

Research Article

Quality Assessment of Biscuits Made from Blend of Wheat and Baobab Leaf Powder

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Abstract

Biscuit is a nutritive snack produced from unpalatable batter transformed into appetizing product by oven heat. Wheat flour, the major raw material for biscuit production, is deficient in lysine, an essential amino acid. Baobab leaf is rich nutritionally and high in antioxidant properties. Baobab leaf flower was blended with wheat flour to produce biscuits rich in nutrients. Wheat and Baobab flours were respectively blended thus: A (100:0); B (98:2); C (96:4); D (94:6); E (92:8); F (90:10), and mixed with other ingredients for production at 175-180°C for 15-20 minutes, cooled and packaged. The functional properties of the flour, proximate composition, colour, microbial and sensory evaluation were determined using standard methods. Result showed baobab leaf flour to be highly functional, while the Protein, Moisture-Content, Ash, Crude-Fat, Crude-Fibre and Carbohydrate respectively ranged thus: (10.50-12.11%), (2.51-3.28%), (1.38-2.96%), (10.31-12.27%), (1.07-2.16%), and (67.99-73.46%). Addition of baobab leaf flour at 10% level slightly affected the biscuit colour, but the microbial load of the samples with baobab leaf flour was low probably due to the antiviral and antibacterial properties of the leaf. The sensory panelists accepted the samples with baobab leaf flour. Incorporating baobab leaf flour into biscuits production improved nutritional content, quality and prevent malnutrition among consumers.

Keywords: Biscuits; Baobab Leaf; Functional Properties; Nutritional Quality; Sensory Qualities; Wheat Flour

Introduction

Biscuit, as it is known in the United Kingdom, but more generally referred to as “cookies” in USA, is regarded as a confection-food with low moisture content [1]. They are nutritive snacks obtained from single or composite dough, which has been transformed into digestible and more appetizing products through the action of heat in the oven [2]. Bakery products are fast foods loved by every age-group for their ease of transportation, taste, cholesterol-free, containing digestive and dietary principles of vital importance, low cost and more convenient with longer shelf-life [3, 4]. It should be noted that nutrient improvement to biscuits is not the sole thing, but shelf life extension could also work on [5]. They are normally classified based on ingredient composition and processing techniques [1]. Wheat flour, because of its high gluten protein, is the basic ingredient for biscuit production [6]. Gluten protein forms elastic dough during baking and gives high

organoleptic quality to products [7]. Whole grain generally is rich in macro and micronutrients than refined ones, which has mostly starch [8]. High energy and gluten in wheat flour combines strength and elasticity in the production of floury products with desirable texture and flavour [9]. African baobab (*Adansonia digitata* L., Malvaceae) is an important indigenous fruit tree species for food security, nutrition and income generation by the rural populace in Africa [10]. The edible parts of baobab (leaves, seeds, and fruit pulp) are consumed mostly by rural communities who also sell them in local markets, while the non-food parts (timber, fodder, and fibers) are mainly used for income generation in sub-Saharan Africa [11]. *A. digitata* originated in Madagascar, but was introduced to the rest of African countries by long distance dispersal [12], and in Nigeria, it is found in all the ecological zones, as it is widely distributed in the arid, semi-arid zones and savannah regions; the north west and east regions, where it is called Kuka tree [13, 14]. In the southern part of Nigeria, the Yorubas called it Ose tree, Nupe (Muchi), Edo (Usi) [13]. The tree which is normally used in parkland agro-forestry system in Nigeria grows undistributed and survives until their natural death.

Large numbers of people suffer from hidden hunger, a condition caused not by lack of sufficient food, but lack of essential nutrients. Hidden hunger is defined as a nutritional deficiency caused by the lack of balance in an otherwise full diet [15]. People who suffer from hidden hunger are receiving enough calories, but lack micronutrients, which sustain life, and baobab leaves are rich in several minerals; such as calcium, iron, potassium, magnesium, to name a few among which calcium and iron are found to be predominant, as well as vitamins and superior to that of fruit of the tree [16]. Most families in the rural areas use either the fresh and/or dried leaves as food to supply essential nutrients [17], and when compared to fruits, the leaves contain more essential amino acids, minerals and vitamin A. [18] who worked on the antiviral activity of *A. digitata* leaves by extracting with them with water, dimethyl sulfoxide and methanol reported that the leaf extract exhibited a promising activity against influenza virus and herpes simplex virus. As a result of the numerous benefits and qualities of *A. digitata*, this research work, apart from preventing its extinction, tended to increase its utilization by eliminating micronutrient deficiencies among young children in particular by incorporation into products such as biscuit.

Materials and Methods

Materials

The wheat flour used for the research work was purchased at Tanke area, Ilorin, Kwara State, while fresh baobab leaves was sourced from the teak plantation, University of Ilorin permanent site. Other ingredients used were equally sourced from Tanke area, Ilorin, Kwara State. The Oven, milling machine, stirrer, chopping board, rolling pin, weighing balance were sourced from the laboratory.

Preparation of Baobab Leaf Flour and Blends

Fresh baobab leaves harvested were carefully rinsed with water to remove dust, drained and oven dried at 50°C and milled into flour. The wheat flour was blended with 2% to 10% baobab leaf flour and labeled as samples A, B, C, D, E and F respectively, with sample A serving as the control sample (Table 1).

Composite Flour	Wheat Flour (WF) (%)	Baobab Leaf Flour (BLF) (%)
A	100	0
B	98	2
C	96	4
D	94	6
E	92	8
F	90	10

Table 1: Formulation of Wheat and Baobab Flour.

Biscuit Formulation and Production

The method of [19] was adopted for the biscuit production (Figure 1). The recipe for the biscuit include 200 grammes composite flour, 3 teaspoon of milk, 60 grammes margarine, 0.2 grammes salt, 80 grammes sugar, 2 medium sized eggs and 2 grammes baking powder etc.

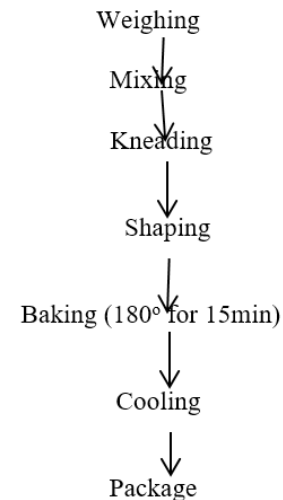


Figure 1: Flowchart for Production of Biscuits. Source: (Oyewole et al., 1996) [19].

Proximate Composition Flour and Biscuit Samples

The proximate composition of the flour and biscuit samples was determined by the method of American Association of Cereal Chemists [20] with number 44.15A.

Functional Properties of Flour samples

Water and oil absorption capacities, bulk density and dispersibility were determined by the methods of [21, 22] respectively.

Sensory Analysis of Biscuit Samples

The taste, appearance, texture, aroma and overall acceptability of the biscuit samples were assessed by a 50-member panelist (untrained but regular consumers of biscuits). They were properly instructed in both written and verbal formats as described by [23]. Each panelist compares the six samples using a nine 9-point hedonic scale with 9 representing -like extremely, and 1 representing dislike extremely.

Colour Determination

The flour colour was measured with a Minolta CR-310 (Minolta camera Co. Ltd, Osaka, Japan) tristimulus colorimeter, recording L, "a" and "b" values. L represented lightness (with

0=blackness, 100=brightness); a, corresponds to the extent of green colour (from negative= green to positive = redness); b represents blue from negative=blue to positive=yellow. The colorimeter was calibrated against a standard white reference tile. Samples were placed in clear glass Petri dish (10 replicates), and measurements done in triplicate.

Mineral Analysis of Biscuit Samples

Digestion and determination of Biscuit Samples for Mineral Analysis. Two-grammes each of the biscuit samples were weighed into a 125 ml Erlenmeyer flask earlier washed with acid and distilled water. About 4 ml perchloric acid, 25ml of conc. HNO₃ and 2ml conc. H₂SO₄ were added under a fume hood. The contents were mixed and heated gently at low heat on a hot plate until dense white fumes appeared, heated strongly for another 30 seconds and cooled. About 40-50ml distilled water was added and the solution boiled for 30 seconds on the same plate at medium heat. The solution that emerged was cooled and filtered completely into a 100ml Pyrex volumetric flask before making-up to mark with distilled water and later filtered with Whatman No 42 filter paper (9cm) [24]. Selected minerals were determined with “Buck scientific Atomic Absorption Spectrophotometer Model 210A”. Standard solutions with optimum range for each element were prepared and strictly followed.

Microbiological Analysis of Biscuit Samples

The microbial (bacteria and fungi) counts of the biscuit samples were determined by the compendium of methods for the microbiological examination of foods [25].

Samples	WAC (%)	OAC (%)	Bulk density (g/cm ³)	Dispersibility (%)
Baobab leaf flour	48.11a±1.80	19.50a±0.99	2.08a±0.08	3.48a±0.07
Wheat flour	14.91b±0.09	10.95b±0.09	0.89b±0.01	1.34b±0.04

*Data are mean values of triplicate determination ± standard deviation

Table 2: Functional Properties of Wheat and Baobab Leaf Flour.

The Bulk Density (BD) of baobab leaf and wheat flour was 2.08 g/cm³ and 0.89 g/cm³ respectively. The particle size and density of the flour generally affect the BD and important in determining the packaging requirement, raw material handling and application in wet processing in the food industry [32]. The lower the BD, the higher the amount of flour particles that can bind together leading to higher energy values [33]. Low BD of the flours was reported to be useful for food formulation, and such products tended to have less retrogradation. It also measures the heaviness of a flour sample [34]. Dispersibility of baobab leaf and wheat flour was 3.48% and 1.34% respectively.

In Table 3, the proximate composition of the flour sample baobab leaf to be rich in protein (13.75%), crude lipids (6.45%)

Statistical Analysis of Biscuit Samples

The sensory evaluation data was statistically analysed using the analysis of variance (ANOVA) and the Duncan Multiple range test with significance level at p<0.05 [7].

Results and Discussion

The functional properties of the flour samples are as shown in Table 2. According to [26], functional property of food is the physico-chemical property/characteristic of the food component, which determines their usefulness and success. The Water Absorption Capacity (WAC) of baobab and wheat flour was 48.11% and 14.91% respectively. The WAC of baobab leaf flour was higher than that of wheat flour, and could be due to the high protein and mucilage contents reported in baobab leaf flour [27]. This was reflected in the dough formation with increasing proportion of baobab leaf flour given hardness to the dough. According to [28], it was stated that WAC enables bakers to quantify the quantity of water added to dough in order to improve its handling characteristics and freshness of the baked products. WAC is the maximum water added to food material to retain formulation condition or relates to dryness and porosity of the material [29]. It could also be the ability of protein in a product to associate and retain water, thus increasing the water absorption and protein content [28]. The Oil Absorption Capacity (OAC) of the baobab leaf and wheat flour was 19.50% and 10.95% respectively. Good OAC of flour in baking improves handling characteristics of products [30], and equally improves biscuit quality by contributing to the soft texture [31].

and ash content (7.73%). The high mineral content recorded made it suitable for combating micronutrient deficiency. The crude fibre was 3.68% and was known to reduce the risk of some prevalent diseases like obesity, diabetes, high blood cholesterol, cardiovascular disease, and numerous gastrointestinal disorders [35]. Lowered blood cholesterol level helps slows the process of absorption of glucose and its control. It also ensures smooth bowel movements, which helps in the ease of flushing out of waste products from the body, increasing satiety and hence impacts some degree of weight management. The protein content was similar to that reported by [36, 37] (between 13-15 %), and that of [17] for protein, carbohydrate and crude lipids; 13-15%, 60-70% and 4-10% respectively.

Samples	Moisture (%)	Ash (%)	Carbohydrate (%)	Total protein (%)	Crude fibre (%)	Crude lipids (%)
Baobab leaf flour	8.16±0.29b	7.73±0.62a	60.23±0.99b	13.75±0.26a	3.68±0.31a	6.45±0.40a
Wheat flour	11.10±0.21a	1.23±0.08b	70.86±0.15a	11.62±0.19b	1.87±0.04b	3.32±0.12b

*Data are mean values of triplicate determination ± standard deviation.

Table 3: Proximate Composition of Wheat and Baobab Leaf Flour.

In Table 4, significant differences were recorded for the proximate composition of the biscuit samples. The protein, ash, fat, fibre and vitamins of the samples increased with increasing addition of baobab leaf flour except for moisture and carbohydrate. The decrease in the moisture content of the biscuits with increasing proportion of baobab leaf flour may be due to the higher oil and water absorption capacities of baobab leaf flour. The ash content of the biscuit samples varied from 1.38 to 3.31%, with the control sample having the least value. Higher ash contents depicted higher mineral content.

Samples	Moisture (%)	Ash (%)	Carbohydrate (%)	Total protein (%)	Crude fibre (%)	Crude lipids (%)
A	3.28±0.05 ^a	1.38±0.04 ^f	73.46±0.09 ^a	10.50±0.07 ^c	1.07±0.03 ^f	10.31±0.04 ^f
B	3.10±0.05 ^b	1.56±0.06 ^e	72.76±0.16 ^b	10.73±0.04 ^d	1.23±0.07 ^e	10.52±0.03 ^e
C	2.98±0.10 ^b	2.37±0.07 ^d	70.78±0.01 ^c	11.41±0.08 ^c	1.43±0.09 ^d	11.03±0.02 ^d
D	2.69±0.03 ^c	2.65±0.06 ^c	69.97±0.13 ^d	11.44±0.11 ^c	1.76±0.05 ^c	11.49±0.09 ^c
E	2.51±0.12 ^d	2.96±0.12 ^b	67.99±0.38 ^e	12.11±0.06 ^b	2.16±0.07 ^b	12.27±0.07 ^b
F	2.39±0.15 ^d	3.31±0.72 ^a	66.92±0.28 ^f	12.33±0.04 ^a	2.43±0.05 ^a	12.60±0.05 ^a

*Data are mean values of triplicate determination ± standard deviation. Mean value with different subscript in the same column are significantly different (P<0.05). Legend as in Table 1.

Table 4: Proximate Composition of Biscuit Samples.

From Table 5, mineral contents increased with increasing quantities of added baobab leaf. Sample F had the highest mineral content, though not significantly different, but the value of zinc and iron was the most abundant in the samples. Highest value of iron (0.58mg/g) was recorded in sample F, showing the rich mineral contents of baobab leaf.

Samples	P(mg/g)	Na(mg/g)	Ca(mg/g)	Mg(mg/g)	Fe(mg/g)	Zn(mg/g)
A	0.012±0.0 ^b	0.005±0.0 ^c	0.12±0.0 ^d	0.12±0.0 ^c	0.27±0.0 ^d	0.02±0.0 ^a
B	0.012±0.0 ^b	0.008±0.0 ^d	0.15±0.0 ^c	0.16±0.0 ^d	0.31±0.0 ^d	0.021±0.0 ^a
C	0.012±0.0 ^b	0.010±0.0 ^d	0.15±0.0 ^c	0.17±0.0 ^d	0.39±0.0 ^c	0.023±0.0 ^a
D	0.016±0.0 ^a	0.014±0.0 ^c	0.18±0.0 ^c	0.19±0.0 ^c	0.46±0.01 ^b	0.024±0.0 ^a
E	0.017±0.0 ^a	0.017±0.0 ^b	0.22±0.0 ^b	0.30±0.0 ^b	0.54±0.0 ^a	0.025±0.0 ^a
F	0.017±0.0 ^a	0.20±0.0 ^a	0.25±0.0 ^a	0.33±0.0 ^a	0.58±0.0 ^a	0.028±0.0 ^a

*Data are mean values of duplicate determination ± standard deviation. Mean value with different subscript in the same column are significantly different (P<0.05). Legend as in Table 1.

Table 5: Mineral Composition of Biscuit Samples.

The L*, a*, b* values of the biscuit samples are shown in Table 6. Statistically, there were significant differences between the mean values of the colour measurements (at $p \leq 0.05$). Lightness (L* values) for the samples was in the range of 46.54 to 55.94. L* value (lightness) decreased with increasing content of baobab leaf flour. The a* values were between -2.41 and 2.25. Though samples A and B had positive values, the biscuit appears greenish with increasing addition of baobab leaf flour. However, the a* values of sample F and A were 2.25 and -1.36 respectively. In terms of the degree of yellowness (b*), incorporation of baobab leaf flour to wheat flour decreased the yellowness.

Samples	L*	a*	b*
A	55.94±2.61 ^a	2.25±0.82 ^a	33.25±1.24 ^a
B	52.29±3.01 ^b	0.79±0.24 ^{ab}	29.84±1.72 ^b
C	49.49±1.20 ^{bc}	-0.61±1.31 ^{bc}	28.68±0.12 ^{bc}
D	47.61±0.34 ^c	-2.41±0.35 ^d	26.76±0.29 ^{cd}
E	48.33±1.49 ^{bc}	-0.91±0.57 ^{cd}	27.51±0.94 ^{cd}
F	46.54±1.47 ^c	-1.39±1.43 ^{cd}	26.54±1.20 ^d

*Data are mean values of triplicate determination ± standard deviation. Mean value with different subscript in the same column are significantly different ($P < 0.05$). Legend as in Table 1.

Table 6: Result of Colour Determination of the Biscuit Samples.

The bacterial and fungal loads of the samples stored for 8 weeks at ambient temperature are shown in Tables 7,8. There was an increase in the total plate counts. The bacterial count of 18×10^2 CFU (Colony forming unit) per gram and fungal count of 3×10^1 CFU per gram were the highest over the period. These values may be due to increase in moisture content of the biscuit leading to increase in its water activity, which may have favoured microbial growth. Of all the samples, control sample (A) had higher load for both bacteria and fungi. Low values recorded for the samples with baobab leaf flour may be due to antiviral and antibacterial properties of the baobab leaf. Ray & Bhunia 2007 [38] reported a maximum bacteria (aerobic) count of 5.30×10^4 cfu/g and 5.0×10^3 cfu/g for cookie sample. The results obtained even after six weeks was lower than that recorded by Ray & Bhunia (2007) [38] for cookie, hence, better shelf life.

Samples	0 day	2weeks	4weeks	6weeks	8weeks
A	0.0±0.0 ^a	2.00±1.00 ^b	8.00±1.00 ^a	9.33±1.53 ^a	18.00±2.65 ^a
B	0.33±0.58 ^a	2.33±0.58 ^{ab}	5.33±1.53 ^{bc}	8.33±0.58 ^{ab}	12.67±2.57 ^{ab}
C	0.33±0.58 ^a	2.33±0.58 ^{ab}	5.00±1.00 ^{bc}	7.00±1.00 ^b	12.33±2.52 ^b
D	0.33±0.58 ^a	2.33±0.58 ^{ab}	4.33±0.58 ^c	6.67±1.16 ^b	13.32±3.06 ^{ab}
E	0.0±0.0 ^a	1.67±0.58 ^b	6.67±1.16 ^{ab}	8.00±1.00 ^{ab}	14.00±3.61 ^{ab}
F	0.33±0.58 ^a	3.67±1.16 ^b	5.67±1.16 ^{bc}	8.67±1.16 ^{ab}	11.00±2.65 ^b

*Data are mean values of triplicate determination ± standard deviation. Mean value with different subscript in the same column are significantly different ($P < 0.05$). Legend as in Table 1.

Table 7: Bacteria Load of Biscuit Samples (cfu×10²).

Samples	0day	2weeks	4weeks	6weeks	8weeks
A	0.00±0.0	0.33±0.12 ^a	0.67±0.22 ^a	1.33±0.21 ^a	3.00±1.73 ^a
B	0.00±0.0	0.30±0.28 ^a	0.33±0.58 ^a	1.33±0.21 ^a	1.67±0.31 ^b
C	0.00±0.0	0.33±0.12 ^a	0.33±0.58 ^a	0.67±0.42 ^{ab}	1.33±0.33 ^b
D	0.00±0.0	0.00±0.0 ^a	0.28±0.51 ^a	0.00±0.0 ^b	0.67±0.58 ^b
E	0.00±0.0	0.33±0.12 ^a	0.00±0.0 ^a	0.67±0.42 ^{ab}	1.33±0.33 ^b
F	0.00±0.0	0.00±0.0 ^a	0.33±0.58 ^a	0.33±0.58 ^{ab}	1.00±0.0 ^b

*Data are mean values of triplicate determination ± standard deviation. Mean value with different subscript in the same column are significantly different ($P < 0.05$). Legend as in Table 1

Table 8: Fungi Load of Biscuit Samples (cfu×10¹).

The sensory evaluation carried out on the samples (Table 9) showed significant differences (at $p < 0.05$) among the samples. Sample A had the highest score (7.98) for appearance, while sample F had the lowest score (4.54). The appearance, which was visual, revealed that the panelists showed preference for the light colour of sample A. The browning appearance of the biscuit could have been due to Maillard-type reactions [8], a reaction between reducing sugars, proteins and amino acids or caramelization,

severe heating during processing [39]. The acceptance level of the biscuit appearance decreased with increasing quantities of baobab leaf flour. It was reported by [40] stated that when colour of a new product differs significantly from the existing one, consumers see it as a sign of spoilage and sometimes reject them. Appearance plays vital role in raw material's suitability for baked goods, provides information about the formulation and quality of the product [41].

Samples	Appearance	Aroma	Crunchiness	Texture	Taste	Overall Acceptability
A	7.98±0.81 ^a	5.68±0.79 ^b	5.90±0.61 ^c	7.56±0.76 ^a	7.70±0.58 ^a	8.08±0.60 ^a
B	7.74±0.80 ^a	7.00±0.81 ^a	6.56±0.58 ^b	7.22±0.65 ^b	7.58±0.50 ^{ab}	7.38±0.73 ^b
C	6.24±0.87 ^b	7.02±0.62 ^a	6.84±0.84 ^{ab}	6.32±0.94 ^c	7.50±0.51 ^{ab}	7.22±0.42 ^{bc}
D	5.46±0.76 ^c	7.06±0.91 ^a	6.90±1.30 ^{ab}	5.68±0.68 ^d	7.44±0.58 ^b	7.22±0.51 ^{bc}
E	4.80±0.88 ^d	7.00±0.83 ^a	6.88±0.87 ^{ab}	5.76±0.89 ^d	7.42±0.58 ^b	7.06±0.59 ^{cd}
F	4.54±0.86 ^d	6.98±0.59 ^a	7.06±0.84 ^a	5.48±0.89 ^d	7.34±0.56 ^b	6.92±0.60 ^d

Mean value with different subscript in the same column are significantly different ($P < 0.05$). Legend as in Table 1.

Table 9: Sensory Evaluation of Biscuit Samples.

The aroma of the biscuits ranged from 5.68 to 7.06. Addition of baobab leaf flour increased the aroma. There was no significant difference ($p \leq 0.05$) in aroma of the samples containing baobab leaf flour, but were significant from the control sample. The results obtained showed that the inclusion of baobab leaf flour had impact on aroma of the biscuits. It was reported by [42] that flavour enhancement could contribute to non-enzymatic browning reactions or development of new flavour complex molecules. Flavours affect the senses of taste and smell and affect aroma [43]. The crunchiness of the biscuits was liked slightly and was significant ($p \leq 0.05$) in all the samples. Biscuit sample with 10% baobab leaf was rated higher in terms of crunchiness. Inclusion of baobab leaf flour to the biscuit had varying effect on the crunchiness. Crunchiness is a desired characteristic that makes customers purchase any biscuit [44]. These attributes are perceived by sounds or noises produced during mastication. A report by [45] showed crisp and crunch demonstrates evidence of a crunchy sound. The disparity is that crispness has a higher pitch and louder than that from crunchiness [46].

The texture values ranged from 5.48 to 7.56, and there was significant difference ($p \leq 0.05$) between the texture of the control and treated samples. Product texture is an essential attribute in consumer's examination and buying judgment and [47] says texture is a "sensory expression for product structure in terms of reaction to stress by the kinesthetic sense. Samples A, B and C had the best taste. Taste of foods is affected more by formulation than processing, and biscuit taste is mainly affected by composition

than baking parameters [48]. The score for overall acceptability of the biscuit samples varies between 8.08 (sample A) and 6.92 (sample F). The least accepted of the samples was liked moderately (approximately 7.0). The result was an indication that baobab leaf enriched biscuit samples were accepted by the panelists. This result agrees with the report of [49] for cookies enriched with 5% dried Moringa leaves.

Conclusion

The research work was able to reveal the improved nutritional composition of the biscuit produced from the blends, that is, biscuits with superior nutritional composition in terms of macro and micronutrients. The macronutrients increased with increase in the addition of baobab leaf flour except for carbohydrate and moisture contents that decreased. Inclusion of the baobab leaf flour improves the crunchiness of the biscuit because of its fibre content, though the colour depreciated slightly. Biscuit with baobab leaf flour had higher WAC, OAC, BD and dispersibility, which rubbed on the nutritional qualities, functional properties and enhanced consumers' acceptability. With the high macro and micro nutrients in the biscuit, it could be adopted in the fight against children protein malnutrition through the snack eating by children, most especially, in the third world countries. Addition of baobab leaf reduces carbohydrate content and enhanced availability of desirable minerals, which was safe for human consumption due to their low microbial content. As a result, incorporating baobab leaf flour into biscuit production would improve nutritional content, prevent malnutrition among children and reduce the cost of production.

Conflict of Interest Statement

The authors, of which I am the lead and corresponding author, declared that there was no conflict of interest whatsoever on the part of individual author or between the authors in any way.

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