

PRODUCTION AND QUALITY EVALUATION OF GLUTEN FREE BISCUITS FROM MAIZE AND SOYBEAN FLOUR BLENDS

Abstract

In recent years, there has been increase in the demand for gluten-free products that are suitable for use by people with celiac disease. The study evaluated the quality of gluten-free biscuits made from maize and soybean flour blends, and compared them with 100% wheat flour biscuit. Flours were prepared from maize and soybean grains. The soybean flour was used to substitute 5, 10, 15, 20 and 30% of maize flour in order to obtain various flour blends. The chemical composition, functional and pasting properties of the flours and their blends were determined. The flour blends were used to prepare biscuits, which were analyzed for the chemical composition, physical and sensory properties. The results showed that the moisture, protein, fat, fiber, ash, and carbohydrate contents of the flours ranged from 10.58-11.37, 7.30-42.23, 1.82-10.15, 0.34-5.20, 0.85-4.00, 26.38-76.36%, respectively. The phosphorus, iron and magnesium contents varied from 1.24-166.96, 2.31-19.54, 0.01-157.56 mg/100g, respectively. The bulk density, foaming capacity, water absorption and oil absorption capacity of the flours and the blends ranged from 0.66-0.68 g/cm³, 3.22-29.25%, 85.00-210.00 g/g and 80.0-145 g/g, respectively. The peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time and pasting temperature decreased with an increase in the level of soybean flour incorporation and ranged from 113.05-127.98, 83.95-97.05, 23.99-41.95, 224.02-409.98, 136.01-325.03, 6.12-7.05, and 55.4-95.0 RVU, respectively. The moisture, protein, fat, fiber, ash, and carbohydrate of the biscuits increased from 4.10-9.19, 34.94-40.38, 27.24-31.39, 6.88-7.93, 0.53-3.79, and 17.32-24.65%, respectively. The phosphorus, iron, and magnesium contents of the biscuits ranged from 47.01-65.91, 3.37-7.08, 0.0018-0.0047 mg/100g, respectively. The sensory scores for the biscuit samples decreased with the level of soybean flour. However, the biscuits containing 10% soybean flour was not significantly different from the 100% wheat flour biscuits in all the sensory attributes evaluated. Therefore, comparable biscuits with 100% wheat biscuit could be produced from the blend of 90% maize flour and 10% soybean flour.

Comment [U1]: more detailed to illustrate the influence of treatment on parameters rather than just the range of data that provides more novelty

Keywords: Gluten-free biscuit, wheat, maize, flour, soybean, celiac disease, chemical composition, functional properties, physical and sensory properties.

Introduction

Biscuits are ready-to-eat foods, traditionally prepared mainly with wheat flour, fats and sugar through the action of heat from oven (Goubgou *et al.*, 2021). They are popularly considered as junk snacks that come in different flavours, and could be eaten anytime of the day. Among baked and ready-to-eat snack foods, biscuits are mostly preferred by all age groups of the population. Valitutti *et al.* (2017) showed that celiac disease patients prefer biscuits and crackers to bread as carbohydrate source. Biscuits are convenient and inexpensive food products, containing digestive and dietary principles of vital importance. They are one of the confectionary food products consumed in Nigeria especially among children with a growing popularity (Okereke *et al.*, 2021^a; Akubor *et al.*, 2023). The production of biscuits involves steps in which ingredient mixing, dough kneading, fermentation and baking are dominant. In all these steps, gluten (protein that gives elasticity to wheat) plays key role (Ziobro *et al.*, 2016). The elastic property allows bread and other risen bakery products to be processed (Arendt and Moore, 2006). The gluten provides structure that allows flour to rise and hold its shape when baked. Gluten exists not only in wheat but to a lesser degree in relatives of wheat such as rye, triticale, barley and kamut.

Unfortunately, wheat flour products like biscuits can pose health risks to consumers, especially celiac patients due to some of their ingredients, particularly wheat flour. Of course, gluten-free foods (suitable for celiac patients) are at high demand, nowadays. Wheat flour (major ingredient in biscuit) constitutes dietary allergies to some consumers of wheat flour-based products due to its constituent protein- gluten (Armstrong *et al.*, 2012; Furlán and Chen, 2017). Besides, wheat flour is low in protein and deficient in essential amino acids such as lysine, threonine and tryptophan (Akubor *et al.*, 2023). According to Okereke (2023), wheat flour is refined and stripped of minerals, vitamins and antioxidants; and has been implicated in health concerns such as weight gain/obesity, diabetes, cardiovascular diseases and digestion problems. Therefore, bakery products that exclude allergen (gluten) in their recipes such as gluten-free bread and biscuits are the preferences of consumers with wheat allergy, celiac disease or gluten sensitivity. The three main forms human can react to gluten intake are allergic (wheat allergy), autoimmune (celiac disease, dermatitis herpetiformis and gluten ataxia) and immune-mediated (gluten sensitivity) (Therdthai *et al.*, 2016). Celiac disease is characterized with trigger of harmful autoimmune response in the small intestine: the reaction to the gluten damages the villi of the small intestine's

lining, leading to impaired absorption of some nutrients such as calcium, iron, folic acid and fat-soluble vitamins (De Re *et al.*, 2013; Caio *et al.*, 2019). The damage of the intestine can cause diarrhea, fatigue, weight loss, bloating, anemia, flatulence, constipation, brain disorders (such as schizophrenia and epilepsy), stomach pain, impaired growth and development in children, and other serious complications (Mavroudi *et al.*, 2005; Di Sabatino and Corazza, 2009; Samaroo *et al.*, 2010; Furlán and Chen, 2017). According to Kvamme *et al.* (2022), studies have placed the population of people with celiac disease worldwide at 1-2%. Shockingly celiac disease has no cure but the symptoms can be managed by following a strict gluten-free diet which will also promote intestinal healing.

Of course, the narrowing thin line existing between nutrition and health has increased the research importance for development of functional foods whose commercial demand is rapidly growing. The worsening food insecurity, and depleting economy of Nigeria, aided partly by low nutritional content of 100% wheat flour-based products and high import bills of wheat respectively, have put our local researchers on their toes towards food formulations using local raw materials. Food Scientists have employed the use of composite flour technology to address the nutritional deficiency common with wheat flour products; and rice, pea, potato, sorghum, buckwheat, and flaxseed flours are the most found substitutes to wheat flour (Goubgou *et al.*, 2021). But celiac disease (i.e. of genetic susceptibility to gluten) imposes the total withdrawal of gluten from consumption. The replacement of wheat flour in regular food products is a challenge because of the absence of viscoelastic network created by glutenous proteins (Tye-Din *et al.*, 2018). Working with non-wheat flours has a number of challenges, which include weak structure, molding problem, inferior sensory and nutritional quality, high production costs and low nutrition in certain ingredients. Gluten-free diets also adversely alter the intestinal flora and cause elevated risk of micronutrient deficiencies in patients with cardiovascular diseases. The other risks linked to gluten-free diet include limited variety of healthy food choices, increased intake of necessary nutrients such as carbohydrates, protein, fiber, folate, iron, vitamin B₃, calcium and increased food cost (Miñarro *et al.*, 2012). Other problems include increased intake of wheat replacements that, have higher glycemic indexes and lower fiber and protein levels than wheat. Thus, in a bid to address these problems, flour blends from legumes, cereals, and root crops have been used to produce gluten free biscuits with good sensory acceptability and high nutritional quality.

In order to develop gluten-free products, food professionals have explored the abilities of different food ingredients/crops as well as adequate technological processes (Padalino *et al.*, 2011; Okereke, 2023) but the quality of biscuit from maize and soybean flour blends has not been assessed. Soybean protein is the only vegetable source of complete protein, in which its quality is comparable to meat and eggs, which contain all the essential amino acids required by humans (Okereke and Banigo, 2021). It also contains potassium, linoleic fatty acid (omega-6), alpha-linolenic fatty acid (omega-3) and polyunsaturated fatty acids that are essential nutrients, and also increasingly recognized to reduce the risk of chronic age-related diseases such as cancer and cardiovascular disease (Deol *et al.*, 2017; Okereke and Banigo, 2021). Soybean has been used effectively in bakery products (e.g. biscuit, bread etc.) to improve their protein contents. Biscuits enriched with protein, usually from soy flour and caseinate, have been developed for special feeding programmes usually for children in developing countries. Research works have been done by Akbar *et al.* (2016), Apotiola and Fashakin (2013), Okoye *et al.*, (2008), and Banureka and Mahendran (2009) to improve the protein content of biscuit. Maize is the second most important cereal crop in Nigeria ranking behind sorghum in the number of people it feeds; they are grown widely throughout the world in a range of agro ecological environment. It is a major staple food in developing countries especially in Nigeria, where it serves as raw materials for the production of some staple foods such as traditional fermented maize porridge (ogi), maize flour (tuwo) and different traditional snacks (robo, adun, donkua and kokoro). Maize is predominantly starch (60-75%), with low protein content (9-12% when compared with legumes) and remains an essential source of various major phytochemicals such as carotenoids, phenolic compounds, and phytosterols (Jiang and Wang, 2005; Kopsell *et al.*, 2009). It is however, rich in methionine and cysteine. Maize kernel (edible and nutritive part of it) contains vitamin C, vitamin E, vitamin K, vitamin B₁ (thiamine), vitamin B₃ (niacin), vitamin B₃ (riboflavin), vitamin B₅ (pantothenic acid), vitamin B₆ (pyridoxine), folic acid, selenium, N-p-coumaryl tryptamine and N-ferrulyl tryptamine.

Hence, formulation of biscuits from soybean and maize flours can provide a remarkable improvement in quality of gluten-free snack foods, which are suitable for people following a gluten-free diet, with additional advantages of strengthened national economy, promotion of local agriculture, increased employment opportunities and fortified food security. Thus, the objective of this study was to investigate the quality of various gluten-free biscuits made by substituting 5, 10, 15, 20 and 30% of maize flour with soybean flour.

Materials and Methods

Materials

Soybean (*Glycinemax L.*), maize (*Zea mays L.*) grains, baking fat, powdered milk, baking powder, sugar, salt and wheat flour were purchased from New market, Wukari, Taraba State, Nigeria. These raw materials were packed in polyethylene bags and stored (under dry condition and ambient room temperature of 27⁰C) in the Laboratory of Food Science and Technology Department, Federal University, Wukari, Taraba State, Nigeria.

Methods

Preparation of Wheat flour: The commercial wheat flour was sieved through a 0.20mm mesh screen, packaged in high density polythene bags and stored in airy clean dry place at ambient room temperature (27⁰C) prior to use.

Preparation of Maize flour: The maize flour was produced as described by Barber *et al.*(2010)with slight modifications, as shown in figure 1.The maize grains were manually sorted to remove unwholesome ones and all extraneous materials.They were washed with portable water and then boiled for 1h.The boiled maize grains were drained of water and dried in an oven (NL9023A, England) at 50⁰C for 12 h. The dried maize grains were milled using an attrition mill (9FC-36, China); sieved through a 150 μ m sieve and then stored in air-tight polythene bags (Ziplock, China) until needed.

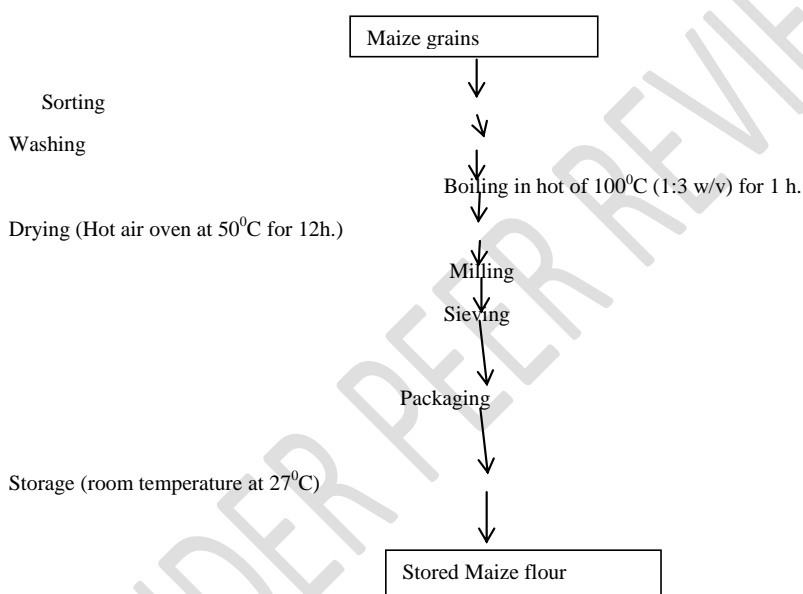


Figure 1. Flow chart for production of maize flour

Source: Modified Barber *et al.* (2010)

Preparation of soybean flour: Soybeans flour was produced using the method of Akubor *et al.* (2013) with slight modifications as shown in figure 2. Soybeans were manually cleaned by hand picking the chaff and the stones before washing them with water in order to remove the adhering dirt.The cleaned soybeans were poured into heated water of 100⁰C to boil for 30 minutes to remove the anti-nutritional factors and beany flavour. The boiled soya beans were dehulled and washed properly. The grains were dried in an oven at 60⁰C for 24 hours to a moisture content of about 10%. The dried grains were milled into flour using attrition milling machine (9FC-36, China), before sieving into fine flour of uniform particle size by passing it through a 150 μ m aperture screen. The soybean flour was packaged in polyethylene bags (Ziplock, China) and kept at room temperature for subsequent use.

Comment [U2]: please make the flowchart more systematic

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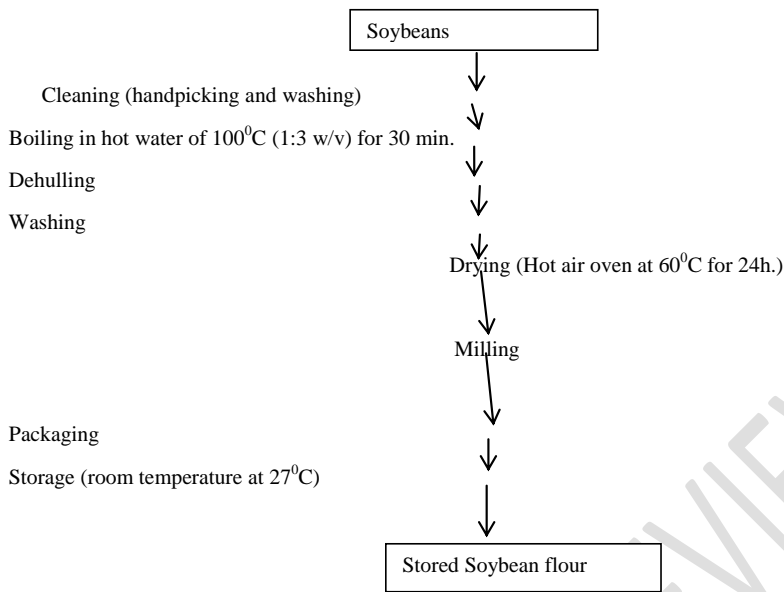


Figure 2. Flow chart for production of soybean flour
Source: Modified Akuboret al. (2013)

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Formulation of the Flour Blends:The soybean flour was used to substitute 5, 10, 15, 20 and 30% of maize flour in a food blender (Philip HR2811 Model) that was operated at full speed for 5 minutes in order to develop various flour blends. The blends were packaged in labeled polyethylene bags (Ziplock, China) prior to use.

Production of the biscuits: The recipe used for the production of gluten-free biscuits is shown in Table 1. The biscuits were produced using the method described by Hussein *et al.* (2020) with modifications. Six samples of biscuits were prepared in which one (Sample WFB) was prepared from 100% wheat flour and served as control; five other samples were prepared from maize and soybean flour blends. The ingredients were weighed, and mixed together using a Philip blender (HR2811 Model) that was operated at full speed for 5 min. Mixing was done to ensure a homogeneous mixture of the samples. The baking fat was rubbed in and mixed until dough was formed. The resultant dough was kneaded and rested for about 5 minutes. The rested dough was rolled out into sheets and cut into shapes, using biscuit cutters. The dough was placed on greased baking trays and baked for 15 minutes in an oven pre-heated at 180°C, allowed to cool, then was packaged in high density polyethylene bags in an air-tight container. The flow chart for the production of biscuit is shown in Figure 3.

Table 1: Recipe for production of gluten-free biscuits from the various blends of maize flour and soybean flour

Biscuit Sample	Flour blend (MF: SF) (g)	Baking fat (g)	Baking powder (g)	Egg (g)	Salt (g)	Powdered Milk (g)
95:5	95.0: 5.0	40.0	1.5	30.0	0.6	10.0
90:10	90.0: 10.0	40.0	1.5	30.0	0.6	10.0
85:15	85.0: 15.0	40.0	1.5	30.0	0.6	10.0
80:20	80.0: 20.0	40.0	1.5	30.0	0.6	10.0

70:30	70.0: 30.0	40.0	1.5	30.0	0.6	10.0
WFB	100	40.0	1.5	30.0	0.6	10.0

Source: Hussein *et al.* (2020) modified. MF= Maize flour, SF=soybean flour, WFB = 100% Wheat flour biscuit (Control)

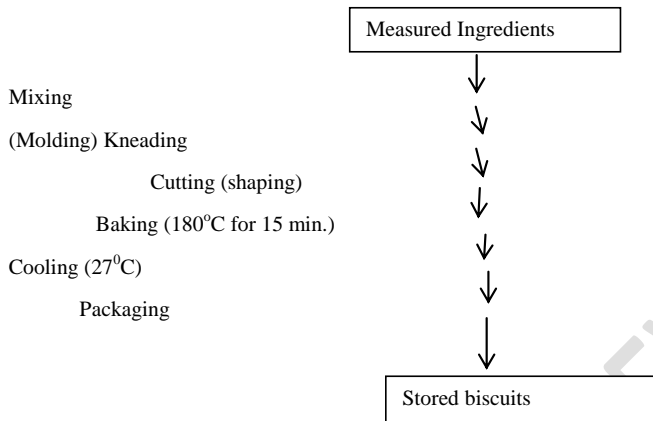


Figure 3. Flow chart for production of biscuits

Source:Hussein *et al.* (2020)modified.

Determination of the proximate composition of the wheat flour, maize flour, soybean flour and biscuits

Proximate analyses were carried out on the samples of the flours (maize flour and soybean flour) and biscuits to determine the moisture, ash, crude fibre, fat, protein and carbohydrate contents using the method outlined by the Association of Official Analytical Chemists (2010).

Moisture Content

The moisture contents of the flours (wheat flour, maize flour and soybean flour) and biscuits were determined by hot air oven method as described by AOAC (2010). The sample (2 g) was weighed into an empty dish. This was placed into the hot air oven to dry for 24 hours at 100°C. The dish and its contents were cooled in the desiccator and their weights taken. The loss in weight was recorded as moisture content and expressed as percentage of the original weight of the sample. This experiment was carried out in triplicates.

$$\% \text{ Moisture Content} = \left(\frac{W_2 - W_3}{W_2 - W_1} \right) \times 100$$

W_1 = weight of cooled empty dish

W_2 = weight of empty dish + undried sample

W_3 = weight of dish + dried sample

Ash Content

The Ash contents of the flour/biscuit samples were determined using the method of AOAC (2010). The sample (5 g) was weighed into empty crucible and then the sample was incinerated in a muffle furnace at 550°C until a light grey ash was observed and a constant weight obtained. The sample was cooled in the desiccator to avoid absorption of moisture and weighed to obtain ash content. The percentage ash content was expressed as percentage of the original weight of the sample on dry basis. The experiment was done in triplicates.

$$\% \text{ Ash Content} = \left(\frac{W_3 - W_1}{W_2 - W_1} \right) \times 100$$

W_1 = weight of cooled empty crucible

W_2 = weight of empty crucible + undried sample

W_3 = weight of crucible + dried sample

Crude Fibre Content

Crude fibre content of the flour/biscuits sample was determined using the method of AOAC (2010). The sample (5 g) was weighed into a 500 ml Erlenmeyer flask and 100 ml of TCA digestion reagent was added. It was then brought to boiling and refluxed for exactly 40 minutes counting from the start of boiling. The flask was removed from the heater, cooled a little then filtered through a 15.0 cm number 4 Whatman paper. The residue was washed with hot water, stirred once with a spatula and transferred to a porcelain dish. The sample was dried overnight at 105°C. After drying, it was transferred to a desiccator and weighed as W_1 . It was then burnt in a muffle furnace at 500°C for 6 hours, allowed to cool, and reweighed as W_2 .

$$\% \text{ Crude fibre Content} = \left(\frac{W_2 - W_1}{W_0} \right) \times 100$$

W_1 = Weight of crucible + fiber + ash

W_2 = Weight of crucible + ash

W_0 = Dry weight of food sample

Crude Fat Content

The soxhlet extraction method described by AOAC (2010) was used in determining fat contents of the flours and biscuits. Two grams (2 g) of the flour/biscuit sample were weighed into a weighed flat bottom flask, with the extractor mounted on it. The thimble was held half way into the extractor and the weighed sample. Extraction was carried out using boiling point of hexane (40 - 60°C). The thimble was plugged with cotton wool. At completion of extraction which lasted for 8 hours, the solvent was removed by evaporation on a water bath and the remaining part in the flask was dried at 80°C for 30 minutes in the air oven to dry the fat and then cooled in a desiccator. The flask was reweighed and percentage fat content calculated as follows

$$\% \text{ Crude fat Content} = \left(\frac{\text{weight of fat}}{\text{weight of sample}} \right) \times 100$$

Protein Content

The micro Kjeldahl method as described by AOAC (2010) was used to determine crude protein of the flours and biscuits. The flour/biscuit sample (2 g) was weighed into the digestion flask. Ten grams (10 g) of copper sulphate and sodium sulphate (catalyst) in the ratio of 5:1 respectively and 25 mL concentrated sulphuric acid were added to the digestion flask. The flask was placed into the digestion block in the fume cupboard and heated until frothing ceased giving clear and light blue green coloration. The mixture was then allowed to cool and diluted with distilled water until it reached 250 mL of volumetric flask. Distillation apparatus was connected and 10 mL of the mixture was poured into the receiver of the distillation apparatus. Also 10 mL of 40% sodium hydroxide was added. The released ammonia by boric acid was then treated with 0.02 N of hydrochloric acid until the green color changed to purple. Percentage of nitrogen in the sample was calculated using the formula below

$$\text{Nitrogen (\%)} = \left(\frac{(\text{Titre} - \text{blank}) \times 14.008 \times \text{Normality} \times 100}{\text{Weight of Sample}} \right) \times 100$$

$$\% \text{ Protein Content} = \% \text{ N} \times 6.25$$

Carbohydrate Content

The carbohydrate contents of the flours and biscuit were calculated by difference method according to Ihekoronye and Ngoddy(1985). This was done by summing up the moisture, crude protein, crude fat, crude fibre and ash contents and then subtracting from 100.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Crude Protein} + \% \text{ Crude Fat} + \% \text{ Crude Fibre} + \% \text{ Ash})$$

Determination of minerals (magnesium, iron and phosphorous) contents of the flours (wheat flour, maize flour and soybean flour) and biscuitsamples

Magnesium content

The magnesium content of the flour/biscuit sample was determined using the method described by AOAC (2010). The ash (2 g) obtained from the ash analysis earlier was boiled in a beaker with 10 ml of 20% HCl and then filtered into 100 ml standard flask. This was made up to the mark with de-ionized water. The magnesium content was determined by using the Unicam Solar Spectrophotometer (Model 969 Mk 11, Unicam Ltd, Cambridge, UK) to measure the absorbance at 285.2 nm wavelength.

Iron content

The iron content of the flour/biscuit sample was determined using the method described by AOAC (2010). The ash (2 g) obtained from the ash analysis earlier was boiled in a beaker with 10 mL of 20% HCl and then filtered into 100 mL standard flask. This was made up to the mark with de-ionized water. The iron content was determined by using the Unicam Solar Spectrophotometer (Model 969 Mk 11, Unicam Ltd, Cambridge, UK) to measure the absorbance at 248.3 nm wavelength.

Phosphorus content

The phosphorus content of the flour/biscuit sample was determined using the method described by AOAC (2010). The ash (2 g) obtained from the ash analyses earlier was boiled in a beaker with 10 ml of 20% HCl and then filtered into 100 ml standard flask. This was made up to the mark with de-ionized water. The total phosphorus content was obtained using ascorbic blue colour procedure of Okalebo et al. (2002) by reading the absorbance at a wavelength of 880 nm on a Helia Gamma Spectrophotometer (Helios Gamma UV-vis Spectrophotometer, thermo Spectronic, Cambridge, UK).

Evaluation of functional properties of the flours and their blends

The following functional properties- water absorption capacity, oil absorption capacity, bulk density, foaming capacity, foaming stability, least gelation concentration(LGC) of maize flour, soybean flour and the maize-soybean flour blends were determined.

Water absorption capacity

The water absorption capacities of the samples were determined as described by Onimawo and Akubor, (2012). One gram of the sample was mixed with 10mL distilled water and allowed to stand at ambient temperature for 30minutes and then was centrifuged for 30minutes at 2000rpm. Water absorption capacity was expressed as percent water bound per gram sample.

$$\text{Water absorption capacity (g/g)} = \left(\frac{\text{Weight of sample after centrifuge} - \text{Weight of tube}}{\text{Absorbance of standard solution}} \right)$$

Oil absorption capacity

The oil absorption capacities of samples were determined as described by Onimawo and Akubor, (2012). One gram of the sample was mixed with 10mL distilled water and allowed to stand at ambient temperature for 30minutes and then was centrifuged for 30minutes at 2000 rpm. Oil absorption capacity was expressed as percent oil bound per gram sample.

$$\text{Water absorption capacity (g/g)} = \left(\frac{\text{Weight of sample after centrifuge} - \text{Weight of tube}}{\text{Absorbance of standard solution}} \right)$$

Bulk density

The bulk density was determined as described by Onimawo and Akubor (2012). A 50g of sample was put into a 100mL graduated cylinder. The cylinder was taped 40-50 times and the volume of the flour was read. The bulk density was calculated as:

$$\text{Bulk density (g/mL)} = \left(\frac{\text{Weight of sample}}{\text{Volume of sample}} \right)$$

Pasting properties of flours and flour blends

The pasting characteristics were determined with a Rapid Visco Analyzer (RVA), (Model RVA3Dt, Network Scientific, and Australia). The flour sample (2.5g) was weighed into dried empty canisters. Then, 25 l of distilled

water was dispensed into the canister containing the sample. The solution was thoroughly mixed and the canisters were fitted into a Rapid Visco Analyzer (RVA) as recommended. The slurry was then heated from 50°C to 60°C with holding time of two minutes followed by cooling to 50°C with 2 minutes holding time. The rate of heating and cooling was at a constant rate of 11.25°C per min. The pasting temperature, viscosity at 95°C, stability, cooking time and setback viscosities were read off the amylograph.

Evaluation of physical properties of biscuits

All the biscuits including wheat flour were analyzed for weight, height, length, density, volume, spread ratio and break strength.

Weight

The weight and diameter of the baked biscuits were determined by weighing on a weighing balance and measuring with a calibrated ruler, respectively (Ayo *et al.*, 2007).

Spread ratio

The spread ratio was determined using the method of Gomez *et al.* (1997). Three rows of five well-formed biscuits were made and the height was measured. Also, the same was arranged horizontally edge to edge and the sum diameter was measured. The spread ratio was calculated as diameter/ height.

Break strength

The break strength of the biscuit was determined by modified method of Adeola and Ohizua (2018). Biscuit of known thickness was placed centrally between two parallel metal bars (3 cm apart). The weights were added on the biscuit until the biscuit snapped. The least weight that caused the breaking of the biscuit was regarded as the break strength of the biscuit.

Diameter and Thickness

The biscuit diameter and thickness were measured using vernier calipers (Ayo *et al.*, 2007).

Sensory evaluation of biscuits

The sensory characteristics of the prepared biscuits were assessed in the Sensory Evaluation Laboratory of the Department of Food Science and Technology, Federal University Wukari, under controlled conditions of adequate lighting and ventilation. The samples were evaluated for appearance, texture, taste, flavor and overall acceptability using a 9-point Hedonic scale as described by Larmond (1991) where 9 = like extremely and 1 = dislike extremely. The panelists were selected randomly from students of the Department based on their familiarity with biscuits. The biscuits were presented to the panelists in 3-digit coded white plastic plates in randomized order. Portable water was used to neutralize the taste between samples testing.

Experimental design

The experiments were fit into a one-way Analysis of variance (ANOVA). Three (3) samples of flours were generated in triplicates for each experiment on the nutritional composition of flours, yielding nine (9) samples per experiment analyzed; and eight (8) samples of flour and flour blends were generated for each experiment on the functional properties, yielding 24 samples per experiment analyzed. Then, four (4) samples of biscuits were generated in triplicates for each experiment on the nutritional composition of biscuits, yielding twelve (12) samples per experiment analyzed; and six (6) samples of biscuits were generated in triplicates for each experiment on the physical and sensory properties of the biscuits, yielding 18 samples per experiment analyzed.

Statistical analysis

Data obtained were analyzed as means of triplicate values by one way analysis of variance (ANOVA) in completely randomized design using Statistical Package for Social Science (SPSS) Version 16.00. The statistically significant differences were separated using the Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

Results and discussion

Table 2. Proximate composition (%) of maize flour, soybean flour and wheat flour

Sample	Moisture	Protein	Crude fat	Crude fiber	Ash	Carbohydrate
MF	10.79 ^a ±0.01	7.30 ^b ±0.01	2.98 ^c ±0.01	0.81 ^c ±0.01	1.71 ^c ±0.01	76.36 ^c ±0.01
SF	10.58 ^b ±0.01	42.23 ^a ±0.04	10.15 ^a ±0.01	5.20 ^b ±0.01	4.00 ^b ±0.01	27.79 ^a ±0.01

Comment [U6]: You do not need to conduct a difference test on the raw materials, nor do you need to provide the superscript.

WF 11.37^c±0.01 12.96^c±0.01 1.82^b±0.02 0.34^a±0.01 0.85^a±0.01 26.38^b±0.01

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different (P ≤ 0.05).MF=maize flour, SF=soybean flour, WF= wheat flour

Proximate composition of flours

Table 2 shows the proximate composition of maize flour, soybean flour and wheat flour. The moisture contents of soybean flour, maize flour and wheat flour varied in the range of 10.58-11.37%, with soybean flour having the least value of 10.58% while the wheat flour had the highest value of 11.37%. Lower moisture contents in soybean flour of 8.08% and 9.98% have been reported by Ogbemudia *et al.* (2018), and Okereke and Banigo (2021) respectively. The drying process removed free moisture on the surface of the food product. Then, it removes bound moisture in the inside or matrix of the food product until the lowest moisture is reached. Thus, heat and mass transfer operations are involved. This is an indication that the soybean flour cannot be stored for a very long time since moisture, which is an important medium for multiplication of microorganisms was high compared to the previous reports. The low moisture level is important for the flours to maintain long shelf life. The levels of moisture in the flours were below the recommended moisture level of 14 % for safe storage (WQCR, 2020), suggesting that all the flours would have good shelf stability.

The protein contents of maize flour, soybean flour ranged from 7.30-42.23%: maize flour had the least protein content while soybean flour had the highest protein content. The protein content of soybean flour was higher than the reported values of 37.69% by Siulapwa and Mwambungu (2014), 39.24% by Bayero *et al.* (2019) and slightly lower than the reported value of 43.02% by Okereke and Banigo (2021). This specie of soybean is of nutritional importance to sub-Sahara Africa (Nigeria) where protein energy malnutrition is a menace. They can be incorporated into diet formulations for weaning foods in infant, bakery products to improve nutrient value and as an alternative to animal proteins, which has the problem of low density lipoproteins that have adverse health effect such as coronary diseases. The protein content of 7.30% for maize flour was within the range (7-10.23%) reported by Shah *et al.*, (2015) and Gopalan *et al.*, (2007).

The crude fat contents of the maize flour, soybean flour and wheat flour varied from 1.82-10.15%; wheat flour had the lowest crude fat content of 1.82% while soybean flour contained highest fat content of 10.15%. This value of 10.15% oil content of soybean flour is lower than the reported value of 19.50% by Okereke and Banigo (2021). The high crude fat content of 10.15% suggested that soybeans maybe a viable source of oil, therefore maybe used for oil production. Most legumes contain 1.5% crude fat. Soybean crude fat is very high compared to most legumes because it is an oilseed.

The crude fiber contents of the flours ranged from 0.34%-5.40%; wheat flour contained the least amount of fiber while soybean contained the highest amount of fiber. The 5.40% fiber content of the soybean flour was relatively low but the value was in line with 5.44% fiber reported by Ogbemudia *et al.* (2018). Shah *et al.* (2015) and Gopalan *et al.* (2007) reported (2.15%) for maize. The presence of fiber in food is beneficial. Fiber is important for the removal of waste from the body thereby preventing constipation and many health disorders. Consumption of vegetable fiber has been shown to reduce the cholesterol level, risk of coronary heart diseases, colon and breast cancers and hypertension (Okereke *et al.*, 2021^a; Akubor *et al.*, 2023). It also enhances glucose tolerance and increases insulin sensitivity.

Ash contents of maize flour, soybean flour and maize flour were in the range of 0.34-4.00%, with wheat flour containing the least value of 0.34% and soybean flour having the highest value of 4.00%, which was in close range to 4.29% reported by Ogbemudia *et al.* (2018). Shah *et al.* (2015) and Gopalan *et al.* (2007) reported (2.33%) for maize. High ash content indicated that the flour sample could be important sources of minerals. The carbohydrate contents of maize flour, soybean flour and wheat flour varied from 26.38-76.36%. Wheat flour had the least value of 26.38% while maize flour had the highest value of 76.36%, which was in close range to (71.88%) reported by Shah *et al.*, (2015) and Gopalan *et al.*, (2007). The high quantity of carbohydrate 27.79% in the soybean flour was in contrast with 16.31% reported by Ogbemudia *et al.* (2018). High carbohydrate content of the flour sample suggested that the flour sample could be used in managing protein-energy malnutrition since there is enough quantity of carbohydrate to give energy while the protein can be used for its primary function of building the body and repairing worn-out tissues.

Table 3. Mineral composition (mg/100g) of maize flour, soybean flour and wheat flour

Flour Sample	Phosphorus	Iron	Magnesium
MF	166.96 ^a ±0.04	19.54 ^a ±0.01	157.56 ^a ±0.00
SF	15.25 ^a ±0.35	2.31 ^a ±0.01	0.01 ^b ±0.00
WF	1.24 ^a ±0.02	10.93 ^a ±0.03	3.88 ^c ±0.00

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different (P ≤ 0.05).MF=maize flour, SF=soybean flour, WF= wheat flour

Comment [U7]: You do not need to conduct a difference test on the raw materials, nor do you need to provide the superscript.

Mineral composition of flours

The mineral composition of maize flour, soybean flour and wheat flour are shown in Table 3. The phosphorus contents of maize flour, soybean flour and wheat flour were in the range of 1.24- 166.96%. Maize flour had the highest value of phosphorus; high value of phosphorus for maize flour has been reported by Shah *et al.* (2015). Phosphorus performs regulatory function (enzyme co-factors) and building up biological structures (bones, bio-membranes) (Gopalan *et al.*, 2007).

The iron contents of maize flour, soybean flour and wheat flour were in the range of 2.31- 19.54%. Maize flour was higher in iron content.

The magnesium contents of maize flour, soybean flour and wheat flour ranged from 0.01-157.56%. Maize flour had the highest value of magnesium, which was comparable to reports by Kumar and Jhariya (2013). Minerals are essential for the maintenance of the overall mental and physical wellbeing. They are important constituents for the development and maintenance of bones, teeth, tissues, muscles, blood, and nerve cells. They aid acid base balance, response of the nerves to physiological stimulation and blood clotting, and also play key role in various physiological functions of the body, especially in the building and regulation processes (Okereke, 2023).

Table 4. Functional properties of maize flour, soybean flour, wheat flour and various maize-soybean flour blends

Flour/flour blends (MF:SF)	Bulk density (g/cm ³)	Foaming capacity (%)	WAC (g/g)	OAC (g/g)
100:0	0.03 ^b ±0.02	12.21 ^e ±0.13	205.00 ^a ±7.07	160.00 ^b ±14.14
0:100	1.28 ^a ±0.04	16.17 ^f ±0.05	210.00 ^a ±14.14	145.00 ^{ab} ±7.07
95:5	0.67 ^b ±0.04	3.22 ^a ±0.12	200.00 ^a ±14.14	145.00 ^b ±7.07
90:10	0.66 ^b ±0.01	9.15 ^a ±0.08	160.00 ^a ±14.14	140.00 ^{ab} ±14.14
85:15	0.67 ^b ±0.01	3.27 ^a ±0.33	165.00 ^a ±7.07	105.00 ^a ±7.07
80:20	0.67 ^b ±0.01	6.04 ^b ±0.03	210.00 ^a ±14.14	110.00 ^b ±14.14
70:30	0.68 ^b ±0.03	6.58 ^c ±0.18	180.00 ^a ±14.14	80.00 ^b ±14.14
WF	0.67 ^b ±0.01	29.25 ^e ±0.08	85.00 ^a ±7.07	95.50 ^a ±6.36

Values are mean ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different (P < 0.05)

WAC= water absorption capacity, OAC= oil absorption capacity, MF=maize flour, SF=soybean flour, WF= wheat flour

Comment [U8]: Please double-check the superscript in foaming capacity, WAC; there are detectable errors there.

Functional properties of flours

Table4 shows the functional properties of the wheat flour, soybeans flour, maize flour and maize-soybean flour blends. Functional properties are the characteristics that determine the suitability of the food material for specific purpose. They are those characteristics that govern the behavior of food constituents during processing, storage and preparation as they affect food quality and acceptability (Olapade and Adeyemo, 2014. Functional properties are characteristics of flour that affect its behavior and that of the products to which it is added during food processing.

The bulk densities of the individual flours varied from 0.03-1.28g/cm³ bulk, and those of the composite flours varied from 0.66-0.68g/cm³. Theblend containing 10% soybean flour blend had the least bulk of 0.66g/cm³while that containing 30 had the highest value of 0.68g/cm³. The bulk density increased with an increase in soybean flour. However, soybean had a higher value of bulk 1.28% while maize flour had the least value of bulk (0.03g/cm³). Bulk density is a measure of the heaviness of flour. It also measures the porosity of a material and affects the design of a package. In this regard, bulk density would be useful for determining the packaging requirement of flour as well as in material handling and application in wet processing of food. Thus, the low bulk densities of the flours evaluated in this study makes their packaging economical. The complementary foods that would be prepared from the flour blends will have low dietary bulk. This is important for such foods because high bulk limits calorie and nutrients intake from food, and infants will not be able to consume enough of high dietary bulk foods to meet their energy and nutrients requirements. A diet with low dietary bulk would not form thick gel with high viscosity, plasticity and elasticity (Onimawo and Akubor, 2012).

Foaming capacities of maize flour, wheat flour and soybean flour were 12.21%, 16.17%, and 29.25%, respectively. The foaming capacity of the composite flours increased from 3.22% to 6.58%.The sample containing 10% soybean flour had the highest foaming capacity and the sample containing 5% soybean flour had the least value. The higher the foaming capacity, the better the re-constititional properties of the flour,

Comment [U9]: If the statistics reveal no disparities, it should not be reported highest, lowest...There are no variations in bulk density...review your data

which has a marked effect on the kneading quality (Mbwana *et al.*, 2016; Wang *et al.*, 2002). The soybean flour had higher capacity to form and stabilize foam probably due to its higher protein content over that of wheat flour (Table 2). Akubor and Eze (2016) noted the dependent of foaming capacity and foam stability on protein concentration and protein solubility among other factors. Foaming capacity and foaming stability of the flour blends decreased with increase in the amount of soybean flour. This was in agreement with the report by Akubor (2017) that foaming properties are not additives. Foamability is related to the ability of proteins to adsorb rapidly at the air- water interface during bubbling and also on the ability of proteins to undergo rapid conformational change and arrangement at the interface (Igbokwe *et al.*, 2016). For food to form stable foam, the proteins must have the capacity to form cohesive viscoelastic film through intermolecular interactions during processing (Eguono and Akubor, 2016). Foam formation and foam stability are influenced by the type of protein, pH, processing methods, viscosity and surface tension (Eguono and Akubor, 2016). Akubor and Chukwu (1999) reported that foams improve the texture and appearance of processed foods.

Water absorption capacity is a function of water holding ability of the flour sample. It is an important processing parameter that has implication in viscosity. The water absorption capacities of the individual flours were 85%, 205% and 210% for wheat flour, maize flour and soybean flour. The water absorption capacities ranged from 160% - 210% for composite flours. The high values for the water absorption capacity of the composite flours indicated higher affinity for water. The loose structure of starch polymers causes it to possess high water absorption capacity while, products with low value indicated the compactness of such product structure (Adebowale *et al.*, 2012). The soybean flour contained higher amounts of hydrophilic constituents such as protein and crude fiber than wheat flour and maize flour (Table 2). Akpata and Akubor (1999) showed that water absorption capacity is influenced mainly by the nature of the hydrophilic constituents and to some extent the pH and nature of the protein (Damak *et al.*, 2022). The low oil absorption capacity of soybean flour may have enhanced its water absorption capacity. High water absorption capacity is also useful in the development of ready-to eat foods where it promotes product cohesiveness.

The oil absorption capacities of the individual flours varied from 95.50 -160% while those of the composite flours ranged between 80-145%. The oil absorption capacity decreased with an increase in soybean flour in the blends. Oil absorption capacity is due to binding of fat by non-polar side chains of proteins. High oil absorption of the protein is required in baked foods for improve taste (Tizazu and Emire, 2010; Okereke *et al.*, 2022^a). This suggested that, the blend containing 5% soybean flour will taste better than products from 100% wheat flour and other samples due to its high oil absorption capacity. Onimawo and Akubor (2012) ascribed oil absorption capacity to mainly physical entrapment of oil which indicated the rate at which proteins bind to fat in food formulations. High oil absorption capacity has been suggested to be useful in food formulations because fats improve flavor and mouth feel of foods (Akubor, 2017; Okereke *et al.*, 2022^a).

Table5: Pasting properties of wheat flour, maize flour, soybean flour, and maize-soybean flour blend

Flour/flour blend (MF:SF)	Peak (RVU)	Trough (RVU)	Break down (RVU)	Final viscosity (RVU)	Setback (RVU)	Peak Time (mins)	Pasting Temperature (°C)
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Comment [U10]: cek the data, the superscript symbol is mistake

WF	1206.25 ^a ±0.35	654.05 ^a ±0.07	551.99 ^a ±0.01	1366.50 ^a ±0.71	713.00 ^a ±0.01	5.72 ^c ±0.02	89.55±0.00
SF	118.90 ^e ±0.14	99.05 ^c ±0.07	20.02 ^c ±0.02	139.05 ^b ±0.71	40.01 ^b ±0.01	6.40 ^b ±0.01	89.55±0.00
MF	474.45 ^b ±0.64	264.05 ^b ±0.07	437.01 ^b ±0.01	577.95 ^b ±0.71	540.99 ^b ±0.02	5.73 ^c ±0.01	87.68±0.00
95:5	118.95 ^d ±0.07	85.05 ^e ±0.07	33.99 ^e ±0.02	409.98 ^c ±0.34	325.03 ^c ±0.04	7.05 ^a ±0.01	55.45±0.00
90:10	125.95 ^d ±0.07	83.95 ^b ±0.07	41.95 ^b ±0.07	224.02 ^e ±0.02	139.99 ^f ±0.02	6.12 ^d ±0.04	95.05±0.00
85:15	127.90 ^c ±0.14	97.05 ^d ±0.07	30.99 ^d ±0.01	262.00 ^d ±0.01	165.03 ^d ±0.04	5.80 ^a ±0.01	89.00±0.00
80:20	114.10 ^f ±0.14	88.03 ^e ±0.34	25.99 ^e ±0.01	229.99 ^e ±0.01	141.99 ^e ±0.01	7.00 ^a ±0.01	88.56±0.00
70:30	113.05 ^e ±0.07	88.98 ^f ±0.34	23.99 ^f ±0.01	225.02 ^f ±0.03	136.01 ^e ±0.01	7.01 ^a ±0.01	86.78±0.00

Values are mean ± standard deviation of 3 replications. Means within each column not followed by the same superscript are significantly different (P < 0.05)

WAC= water absorption capacity, OAC= oil absorption capacity, MF=maize flour, SF=soybean flour, WF= wheat flour

Pasting properties of wheat flour, maize flour, soybean flour and maize-soybean flour blends

The pasting properties of wheat flour, maize flour, soybean flour and maize-soybean composite flours are shown in Table 5. Pasting properties is an important index in determining the cooking and baking qualities of flours. The peak viscosities varied for the individual flours from 118.90RVU in soybean flour to 1206.25RVU in wheat flour. The addition of soybean flour to the maize flour significantly (p<0.05) reduced the peak, trough, breakdown, final and setback viscosities, and increased the peak time and pasting temperature of the composite flours. This could be attributed to the reduction in starch gelatinization (Okereke, 2023). This affirmed why 100% wheat flour had the highest peak viscosity than all the composite flours. The value suggested that 100% wheat flour will swell freely than the other composite flours. Peak viscosity is an index of the ability of starch-based foods to swell freely before their physical break down (Okereke, 2023).

Peak viscosity indicates the water binding capacity of the starch grains and the flimsiness of swollen granules (Obinna-Echem, 2017). The decrease in the peak viscosity with increase in soybean suggest that the soybean flour may have more water binding ability as evidenced in the water absorption capacities of the samples. Peak viscosity also reflects the ease of cooking of the starch fraction and a good texture of cooked starch (Ikegwu *et al.*, 2010; Okereke *et al.*, 2022^b).

The trough viscosity which is also known as the holding strength is the minimum viscosity after the peak, normally occurring around the commencement of sample cooling. The values varied from 83.95 – 654.05 RVU. The blend containing 10% soybean flour and the control (wheat flour) had the least and highest values, respectively. As with the final viscosity, it implies that the samples with the soybean flour may not form very stiff and firm dough compared to the control.

The breakdown viscosities of the samples varied from 20.02 in soybean flour to 551.99 RVU in wheat flour. Breakdown viscosity reflects the ability of the sample to withstand sheer stress and heating during cooking (Okereke *et al.*, 2022^b). This implied that the starch grains of the maize with high levels of soybean flour lacked the ability to withstand stress from the mechanical agitation by the visco analyzer and therefore, ruptured earlier leading to decreased viscosity. The blends containing 20% and 30% soybean flour may be able to withstand cooking/baking without losing firmness.

Setback viscosity ranged from 136.01 for the blend containing 30% soybean flour -713.00 RVU for the wheat flour. Setback viscosity is related to the degree of polymerization of the amylose fraction leached during swelling, hence it is an indication of resistance of the starch to retrograde (Chung *et al.*, 2003; Sanni *et al.*, 2002; Okereke *et al.*, 2022^b). The addition of soybean flour may have lowered the degree of polymerization of the maize starch, which gave the blends lower set back viscosity.

The final viscosity varied significantly from 139.05 -1366.50 RVU. The wheat flour had the highest final viscosity and soybean flour had the least. The final viscosities of the maize and soybean flour samples were higher than their peak viscosities. Final viscosity marks the ability of starch to form viscous paste after cooking and cooling (Obinna-Echem, 2016; Okereke *et al.*, 2022^b).

Peak time and pasting temperature correspond to time in minutes, and temperature (°C) at which the peak viscosity occurred. The peak times (5.72 – 7.05 min.) and pasting temperatures (55.45 – 95.05°C) increased with the addition of the soybean flour, this could be as a result of the protein content of the flour. The peak time is a measure of the cooking time (Okereke *et al.*, 2022^b). High pasting temperature is an indication of higher water binding capacity, higher gelatinization tendency and lower swelling property of starch-based flour as a result of high degree of association between starch granules (Adebowale *et al.*, 2008).

Table 6: Proximate composition (%) of biscuits from maize-soybean flour blends and 100% wheat flour

Biscuit sample	Moisture	Crude protein	Crude Fat	Crude fiber	Ash	Carbohydrate	Comment [U11]: carbohydrate by difference
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90:10	4.10 ^a ±0.14	40.38 ^a ±0.78	28.66 ^c ±0.34	2.89 ^b ±0.16	0.53 ^b ±0.04	24.65 ^b ±0.84
80:20	4.98 ^b ±0.02	34.94 ^b ±0.08	31.39 ^d ±0.02	6.88 ^c ±0.17	3.79 ^d ±0.16	17.90 ^a ±0.01
70:30	9.19 ^c ±0.03	37.10 ^c ±0.14	27.24 ^b ±0.08	7.93 ^d ±0.99	1.55 ^c ±0.03	17.32 ^c ±0.05
WFB	7.97 ^d ±0.05	8.91 ^d ±0.13	18.41 ^a ±0.57	1.63 ^a ±0.39	0.99 ^e ±0.02	62.16 ^d ±0.06

Values are mean ± standard deviation of 3 replications. Means within each column not followed by the same superscript are significantly different (P < 0.05); WFB= biscuit of 100% wheat flour

Proximate composition of biscuits

The proximate composition of biscuits prepared from maize and soybean flour blends and 100% wheat flour is shown in Table 6. The maize-soybean composite flour biscuits contained moisture contents of 4.10 - 9.19%, and the 100% wheat flour biscuit contained 7.97% moisture. The moisture content increased with increase in soybean flour in the biscuits. The difference in moisture content was due to the difference in the water holding capacity and composition of flours (Okereke, 2023; Akubor *et al.*, 2023). Higher moisture content produces an extensive gluten structure that results in harder biscuits and favored mold growth in baked products. These low values may aid the biscuits' storage and subsequent prevention of triglyceride degradation during the storage.

The maize-soybean composite flour biscuits contained protein contents in the range of 37.10 - 40.38% and 100% wheat flour biscuit contained 8.91% protein. The protein content of the maize-soybean flour biscuits decreased from 40.38%-34.94% and then, increased to 37.10% with addition of soybean flour. The sudden decrease and increase could be as a result of the chemical used for the analysis. The highest protein content was obtained in the biscuit containing 90% maize flour. Proteins play important role in the organoleptic properties of food products and acts as a source of amino acids in the food. Proteins are immune boosters and can help in cell division as well as growth (Okereke, 2023).

The maize-soybean composite biscuit contained crude fat contents of 27.24 to 31.39% and 100% wheat flour biscuit contained 18.41% crude fat. The fat contents increased with increase in soybean flour. But low fat content would enhance storage stability as they are less likely to develop rancid flavor.

The maize-soybean composite biscuit contained crude fiber contents of 2.89 to 7.93% and 100% wheat flour biscuit contained 1.63% crude fiber. The fiber content of maize-soybean biscuits increased with increase in soybean flour. This indicated that soybean flour enrichment with maize flour improved the crude fiber content. The maize-soybean composite biscuit contained ash contents in the range of 0.53% to 3.79% and 100% wheat flour biscuit contained 0.99% ash. Ash contents of the biscuits increased from 0.53% to 3.79% and then decreased to 1.55% as the soybean flour substitution increased. The values agreed with results of other research studies (Ndife *et al.*, 2011; Okereke *et al.*, 2011^b). The increase in ash content could be due to the higher ash content of the soybean flour than in the maize; soybean has been reported to contain an appropriate quantity of minerals and fat (Okereke and Banigo, 2021).

The maize-soybean composite flour biscuits contained carbohydrate contents in the range of 17.32-24.65% and 100% wheat flour biscuit contained 62.16% carbohydrate. As the substitution of soy flour increased, the carbohydrate content decreased. The 100% wheat flour biscuit had the highest value for carbohydrate. Carbohydrate provides energy; these biscuits will serve as a good source of energy. Carbohydrate provides the body with energy for daily tasks and are the primary fuel source for the brain's high energy demands (Nacer, 2010).

Table 7. Mineral composition (mg/100g) of biscuits from maize-soybean flour blends and 100% wheat flour

Biscuit sample	Phosphorus	Iron	Magnesium
90:10	58.10 ^b ±0.14	6.25 ^b ±0.35	0.0040 ^a ±0.00
80:20	65.91 ^b ±0.13	3.37 ^a ±0.05	0.0047 ^a ±0.00
70:30	47.01 ^a ±0.01	6.35 ^b ±0.07	0.0018 ^a ±0.00
WFB	124.59 ^c ±6.28	7.08 ^c ±6.03	33.89 ^b ±0.02

Values are mean ± standard deviation of 3 replications. Means within each column not followed by the same superscript are significantly different (P < 0.05); WFB= biscuit of 100% wheat flour

Mineral composition of biscuits

The Mineral composition of biscuits prepared from maize and soybean flour blends and 100% wheat flour is shown in Table 7. The maize-soybean flour biscuits contained phosphorus that ranged from 47.01 to 65.91 mg/100g while the 100% wheat biscuit had 124.59 mg/100g. This result is in disagreement with the findings of Salguero *et al.* (2017) that reported increased phosphorus contents in composite cookies of carrot, lupine and barley. Phosphorus helps to activate enzymes and substrates in enzyme catalyzed reactions. It is needed in the formations of ATP (Adenosine triphosphate), creatine phosphate, DNA (Deoxyribonucleic acid), RNA

Comment [U12]: please double-check Protein is greater in the data than carbohydrates...Please clarify the product's formulation, which is dominated by carbohydrates.

(Ribonucleic acid), phospholipids, strong bone and cartilage, and active transport (Sahay *et al.*, 2017; Okereke *et al.*, 2021^b). However, excess of phosphorus intake can reduce body store of calcium (Okereke *et al.*, 2021^b). Maize-soybean flour biscuit contained 3.37-6.35 mg/100g iron and 100% wheat biscuit contained 7.08 mg/100g iron. This result is in conflict with the results of Okereke *et al.* (2021^a) who reported significant improvements on the iron contents of composite bread made with wheat flour, white yam starch, trifoliolate yam starch, sweet potato starch and *Moringa oleifera* seed flour. Iron is necessary for growth, development, normal cellular functioning, and synthesis of some hormones and connective tissues (Okereke, 2023). The maize-soybean biscuit had magnesium content of 0.0018-0.0047 mg/100g and 100% wheat flour biscuit had magnesium content of 33.89 mg/100g. The result is in agreement with the reports of Inyang *et al.* (2018), Okereke *et al.* (2021^b) and Okereke *et al.* (2021^b) that showed decreased iron contents of composite cookies, composite bread and composite cookies respectively, in their studies. The maize and soybean flour biscuits were lower in magnesium; this could be as a result of the species of soybean used. Magnesium content decreased with increase in soybean flour in the biscuits. Magnesium aids in blood coagulation (Ca-fibrinogen fibrin transformation) and building up of bones.

Table 8: Physical properties of biscuits from maize and soybean flour blends and 100% wheat flour.

Biscuit sample	Thickness (cm)	Diameter (cm)	Weight (g)	Break strength (kg)	Spread Ratio
95:5	0.40 ^a ±0.06	4.99 ^{ab} ±0.12	16.05 ^a ±3.47	352.50 ^b ±3.54	14.28 ^c ±0.40
90:10	0.39 ^a ±0.03	4.85 ^{ab} ±0.11	19.70 ^a ±3.68	255.00 ^{ab} ±7.07	11.44 ^b ±0.35
85:15	0.58 ^b ±0.08	5.06 ^{bc} ±0.07	18.10 ^a ±2.69	162.50 ^a ±159.10	11.29 ^a ±0.84
80:20	0.45 ^a ±0.01	4.86 ^c ±0.07	19.55 ^a ±0.64	355.00 ^b ±7.07	13.78 ^b ±0.26
70:30	0.42 ^a ±0.50	4.87 ^c ±0.07	17.95 ^a ±1.63	352.50 ^b ±3.54	13.80 ^b ±0.25
WFB	0.80 ^a ±0.21	4.86 ^a ±0.07	21.45 ^a ±0.21	625.00 ^c ±35.36	5.03 ^c ±0.43

Values are mean ± standard deviation of 3 replications. Means within each column not followed by the same superscript are significantly different (P < 0.05); WFB= biscuit of 100% wheat flour

Physical properties of biscuits

The physical properties of biscuits prepared from maize and soybean flour blends and 100% wheat flour are shown in Table 8. The composite flour biscuits had thickness ranging from 0.3 to 0.58 cm and 100% wheat biscuits had 0.80 cm thickness. Gluten free biscuits had less thickness than the 100% wheat biscuit due to the substitution effects of wheat flour with non-wheat flours (Okereke *et al.*, 2021^b). The maize and soybean composite flour biscuits had diameters, which ranged from 4.85-5.06 cm and 100% wheat biscuit had diameter of 4.86 cm. The gluten free biscuits had higher diameters than 100% wheat biscuit due to increased hydrophilic sites of the starch granules of the non-wheat flours leading to moisture absorption and subsequent diameter increase (Okereke *et al.*, 2021^b). The maize-soybean flour biscuits had weight ranging from 17.95-19.70 g and 100% wheat had 21.45 g weight. Gluten free biscuits had less weight when compared to 100% wheat biscuit. The Maize-soybean biscuit had break strength of 162.50-355.00% and 100% wheat biscuit had 625.00% break strength. Gluten-free biscuits had less break strength when compared to 100% wheat biscuit. The values of this study agreed with Ayo *et al.* (2007) who reported that the use of composite flour for making biscuits reduces its break strength. The maize-soybean biscuit had spread ratios that ranged from 11.29-14.28% and 100% wheat biscuit had 5.03% spread ratio. Gluten free biscuits had higher spread ratio when compared to 100% wheat biscuit, and could be attributable to the effects of non-wheat flour fractions of the flour blends on the composite biscuits (Igbabul *et al.*, 2018; Okereke *et al.*, 2021^b). The increased spread ratio or factor observed could be traced further to the increased number of hydrophilic sites in the dough leading to increased water absorption and swelling index (Igbabul *et al.*, 2018; Okereke *et al.*, 2021^b).

Table 9: Sensory properties of biscuits from maize and soybean flour blends and 100% wheat flour

Biscuit sample	Appearance	Mouthfeel	Aroma	Texture	Taste	General acceptability
95:5	7.25 ^{ab} ±1.48	6.79 ^{ab} ±1.41	6.94 ^{ab} ±1.20	6.38 ^b ±1.71	6.67 ^a ±2.04	7.29 ^{abc} ±1.40
90:10	7.40 ^{ab} ±1.38	6.72 ^{ab} ±1.62	6.60 ^{ab} ±1.63	5.88 ^b ±1.88	6.92 ^a ±1.75	7.48 ^{ab} ±1.26
85:15	6.76 ^{ab} ±1.99	6.40 ^b ±1.58	6.44 ^b ±1.50	6.64 ^b ±1.19	6.80 ^a ±1.98	7.24 ^{abc} ±1.30
80:20	7.16 ^{ab} ±1.34	6.56 ^{ab} ±1.78	6.72 ^{ab} ±1.49	6.64 ^b ±2.04	6.76 ^a ±1.45	6.84 ^{bc} ±1.75

70:30	6.64 ^a ±1.71	6.32 ^b ±1.41	6.40 ^b ±1.58	6.32 ^b ±1.63	6.16 ^a ±2.14	6.48 ^c ±1.42
WFB	7.69 ^b ±1.23	7.65 ^a ±0.94	7.46 ^a ±1.14	7.65 ^a ±1.02	7.23 ^a ±1.70	7.89 ^a ±0.95

Values are means ± standard deviation of 3 replications. Means within each column not followed by the same superscript are significantly different ($P < 0.05$); WFB= biscuit of 100% wheat flour

Sensory properties of biscuits

Sensory quality is considered a key factor in food acceptance because consumers look out for food with specific sensory characteristics (Bello *et al.*, 2018). Table 9 shows the sensory properties of biscuits from maize-soybean flour blends and 100% wheat biscuits.

The maize-soybean flour biscuits had scores for appearance that ranged from 6.64-7.40 and 100% wheat biscuit had score of 7.46 for appearance. Increase in soybean flour decreased the mean score for appearance. The appearance score was highest (7.40) for 10% Soybean substitution and lowest (6.64) for 30% Soybean flour substitution. A similar trend was also reported by Banureka and Mahendran (2009).

The scores for the mouthfeel of the composite biscuits ranged from 6.32-6.79 and the 100% wheat flour biscuit had score of 7.65. The mouthfeel of the biscuit decreased with an increase in soybean flour; biscuit with 30% soybean flour rated poorest. The control had the highest value for mouthfeel.

The maize-soybean flour had score for aroma that ranged from 6.40-6.94 and 100% wheat biscuit had 7.46. Increase in soybean flour decreased the aroma of the biscuit from maize-soybean flour blends and wheat flour biscuit recorded the highest value of 7.46. This could be due to the beany flavour of soy flour (Akubor and Ukwuru 2005).

Maize-soybean biscuit had texture scores that ranged from 5.88-6.64 and 100% wheat biscuit had 7.65 in taste attribute. The texture of the crust is related to the external appearance of the biscuit top which implies smoothness or roughness of the crust. With the increase in substitution of soybean flour to the biscuits, the texture of crust was decreased from 6.64-5.88.

The maize-soybean biscuit had taste scores that ranged from 6.16-6.92 and 100% wheat biscuit had 7.23. The maize-soybean biscuit had general acceptability scores that ranged from 6.84-7.29 and 100% wheat biscuit had score of 7.89 for general acceptability. All the biscuits produced were acceptable, indicating that maize and soybean blends can be used to produce acceptable biscuits.

Conclusion

The study has shown that maize and soybean flour blends could be used to produce gluten-free biscuits (of acceptable nutritional, physical and sensory qualities) and other food products as revealed by their results on functional, pasting and nutritional properties' investigations. These biscuits are suitable for celiac disease patients, including those following a gluten-free diet. Such gluten-free biscuits (with high nutritional value) made from maize and soybean flours were quite promising, showing similar acceptability rating with the 100% wheat flour biscuits. Generally, all the composite biscuits were acceptable but most preferred was the biscuit of 10% soybean flour substitution. The adoption of production of maize- soybean flour blended biscuits will go a long way in curtailing high import bills of wheat.

Recommendations

The functional and pasting properties of the various maize-soybean flour blends suggest of the utilization of the blends in the formulations of complementary foods, sauces, confectioneries, thickeners, texturizers, energy-giving foods, cheese, gravies and beverage products. The flours and their blends would be useful in energy and time savings (cost effectiveness) at commercial applications. Biscuits produced by blending 90% maize flour and 10% soybean flour is recommended for use.

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