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Evaluation of Storage Stability and Quality Parameters in Biscuit Made from Blends of Wheat, Cassava (*Manihot esculenta*) and Carrot *(Daucus carota)* Flour

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***Abstract*—**Biscuit is one of the most consumed cereal foods in Nigeria and research has shown that locally available tropical crops like cassava, sweet potato can be made into flour and used in the production of biscuits and other pastries. This study investigates some quality parameters in biscuits made from blends of wheat, cassava and carrot flour. The values of result of samples increased with increasing percentage substitution of cassava and carrot flour in some quality parameter like fiber, ash, gluten content, and carbohydrate. The protein content reduced significantly ($P < 0.05$) with increasing percentage substitution of cassava and carrot flour which ranged from 14.80% to 11.80% compared with the control sample which had 15.60%. There was a recorded significant increase ($P < 0.05$) in some mineral composition such as calcium, magnesium, sodium, iron, phosphorus, and vitamin A and C composition as the percentage substitution of cassava and carrot flour increased. During storage stability test, samples stored in the fridge and freezer were found to be the best storage location to preserve the sensory attributes and inhibit microbial growth when compared with storage under the sun and on the shelf. Biscuit made with blends of wheat, cassava and carrot flour can therefore serve as an alternative to biscuits made from 100% wheat flour, as they are richer in vitamin A, vitamin C, carbohydrate, dietary fiber and some essential minerals.

***Keywords*—**Biscuit, carrot, flour blends, storage.

I. Introduction

BISCUIT is one of the oldest commonly consumed non fermented baked snacks [1]. Snacks are referred to as convenience foods which can be eaten in between meals. Most snacks are generally cereal based and the most common cereal used in producing snacks is the wheat grain. Reports showed that the use of 100% wheat flour as the major ingredient for preparing snacks generally tends to result into products high in calories and fat but low in proteins, vitamins and other nutrients [2], [3]. Baked products such as bread, breakfast cereals and particularly biscuits can be considered a convenient vehicle for the addition of micronutrients and protein to meet these consumer health demands [4]. Biscuits are baked dry products usually with a golden brown crust and crispy [5]. Wheat flour is generally used for biscuit production with other ingredients such as margarine, sugar, leavening agent, eggs, milk, salt, and flavors [6]. Recently, the use of composite flour has evolved in the bakery world for cake and biscuits. Composite flour is the name given to combination of two or more types of flour in specific ratio for baking [7]. Both rupturing of dough during sheet and fragility of biscuit samples from composite flour could be reduced by slight modification of the recipe [8]. The availability of our local crops, their reduction in post-harvest losses through processing of some into flour and the cost of importation of wheat flour, led to the use of wheat flour [9].

Biscuits are high in carbohydrates, fat and calories, but low in protein, fiber, vitamin, and mineral which make it unhealthy for daily consumption. It is widely acceptable and consumed by all age groups in all geographical locations both in rural and urban settings; and can be eaten at all times as a result of its relatively long shelf-life, more convenience and good eating quality. Due to itswide acceptability by all age groups, it could be considered a good product for protein fortification and other nutritional improvements [10], [11]. In Nigeria, ready-to-eat baked products (snacks) consumption is continually growing and there has been increasing reliance on imported wheat [12]. Carrot is also an excellent source of calcium pectate; an extraordinary pectin fiber that has the cholesterol lowering properties. It has a property to reduce the risk of high blood pressure, stroke, heart disease and some type of cancer [13]. In recent times, consumption of carrot and its products has gained wide acceptance as a result of its natural antioxidants properties coupled with the anticancer activities of β-carotene in it which is a precursor of vitamin A [14], [15]. Vitamin A deficiency (VAD) has been reported to be one of the major public health problems in developing countries,of which Nigeria is one, hence the need to develop enriched baked products such as biscuits which are widely acceptable and consumed as snacks in order to meet the nutritional needs and improve the health of vulnerable groups [4]. This research is aimed at production and evaluation of the suitability of biscuit using wheat, cassava, and carrot composite flour and to investigate the sensory and some quality parameters of the biscuit during storage.

II. Materials and Methods

A. Materials

The cassava flour used was obtained from the Oamsal

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Nigeria Limited (ONL). Other ingredients such as wheat flour, carrot, sugar, fat, sodium bicarbonate (baking powder) and eggs were all purchased from the Ota market in Ogun State, Nigeria.

Reagents and equipment used were available in the Food Processing and Analytical Laboratories of the Chemical and Food Sciences Department of Bells University of Technology, the Central Teaching and Research Laboratory of Bells University of Technology and SMO Laboratory Services, Ibadan.

B. Methods

Preparation of Carrot Powder

The carrot fruit was washed in portable water, peeled and sliced into 56mm thickness. The sliced carrots were then blanched for 3 minutes in hot water then cooled by exposing them to air and drying them in a cabinet dryer afterwards at 50 ºC for 12 hours. The dried carrots were milled to produce carrot flour which was later cooled, sieved and packaged.

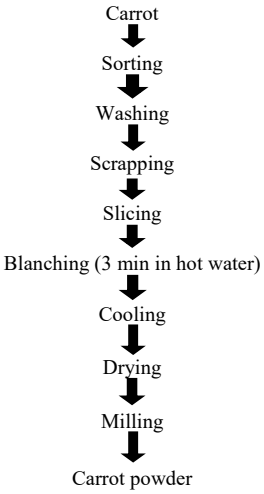

Fig. 1 Flow chart for production of carrot powder [16]

C. Flour Blends

The composite flour blends were formulated from wheat flour, cassava flour and carrot flour. The blends were prepared by mixing wheat flour with cassava flour and carrot flour in the percentage proportions of 80:10:10, 70:15:15, 60:20:20, respectively, using a food processor (Kenwood M907 D England). The control was 100% wheat flour.

D. Production of Biscuit

The composite flour after formulation was weighed out and other ingredients like fat, sugar and baking powder were mixed together with the flour till it looked like bread crumbs. The eggs were then added after this together with water to make dough. The dough was kneaded and the desired shape was cut out using a biscuit cutter and placed in the oven to bake.

E. Proximate Content Determination

Proximate content of the samples were determined using the methods of [17]. Carbohydrate was determined by difference.

TABLE I
RECIPE FOR BISCUIT PRODUCTION

Ingredients	Weight
Flour	500 g
Butter	125 g
Sugar	120 g
Baking Powder	5(1 Tea Spoon)
Egg	2 eggs
Flavor	1 Tea Spoon
Water	100 – 200 ml

Source: Food processing manual [38]

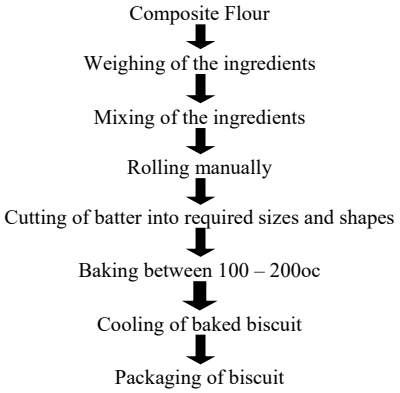

Fig. 2 Production of biscuit. Source: Food processing manual [38]

F. Mineral Content Determination

Magnesium, Calcium, Sodium, Manganese, Iron, Zinc and Copper

The dry ashing procedure was used for mineral content as described by [18]. Five gram of samples each was weighed into porcelain crucibles and pre-ashed until the samples were completely charred on a hot plate. The pre-ashed samples were thereafter ashed in a muffle furnace at 500ºC till the ash was white for 2 hours. After ashing, the crucibles were transferred into the desiccator to cool and reweigh. Each sample was then quantitatively transferred into a volumetric flask by carefully washing the crucibles with 1ml of nitric acid then with portions of nitric acid.

All washings were transferred into individual volumetric flask, repeating the washing procedure twice. The solution was diluted with deionized water and was used for individual mineral determination using the appropriate standards and blanks. The contents of the mineral were determined using an Atomic Absorption Spectrophotometer (AAS). Calculation:

$$\text{Mineral Element Concentration (\%)} = \text{ppm}/1000$$

where parts per million (ppm) of any element = Meter reading × Dilution factor

Phosphorous Determination

Phosphorus will be determined using the method described by [19].

The dry ash of each sample obtained was digested by adding 5 ml of 2 M hydrochloric acid to the ash in the crucible and heated to dryness on a heating mantle. Next, 5 ml of 2M hydrochloric acid was added again, heated to boil and filtered

through a Whatman No. 1 filter paper into a 100 ml volumetric flask. Then, 10 ml of the filtrate solution was pipetted into a 50 ml volumetric flask and 10 ml of Vanadate-molybdate yellow added and the flask was made up to mark with distilled water, stooped and left for 10 minutes for full yellow development. The concentration of the phosphorus was obtained by taking the absorbance on a spectronic 21D (Milton Roy Model Spectrophotometer.

G. Functional Properties

Water Absorption Capacity (WAC)

This was determined using the method described by [20].

Bulk Density

Bulk density was determined by using [21].

Swelling Power and Solubility

This was determined by the method described of [22].

Foam Capacity (FC) and Foam Stability (FS)

The method of [23] was used as described with slight modification. One gram (1 g) of sample was added to 50ml distilled water at 30 ± 2ºC in a graduated cylinder. The suspension was mixed and shaken for 5 minutes to foam. The volume of foam at 30 seconds after whipping was expressed as FC using the formula:

$$\text{FC (\%)} = \frac{\text{volume of foam AW} - \text{volume of foam B}}{\text{volume of foam BW}} \text{x } 100$$

where, AW = After Whipping, BW = Before Whipping.

The volume of foam was recorded one hour after whipping to determine foam stability as percentage of initial foam volume.

Gluten Content Determination

Twenty grams (20 g) of flour was weighed into a beaker and 10 ml of water was added to make a stiff dough and allowed to stand in a beaker of water. The stiff dough was removed, squeezed in the fingers and gently kneaded under a stream of running water until all the starch was washed away and water squeezed out runs out quite clear. The residue of moist gluten was squeezed as dry as possible and weighed. It was torn into tiny pieces and dried in the oven until it was properly dried and then weighed.

$$\text{\%Gluten Content} = \frac{Weight\ of\ Gluten}{Weight\ of\ original\ flour\ sample\ x\ 100}$$

H. Determination of Anti-nutritional Properties

Determination of Saponins

This was done by the double solvent extraction gravimetric method (A.O.A.C., 1990).

Twogram of the processed sample was mixed with 100 ml of 20% aqueous ethanol solution and incubation for 12 hours at a temperature of 55ºC with constant agitation. After that, the mixture was filtered through Whatman No. 42 grade of filter paper. The residue was then re-extracted with 50 ml of the ethanol solution for 30 minutes and the extracts joined together and weighed.

The new extract (40 ml) reduced due to evaporation was transferred to a separating funnel and 40 ml of diethyl ether was added to it. After mixing, the upper layer was discarded, while the lower layer was re-extracted with ether then its pH was reduced with drop-wise addition of NaOH solution to 4.5. Successive extraction with5% of NaCl solution resulted in saponin to be taken up and evaporation with a water bath in a previously weighed evaporated dish. The saponin was dried in the oven at 60ºC, cooled in a desiccator andre-weighed. The saponin content was calculated as:

$$\text{\% Saponin} = \frac{W2 - W1}{W}$$

where W= Weight of sample used, W1 = Weight of empty evaporation dish, W2 = Weight of dish + saponin extract.

Determination of Tannins

The Folin-Denis spectrophotometric method was used. The method is described by [24].

Tannin content was calculated as follows:

$$\text{\% Tannin} = \frac{\text{Au}}{\text{As}}\ x\ C\ x\ \frac{100}{W}\ x\ \frac{Vf}{Va} x\ D$$

where; Au = Absorbance of test sample, As = Absorbance of standard tannin solution, C = Concentration of standard tannin solution, W = Weight of sample used, Vf = Total volume of extract, Va = Volume of extract analysed, D = Dilution factor (if any)

Determination of Flavonoids

The total flavonoid content of crude extract was determined by the aluminum chloride colorimetric method. Crude extract (50 µL)(1 mg/ml ethanol) was made up to 1 ml with methanol, then mixed with 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$ solution; 0.3ml of 10% $AlCl_3$ solution was added after 5 min of incubation, the mixture was allowed to stand for 6 min. Solution of 1 mol/lNaOH (2 ml) was added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The absorbance was measured at 510 nm after the mixture was allowed to stand for 15 min. The total flavonoid content was calculated and the result was expressed as mg rutin equivalent per g dry weight.

Determination of Cyanide

The hydrocyanic acid of the samples was determined using the alkaline picrate method with modifications. Sample (1.0 g) was weighed out using an electronic weighing balance. This was transferred into a 100 ml volumetric flask. Ten milliliter (10 ml) of distilled water was added; mixed together, then let it stand for 30 min, and the temperature maintained at 28 ± 5°C. The supernatant was collected, and then the cyanide in it measured. Sample (1ml) was pipetted into test tube and1 ml of 0.04M picric acid and 1 ml of 0.75 M NaOH. The solution was mixed and incubated for 15 minutes at room temperature. Hydrocyanic acid (HCN) released during hydrolysis of the *Cyanogenic glycosides* reacts with picric acid to produce a

yellow colored solution. Sodium hydroxide preserves the HCN released. Color intensity was measured spectrophotometrically at the wavelength of 540 nm. The same process above was used for all other samples and the reference standard. Calculation:

$$\text{CONC HCN} = \frac{\text{O.Dtest} \times \text{CONC std Volume obtained x } 1000}{O.Dstd\ Vol.of\ sample\ used}$$

O.D STD = Vol. used, O.D = Optical Density/Absorbance, STD = standard, Conc = Concentration.

I. Microbiological Analyses

Total Bacterial Count

Microbiological analysis was carried out according to the Official Methods of Analysis [18]. A measure of 1 ml of each sample was aseptically transferred to 9 ml of sterile water in a separate tube and mixed vigorously. Next, 1ml of the resulting mixture was transferred into 9 ml of sterile water in a separate tube. The process was continued until the third diluent (10^{-3}). Nutrient Agar (NA) was inoculated with a 0.1 ml of appropriately diluted biscuit powder (10^{-3}) by pour plate technique and incubated at 37ºC for 24 hours. Colonies were counted and multiplied by the dilution factor.

$$\text{Bacterial load (cfu/ ml)} = \frac{Nx1xD}{V}$$

where; N= Numbers of colonies, V=Volume of inoculums, D= Dilution factor.

Total Fungi Count

The fungal load was determined in a similar way as the bacteria load. However, Potato Dextrose Agar was used using pour plate technique. A measure of 1 ml of the diluent was used and incubated at 37ºC.

$$\text{Fungi load (cfu/ ml)} = \frac{Nx1xD}{V}$$

where; N= Numbers of colonies, V=Volume of inoculums, D= Dilution factor.

J. Sensory Evaluation

A 9-point hedonic scale, where 1 represents "extremely dislike" and 9 represents "extremely like", was used for this study. The organoleptic evaluation of the biscuit samples was carried out for consumer acceptance and preference using 15 semi-trained panelists; the properties to be evaluated included color, texture, aroma, taste, mouthfeel, crispiness and overall acceptability.

K. Storage Stability Test

Storage stability test was carried out on the biscuit produced from both the flour blends of wheat, cassava, carrot flour and 100% wheat flour which was the control sample. Storage stability was done by storing the biscuit sample in four different storage locations which include, under the sun, on the shelf, in the fridge and in the freezer. Sensory evaluation and microbial test for fungi and bacterial growth were carried out once a week for four weeks of storage. The days which were chosen for storage stability test were Day 0, which was the day of the biscuit production, Day 7, Day 14 and Day 21.

III. Results and Discussion

Proximate Composition of Flour Samples

The result of the proximate composition of the flour samples is presented on Table II.

TABLE II
PROXIMATE COMPOSITION OF THE FLOUR SAMPLES

SAMPLES	PROTEIN (%)	FAT (%)	FIBRE (%)	ASH (%)	MOISTURE (%)	CHO (%)
WCC1	15.60[b]	1.51[a]	0.17[a]	0.67[a]	11.11[a]	71.11[a]
WCC2	14.80[b]	0.75[a]	0.57[a]	1.93[a]	10.10[a]	72.42[a]
WCC3	12.70[ab]	1.17[a]	1.08[a]	2.09[a]	11.46[a]	72.58[a]
WCC4	11.80[a]	0.55[a]	0.92[a]	2.56[a]	11.36[a]	73.73[a]

Figures with same letters in a column are not significantly different (P > 0.05). Figures with different letters in the same column are significantly different at (P < 0.05)

LEGEND**:** CHO: Carbohydrate, WCC1: 100% Wheat flour, WCC2: 80% Wheat flour + 10% Cassava flour + 10% Carrot flour, WCC3: 70% Wheat flour + 20% Cassava flour + 20% Carrot flour, WCC4: 60% Wheat flour + 15% Cassava flour + 15% Carrot flour.

The fiber content of the flour blends ranged from 0.57 to 1.08%. The incorporation of cassava and carrot increased the fiber content of the sample. This is in agreement with the value reported by [25] who worked on the utilization of carrot pomace powder for the production of high fiber biscuit.

The ash content of the flour blends ranged from 1.93% to 2.56% as compared to wheat flour which had an ash content of 0.67%. This shows that the incorporation of cassava and carrot flour increased the amount of mineral content in the flour blend. The ash content was generally low.

The protein content of the flour samples ranges from 11.80% to 14.80% as compared to the value obtained from wheat flour which had a protein content of 15.60%. The protein content decreased as the percentage substitution level of cassava and carrot increased. Similar conclusion was reported by previous researcher [26] who worked on the utilization of cassava and wheat composite flour in the production of biscuit.

The fat content of the flour sample ranges from 0.55% to 1.17% as compared to that of wheat flour which had a fat content of 1.50%. There was no significant difference in the fat content of all the flour samples and this can attributed to the low fat content of both cassava and carrot which were incorporated into the wheat flour.

The moisture content of the flour sample ranges from 10.10% to 11.36% as compared to wheat flour which had a moisture content of 11.11%. There was no significant difference in the moisture content of all the flour samples. These values did not exceed the maximum standard of 15.5% m/m [27].

The carbohydrate content of the flour samples ranges from 72.42% to 73.73% as compared to wheat flour which had a carbohydrate content of 71.11%. The carbohydrate content increased as the percentage of cassava and carrot flour

increased and this can be said to occur because cassava and carrot are rich sources of carbohydrate. The values of the formulated biscuit are similar to values reported in earlier studies on composite biscuits [28]-[30].

Functional Properties of Flour Samples

The results of functional properties of the flour samples are presented in Table III.

TABLE III
FUNCTIONAL PROPERTIES OF THE FLOUR SAMPLES

SAMPLES	WAC (%)	BD (%)	SP (%)	Solubility (%)	FS (%)	FC (%)	Gluten (%)
WCC1	108.60^{a}	0.5795^{a}	18.3^{b}	32.75^{a}	7.40^{a}	15.60^{a}	13.40^{c}
WCC2	122.60^{c}	0.5975^{a}	12.1^{a}	37.89^{b}	6.90^{a}	14.80^{a}	11.20^{bc}
WCC3	115.70^{b}	0.6104^{a}	13.6^{a}	41.92^{c}	6.30^{a}	12.70^{a}	8.90^{ab}
WCC4	111.30^{a}	0.6184^{a}	15.2^{ab}	44.86^{c}	5.80^{a}	11.80^{a}	6.10^{a}

Figures with same letters in a column are not significantly different ($P > 0.05$). Figures with different letters in the same column are significantly different at ($P < 0.05$).

LEGEND**:** BD: Bulk Density, SP: Swelling Power.

Water absorption is the ability of flour particles to entrap large amounts of water such that exudation is prevented [30]. There was a significant difference ($P < 0.05$) in the water absorption of the flour samples with sample WCC 2 having the highest value of 122.60% and therefore has the highest affinity. There was no significance difference in the bulk densities of the varying samples. There are significance differences in the SP and solubility of the samples with WCC2 and WCC3 having no significant difference in SP and WCC3 and WCC4 having no significant difference in solubility. There is also a significance difference between the FC and the FS of the flour samples. WCC1 has both the highest value for both FS and FC and WCC4 has the least value for both FS and FC. Proteins can help the foaming because of their surface active property. Food ingredients with good foaming capacity and stability are required in bakery products [31]-[33]. The gluten content ranges from 13.40% to 6.10% with WCC 1 having the highest value and WCC4 having the least value. The gluten content reduced as the percentage of the cassava and carrot flour increased. This can be said to be because of the low gluten content of both the cassava flour and the carrot flour present in the composite flour and high gluten content of wheat flour [37].

Mineral Composition of Flour Samples

The results of the mineral composition of the flour samples are represented on the Table V. Minerals are inorganic substances necessary for maintaining good health, regulation of fluid and acid base, water balance in the body depends to a great extent on certain mineral balance in the body. Calcium is necessary for normal ossification of bones and normal nerve impulse transmission. There are significance differences in the calcium content of the composite flour sample with WCC1 having the least value of 1025 mg/kg to WCC4 having the highest value of 2775 mg/kg. The presence of cassava and carrot flour has increased the calcium content of the composite flour. Magnesium plays an important role in calcium and phosphorus metabolism in man. The values range from 1058.75 mg/kg to 2242.50 mg/kg. The addition of the composite flour increased the magnesium content of the samples except in the WCC sample.

Sodium is essential for normal functioning of the body and it plays a role in the regulation of the acid base balance and water metabolism in the body. There is a significance difference in the sodium content of the samples with values ranging from 1004.50 mg/kg to 2242.50 mg/kg. The addition of cassava and carrot flour into wheat flour increased the value of sodium content in the composite flour.

Zinc is found in cells throughout the body. It is needed for body's defensive (immune) system to properly work. It plays a role in cell division, cell growth, wound healing, and breakdown of carbohydrates. Zinc is also needed for the senses of smell and taste. The zinc content ranged from 101.60 mg/kg in WCC3 to 127.45 mg/kg in WCC2 sample.

The copper content increased and decreased with the increasing levels of cassava and carrot flours, respectively, but not significantly different. Manganese aids in the formation of connective tissue, bones, blood clotting factors, and sex hormones. The presence of cassava and carrot flour significantly increased the manganese content as compared with the control sample WCC1 which had a content of 9.75 mg/kg.

Iron is an important component of hemoglobin, the substance in red blood cells that carries oxygen from the lungs to transport throughout your body. The addition of cassava and carrot flour into the wheat flour significantly increased and decreased the iron content in the composite flour sample. Phosphorus in the body is essential for the formation of bones and teeth. There was a significant increase in the phosphorus content as the percentage composition of cassava and carrot increased. The addition of cassava and carrot flour into the wheat flour increased the phosphorus content of the composite.

Anti-Nutritional Content of Flour Samples

The results of the ant nutritional contents are presented on Table V. Saponins are glucosides with foaming characteristics. Saponin helps in cholesterol reduction, reduces cancer risk, serves as immunity boosters, reduces bone loss and also serves as an anti-oxidant. The value ranges from 0.221 mg/kg to 0.334 mg/kg, with no significant difference between them and the control sample which is WCC1. The saponin value increased slightly as the percentage of cassava and carrot flour increased. The values range from 0.0021 mg/kg to 0.0039 mg/kg. The value of tannins increases slightly with increasing percentage of cassava and carrot flour.

Cyanide prevents cells from using oxygen and eventually these cells die. The heart, the respiratory system and the central nervous system are most susceptible to cyanide poisoning. There is a significant difference ($P < 0.05$) in the values of cyanide as compared with the control sample. The value of cyanide in the samples increases with increasing percentage of cassava and carrot flour. This can be attributed to the high cyanide content of cassava flour. The cyanide

values obtained in this study were below the maximum recommended safe level of 10 mg HCN kg-1 dry weight basis [34]. The flavonoid value present in the sample ranges with no significant difference (P > 0.05) in the values. The flavonoid value increases with an increasing percentage of cassava and carrot flour.

Vitamin Composition of Flour Samples

The result of the vitamin analysis of the flour samples are presented in Table VI. The value of vitamin A content significantly increased from a value ranging from 287.30 mg/100 g in the control sample to 726.40 mg/100 g in WCC4. The value of vitamin A significantly increased with increasing percentage of cassava and carrot flour. This can be attributed to the high vitamin A content of carrot flour. A similar result was reported by previous researchers [26] who worked on the utilization of cassava and wheat composite flour in the production of biscuit.

Vitamins are essential in wound healing and forming scar tissues. The value of vitamin C content did not significantly increase (P > 0.05) but it ranges from a value of 11.69 mg/100 g in the control sample to 19.77 mg/100 g in WCC4. The value of vitamin C increased but not significantly with increasing percentage of cassava and carrot flour.

TABLE IV
MINERAL COMPOSITION OF FLOUR SAMPLES

SAMPLES	Ca (Mg/Kg)	Mg (Mg/Kg)	Na (Mg/Kg)	Zn (Mg/Kg)	Cu (Mg/Kg)	Mn (Mg/Kg)	Fe (Mg/Kg)	P (Mg/Kg)
WCC1	1025.00^{a}	1171.30^{b}	1004.50^{a}	112.85^{b}	4.00^{a}	9.75^{a}	144.50^{b}	128.69^{a}
WCC2	1975.00^{b}	2242.50^{d}	1947.50^{d}	127.45^{c}	4.25^{a}	12.50^{a}	149.50^{c}	132.48^{a}
WCC3	2600.00^{c}	1058.75^{a}	1783.52^{b}	101.60^{a}	3.50^{a}	10.75^{a}	151.25^{c}	141.64^{b}
WCC4	2775.00^{c}	2153.75^{c}	1845.02^{c}	110.40^{b}	3.51^{a}	10.75^{a}	136.00^{a}	175.70^{c}

Figures with same letters in a column are not significantly different (P > 0.05). Figures with different letters in the same column are significantly different at (P > 0.05).

LEGEND: Ca: Calcium; Mg: Magnesium; Na: Sodium; Zn: Zinc; Cu: Copper; Mn: Manganese; Fe: Iron; P: Phosphorus.

TABLE V
ANTI-NUTRITIONAL PROPERTIES OF THE FLOUR SAMPLES

SAMPLES	Saponin (Mg/Kg)	Tannin (Mg/Kg)	Cyanide (Mg/Kg)	Flavonoid (Mg/Kg)
WCC1	0.221^{a}	0.0021^{a}	2.66^{a}	0.016^{a}
WCC2	0.296^{a}	0.0026^{a}	2.84^{b}	0.025^{ab}
WCC3	0.329^{a}	0.0034^{a}	3.92^{c}	0.037^{bc}
WCC4	0.334^{a}	0.0039^{a}	4.37^{d}	0.049^{c}

Figures with same letters in a column are not significantly different (P > 0.05). Figures with different letters in the same column are significantly different at (P < 0.05).

TABLE VI
VITAMIN COMPOSITION OF FLOUR SAMPLES

SAMPLES	Vitamin A (mg/100g)	Vitamin C(mg/100g)
WCC1	287.30^{a}	11.69^{a}
WCC2	526.50^{b}	14.62^{a}
WCC3	714.60^{c}	18.88^{b}
WCC4	726.40^{d}	19.77^{b}

Figures with same letters in a column are not significantly different (P > 0.05). Figures with different letters in the same column are significantly different at (P < 0.05).

Storage Stability and Acceptability of the Flour Samples

Storage Stability and Acceptability Test (Week 1)

The result for sensory evaluation is presented on Table VII.A. All the samples scored high value in the attributes they were evaluated for at week one. Sample A and sample B had the highest values and their values were closely related, especially in attributes like texture, taste, mouthfeel and overall acceptability. A similar result was reported by [34] who also found no significant difference in the sensory values obtained from his control sample and biscuit samples incorporated with 4% and 8% carrot pomace.

Sample B scored the highest value for color showing it that it was more preferable than the control sample which is sample A. The result for color was significantly different in all the samples.

Sample A scored the highest value in texture but was not significantly different from sample B. This shows that the texture of both sample A and B were the most preferable. Sample C and sample D also had values that were not significantly different from each other.

Sample A with a value of 8.89 had the highest value rating for Aroma. The results for the evaluation of the aroma attributes were significantly different from each other except in sample B and sample C with values that were closely related. There was no significant difference between values of sample A and sample B in the result for taste attribute. Sample C and sample D also had values that were not significantly different from each other.

Sample B had the highest value for the mouthfeel rating which shows that it was preferred than sample A but the values of sample A and sample B were not significantly different from each other. Samples C and D followed with values less significantly different from each other and the control sample. The values for crispiness were significantly different from each other. Sample A had the highest value and the value for each sample decreased as the percentage of cassava and carrot flour in the flour sample increased.

For the overall acceptability, sample A was the most preferably with a value of 8.22 which was closely followed and was also not significantly different from sample B with a value of 8.21. Sample C came third in the rating and sample D forth. The overall acceptability reduced with increasing percentage of cassava and carrot flour.

Cassava flour and carrot flour and similar sensory characteristics was reported by [35] who also noticed decrease

in general sensory properties with increasing substitution level of blended flour.

Storage Stability and Acceptability Test (Week 2)

From the result of the week 2 storage stability test (Table VII.B), it was observed that sample A, which is the control sample, had the highest ratings in most sensory attributes and sample B had the next highest rating in most attributes after sample A. Sample D had the least values for storage stability in most attributes when compared with those of sample A, sample B and sample C.

Samples that were stored in the fridge had the highest value rating in all attributes in color, texture, aroma, taste, mouthfeel and overall acceptability. It was also observed that samples stored in the freezer had the second highest rating in all sensory attributes.

TABLE VII.A
STORAGE STABILITY AND ACCEPTABILITY TEST (WEEK 1)

Samples	Color	Texture	Aroma	Taste	Mouthfeel	Crispiness	Overall acceptability
A	8.72[b]	8.14[a]	8.89[a]	8.33[a]	7.78[a]	8.42[a]	8.22[a]
B	9.69[a]	7.93[a]	8.12[b]	8.11[a]	8.09[a]	7.39[b]	8.21[a]
C	7.27[c]	7.34[c]	7.71[b]	7.61[c]	7.22[c]	6.10[c]	7.43[c]
D	6.46[d]	6.97[c]	7.20[c]	7.30[c]	6.41[d]	5.61[d]	6.72[d]

TABLE VII.B
STORAGE STABILITY AND ACCEPTABILITY TEST (WEEK 2)

Samples	Color	Texture	Aroma	Taste	Mouthfeel	Crispiness	Overall acceptability
A/Sun	7.02[a]	6.87a	7.91[a]	7.11[a]	6.72[a]	7.22[a]	7.04[a]
A/Shelf	7.84[b]	7.43[b]	8.14[b]	8.20[c]	7.11[b]	7.85[b]	7.67[d]
A/Fridge	8.06[d]	7.70[d]	8.23[b]	8.38[c]	7.84[d]	8.00[b]	8.33[d]
A/Freezer	7.76[b]	7.15[b]	8.06[b]	7.72[b]	7.25[b]	8.12[b]	8.14[d]
B/Sun	7.19[a]	6.98[b]	6.71[a]	5.32[a]	6.93[a]	7.14[a]	7.34[a]
B/Shelf	7.11[a]	6.78[b]	7.48[b]	7.14[c]	6.89[a]	7.37[a]	7.23[a]
B/Fridge	7.67[a]	7.07[d]	7.80[b]	7.13[c]	7.47[c]	7.40[a]	7.87[a]
B/Freezer	7.43[a]	6.10[a]	8.70[d]	6.84[b]	7.27[c]	7.29[a]	7.77[a]
C/Sun	7.14[a]	6.89[a]	6.92[a]	6.71[a]	6.49[a]	6.04[b]	6.80[a]
C/Shelf	7.21[a]	7.11[c]	7.63[c]	6.92[a]	6.84[a]	6.29[b]	6.78[a]
C/Fridge	7.40[a]	7.13[c]	6.53[a]	6.83[a]	6.98[a]	5.21[a]	6.98[a]
C/Freezer	7.39[a]	7.09[a]	7.77[c]	6.31[a]	6.72[a]	6.92[b]	6.82[a]
D/Sun	6.42[a]	6.01[a]	6.47[a]	5.39[d]	6.49[c]	5.22[a]	5.13[a]
D/Shelf	6.69[a]	6.29[a]	6.69[a]	6.52[a]	6.31[c]	5.75[a]	5.16[a]
D/Fridge	6.72[a]	6.26[a]	6.92[a]	6.61[a]	6.01[a]	6.54[d]	6.36[d]
D/Freezer	6.88[a]	6.11[a]	6.84[a]	6.11[a]	5.99[a]	5.80[a]	5.71[c]

TABLE VII.C
STORAGE STABILITY AND ACCEPTABILITY TEST (WEEK 3)

Samples	Color	Texture	Aroma	Taste	Mouthfeel	Crispiness	Overall acceptability
A/Sun	6.71[b]	6.24[a]	6.98[b]	6.04[a]	6.20[b]	6.09[a]	5.96[a]
A/Shelf	6.52[a]	6.63[a]	6.25[a]	6.32[b]	6.19[b]	6.27[b]	6.39[b]
A/Fridge	6.83[b]	6.35[a]	7.55[d]	6.72[d]	6.31[b]	7.33[d]	6.98[c]
A/Freezer	6.74[b]	6.41[a]	7.11[b]	6.54[b]	6.15[a]	7.16[c]	7.22[d]
B/Sun	6.33[b]	5.02[a]	5.32[a]	5.52[b]	5.98[a]	6.19[b]	5.42[a]
B/Shelf	5.49[a]	6.13[b]	6.55[b]	5.43[a]	5.43[a]	6.11[a]	5.55[a]
B/Fridge	6.67[c]	6.27[b]	6.90[b]	6.73[c]	6.43[b]	6.30[b]	6.78[c]
B/Freezer	6.53[c]	6.25[b]	6.73[b]	6.25[c]	6.11[b]	6.29[b]	6.75[c]
C/Sun	6.38[a]	6.17[a]	5.15[a]	5.25[b]	5.26[b]	4.98[a]	5.20[a]
C/Shelf	6.49[a]	6.24[b]	5.38[b]	5.57[b]	5.21[a]	5.14[b]	5.30[b]
C/Fridge	6.90[c]	6.54[b]	6.53[c]	5.83[d]	5.98[d]	5.21[b]	5.98[d]
C/Freezer	6.87[c]	6.33[b]	6.49[c]	5.20[a]	5.38[b]	5.23[b]	5.40[c]
D/Sun	5.02[a]	5.20[a]	5.32[a]	5.10[a]	5.69[a]	5.00[a]	5.21[a]
D/Shelf	5.30[b]	5.60[b]	5.52[b]	5.29[b]	5.70[b]	5.29[b]	5.43[b]
D/Fridge	5.70[c]	5.69[b]	5.70[d]	5.41[b]	5.80[b]	5.36[b]	5.68[c]
D/Freezer	5.79[c]	5.49[b]	5.50[b]	5.32[b]	5.78[b]	5.10[b]	5.87[d]

TABLE VII.D
STORAGE STABILITY AND ACCEPTABILITY TEST (WEEK 4)

Samples	Color	Texture	Aroma	Taste	Mouthfeel	Crispiness	Overall acceptability
A/Sun	3.21[a]	3.84[a]	4.12[a]	4.24[b]	4.37[b]	4.45[b]	3.90[a]
A/Shelf	4.18[b]	4.41[b]	4.84[b]	3.91[a]	3.29[a]	3.65[a]	3.84[a]
A/Fridge	5.84[c]	5.92[c]	5.76[c]	5.32[c]	5.11[d]	5.00[d]	5.59[c]
A/Freezer	5.24[c]	5.33[c]	5.66[c]	5.00[c]	4.84[c]	4.54[b]	5.32[c]
B/Sun	3.95[a]	3.69[a]	4.62[a]	3.55[b]	4.32[b]	4.29[b]	3.79[a]
B/Shelf	4.07[b]	3.74[b]	4.78[b]	3.75[b]	3.54[a]	3.88[a]	3.53[a]
B/Fridge	5.12[c]	4.98[c]	5.00[c]	5.09[d]	4.84[c]	4.57[b]	4.79[c]
B/Freezer	5.00[c]	4.82[c]	4.95[c]	4.91[a]	4.72[c]	4.47[b]	4.26[c]
C/Sun	3.24[a]	3.84[a]	3.74[c]	4.09[b]	4.14[b]	3.27[b]	3.55[a]
C/Shelf	4.19[b]	3.40[b]	2.95[a]	3.16[a]	3.20[a]	3.15[b]	3.16[a]
C/Fridge	4.20[b]	4.85[c]	4.15[d]	4.21[c]	4.30[d]	4.01[d]	4.20[c]
C/Freezer	4.01[b]	4.39[c]	3.27[b]	4.11[c]	4.24[b]	3.04[a]	4.32[c]
D/Sun	2.96[a]	3.14[a]	3.00[a]	3.24[a]	3.11[a]	2.64[a]	3.15[a]
D/Shelf	3.64[b]	3.28[a]	3.21[c]	3.42[b]	3.09[a]	2.93[a]	3.05[a]
D/Fridge	3.76[b]	3.28[a]	4.11[d]	3.54[b]	3.15[a]	3.05[a]	3.00[a]
D/Freezer	3.46[b]	3.12[a]	3.02[a]	3.47[b]	3.07[a]	2.54[a]	3.34[a]

Figures with same letters in a column are not significantly different ($P > 0.05$). Figures with different letters in the same column are significantly different at ($P > 0.05$).

LEGEND: A: 100% Wheat flour, B: 80% Wheat flour + 10% Cassava flour + 10% Carrot flour, C: 70% Wheat flour + 20% Cassava flour + 20% Carrot flour, D: 60% Wheat flour + 15% Cassava flour + 15% Carrot flour.

Storage Stability and Acceptability Test (Week 3)

The result of week 3 sensory evaluation (Table VII.C) shows that sample A stored in the fridge had the highest value rating in color, aroma, taste, mouthfeel and crispiness. Sample A stored in the freezer was closely related to sample A stored in the fridge and scored higher values in texture and overall acceptability. The values of sample A stored on the freezer and those stored in the fridge are not significantly different and these samples scored the highest value in the week 3 sensory evaluation but had lower values compared with the results of week 1. Samples B had the next highest value rating after the control sample which is sample A. Sample D scored the least as compared with the control sample.

Storage Stability and Acceptability Test (Week 4)

From the week 4 result of the sensory and storage stability test, it is seen that the values of each attributes at each storage condition are at the lowest here when compared with the values of week 1, week 2 and week 3. The values of sample A stored on the shelf had the highest number of value rating most especially in color, taste, mouthfeel and crispiness. Sample B stored in the fridge was closely related and scored high in texture, aroma and overall acceptability. The sample A stored in the fridge and on the shelf scored the highest as compared with other samples stored in various storage locations. Sample B scored the second highest in storage stability for week 4 when compared with sample A which is the control sample. Sample D scored the least in storage stability at the various storage location when compared with the values of the highest rating which is sample A. Reference [35] also recorded a decrease in general sensory attributes of composite biscuits over a period of days ranging from day 0 to day 20 to day 40 and then to day 60.

Microbiological Studies of Biscuits Made from the Flour Blends

The total viable count indicated the microbiological quality of any food product and the presence of a high number of total viable counts is an indication for low expected shelf life of the product. Microorganisms play a significant role in the determination of shelf lives of food products. They are usually responsible for the spoilage of many food items. A high aerobic plate count could indicate the presence mixed population of microorganisms, which may consist of spoilage types [36].

Week 1 result for microbial analysis during storage stability (Table VIII.A) shows that there was no growth both in the nutrient agar plates for bacteria count and in the potato dextrose agar plate for fungi count. The absence of microbial can be attributed to the Good Manufacturing Practice (GMP) during the production of the biscuit and the heat involved in its production. A similar conclusion was reported by [35] who concluded that it was an indication that the cookies were prepared under good hygienic condition and the integrity of the packaging material used was not compromised.

Week 2 result for microbial analysis during storage depicts that total viable count of the biscuits are within the microbial limit of 10^4 to less than 10^6cfu/g of ready to eat food product [37]. The microbial load for fungi ranged from 0 to 0.8×10^3 and that of bacterial ranged from 0 to 1.3×10^2. All the samples stored in the various storage locations had few microbial count but the samples stored in the fridge and freezer had slightly less bacteria and fungi count than those stored under the sun and on the shelf.

The week 3 result for microbial analysis during storage stability showed an increase in the microbial counts of both fungi and bacteria plates. The bacteria count ranged from 1.5×10^2 to 2.5×10^4 and the fungi count ranged from 0.1×10^2 to 2.9×10^3. During this week, the result showed that the

microbial load is still within the acceptable limit [37].

The week 4 result for the microbial analysis during the storage stability test also showed that the microbial range was still within the required microbial limit. The microbial growth for bacteria ranged from 2.6×10^2 in sample D stored in the fridge to 1.3×10^4 in sample C stored in the fridge, while that of fungi ranged from 1.2×10^2 in sample B stored in the fridge to 1.8×10^4 in sample C stored on the shelf.

TABLE VIII.A
MICROBIAL COUNT OF BISCUIT SAMPLES DURING STORAGE STABILITY (WEEK 1)

Samples	Bacteria count (cfu/g)	Fungi count (cfu/g)
A	0	0
B	0	0
C	0	0
D	0	0

TABLE VIII.B
MICROBIAL COUNT OF BISCUIT SAMPLES DURING STORAGE STABILITY TEST (WEEK 2)

Sample/Storage	Bacteria count (cfu/g)	Fungi count (cfu/g)
A/Sun	0.3×10^2	0.1×10^3
A/Shelf	0.3×10^3	0.2×10^2
A/Fridge	0.1×10^2	0.1×10^2
A/Freezer	0.2×10^2	0
B/Sun	0.1×10^2	0.1×10^2
B/Shelf	0.2×10^4	0.1×10^2
B/Fridge	0.1×10^2	0
B/Freezer	0	0.1×10^2
C/Sun	1.3×10^2	0.8×10^3
C/Shelf	0.2×10^3	0.3×10^2
C/Fridge	0.1×10^2	0.1×10^2
C/Freezer	0.3×10^2	0.2×10^2
D/Sun	0.6×10^2	0.3×10^3
D/Shelf	0.9×10^3	0.1×10^2
D/Fridge	0.1×10^2	0.1×10^2
D/Freezer	0.3×10^3	0.1×10^2

TABLE VIII.C
MICROBIAL COUNT OF BISCUIT SAMPLES DURING STORAGE STABILITY TEST (WEEK 3)

Sample/Storage	Bacteria count (cfu/g)	Fungi count (cfu/g)
A/Sun	3.3×10^2	1.4×10^2
A/Shelf	3.7×10^2	1.5×10^3
A/Fridge	2.3×10^3	1.1×10^2
A/Freezer	1.9×10^2	1.0×10^2
B/Sun	3.6×10^2	2.0×10^2
B/Shelf	3.2×10^3	2.0×10^2
B/Fridge	2.7×10^2	1.8×10^2
B/Freezer	1.0×10^3	1.2×10^2
C/Sun	3.7×10^3	4.1×10^2
C/Shelf	3.5×10^3	2.5×10^3
C/Fridge	1.5×10^2	1.4×10^2
C/Freezer	2.7×10^2	1.1×10^3
D/Sun	2.5×10^4	3.4×10^2
D/Shelf	3.9×10^3	2.9×10^3
D/Fridge	2.0×10^2	1.4×10^2
D/Freezer	1.8×10^2	1.0×10^2

TABLE VIII.D
MICROBIAL COUNT OF BISCUIT SAMPLES DURING STORAGE STABILITY (WEEK 4)

Sample/Storage	Bacteria count (cfu/g)	Fungi count (cfu/g)
A/Sun	7.2×10^2	1.5×10^3
A/Shelf	5.6×10^2	1.2×10^3
A/Fridge	3.9×10^2	1.3×10^3
A/Freezer	2.8×10^3	1.0×10^3
B/Sun	4.8×10^2	1.3×10^2
B/Shelf	5.7×10^3	2.1×10^2
B/Fridge	3.7×10^2	1.2×10^2
B/Freezer	3.2×10^2	2.3×10^2
C/Sun	7.0×10^2	5.0×10^2
C/Shelf	6.7×10^2	1.8×10^4
C/Fridge	1.3×10^4	1.5×10^2
C/Freezer	2.9×10^2	2.3×10^2
D/Sun	6.4×10^2	4.4×10^2
D/Shelf	5.0×10^2	2.9×10^2
D/Fridge	2.6×10^2	1.3×10^3
D/Freezer	3.0×10^3	2.7×10^2

LEGEND: A: 100% Wheat flour, B: 80% Wheat flour + 10% Cassava flour + 10% Carrot flour, C: 70% Wheat flour + 20% Cassava flour + 20% Carrot flour, D: 60% Wheat flour + 15% Cassava flour + 15% Carrot flour.

In general, the microbial load of samples stored in the fridge and freezer in all the weeks of storage was found to be lower than the remaining storage condition.

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