www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 637.147.3:582.736.3]:664.55
Original research paper
https://doi.org/10.5937/ffr0-63281

NUTRACEUTICAL, PROXIMATE, AND SENSORY QUALITIES OF READY-TO-CONSUME BAMBARA GROUNDNUT DAIRY SUBSTITUTE SUPPLEMENTED WITH BULLOCK AND GINGER POWDER

Emmanuel A. Irondi[*1], Hope T. Aina[1], Abdulhakeem O. Sulyman[1], Yunus T. Imam[1,2], Wasiu Awoyale[3], Emmanuel O. Ajani[1]

[1]Kwara State University, Department of Biochemistry, Malete, P.M.B. 1530, Ilorin, Nigeria
[2]University of Abuja, Department of Biochemistry, P.M.B. 117, Abuja, Nigeria
[3]Kwara State University, Department of Food Science and Technology, Malete, P.M.B. 1530, Ilorin, Nigeria

Abstract: Plant-based dairy substitutes have gained significant attention recently due to consumers' growing demand for healthier food options. This study evaluated the nutraceutical, proximate and sensory qualities of ready-to-consume Bambara groundnut milk substitute (BGMS) supplemented with bullock and ginger, as natural supplements. BGMS was formulated from Bambara groundnut flour and supplemented with an optimised proportion of one of the bullock, ginger, and bullock-ginger blends to obtain milk substitutes, coded BGMS-B, BGMS-G, and BGMS-BG, respectively, with BGMS (without any natural additive) as the control. Each sample was freeze-dried to obtain a ready-to-consume BGMS. Bioactive constituents (total flavonoids, tannins, total phenolics and total saponins), enzyme (pancreatic lipase, alpha-amylase, alpha-glucosidase and angiotensin I-converting enzyme (ACE)) inhibitory and antioxidant (ferric reducing power, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$-scavenging) activities of the ready-to-consume BGMS samples were analysed. BGMS-B had the highest total flavonoids (0.98±0.01 mg/g), tannins (12.57±0.02 mg/g), total phenolics (23.49±0.52 mg/g) and saponins (25.82±1.71 mg/g) levels. It also exhibited the most potent inhibitory effects against pancreatic lipase (IC_{50}: 329.53±0.03 µg/mL), alpha-glucosidase (IC_{50}: 116.03±0.03 µg/mL), and ACE (IC_{50}: 370.90±0.01 µg/mL), displaying the strongest antioxidant capacity. BGMS-G had the highest protein content (24.50±0.85%), while BGMS-B had the lowest fat (4.47±0.35%), ash (3.49±0.00%), and moisture content (5.99±0.78%). All the samples had overall sensory attributes acceptability above 5 points. Hence, supplementing the ready-to-consume BGMS with bullock and ginger enhanced its bioactive constituents, enzyme inhibitory and antioxidant capacities, making it a potential functional food.

Key words: *plant-based milk, natural additive, bioactivity, antioxidant activity, enzyme-inhibitory activity, sensory quality*

INTRODUCTION

Increasing consumer awareness of the relationship between nutrition and non-communicable diseases—such as diabetes, obesity, hypertension, and certain cancers—has led to a growing preference for diets rich in plant-based foods rather than animal-derived products (Pahane, Tatsadjieu, Bernard, & Njintang, 2017; Irondi et al., 2025). However, there is growing pressure on the world's food supply due to the growing global population, which is predicted

Corresponding author: Phone: +234 803 487 0657
E-mail address: emmanuel.irondi@kwasu.edu.ng

to reach roughly 10 billion people by 2050, the migration of people from rural to urban areas, who are becoming increasingly wealthy, and the changes in nutrition (McClements, Newman & McClements, 2019). The Ukraine and Russia war (Irondi, Imam, Ajani & Alamu., 2023; Kubatko, Merritt, Duane & Piven., 2023) and climate change (Farooq, Farooq, Akbar, Hassan, & Gheewala 2023; Mirón, Linares & Díaz, 2023) have recently put the world's food supply in unprecedented danger, working against the United Nations' sustainable development goals on food security. These advancements necessitate a more environmentally friendly food system that can mitigate pollution and climate change, while also enhancing the health of both people and animals. In this context, plant-based diets, such as plant-based milk substitutes (PBMS), are a promising option, as they improve human health, promote food sustainability, and reduce environmental pollution, water, and land use (McClements, Newman & McClements, 2019; Irondi, Imam, Ajani & Alamu, 2023).

PBMS are made from plant sources and have a continuous phase colloidal system consisting of water and the dispersed phase of particles. The dispersed particles consist of solid portions of plant matrices, lipid droplets, protein fractions, and starch granules (Briviba, Gräf, Walz, Guamis & Butz, 2016; Bocker & Silva, 2022). These are plant extracts that dissolve in water and are designed to resemble animal milk. They are a sustainable dietary option beneficial to human health, animal welfare, and the environment (Haas, Schnepps, Pichler & Meixneret, 2019; McClements & Grossmann, 2021; Rasika et al., 2021; Reyes-Jurado et al., 2023). The PBMS has different nutritional, nutraceutical, and sensory attributes. Several reports have demonstrated the potential of the PBMS to meet human dietary needs and promote health. For instance, they are an important source of moisture, carbohydrates, protein, fat, unsaturated fatty acids, fiber (Aydar, Tutuncu & Ozcelik, 2020), vitamins, minerals, and essential fatty acids (Reyes-Jurado et al., 2023). The nutraceutical benefits of PBMS are also well-known. They are a source of bioactive compounds, such as phenolic compounds and peptides, with health-beneficial qualities like antioxidant, immunostimulatory, anti-hypertensive, anti-microbial, anti-cancer, and anti-anaemic activities (Zujko & Witkowska, 2014; Aydar et al., 2020; Paul, Kumar, Kumar & Sharma, 2020; Reyes-Jurado et al., 2023).

Bambara groundnut (*Vigna subterranean*) is a legume belonging to the family and sub-family of Fabaceae and Faboidea, respectively (Ibny, Jaiswal, Mohammed & Dakora, 2019). Bambara groundnut (BG) is a leguminous crop native to Africa, with its origin generally traced to the region spanning West and Central Africa (Temegne et al., 2018). It grows in the wild in northern Nigeria and eastwards to southern Sudan and is currently grown across tropical Africa and to a smaller range in tropical portions of America, Asia, and Australia (Adeleke, Adiamo & Fawale, 2018). BG is the third most significant legume after groundnut (*Arachis hypogaea*) and cowpea (*Vigna hypogaea*) (Arise, Amonsou & Ijabadeniyi, 2015; Oyeyinka & Oyeyinka, 2018). It is rich in protein (18.0–24.0%) and could help alleviate protein deficiency globally if combined with other native protein sources (Mbosso et al., 2020; Ramatsetse, Ramashia & Mashau, 2023). BG is a proper healthy food due to its high iron (4.9–48 mg/100 g) and protein contents, when compared to other legume products, with a high amount of amino acids content, fiber (5.0–12%), fat (5.0–7.0%), carbohydrate (57.43–63.09%) calcium (95.8–99 mg/100 g), potassium (1144–1935 mg/100 g), and sodium (2.9 – 12.0 mg/100 g) (Mbosso et al., 2020; Tan et al., 2020). BG's diverse nutritional composition suggests that it can meet the dietary needs of people (Maphosa, Jideani & Maphosa, 2020). It also contains several polyphenols, such as flavonoids (medioresinol, catechin, catechin dimer anthocyanin, and epicatechin) and phenolic acids (quinic acid, chlorogenic acid, caffeic acid, and ellagic acid) that have health benefits and strengthen the immune system against infectious diseases (Nyau, Prakash, Rodrigues & Farrant, 2015; Harris, Jideani & Le Roes-Hill, 2018; Ramatsetse et al., 2023).

Furthermore, antioxidants from plant extracts, including BG, have also been reported to inhibit lipid oxidation in food products, thereby preventing transition metals and oxygen from reacting with food (Oyeyinka & Oyeyinka, 2018; Manessis et al., 2020; Okafor, Jideani, Meyer & Le Roes-Hill, 2022). A study of the flavour and composition of BG milk in comparison to other plant-based milk prepared from other legumes, such as cowpea, pigeon pea and soybean, has been previously investigated. The acceptability of BG milk was ranked high compared to other plant milks; also, the colour and flavour of BG milk were the most preferred (Murevanhema & Jideani, 2015; Harris, 2017). Hence, BG can be an appealing plant-based milk source.

The processing techniques applied to PBMS significantly influence its characteristics, including its micronutrient composition. Water-soluble vitamins may be lost when the raw material is soaked before production. However, its nutritional profile may be enhanced by supplementing with natural food additives (Silva, Silva & Ribeiro, 2020). Food additives are natural or synthetic substances added intentionally to food during its processing, packaging, and transition for different purposes, such as preservation, sweetening, and flavour enhancement (Elshama, 2020). Various studies have reported that synthetic additives contribute little to the nutritional value of food, thus providing limited nutritional advantages for consumers (Savin et al., 2022). Some studies have also suggested that synthetic additives may increase consumers' susceptibility to certain diseases, such as diabetes and cancer, and may cause adverse reactions in the gastrointestinal, respiratory, dermatological, and neurological systems (Savin et al., 2022; Elshreif, Elkhoudary, Moustafa, Hadad & el-Gendy, 2023).

These limitations of the synthetic additives, coupled with consumers' growing demand for healthier food options, have prompted the exploration of natural additives in food production. Over the past several decades, natural food additives have attracted increasing attention from both consumers and food manufacturers. In general, consumers tend to prefer foods that are either free from additives or contain natural additives, rather than those formulated with synthetic alternatives. (Carocho, Barreiro, Morales & Ferreira, 2014).

This study focused on formulating and determining some quality attributes of ready-to-consume BG milk substitute (BGMS) supplemented with natural ingredients. The selected natural ingredients, namely bullock and ginger, used in this study as natural antioxidants and flavouring agents, respectively, are known to possess some health-benefiting properties.

Bullock *(Annona reticulata)* is a perennial herbaceous plant belonging to the Asclepiadaceae family. The plant is found in the tropical regions of Africa, across Senegal to Nigeria, and over the Congo basin to southern tropical Africa (Kayode, Ohanaka, Femi-Olabisi, Agboola & Oyebanji, 2022). Bullock leaf extract was reported to possess high nutritional quality, including high crude protein, mineral (especially calcium and potassium), and vitamin content (Olumide et al., 2022). According to the report of Awoniyi, Abidakun and Oladiji (2022), bullock leaf infusion is a food-based approach for alleviating diet-induced iron deficiency in weanling rats. Several pharmacological activities, including haematological and antianemic activity, antioxidant, antidiabetic, antipyretic, anti-inflammatory, antiasthmatic, analgesic, antimicrobial, memory-enhancing, and antilipidemic activities, have been attributed to *P. nigrescens.* These pharmacological activities were attributed to phytochemical constituents in bullock, including tannins, flavonoids, alkaloids, steroids, cardiac glycosides, coumarin, terpenoids, saponins, and anthraquinones (Adase et al., 2022).

Ginger (*Zingiber officinale*) is a plant that has been used for medicinal purposes for centuries. Ginger has a wide range of bioactive compounds that are responsible for its various health benefits. Studies have shown that ginger has anti-inflammatory properties and can be useful in reducing pain, especially in conditions such as osteoarthritis and rheumatoid arthritis (Mutthuraj et al., 2020). It has also been found to have antioxidant effects, which can help reduce oxidative stress in the body (Mustafa & Chin, 2023). In addition, ginger has been shown to have potential benefits for digestive health, such as reducing symptoms of nausea and vomiting, and improving indigestion (Akrami, Naseri & Kianbakht, 2021). It has also been found to have a blood-sugar-lowering effect, which may make it useful in the management of type 2 diabetes (El Gayar, Aboromia, Ibrahim & Hafiz, 2019).

Although some researchers have reported on the quality of BG milk (Murevanhema & Jideani, 2015; Okudu, Ojinnaka & Iloh, 2016; Pahane et al., 2017), there is a paucity of information on the effect of natural additives, specifically bullock and ginger, supplementation on the nutraceutical, proximate, and sensory qualities of ready-to-consume BG milk. Therefore, this study was designed to evaluate the nutraceutical, proximate and sensory attributes of BGMS supplemented with bullock and ginger.

MATERIALS AND METHODS

The materials (BG, bullock leaves, and ginger rhizome) used in this study were obtained from a local market in Kwara State, Nigeria. Analytical grades of chemicals and reagents were used.

Preparations of samples

The BG flour was prepared as described by Kaptso et al. (2015), with minor modifications. Dried BG seeds were sorted, washed, and soaked in clean water in a ratio of 1/5 (w/v) for 24 h at ambient temperature. Thereafter, the seeds were drained, manually dehulled, and blanched in boiled water (100 °C) for 20 s, and air-dried.

The dried sample was milled in a hammer milling machine (Zhecheng Hong Xin Machinery Factory, HXXM-250, China) with a 500 µm mesh sieve. The bullock leaves sample was rinsed with potable water and air-dried for seven days. The ginger rhizome was carefully washed, peeled, and sliced thinly, after which it was air-dried for one week. The bullock leaves and ginger rhizome samples were ground into powder using a HXXM-250 hammer milling machine, packed in sealed polythene bags, appropriately labelled, and stored at ambient temperature for analysis.

Preparation of natural additive blend

The natural additives (bullock leaves and ginger rhizome powder) blends used in this study were formulated using the ratios derived from the Response Surface Methodology Central Composite Rotatable Design of the Design Expert software (Version 6.0). Total phenolics, as well as total flavonoids, being key bioactive compounds with antioxidant and digestive enzyme-inhibitory properties, were maximized, while tannin was minimized due to its anti-nutrient and astringent properties. Nine combination ratios of the individual natural additives were derived using the Design Expert software as shown in Table 1.

Subsequently, the combination ratio with the best desirability, as determined by the Design Expert software, was used for formulating the natural additives blend used for BGMS production. Ginger was used as a natural flavouring agent, while bullock was used as a natural antioxidant.

Table 1.
Central composite rotatable design of the selected natural ingredient combinations (bullock and ginger) used for the ready-to-consume Bambara groundnut milk substitute production

Runs	Bullock (g)	Ginger (g)
1	1.98	3.75
2	3.75	3.75
3	5.52	3.75
4	2.50	5.00
5	2.50	2.50
6	3.75	5.52
7	3.75	1.98
8	5.00	2.50
9	5.00	2.00

BGMS production

The extraction of BGMS was based on the method reported by Mang et al. (2016), with some modifications. Initially, 500 g of BG flour was suspended in 1.5 L of potable water and stirred using an electric blender. The mixture was then filtered through a double-layer muslin cloth. The sediment was subjected to a second extraction under the same conditions.

The first and second filtrates were combined, followed by the addition of natural additives (2.28 g of bullock and 3.38 g of ginger) and 7.5 g of sugar. The BGMS samples were then pasteurized for 30 minutes at 65 °C.

After pasteurization, the samples were cooled to ambient temperature. BGMS sample supplemented with bullock was coded BGMS-B; the BGMS sample supplemented with ginger was coded BGMS-G, while the BGMS sample supplemented with bullock and ginger blend was coded BGMS-BG. The BGMS sample without any additive served as the control (Table 2).

The BGMS samples were packed in 2-liter plastic containers and stored in a refrigerator. Thereafter, the BGMS samples were freeze-dried using a lyophilizer (Searchtech Instruments, LGJ-10, UK). The freeze-dried BGMS samples (Fig. 1) were subsequently pulverized into powder to obtain the ready-to-consume BGMS.

Table 2.
Ingredients for the production of ready-to-consume Bambara groundnut plant milk

Plant milk samples	**Bambara groundnut flour** (g)	**Water** (L)	**Bullock** (g)	**Ginger** (g)	**Sugar** (g)
BGMS (control)	500	2.5	_	_	7.5
BGMS-B	500	2.5	2.28	_	7.5
BGMS-G	500	2.5	_	3.98	7.5
BGMS-BG	500	2.5	2.28	3.98	7.5

BGMS: Bambara groundnut milk substitute; BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitutes with bullock and ginger

Figure 1. Lyophilised plant-based milk samples. BGMS (Control): Bambara groundnut milk substitute without bullock and ginger; BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitute with bullock and ginger.

Preparation of BGMS extract

A sample (0.2 g) of the ready-to-consume BGMS was soaked with 20 mL of methanol for 24 h, after which it was filtered through Whatman (No. 1) filter paper.

The filtrate (referred to as extract hereafter) was used for bioactive components and bioactivity assays.

Determination of bioactive constituents

Bioactive constituents, including total flavonoid content (TFC), tannin content (TC), total phenolic content (TPC), and total saponin content (TSC), in BGMS samples were quantified using established spectrophotometric methods.

The TPC was quantified using Folin-Ciocalteu reagent and expressed on the basis of gallic acid standard, as described by Kareem et al. (2022).

The TC was quantified using Folin-Denis reagent and calculated on the basis of tannic acid standard as per Olatoye, Irondi, Awoyale and Adeyemo (2023). TFC was determined using $AlCl_3$ reagent and expressed on the basis of quercetin, in line with Kareem et al. (2023). The TSC was quantified using vanillin reagent and expressed on the basis of diosgenin standard, as outlined by Makkar, Siddhuraju and Becker (2017).

Pancreatic lipase inhibition assay

BGMS extract's pancreatic lipase inhibition assay was assessed *in vitro* (Eom, Lee, Lee, Kim & Kim, 2013), with p-nitrophenyl butyrate and orlistat serving as the substrate and positive control, respectively. To prepare the enzyme solution, 30 μL (10 units) of porcine pancreatic lipase was combined with 10 mM morpholine propane sulphonic acid and 1 mM EDTA at pH 6.8. This mixture was then added to 850 μL of Tris buffer, containing 100 mM Tris-HCl, and 5 mM $CaCl_2$, at a pH of 7.0. This was followed by incubating a mixture of 100 μL of different dilutions of the extract and the enzyme solution (880 μL) at 37 °C for 10 min. Subsequently, the hydrolysis reaction was initiated by adding 20 μL of a 10 mM p-nitrophenyl butyrate solution in dimethyl formamide and incubating the mixture at 37 °C for 20 min. Afterward, the absorbance reading of the p-nitrophenol produced from p-nitrophenyl butyrate hydrolysis was taken at 405 nm in a UV-Visible spectrophotometer (Lasany, LI-722, UK). The pancreatic lipase percentage inhibition of the BGMS extract was calculated, and the IC_{50}, the concentration of BGMS extract causing 50% PL activity inhibition, was determined using the concentration-inhibition linear regression equation of the BGMS extract.

Alpha-amylase inhibition assay

BGMS extract's alpha-amylase inhibition assay was performed using porcine pancreas α-amylase (EC 3.2.1.1), with soluble starch and acarbose as substrate and reference inhibitor, respectively. Different dilutions of the BGMS extract, totaling 500 μL, were mixed with 500 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/mL of porcine pancreas α-amylase solution. The mixture was then incubated at 37 °C for 10 min, before adding 500 μL of 1% starch solution in 0.02 M sodium phosphate buffer. The mixture was further incubated at 37 °C for 15 min. Next, 1.0 mL of 3,5-dinitrosalicylic acid reagent was added to the mixture to terminate the alpha-amylase-catalyzed starch hydrolysis. After that, further incubation of the reaction mixture in a boiling water bath (Searchtech instruments, DK-600, UK) for 5 minutes was performed. This was followed by cooling the reaction mixture to ambient temperature and diluting it with 10 mL of distilled H_2O, before reading the absorbance at 540 nm in a UV-Visible spectrophotometer (Lasany, LI-722, UK). The IC_{50} of BGMS extract against alpha-amylase was calculated using the concentration-inhibition linear regression equation of the extract (Kareem et al., 2023).

Alpha-glucosidase inhibition assay

BGMS extract's alpha-glucosidase inhibition assay was conducted by reacting 0.05 mL of the sample's extract and 0.05 mL of alpha-glucosidase (5 units) and subjecting the mixture to a 10-minute incubation at 37 °C. Next, 0.1 mL of *p*-nitrophenyl glucopyranoside (PNPG; 3 mmol/L in 0.02 M phosphate buffer; pH of 6.9) was dispensed into the mixture, which was further incubated at 37 °C for 20 minutes. Subsequently, 2 mL of 0.1 M Na_2CO_3 was added to the mixture to terminate alpha-glucosidase-catalysed PNPG hydrolysis, after which the absorbance reading of the *p*-nitrophenol formed was taken at 400 nm (Kareem et al., 2023).

Angiotensin 1-converting enzyme (ACE) inhibition assay

To assay for ACE inhibitory activity of the BGMS samples' extract, the procedure outlined by Xu, Singh and Hillier (2006), was adopted. The sample extract (50 μL) was reacted with 50 μL of ACE solution (4 mU/mL), and incubated for 15 minutes at 37 °C. Next, 150 μL of hippuryl-histidyl-leucine (8.33 mM) in Tris-HCl buffer (125 mM; pH 8.3) was dispensed into the reaction mixture, followed by a 30-minute incubation at 37 °C. The reaction was halted by adding 250 μL of HCl (1 M), and the hippuric acid formed was extracted with 1500 μL of ethyl acetate. After centrifugation, 1000 μL of the ethyl acetate layer was placed in a clean test tube and evaporated to dryness in a hot-air oven. The resulting hippuric acid residue was re-dissolved with 1000 µL of deionized H_2O, and the absorbance was read at 228 nm in a UV-Visible spectrophotometer (Lasany, LI-722, UK).

Determination of reducing power

The ferric reducing power was assayed by mixing 2500 µL of the BGMS extract with 2500 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 2500 µL of 1% potassium ferricyanide. After a 20-minute incubation at 50 °C, 2500 µL of 10% (w/v) trichloroacetic acid solution added and the mixture was centrifuged for 10 minutes at 650 rpm.

Subsequently, 2500 µL of the supernatant was mixed with 2500 µL of distilled H_2O, followed by 1000 µL of 0.1% ferric chloride solution addition. The sample's absorbance, read at 700 nm, was used to calculate its ferric reducing power based on a gallic acid standard curve (Elemosho et al., 2021).

Determination of ABTS$^{•+}$ [2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation] scavenging capacity

ABTS$^{•+}$ scavenging capacity of the BGMS samples was analysed by reacting 200 µL of each sample's extract with 2000 µL of ABTS$^{•+}$ reagent. After incubating at room temperature for 30 minutes, the sample's absorbance was recorded at 734 nm in a UV/Visible spectrophotometer (Lasany, Visible LI-722, UK). Before use, the ABTS$^{•+}$ reagent, containing an equal volume of 0.007 M ABTS and 0.00245 M $K_2S_2O_8$, was incubated in a dark cupboard for 16 hours at an ambient temperature, after which its absorbance (at 734 nm) was adjusted to 0.7 ± 0.02 using 95% ethanol. The sample's ABTS$^{•+}$-scavenging capacity was calculated based on Trolox standard (Kareem et al., 2023).

Determination of DPPH$^{•-}$ scavenging activity

To assay for DPPH radical scavenging capacity, 1000 µL of the sample's extract was reacted with 3000 µL of DPPH radical solution (0.06 mM in methanol). This was followed by a 30-minute incubation of the reaction mixture at ambient temperature, after which the absorbance was recorded at 517 nm (Kareem et al., 2023).

Proximate content and metabolisable food energy determination

Proximate composition (moisture, crude fat, crude protein, ash, and total carbohydrate) of the ready-to-consume BGMS was determined by the AOAC (2025) method. Moisture content was determined by oven-drying 5 g of the sample for 24 hours at 105 °C in a Fisher Scientific Co. oven (model 655F, USA). For ash content determination, 2 g of the sample, contained in a pre-weighed crucible was incinerated in a muffle furnace (Fisher Scientific Co. USA, model m186A) at 600 °C for 6 hours.

The sample's crude protein level was analysed by digesting 0.25 g of the sample at 420 °C for 1 hour. Thereafter, 40% NaOH was added to the digestate, which was heated to liberate NH_3 into a boric acid receiver solution. The NH_3 was titrated against 0.1 M HCl using an automated Kjeldahl analyser (Kjeltec 2300). The nitrogen content (% N) obtained from the titration was multiplied by 6.25 to convert it to protein content (%).

Crude fat content was analysed by extracting 3 g of the sample in a dry thimble with 50 mL of normal hexane using a Soxtec HT unit. The extraction took place in boiling mode for 15 minutes, and in rinsing mode for 30-45 minutes. Total carbohydrate level of the sample was calculated by difference, according to the following formula:

Total carbohydrate level (%) = 100 - (% Moisture + % Protein + % Fat + % Ash)

Metabolisable energy levels of the samples were calculated using Atwater factors (Sarker, Chakraborty & Roy, 2020) for protein, fat, and carbohydrates thus:

Metabolisable energy (kCal/100 g) = (% Crude protein × 4) + (% Total carbohydrate × 4) + (% Fat × 9)

Sensory evaluation

Ethical clearance (KWASU/CRandD/REA/-2024/0035) for the sensory evaluation of the BGMS samples was obtained from the institutional Research Ethics Board of Kwara State University, Malete, Nigeria. Informed consent was obtained from all the sensory panellists who participated in the BGMS sensory study.

Following the protocol described by Uchoa et al. (2009), the sensory evaluation was conducted in a sensory laboratory using a 9-point hedonic scale (1 - extremely dislike, 2 - dislike very much, 3 - dislike moderately, 4 - dislike slightly, 5 - neither like nor dislike, 6 - like slightly, 7 - like moderately, 8 - like very much, and 9 - extremely like). The samples' colour, taste, flavour, viscosity, aroma, appearance, mouth feel, and overall acceptability were evaluated. Fifty informed panellists (30 females and 20 males in the age range of 18 to 25), representing Kwara State University, Malete, students participated in the evaluation. The BGMS samples were coded randomly and served to the panellists in a single session. The panellists were provided potable water for palate-cleansing between different BGMS evaluation.

Statistical analysis of data

Triplicate results of each test were subjected to one-way analysis of variance (ANOVA). Comparison of means was performed using Duncan's Multiple Range Test at 95% level of confidence. Statistical Package for Social Sciences (version 21) was used for data analysis.

RESULTS AND DISCUSSION

Optimisation of natural additive for BGMS production

The criteria for optimising the natural additives (bullock leaves and ginger rhizomes powder) blend used for BGMS production are presented in Table 3. Bullock and ginger had constraints that were in the acceptable range. For optimisation, total phenolics and total flavonoids levels were maximized, considering their health-promoting properties. However, tannin content was minimized due to their astringency. The combination outcome having the best desirability (0.54; that is, 54%), consisting of bullock (2.28 g) and ginger (3.98 g), was then used for the BGMS production.

Bioactive constituents of the BGMS samples

The analysis of the bioactive components in the ready-to-consume BGMS samples (Table 4) revealed that the BGMS supplemented with natural additives (BGMS-B, BGMS-G, and BGMS-BG) had higher ($p<0.05$) levels of total phenolics, tannins, total flavonoids, and saponins, compared to the control (BGMS without any natural additive). Among the BGMS with natural additives, BGMS-B consistently exhibited the highest concentrations of total phenolics (23.49±0.52 mg/g), tannins (12.57±0.02 mg/g), and total flavonoids (0.98±0.01 mg/g), followed by BGMS-G and BGMS-BG. This may reflect higher levels of these bioactive constituents in bullock in comparison with ginger. A preliminary bioactive constituent analysis (data not shown) of the bullock and ginger powders used in this study revealed that bullock had 27.16±0.08 and 12.11±0.19 mg/g, while ginger had 24.40±0.03 and 9.56±0.05 mg/g of total phenolics and flavonoids, respectively. However, the total saponin concentrations in BGMS-B and BGMS-G were not significantly different ($p>0.05$). Supplementing food products with natural additives rich in bioactive constituents has been reported as an approach to improving their nutritional and health-promoting qualities.

For example, adding a natural additive blend of sweet detar seed, ginger rhizome, and hibiscus calyx powder enhanced the nutritional and health-promoting qualities of instant bio-yoghurts (Irondi, Bankole, Awoyale, Ajani & Alamu, 2024). Furthermore, the bioactive constituents detected in the BGMS supplemented with natural additives in this study are well-documented for their health benefits, such as starch-digesting enzyme inhibitory and antioxidant properties (Abdulrazaaq, Imam, Irondi & Ajani, 2024).

Table 3.
Optimisation of the desired plant milk attributes and supplementation levels in the production of ready-to-consume Bambara groundnut (BG)-based milk substitute

Parameters	Constraints	Lower limit	Upper limit	Solution
Bullock supplementation level (g)	Within the range	2.5	5	2.28
Ginger supplementation level (g)	Within the range	2.5	5	3.98
Total phenolics (mg)	Maximize	26.83	32.03	29.76
Tannins (mg)	Minimize	18.35	20.84	19.07
Flavonoids (mg)	Maximize	2.01	2.60	2.25
Desirability				**0.54**

Table 4.
Content of bioactive constituents in ready-to-consume bambara groundnut plant milk samples

Samples	**Total phenolics** (mg/g)	**Total flavonoids** (mg/g)	**Tannins** (mg/g)	**Total saponins** (mg/g)
BGMS (Control)	1.14±0.11[a]	0.44±0.01[a]	0.11±0.03[a]	11.63±0.03[a]
BGMS-B	23.49±0.52[d]	0.98±0.01[d]	12.57±0.02[d]	25.82±1.71[c]
BGMS-G	19.32±0.18[c]	0.77±0.01[c]	7.23±0.01[c]	25.44±0.41[c]
BGMS-BG	11.98±0.87[b]	0.59±0.01[b]	4.63±0.03[b]	15.12±0.22[b]

Data are the mean values ± SD of triplicate determinations. Data with the different letters in the same column are significantly different (p<0.05). BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitute with bullock and ginger

Antioxidant activities of the BGMS samples

Figures 2-4 display the antioxidant activity of the BGMS samples. $ABTS^{•+}$-scavenging activity (Fig. 2) varied significantly ($p<0.05$) among the samples, with BGMS-B having the strongest activity, followed by BGMS-G, BGMS-BG, and BGMS. The SC_{50} (half-maximal scavenging concentration) of the samples against $DPPH^{•}$ also differed significantly, such that BGMS-B (297.79±0.04 μg/mL) < BGMS-G (308.32±0.01 μg/mL) < BGMS-BG (335.18±0.06 μg/mL) < Control (350.71±0.11 μg/mL) (Fig. 3).

Thus, the $DPPH^{•}$-scavenging strengths of the samples were in the same order as their $ABTS^{•+}$-scavenging activity, since a lower SC_{50} indicates a stronger scavenging power.

The ferric reducing powers of the BGMS samples were in the following order: BGMS-B (7.31±0.06 mg GAE/g) > BGMS-G (6.39±0.72 mg GAE/g) > BGMS-BG (5.78±0.08 mg GAE/g) > BGMS (4.67±0.12 mg GAE/g). BGMS-B exhibited the highest ferric reducing power ($p<0.05$), which was statistically similar to that of BGMS-G. Additionally, the ferric reducing power of BGMS-G was comparable ($p>0.05$) to that of BGMS-BG (Fig. 4).

The stronger antioxidant capacity observed in BGMS-B relative to BGMS-G, BGMS-BG, and BGMS could be attributed to its higher levels of bioactive constituents. Flavonoids and saponins, for instance, are renowned for their antioxidant activity exhibited through diverse mechanisms, such as free radical scavenging, peroxide decomposition, metal-reducing capacity, prevention of continued hydrogen abstraction, and chain initiation transition metal ion catalysts binding (Arslan and Çelik, 2013; Irondi, Adewuyi & Aroyehun, 2022).

Several studies have reported that adding natural supplements enhances the antioxidant properties of plant-based food. Ogidi, Oladeji, Karigidi, Oluwasina and Adetuyi (2025) observed an increased antioxidant activity in PBMS when supplemented with mustard seed, date fruit, and turmeric. Similarly, the increase in the antioxidant capacity of the natural additive-supplemented BGMS observed in the present study is consistent with that reported by Balbino, Cvitković, Skendrović and Dragović-Uzelac (2023), who found improved antioxidant activity in almond-based non-dairy milk alternative enriched with herbs, including myrtle, fennel, and bay leaf extracts.

Enzyme-inhibitory activity of BGMS samples

As presented in Table 5, BGMS-B exhibited the lowest IC_{50} values against α-glucosidase, ACE, and pancreatic lipase, measuring 116.03±0.03 µg/mL, 370.90±0.01 µg/mL, and 329.53±0.03 µg/mL, respectively. BGMS-G had the lowest IC_{50} value against α-amylase at 295.18±0.04 µg/mL. However, the IC_{50} values of the standard inhibitors of the various enzymes, including acarbose (1.60±0.64 and 0.53±0.37 µg/mL for alpha-amylase and alpha-glucosidase, respectively, captopril (0.06 ± 0.04 µg/mL for ACE), and orlistat (0.20 ± 0.08 mg/g for pancreatic lipase) were lower than those of the BGMS samples.

Alpha-amylase and alpha-glucosidase hydrolyse starch molecules to form glucose, which is then absorbed and used by the body cells for energy production and other metabolic functions (Navik, Tomar & Shrivastava, 2022).

The BGMS supplemented with bullock and ginger demonstrated the ability to decelerate starch hydrolysis by inhibiting these digestive enzymes, representing an important strategy for mitigating postprandial hyperglycaemia (Pas-mans, Meex, van Loon & Blaak, 2022).

In this study, the ready-to-consume BGMS supplemented with natural additives exhibited a stronger inhibitory effect on ACE compared to the control sample without additives. The inhibition of ACE by the BGMS supplemented with the natural additives and the associated diminution in angiotensin II production may indicate its anti-hypertensive potential (Pra-veen et al., 2023). Furthermore, the pancreatic lipase inhibitory activity of ready-to-consume supplemented BGMS suggests its potential to reduce the rate of fatty acid formation, ab-sorption, and accumulation from dietary fat di-gestion, which is an important approach for controlling obesity (Point et al., 2016).

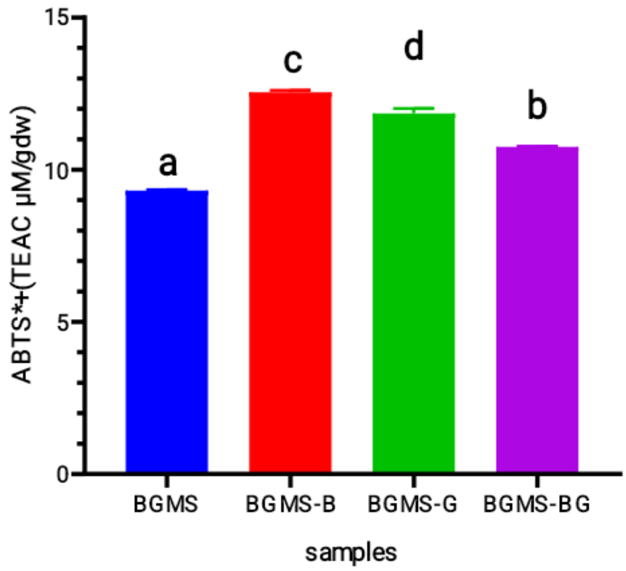

Figure 2. ABTS$^{•+}$-scavenging activity of ready-to-consume Bambara groundnut milk substitute samples.Bars represent the mean ± SD of triplicate determinations. The mean values of bars with different letters are significantly different (p<0.05). BGMS (Control): Bambara groundnut milk substitute without bullock and ginger; BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitute with bullock and ginger

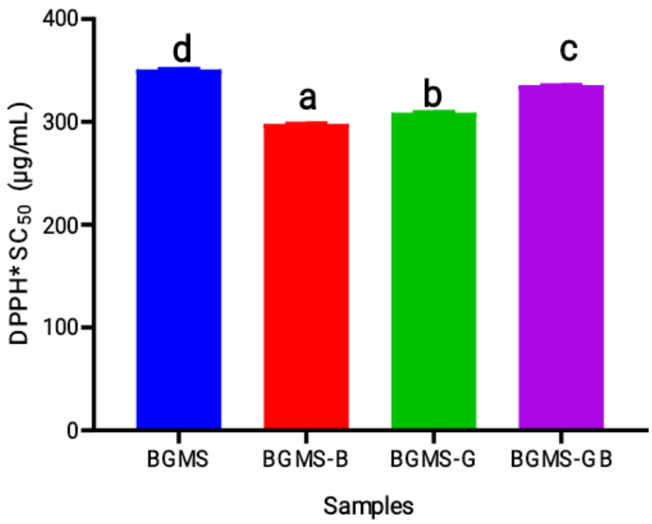

Figure 3. DPPH$^{•}$-scavenging activity of ready-to-consume Bambara groundnut milk substitute samples. Bars represent the mean ± SD of triplicate determinations. The mean values of bars with different letters are significantly different (p<0.05). BGMS (Control): Bambara groundnut milk substitute without bullock and ginger; BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitutes with bullock and ginger

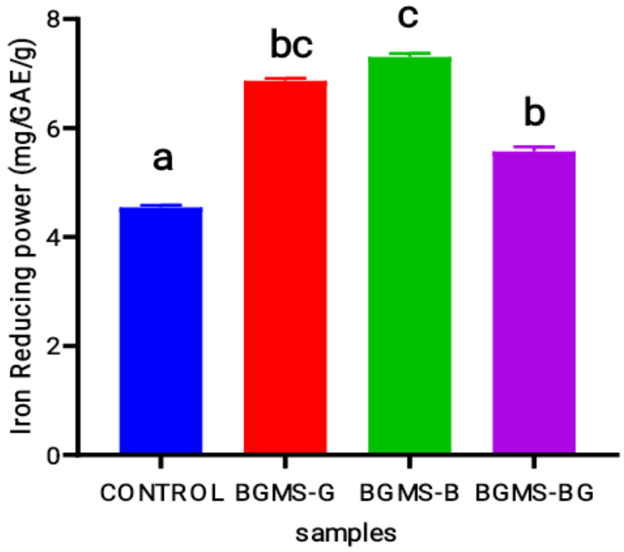

Figure 4. Ferric reducing power of ready-to-consume Bambara groundnut milk substitute samples. Bars represent the mean ± SD of triplicate determinations. The mean values of bars with different letters are significantly different (p<0.05). BGMS (Control): Bambara groundnut milk substitute without bullock and ginger; BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitute with bullock and ginger

Table 5.
Enzyme-inhibitory activity of ready-to-consume Bambara groundnut-based plant milk

Samples	Inhibitory activity			
	α-amylase IC_{50} (µg/mL)	**α-glucosidase** IC_{50} (µg/mL)	**ACE** IC_{50}(µg/mL)	**Pancreatic lipase** IC_{50} (µg/mL)
BGMS (Control)	586.39±0.02^{e}	358.34±0.02^{e}	879.29±0.03^{e}	496.69±0.01^{e}
BGMS-B	369.59±0.01^{c}	116.03±0.03^{b}	370.90±0.01^{b}	329.53±0.03^{b}
BGMS-G	295.18±0.04^{b}	158.28±0.03^{c}	453.32±0.02^{c}	351.39±0.02^{c}
BGMS-BG	430.02±0.03^{d}	176.29±0.01^{d}	520.41±0.01^{d}	415.96±0.01^{d}
Acarbose	1.60±0.64^{a}	0.53±0.37^{a}	-	-
Captopril	-	-	0.06±0.04^{a}	-
Orlistat	-	-	-	0.20±0.08^{a}

Data are the mean values ± SD of triplicate determinations. Data with the different letters in the same column are significantly different (p<0.05). BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitute with bullock and ginger; ACE: Angiotensin 1-converting enzyme

Proximate composition and metabolisable food energy of BGMS samples

In Table 6, the proximate composition and metabolisable energy of the BGMS samples are presented. The control sample had the highest ($p<0.05$) moisture level, followed by BGMS-BG, whereas the moisture levels of BGMS-B and BGMS-G were similar ($p>0.05$). The crude fat contents of the samples varied significantly, with BGMS-B having the lowest fat content (4.47±0.35%) and the control having the highest (7.57±0.43%). Ash contents were comparable in BGMS-B, BGMS-G, and BGMS-BG, but these were significantly lower than the ash content of the control.

Protein content varied significantly among the samples, ranging from 19.77±0.16% in the control to 24.50±0.85% in the BGMS-G. The BGMS-B had the highest total carbohydrate content (64.11±0.36%), while BGMS-G had the least (59.74±0.08%). The metabolisable energy content ranged from 387.46±0.73 kCal/100 g in the control to 392.41±0.23

kCal/100 g in the BGMS-G, varying significantly among the samples.

The low-level moisture content of the ready-to-consume BGMS supplemented with bullock and ginger suggests better storage stability when compared with the control sample. It is well known that a lower moisture level stalls microbial (bacterial and fungal) growth on food (Sarker, Chakraborty & Roy, 2020; Irondi et al., 2024).

The decrease in the crude fat contents of the BGMS supplemented with bullock and ginger, relative to the control, may confer a dietary advantage to the supplemented ready-to-consume BGMS samples. As affir-med by the report of Mozaffarian (2019), low-fat foods are beneficial in managing body weight, reducing the risk of chronic diseases, enhancing insulin sensitivity, and improving cardiovascular health.

The range of protein observed in this study (19.77±0.16 - 24.50±0.85%) is consistent with the 20.80% protein level in PBMS from Bambara groundnut reported by Chude et al. (2023). The high protein contents of the BGMS samples supplemented with bullock and ginger could contribute to their nutritional benefits to consumers.

High-protein food promotes growth and development, body maintenance, weight management, and improves blood glucose maintenance, among other important metabolic and physiological functions (Olatoye et al., 2023; Irondi et al., 2024). The metabolisable energy values of the ready-to-consume BGMS samples in this study are within the range (368.10 - 425.10 kCal/100 g) reported by Chude et al. (2023) in PBMS made from Bambara groundnut.

Sensory attributes of BGMS samples

The sensory attributes of the ready-to-consume BGMS samples are presented in Table 7. Significant differences ($p<0.05$) were observed in the samples' colour, taste, flavour, thickness, aroma, consistency, mouth-feel, and overall acceptability. Generally, the control sample (without bullock and ginger) had higher ratings compared to the samples supplemented with bullock (BGMS-B), ginger (BGMS-G), and a combination of both (BGMS-BG).

However, all supplemented samples achieved overall acceptability scores above 5 points, which represent the limit for sensory acceptability in this study, consistent with previous reports (Irondi et al., 2024). In this study, the overall acceptability of BGMS samples ranged from 5.92 ± 2.02 to 7.92 ± 1.16, aligning with the values of 6.2 and 6.1 reported by Okudu et al. (2016) for PBMS prepared from white and mixed-coloured Bambara groundnut varieties, respectively. In terms of taste and mouthfeel, BGMS-B received significantly ($p<0.05$) lower ratings (4.20±2.16 and 4.68±2.12, respectively) than the control and the BGMS-G. This observation could be attributed to the higher level of tannins in BGMS-B relative to the control and BGMS-G. Tannins possess a bitter and astringent property that can interfere with the palatability and acceptance of food products (Oliveira et al., 2020; Irondi et al., 2024).

Table 6.
Proximate composition and metabolisable food energy of ready-to-consume Bambara groundnut-based plant milk samples

Sample	**Moisture** (%)	**Fat** (%)	**Ash** (%)	**Protein** (%)	**Total carbohydrates** (%)	**Metabolisable energy** (kCal/100 g)
BGMS (Control)	8.67±0.39[c]	7.57±0.43[c]	3.93±0.04[b]	19.77±0.16[a]	60.08±0.95[a]	387.46±0.73[b]
BGMS-B	5.99±0.78[a]	4.47±0.35[a]	3.49±0.00[a]	21.95±0.09[c]	64.11±0.36[c]	385.92±0.08[a]
BGMS-G	6.08±0.03[a]	6.17±0.02[b]	3.52±0.01[a]	24.50±0.85[d]	59.74±0.08[a]	392.41±0.23[d]
BGMS-BG	7.11±0.04[b]	6.51±0.12[b]	3.56±0.06[a]	21.11±0.19[b]	61.73±0.04[b]	390.38±0.19[c]

Data are the mean values ± SD of triplicate determinations. Data with the different letters in the same column are significantly different ($p<0.05$). BGMS-B: Bambara groundnut milk substitutes with bullock; BGMS-G: Bambara groundnut milk substitutes with ginger; BGMS-BG: Bambara groundnut milk substitutes with bullock and ginger

Table 7.
Sensory attributes of ready-to-consume Bambara groundnut milk substitute samples

Samples	Colour	Taste	Flavour	Thickness	Aroma	Consis-tency	Mouth-feel	Overall accepta-bility
BGMS (Control)	8.12±0.92[b]	7.26±1.60[c]	7.18±1.57[c]	7.16±1.40[b]	6.32±1.95[b]	7.02±1.55[b]	6.98±1.49[c]	7.92±1.16[c]
BGMS-B	5.72±2.01[a]	4.20±2.16[a]	4.46±2.17[a]	5.78±2.11[a]	5.04±2.21[a]	5.68±2.24[a]	4.68±2.12[a]	5.94±1.79[a]
BGMS-G	7.60±1.34[b]	6.16±2.26[b]	6.56±1.64[b]	6.04±1.54[a]	5.66±1.99[ab]	6.18±2.34[ab]	6.08±2.05[b]	6.98±1.44[b]
BGMS-BG	5.50±2.14[a]	4.40±1.92[a]	4.56±2.09[a]	6.22±2.033[a]	4.92±2.28[a]	6.34±1.75[ab]	5.12±1.87[a]	5.92±2.02[a]

Data are the mean values ± SD of triplicate determinations. Data with the different letters in the same column are significantly different (p<0.05). BGMS-B: Bambara groundnut milk substitute with bullock; BGMS-G: Bambara groundnut milk substitute with ginger; BGMS-BG: Bambara groundnut milk substitute with bullock and ginger

CONCLUSIONS

The findings of this study indicate that fortifying Bambara groundnut milk substitute with bullock and ginger significantly increased its levels of bioactive constituents and enhanced its antioxidant activity, as well as its inhibitory effects on key metabolic enzymes (α-amylase, α-glucosidase, pancreatic lipase, and angiotensin-converting enzyme). Supplementation with bullock produced greater enhancements in the antioxidant activity and enzyme inhibitory capacities of the Bambara groundnut milk substitute compared to supplementation with ginger.

In contrast, ginger supplementation led to increases in the protein content and metabolisable energy values of the milk substitute. In conclusion, the incorporation of bullock and ginger improved the nutraceutical profile, proximate composition, and sensory attributes of the ready-to-consume Bambara groundnut milk substitute, thereby positioning it as a promising candidate for development as a functional food.

AUTHOR CONTRIBUTIONS

Conceptualization, E.A.I.; Methodology, H.T.A., E.A.I. and W.A.; Software, H.T.A. and W.A.; Validation, E.O.A.., Y.T.I. and W.A.; Formal analysis, H.T.A. and Y.T.I.; Investigation, H.T.A; Resources, E.A.I., H.T.A. and E.O.A.; Data curation, H.T.A, E.A.I. and Y.T.I.; Writing—original draft preparation, H.T.A. and E.A.I.; Writing—review and editing, W.A., E.O.A. and A.O.S.; Visualization, H.T.A. and W.A.; Supervision, E.A.I., A.O.S. and E.O.A.; Project administration, E.A.I.

DATA AVAILABILITY STATEMENT

Data contained within the article.

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Biochemistry, Kwara State University, Malete, Nigeria, for providing the laboratory facilities and consumables used in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdulrazaaq, K. O., Imam, Y. T., Irondi, E. A., & Ajani, E. O. (2024). Bioactive composition and anti-hyperglycemic properties of biofortified yellow maize-based gluten-free cookies. *Frontiers in Food Science and Technology, 4,* 1365021. https://doi.org/10.3389/frfst.2024.1365021

Adase, E., Ankutse, P., Kumadoh, D., Archer, M. A., Kyene, M. O., Yeboah, G. N., & Asamoah Agyare, D. O. (2022). A review of *Parquetina nigrescens* (Afzel.) Bullock, a plant for traditional medicine: Phytochemical and pharmacological properties. *Evidence-Based Complementary and Alternative Medicine, 2022*(1), 6076707. https://doi.org/10.1155/2022/6076707

Adeleke, O. R., Adiamo, O. Q., & Fawale, O. S. (2018). Nutritional, physicochemical, and functional properties of protein concentrate and isolate of newly-developed Bambara groundnut *(Vigna subterranea* L.*)* cultivars. *Food Science & Nutrition, 6*(1), 229-242. https://doi.org/10.1002/fsn3.552

Akrami, M., Naseri, M., & Kianbakht, S. (2020). The effects of ginger consumption on weight loss and metabolic profiles among overweight and obese adults: A systematic review and meta-analysis of randomized controlled trials. *Complementary Therapies in Medicine, 49*, 102098. https://doi.org/10.1080/10408398.2018.1427044

Arise, A. K., Amonsou, E. O., & Ijabadeniyi, O. A. (2015). Influence of extraction methods on functional properties of protein concentrates prepared from South African bambara groundnut landraces. *International Journal of Food Science and Technology*, *50*(5), 1095-1101. https://doi.org/10.1111/ijfs.12746

Arslan, I., & Çelik, A. (2013). Saponin-rich fractions (SRPs) from soapwort show antioxidant and hemolytic activity. *APCBEE Procedia*, *7*, 103-108. https://doi.org/10.1016/j.apcbee.2013.08.019

(AOAC) Association of Official Analytical Chemists. (2005). *Official methods of analysis* (18th ed., pp. 152-154). Washington D.C.: Association of Analytical Chemists International.

Awoniyi, A. J., Abidakun, O. M., & Oladiji, A. T., (2022). *Parquetina nigrescens* leaf infusion: a food-based approach for the management of diet-induced iron deficiency in weanling rats. *Notulae Scientia Biologicae*, *14*(3), 11275. https://doi.org/10.55779/nsb14311275

Aydar, E. F., Tutuncu, S., & Ozcelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *Journal of Functional Foods*, *70*, 103975. https://doi.org/10.1016/j.jff.2020.103975

Balbino, S., Cvitković, D., Skendrović, H., & Dragović-Uzelac, V. (2023). Optimisation of almond-based dairy-free milk alternative formulation fortified with myrtle, bay leaf and fennel extracts. *Food Technology and Biotechnology*, *61*(3), 378-388. https://doi.org/10.17113/ftb.61.03.23.8002

Bocker, R., & Silva, E. K. (2022). Innovative technologies for manufacturing plant-based non-dairy alternative milk and their impact on nutritional, sensory and safety aspects. *Future Foods*, *5*, 100098. https://doi.org/10.1016/j.fufo.2021.100098

Briviba, K., Gräf, V., Walz, E., Guamis, B., & Butz, P. (2016). Ultra-high pressure homogenization of almond milk: Physico-chemical and physiological effects. *Food Chemistry*, *192*, 82-89. https://doi.org/10.1016/j.foodchem.2015.06.063

Carocho, M., Barreiro, M. F., Morales, P., & Ferreira, I. C. (2014). Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Comprehensive Reviews in Food Science and Food Safety*, *13*(4), 377-399. https://doi.org/10.1111/1541-4337.12065

Chude, C. O., Nwagbo, C. C., Dibua, N. A., Okoye, E. C. S., Okpalanma, E. F., & Okoyeuzu, C. F. (2023). Microbial lactic acid fermentation improves nutritional and organoleptic profile of non-dairy milk made from Bambara groundnut. *American Journal of Food Science & Technology*, *2*(1), 5-15. https://doi.org/10.54536/AJFST.V2I1.1025

Elemosho, A. O., Irondi, E. A., Alamu, E. O., Ajani, E. O., Menkir, A., & Maziya-Dixon B. (2021). Antioxidant, and starch-hydrolyzing enzymes inhibitory properties of striga-resistant yellow-orange maize hybrids. *Molecules*. 26, 6874. https://doi.org/10.3390/molecules26226874

El Gayar, M. H., Aboromia, M. M., Ibrahim, N. A., & Hafiz, M. H. A. (2019). Effects of ginger powder supplementation on glycemic status and lipid profile in newly diagnosed obese patients with type 2 diabetes mellitus. *Obesity Medicine*, *14*, 100094. https://doi.org/10.1016/j.obmed.2019.100094

Elshama, S. S. (2020). Synthetic and natural food additives: Toxicological hazards and health benefits. *Journal of Toxicology*, *4*(4), 555643. http://dx.doi.org/10.19080/OAJT.2020.04.555643

Elshreif, H., Elkhoudary, M., Moustafa, R., Hadad, G., & el-Gendy, A. (2023). A review on food additives from the definition, and types to the method of analysis. *Records of Pharmaceutical and Biomedical Sciences*, *7*, 48-64. https://doi.org/10.21608/rpbs.2023.190784.1210

Eom, S. H., Lee, M. S., Lee, E. W., Kim, Y. M., & Kim, T. H. (2013). Pancreatic lipase inhibitory activity of phlorotannins isolated from *Eiseniabicyclis*. *Phytotherapy Research, 27*, 148-151. https://doi.org/10.1002/ptr.4694

Farooq, A., Farooq, N., Akbar, H., Hassan, Z. U., & Gheewala, S. H. (2023). A critical review of climate change impact at a global scale on cereal crop production. *Agronomy*, *13*(1), 162. https://doi.org/10.3390/agronomy13010162

Haas, R., Schnepps, A., Pichler, A., & Meixner, O. (2019). Cow milk versus plant-based milk substitutes: A comparison of product image and motivational structure of consumption. *Sustainability*, *11*(18), 5046. https://doi.org/10.3390/su11185046

Harris, T. (2017). Bambara groundnut (*Vigna subterranean*) from Mpumalanga province of South Africa: phytochemical and antimicrobial properties of seeds and product extracts (Doctoral dissertation). Cape Peninsula University of Technology, South Africa.

Harris, T., Jideani, V., & Le Roes-Hill, M. (2018). Flavonoids and tannin composition of Bambara groundnut (*Vigna subterranea*) of Mpumalanga, South Africa. *Heliyon*, *4*(9). https://doi.org/10.1016/j.heliyon.2018.e00833

Ibny, F. Y., Jaiswal, S. K., Mohammed, M., & Dakora, F. D. (2019). Symbiotic effectiveness and ecologically adaptive traits of native rhizobial symbionts of Bambara groundnut (*Vigna subterranea* L. Verdc.) in Africa and their relationship with phylogeny. *Scientific Reports*, *9*(1), 12666. https://doi.org/10.1038/s41598-019-48944-1

Irondi, E. A., Adewuyi, A. E., & Aroyehun, T. M. (2022). Effect of endogenous lipids and proteins on the antioxidant, in vitro starch digestibility, and pasting properties of Sorghum flour. *Frontiers in Nutrition*, *8*, 809330. https://doi.org/10.3389/fnut.2021.809330

Irondi, E. A., Aina, H. T., Imam, Y. T., Bankole, A. O., Anyiam, A. F., Elemosho, A. O., Kareem, B., & Adewumi, T. O. (2025). Plant-based milk substitutes: sources, production, and nutritional, nutraceutical and sensory qualities. *Frontiers in Food Science & Technology*, 5, 1593870. https://doi.org/10.3389/frfst.2025.1593870

Irondi, E. A., Bankole, A. O., Awoyale, W., Ajani, E. O., & Alamu, E. O. (2024). Antioxidant, enzymes inhibitory, physicochemical and sensory properties of instant bio-yoghurts containing multi-purpose natural additives. *Frontiers in Nutrition, 10*, 1340679. https://doi.org/10.3389/fnut.2023.1340679

Irondi, E. A., Imam, Y. T., Ajani, E. O., & Alamu, E. O. (2023). Natural and modified food hydrocolloids as gluten replacement in baked foods: functional benefits. *Grain & Oil Science and Technology*, *6*, 163-171. https://doi.org/10.1016/j.gaost.2023.10.001

Kaptso, K. G., Njintang, Y. N., Nguemtchouin, M. M. G., Scher, J., Hounhouigan, J., & Mbofung, C. M. (2015). Physicochemical and micro-structural properties of flours, starch and proteins from two varieties of legumes: bambara groundnut (*Vigna sub-*

terranea). *Journal of Food Science and Technology*, *52*, 4915-4924. https://doi.org/10.1007/s13197-014-1580-7

Kareem, B., Irondi, E. A., Alamu, E. O., Ajani, E. O., Abass, A., Parkes, E., & Maziya-Dixon, B. (2023). Antioxidant, starch-digesting enzymes inhibitory, and pasting properties of elite yellow-fleshed cassava genotypes. *Frontiers in Sustainable Food Systems*, *7*, 1129807. https://doi.org/10.3389/fsufs.2023.1129807

Kareem, B., Irondi, E. A., Alamu, E. O., Ajani, E. O., Abass, A., Adesokan, M, Parkes, E., & Maziya-Dixon B. (2022). Influence of traditional processing on the antioxidant, starch-digesting enzymes inhibitory activities and glycaemic index of yellow-fleshed cassava genotypes. *Frontiers in Nutrition*, *9*, 894843. https://doi.org/10.3389/fnut.2022.894843

Kayode, O. T., Ohanaka, N. J., Femi-Olabisi, F. J., Agboola, A. O., & Oyebanji, E. O. (2022). *Parquetina nigrescens* Species: A Concise Review of Phytochemistry and Pharmacology. *Tropical Journal of Natural Product Research*, *6*(4). https://doi.org/10.26538/tjnpr/v6i4.1

Kubatko, O., Merritt, R., Duane, S., & Piven, V. (2023). The impact of the COVID-19 pandemic on global food system resilience. *Mechanism of an Economic Regulation*, *1*(99), 144-148. https://doi.org/10.32782/mer.2023.99.22

Makkar, H. P. S, Siddhuraju, P., & Becker, K. (2007). Plant secondary metabolites. Totowa, NJ, USA: Humana Press Inc.

Manessis, G., Kalogianni, A. I., Lazou, T., Moschovas, M., Bossis, I., & Gelasakis, A. I. (2020). Plant-derived natural antioxidants in meat and meat products. *Antioxidants*, *9*(12), 1215. https://doi.org/10.3390/antiox9121215

Mang, Y. D., Njintang, Y. N., Abdou, B. A., Scher, J., Bernard, C., & Mbofung, M. C. (2016). Dehulling reduces toxicity and improves in vivo biological value of proteins in vegetal milk derived from two mucuna (*Mucuna pruriens* L.) seeds varieties. *Journal of Food Science and Technology*, *53*, 2548-2557. https://doi.org/10.1007/s13197-016-2211-2

Maphosa, Y., Jideani, V. A., & Maphosa, L. (2022). Bambara groundnut production, grain composition and nutritional value: opportunities for improvements. *The Journal of Agricultural Science*, *160*(6), 448-458. https://doi.org/10.1017/S0021859622000521

Mbosso, C., Boulay, B., Padulosi, S., Meldrum, G., Mohamadou, Y., Berthe-Niang, A., Coulibaly, H., Koreissi, Y., & Sidibé, A. (2020). Fonio and bambara groundnut value chains in Mali: issues, needs, and opportunities for their sustainable promotion. *Sustainability*, *12*(11), 4766. https://doi.org/10.3390/su12114766

McClements, D. J., & Grossmann, L. (2021). The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety*, *20*(4), 4049-4100. https://doi.org/10.1111/1541-4337.12771

McClements, D. J., Newman, E., & McClements, I. F. (2019). Plant-based milks: A review of the science underpinning their design, fabrication, and performance. *Comprehensive Reviews in Food Science and Food Safety*, *18*(6), 2047-2067. https://doi.org/10.1111/1541-4337.12505

Mirón, I. J., Linares, C., & Díaz, J. (2023). The influence of climate change on food production and food safety. *Environmental Research*, *216*, 114674. https://doi.org/10.1016/j.envres.2022.114674

Mozaffarian, D. (2019). Dairy foods, obesity, and metabolic health: the role of the food matrix compared with single nutrients. *Advances in Nutrition*, *10*, 917S–923S. https://doi.org/10.1093/advances/nmz053

Murevanhema, Y. Y., & Jideani, V. A. (2015). Production and characterization of milk produced from Bambara Groundnut (*Vigna subterranea*) Varieties. *Journal of Food Processing and Preservation*, *39*(6), 1485-1498. https://doi.org/10.1111/jfpp.12368

Mustafa, I., & Chin, N. L. (2023). Antioxidant properties of dried ginger (*Zingiber officinale* Roscoe) var. *Bentong*. *Foods*, *12*(1), 178. https://doi.org/10.3390/foods12010178

Mutthuraj, D., Vinutha, T., Gopenath, T. S., Kaginelli, S. B., Karthikeyan, M., Ashok, G., Ranjith, M. S., Palanisamy, P., & Basalingappa, K. M. (2020). Inhibition of pro-inflammatory molecules by ginger (*Zingiber officinale* Roscoe) and its anti-inflammatory effects on arthritis patients. *Journal of Drug Delivery & Therapeutics*, *10*(2s), 125-139. https://doi.org/10.10.22270/jddt.v10i2-s.3963

Navik, P., Tomar, R., & Shrivastava, V. (2022). Role of alpha amylase inhibitor in diabetes mellitus. *International Journal of Biology, Pharmacy and Allied Sciences*, *11*(11), 5062-5079. https://doi.org/10.31032/IJBPAS/2022/11.11.6547

Nyau, V., Prakash, S., Rodrigues, J., & Farrant, J. (2015). Antioxidant activities of Bambara groundnuts as assessed by FRAP and DPPH assays. *American Journal of Food and Nutrition*, *3*(1), 7-11. https://doi.org/10.12691/ajfn-3-1-2

Ogidi, C. O., Oladeji, O. A., Karigidi, K. O., Oluwasina, O. O., & Adetuyi, F. O. (2025). Fortification of non-dairy milk with date fruit, mustard seed, and turmeric: nutritional quality, probiotics viability, antimicrobial and antioxidant potentials. *Scientific Reports*, *15*(1), 34129. https://doi.org/10.1038/s41598-025-17960-9

Okafor, J. N., Jideani, V. A., Meyer, M., & Le Roes-Hill, M. (2022). Bioactive components in Bambara groundnut (*Vigna subterranea* (L.) Verdc) as a potential source of nutraceutical ingredients. *Heliyon*, *8*(3), 1-11. https://doi.org/10.1016/j.heliyon.2022.e09024

Okudu, H. O., Ojinnaka, M. C., & Iloh, C. (2016). Chemical and sensory properties of milk developed from two varieties of bambara groundnut (Vigna subterranean). *Journal of Biological Sciences and Bioconservation*, *8*(1), 1-13.

Olatoye, K. K., Irondi, E. A., Awoyale, W., & Adeyemo, O. I. (2023). Nutrient composition, antioxidant properties, and sensory characteristics of instant Kunu from pearl millet supplemented with African locust bean pulp. *Journal of Ethnic Food*, *10*, 21. https://doi.org/10.1186/s42779-023-00188-1

Oliveira, F. C. E. D., Pontes, J. P., Queiroz, V. A. V., Roncheti, E. F. S., Dutra, V. L. M., Correia, V. T. D. V., & Ferreira, A. A. (2020). Yogurt griego adicionado de harinas integrales de sorgo: potencial antioxidante y evaluación sensorial. *Revista chilena de nutrición*, *47*(2), 272-280.

http://dx.doi.org/10.4067/S0717-75182020000200272

Olumide, M. D., Akintunde, A. O., Ndubuisi-Ogbonna, L. C., Shobo, B. A., Oreagba, T., & Isiadinso, I. (2022). Nutritional and ethnomedicinal potentials of *Parquetina nigrescens* leaf extracts in livestock production. *Tropical Animal Production Investigation, 25*, 15-26.

Oyeyinka, S. A., & Oyeyinka, A. T. (2018). A review on isolation, composition, physicochemical properties and modification of Bambara groundnut starch. *Food Hydrocolloids*, *75*, 62-71. https://doi.org/10.1016/j.foodhyd.2017.09.012

Pahane, M. M., Tatsadjieu, L. N., Bernard, C., & Njintang, N. Y. (2017). Production, nutritional and biological value of bambara groundnut (Vigna subterranea) milk and yoghurt. *Journal of Food Measurement and Characterization*, *11*(4), 1613-1622. https://doi.org/10.1007/s11694-017-9541-2

Pasmans, K., Meex, R. C., van Loon, L. J., & Blaak, E. E. (2022). Nutritional strategies to attenuate postprandial glycemic response. *Obesity Reviews*, *23*(9), e13486. https://doi.org/10.1111/obr.13486

Paul, A. A., Kumar, S., Kumar, V., & Sharma, R. (2020). Milk Analog: Plant-based substitutes to conventional milk, production, potential and health concerns. *Critical Reviews in Food Science and Nutrition*, *60*(18), 3005-3023. https://doi.org/10.1080/10408398.2019.1674243

Point, V., Bénarouche, A., Zarrillo, J., Guy, A., Magnez, R., Fonseca, L., & Cavalier, J. F. (2016). Slowing down fat digestion, and absorption by an oxadiazolone inhibitor targeting selectively gastric lipolysis. *European Journal of Medicinal Chemistry*, *123*, 834-848. https://doi.org/10.1016/j.ejmech.2016.08.009

Praveen, A., Biswas, V., Goswami, S., Marisetti, A. L., Tiwari, A., Tiwari, V., Savita, N., Kumar, V., Gupta, M., Bhatia, S., & Sharma, A. (2023). Usefulness of medicinal plants in treating various diseases. In Kesharwani, P., & Gajbhiye, V. (Eds.). *Advances in phytonanotechnology for treatment of various diseases* (pp. 21-65). CRC Press. https://doi.org/10.1201/9781003231721

Ramatsetse, K. E., Ramashia, S. E., & Mashau, M. E. (2023). A review on health benefits, antimicrobial and antioxidant properties of Bambara groundnut (*Vigna subterranean*). *International Journal of Food Properties*, *26*(1), 91-107. https://doi.org/10.1080/10942912.2022.2153864

Rasika, D. M., Vidanarachchi, J. K., Rocha, R. S., Balthazar, C. F., Cruz, A. G., Sant'Ana, A. S., & Ranadheera, C. S. (2021). Plant-based milk substitutes as emerging probiotic carriers. *Current Opinion in Food Science*, *38*, 8-20. https://doi.org/10.1016/j.cofs.2020.10.025

Reyes-Jurado, F., Soto-Reyes, N., Dávila-Rodríguez, M., Lorenzo-Leal, A. C., Jiménez-Munguía, M. T., Mani-López, E., & López-Malo, A. (2023). Plant-based milk substitutes: Types, processes, benefits, and characteristics. *Food Reviews International*, *39*(4), 2320-2351. https://doi.org/10.1080/87559129.2021.1952421

Sarker, A., Chakraborty, S., & Roy, M. (2020). Dark red kidney bean (*Phaseolus vulgaris* L.) protein hydrolysates inhibit the growth of oxidizing substances in plain yogurt. *Journal of Agriculture and Food Research*, *2*, 100062. https://doi.org/10.1016/j.jafr.2020.100062

Savin, M., Vrkatić, A., Dedić, D., Vlaški, T., Vorgučin, I., Bjelanović, J., & Jevtic, M. (2022). Additives in children's nutrition – A review of current events. *International Journal of Environmental Research and Public Health*, *19*(20), 13452. https://doi.org/10.3390/ijerph192013452

Silva, A. R., Silva, M. M., & Ribeiro, B. D. (2020). Health issues and technological aspects of plant-based alternative milk. *Food Research International*, *131*, 108972. https://doi.org/10.1016/j.foodres.2019.108972

Tan, X. L., Azam-Ali, S., Goh, E. V., Mustafa, M., Chai, H. H., Ho, W. K., Mayes, S., Mabhaudhi, T., Azam-Ali, S., & Massawe, F. (2020). Bambara groundnut: An underutilized leguminous crop for global food security and nutrition. *Frontiers in Nutrition*, *7*, 601496. https://doi.org/10.3389/fnut.2020.601496

Temegne, N. C., Gouertoumbo, W. F., Wakem, G. A., Nkou, F. T. D., Youmbi, E., & Ntsomboh-Ntsefong, G. (2018). Origin and ecology of Bambara groundnut (*Vigna subterranea* (L.) Verdc): A review. *Journal of Ecology & Natural Resources*, *2*(4), 000140.

Uchoa, A. M. A., Correia da Costa, J. M., Maia, G. A., Meira, T. R., Sousa, P. H. M., & Montenegro Brasil, I. (2009). Formulation and physicochemical and sensorial evaluation of biscuit-type cookies supplemented with fruit powders. *Plant foods for human nutrition*, *64*(2), 153-159. https://doi.org/10.1007/s11130-009-0118-z

Xu, X., Singh, T. K., & Hillier, A. J. (2006). An HPLC assay for the determination of ACE inhibitory activity of food protein derived peptides. *Australian Journal of Dairy Technology*, *61*(2), 228.

Zujko, M. E., & Witkowska, A. M. (2014). Antioxidant potential and polyphenol content of beverages, chocolates, nuts, and seeds. *International Journal of Food Properties*, *17*(1), 86-92. https://doi.org/10.1080/10942912.2011.614984

NUTRITIVNI I HEMIJSKI SASTAV I SENZORSKI KVALITET GOTOVOG BILJNOG MLEKA NA BAZI BAMBARA ZEMLJANOG ORAHA OBOGAĆENOG DODATKOM PRAHA LISTA *ANNONA RETICULATA* I KORENA ĐUMBIRA

Emmanuel A. Irondi[*1], Hope T. Aina[1], Abdulhakeem O. Sulyman[1], Yunus T. Imam[1,2], Wasiu Awoyale[3], Emmanuel O. Ajani[1]
[1]Državni univerzitet Kuara, Departman za biohemiju, Malet, Ilorin, Nigerija
[2]Univerzitet u Abudži, Departman za biohemiju, Abudža, Nigerija
[3]Državni univerzitet Kuara, Departman za nauku o hrani i prehrambene tehnologije, Malet, Ilorin, Nigerija

Biljne zamene za mleko su u poslednje vreme privukle značajnu pažnju zbog sve većeg interesovanja potrošača za zdravijim prehrambenim navikama. Ova studija je ispitivala nutritivni i hemijski sastav kao i senzorska svojstva gotovog napitka od bambara zemljanog oraha kao zamene za mleko (BGMS), obogaćenog listom *Annona reticulata* i korenom đumbira u prahu kao prirodnim suplementima. Kontrolni uzorak BGMS je formulisan od brašna bambara oraha. Njegovom suplementacijom optimizovanim proporcijama *A. reticulata* (B), đumbira (G) ili njihove mešavine (BG), dobijeni su uzorci biljnog mleka označeni kao BGMS-B, BGMS-G i BGMS-BG, respektivno. Svaki uzorak je liofilizovan kako bi se dobio napitak BGMS gotov za konzumaciju. Analizirani su bioaktivni sastojci (ukupni flavonoidi, tanini, ukupni fenoli i ukupni saponini), enzimska inhibicija (pankreasna lipaza, alfa-amilaza, alfa-glukozidaza i angiotenzin I-konvertujući enzim – ACE) i antioksidativne aktivnosti (redukcioni potencijal gvožđa, ABTS•+ i DPPH•- skevindžer) gotovih BGMS uzoraka. Biljno mleko BGMS-B je imalo najviši sadržaj ukupnih flavonoida (0.98±0.01 mg/g), tanina (12.57±0.02 mg/g), ukupnih fenola (23.49±0.52 mg/g) i saponina (25.82±1.71 mg/g). Takođe, BGMS-B je imalo najjače inhibitorne efekte protiv pankreasne lipaze (IC50: 329.53±0.03 µg/mL), alfa-glukozidaze (IC50: 116.03±0.03 µg/mL) i ACE (IC50: 370.90±0.01 µg/mL), uz najjači antioksidativni kapacitet. Uzorak biljnog mleka BGMS-G je imao najveći sadržaj proteina (24.50±0.85%), dok je BGMS-B imao najniži sadržaj masti (4.47±0.35%), pepela (3.49±0.00%) i vlage (5.99±0.78%). Svi uzorci su imali ukupnu prihvatljivost senzorskih atributa iznad 5 bodova. Prema tome, suplementacija biljnog mleka na bazi bambara oraha (BGMS) sa listom *A. reticulata* i korenom đumbira poboljšala je njegov bioaktivni sastav, enzimske inhibitorne i antioksidativne kapacitete, čineći ga potencijalnom funkcionalnom hranom.

Ključne reči: *zamena za mleko, prirodni dodaci, bioaktivnost, antioksidaciona aktivnost, enzimsko-inhibitorna aktivnost, senzoski kvalitet*

Received: *06 December 2025* / ***Received in revised form:*** *12 February 2026* / ***Accepted:*** *26 February 2026*

Available online: *March 2026*