



Physicochemical Properties of Flours from Blends of Wheat, Fermented and Roasted Bambara Nut Flours

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Abstract

Bambara nut (*Voandzeia subterreneae* (L.) is a cheap source of leguminous protein with a good potential as substitute for relatively expensive animal protein, in several food products such as cookies, bread and biscuits, particularly in developing countries. This study aimed at assessing the functional Properties, pasting properties, proximate composition and antinutrient composition of flour blends from wheat, fermented and roasted Bambara nut. Flour blends were formulated in respective percentages of 100:0:0, 90:10:0, 90:0:10, 80:10:10, and 70:15:15 from wheat and Bambara nut. Analysis of the flour blends indicated that functional properties of flour blends of wheat, fermented Bambara nut and roasted Bambara nut showed Oil absorption capacity, bulky density, water absorption capacity, swelling capacity, reconstitution index, and foaming capacity ranged from 75.44-84.44 %, 0.59-0.68 g/m³, 71.55-76.87 %, 14.35-21.34 %, 14.66-23.66 %, and 18.36-22.80 % respectively. The pasting properties revealed Peak Viscosity, Final viscosity, Setback, Breakdown, pasting time, pasting temperature and trough ranged 168.60-178.50 RVU, 235.40-241.10 RVU, 70.03-88.97 RVU, 13.11-16.93 RVU, 7.45-9.86 min, 54.16-66.75 °C, and 151.60-160.40 RVU respectively. Proximate composition of flour blends showed protein, fat, fibre, ash, moisture, carbohydrate and energy value ranged from 11.14-16.02 %, 2.00-2.96 %, 1.23-1.55 %, 5.30-5.93 %, 7.56-7.76 %, and 65.78-72.77 % respectively. Antinutrient composition of flour blends indicated that trypsin inhibitors, oxalates, tannins, haemagglutinins and phytate contents ranged from 18.21-31.34, 1.47-6.13, 7.35-

13.91,31.23-57.45, and 1.88-4.86 mg/100 g respectively. Consequently, the potentials of fermented and roasted bambara nut in composite flour production was established, hence enhancing the utilization of fermented and roasted bambaranut and reducing overdependence on wheat flour for baking products.

Keywords: Physicochemical, Properties, Flours, Fermented, Roasted Bambara nut

Introduction

Wheat flour used for the production of biscuits and other bakery products is insufficient in many regions of the world resulting in importation of the flour by regions with limit supplies (Zouariet *al*, 2016). There is therefore, a compelling need to develop an adequate substitute for wheat flour. This substitute flour should be readily available, cheap and capable of replacing wheat in functionality. In many parts of Sub-Saharan Africa and most especially Nigeria, advancing prosperity and urbanization coupled with tremendous increase in population in recent years have led to an increase in the consumption of wheat-based products especially biscuits and breads. Composite flour has the added advantages of improving the nutrient value of biscuits and other bakery products especially when cereals are blended with legumes e.g Bambara nut (Adeyeye 2009). Bambara nuts are edible seed from the Leguminosae family (Basuet *al* 2007). It is a promising crop which needs more awareness as a food. As a legume, bambara nut is high in protein that play important role in human nutrition which makes the crop an excellent source of supplementing proteins in the diet.

Bambara nut is highly nutritive in vitamin and mineral contents like iron, phosphorous and calcium. It also has a high content of crude fibre and high level of sulphur containing amino acids which are limited in cereals (Okpala 2011). Bambara nut are grossly underutilized. Therefore, with the ban on importation of wheat used for baking in confectionery industries. It is hoped that indigenous crop such as Bambara nut will be used as substitutes for imported ones. Bambara nut (*Vigna subterranean L.*) has been used by researchers as composite flour for baking because of its nutritional value. Bambara nut is high in protein that plays important role in human nutrition. Study shows that it contains 20-26% crude protein (high in lysine; 6.6%); and makes an excellent source of supplementing protein in diet. (Nwosu 2013). Bambara nut like most legumes has strong beany flavor, bitter taste, antinutritional factors and hard-to-cook defects which limit their utilization in food formulation (Uvereet *al* 1999). Different processing treatments like roasting brings about changes in aroma which are described as nutty, burnt and coffee like due to the formation of

pyrazine compounds in the roasted food. It has been reported that the level of pyrazine compounds is also related to the extent of browning (Powrie 1981). This work therefore seeks to produce flour blends of wheat, roasted and fermented bambara nut flour blends with the aim of producing products of high nutritional quality and the optimum level of Bambara nut flour substitution that would give an acceptable biscuit using sensory parameters

MATERIALS AND METHODS

Source of Materials

Raw materials including wheat flour, butter, eggs, salt, sugar, baking powder and were purchased from Makurdi modern market. Bambara nut will be purchased from Wurukum market Makurdi, Benue State and were taken to the department of Chemistry, Benue state University Makurdi for further processing. All laboratory materials and reagents used were of analytical grade.

Sample Preparation

Production of Roasted Bambara nut Flour

Bambara nut flour will be prepared according to methods described by Okafor et al. (2015) with modifications. Bambara nut seeds (Bukina) were sorted and the bean soaked for 24 h in distilled water and dehulled manually and chaffs removed from the seed. The dehulled seeds were further boiled for 10 min (1:4 beans to water ratio); in a stainless steel pot, drained and dried at 50°C in an air circulating oven for 9 h. The dried samples were roasted at 140°C for 20 min using In-Lab electric roaster (In-Lab Furnaces, Mysore, India). The roasted seeds were cooled and milled into flour using hammer mill and sieved into flour using 0.05 mm sieves. The resulting roasted Bambara nut flour sample will be stored sealed in high density polyethylene bags prior to further use. This is as shown in Figure 1.

Production of fermented Bambara nut Flour

Bambara nut were fermented using the method described by Ola and Adewole (2019) with modifications. Bambara nut will be cleaned and washed. It will be steeped in water for 24 h in an open space at room temperature and allowed to ferment after which the seeds were dehulled. The steeped seeds were boiled in the steeped water for 15 mins, drained and spread out to dry a little at room temperature. The seeds were dried in an oven maintained at 55°C for 24 h, cooled and finely ground using hammer mill to obtain a fine particle flour. The flour samples were kept in airtight containers. Production of fermented bambara nut flour is as shown in **Figure 2**.

Blend Formulation

Blends with different treatment of Bambara nut and wheat were prepared as shown in Table 1.

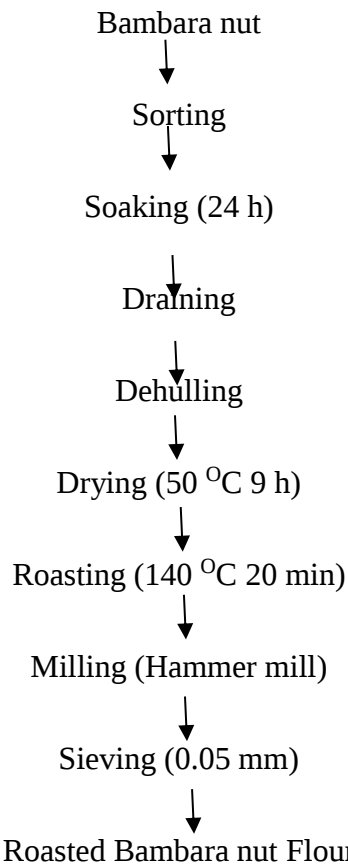
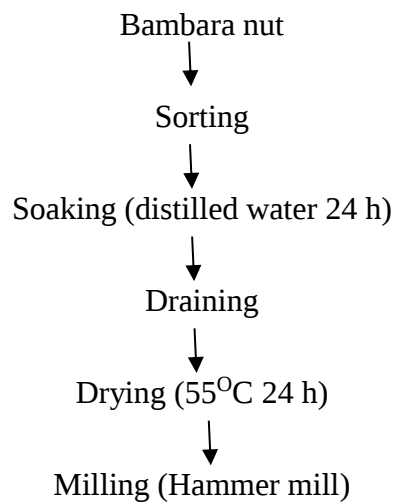


Figure 1: Flow Chart for the Production of roasted Bambara nut Flour

Source: (Okafor *et al.*, 2015) Modified



Sieving (0.005 mm)



Fermented Bambara nut Flour

Figure 2: Flow Chart for the Production of fermented Bambara nut Flour

Source: (Ola and Adewole, 2019) Modified

Table 1: Formulation Blends of the Composite Flour (%)

% Flour (w/w)			
Sample Code	Wheat	FBF	RBF
A	100	0	0
B	90	10	0
C	90	0	10
D	80	10	10
E	70	15	15

Key:FBF= Fermented Bambara nut flour, RBF= Roasted Bambara nut flour

A= 100% Wheat flour + 0% Fermented Bambara nut flour + 0% Roasted Bambara nut flour

B= 90% Wheat flour + 10% Fermented Bambara nut flour + 0% Roasted Bambara nut flour

C= 90% Wheat flour + 0% Fermented Bambara nut flour + 10% Roasted Bambara nut flour

D= 80% Wheat flour + 10% Fermented Bambara nut flour + 10% Roasted Bambara nut flour

E= 70% Wheat flour + 15% Fermented Bambara nut flour + 15% Roasted Bambara nut flour

Functional Properties of the Flour Blends

Water absorption capacity

The method described by Onwuka (2005) was used to determine the water absorption capacity.

WaterAbsorptionCapacity

$$= \frac{\text{Amount of water added} - \text{Free water}}{\text{Weight of sample}} \times \text{density of water} \times 100$$

Oil absorption capacity

The method as described by Onwuka (2005) was used to determine oil absorption capacity

Oil Absorption Capacity

$$= \frac{\text{Amount of oil added} - \text{Free oil}}{\text{Weight of sample}} \times \text{density of oil} \times 100$$

Bulk density

The method described by Onwuka (2005) was adopted for determination of bulk density. Bulk density was expressed as:

$$\text{Bulk Density (g/ml)} = \frac{\text{weight of sample (g)}}{\text{volume of sample (cm}^3\text{)}}$$

Foaming capacity

Foaming capacity (FC) was determined according to the method described by Onwuka

$$(2005). \text{Foaming Capacity (\%)} = \frac{\text{Volume after whipping} - \text{Volume before whipping}}{\text{Volume before whipping}} \times 100$$

Swelling index

This was determined using the method described by Onwuka (2005). The volume was measured after 10 mins and swelling index was calculated as:

$$\text{Swelling Index} \left(\frac{\text{mL}}{\text{g}} \right) = \frac{\text{Change in Volume of Sample (ml)}}{\text{Original weight of Sample}}$$

Reconstitution Index

This was determined using the method described by Onwuka, (2005). About 5.0 g of each of the samples were dissolved in 50ml of boiling water and the mixture was agitated for 90 seconds. Thereafter, it was transferred into a 50ml graduated cylinder and the volume of the sediment was recorded after settling for 10 minutes.

Pasting properties of the flour blends

Pasting properties of flour blends were evaluated with Rapid Visco-Analyzer (Model IVisco 20 Dinglin Global Sterling England, 2014). Test profile STDI (Newport Scientific Method1, Version 5, 1997) was used for determination of pasting characteristics. The pasting curve obtained were analyzed using a RVA Starch Master Software setup Tool (SMST) to obtain the characteristic parameters: peak viscosity (PV, maximum paste viscosity achieved in the heating stage of the profile); hot paste viscosity (HPV, minimum paste viscosity at 95°C); cool paste viscosity (CPV, final viscosity at 50 °C) set back viscosity (SB = CPV-PV), pasting temperature (Ptemp), and pasting time. Measurements were taken in duplicates.

Pasting properties derivatives: Pasting properties derivatives, namely, breakdown viscosity (BD = PV-HPV); consistency (CS = CPV-HPV), stability ratio (HPV/PV) and set back ratio (CPV/HPV) were computed, accordingly (Bhupenderet *al.*, 2013).

Determination of the Proximate Composition

The proximate composition of the flour samples was determined according to the methods of AOAC (2015) and Carbohydrate content was determined by difference according to Ihekoronye and Ngoddy (1985).

Determination of the Anti-Nutritional Content of Flour Blends

Oxalate was determined by the method described by Onwuka (2005). Phytate was determined using the method described by Oberleas (1973). Tannins was determined using the Folin-Denis spectrophotometer method described by Pearson (1976). Trypsin inhibitor was determined by spectrophotometry method described by Arntfield *et al.* (1985). Hemagglutinin was determined according to the method described by Onwuka (2005).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) followed by Fishers least Significance test to compare treatment means, differences were considered significant at 5 % level of probability.

Results and Discussion

Presented in Table 2 are the data on the functional properties of flour blends of wheat, fermented Bambara nut and roasted Bambara nut. The Oil absorption capacity, bulky density, water absorption capacity, swelling capacity, reconstitution index, and foaming capacity ranged from 75.44-84.44 %, 0.59-0.68 g/m³, 71.55-76.87 %, 14.35-21.34 %, 14.66-23.66 %, and 18.36-22.80 % respectively. There was significant ($p < 0.05$) difference in the oil absorption capacity, swelling capacity, reconstitution index, and foaming capacity. Functional properties of a food material are parameters that determine its application and end use (Adeleke and Odedeji, 2010). Functional properties of food substances depend on the quality attributes of their macro molecules such as protein, starch, carbohydrate, sugars, fibre and fat, and influence their utilization and industrial applications (Fetuga *et al.*, 2014). According to Bhat and Yahya (2014) functional properties of legume flour and cereal flour mixed with legume flour are regarded as very important parameters to consider in food product development due to their effects on texture and mouth feel as well as the consistency of the product. Understanding some of these intrinsic behaviors helps in setting the parameters for producing different products (Rahman *et al.*, 2011). The result of the oil absorption capacity showed a significant increase with increase in the substitution of wheat flour with fermented and roasted Bambara nut carried out in this study. The least oil absorption capacity of (75.44 %) was observed in sample A (100:0:0) while the highest oil absorption capacity (84.44 %) was recorded in sample E (70:15:15).

There was significant difference ($p < 0.05$) among the blends. This result disagrees with data reported by Ubbor et al. (2022) who examined the physiochemical and sensory evaluation of cookies produced from composite flours of wheat, bambara nut and orange fleshed sweet potato. Higher oil absorption capacity was reported in 100% wheat flour as compared to Bambara nut composites (Ubboret *et al.*, 2022). Findings from this present study suggests that pretreatment such as fermentation and roasting of Bambara nut which was carried out significantly improved oil absorption capacity of composite flours. Oil flavours and gives soft texture to food as absorption of oil by food products improves mouth feel and flavour retention. The increase in oil absorption may be attributed to the presence of more hydrophobic proteins which show superior binding of lipids (Issoufou *et al.*, 2017). The bulk density of wheat flour and sample D (80:10:10) level of substitution the formulated cereal–legume composite flours were similar. Bulk density depends on the combined effects of interrelated factors such as the intensity of attractive inter-particle forces, particle size, and number of contact points. The result of the bulk density showed a progressive decrease with increase in substitution of wheat flour with fermented and roasted Bambara nut flour as the least bulk density (0.59 g/cm^3) was recorded in sample E (70:15:15). The findings of the bulk density of wheat-Bambara nut composite flours collaborates to that of Touzouet *et al.* (2021) who reported that fermentation leads to decrease in the bulk density of foods. The decrease in bulk density of the fermented flour would improve the fluidity of the slurry. Fermentation would therefore be a useful method for the preparation of weaning feeds. Determining the bulk density of flours and flour blends is important for consumer expectation of a filled package as well as for shipping purposes (Yeboah-Awudziet *et al.*, 2018). The water adsorption capacity of flour Increased with increase in fermented Bambara nut flour, higher water absorption capacity was recorded in sample B (90:10:0) as compared to the control. This result agrees to the work of Malomoet *et al.* (2012) where the WAC decreases with increasing protein content. Fermentation significantly ($p < 0.05$) increased the water absorption capacity of wheat flour substitution by Bambara nut flours. The amount and nature of hydrophilic constituents such as proteins and to some extent the pH value favorably influence the water absorption capacity of a flour (Onimawo and Akubor, 2012). Water absorption characteristic represents the ability of the product to associate with water under conditions when water is limiting such as dough and pastes. Water absorption capacity is important in the development of ready to eat foods. It has been observed that a high absorption capacity assure product cohesiveness (Houson and Ayenor, 2002). Nibaet *et al.* (2001) also reported that water absorption capacity is important in bulking and consistency of products as well as baking applications. Water absorption capacity represents the ability of the products to associate with water under conditions when water is limiting such as dough's and pastes.

Foaming capacity is used to determine the ability of flour to foam which is dependent on the presence of the flexible protein molecules which decrease the surface tension of water (Asif-Ul- Alamet *et al.*, 2014). Proteins have been reported to enhance foam formation (Ojinnaka *et al.*, 2016) and hence, the highest foaming capacity recorded in sample E (70:15:15) could be attributed to its high flour protein content. Protein foams are important in many processes in the beverage and food industries and this has stimulated interest in their formulation and stability. Foams are used to improve texture, consistency and appearance of foods (Ajani *et al.*, 2016). Good foam capacity is a desirable attribute for flours in the food system due to its high percentage of porosity intended for the production of a variety of baked products such as ice cream, cakes, muffins, *akara*, and also act as functional agents in other food formulations (Onabanjo and Ighere-Dickson, 2014). The swelling capacity of flour granules is an indication of the extent of associative forces within the granule or it is the indication of the water absorption index of the granules during heating (Loos *et al.*, 1981). Increase in the swelling index could be due to strong bond forces in wheat-bambara nut flour and reduction in the carbohydrate content of the composite flours (Adebowale *et al.*, 2008b). The swelling capacity of flours depends on size of particles, types of variety and types of processing methods or unit operations. Swelling capacity of the composite flour increased with increase in the level of substitution.

Table 2: Functional Properties of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut.

Samples	Oil Absorption Capacity %	Bulk density g/m ³	Water Absorption Capacity %	Swelling capacity %	Reconstitution index %	Foaming capacity %
A	75.44 ^a ±0.01	0.67 ^a ±0.01	76.87 ^a ±7.06	14.35 ^a ±0.01	14.66 ^a ±0.01	18.36 ^a ±0.01
B	78.22 ^d ±0.02	0.62 ^b ±0.01	77.17 ^a ±2.11	15.16 ^d ±0.02	18.44 ^d ±0.02	19.56 ^d ±0.02
C	79.34 ^c ±0.01	0.63 ^b ±0.01	73.44 ^a ±4.23	16.35 ^c ±0.04	18.59 ^c ±0.01	19.94 ^c ±0.01
D	81.94 ^b ±0.01	0.68 ^a ±0.01	76.56 ^a ±2.84	18.44 ^b ±0.02	19.72 ^b ±0.01	21.13 ^b ±0.01
E	84.44 ^a ±0.01	0.59 ^c ±0.00	71.55 ^a ±0.00	21.34 ^a ±0.01	23.66 ^a ±0.02	22.80 ^a ±0.00
LSD	0.03	0.03	10.29	0.05	0.04	0.03

Values are means ± standard deviations of duplicate determinations. Means in the same column with different superscripts are significantly (p<0.05) different

Key: A= 100% Wheat flour + 0% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, B= 90% Wheat flour + 10% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, C= 90% Wheat flour + 0% Fermented Bambara nut flour

+ 10% Roasted Bambara nut flour, D= 80% Wheat flour + 10% Fermented Bambara nut flour + 10% Roasted Bambara nut flour, E= 70% Wheat flour + 15% Fermented Bambara nut flour + 15% Roasted Bambara nut flour, LSD = least significance difference

Pasting properties of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut.

Table 5 showed the pasting properties of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut. The result showed that there was significant ($p < 0.05$) difference among the samples in their pasting properties. The Peak Viscosity, Final viscosity, Setback, Breakdown, pasting time, pasting temperature and trough ranged 168.60-178.50 RVU, 235.40-241.10 RVU, 70.03-88.97 RVU, 13.11-16.93 RVU, 7.45-9.86 min, 54.16-66.75 °C, and 151.60-160.40 RVU respectively. Pasting characteristics is one of the most important properties that determine quality and aesthetic considerations. In the food industries, such properties affect texture, digestibility, and the applicability of starch-based food commodities (Adebawale *et al.*, 2011). When an aqueous suspension of starch is heated above a critical temperature, granules swell irreversibly and amylose leaches out into the aqueous phase resulting into increased viscosity pasting (Brabet *et al.*, 1998). The result of the pasting properties in this study shows that substitution with fermented and roasted Bambara nut flour did significantly ($p \leq 0.05$) influenced Peak viscosity, final viscosity, setback, breakdown, pasting time, pasting temperature, and trough. Peak viscosity indicates the water-binding capacity of the starch. It is the point at which gelatinized starch reaches its maximum viscosity during heating in water (Shimelis *et al.*, 2006). In this study, samples showed significant difference. This implies that different treatment given and the blending ratio influenced the peak viscosity. High peak viscosity maybe suitable for products requiring high gel strength and elasticity. The result obtained in this study (168.60–178.50 RVU) is not suitable for infants who require low viscosity gruel. Trough measures the ability of the paste to withstand breakdown during cooling. It is the minimum viscosity value in the constant temperature phase of the RVA pasting profile (Adebawale *et al.*, 2011). Treated blends had the highest values, which implies that the blends have the ability to remain undisrupted when subjected to hold period of constant high temperature and mechanical stress by rapid and continuous mixing. Infant food is expected to remain uniform without separation of layers either hot or at room temperature. Breakdown measures the degree of disintegration of granules or paste stability. Zaidul *et al.* (2007) reported that, at breakdown, swollen granules disrupt further and amylose molecules leach into the solution. In this study, samples did not show any variability. This implies that blending ratio and treatment had no influence on breakdown values. The higher the breakdown value, the lower the ability of the samples to withstand heating and shear stress during cooking. The result obtained in this study (13.11–16.93 RVU) was low compared to 41.58 RVU and 56.50–107.75 RVU for *Brachysteriaeurylom*a flour and cassava starch isolate, respectively (Ikegwuet *et al.*, 2009; Ikegwuet *et al.*, 2010).

Starches with high breakdown are likely to produce unsuitable paste (Singh *et al.*, 2006). As low breakdown values are associated with stable gruels, the low values reported in this study would be suitable for infant food formulation. Final viscosity defines the quality of a particular flour resistance during stirring. It also indicates the ability of starch to form paste or gel after cooling. Less stable starch paste is commonly accompanied with high value of breakdown. Samples A and B showed significantly ($p < .05$) high values, while various blends showed significantly ($p < .05$) low values. High values of the two samples could be attributed to non-inclusion and low inclusion of Bambara nut flour in them. Low final viscosity implies that the infant diet will form a low viscous paste rather than a thick gel on cooking and cooling. Also, the gruel will be a high caloric density food per unit volume. Hence, low value reported in this study is desirable for infant food preparation. Setback viscosity is the phase in the pasting curve after cooling the paste that shows the tendency of the starch to associate and retrograde (James and Nwabueze, 2014; Ogundele *et al.*, 2015). High setback values have been associated with a cohesive paste, while a low setback is an indication that the paste is not cohesive. Therefore, the higher the setback viscosity, the lower the retrogradation during cooling and the lower the starting rate of the product made from the flour (Adebowale and Lawal, 2003). In this study, samples differ significantly in setback value; however, the values (70.03-88.97 RVU) obtained were high implying high susceptibility of the flour to retrogradation during freeze-thaw cycles. Pasting time is a measure of the cooling time. Sample A and B had significantly high values, while blended samples had significantly low values. Inclusion of treated Bambara nut (roasting and fermenting) in the blend could be thought to decrease the pasting time of the blend. The value recorded here were high when compared to 5.94–6.37 min for extruded African breadfruit snack (James and Nwabueze, 2014). Pasting temperature provides an indication of the minimum temperature required to cook a given flour. High pasting temperature indicates high water-binding capacity, gelatinization tendency, and lower swelling property of starch-based flour due to high degree of association between starch granules. Samples in this study were significantly different. The value (54.16-66.75°C) was lower than 73.1–75.2°C for cassava starch isolate and 67.74–75.82°C for complementary extrudates reported by Ikegwue *et al.*, 2009; James and Nwabueze, 2014 respectively.

Table 3: Pasting properties of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut.

Samples	Peak Viscosity (RVU)	Final viscosity (RVU)	Setback (RVU)	Breakdown (RVU)	Pasting time (Min)	Pasting temperature (°C)	Trough (RVU)
A	168.60 ^a ±0.01	241.10 ^a ±0.01	88.97 ^a ±0.02	16.93 ^a ±0.01	9.86 ^a ±0.01	54.16 ^a ±0.01	151.60 ^b ±0.01
B	169.10 ^d ±0.01	240.90 ^b ±0.01	88.62 ^b ±0.01	16.84 ^b ±0.02	9.34 ^b ±0.01	58.92 ^d ±0.02	152.30 ^{ab} ±0.02
C	170.00 ^c ±0.02	240.70 ^c ±0.02	85.43 ^c ±0.01	14.70 ^c ±0.03	8.57 ^c ±0.01	62.36 ^c ±0.01	155.30 ^{ab} ±0.02
D	174.40 ^b ±0.01	240.60 ^d ±0.01	81.24 ^d ±0.01	14.56 ^d ±0.03	7.98 ^d ±0.01	64.20 ^b ±0.00	159.90 ^a ±0.02
E	178.50 ^a ±0.01	235.40 ^e ±0.01	70.03 ^e ±0.01	13.11 ^e ±0.01	7.45 ^e ±0.00	66.75 ^a ±0.00	160.40 ^a ±7.05
LSD	0.03	0.04	0.04	0.06	0.03	0.03	8.11

Values are means $\pm$ standard deviations of duplicate determinations. Means in the same column with different superscripts are significantly ($p < 0.05$) different

Key: A= 100% Wheat flour + 0% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, B= 90% Wheat flour + 10% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, C= 90% Wheat flour + 0% Fermented Bambara nut flour + 10% Roasted Bambara nut flour, D= 80% Wheat flour + 10% Fermented Bambara nut flour + 10% Roasted Bambara nut flour, E= 70% Wheat flour + 15% Fermented Bambara nut flour + 15% Roasted Bambara nut flour, LSD = least significance difference

Proximate composition of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut.

The result of the proximate composition of flour is presented in Table 4. The result showed a significant ($p < 0.05$) increase with the increase substitution of fermented and roasted bambara nut flour. The protein, fat, fibre, ash, moisture, carbohydrate and energy value ranged from 11.14-16.02 %, 2.00-2.96 %, 1.23-1.55 %, 5.30-5.93 %, 7.56-7.76 %, and 65.78-72.77 % respectively. The fat contents of samples B and C are not significantly ($p > 0.05$) different. The ash contents of samples B, C and D are not significantly ($p > 0.05$) different. Similarly, the moisture contents of samples A, B, and C are not significantly ($p > 0.05$) different.

The protein content of the flour sample increase with increasing fermented and roasted Bambara nut flour. This was expected since Bambara nut is a legume while wheat is a cereal grain and legumes naturally contain more protein than cereals although the prevalent protein in wheat occurs as gluten which is needed in baking. The protein content of wheat-bambara nut are lower the values (21–28 g 100 g⁻¹) reported from past studies (Arise *et al.*, 2015; Oyeyinka *et al.*, 2018). The least value of protein in sample A (100:0:0) could be attributed to non-incorporation of legume (Bambara nut) which contains substantial amount of protein. Protein is crucial to the regulation and maintenance of the body. Wheat-Bambara nut composite flour (sample B-E) contained highest amount of fat (2.51- 2.96 %) as compared to wheat flour (2.00 %). Results of the present study show a progressive increase in fat composition of flour and biscuits with increase in level of substitution of wheat flour with fermented and roasted Bambara nut flour. The fat content could be a good source of energy supply to the body (Alozie *et al.*, 2009). The level of rancidity would be low because of the moderate fat content of the product which is important in extending the shelf life of the flours. Lipids are very important in food substances since they are vital to the structure and biological function of cells and contribute significantly to the energy value of foods (Eleazy and Ironua, 2013). In addition, fat is a rich source of energy and is essential as carriers of fat-soluble vitamins, A, D, E and K. However high levels of fat

in food products should be <25%, since this could lead to rancidity in foods and development of unpleasant and odorous compounds (Ihekoronye and Ngoddy, 1985, Ikumola *et al.*, 2017). Fats acts as flavours retainers and helps improves sensory properties of baked products. However, diets high in fat predispose consumers to different illness such as obesity and coronary heart diseases (Okpala and Chinyelu, 2011).

The highest moisture content of the flour was 6.47 in sample A (100% wheat). The low moisture contents reported here is very important for the flours to maintain long shelf life. The level of moisture content in the flour samples were below the recommended moisture levels 14 % for safe storage. The moisture content should be below 14% to prevent microbial growth and chemical changes during storage (Aryeet *et al.*, 2006) and low levels of moisture in food products enhances more concentrated forms of nutrients (Zakpaa *et al.*, 2010). This implies that, all the flour samples investigated have good self-stability (Polycarp *et al.*, 2012). Most spoilage microorganisms may not thrive in relatively low moisture content biscuit and the biochemical and enzymatic reactions could be minimal which could be an advantage in extending the shelf life of the product (Ayo *et al.*, 2010). The highest crude fibre content in sample E (70:15:15) could be attributed to the high crude fibre contents of Bambara nut. The high crude fiber content 1.55 % (flour) will help digestion better in the body (Ayo and Andrew, 2016). The increased fiber content was due to the increase in fermented and roasted Bambara nut, confirming that Bambara nut is a good source of dietary fiber. In contrast, the present crude fiber contents obtained in this study (1.55) is higher than the 1.47% reported by Oyeyinka *et al.*, 2018 and this could be influence by difference source, origin and processing conditions respectively. The increase in fibre was observed as an improvement in the nutrient status since they are agents in food which aids in absorption during digestion process (Ubboret *et al.*, 2022). The wheat Bambara nut (composite) formulated flours had greater ash content as compared to flour from 100% wheat, and increased with an increase in fermented and roasted Bambara groundnut percentage. The higher ash content in rice–bambara groundnut flour implies that it contains relatively greater mineral content, which could be attributed to the Bambara nut flour as it is known to have high values for ash content. Similar results were reported by Yousif and Safaa (2014) where rice flour had lower ash content compared to chickpea and sweet flour. Bambara groundnut and chickpea flour have been reported to have greater nutritional value than wheat flour due to greater protein and iron levels (Madukweet *et al.*, 2013). This is also in agreement with research done by Kohajdova' *et al.* (2013) on pea flour. The ash content of the flours which is an indication of their mineral profile of the flours increased progressively as the level of supplementation increased. Increase in the ash content

indicates that the samples with high percentage of ash will be good sources of minerals (Shan and Kehong, 2019).

Carbohydrate content decreased from sample A to E with increased substitution. There was significant difference ($p>0.05$) between the carbohydrates content of the flour samples, the reason of reduction in carbohydrate content of the flour could be an increasing in protein, moisture, fat, ash and fiber content of the flour and biscuits as the proportion of fermented and roasted Bambara nut flour in the formulation was increased which leads a reduction in carbohydrate content since carbohydrate is calculated by difference. Decreasing trend of carbohydrate content as the substitution level increases is in accordance with the findings of Okaka *et al.*, (2009) as well as Okoye and Obi (2017). The relatively low carbohydrate content in composite flours could be from the substitution of wheat flour with fermented and roasted Bambara nut flour.

Table 4: Proximate composition (%) of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut.

Samples	Crude Protein	Fat	Crude Fibre	Ash	Moisture	Carbohydrates
A	11.14 ^e ±0.05	2.00 ^d ±0.00	1.23 ^a ±0.01	5.30 ^c ±0.08	7.56 ^c ±0.01	72.77 ^a ±0.03
B	12.82 ^d ±0.01	2.51 ^c ±0.01	1.25 ^d ±0.01	5.75 ^b ±0.00	7.56 ^c ±0.01	70.11 ^b ±0.02
C	14.14 ^c ±0.01	2.52 ^c ±0.02	1.39 ^c ±0.01	5.82 ^b ±0.00	7.56 ^c ±0.01	68.57 ^c ±0.04
D	15.64 ^b ±0.01	2.65 ^b ±0.00	1.42 ^b ±0.00	5.83 ^b ±0.01	7.62 ^b ±0.01	66.84 ^d ±0.06
E	16.02 ^a ±0.01	2.96 ^a ±0.01	1.55 ^a ±0.00	5.93 ^a ±0.00	7.76 ^a ±0.01	65.78 ^e ±0.02
LSD	0.06	0.03	0.01	0.09	0.03	0.10

Values are means ± standard deviations of duplicate determinations. Means in the same column with different superscripts are significantly ($p<0.05$) different

Key: A= 100% Wheat flour + 0% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, B= 90% Wheat flour + 10% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, C= 90% Wheat flour + 0% Fermented Bambara nut flour + 10% Roasted Bambara nut flour, D= 80% Wheat flour + 10% Fermented Bambara nut flour + 10% Roasted Bambara nut flour, E= 70% Wheat flour + 15% Fermented Bambara nut flour + 15% Roasted Bambara nut flour, LSD = least significance difference

Anti-nutritional content of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut.

Table 5 showed the proximate composition of the Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut. The result showed that there was significant

($p < 0.05$) difference among the flour blends in their Antinutrient composition. The trypsin inhibitors, oxalates, tannins, haemagglutinins and phytate contents ranged from 18.21-31.34, 1.47-6.13, 7.35-13.91, 31.23-57.45, and 1.88-4.86 mg/100 g respectively. Anti-nutritional factors are generally toxic and may negatively affect the nutrient value of seeds by impairing protein digestibility and mineral availability. Fermentation reduces anti-nutrients and helps absorb the full benefits from grains (Ekwereet *al.*, 2017). When Bambara nut grains are subjected to certain processing methods, the antinutrients could be significantly reduced to a safe or negligible level. Nwadi and Uvere (2019) produced complementary food with Bambara nut as one of the major ingredients. They reported that malting and fermentation effectively reduced antinutrients in Bambara nut which collaborates with López-Martínez et al. (2017). Anti-nutritional factors are reduced in foods by processing using methods such as soaking/steeping, malting, fermentation and heat treatment such as cooking/boiling (Danhassanet *al.*, 2018; Uvereet *al.*, 2010). Oxalates affect calcium and magnesium metabolism and react with protein to form complexes which have an inhibitory effect on peptic digestion (McDonald *et al.*, 1995). Oxalates complex with calcium forming calcium crystals which get deposited as stones and are associated with blockage of renal tubules (Banso and Adeyemo, 2007). Oxalates also prevent the body's absorption of calcium ions by forming insoluble calcium-oxalate complex (Adeniyi *et al.* 2009). Phytates form stable complexes with mineral ions like Ca, Fe, Mg and Zn and lower their bioavailability for intestinal absorption (Walter *et al.* 2002; Banso and Adeyemo, 2007). The oxalate content of flour showed an increased value with increased level of fermented and roasted Bambara nut substitution. The least was in the sample 100:0:0 which is the control. Generally, small amounts (as reported in all the samples) of oxalate may occur in many vegetables and fruits but do not pose nutritional problems (Oyelekeet *al.*, 2013). Consumption of large doses of oxalic acid causes corrosive gastroenteritis, shock, convulsive symptoms, low plasma calcium, high plasma oxalates and renal damage. The toxic range or lethal dose of oxalates has been reported to be between 3-5 g for man (Obotet *al.*, 2008). This study therefore reveals a safe margin for the analyzed flour samples. Edet, (2005), however stated that there was no incidence of oxalic acid poisoning reported in Nigeria. Chinma et al. (2016) prepared bread by substituting wheat flour with different levels (0, 5:5, 10:10 and 15:15) of sourdough flour (acha and Bambara nut). Tannins are phenolic compounds which form complexes with proteins, precipitating them and making them insoluble. Tannins inhibit and inactivate enzymes which act upon proteins causing dysfunctional digestion of proteins (Ekwereet *al.*, 2017). Tannins reduce protein quality by decreasing digestibility. They can bind to several macromolecules like starch and reduce the nutritional value of food (Igileet *al.*, 2013). Sample E (70:15:15) had the highest tannin content followed by sample D (80:10:10) and the least were

reported in sample A (100:0:0). Trypsin inhibitor is a protein that interferes with nutrient absorption by reducing the activity of proteolytic enzymes trypsin and chymotrypsin. The anti-nutritional contents of the flours samples increase with increased level of Bambara nut substitution as Bambara nut has been reported to be good source of anti-nutrients. Sample E (70:15:15) is significantly different and higher than other samples in their anti-nutritional contents due to the level of fermented and roasted Bambara nut substitution in the blends. This is attributed to the high anti-nutritional contents of Bambara nut as reported by Nwadiet *al.* (2019) and Adegunwaet *al.* (2013). The result of the anti-nutritional contents of the flour samples corroborated the finding of Okafor et al. (2014). Phytic acid is an organic acid in which phosphorus is bound. It is commonly found in the outer hulls of nuts, seeds and grains. Low levels of Phytate was recorded in the biscuits and flours but sample E (70:15:15) which had the highest level of Bambara nut substitution was significantly ($p<0.05$) higher than other biscuits and flours in their anti-nutritional contents. The levels of Phytic acid reported in flour samples in this present study are lower than those reported by Phytic acid has a strong binding affinity to minerals elements such as calcium, magnesium, copper, and particularly zinc and iron. Phytates combine with these minerals in the intestinal tract and precipitates them as well as block them from being absorbed, and make them unavailable for absorption in the intestines (Ekholmet *al.*, 2003). Haemagglutinins are known to exert deleterious effects via structural and functional disruptions of the intestinal microvilli resulting in reduced nutrient absorption (Emiola, 2011). The result of the haemagglutinin content of the biscuits and flour showed that sample E (70:15:15) was significantly different from all other samples. haemagglutinins was very low in the control sample but higher in flour blends due to the presence of Bambara nut. Okafor et al. (2014) reported that roasting, germination and a combination of the two processes reduced haemagglutinins (6.50 Hu/mg) which can be reduced by roasting (3.80 Hu/mg), germination (5.20 Hu/mg). The levels of these anti-nutrients in all the samples were relatively low, below toxic levels and may not hinder the bioavailability of essential nutrients in the flours.

Table 5: Anti-nutrient Composition (mg/100g) of Flour Blends of Wheat, Fermented Bambara nut and Roasted Bambara nut.

Samples	Trypsin inhibitors	Oxalates	Tannins	Haemagglutinins	Phytates
A	18.21 ^e ±0.01	1.47 ^e ±0.02	7.35 ^e ±0.00	31.23 ^e ±0.01	1.88 ^e ±0.01
B	21.59 ^d ±0.01	2.88 ^d ±0.01	9.52 ^d ±0.01	33.50 ^d ±0.02	2.15 ^d ±0.00
C	23.95 ^c ±0.01	3.40 ^c ±0.00	12.51 ^c ±0.01	41.77 ^c ±0.01	3.23 ^c ±0.01
D	25.66 ^b ±0.01	4.23 ^b ±0.01	12.77 ^b ±0.00	48.78 ^b ±0.02	4.13 ^b ±0.01
E	31.34 ^a ±0.02	6.13 ^a ±0.01	13.91 ^a ±0.01	57.45 ^a ±0.01	4.86 ^a ±0.01

LSD	0.03	0.03	0.02	0.02	0.02
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Values are means $\pm$ standard deviations of duplicate determinations. Means in the same column with different superscripts are significantly ($p < 0.05$) different

Key: A= 100% Wheat flour + 0% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, B= 90% Wheat flour + 10% Fermented Bambara nut flour + 0% Roasted Bambara nut flour, C= 90% Wheat flour + 0% Fermented Bambara nut flour + 10% Roasted Bambara nut flour, D= 80% Wheat flour + 10% Fermented Bambara nut flour + 10% Roasted Bambara nut flour, E= 70% Wheat flour + 15% Fermented Bambara nut flour + 15% Roasted Bambara nut flour, LSD = least significance difference

CONCLUSION

In conclusion, the functional, pasting properties, proximate and antinutrient composition of flour blends from wheat, roasted and fermented Bambara nut indicate that good and acceptable composites could be produced from the blends up to 15% substitution of the Bambara nut flour. Production of composite flour from wheat and Bambara nut flours should thus be encouraged in developing countries of the tropics such as Nigeria where wheat crops do not usually thrive well for climatic reasons. The practice would promote utilization of local raw materials, especially Bambara nut, in the production of baked food products. This may help in the avoidance of wastage that is usually associated with gross underutilization of vegetables in many developing countries such as Nigeria. This would also partially reduce dependence on importation of wheat flour and conserve foreign exchange that is usually associated with wheat importation.

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