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Effect of Blending Ratios and Baking Temperatures on Nutritional and Sensory Properties of Biscuit Made from Wheat, Pigeon Pea, and Moringa Leaf Flour Blends

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Abstract

The study assessed the effect of blending ratios and baking temperatures on nutrient composition and sensory acceptability of biscuits made from wheat, pigeon, and moringa leaf flour blends. The study compared proximate composition (moisture, carbohydrate, fat, protein, crude fiber, and ash); micronutrients (zinc, iron, vitamin C, and pro-vitamin A) and sensory attributes (taste, color, appearance, flavor, and texture) of biscuits made from different ratios of flour blends baked at 180⁰C and 200⁰C. Flours made from wheat, pigeon pea, and moringa leaves were used to produce blends for biscuit production in the following ratios in percentage: 100:0:0 (Control), 80:15:5, and 70:20:10. Proximate, micronutrient, and sensory characteristics were determined using standard methods. Data were analyzed with means, standard deviations and analysis of variance. Control biscuits baked at 200⁰C recorded the lowest protein (9.90%), ash (2.00%), crude fiber (0.50%) and fat (6.00%) content and highest carbohydrate content (66.79%). However, highest ash (4.50%), protein (17.86%), moisture (21.5%), crude fiber (9.8%) and fat (9.50%) content and lowest carbohydrate (42.23%) content were recorded in blending ratio of 70:20:10 baked at 180⁰C. Zinc increased from 0.30mg in control to 4.58mg in 70:20:10 baked at 180⁰C. Iron increased from 0.77mg in control baked at 200⁰C to 1.82mg in 70:20:10 baked at 180⁰C. Vitamin C (mg) increased from 13.76 in 80:15:5 baked at 200⁰Cto 30.96 in control baked at 180⁰C. Pro-vitamin A (iu) increased from 164.54 in 80:15:5 at 200⁰Cto 434.04 in control at 180⁰C. Biscuits made from 80:15:5 baked at 200°C, emerged as the most favored and well-received sample, exhibiting the highest mean scores in all sensory attributes. Control biscuits baked at 200°C, received the lowest scores in most attributes. The study has shown that biscuits rich in nutrients, especially protein, zinc, and iron, and acceptable biscuits could be produced from the partial substitution of wheat with pigeon pea flour and moringa leaf flour. Enriching biscuits with pigeon pea and moringa leaf flour can improve their sensory and nutrient attributes.
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Keywords: Pigeon pea flour, Moringa leaf flour, Wheat flour, Blends, Biscuits.

Introduction

Biscuit is a small flat, crisp cake made from flour. They are typically hard, flat, and unleavened hence, regarded as dried products (Idoko & Nwajiaku, 2013). Biscuits represent a fast-growing segment of food in most parts of the world because of consumer demands for safe, tasty, convenient, and nutritious food products (Lubna & Vidhu, 2012). They are considered an ideal food for traveling as they remain fresh for a longer time (Hussein et al., 2006). Biscuits are mostly produced from wheat flour. Apart from its major constituent starch, wheat flour also contains many other types of substances such as gluten and non-starch polysaccharides. The protein called gluten helps to make the biscuit dough stick together thereby giving it the ability to retain gas (Goesaert et al., 2005).

Wheat flour, which is the main ingredient used to produce biscuits, cannot be grown commercially in many regions of the world due to various climatic conditions. Therefore, industry can only survive by utilizing available local crops which can be substituted with wheat in food products without adversely altering the quality of the product. wheat is a good source of calories, but it is considered poor, cereal is deficient in essential amino acids, namely lysine and threonine. Thus, the addition of wheat flour with other inexpensive staples like cereals and pulses benefits the nutritional value of wheat products (Banua et al, 2021). Composite flour is defined as a mixture of several flours obtained from roots, tubers, cereals, and legumes with or without the addition of wheat flour (Mitaigiri et al, 2021). The purpose of composite flour is to improve the protein profile of foods because some essential amino acids (lysine, threonine, and tryptophan) are absent in cereals but present in legumes while some essential amino acids (lucine, methionine, and cysteine) are present in cereals (Abdelghafor et al, 2011). The main ingredient generally used for biscuit production is wheat flour and other ingredients such as margarine, sugar, leavening agents, eggs, milk, salt, and flavour (Wade, 2018).

Pigeon pea (*Cajanus cajan* (L) Huth) is a legume belonging to the family of Fabaceae. Pigeon pea is fast-growing, hardy, widely adaptable, and drought resistant. Due to its drought resistance, it can be considered of utmost importance for food security in regions where rainfall does not often occur. Also because of its high protein value, it is a very important crop for poor people in Africa who cannot afford expensive animal protein (Sarkar et al, 2020). Although it has a very high nutritional value, it is still neglected and under-utilized in Nigeria. As a legume, pigeon pea is high in protein and plays an important role in human nutrition which makes the crop an excellent source of supplementing proteins in the diet, it is also highly nutritive in vitamin and mineral contents like iron, phosphorous, calcium, and magnesium, zinc and potassium, but low sodium content and has a low glycemic index (Nwosu et al.,2013).

Rockwood et al. (2013) reported that moringa provides seven times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas, and 25 times more iron than spinach. Moringa leaves contain fiber, fat proteins and minerals like Ca, Mg, P, K, Cu, Fe, and S. Vitamins like Vitamin A (Beta-carotene), vitamin B-choline, vitamin B1-thiamine, riboflavin, nicotinic acid and ascorbic acid are present. Various amino acids like Arginine, Histidine, Lysine, Trypsin, Phenolylinine, Thripcine, Leucine, Meteonine, and Valine is present. Phytochemicals like tannins, sterols, saponins, trepenoids, phenolics, alkaloids, and flavanoids like quercitin, isoquercitin, kaemfericitin, isothiocyanates, and glycoside compounds are present (Williams et al., 2013).

Many compounds found in moringa leaves might be involved in glucose homeostasis (Fahey, 2005). For example, isothiocyanates have been reported to reduce insulin resistance as well as hepatic gluconeogenesis. Phenolic acids and flavonoids increase insulin sensitivity in peripheral tissues. Phenolic compounds, flavonoids, and tannins also inhibit intestinal sucrase and to a certain extent, pancreatic α-amylase activities (Fahey, 2005). The hypoglycemic and antihyperglycemic activity of the leaves of moringa leaves might be due to the presence of terpenoids, which are involved in the stimulation of β-cells and the subsequent secretion of insulin (Makkar et al., 2016). Moringa leaf powder contains high concentrations of vitamin A, which can help to prevent night, cataracts, and eye problems (Anwar et al., 2007).

The presence of flavonoids gives the leaves antidiabetic, anticancer, and antioxidant properties. Moringa leaf has been studied for its chemopreventive properties and has been shown to inhibit the growth of several human cancer cells. The extract of moringa leaves inhibited the viability of acute myeloid leukemia, acute lymphoblastic leukemia, and hepatocellular carcinoma cells (Fahey, 2005). Several bioactive compounds, including 4-(α-l-rhamnosyloxy) benzyl isothiocyanate, niazimicin, and β-sitosterol-3-O-β-d-glucopyranoside present in moringa leaves, may be responsible for its anti-cancer properties (Nambiar et al., 2010). The objective of this study is to determine the effect of blending ratios and baking temperatures on nutrient composition and sensory properties of biscuits made from blends of wheat flour, pigeon pea flour, and moringa leaf flour

Objectives of the Study

The general objective of the study was to determine the effect of blending ratios and baking temperatures on nutrient composition and sensory properties of biscuits made from blends of wheat flour, pigeon pea flour, and moringa leaf flour.

Specific Objectives

The specific objectives of this study were to;

1. determine the effect of blending ratio and baking temperature on the proximate composition (moisture, carbohydrate, fat, protein, crude fiber, and ash) of biscuit samples made from

wheat, pigeon pea, and moringa leaves.
2. determine the effect of blending ratio and baking temperature on the micronutrient (zinc, iron, vitamin C, and pro-vitamin A) contents of biscuit samples
3. compare the sensory attributes of the biscuit samples made from different blending ratios and baking temperatures.

Materials and Methods

Study Design:
The study design used was quasi-experimental. The research design employed in this study will be quasi-experimental. The quasi-experimental method is suitable for this study because the study aims to compare various parameters between samples without random assignment to treatment (Thomas, 2020).

Procurement of Raw materials
One kilogram (1kg) of white seeds of pigeon pea (*Cajanus cajan*) and processed wheat flour was obtained from Ogige market in Nsukka Local Government Area, Enugu state. The fresh leaves of moringa were obtained from a farm in Opi in Nsukka. Other materials such as margarine, sugar, baking powder, eggs, milk, salt, and flavour were also purchased from the same market.

Production of Pigeon pea flour: The pigeon pea flour was prepared by modifying the method of Okorie and Okoro in 2009. The pigeon pea seeds were sorted and made free from dirt or other foreign particles such as sticks, stones, and leaves. The seeds were thoroughly cleaned and boiled in liters of potable water at 100 ∘ C for about 15 minutes. The partially cooked seeds were drained, cooled, and de-hulled manually; this was done by rubbing them in between palms to remove the hulls. The de-hulled seeds were spread on the trays and sun-dried for some days. The dried seeds were milled in a locally fabricated attrition mill and sieved through a 300 μm mesh sieve. The flour that was produced was packaged in an airtight plastic container, labeled, and stored at room temperature until when needed for further use (Okorie and Okoro, 2009).

Production of moringa leaf flour: One kilogram of freshly plucked moringa leaves to get any extra dirt/dust off. Let the washed leaves dry for a few days using the shade drying method. Collect the dried moringa leaves into bowls or onto a sheet. Place leaves in blender or grinder until the desired consistency is achieved. The grounded leaves will be sieved using a 300 μm mesh size sieve to obtain fine flour which is packaged in an airtight plastic container, labeled, and stored for further use (Makkar et al., 2016).

Table 1: Sample Description According to Blending Ratios and Baking Temperature

Ratios	T1 (180°c)	T2 (200°c)
100:0:0 = Control	CnlT1	CnlT2
80:15:5 = 80% wheat, 15% pigeon pea, 5% moringa.	WPM1T1	WPM1T2
70:20:10 = 70% wheat, 20% pigeon pea, 10% moringa.	M2T1	WPM2T2

Biscuit production

Recipe of biscuit

The modified recipe by FAO, (2014) adopted after preliminary experimentation was as follows: 250g soft wheat flour, which was replaced by the blending ratios in use, 65g margarine, 7g baking powder, a pinch of salt, one (1) medium-sized egg, 20g sugar, 15g milk or water. This recipe yielded a minimum of 25 medium size biscuits.

Method of preparation

Sieve the flour through a 1mm sieve to remove lumps and impurities and incorporate air into the flour.

Mixing and beating: The ingredients were well mixed to form a dough that was kneaded to make a smooth finish. The dry ingredients are mixed first before pouring in the wet ingredients.

Roll and cut: Biscuit dough was rolled out to about 3-4mm thickness. Shaped cutters were used to cut the biscuit into different shapes.

Baking: Biscuits were baked in a hot oven (for 180°c and 200°c) for 15 to 20 minutes. The biscuit is allowed to cool and then readied for sensory evaluation, physics properties, and proximate analysis (Suleiman et al., 2013)

Chemical Analysis: Analysis of biscuits from blends of wheat, pigeon pea, and moringa flour.

The biscuits were analyzed for their proximate composition, micronutrient content, and sensory properties.

Determination of proximate composition of biscuit sample

Determination of moisture content

Moisture content was determined according to the methods of AOAC (2010). Stainless steel oven dishes were cleaned and dried in the oven at 100°C for 1 hour to achieve a constant weight. The dishes were then cooled in a desiccator and weighed, (W_1). Two grams (2g) of the sample was placed in each dish and weighed, W_2. The samples in the dishes were dried in the oven at 105°C for 24hours until constant weight was achieved. The dishes together with the sample were cooled in a desiccator and weighed, W_3.

$$\%\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where W_1= Weight of dish; W_2 = Weight of dish+ sample before it was dried and W_3 = Weight of dish + sample after it was dried.

Determination of crude protein

The crude content of the sample was determined using Kjeldhal method according to the method of AOAC (2010). The samples were digested, distilled, and titrated.

(a) **Digestion of samples**: Two grams (2g) of the samples were weighed into the kjeldhal flask. There was an addition of 25ml of concentrated tetraoxosulphate (IV) acid (conc H_2SO_4) which was done with a few boiling chips. The flask with the content was heated in the fume chamber until sample solution becomes clear. The solution was then cooled at room temperature after which it was transferred into 250ml volumetric flask and was made up to 100ml with distilled water.

(b) **Distillation**: The distillation unit will be cleaned and the apparatus set up. A 100 ml conical flask (receiving flask) that contained 5 ml of 2% boric acid solution with a few drops of methyl red.

(c) **Titration:** The indicator was placed under the condenser, then, 5 ml of the sample digest was pipetted into the apparatus through a small funnel and washed down with distilled water. The digestion flask was heated until distillate (aluminum sulphite) remains 100 ml to be collected in the receiving flask. The solution in the receiving flask was then titrated with 0.049M H_2SO_4 to pink colored end point. A blank filter paper also was subjected to the same procedure.

Calculation:

$$\% \text{ Nitrogen sample } (\%) = \frac{V_S - V_B \times M \text{ acid} \times 0.01401 \times 100}{W}$$

Where: V_S = Volume (ml) of acid required to titrate the sample; V_B= Volume (ml) of acid required to titrate the blank; M acid= Molarity of acid (0.1M); W= Weight of sample in gram.
%Crude protein =% N x 6.25 (conversion factor).

Determination of ash content

The ash content of the samples was determined according to the method of AOAC (2010). Empty crucibles were cleaned, heated, cooled, and weighed, W_1. Two grams (2g) of the sample were put into the preheated cooled crucibles and weighed, W_2. The samples were charred in a Bunsen flame inside a fume cupboard. The charred samples were transferred into a preheated muffle furnace at 600°C for 6 hours until a white light grey ash was obtained. The samples was then cooled in a desiccator and weighed, W_3. The ash content will be calculated as follows:

$$\%\text{Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where W_1= Weight of empty crucibles; W_2= Weight of crucible + Weight of sample before ashing and W_3= Weight of crucible + Weight of sample after ashing.

Determination of crude fiber content

The crude fiber content of the samples was determined using the standard method of AOAC (2010). N-Hexane was used to de-fat 2g of the sample. This was added in oiled 200ml of 1.25% H_2SO_4 and boiled for 30 minutes. The solutions were filtered through linen or muslin cloth on a fluted funnel. The samples were further washed with 1% HCL and boiling water, to free the samples of hydrochloric acid. The residues were returned to 200ml boiling NaOH and allowed for 30 minutes. The final residues were drained and transferred to a silica ash crucible (porcelain crucible), dried in the oven at 100°C for 2 hours, and cooled until a constant weight was obtained. The cooled samples were weighed and incinerated (ashed) in a muffle furnace at 600°C for 5 hours, cooled in a desiccator, and weighed.

Calculation

% Crude fiber =

$$\frac{\text{Loss of weight after ignition}}{\text{Weight of original sample}} \times \frac{100}{1}$$

Determination of fat content

The crude fat content of the sample was determined using Soxhlet extraction method as described by AOAC (2010). The sample underwent three phases: boiling phase, rinsing phase, and sample extraction. The soxhlet extractor was fixed with a reflux condenser and a 500 ml round-bottomed flask. The samples were folded in filter paper and placed within labeled thimbles. The extraction thimble was sealed with cotton wool. Two grams of sample were weighed accurately and placed inside an extraction thimble. Thimbles were attached to the extraction columns of the fat analyzer. The thimble was placed inside the soxhlet extractor, and the extractor flask was filled with 300 ml petroleum ether which was connected to the soxhlet unit and then to the condenser. The apparatus was allowed to reflux for about 6 hours after which the thimble was removed with care and the petroleum ether recovered. The oil collected from the flask was dried at 105°C for one hour in the hot air oven. The extracted oil was cooled and weighed, and the fat percentage was calculated as

Calculation:

$$\%Fat = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of carbohydrate content

The carbohydrate content of the sample was determined as nitrogen-free extraction calculation by difference as described by AOAC (2010) using the formula below:

% Carbohydrate =100 - (moisture% + protein% + ash% + crude fiber% + fat%)

Determination Selected Micronutrients

Determination of iron

The iron content of the sample was obtained using the AOAC 2010 method. An aliquot of ash solution (10 ml) was pipetted into 250 ml volumetric flask, and 1 ml hydroxylamine hydrochloride was added. After 5 min, 5 ml buffer solution and 1 ml o-phenanthroline solution or 2 ml of dipyridyl solution were added and diluted to volume. The absorbance of the solution at 510 nm was determined. The iron (Fe) content present in aliquot of ash solution was determined from absorbance reading by taking reference to the standard curve (AOAC 2010).

Determination of zinc

The AOAC (2010), method was used. One gram (1g) of the sample was used to make an aliquot of the sample solution which was diluted with 0.4M HCl to obtain a solution containing 0.5Ug/ml of zinc. The atomic absorption spectrometer was adjusted to the steady zero and the same suitable maximum readings were obtained with the zinc working standard solutions containing 0.5Ug/ml of zinc. Each diluted solution was nebulized, and the galvanometer reading was noted.

$$\% \text{Zinc} = A/B \times C/D \times 100/1$$

Where A = Quantity of zinc in aliquot of ash solution

B = Aliquot of ash solution taken for determination

C = Total volume of ash solution

D= weight of the sample taken for ashing

Determination of vitamin C

The AOAC (2010) method was used. Five grams (5g) of the sample was added to 60ml of the extraction solution and the mixture was homogenized with an electric high-speed homogenizer. The mixture was filtered under suction. The filtrate was poured into 250ml volumetric flask and made up to 100ml with distilled water. 10ml of the resulting solution was pipetted into a conical flask and titrated against the standard indophenols solution and the titre value (Y) was recorded.

Determination of pro-vitamin A
The method to use was Pearson (1976) as modified by AOAC (2010). 2ml of the sample was put into a film container and 20ml of petroleum ether was added. The solution was filtered through Whatman filter paper No 42. The filtrate was evaporated to dryness, after which it was dissolved with 0.2ml of chloroform acetic anhydride, 2ml of orthophosphoric acid was added and read at 620nm using a spectrophotometer.

Sensory Evaluation
Sensory evaluation of the biscuits was conducted using randomly selected fifteen panelists from among the students in the Department of Nutrition and Dietetics, University of Nigeria Nsukka. These panelists were familiar with the quality attributes of biscuits. The sensory evaluation was done around 10 am when the panelist was neither hungry nor full. The samples were coded and presented in identical containers to avoid biased judgment from the panelists. Room temperature clean water was provided to serve as plate cleanser to prevent carryover effects. A nine-point Hedonic scale was used, where 9 is "extremely like" and 1 is "extremely dislike". Each of the samples was rated for color, appearance, flavor, taste, texture, and general acceptability.

Statistical Analysis: The data was analyzed using Statistical Package for Service Solution (IBM-SPSS, version 26.0). Descriptive statistics such as means and standard deviation were used. Analysis of variance (ANOVA) was used to test for differences between means. Mean separation was done using Duncan's New Multiple Range Test (DNMRT). Significance was accepted at (P≤ 0.05).

Results

Proximate Composition of Biscuit
Table 2 shows the proximate composition of biscuits produced from wheat flour, pigeon pea flour, and moringa leaf flour blends. Protein content (%) ranged from 9.81 - 14.71%, while ash content (%) ranged from 2.00 - 5.00, fibre content (%) ranged from 0.40 - 10.00, fat content (%) ranged from 6.00 - 10.00, moisture content (%) ranged from 10.00 - 22.00 and carbohydrate content (%) ranged from 49.39 - 65.89. Control samples CnlT2 (100% wheat flour at 200°c) and CnlT1 (100% wheat flour at 180°c) have the lowest protein content (9.9%) and (10.75%), while sample WPM1T1 (70:20:10 of Wheat flour, pigeon pea flour and moringa leaf powder at 180°c) has the highest protein (17.86%). The protein content of WPM1T2 (70:20:10 of Wheat flour, pigeon pea flour, and moringa leaf powder at 200°c) was 14.71%, WPM2T1 (80:15:5 of Wheat flour, pigeon pea flour and moringa leaf powder at 180°c) was 12.61% while WPM2T2 (80:15:5 of Wheat flour, pigeon pea flour and moringa leaf powder at 200°c) was 11.21%. Sample WPM1T1 has the highest moisture content (21.5%), while CnlT2 has the lowest (10.00%). Sample WPM1T1 has the highest ash content (4.50%), while sample CnlT2 has the lowest (2.00%). Sample WPM1T1 has the highest fiber content (9.8%), while sample CnlT2 has the lowest (0.50%). Sample WPM1T1 has the highest fat content (9.50%), while sample CnlT2 has

the lowest (6.00%). Sample CnlT2 has the highest carbohydrate content (66.79%), while sample WPM1T1 has the lowest (42.23%).

Table 2: Proximate composition (%) of biscuits samples

Sample code	Moisture	Ash	Crude fibre	Protein	Fat	Carbohydrate
WPM1T1	21.50±0.50[d]	4.50±0.50[a]	9.80±0.20[d]	17.86±0.00[e]	9.50±0.00[c]	42.23±0.92[a]
WPM1T2	13.50±0.50[b]	3.50±0.50[a]	8.70±0.30[c]	14.71±0.00[c]	8.00±0.17[b]	54.80±0.18[c]
WPM2T1	19.50±0.50[c]	4.50±0.50[a]	8.20±0.20[b]	12.61±0.00[c]	9.00±0.58[c]	48.25±1.42[b]
WPM2T2	12.50±0.50[b]	2.50±0.50[b]	7.80±0.20[b]	11.21±0.00[b]	8.00±0.08[b]	59.30±1.28[d]
CnlT1	11.00±0.00[a]	3.50±0.50[a]	0.60±0.00[a]	10.75±0.00[a]	6.50±0.00[a]	60.54±1.00[d]
CnlT2	10.00±0.00[a]	2.00±0.00[b]	0.50±0.10[a]	9.90±0.00[a]	6.00±0.00[a]	66.79±0.90[e]

Values are presented in mean ± standard deviation of n=2. Sample means on the same column with different superscripts are significantly ($p<0.05$) different

Key:
CnlT1 = 100% wheat flour at 180°c. CnlT2 = 100% wheat flour at 200°c. WPM1T1 = 70:20:10 (Wheat flour, pigeon pea flour and moringa leaf powder) at180°c. WPM1T2= 70:20:10 (Wheat flour, pigeon pea flour and moringa leaf powder)at 200°c. WPM2T1= 80:15:5 (Wheat flour, pigeon pea flour and moringa leaf powder) at 180°c. WPM2T2=80:15:5 (Wheat flour, pigeon pea flour and moringa leaf powder) at 200°c.

Micronutrient composition of the biscuit samples

Table 3 presents the nutrient levels of pro Vitamin A, Vitamin C, Iron, and Zinc indifferent samples. Vitamin A content ranges from 17.86 µg/dl to 434.04 µg/dl, with standard deviations varying from 0 to 7.1µg/dl. There are significant differences in Vitamin A levels among the different sample codes. The mean Vitamin A levels in decreasing values in samples WPM1TI (70:20:10: Wheat flour, pigeon pea flour, and moringa leaf powder at 180°c), WPM1T2 (70:20:10: Wheat flour, pigeon pea flour, and moringa leaf powder at 200°c), WPM2T1 (80:15:5: Wheat flour, pigeon pea flour, and moringa leaf powder at 180°c), and WPM2T2 (80:15:5: Wheat flour, pigeon pea flour, and moringa leaf powder at 200°c) are 235.46 µg/dl, 207.09 µg/dl, 185.82 µg/dl, and 164.54 µg/dl, respectively. All these values are significantly lower than the mean pro Vitamin A levels in the control samples, which are 434.04 µg/dl for CnlT1 and 249.65 µg/dl for CnlT2. Vitamin C content ranges from 13.76 mcg/100g to 30.96 mcg/100g. Mean Vitamin C levels in samples WPM1TI, WPM1T2, WPM2T2, and CnlT2 are 27.52 mcg/100g, 17.20 mcg/100g, 13.76 mcg/100g and 20.64 mcg/100g respectively. These values are significantly lower than the mean Vitamin C levels in the control sample CnlT1 and WPM2T1, which are both 30.96 mcg/100g. Iron content ranges from 0.77 mg to 1.82 mg, with standard deviations varying from 0 to 0.08 mg. There are variations in iron levels among the biscuit formulations. The mean Iron levels in samples WPM1TI, WPM1T2, WPM2T1, and WPM2T2 are 1.82 mg/100g, 1.65 mg/100g, 1.47 mg/100g, and 1.39 mg/100g, respectively. All these values are significantly higher than the mean Iron

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levels in the control samples, which are 0.95 mg/100g for CnlT1 and 0.77 mg/100g for CnlT2. Zinc content ranges from 0.3 mg to 4.58 mg, with standard deviations varying from 0 to 0.33 mg. The zinc content varies across the different biscuit formulations. The mean Zinc levels in samples WPM1TI, WPM1T2, WPM2T1, and WPM2T2 are 4.58 mg/100ml, 1.96 mg/100ml, 3.59 mg/100ml, and 1.28 mg/100ml, respectively. All these values are significantly higher than the mean Zinc levels in the control samples, which are 0.40 mg/100ml for CnlT1 and 0.30 mg/100ml for CnlT2.

Table 3: Micronutrient composition of the biscuit samples

Sample code	Zinc	Iron	Pro Vitamin A	Vitamin C
WPM1T1	4.58±0.00[e]	1.82±0.00[e]	233.46±0.00[d]	27.52±0.00[d]
WPM1T2	1.96±0.00[c]	1.65±0.00[d]	207.09±0.00[c]	17.20±0.00[b]
WPM2T1	3.59±0.33[d]	1.47±0.00[c]	185.81±7.10[b]	30.96±0.00[e]
WPM2T2	1.27±0.04[b]	1.38±0.08[c]	164.54±0.00[a]	13.76±0.00[a]
CnlT1	0.40±0.06[a]	0.93±0.00[b]	434.04±0.00[f]	30.96±0.00[e]
Cnlt2	0.30±0.04[a]	0.77±0.00[a]	249.65±0.00[e]	20.64±0.00[c]

Values are presented in mean ± standard deviation of n=2. Sample means on the same column with different superscripts are significantly ($p<0.05$) different

Key:
CnlT1 = 100% wheat flour at 180°c
CnlT2 = 100% wheat flour at 200°c
WPM1T1 = 70:20:10 (Wheat flour, pigeon pea flour and moringa leaf powder) at180°c
WPM1T2= 70:20:10 (Wheat flour, pigeon pea flour and moringa leaf powder)at 200°c
WPM2T1= 80:15:5 (Wheat flour, pigeon pea flour and moringa leaf powder) at 180°c
WPM2T2=80:15:5 (Wheat flour, pigeon pea flour and moringa leaf powder) at 200°c

Sensory properties of the samples

In Table 4 below, the sensory properties of the samples (WPM1TI, WPM1T2, WPM2T1, WPM2T2, CnlT1, and CnlT2) are presented and analyzed. Each sample was evaluated for taste, color, appearance, flavor, texture, and general acceptability, and the mean scores along with the standard deviations are provided. The findings indicate that there are significant differences in sensory properties among the samples. Sample WPM2T2 (80:15:5 of Wheat flour, pigeon pea flour and moringa leaf powder at 200°c) showed the highest scores in taste, color, appearance, flavor, texture, and general acceptability, indicating that it was well-liked in all aspects. The control sample CnlT1 (100% wheat flour at 180°c) had the lowest scores in most attributes, suggesting it was less preferred in terms of taste, color, appearance, flavor, and texture. The standard deviations show the degree of variability within each sample group, indicating how consistent or varied the sensory responses were for each attribute.

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Table 4: Sensory properties of the samples

Sample	Taste	Color	Appearance	Flavor	Texture	General acceptability
WPM1TI	2.75±1.05[a]	3.25±2.71[a]	3.25±2.01[a]	3.75±1.03[a]	3.10±1.01[a]	3.25±2.71[a]
WPM1T2	3.75±2.04[a]	2.85±1.21[a]	3.15±2.06[a]	2.95±2.79[a]	2.95±1.68[a]	3.75±1.68[a]
WPM2T2	5.65±2.21[a]	5.45±1.12[a]	4.75±1.99[a]	4.30±2.79[a]	5.65±1.76[a]	5.75±2.78[a]
WPM2T1	3.15±1.42[a]	2.75±1.27[a]	3.25±2.06[a]	3.95±1.05[a]	3.15±1.20[a]	3.75±1.44[a]
CnlT2	3.55±1.90[a]	2.35±1.46[a]	3.60±1.90[a]	4.20±2.16[a]	4.05±1.07[a]	4.20±2.26[a]
CnlT1	2.70±1.92[a]	2.00±1.69[a]	2.00±1.90[a]	2.75±1.20[a]	2.00±1.96[a]	2.75±1.53[a]

Data represent mean ± standard deviation of four replicates, values with "a" superscript are statistically significant ($p<0.05$)

Key:
CnlT1 = 100% wheat flour at 180°c
CnlT2 = 100% wheat flour at 200°c
WPM1T1 = 70:20:10 (Wheat flour, pigeon pea flour and moringa leaf powder) at180°c
WPM1T2= 70:20:10 (Wheat flour, pigeon pea flour and moringa leaf powder)at 200°c
WPM2T1= 80:15:5 (Wheat flour, pigeon pea flour and moringa leaf powder) at 180°c
WPM2T2=80:15:5 (Wheat flour, pigeon pea flour and moringa leaf powder) at 200°c

Discussion

Proximate composition of the sample

Proximate analysis is an essential technique for assessing the nutritional composition of food products. In this study, the proximate analysis of different biscuit samples produced with varying proportions of wheat flour, pigeon pea flour, and moringa leaf flour at different temperatures was analyzed. The proximate analysis measures the amount of ash, fiber, protein, moisture, fat, and carbohydrates in the samples. The protein content (%) of the biscuit samples ranged from 9.81 - 14.71 and significantly increased as wheat was substituted with pigeon pea and moringa leaves in the samples. The protein content of the biscuits decreased as the oven temperature increased from 180°c to 200°c. This signified that the addition of pigeon pea flour increased the protein content of the biscuits. The amino acid profile of pigeon pea seed proteins is similar to that of soybeans, with about 43 wt. % of the total amino acids being essential amino acids. The presence of valine, leucine, isoleucine, glutamic acid, phenylalanine, and lysine stands out. The contents of these last three amino acids were found to be higher in pigeon peas than in chickpeas; however, it is low in methionine and cysteine, two sulfur-rich amino acids (Locali-Pereira et al, 2023). The observed increase in the protein content of the samples is not surprising as pigeon pea had been reported to be a rich source of protein (Torres et al., 2007; Adebowale & Maliki, 2001). Furthermore, m*oringa oleifera* leaf dry matter content of protein is usually as high as approximately 20% or more (Peñalver et al., 2022). Therefore, *Moringa oleifera* leaf is one of the most well-known and important sources of plant protein.

An increase in moringa leaf flour addition and an increase in pigeon pea levels led to a significant increase in the ash content of the biscuit samples. The ash content of the biscuit samples increased as moringa leaf flour addition increased. This could be because moringa leaf flour contains high ash contents, so as its addition was increased, the ash content of the biscuit samples increased. The high ash content of the biscuits is an indication that they are a good source of minerals (Eneche, 1999). Fibre content (%) of the control biscuit samples (ranging from 0.50 - 0.60) was the lowest, while the composite samples (ranging from 7.80 - 9.80) were higher. Sultana (2020) recorded a similar crude fiber content of *Moringa* leaves (6.00–9.60%). Increasing the oven temperature from 180°c to 200°c showed a significant decreasing effect on protein, crude fiber and ash content of the biscuits made from the two composite flour. The addition of pigeon pea flour and moringa leaf flour has shown a significant increasing effect on protein and crude fiber content of the biscuits made from the two composite flour.

Further findings showed that the addition of pigeon pea flour and moringa leaf flour has a significantly increasing effect on the fat content of the biscuit samples when compared to the control sample. The fat content of the biscuits increased as the proportion of pigeon pea flour increased. This is in line with reports that pigeon peas are relatively low in oil content but higher than wheat (Onu and Okongwu, 2006). Moisture content (%) of the biscuit decreased as the baking temperature increased from 180^{0}C to 200^{0}C. This was reflected in the finding that samples baked at a higher temperature had a lower level of moisture content. The addition of pigeon pea flour and moringa leaf powder caused a significant increase in the moisture content of the biscuit samples. This increase could be due to the high moisture content of pigeon pea flour since the pigeon pea was soaked in water for hours for easy dehulling before drying and blending it into flour. The moisture content of the wheat-pigeon-moringa biscuits was comparable with reports on the moisture content of biscuits. Moisture contents above 10% are likely to affect the storage stability of the products (Echendu et al., 2008; Okpala and Okoli, 2011).

The carbohydrate content (%) of the biscuit samples ranged from 42.23 - 66.79. There was a significant decrease in the carbohydrate content of the biscuit samples when compared to the control sample and a decrease among the samples baked at 180°c. Substituting wheat with pigeon pea and moringa leaf led to a decrease in the carbohydrate content range of 42.23% to 59.5%. These biscuits are appropriate snacks for diabetes patients. Lower-carbohydrate (20%) diet improves glycaemia control than higher-carbohydrate diet (Boden et al, 2005). Lowering carbohydrates to 45% of total calories significantly reduced blood glucose among type 2 diabetes patients studied. Carbohydrate restriction to 45% could be recommended for optimal glycaemic control among type 2 diabetes patients (Nnadi et al, 2012). Low carbohydrate foods may lead to improvements in markers of glycaemic control such as insulin, C-peptide, and leptin. Low-quality carbohydrates may be associated with increased fullness and satiety (Nnadi, 2021).

Vitamins and Minerals contained in the samples

The mineral composition of biscuits produced from wheat flour, pigeon pea flour, and moringa leaf flour was shown. The zinc (mcg) and iron (mcg) composition of the biscuit samples ranged from 0.30 - 4.58 and 0.77 - 1.82 respectively. The addition of pigeon pea flour and moringa leaf flour led to a significant increase in zinc and iron. When compared to the control sample, pigeon pea is a good source of minerals such as phosphorus, magnesium, zinc, iron, calcium, sulfur, and potassium but low in sodium. Zinc is also associated with proteins and plays an important role in lowering the rate of protein synthesis in zinc deficiency (Yashona et al., 2018). *Moringa* administration has measurable beneficial effects that are comparable to those of iron supplementation (Rotella et al., 2023). Vitamin C (mg) and Vitamin A (IU) composition of the biscuit samples range from 13.76 - 30.96 and 434.04 - 164.54 respectively. The control sample baked at 180°c and the sample with a reduction in pigeon pea and moringa leaf powder baked at 180°c significantly showed increased vitamin C content which indicates the loss of vitamin C to high temperatures. Vitamin C is a water-soluble and temperature-sensitive vitamin, so it is easily degraded during cooking, and elevated temperatures and long cooking times have been found to cause particularly severe losses of vitamin C (Tian et al., 2016). The control samples showed a higher content of vitamin A than the composite flour samples.

Sensory properties of the samples

The study explores the sensory properties of various biscuit samples produced with different proportions of wheat flour, pigeon pea flour, and moringa leaf flour at different baking temperatures. The sensory evaluation involved attributes such as taste, color, appearance, flavor, texture, and general acceptability. In the study, the biscuit sample made from 80% Wheat flour, 15% pigeon pea flour, and 5% moringa leaf powder and baked at 200°c, emerged as the most favored sample, exhibiting the highest mean scores in all sensory attributes, including taste, color, appearance, flavor, texture, and general acceptability. This result suggests that the formulation involving 80% wheat flour, 15% pigeon pea flour, and 5% moringa leaf flour at a baking temperature of 200°C was well-received by the sensory panel. On the other hand, the control sample made from 100% wheat flour and baked at a higher temperature of 200°c, received the lowest scores in most attributes. These findings imply that the addition of alternative flours increased the sensory characteristics while an increase in baking temperature decreased the sensory attributes of the biscuits.

The outcomes align with the study of the sensory properties of multi-grain cookies with varying proportions of flours by Yamsaengsung, et al. (2012) who reported that cookies with a higher proportion of alternative flour, such as chickpea and quinoa flour, demonstrated improved sensory attributes compared to control samples made with only wheat flour. This corroborates with the current study's observations, where samples containing pigeon pea flour and moringa leaf flour exhibited enhanced sensory characteristics in comparison to the wheat flour-based controls. Furthermore, the study conducted by Chopra et al. (2018) examined the sensory properties of cookies produced at different baking temperatures and found that higher baking temperatures led to improved sensory attributes in terms of flavor and texture. This corroborates with the findings of this study as biscuits made from different blends and baked at a higher temperature, received higher scores in flavor and texture.

Conclusion

The study has shown that nutritionally enriched and acceptable biscuits could be produced from the partial substitution of wheat with pigeon pea flour and moringa leaf flour. The incorporation of pigeon pea flour and moringa leaf flour will help to produce biscuits rich in nutrients, especially protein, vitamins, and minerals such as vitamin A, zinc, and iron. The study also shows that biscuits baked at 180°c had more nutrient content as some of the nutrients were lost to high heat, especially the water-soluble vitamin but the biscuit baked at 200°c has less moisture content. The biscuit samples made with a combination of flours and baked at 200°C, received the highest scores for taste, color, appearance, flavor, texture, and general acceptability. In contrast, the blended sample baked at 180°C, had relatively lower sensory attributes. Further studies should be carried out on the storage stability of biscuits baked at 180°c and 200°c to ascertain the possible shelf life of the biscuits.

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