal seconds.

Enzyme Activity

Table 2 shows the enzyme activity, including amylase, protease, and lipase, result revealed that the activity remained constant and there was no significant difference ($p > 0.05$) during fermentation for all enzymes.

Table 2. Effect of fermentation time on enzyme activity of yoghurt supplemented with baobab pulp

S/N	Fermentation time (hours)	Parameters (mg/mL)		
		Amylase	Protease	Lipase
1	0	2.72±0.01 ^a	31.65±0.01 ^a	0.37±0.01 ^a
2	3	2.72±0.01 ^a	31.65±0.01 ^a	0.37±0.01 ^a
3	6	2.72±0.01 ^a	31.65±0.01 ^a	0.37±0.01 ^a
4	9	2.72±0.01 ^a	31.65±0.01 ^a	0.37±0.01 ^a
5	12	2.72±0.01 ^a	31.65±0.01 ^a	0.37±0.01 ^a
6	15	2.72±0.01 ^a	31.65±0.01 ^a	0.37±0.01 ^a

Values are means ± SD. Means having similar superscript letter in a column are not significantly different ($p < 0.05$). Key word(s); mg/mL – milligram per milliliter.

Discussions

Titrate acidity is the total acids in a solution, it measure both free and bound acids. Fruit pulp is rich in organic acids such as ascorbic, citric, tartaric, malic acids. The titratable acidity at initial time 0, is 0.71 at 3 hours is 0.89, at 6 hours is 0.12, at 9 hours is 0.40, at 12 hour is 0.69, and at 15 hours is 0.66. the value 0.66 at fermentation time of 15 hours closely agrees with the findings of Eke at al., (2013) of 0.6 while others values obtained of the fermentation time 0, 3, 6, 9, 12 do not agrees with finding of (Eke *at al.*, 2013). This might be attributed to the differences in the fermentation time. The pH which is a determining factor in the decrease or increase in the titratable acidity of the yoghurt sample ranges between 3.83-4.67 which is

within the recommended pH value range of 3.9-4.5 (Eke *et al.*, 2013). The presence of organic acid is responsible for the low pH values of the baobab pulp yoghurt samples.

The viscosity of the samples was between 257.30-876.20mpa.s however; the viscosity of the samples at different fermentation times (0, 3, 6, 9, 12, and 15 hours) do not compliance with favorable limit for viscosity of 4800-10200mpa.s reported by (Eke *et al.*, 2013). The titrable acidity of the yoghurt with baobab pulp found in the present study was lower the values reported by Mwangi (2023) except that at 3 hour fermentation which is in agreement with their finding. Similarly, Aisha *et al.*, (2017) reported a higher TTA of baobab fruit yoghurt.

The enzymes (amylase, protease and lipase) are a very important factor of yoghurt. The activity of amylase enzyme across the fermentation time (0, 3, 6, 9, 12, and 15 hours) was recorded to be 2.72 throughout and these value are not significantly difference at ($p < 0.05$); Also the activity of protease enzyme across the fermentation time (0, 3, 6, 9, 12, and 15hours) was recorded to be 31.65 throughout and these value are not significantly difference at ($p < 0.05$); again, the activity of lipase enzymes across the fermentation time (0, 3, 6, 9, 12, and 15hours) was recorded to be 0.37 throughout and these value are not significantly difference at ($p < 0.05$).

Conclusion

Results obtained indicated that fermentation time significantly affected titratable acidity, pH, viscosity, sugar content, and temperature of the different yoghurt samples. The TTA values were generally variable within the fermentation periods; however, the pH values were within the acceptable limit for fermented yoghurt, suggesting a good acidification property due to organic acid in nature within the baobab pulp. Viscosity varied significantly across fermentation time but did not fall within the recommended minimum standard range, indicating that baobab pulp may affect the textural properties depending on the fermentation time. Sugar content gradually increased with fermentation, while temperature decreased slightly. On the other hand, activities of enzymes-amylase, protease, and lipase-remained similar among all fermentation periods without showing any significant variation ($p < 0.05$). This means that neither the baobab pulp supplementations nor its fermentation duration could affect the metabolic enzyme profile of the yoghurt.

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