

DOI: 10.47745/ausal-2025-0002

Effect of sprouting on the phytochemical and physicochemical properties of bambara nut (*Vigna subterranea* (L.) Verdc.) cheese analogue

A. T. Omidiran^{1,2} e-mail: omidiranat@funaab.edu.ng

M. O. Abdul-Salam¹ e-mail: maryamo@gmail.com

K. M. Amusa¹ e-mail: amohtunrayo@gmail.com

O. O. Akinbule³ e-mail: akinbuleoo@funaab.edu.ng

A. F. Adegoke¹

e-mail: adegokeaf@funaab.edu.ng

¹Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, Nigeria

²Laboratorio de Ciencia da Carne, Departamento de Zootecnia, Universidade Federal de Viçosa, Brazil

³ Department of Nutrition and Dietetics, Federal University of Agriculture, Abeokuta, Nigeria

Abstract. The purpose of this study was to evaluate the effect of sprouting on the phytochemical and physicochemical properties of bambara nut cheese analogue. The produced sprouted nut cheese analogue had a pH (4.83–5.44) and was compared with the pH value (4.8) of the control (obtained with unsprouted bambara nut). Total titratable acidity, total soluble solid, total dietary fibre, and yield were measured and compared with control samples. The main bioactive phytochemical content (anthocyanins, tannins, phytate, alkaloids, saponins, flavonoids, and polyphenols) was also measured. Sprouting significantly influenced the nutritional composition of bambara nut cheese analogues, whereas in 3 days favourable phytochemicals content and improved physicochemical properties were obtained. The research could reduce the hard-to-cook stigma and promote its use in innovative food product development.

Keywords: plant-based proteins, phytochemicals, sprouting, plant cheese analogues

1. Introduction

One of the best ways to curb food insecurity, according to the food and agricultural organizations, is nutritional diversity. Over the past few years, food consumption patterns have drastically changed because of an aging population, increased awareness to live healthier, the urge to cater for individuals with specific nutritional needs, and people's desire to globally reduce dependency on animal food products. Several studies have been conducted on plants as alternatives for animal-based protein. This tendency has led to the exploration of several strategies by researchers and the food industry to develop alternative milk, cheese, meat, and egg products from various plant-based sources (*Tachie et al.*, 2023).

Cheese analogue is the term that can be described as a cheese-like product produced from the partial or complete substitution of components such as milk, milk fat, or milk protein and the incorporation of vegetable-based raw materials. Plant-based cheese alternatives might also fit into the diets of people with special dietary needs such as those with cow milk allergy or lactose intolerance, and those with concerns about cow milk hormones or cholesterol. Plants (legume) proteins have good technological properties and are cheap, which gives them a strong commercial potential to be used in plant-based cheese-like products (*Mefleh et al.*, 2021). Augmented interest in plant-based foods has increased due to concerns related to health, sustainability, and animal welfare. The study intends to fill the knowledge gap on the use of bambara nut in the production of plant-based cheese analogue.

Bambara groundnut is a grain legume grown mainly by subsistence farmers in sub-Saharan Africa. It is a sustainable, low-cost source of complex carbohydrate, plant-based protein, unsaturated fatty acids, and essential nutrients. It is an underutilized legume, a hardy crop and has been recognized as an important nutritious food source when food is scarce (*Mbosso et al.*, 2020). As a "complete food", this crop has recently been treated as a new-millennium crop, and, furthermore, it is more adjusted to poor soil and climatic conditions than other dominant crops, with a good potential in bridging the gap of nutrition deficit.

In common with other legumes, in bambara groundnut, several phytochemicals (tannins, phytates), so-called antinutrients, were identified (*Pretorius et al.*, 2023). Several processing methods are available to reduce or eliminate their antinutrient activity (*Mashau et al.*, 2025), simultaneously increasing the bioavailability of the essential nutrients. Sprouting induces the activation and *de novo* synthesis of hydrolytic enzymes that make nutrients available for plant growth and development. Consumption of sprouted grains is suggested to be beneficial for human health. Therefore, the objective of this study was to evaluate the phytochemicals and their properties of bambara nut cheese analogue. The food industry may benefit from this

research by formulating improved cheese analogues with greater market appeal. This could also lead to the creation of innovative products aligned with consumer demand for healthier options.

2. Materials and methods

2.1. Materials

Bambara nuts (cream-coloured variety) *Vigna subterranea* (L.) Verdc., alum, and starter culture were purchased at Osiele market, Abeokuta, Ogun State.

2.1.1. Bambara nut

The Bambara groundnut (*Vigna subterranea* L. Verdc.; Syn: *Voandzeia subterranean* L. Thouars) represents an underutilized legume species that enjoys widespread cultivation across Africa (*Ntundu et al.*, 2006). Ranked as the third most prevalent major legume on the African continent after groundnuts (*Arachis hypogea*) and cowpeas (*Vigna unguiculata*), this crop plays a vital role in promoting nourishment, enhancing food security, facilitating pastoral development, and supporting sustainable land management practices. The seeds of the bambara groundnut come in various colours and textures across different accessions.

2.1.2. Alum

Alum is a non-acidic compound commonly used in water purification and the leather industry for treating effluents ($Symons\ et\ al.$, 2010). Chemically, it is a double sulphate of aluminium and potassium ($KAl(SO_4)_2\cdot 12H_2O$) and is readily available in both powdered and crystalline forms. When added to water, alum binds to impurities, facilitating their separation and resulting in cleaner water. In the leather industry, it similarly binds to contaminants such as dyes, aiding in effluent purification ($Glick\ et\ al.$, 2005); when added to milk, alum has been found to induce coagulation, leaving behind a clear whey ($Dwarakanath\ et\ al.$, 2020).

2.1.3. Starter culture

Starter culture refers to selected strains of food-grade microorganisms with known and stable metabolic activities, used in the production of fermented foods that exhibit desirable appearance, texture, body, and flavour (*Elhadi*, 2022). Another definition describes a starter culture as microorganisms chosen for their ability

to produce lactic acid – essential for curd formation – and to lower pH levels to inhibit spoilage. These cultures may also generate metabolites that enhance flavour or enzymes that aid in the ripening of dairy products (*Laranjo et al.*, 2019).

2.2. Sample preparation

2.2.1. Preparation of sprouted bambara nut

Sprouted bambara nut (*Figure 1*) was prepared according to the modified version of *Nyau et al.* (2017). Four portions of 1,400 g seeds of bambara nut labelled as S_0 to S_4 was soaked in water at a ratio of 2:1 for 8 hrs until fully imbibed (while water used in soaking was changed every 4 hrs during the soaking period to avoid fermentation). A portion was sprouted for one day and labelled as S_1 , another portion was sprouted for two days (S_2), and the remaining two portions were sprouted for three and four days respectively (labelled as S_3 and S_4).

2.2.2. Preparation of sprouted bambara nut milk

The method described by *Balogun et al.* (2016) was used. The sprouts were removed, the seed was dehulled by rubbing it with the palm, and the husks were sieved out of the water and subsequently wet milled with water at a ratio of 1:3. Muslin cloth was used in the extraction of milk from the groundnut mash and double filtered and kept in a container for further use.

2.2.3. Preparation of unsprouted bambara nut milk

The method of *Balogun et al.* (2016) was used to prepare bambara nut milk. 1,400 g of bambara groundnut seeds was manually sorted, cleaned with potable water, and soaked in water for 8 hrs, while water used in soaking was changed every 4 hrs during the soaking period. The seed coat was dehulled after 8 hrs of soaking, the husks sieved out of the water and subsequently wet milled with water at a ratio of 1:3. Muslin cloth was used in the extraction of milk from the groundnut mash and triple filtered and stored in a sterile white container for further use.

2.2.4. Preparation of the coagulant

The coagulant used was alum. Before the start of the production, 80 g of alum was dissolved in 130 mL of water (*Ndife et al.*, 2019). The dissolved alum served as the coagulant. 0.6 mL of starter culture was also used.

2.2.5. Preparation of bambara nut cheese

The method from literature (*Abiola et al.*, 2017) with some modifications was used to produce the cheese. Bambara nut milk was heated to a temperature of 40 °C, then a starter culture was first introduced into the milk – the addition of starter culture was to improve the overall flavour, taste, and texture – and then inoculated for 10 min. The coagulant was added to the nut milk (10 mL to 1 L) and stirred intermittently until boiling point and coagulation. The curd formed was transferred into a muslin cheese cloth (*Figure 2*) and weight was placed on it to drain out the whey. After pressing and draining, the cheese was cut into different shapes (*Figure 3*).



Figure 1. Sprouted bambara groundnut



Figure 2. Unsliced bambara nut cheese analogue



Figure 3. Sliced bambara nut cheese analogue

2.3. Methodology

2.3.1. Phytate determinations

The *Wheeler and Ferrel* (1971) method was followed to determine the phytate content in the sample. This method relies on the solubility of phytate by dilute acid

and the subsequent precipitation of the phytate with ferric ion (Fe³+). About 4 g of the sample was soaked in 100 mL of 2% HCl for 3 hrs and then filtered; then, an amount of 25 mL of filtrate was dispensed into a conical flask, and 5 mL of 0.3 mL of ammonium thiocyanate (NH $_4$ SCN) solution was added as indicator. Thereafter, 53.5 mL of distilled water was added to the mixture to give it a proper acidity, and this was titrated with standard ferric chloride solution – which contained about 1.95 milligram (mg) of iron per millilitre (mL) – until a brownish-yellow colour persisted for 5 minutes.

2.3.2. Tannin determinations

Quantitative estimation of tannins was carried out using the modified vanillin-HCl method (*Abdelseed et al.*, 2011). A 200 mg of sample was extracted using 10 mL of 1% (V/V) concentrated HCl in methanol for 20 minutes in capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 mL), and the absorbance of the developed coloured solution after 20 minutes at a temperature of 30 °C was measured at λ = 500 nm. A standard curve was prepared to express the results of catechin equivalents, and then the tannin content was calculated and expressed in mg·100 g⁻¹.

2.3.3. Saponin determinations

The spectrophotometric method described by Brunner (1984) was used for saponin analysis. An amount of 0.5 g of finely ground sample was weighed into 20 mL test tubes, and 10mL of 80% ethanol was added. The mixture was shaken in a shaker for 5 hrs to ensure uniform mixing. The mixture was filtered through a filter paper into a 100 mL beaker, and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained with saturated MgCO $_3$ was again filtered to obtain a clear, colourless solution. 1 mL of the colourless solution was pipetted into 50 ml volumetric flask, and 2 mL of 5% FeCl $_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 minutes for a blood red colour to develop. Standard solutions (0–10 ppm) of saponin were prepared from stock solution. The standard solutions were treated similarly with 2 mL of 5% FeCl $_3$ solution, as done for the 1 mL sample 3 above. The absorbance of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2lD spectrophotometer at a wavelength of $\lambda = 380$ nm. The calculation was made in conformity with Equation 1.

$$c_s = \frac{10^{-4} \cdot c \cdot V \cdot f_d}{m}, \frac{mg \ saponine}{g \ probe},\tag{1}$$

where: c_s – concentration of saponin (mg/g probe); c – concentration of standard saponin solution (µg/ml); V – volume of the probe (mL); f_d – dilution factor; m – weight of the probe (g).

2.3.4. Polyphenol determination

To the 3 mL of extracts, 10 mL of ethanol was added, and they were warmed up in a water bath for 15 minutes. A few drops of freshly prepared ferric cyanide $(Fe(CN)_3)$ solution was then added to the mixture. The formation of blueish-greenish colour indicates the presence of polyphenols. To 1 mL of extract, a few drops of 5% solution of lead acetate $((CH_3COO)_2Pb)$ was added. The appearance of yellow precipitate indicates the positive results for polyphenols. To the 5 mL of ethanolic extract, 3 mL of 0.1% gelatine solution was added. The formation of precipitate is positive for polyphenols.

2.3.5. Flavonoid determination

Total flavonoid content was measured according to a colorimetric assay (*Zhishen et al.*, 1999). An aliquot of 1 mL standard solution of catechin at different concentrations, or appropriately diluted samples, was added to a 10 mL volumetric flask containing 4 mL double-distilled water. Then, 0.3 mL of 10% $AlCl_3$ was added. After 6 minutes, 2 mL of 1M NaOH was added to the mixture; the solution was immediately diluted to a volume of 10 mL with double-distilled water and thoroughly mixed. Absorbance was read at $\lambda = 510$ nm spectrophotometrically.

2.3.6. Alkaloid determination

The alkaloid content of the test sample was determined by the gravimetric method as described by Onwuka (2005). About 5.0 g of the test samples was dispersed into 50 mL of 10% acetic acid solution in ethanol. The mixture was properly shaken and allowed to stand for 4 hrs before filtration. The filtrate was allowed to evaporate, and a drop of the cone NH_4OH was added to the remaining filtrate to precipitate the alkaloids. The precipitate was filtered off with a weighed filter paper and then washed with 1% NH_4OH solution. The precipitate on the filter paper was dried in an oven at 60 °C for 50 minutes and then reweighed.

2.3.7. Anthocyanin detection

2 ml of aqueous extract was taken, to which 2 N HCl was added and then followed by the addition of ammonia; the conversion of pink-red into blue-violet indicates the presence of anthocyanins.

2.3.8. Colour analysis

A colorimeter was used to measure the colour of three samples. The equipment was calibrated with a standard light white reference tile, and the measurements were accomplished under standard illuminant. The achieved results were stated in terms of L*, brightness (L* ¼ 0, black; L* ¼ 100, white), a*, redness to greenness (positive to negative values, respectively), and b*, yellowness to blueness (positive to negative values, respectively) values using the Lab Colour System.

2.3.9. Texture analysis

The texture profile analysis was conducted on a computerized testometric material testing machine (machine number 0500-10080). The condition setup in the Texture Analyser for measuring textural properties was as follows: speed: 102 mm/min; preload test speed: 60 mm/min; post-test speed: 1.0 mm/s; count: 2; deflection: 3 mm (50% strain); preload (trigger) force: 0.200 N; acquisition rate: 200 pp/s; load cell: 50 kg; probe diameter: 100 mm.

2.3.10. Determination of pH

The pH and acidity were assessed in the final products as previously described (*AOAC* 2000). Briefly, the pH of 20 g of cheese in 20 mL of distilled water was measured using a pH meter.

2.3.11. Determination of percentage yield

The yield was determined by a method described by *Igyor et al.* (2006) and revised by *Balogun et al.* (2016). The yield of cheese from nut milk samples was determined by calculation as follows (equation 2):

$$Y_{ch} = 100 \cdot \frac{V_m}{m_{ch}}, \%, \tag{2}$$

where: Y_{ch} – yield of bambara nut cheese (%), V_{m} – volume of bambara nut milk (mL), m_{ch} – weight of obtained bambara nut cheese (g), assuming that 1 g = 1 mL.

2.3.12. Total titratable acidity

This was determined using the AOAC 942.15 official method (AOAC 2000) as follows: an amount of 5 g sample was dissolved in 25 mL of distilled water in a beaker and stirred. The mixture was then filtered. The filtrate was made up to

a volume of 100 ml. A volume of 10 mL of the filtrate was pipetted into a beaker, and 1 drop of phenolphthalein was added. The mixture was then titrated against the standard 0.01 N sodium hydroxide solution until a light pink colour was attained. The reading of the burette was recorded, and the measurements were made in triplicate. The results were expressed as g citric acid / kg sample, according to the following equation:

$$A_T = 100 \cdot \frac{N_{NaOH} \cdot t \cdot A_{LA} \cdot f_d}{m_s}, \frac{g \, acid}{100 \, g \, product'}$$
(3)

where: A_T – titratable acidity (g acid/100 g product); N_{NaOH} – normality of NaOH solution (0.01); t – titre value; A_{LA} – lactic acid value (0.09); f_d – dilution factor (10); m_s – mass of the sample (5 g).

2.3.13. Total soluble solid

Cheese samples (approximately 50 g) were blended with 200 ml of deionized water using a blender. The total soluble solids were determined using a digital refractometer (ERMA, Japan) with a scale of 0–32 °Brix (least count 0.2 °Brix) at room temperature (~30 °C) and the °Brix value calculated using a dilution factor (adapted from AOAC, 2000).

2.3.14. Dietary fibre

Gravimetric dietary fibre analysis – exemplified by AOAC Method 985.29 – is a precise technique for quantifying dietary fibre in food samples. The cheese sample was weighed and treated to dissolve soluble fibres, leaving insoluble ones behind. After filtration and washing, the insoluble fraction was dried, and its weight was determined. Next, acid hydrolysis was performed to break down complex carbohydrates, followed by filtration, washing, and drying of the hydrolysed carbohydrates. The dried hydrolysed carbohydrates were weighed. The dietary fibre content was calculated as the difference between the initial weight of insoluble fibre and the weight of hydrolysed carbohydrates.

2.3.15. Statistical analysis

SPSS 16.0 was used to statistically analyse the data obtained from the study. Results obtained are triplicate determinations and were subjected to an analysis of variance to determine the significant differences among the samples, and the means were separated using Duncan's test.

3. Results and discussions

3.1. Phytochemical composition of sprouted and unsprouted bambara nut cheese analogue

The phytochemical properties of cheese analogue prepared from unsprouted and sprouted bambara nut at different periods are represented in *Table 1*. The anthocyanin values varied from 1.49 to 3.36. S_1 had the highest and S_3 the lowest value. There was a significant ($p \le 0.05$) decrease in anthocyanin content in the cheese analogue sprouted for 3 days. (There was significant difference in the value of tannin for each treatment, with values ranging from 0.10 to 0.26). Sample S_2 had the highest value, while sample S_1 had the lowest value. This can be attributed to the effect of sprouting on the tannin content of the bambara nut seeds. Although tannins are important in protecting seeds grown in unfavourable environments, they lower the palatability of the crops by causing bitter taste in plants (*Rauf et al.*, 2019). Low tannin levels ensure the absorption of essential micronutrients and digestion of protein (*Khazaei et al.*, 2019).

The phytate values of the samples varied from 0.06 to 0.24. The highest was sample S_2 , and the lowest was sample S_0 . There was no significant difference between samples S_2 and S_4 . Alkaloid values varied from 0.53 to 1.09, with no significant difference between samples S_1 and S_3 . Dirya et al. (2020) reported an increase in alkaloids in sprouted mung bean, horse gram, black gram, and chickpeas. Increased concentration of alkaloids may improve the biological activity of the cheese analogue. Alkaloids have been reported to have anti-cancer (Gupta et al., 2015) and anti-malarial activity (Ntie-Kang et al., 2014) and to help prevent stroke (Kumar et al., 2012). Saponin values varied from 0.08 to 0.22, the highest being sample S_0 and the lowest sample S_2 . There was no significant difference between samples S_1 and S_4 .

Flavonoid values ranged from 0.17 to 0.31. Sample S_1 had the highest while sample S_0 the lowest value. There was no significant difference between samples S_3 and S_4 . Polyphenol values varied from 0.29 to 0.12. Sample S_1 had the highest while sample S_0 the lowest value. There were significant differences between the samples.

3.2. Physicochemical properties of sprouted and unsprouted bambara nut cheese analogue

The physicochemical properties of bambara nut cheese analogue – namely, total titratable acidity (TTA), total soluble solids (TSS), pH, and percentage yield (% yield) – are represented in *Table 2*. There were significant differences (p < 0.05) in TTA, TSS, Ph, and % yield. The TTA of the bambara nut cheese analogue

samples ranged from 0.02 to 0.04. The lowest TTA value was recorded in S_0 and the highest in samples S_1 , S_2 , S_3 , and S_4 . There was no significant difference between samples S_2 and S_4 .

Table 1. Phytochemical content of cheese analogue from sprouted and unsprouted bambara nut

Samples (g)	Anthocyanin (mg/g)	Tannin (mg/g)	Phytate (mg/g)	Alkaloid (mg/g)	Saponin (mg/g)	Flavonoid (mg/g)	Polyphenol (mg/g)
S_0	$2.81 \pm 0.03^{\circ}$	0.10 ± 0.01^{a}	0.06 ± 0.00^{a}	0.57 ± 0.05^{ab}	0.22 ± 0.00^{d}	0.17 ± 0.02^{a}	0.12 ± 0.01^{a}
S_{1}	$3.36 \pm 0.04^{\rm e}$	$0.26 \pm 0.00^{\rm e}$	0.24 ± 0.00^{d}	0.64 ± 0.03^{b}	$0.14 \pm 0.00^{\rm b}$	0.31± 0.06°	$0.29 \pm 0.00^{\rm e}$
S_2	$1.76 \pm 0.06^{\rm b}$	0.18 ± 0.01^{c}	0.08 ± 000^{b}	0.53 ± 0.04^{a}	0.08 ± 0.03^{a}	0.25 ± 0.06^{bc}	0.02 ± 0.01^{c}
S_3	1.49 ± 0.00^{a}	0.23 ± 0.00^{d}	$0.15 \pm 0.00^{\circ}$	$0.62 \pm 0.00^{\rm b}$	$0.17 \pm 0.02^{\circ}$	0.19 ± 0.01^{ab}	$0.26 \pm 0.00^{\rm d}$
$S_{_4}$	3.02 ± 0.01^{d}	0.13 ± 0.00^{b}	$0.08 \pm 0.00^{\rm b}$	$1.09 \pm 0.07^{\circ}$	0.13 ± 0.00^{b}	0.18 ± 0.00^{ab}	$0.15\pm0.00^{\rm b}$
Notes: Values are mean ± standard deviation of duplicate determinations. Mean values							
along the same column with different superscripts are significantly different (p < 0.05).							
Keys (for all tables): S_0 – cheese analogue prepared from unsprouted bambara nut; S_n – cheese analogue prepared from n-days sprouted bambara nut (n = 1–4).							

This range of TTA values is lower than that of the cheese analogue produced from blends of cashew nut and soybean, as reported by *Oyeyinka et al.* (2019). Titratable acidity acts as preservative, and it also imparts the taste of the bambara nut cheese analogue.

The TSS of the bambara nut cheese analogue samples varied from 2.27 to 4.02 (g/L). The lowest value of TSS was recorded in sample S_0 and the highest value in sample S_1 . The TSS for the cheese analogue samples prepared from sprouted bambara were higher than that of the reference sample. This is as a result of the effect of sprouting on the water-soluble solids in the cheese analogue samples. The total soluble solids in food drinks represent the dissolved solids as sugar.

The pH of the cheese analogue samples was found to be slightly acidic. It ranged from 4.80 to 5.44, sample S_0 having the lowest and sample S_3 the highest value. There was no significant difference between the pH values of S_1 and S_2 . There was a significant decline between the pH values of sample S_4 , which can be attributed to the extended period of sprouting. The pH values were slightly higher than the ones prepared from blends of cashew nut and soybean, as reported by *Oyekinka et al.* (2017), in the range of 4.65–5.25.

The yield of the bambara nut cheese analogue samples varied from 27.18 to 35.45 %. Sample S_4 had the lowest, while sample S_2 had the highest yield. This

might be due to the leaching of 60% water and carbohydrate, 6% lipids and crude protein, and also that components can be metabolized during germination, which, therefore, reduce that verified content (*Nowshin et al.*, 2018).

		1		
Samples	Total titratable acidity	Total soluble solid (°Brix)	pН	% Yield
S_{0}	0.02 ± 0.01^{a}	2.27 ± 0.15^{a}	$4.80 \pm 0.03^{\circ}$	$31.90 \pm 0.05^{\rm b}$
$\mathbf{S}_{_{1}}$	0.03 ± 0.00^{ab}	$4.20\pm0.00^{\rm d}$	$4.83 \pm 0.03^{\circ}$	34.97 ± 0.05^{d}
S_{2}	0.03 ± 0.01^{bc}	4.03 ± 0.29^{a}	5.44 ± 0.04^{d}	$35.45 \pm 0.05^{\rm e}$
$\mathbf{S}_{_{3}}$	$0.04 \pm 0.00^{\circ}$	$3.57 \pm 0.12^{\circ}$	4.56 ± 0.01^{a}	$34.45 \pm 0.05^{\circ}$
S,	0.04 ± 0.01 bc	2.83 ± 0.06^{b}	4.96 ± 0.04^{b}	27.18 ± 0.05 ^a

Table 2. Physicochemical properties of cheese analogue from sprouted and unsprouted bambara nut

3.3. Effect of sprouting on the colour properties of sprouted and unsprouted of bambara nut cheese analogue

The colour parameters (L*, a*, b* value) of the five cheese analogue samples prepared from sprouted and unsprouted bambara nut are represented in *Table 3*. L* (lightness) values ranged from 48.43 to 58.14. Lightness (L*) values of S4 were the highest, followed by S_3 , S_2 , S_1 , and S_0 . There was no significant difference between samples S_0 and S_1 .

The a* (Redness) value ranged from -1.00 to -0.19. Samples S_0 and S_4 were not significantly different, just as samples S_1 and S_4 . The a* (Redness) value ranges indicated that all of the samples were not significantly reddish, as the values were negative.

Sample	L*(lightness)	a*(redness)	b*(yellowness)
$S_{_0}$	51.63 ± 0.39^{b}	0.97 ± 0.01^{a}	10.08 ± 0.09^{b}
$S_{_1}$	52.30 ± 0.08^{b}	0.77 ± 0.03^{b}	9.31 ± 0.03^{a}
S_{2}	48.43 ± 0.28^{a}	0.19 ± 0.03^{d}	11.19 ± 0.16^{d}
S_3	$53.54 \pm 0.32^{\circ}$	0.82 ± 0.01^{b}	11.08 ± 0.09^{d}
$\overline{S_4}$	58.14 ± 0.71^{d}	1.00 ± 0.03 ^a	10.51 ± 0.04°

Table 3. Colour properties of cheese analogue from sprouted and unsprouted bambara nut cheese analogue

From *Table 3*, it can be seen that the b* (Yellowness) value of the samples ranged from 11.91 to 10.08. As the period of sprouting of the samples varied, there was a

significant difference (p > 0.05). Sample S_2 had the highest value of 11.19, while sample S_1 had the lowest value of 9.31.

3.4. Dietary fibre composition of sprouted and unsprouted bambara nut cheese analogue

Table 4 shows the dietary fibre composition of the bambara nut cheese analogue samples. The soluble dietary fibre of the cheese analogue samples varied from 1.37 to 2.29 %. The lowest value was recorded in sample S_0 and the highest value in sample S_3 . There was no significant difference in samples S_2 and S_4 . The insoluble dietary fibre of the cheese analogue samples ranged from 0.59 to 1.75 %. The lowest value was recorded in sample S_0 and the highest value in sample S_3 . There was no significant difference between samples S_2 , S_3 , and S_4 .

The total dietary fibre of the cheese analogue samples varied from 1.69 to 4.05 %, with significant differences. The lowest value was recorded in sample S_0 and the highest value in sample S_3 . Megat et al. (2016) reported an increase in the soluble dietary, insoluble dietary, and total dietary fibre of sprouted soybeans, peanuts, kidney beans, and mung beans. The observed increase in the values of the total dietary fibre as the days of sprouting increased could be attributed to the effect of sprouting on the complex sugar, which resulted in the development of more fibre structure in the nuts. The dietary fibre value of sample S3 was in the same range of values as the bread prepared from blends of wheat flour and germinated bambara nut flour at a ratio of 95: 5 (Chiemela et al., 2023).

The observed decrease in the value of the total dietary fibre of sample S_4 can be attributed to increased sprouting duration (4 days). As sprouting continues, the enzyme responsible for breaking down complex carbohydrates and fibre may further degrade the fibre. This may also be attributed to the nut energy reserve to start the growth of a new plant.

Bullibura Irat							
Sample	Soluble dietary fibre %						
S_0	1.37± 0.01ª	0.59 ± 0.03^{a}	1.96 ± 0.01^{a}				
$S_{_1}$	$1.61 \pm 0.01^{\rm b}$	$1.14 \pm 0.00^{\rm b}$	2.75 ± 0.01^{b}				
S_{2}	$2.23 \pm 0.02^{\circ}$	$1.73 \pm 0.00^{\circ}$	3.95 ± 0.01^{d}				
S_3	$2.29 \pm 0.04^{\rm d}$	$1.75 \pm 0.01^{\circ}$	$4.05 \pm 0.02^{\rm e}$				
S	2.17 ± 0.01°	1.71 ± 0.03°	$3.88 \pm 0.04^{\circ}$				

Table 4. Dietary fibre of cheese analogue from sprouted and unsprouted bambara nut

3.5. Textural properties of sprouted and unsprouted bambara nut cheese analogue

The textural parameters of the cheese analogue prepared from sprouted and unsprouted bambara nut are illustrated in *Table 5*. The hardness of the samples was in the range of 1.74–4.79. The hardness of bambara nut cheese analogue was significantly lower in sample S_4 . There was no significant (p > 0.05) difference between the samples S_1 and S_3 . The springiness of the samples was in the range of 0.28–1.00. Sample S_3 prepared from 3 days sprouted bambara had lower springiness than others. There was no significant (p > 0.05) difference between samples S_1 , S_2 , and S_3 .

The adhesiveness of the samples was in the range of 0.08–0.43. Sample S_0 had lower springiness than others. There was no significant (p > 0.05) difference between the samples. Therefore, sprouting did not have significant effect on the adhesiveness of the bambara nut cheese analogue samples. The capacity of any food material to attach to itself is measured by its cohesiveness, which quantifies the food structure's internal resistance.

Table 5. Textural properties of cheese analogue prepared from sprouted and unsprouted bambara nut

Sample	HRD (N)	SPG	ADH (N.s)	СОН	CHW (N)	FRC (N)	GUM (N)	ETP (N.m)	STR (mm)
S	4.79 ± 0.07 ^{bc}	0.28 ± 0.05 ^a	0.08 ± 0.13 ^a	0.31 ± 0.01 ^a	0.39 ± 0.11 ^a	0.69 ± 0.34 ^a	1.49 ± 0.67^{ab}	$0.02 \pm 0.00^{\rm b}$	10.76± 0.61°
S ₁	2.42 ± 0.22 ^{ab}	1.00± 0.00 ^b	0.32 ± 0.28 ^a	0.42 ± 0.09 ^a	1.02 ± 0.09 ^a	0.32 ± 0.00°	1.01 ± 0.14 ^{ab}	0.01 ± 0.00°	7.84 ± 0.34 ^a
S_2	7.14 ± 0.82°	0.33± 0.04ª	0.09 ± 0.12 ^a	0.27 ± 0.00 ^a	0.66 ± 0.16 ^a	0.45 ± 0.04 ^a	1.98 ± 0.25 ^b	0.04 ± 0.00°	10.62 ± 0.04°
S_3	4.39 ± 0.21 ^{ab}	0.23 ± 0.02 ^a	0.32 ± 0.03^{a}	0.29 ± 0.00^{a}	0.29 ± 0.02^{a}	0.59 ± 0.38^{a}	1.31 ± 0.07^{ab}	0.01 ± 0.00^{ab}	9.54 ± 0.04 bc
S_4	2.42 ± 0.22^{ab}	1.00± 0.00 ^b	0.32 ± 0.28 ^a	0.42 ± 0.09 ^a	1.02 ± 0.09 ^a	0.32 ± 0.00°	0.75± 0.33ª	0.01± 0.00a	8.39 ± 0.78 ^{ab}

Notes: HRD – Hardness, SPG – Springiness, ADH – Adhesiveness, COH – Cohesiveness, CHW – Chewiness, FRC Fracturability, GUM – Gumminess, ETP – Energy to peak, STR – Stringiness.

The cohesiveness of different tofu was in the range of 0.27–0.42. However, there was no significant (p > 0.05) difference between the samples in terms of cohesiveness of the bambara nut cheese analogue. Chewiness was in the range of 0.29–1.02. Sample S_3 had lower chewiness than others. There was no significant

(p > 0.05) difference between the samples. Therefore, sprouting does not have a significant effect on the chewiness of the bambara nut cheese analogue samples.

Fracturability was in the range of 0.32–0.69. Sample S_1 had lower fracturability than others. There was no significant (p > 0.05) difference between the samples. Therefore, sprouting them did not have significant effect on the fracturability of the bambara nut cheese analogue samples. The gumminess of the samples was in the range of 1.01–1.98. Sample S_4 had lower gumminess, while sample S_2 had the highest gumminess. There was no significant (p > 0.05) difference between samples S_0 , S_1 , and S_3 .

The energy to crack of the samples was in the range of 0.01–0.04. Sample S_1 prepared had lower energy to crack than others. There was no significant (p > 0.05) difference between samples S_1 and S_4 . The stringiness of the samples was in the range of 7.84–10.76. Sample S_1 had lower stringiness than others. There were significant (p < 0.05) differences between the samples. Sample S_1 had the lowest while sample S_0 the highest stringiness.

4. Conclusions

The study revealed that both sprouting and the duration of sprouting had significant impact on the phytochemical and physicochemical properties of bambara nut cheese analogues. Samples from sprouted bambara nuts exhibited notable differences in phytochemical composition, pH, yield, total soluble solids, and total titratable acidity when compared to the control sample made from unsprouted nuts. Sprouting increased the dietary fibre content of the cheese in terms of soluble and insoluble dietary fibre.

Samples prepared from bambara nuts sprouted for three days (sample S3) recorded the highest levels of dietary fibre content compared to other samples, indicating that a three-day sprouting period may offer an optimal balance between improved nutritional quality and desirable physicochemical properties. These findings suggest that sprouting serves as an effective pre-processing technique for improving the functional and nutritional attributes of bambara nut. Future studies may explore the sensory attributes, shelf life, and consumer acceptance to further validate their viability as alternatives in the growing plant-based food sector.

References

[1] Abdelseed, B. H., Abdalla, A. H., Mohamed, A. I., Babiker, E., Some nutritional attributes of selected newly developed lines of sorghum (*Sorghum bicolor*) after fermentation. *Journal of Agricultural Science and Technology* (2011).

- [2] Abiola, S. S., Adewumi, O. O., Oyawale, M., Takunbe, O. F., Comparative evaluation of sodom apple extract and lemon juice as vegetable coagulants in the manufacture of homemade cheese. *Journal of Agricultural Science and Environment*, 17. 2. (2017) 13–19. DOI: 10.51406/jagse.v17i2.1829.
- [3] AOAC, Official methods of analysis. 17th ed, The Association of Official Analytical Chemists Methods 925.10, 65.17, 974.24, 992.16. Gaithersburg. (2000).
- [4] Balogun, M. A., Kolawole, F. L., Joseph, J. K., Adebisi, T. T., Ogunleye, O. T., Effect of fortification of fresh cow milk with coconut milk on the proximate composition and yield of warankashi, a traditional cheese. *Croatian Journal of Food Science and Technology*, 8. 1. (2016) 10–14. DOI: 10.17508/CJFST.2016.8.1.02.
- [5] Brunner, J. H., Direct spectrophotometer determination of saponin. *Analytical Chemistry*, 34. 396. (1984) 1314–1326.
- [6] Campus, M. et al., Impact of almond variety on "Amaretti" cookies as assessed through image features modeling, physical chemical measures and sensory analyses. *Foods*, 9. (2020) 1272. DOI: 10.3390/foods9091272.
- [7] Chinma, C. E. et al., Germinated bambara groundnut (*Vigna subterranea*) flour as an ingredient in wheat bread: Physicochemical, nutritional, and sensory properties of bread. *Journal of Food Science*, 88. 6. (2023) 2368–2384. DOI: 10.1111/1750-3841.16585.
- [8] Divya, D. K, Ravi Varma, B., Nagamani, T. S., Effect of sprouting on the anti-nutritional factors in Vigna radiate (L), Macrotyloma uniflorum (L), Vigna mungo (L) and Cicer arietinum (L). International Journal of Innovative Science and Research Technology, 5. 6. (2020) 1451–1453. DOI: 10.38124/ IJISRT20JUN1071.
- [9] Elhadi Sulieman, A. M., Starter cultures: Nature, types, characteristics, preparation and maintenance. In: Elhadi Sulieman, A. M., Mariod, A. A. (eds.), African fermented food products New trends. Springer Nature Switzerland, Cham. (2022) 15–30. DOI: 10.1007/978-3-030-82902-5.
- [10] Glick, T., Alum. In: Glick, T., Livesey, S. J., Wallis, F. (eds.), *Medieval science, technology, and medicine An encyclopedia*. Routledge Taylor & Francis, London. (2005) 32.

- [11] Gupta, A. P. et al., Alkaloids: A source of anticancer agents from nature. Studies in Natural Products Chemistry, V. 46. (2015) 341–445. DOI: 10.1016/B978-0-444-63462-7.00009-9.
- [12] Halimi, A. R.; Mayes, S., Barkla, B., King, G., The potential of the underutilized pulse bambara groundnut [*Vigna subterranea* (L.) Verdc.] for nutritional food security. *Journal of Food Composition*, 77. (2019) 47–59. DOI: 10.1016/j. jfca.2018.12.008.
- [13] Igyor, M. A., Igbian, E. K., Iorbo, C. I., Effects of soymilk supplement on the yield and quality of "Warankasi". *Nigerian Food Journal*, 24. 1. (2006) 92–97. DOI: 10.4314/nifoj.v24i1.33638.
- [14] Khazaei, H. et al., Eliminating vicine and convicine, the main anti-nutritional factors restricting faba bean usage. *Trends in Food Science & Technology*, 91. (2019) 549–556. DOI: 10.1016/j.tifs.2019.07.051.
- [15] Kumar, G., Khanum, F., Neuroprotective potential of phytochemicals. *Pharmacognosy Reviews*, 6. 12. (2012) 81–90. DOI: 10.4103/0973-7847.99898.
- [16] Laranjo, M., Potes, M. E., Elias, M., Role of starter cultures on the safety of fermented meat products. Frontiers in Microbiology, (2019). DOI: 10.3389/ fmicb.2019.00853.
- [17] Mefleh, M., Pasqualone, A., Caponio, F., Faccia, M., Legumes as basic ingredients in the production of dairy-free cheese alternatives. *Journal of the Science of Food and Agriculture*, 102. 1. (2021) 8–18. DOI: 10.1002/jsfa.11502.
- [18] Mashau, M. E., Ramatsetse, K. E., Takalani, T., Bamidele, O. P., Ramashia, S. E., Effect of bioprocessing on the nutritional composition, antinutrients, functional properties, and metabolites of bambara groundnut and its prospective food applications: A review. *Molecules*, 30. 11. (2025) 2356. DOI: 10.3390/molecules30112356.
- [19] Mbosso, C. et al., Fonio and bambara groundnut value chains in Mali: Issues, needs, and opportunities for their sustainable promotion. *Sustainability*, 12. 11. (2020) 4766. DOI: 10.3390/su12114766.
- [20] Megat, M. R. M., Azrina, A., Norhaizan, M. E., Effect of germination on total dietary fibre and total sugar in selected legumes. *International Food Research Journal*, 23. 1. (2016) 257–261.

- [21] Ndife, J., Imade, I., Ubbor, S., Production and quality evaluation of soy cheese (tofu) using selected coagulants. *Journal of Sustainable Agriculture and the Environment*, 17. 2. (2019) 212–224. DOI: 10.17508/CJFST.2021.13.1.05.
- [22] Nowshin, H., Devnath, K., Begum, A. A., Mazumder, M. A.R., Effects of soaking and grinding conditions on anti-nutrient and nutrient contents of soy milk. *Journal of Bangladesh Agricultural University*, 16. 1. (2018) 41–46. DOI:10.3329/jbau.v16i1.36497.
- [23] Ntie-Kang, F. et al., The potential of anti-malarial compounds derived from African medicinal plants, part II: A pharmacological evaluation of non-alkaloids and non-terpenoids. *Malaria Journal*, 13. 81. (2014) DOI: 10.1186/1475-2875-13-81.
- [24] Ntundu, W. H.; Shillah, S. A., Marandu, W. Y. F., Christiansen, J. L., Morphological diversity of Bambara groundnut [Vigna subterranea (L.) Verdc.] landraces in Tanzania. Genetic Resource Crop Evolution, 53. (2006) 367–378. DOI: 10.1007/s10722-004-0580-2.
- [25] Nyau, V., Prakash, S., Rodrigues, J., Farrant, J., Profiling of phenolic compounds in sprouted common beans and bambara groundnuts. *Journal of Food Research*, 6. 6. (2017) 74–82. DOI: 10.5539/jfr.v6n6p74.
- [26] Onwuka, G. I., Food analysis and instrumentation: Theory and practice. Naphthali Print, Lagos. (2005) 133–137.
- [27] Oyeyinka, A. T., Odukoya, J. O., Adebayo, Y. S., Nutritional composition and consumer acceptability of cheese analog from soy and cashew nut milk. *Journal of Food Processing and Preservation*, 43. 5. (2019) 1745–4549. DOI: 10.1111/jfpp.14285.
- [28] Pretorius, B., Otto, M., Schönfeldt, H. C., Antinutrients and metabolomic compounds of bambara groundnut (*Vigna subterranean*) as affected by traditional processing by smallholder farmers. *Journal of Food Science*, 88. 8. (2023) 3435–3444. DOI: 10.1111/1750-3841.16698.
- [29] Rauf, A., Imran, M., Abu-Izneid, T., Patel, D., Pan, X. E., Proanthocyanidins: A comprehensive review. *Biomedicine and Pharmacotherapy*, 116. (2019) 108999. DOI: 10.1016/j.biopha.2019.108999.

- [30] Symons, J. M., *Plain talk about drinking water*. American Water Works Association, Denver. (2010).
- [31] Tachie, C., Nwachukwu, I. D., Aryee, A. N., Trends and innovations in the formulation of plant-based foods. *Food Production, Processing and Nutrition*, 5 (2023). DOI: 10.1186/s43014-023-00129-0.
- [32] Wheeler, R. L., Ferrel, R. E., A method for phytic acid determination in and wheat fractions. *Cereal Chemistry*, 48. 3. (1971) 312–320.
- [33] Zhishen, J., Mengcheng, T., Jianming, W., The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64. 4. (1999) 555–559. DOI: 10.1016/S0308-8146(98)00102-2.