

Effect of Bambara Groundnut Flour Incorporation on the Nutritional, Phytochemical, and Sensory Characteristics of Tapioca

Oluwatoyin H. Animashaun * and Oluseun T. Orelaja

Food Technology Department, Moshood Abiola Polytechnic, Abeokuta 110104, Nigeria

* Correspondence: toyinraji33@gmail.com; Tel.: +234-703-248-3930

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Abstract: Nutritional, phytochemical, sensory, and microbial quality of tapioca grits fortified with Bambara groundnut flour was investigated. The nutrient content of Bambara groundnut makes it suitable for fortification of tapioca. Cassava roots were processed into cassava starch while Bambara groundnut was processed into flour. Tapioca grits were prepared by substituting Bambara groundnut flour into cassava starch at levels between 10 and 50%. The fortified tapioca grits were evaluated for functional, proximate, mineral, anti-nutritional, and microbiological quality according to standard methods. The analysis's findings indicated that the contents of moisture, ash, fat, fiber, protein, and carbohydrates ranged from 6.96 to 9.19%, 1.16 to 1.99%, 7.05 to 11.40%, 0.00 to 1.12%, 5.43 to 17.29%, and 59.54 to 79.40%, respectively. The functional properties were improved by the addition of Bambara groundnut flour to tapioca grits. The values for calcium, magnesium, potassium, iron, and zinc ranged from 0.033 to 0.068%, 0.030 to 0.148%, 0.026 to 0.258%, 40.57 to 336.71 mg/kg, and 1.06 to 13.03 mg/kg, respectively. The values for cyanide, alkaloid, tannin, flavonoid, and trypsin inhibitor ranged from 5.65 to 4.77 mg/kg, 3.076 to 3.724%, 0.069 to 0.183 mg/g, 1.290 to 1.778 mg/g, and 0.023 to 0.613 TIA/mg, respectively. The total viable count ranged from 1.20×10^2 to 2.50×10^2 cfu/g for freshly produced tapioca substituted with BGF and 1.00×10^2 to 1.37×10^2 cfu/g for those stored for 30 days. The fungi count ranged from 0.60×10^2 to 1.32×10^2 cfu/g and 2.05×10^2 to 12.65×10^2 cfu/g for freshly produced and stored samples, respectively. The fortified tapioca samples have fungal and total aerobic plate counts that are within the permissible range, which shows that the samples are safe for consumption. Fortifying tapioca grits with Bambara groundnut flour helps to combat protein deficiency, improves functional properties, and turns a low-protein meal into a nutrient-dense meal. However, increasing BGF progressively decreased overall acceptance.

Keywords: Bambara nut; tapioca grits; cassava; nutritional quality; microbial count

1. Introduction

Tapioca made from cassava (*Manihot esculenta*), is an irregular starch-grit that has been partially gelatinized. It is essentially a starchy, flavorless meal that is typically consumed as milk pudding in many parts of Africa [1]. During the 20th century, tapioca meal originated in the southern region of Nigeria, primarily among the residents of Lagos and its surroundings [2]. It is prepared by peeling, grating, and extracting the starch from the roots. The starch then undergoes partial enzymatic hydrolysis (liquefaction) or partial acid hydrolysis to sugar and gel particles. All of these procedures lower the cyanide content of the tapioca and render it fit for human consumption [1].



Cassava is a perennial vegetable propagated by tubers that is grown all over the tropics. It is a crucial staple dish for Africans, Nigerians inclusive. It is the third most important source of carbohydrates for humans globally, with about 500 million people relying on it [3]. Nigeria is the world's greatest producer of cassava, accounting for 34 million of the 960 million tons of fresh roots produced worldwide [4]. Due to its rapid deterioration within three to four days of harvest, cassava tubers have a limited shelf life. As a result, it must be processed into forms like tapioca, starch, flour, "garri", "fufu", "lafun", and "pupuru."

Nigeria is one of the main growers of the Bambara groundnut (*Vigna subterrenea*), also called Bambara nut, which is a member of the Fabaceae family and is extensively dispersed throughout Africa. Due to its high production, nutritious content, resilience to pests and diseases, and drought tolerance, it has drawn the attention of numerous researchers [5]. The seeds are high in protein (including lysine and methionine) and iron (4.9 to 48 mg). The primary raw material used to make tapioca, cassava, is a rich source of energy with a high carbohydrate content (78 to 96%), but it is poor in protein (0.31 and 1.20%, depending on the type), vitamins, and minerals, making it nutritionally inadequate [6]. The use of cassava for both domestic and industrial purposes has become much more widely known. Bambara groundnuts are utilized in plant-based substitutes, whole-seed recipes, milled flours, roasted snacks, and soups.

The nutrient content of Bambara groundnut makes it suitable for fortification of tapioca, which is another of several ways to exploit the nutritional potential of Bambara groundnut and enhance value addition in tapioca. Bambara nut and cassava processing together may greatly enhance composite functional characteristics, balance nutrients, and lower the effective concentration of cyanides or residual anti-nutrients present in raw cassava. Various studies have worked on the fortification of cassava starch/tapioca grits with under-utilized food crops to improve its nutritional value, provide health benefits, and sensory quality [7–9]. Samuel et al. [9] evaluated the improvement of tapioca by supplementation with full-fat soybean flour and reported that the 15% level of full-fat soy flour did not differ from the control sample in terms of the functional properties; rather, there was increased crude protein, crude fiber, and lower crude fat, which can make it suitable for longer storage periods. Adeboye et al. [7] evaluated the effect of almond seed flour inclusion in tapioca and found that at a 10% level of almond flour inclusion, tapioca had improved protein content and higher overall product acceptability. Adebawale and Ajiboye [8] reported that fortifying cassava starch with coconut residue improved the protein and fibre contents and enhanced the sensory quality. Olatunde et al. [10] in their work revealed that fortifying fermented bambara flour with tapioca led to acceptable tapioca meal with improved swelling power and nutritional value. Eke-ejofo et al. [11] focus on cassava-bambara groundnut flour. While Olatunde et al. [10] worked on fortification of tapioca with fermented Bambara nut and Eke-Ejiofor et al. [11] on cassava-bambara flour, to the best of our knowledge, there is limited information on the mineral, phytonutrient, and microbial quality of tapioca fortified with Bambara nut flour. The purpose of this study, therefore, is to examine the nutritional, functional, phytochemical, sensory, and microbiological quality of tapioca grits enhanced with Bambara groundnut flour.

2. Materials and Method

2.1. Materials

Fresh cassava roots were obtained from Ojere village, Ojere (7.13° N, 3.32° E) while Bambara groundnut was obtained from Lafenwa (7.16° N, 3.32° E) market, both in Abeokuta, Nigeria.

2.2. Methods

2.2.1. Preparation of Cassava Starch (CS)

The procedure outlined by Samuel et al. [9] was used to prepare CS. To get rid of dirt and other earthy foreign objects, the peeled cassava roots were cleaned with potable water. The cleaned roots were mechanically ground using a grating machine (GM-500). The resulting slurry was then sieved using a muslin cloth (with pore size 0.1 mm) to separate the shaft from the starchy solution. After allowing the filtrate to settle for 5 h, the starch sediment was dried using the cabinet dryer at 60 °C for 6 h. A laboratory mill equipped with a 500 µm sieve size was used to grind the dried starch granules, which were then packaged in ziplock bags and stored at 4 °C until needed.

2.2.2. Preparation of Bambara Groundnut Flour (BGF)

Bambara groundnut seeds were sifted, cleaned, and soaked in water at room temperature for 24 h, changing the water every 12 h. By rubbing between the palms and fingers, the seeds were manually dehulled. The de-hulled seeds were dried in an oven (NYC-101 oven, FCD-3000 serials, Medical and Scientific, London, UK) @ 55 °C for 24 h, ground, and sieved [8].

2.2.3. Preparation of Fortified Tapioca from CS-BGF Composite

CS was combined with varying amounts of BGF (10, 20, 30, 40, and 50%), the blends were separately conditioned to 20% moisture, roasted (120 ± 10 °C) in a stainless steel pan, and stirred constantly for 20 min. The resulting tapioca was spread to cool (27 ± 2 °C) on a stainless tray and packaged in zip-lock bags for storage (4 °C) until needed for further analyses. For control, tapioca devoid of BGF was created.

2.3. Analysis

2.3.1. Functional Properties

Determination of pH

The pH value was determined by dissolving 1 g of the tapioca samples in little amounts of distilled water and then adding up to 10 mL. After allowing the dispersion to stand for 30 min, the pH meter's electrode was placed and left to stand until a steady reading was achieved. The pH value was then recorded.

Determination of Bulk Density (Loose and Tapped)

The Malomo et al. [12] method was used to calculate the bulk density of the tapioca samples. A 50 g sample was weighed into a graduated measuring cylinder with a 100 mL capacity. The cylinder was gently tapped on the laboratory flat surface for approximately 5 min, or until the contents were firmly packed. Bulk density was calculated as:

$$\text{Trapped bulk density (g/ml)} = \frac{\text{weight of sample (g)}}{\text{volume of sample (mL)}}$$

$$\text{Loose bulk density (g/ml)} = \frac{\text{weight of loosely pack (g)}}{\text{volume of sample (mL)}}$$

Determination of Water Absorption Capacity

In a 50 mL centrifuge tube, 2.5 g of tapioca and 30 mL of distilled water were added and the mixture was allowed to sit at room temperature (30 ± 2 °C) for 1 h. To create a gel, the centrifuge (MICRO series 220 classic) was then operated for 15 min at 4500 rpm. After carefully decanting the supernatant into a moisture tin of known weight, it was dried for 8 h at 100 °C [13]. The water absorption capacity was calculated as follows:

$$\text{water absorption capacity} = \frac{\text{weight of gel obtained}}{\text{weight of tapioca sample}}$$

Determination of Oil Absorption Capacity

The technique outlined by Adeleke and Odedeji [14] was used to calculate the oil absorption capacity. 5 g of sample was mixed with 10 mL of oil in a pre-weighed centrifuge tube. To ensure that the sample was completely dispersed in the oil, the tube was then shaken for one minute. The tube was centrifuged for 25 min at 3,000 rpm following a 30-min holding period. Before being weighed again, the supernatant was pipetted, and the tube was inverted for 25 min to drain the oil. The amount of oil absorbed was measured in grams per gram of flour.

Determination of Swelling Power

A dry graduated measuring cylinder with a 100 mL capacity was filled with the tapioca sample (10 g), which was then lightly tapped. The volume occupied by the sample was noted. After adding 50 mL of distilled water, the mixture was let to stand for four hours. The ratio of the initial volume to the final volume was used to compute the swelling index [15]. The swelling index was calculated as shown:

$$\text{Swelling index} = \frac{\text{initial volume of sample after tapping}}{\text{final volume occupied by sample mixed with 50 mL}}$$

2.3.2. Proximate Composition

The AOAC [16] technique was used to determine the samples' proximate compositions. The samples were tested in triplicate for moisture, protein, crude fat, ash, and crude fiber content. The micro Kjeldahl nitrogen method was used to quantify the crude protein content, and a 6.25 conversion factor was used to convert the nitrogen content to protein. The difference between 100 and the total of all other proximate factors (moisture + ash + protein + fiber + fat) was used to determine the % carbohydrate content.

2.3.3. Determination of Mineral Composition

Mineral contents (calcium, magnesium, potassium, iron, zinc) were determined using the atomic absorption spectrophotometer method of AOAC [17].

2.3.4. Anti-Nutritional Composition

Determination of Cyanide Contents

The alkaline picrate approach, as outlined by Akande et al. [18], was used. Five grams (5 g) of tapioca grits was dissolved in 50 mL of distilled water and left overnight. After filtering, 1 mL of the extract was added to 4 mL of alkaline picrate solution, which was then heated to 37 °C for 15 min to generate color. The cyanide concentrations were calculated from the standard curve by measuring the absorbance at 490 nm against the reagent blank (1 mL of distilled water and 4 mL of alkaline picrate solution) using a UV-Vis Spectrophotometer.

Determination of Alkaloids

The alkaline precipitation gravimetric method outlined by Harborne [19] was used to accomplish this. A 1:10 (10%) ratio was created by dispersing 2 g of the material in a 10% acetic acid solution in ethanol. The mixture was filtered after being left to stand for 4 h at 28 °C. After evaporating the filtrate to a quarter of its initial volume, concentrated aqueous NH₄OH was added dropwise until the alkaloid precipitated. After receiving the precipitated alkaloid on a weighed filter paper, it was washed with a 1% ammonia solution and dried in an oven (NYC-101, FCD-3000 serials, Medical and Scientific, London, UK) at 80 °C. Alkaloid content was computed and reported as a percentage of the sample's weight.

Determination of Tannins

The Van-Burden and Robinson [20] method was used to quantify tannin. A 50 mL plastic bottle was filled with 0.5 g of the sample and 50 mL of distilled water was added. The total volume was then agitated for an hour in a mechanical shaker and filtered. Next, 2 mL of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide were combined with 5 mL of the filtrate in a test tube. After 10 min of incubation, the absorbance was measured at 720 nm.

Determination of Flavonoids

The Mahajan and Badujar [21] method was used. To a conical flask, 50 mL of 80% methanol was added to 1 g of the sample. The flask was shaken and kept on a hot plate at a low temp for 30 min. It was then cooled and filtered into a volumetric flask after 30 min, and 80% methanol was added to make up the remaining volume. A test tube was filled with 3 mL of the filtrate, 0.1 mL of 10% aluminum chloride, 0.1 mL of sodium potassium tartrate, and 3 mL of distilled water. Using 80% methanol as a blank, the test tube was shaken, and the absorbance was measured at 415 nm using an ultraviolet-visible spectrophotometer.

Determination of Trypsin Inhibitor Activity

To measure trypsin inhibitor activity, the Kakade et al. [22] method was used. For 1 h, 50 mL of 0.01N NaOH was used to extract 1 g of the sample. The suspension's pH was ascertained. The slurry was diluted until 40–60% trypsin inhibition was achieved with 1 mL. The test tubes were filled with 2 mL of trypsin solution and put in a water bath at 37 °C. After adding 5 mL of BAPA solution that had been heated to 37 °C for precisely 10 min, the reaction was stopped by adding 1 mL of 30% acetic acid. Each tube's contents were thoroughly mixed, filtered and the filtrate's absorbance was measured at 410 nm.

2.3.5. Microbiological Analysis of Tapioca Grits

Microbial counts were determined at day 0 and day 30. The tapioca grits were sealed in high-density polyethylene (HDPE) packaging, stored at a temperature of 25 °C and a relative humidity of 65%. Pour plating techniques as outlined by Harrigan and McCance [23] were used for the microbiological analysis. One gram of each sample was homogenized using 9 mL of sterile distilled water. Further serial dilution was carried out using the ten-fold serial dilution method. To count the total bacteria, coliforms, and fungi, respectively, 1 mL of serially diluted homogenized samples was plated on sterile Nutrient agar, MacConkey agar, and Potato Dextrose agar (PDA). Every medium was prepared in compliance with the manufacturer's guidelines. Bacterial cultures were incubated at 37 °C for 24 to 48 h, whereas fungal plates were cultured at 28 °C for 2 to 5 days.

2.3.6. Sensory Evaluation

The ethical approval was obtained before the sensory examination. Adebowale and Ajibode's procedure [8] was followed in conducting the sensory evaluation. The sensory qualities of tapioca meals were assessed by panelists ($n = 50$), 20 men and 30 women between the ages of 18 and 30. In two sessions over the course of a week, the panelists received two hours of training in food sensory evaluation and the interpretation of a nine-point hedonic scale. Before cooking, tapioca grits were hydrated and softened by soaking them in potable water for four hours at a ratio of 1:5. The pre-soaked grits were added to 100 mL of boiling water and cooked for 15 min while being constantly stirred. The panelists were provided cooked tapioca meal (~40 g) at 50 ± 2 °C. Six meals were delivered, one at a time, with 10 milliliters of liquid milk and 4 g of sugar, spaced five minutes apart. A 9-point hedonic scale of preference was used to rate meals, with 1 representing extreme dislike and 9 representing extreme liking. In between each assessment, drinking water was made available to clear the palate. Color, taste, aroma, appearance, thickness, and overall acceptance of the samples were all assessed.

2.3.7. Statistical Analysis

All analyses were performed in triplicate, and the mean and standard deviation of the triplicate determinations were used to reflect the results. Analysis of variance (ANOVA) was performed on the collected data using SPSS 22 software (IBM, Armonk, NY, USA). Significant F tests are provided at probability values of $p < 0.05$. ANOVA was performed on the models at a 5% confidence level using Minitab 16 statistical software (Minitab Ltd., Coventry, UK).

3. Result and Discussion

3.1. Functional Properties (Table 1)

The characteristics of food ingredients other than their nutritional value that significantly influence their use are known as food functioning. According to Hasmadi et al. [24], the functional characteristic dictates how food material is used for different food items. pH quantifies the acidity intensity in food, whereas titratable acidity represents the total acid accessible for reaction with sodium hydroxide during titration. Addition of Bambara groundnut flour (BGF) into cassava starch (CS) increased the pH value of tapioca samples. The increase may be due to BGF having a naturally higher, more basic pH and higher mineral and alkaline protein content than native tapioca starch. There was a significant difference ($p < 0.05$) among the samples (see Supplementary Material). The values obtained for pH of tapioca samples were within the acceptable range as reported by Oluwafemi and Udeh [25], and NIS, where a standard of 5 to 7 units of pH was set for edible cassava products [26]. The starch's particle packing and flow properties are indicated by its loose bulk density (LBD) and tapped bulk density (TBD). Better packing and expandability qualities of the starch are indicated by higher LBD and TBD [27]. Surface characteristics and particle size also influence BD. LBD values decreased as a result of BGF addition to CS, except for 10% and 20% BGF addition, while TBD values increased at different levels of BGF addition. While low BD would be helpful in the formulation of complementary foods, high BD flours indicate their potential for use in food preparations [28]. This may be due to LBD being primary indicator of high-quality weaning formulations indicating the ability of the flour to absorb water without swelling into a thick, unmanageable gel ensuring the gruel is easy to swallow [29,30] while, HBD ensure food particles fit tightly together, leaving minimal empty air space in the matrix, indicates superior packaging efficiency and better expandability. Research has indicated that the initial moisture level of flours may have an impact on their bulk density (BD) [31–33]. High bulk density directly increases a flour's water absorption. Water absorption capacity (WAC) is the ideal amount of water that must be added to flour before it gets overly sticky during processing [34]. Substitution of BGF into CS decreased the WAC of the samples except for 10% BGF. This could be attributed to the alteration of the physical and

chemical matrix of the mixture, as the starchy portion mixed with a non-starch ingredient reduces the overall capacity of the blend to absorb water. This may result in the cooked fortified tapioca becoming much thinner and less viscous.

One crucial functional characteristic that helps improve mouthfeel while preserving the flavor of food products is the oil absorption capacity (OAC) [35]. The 100% cassava starch had the lowest value because OAC rose as the amount of BGF added increased. The rise in OAC happens because the proteins in Bambara contain lipophilic (fat-loving) amino acid side chains that physically bind and trap fat molecules, improving mouthfeel and flavor retention and causing a rise in OAC [36]. Similar values were reported by Adeboye et al. [7] for tapioca with almond seed flour blends. Swelling capacity (SC) measures the starch's capability to absorb water and swell as well as the degree of associative forces present in the starch granules. Foods and flours with a high starch content increase the SC, particularly when the starch contains more branching amylopectin [35]. There was a decrease in the SC of the samples with progressive increase in BGF addition. The unfortified tapioca (100% CS) had the highest SC, because it contains more starch than the BGF-replaced samples. Cassava starch has a very high swelling capacity because its loose molecular structure allows amylose and amylopectin to absorb heat and water, expanding like tiny balloons, while Bambara flour introduces proteins and fibers that physically restrict this expansion [37].

Table 1. Functional properties of tapioca grits fortified with Bambara groundnut flour.

Samples	pH	LBD (g/cm ³)	TBD (g/cm ³)	WAC (g/g)	OAC (g/g)	SC (g/g)
100CS	3.64 ± 0.04 ^a	0.50 ± 0.00 ^d	0.57 ± 0.00 ^a	5.53 ± 0.02 ^c	0.43 ± 0.04 ^a	8.82 ± 0.53 ^e
90CS:10BGF	4.52 ± 0.00 ^b	0.61 ± 0.00 ^f	0.69 ± 0.01 ^c	5.61 ± 0.32 ^c	0.67 ± 0.01 ^b	7.96 ± 0.01 ^d
80CS:20BGF	5.01 ± 0.01 ^c	0.56 ± 0.00 ^e	0.69 ± 0.00 ^c	4.05 ± 0.70 ^b	0.78 ± 0.04 ^{b,c}	7.21 ± 0.01 ^c
70CS:30BGF	5.24 ± 0.01 ^d	0.48 ± 0.00 ^c	0.68 ± 0.00 ^c	3.01 ± 0.01 ^a	0.89 ± 0.03 ^{b,c}	6.29 ± 0.07 ^b
60CS:40BGF	5.38 ± 0.01 ^e	0.42 ± 0.01 ^a	0.65 ± 0.01 ^b	2.23 ± 0.24 ^a	0.89 ± 0.20 ^{b,c}	5.51 ± 0.15 ^a
50CS:50BGF	6.04 ± 0.00 ^f	0.45 ± 0.01 ^b	0.70 ± 0.00 ^c	2.49 ± 0.08 ^a	0.98 ± 0.01 ^c	5.09 ± 0.15 ^a

Values with different superscript letters in the same column are significantly different ($p < 0.05$). Keys: LBD—Loose Bulk Density; TBD—Tapped Bulk Density; WAC—Water Absorption Capacity; OAC—Oil Absorption Capacity; SC—Swelling Capacity; SI—Solubility Index; CS—Moist Cassava; BGF—Bambara Groundnut Flour.

3.2. Proximate Composition (Table 2)

Foods' proximate composition consists of their moisture, ash, fat, fibre, protein, and carbohydrate content. These food ingredients are relevant for quality assurance, industry development, and regulatory objectives [38]. Moisture content is an essential indicator of how long a product may be stored without deteriorating [39]. The moisture content of tapioca grits was observed to increase as a result of the inclusion of BGF, and this could be caused by the high protein and fiber content of BGF, which have potent hydrophilic qualities that actively bind, trap, and hold water in the mixture. There was a significant difference ($p < 0.05$) among the samples (see Supplementary Material). According to Emebu and Anyika [40], foods with high moisture contents are more conducive to the growth of bacteria that promote food spoilage. The minimum and maximum global standards for edible cassava products are 10% and 13%, respectively [26]. All of the tapioca samples, however, had moisture contents below 10% (which is the minimum standard commercial moisture content for tapioca), indicating they can withstand microbial growth and have comparatively great storage stability. The values were within those obtained by Adeboye et al. [7] for moisture content of tapioca fortified with almond seed flour blends.

Ash content is an indicator of the original food's total mineral content [41]. It is the residue that remains in food after all moisture has been eliminated and organic materials (such as fat, protein, carbs, vitamins, organic acids, etc.) have been burned at a temperature of roughly 500 °C [42]. Substitution of BGF into tapioca grits increased the ash content of all the samples, with 20% BGF having the highest value. The increase could be attributed to the contributory effect of BGF, since legumes are naturally richer in inorganic minerals than pure cassava starch, which subsequently increased the ash content of the samples. Similar values were reported by Olatunde et al. [10] for tapioca substituted with fermented Bambara flour. Fats enhance the flavor, texture, and aroma of food, making it more enticing. It serves as an excellent source of energy, helps transport fat-soluble vitamins, protects and insulates internal tissues, and supports vital cell functions [43]. The fat content of the tapioca grits increased with a progressive increase in the substitution of BGF. This is due to bambara groundnut naturally including a large quantity of crude lipids, usually 5% to 7% fat within its cotyledons, compared to cassava starch that is nearly fat-free at less than 0.5% fat [44]. The values were within those obtained by Eke-Ejiofor et al. [11] for cassava-Bambara groundnut flour blends.

Crude fiber is the leftover plant material after solvent extraction and digestion with diluted acid and alkali [45]. The amount of indigestible cellulose, pentosans, lignin, and other substances in food is measured as crude fiber [46].

The cell walls and seed coat of bambara groundnuts are rich in structural carbohydrates like cellulose and hemicellulose [47,48]. The crude fibre content of the tapioca grits increased as the level of BGF increased. The progressive increase in the fibre content could be a result of the contributory effect of BGF [49]. The results were within the range reported by Adeboye et al. [7] for crude fibre of tapioca with almond seed flour blends. Proteins are crucial to nutrition because they catalyze, regulate, protect, and supply energy [50]. The protein content of all tapioca grits increased as a result of substitution of BGF. The gradual rise in protein content may be due to the BGF's contributing effect, as cotyledons of the legume Bambara groundnut contain a high concentration of storage proteins (usually between 16% and 25% crude protein) [51]. Elevating the protein content from 5.43% to 17.29% marks a significant macronutrient optimization, increasing the percent Daily Value (%DV) per 100 g serving to 30.9% and satisfying nearly one-third of the adult Recommended Dietary Allowance (RDA). Similar results were reported by Elochukwu et al. [52] and Nwadi et al. [53]. Carbohydrates contain glucose that is used by the body as the primary energy source. As the amount of BGF added increased, the tapioca's carbohydrate content dramatically dropped ($p < 0.05$ (see Supplementary Material)). The study's carbohydrate content results are comparable to those of Ocheme et al. [54] for mixes of wheat and groundnut protein concentrate flour.

Table 2. Proximate composition of tapioca grits fortified with Bambara groundnut flour.

Samples	MC (%)	Ash (%)	Fat (%)	Fibre (%)	Protein (%)	CHO (%)
100CS	6.96 ± 0.26 ^a	1.16 ± 0.28 ^a	7.05 ± 0.45 ^a	0.00 ± 0.00 ^a	5.43 ± 0.04 ^a	79.40 ± 0.41 ^f
90CS:10BGF	7.02 ± 0.41 ^{a,b}	1.32 ± 0.28 ^a	7.55 ± 0.05 ^{a,b}	0.16 ± 0.04 ^b	7.88 ± 0.04 ^b	76.07 ± 0.48 ^e
80CS:20BGF	7.42 ± 0.19 ^b	1.99 ± 0.50 ^b	8.10 ± 0.30 ^b	0.31 ± 0.01 ^c	9.69 ± 0.02 ^c	72.49 ± 0.47 ^d
70CS:30BGF	7.44 ± 0.11 ^b	1.74 ± 0.42 ^{a,b}	8.80 ± 0.40 ^c	0.52 ± 0.02 ^d	13.57 ± 0.04 ^d	67.92 ± 0.24 ^c
60CS:40BGF	8.25 ± 0.14 ^c	1.31 ± 0.12 ^a	10.55 ± 0.35 ^d	0.73 ± 0.09 ^e	16.02 ± 0.04 ^e	63.14 ± 0.51 ^b
50CS:50BGF	9.19 ± 0.17 ^d	1.46 ± 0.01 ^{a,b}	11.40 ± 0.30 ^e	1.12 ± 0.08 ^f	17.29 ± 0.04 ^f	59.54 ± 0.29 ^a

Values with different superscript letters in the same column are significantly different ($p < 0.05$). Keys: MC—Moisture Content; CHO—Carbohydrate; CS—Moist Cassava Starch; BGF—Bambara Groundnut Flour.

3.3. Mineral Composition (Table 3)

Minerals are essential for the operation of numerous bodily systems [55]. Calcium regulates nerve and muscle function and is a component of bones and teeth; it is essential for the activation of enzymes. Osteoporosis, which causes the bone to degrade and increases the risk of fractures, can result from its shortage [56,57]. The increase could be as a result of Bambara nut being rich in calcium. The BGF's high calcium content may be the cause of the rise. Tapioca with 100% cs and 10% BGF differed considerably from the others ($p > 0.05$) (see Supplementary Material). Nwadi et al. [53] obtained a similar outcome regarding the calcium content of blends of cowpea and wheat-cassava flour.

Table 3. Mineral content of tapioca grits fortified with Bambara groundnut flour.

Samples	Calcium (%)	Magnesium (%)	Potassium (%)	Iron (mg/kg)	Zinc (mg/kg)
100CS	0.033 ± 0.003 ^a	0.030 ± 0.001 ^a	0.026 ± 0.001 ^a	40.57 ± 0.13 ^a	1.06 ± 0.01 ^a
90CS:10BGF	0.039 ± 0.002 ^a	0.049 ± 0.002 ^b	0.095 ± 0.007 ^b	128.06 ± 0.05 ^b	4.39 ± 0.23 ^b
80CS:20BGF	0.066 ± 0.005 ^{b,c}	0.064 ± 0.001 ^c	0.151 ± 0.001 ^c	171.07 ± 0.21 ^c	8.31 ± 0.18 ^c
70CS:30BGF	0.068 ± 0.003 ^c	0.075 ± 0.003 ^d	0.214 ± 0.004 ^d	256.05 ± 1.02 ^d	10.21 ± 0.65 ^d
60CS:40BGF	0.058 ± 0.003 ^b	0.080 ± 0.002 ^d	0.229 ± 0.002 ^e	264.11 ± 0.32 ^e	10.75 ± 0.29 ^d
50CS:50BGF	0.061 ± 0.003 ^{b,c}	0.148 ± 0.003 ^e	0.258 ± 0.005 ^f	336.71 ± 1.64 ^f	13.03 ± 1.11 ^e

Values with different superscript letters in the same column are significantly different ($p < 0.05$). Keys: CS—Moist Cassava Starch; BGF—Bambara Groundnut Flour.

Magnesium strengthens bones, supports nerve, heart, and enzyme functions, lowers blood pressure, and helps avoid stroke, cardiac arrest, and sudden heart attack [58,59]. The incorporation of BGF into cs results in a progressive increase in the magnesium content of the tapioca. Eke-Ejiofor and Kporna [60] obtained similar results regarding the calcium and magnesium content of blends of cowpea and acha flour. The potassium content of the tapioca grits increased progressively as the level of BGF addition increased. Significant differences ($p < 0.05$) exist among the samples. As the amount of BGF added increased, the tapioca potassium content gradually increased. The samples exhibit a significant difference ($p < 0.05$). High potassium levels have been shown to improve iron utilization [61] and are advantageous for those who take diuretics to manage hypertension and have excessive potassium excretion through bodily fluids [62]. A comparable outcome was reported by Olapade and Adeyemo [63] for the potassium content of wheat-cassava and cowpea flour blends. The iron and zinc concentrations in tapioca grits rose with higher levels of BGF incorporation. The samples differed significantly ($p < 0.05$). The iron

and zinc concentrations are comparable to what Olayiwola et al. [64] found for cocoyam-based recipes enhanced with cowpea flour.

3.4. Anti-Nutritional and Phytonutrients Composition (Table 4)

Anti-nutrients are characterized as natural or manmade substances that impede nutrient absorption, hence hindering their usage [65]. Cyanide is a chemical that frequently combines with other substances to form compounds that could be harmful to both people and animals [66]. The cyanide content was noted to decrease by 15.58% (from 5.65 mg/kg to 4.77 mg/kg) when tapioca was substituted with different levels of BGF. This could be the effect of substituting a naturally non-cyanogenic legume for cyanogenic cassava starch, which lowers the overall concentration of cyanogens per unit mass. The cyanide content was within the permissible intake range because it was below the 10.0 mg/kg maximum threshold set for cassava products [67]. This indicated that tapioca made with 10–50% BGF replacement is comparatively safe to eat.

The alkaloid content was shown to rise as the BGF level gradually increased. The samples differed significantly ($p < 0.05$) (see Supplementary Material). The lethal dose of 20 mg/100g reported by Inuwa et al. [68] is higher than the alkaloid concentration found in this investigation. The tannin content of the tapioca grits increased at different levels of BGF addition. According to Thakur et al. [66], tannins are known to be present in food items and reduce the protein quality of foods, impede the absorption of dietary iron, and inhibit the activities of trypsin, chymotrypsin, amylase, and lipase. Tannin levels reported are below the 30 mg/g fatal threshold. Flavonoid concentration was reduced when BGF was substituted into CS at various addition levels; however, there was no significant difference ($p > 0.05$) between 10 and 30% BGF inclusion. The study's findings are consistent with the Chowdhury et al. [69] research. At varying BGF addition levels, the samples' trypsin inhibitor concentration rose. Although legume proteins increase trypsin inhibitors, bioprocessing via heat denatures them to non-hazardous levels. Since legumes naturally concentrate protein-based anti-nutrients inside their storage tissue, adding BGF to CS enhances the trypsin inhibitor concentration. Several studies have shown that adding leguminous flours, such as Bambara groundnut, to starchy matrices, such as cassava or cereal grains, increases trypsin inhibitors [70–73].

Table 4. Antinutritional and phytonutrient content of tapioca grits fortified with Bambara groundnut flour.

Samples	Cyanide (mg/kg)	Alkaloid (%)	Tannin (mg/g)	Flavonoid (mg/g)	Trypsin Inhibitor (TIA/mg)
100CS	5.65 ± 0.04 ^f	3.076 ± 0.025 ^a	0.069 ± 0.003 ^a	1.778 ± 0.028 ^d	0.023 ± 0.002 ^a
90CS:10BGF	5.48 ± 0.15 ^e	3.371 ± 0.001 ^b	0.097 ± 0.005 ^b	1.570 ± 0.007 ^c	0.209 ± 0.001 ^b
80CS:20BGF	5.33 ± 0.07 ^d	3.526 ± 0.020 ^c	0.096 ± 0.003 ^b	1.538 ± 0.007 ^c	0.211 ± 0.001 ^b
70CS:30BGF	5.14 ± 0.06 ^c	3.573 ± 0.012 ^d	0.122 ± 0.002 ^d	1.539 ± 0.005 ^c	0.295 ± 0.006 ^c
60CS:40BGF	4.92 ± 0.10 ^b	3.608 ± 0.017 ^e	0.109 ± 0.008 ^c	1.378 ± 0.028 ^b	0.549 ± 0.001 ^d
50CS:50BGF	4.77 ± 0.21 ^a	3.724 ± 0.018 ^f	0.183 ± 0.004 ^e	1.290 ± 0.008 ^a	0.613 ± 0.018 ^e

Values with different superscript letters in the same column are significantly different ($p < 0.05$). Keys: CS—Moist Cassava Starch; BGF—Bambara Groundnut Flour.

3.5. Microbial Quality (Table 5)

Microbial product quality pertains to organisms and their metabolic products that are present in certain food sources at defined quantities, which can be utilized to evaluate current quality or forecast product shelf life [74]. Total viable counts serve as an indicator of the extent of bacterial contamination in food [75]. Higher substitution levels of BGF considerably ($p < 0.05$) (see Supplementary Material) changed the microbial counts of the tapioca blends, according to the 30-day microbiological examination. All samples showed remarkably low total viable bacterial counts (TVC) and fungal counts ($<10^2$ CFU/g) at Day 0, indicating that the high-temperature roasting and gelatinization stage successfully pasteurized the product by eliminating vegetative cells. Due to the dry matrix and low water activity of the flours, which prevent bacterial growth, the TVC in all formulations dramatically dropped as storage went on. On the other hand, the total fungal count (moulds and yeasts) grew linearly over time, with the 50:50 blend showing the greatest growth (12.65×10^2 CFU/g) as opposed to the 100% CS (2.05×10^2 CFU/g). Adding BGF to CS introduces highly hydrophilic proteins that significantly increase the hygroscopicity of the tapioca grits during storage, causing them to absorb ambient moisture, clump, and trigger fungal growth. However, even at maximum substitution, the final fungal loads remained well below the international safety threshold of 10^4 CFU/g [76], verifying that the composite meal maintains short-term microbial safety in airtight packaging. The total aerobic counts for freshly prepared and stored tapioca are lower than those obtained by Gacheru et al. [77] for cassava flour from the marketplaces in Mombasa and Nairobi. While the quantification of

total microbial counts successfully demonstrates the overall microbial load, a limitation of this study is that individual microbial taxa were not isolated or identified. Future research utilizing molecular techniques, such as 16S rRNA gene sequencing, is essential to characterize the specific taxonomic shifts and confirm the exact microbial species involved.

These results fell within the range of microbial counts reported by Adebayo-Oyetoro et al. [78] for fermented cassava flour known as “lafun”, sold in Nigeria’s Ogun and Oyo States. According to Olopade et al. [79], plate counts of 10^3 are acceptable, counts of 10^4 to 10^5 are reasonable, and counts of 10^6 are unacceptable. The tapioca samples have total aerobic plate counts and fungal counts within permissible limits.

Table 5. Microbial count of tapioca grits fortified with Bambara groundnut flour.

Samples	Freshly Produced (Day 0)		Day 30	
	Total Viable Bacterial Count (cfu/g)	Total Fungi Count (cfu/g)	Total Viable Bacterial Count (cfu/g)	Total Fungi Count (cfu/g)
100CS	1.20 ± 0.05^a	0.60 ± 0.02^a	1.00 ± 0.04^a	2.05 ± 0.09^a
90CS:10BGF	1.44 ± 0.06^b	0.75 ± 0.03^b	1.10 ± 0.05^b	3.15 ± 0.14^b
80CS:20BGF	1.67 ± 0.08^c	0.88 ± 0.04^c	1.18 ± 0.06^c	5.08 ± 0.22^c
70CS:30BGF	1.92 ± 0.11^d	1.02 ± 0.06^d	1.23 ± 0.05^c	6.94 ± 0.31^d
60CS:40BGF	2.17 ± 0.12^e	1.15 ± 0.06^e	1.31 ± 0.06^d	9.14 ± 0.42^e
50CS:50BGF	2.50 ± 0.15^f	1.32 ± 0.08^f	1.37 ± 0.06^d	12.65 ± 0.61^f

Keys: CfU/g—Colony Forming Unit per gram; CS—Moist Cassava Starch; BGF—Bambara Groundnut Flour.

3.6. Sensory Evaluation (Table 6)

A product’s shelf life, acceptability, and nutritional value are all determined by sensory evaluation; a product with superior nutritional value but poor taste and flavor acceptability has no commercial value [80]. Consumer initial acceptance of food is highly reliant on the colour of the food. The mean score for colour decreased as a result of BGF addition. The tapioca with BGF did not differ significantly ($p > 0.05$) (see Supplementary Material). The taste of food samples describes the delicious feeling that the sample produces in the mouth [63]. Substitution of BGF into tapioca at different levels decreased the average taste score. The mean scores obtained for colour and taste are within the range obtained by Adeboye et al. [7] for tapioca sample substituted with almond seed flour.

Table 6. Sensory attributes of tapioca grits fortified with Bambara groundnut flour.

Samples	Colour	Taste	Appearance	Aroma	Thickness	Overall Acceptability
100CS	8.32 ± 1.06^b	7.84 ± 1.90^b	8.36 ± 0.86^c	8.00 ± 1.08^c	8.00 ± 0.86^b	7.96 ± 1.20^b
90CS:10BGF	6.76 ± 1.80^a	$7.00 \pm 1.29^{a,b}$	7.12 ± 1.50^b	$7.32 \pm 1.28^{b,c}$	7.08 ± 1.11^a	7.08 ± 1.47^a
80CS:20BGF	6.40 ± 0.91^a	6.12 ± 1.36^a	7.24 ± 1.16^b	6.28 ± 1.40^a	6.68 ± 1.43^a	6.68 ± 1.34^a
70CS:30BGF	6.24 ± 2.04^a	6.52 ± 2.04^a	6.00 ± 1.93^a	$7.04 \pm 1.48^{a,b}$	6.52 ± 1.50^a	6.84 ± 1.37^a
60CS:40BGF	6.84 ± 1.62^a	6.76 ± 1.85^a	7.00 ± 1.70^b	$7.28 \pm 1.33^{b,c}$	6.36 ± 2.03^a	6.28 ± 1.40^a
50CS:50BGF	7.00 ± 1.78^a	6.60 ± 1.70^a	$6.80 \pm 1.73^{a,b}$	$6.92 \pm 1.97^{a,b}$	6.64 ± 1.95^a	6.24 ± 2.27^a

Values with different superscript letters in the same column are significantly different ($p < 0.05$). Keys: MC—Moist Cassava Starch; BGF—Bambara Groundnut Flour.

The most crucial aspect of food is its appearance, which influences consumers’ decisions to accept or reject the product. It was observed from the results that the mean score for appearance decreased with the inclusion of BGF. Since tapioca is typically thought of as having a white sensory quality, the BGF-supplemented tapioca’s appearance decreased, which affected the sensory evaluation of its other characteristics. The aroma of food is generated when food products are subjected to high temperatures due to the interaction of flavour compounds. The mean score for aroma decreased with various levels of addition of BGF. The mean scores for appearance and aroma are within the range obtained by Olatunde et al. [10] for tapioca substituted with fermented bambara flour. The mean score for thickness of the BGF-supplemented tapioca diminished due to BGF addition, and this may possibly be due to the composite product’s poor past-like feel and reduced particle size. The general or overall acceptability indicates how the product is widely accepted by both panelists and consumers. The panelist preference for the tapioca decreased as a result of substitution of BGF. No significant difference exists among the BGF-substituted tapioca. Tapioca produced from 100% CS was judged most acceptable by the panelists, closely followed by the sample with 10% BGF, while the sample with 50% BGF had the least acceptance. The outcome was in line with the studies of Olatunde et al. [10] and Adeboye et al. [7]. The decrease in the overall acceptability of tapioca at greater substitution levels of BGF is probably due to specific biochemical, physical, and visual

changes that modify the conventional consumer experience of pure cassava starch. De-hulling the seeds to minimize discolouration, prolonging fermentation or de-fating to remove beany off-flavors, and fine-sieving or germinating the flour to remove grittiness and match the smooth mouthfeel of tapioca starch are ways to enhance the sensory profile and increase consumer acceptance [10,81,82].

4. Conclusions

Substituting CS with BGF successfully converts traditional tapioca into a micronutrient-dense functional food by significantly improving its functional properties (pH, tapped bulk density, and oil absorption capacity), proximate parameters, and essential mineral profile. Toxicology confirmed the safety of the blend; residual cyanide decreased by 15.58% (to 4.77 mg/kg), and 30-day microbial loads remained safely beneath the 10^4 CFU/g threshold despite BGF-induced moisture sorption. However, because substitution levels exceeding 20% introduce a beany aroma, grittiness, and dark discoloration, an optimal blending ratio of 10% to 20% is recommended. To achieve maximum consumer acceptability and further reduce anti-nutritional factors, processing modifications such as dehulling, sprouting, and fermentation should be integrated into the flour preparation stage. Further work should be carried out on the identification of the isolated microorganisms to provide the specific taxonomic shifts and confirm the exact microbial species involved.

Supplementary Materials

The additional data and information can be downloaded at: <https://media.sciltp.com/articles/others/2609091139310099/FSP-26060185-SM.pdf>.

Author Contributions

O.H.A.: conceptualization, methodology, visualization, writing—reviewing and editing, supervision, validation; O.T.O.: investigation, software, data curation, writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

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