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Quality Evaluation of Bread Produced from Fermented Cowpea Bran and Wheat Flour Blends

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Abstract

The research evaluated wheat and cowpea bran flour (FCBF) bread. Cowpea bran was fermented with 5% saccharomyces cerevisae for 24 h dried and milled to powder. The fermented cowpea bran was incorporated into wheat flour at varying ratios. Functional attributes (flour blends) while chemical composition, physical and sensory studies were investigated on the products. Nutritional analysis revealed significant increases ($p<0.05$) in protein, lipids, crude fiber, and ash composition across the flour blend formulations from 11.34 to 13.06, 3.31 to 3.34, 2.56 to 2.71 and 8.78 to 8.81%, respectively, while carbohydrate content decreased from 67.87 to 65.05% with an increase in added content of fermented cowpea bran. The effects were generally significant, $p\leq0.05$. Carotenoids, flavonoids and phenols content increased from 6.17 to 8.20, 12.16 to 18.73 and 39.38 to 49.64 mg/100g, respectively. DPPH, FRAP and ABTS content increased from 9.47 to 12.77, 4.31 to 8.28 and 12.42 to 21.39, respectively as FCBF levels increased. Dietary fiber values of composite flours are 6.56 to 9.68 (SDF), 21.38 to 23.42 (IDF) and 27.95 to 33.10g/100g (TDF), respectively with an increase in levels of FCBF. The functional properties analysis including oil absorption capacity (OAC), water absorption capacity (WAC), swelling capacity, with foaming capacity values increased from 1.25 to 1.75 g/ml, 1.75 to 2.00 g/ml, 1.20 to 1.25 g/ml and 0.11 to 0.15 g/ml, respectively while bulk density (BD) reduced from 0.75 to 0.62g/ml with increasing FCBF inclusion level. Loaf weight reduced while volume and volume-to-weight ratio also decreased significantly ($p<0.05$) index as FCBF increased. The bread sample containing 5% FCBF was not significantly different from the 100% wheat bread in terms of sensory attributes. The study revealed that the supplementation of FCBF enhanced the functional characteristics and nutrient composition of the composite bread and the sample containing 5% FCBF was the most preferred.

Keywords: *Quality evaluation, Bread, Fermented cowpea bran, Wheat flour blends*

1.0 INTRODUCTION

Bread is a fundamental food item that is created by baking a mixture of flour and water [1]. The primary ingredient used in bread-making is wheat flour, which contains gluten that imparts the unique viscoelastic characteristics of dough that define the texture of various breads [1]. Despite the beneficial qualities of wheat flour that make it ideal for bread production, studies indicate that it has a lower content of beneficial bioactive compounds such as vitamins, β-carotene, polyphenols, dietary fiber, and flavonoids [2]. Refined wheat bread is also noted to have insufficient levels of essential amino acids like lysine and threonine. Furthermore, certain negative reactions are linked to the consumption of gluten-containing foods (gluten protein), particularly in individuals with celiac disease. Global efforts have been undertaken to investigate alternative high-quality flours that could either partially or fully substitute wheat flour in the creation of wheat flour-based products [2]. Legumes, such as cowpeas, have garnered attention to enhance the quality of bread.

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop that was first discovered in Africa and has since domesticated in South America and Southeast Asia [3]. Cowpea belongs to the Fabaceae family, specifically the sub-family Phaeseolinae, within the Vigna family. The cultivated cowpeas are derived from the *V. unguiculata* subspecies *unguiculata* [4]. This legume is currently cultivated globally, with a significant presence in tropical regions [5]. Cowpea plays a crucial role in food security and contributes positively to the living standards of farmers. It supplies vital calories and protein to the diet [4]. Cowpea addresses the issue of decreasing protein consumption, which has been linked to the limited availability and high costs of animal protein sources, including milk, eggs, meat, and fish.

In the food industry, cowpea grains are used in the production of canned beans and isolated proteins, with various applications such as additives, supplements and functional foods [6] [7]. It is used as an ingredient to enhance gluten-free baked goods such cookies, cheese bread, and cereal bars. Additionally, it can be utilized as a raw ingredient in recipes for pizza dough and other baked goods [8]. It is also used traditionally as a cooked legume and in the production of *moi moi* (a steamed pudding) and *akara* (a fried pudding) [8]. During the industrial processing of cowpea to obtain the highlighted products large volume of wastes is generated, comprising mostly

the bran [9]. The bran consists of the pericarp, the seed coat, and the aleurone layer [10]. The main nutrient found in bran is dietary fiber, which is mostly insoluble, but it also contains notable amounts of soluble fibers like arabinoxylan and beta-glucan. The bran layers are also a principal source of phytochemicals and boost the antioxidant activity of the grains [8]. Cowpea brans are also rich in proteins, vitamins, minerals and bioactive compounds [11]. Due to its inherent nutritional and biofunctional properties, cowpea bran has potential in food product development.

Wheat is deficient in protein and some essential compounds such as phytochemicals, dietary fiber and antioxidants that cowpea bran incorporated in bread can improve the nutrient content of bread. Bread production is dependent on wheat which contains gluten that affects patients with gluten intolerance and celiac disease. In spite of high bio-functional and nutritional properties of cowpea bran, its utilization is low due to certain inherent limitations such as poor techno-functional properties, due to high fibre content, prolonged cooking, beany flavor and high content of antinutrients [11]. Fermentation helps break down cellulose present in bran which is complex needs to be broken down for easy digestibility, reduces antinutrients and improves texture of bran [12].

Incorporating cowpea bran into bread may enhance the protein and antioxidant levels of the bread, as it has been identified as a substantial source of nutrients and phytoactive compounds all of which improve the health benefits and enhance antioxidant activity. Components of cowpea bran (oryzanol) help to decrease cholesterol levels in the bloodstream, resulting in lesser low-density lipoproteins (LDL) and improved high-density lipoproteins (HDL). It is also known to support cardiovascular health. The fermentation of cowpea bran is crucial for increasing the bioavailability of its phenolics by releasing insoluble bound phenolics, reducing antinutrients, shortening cooking time, minimizing beany flavor, and enhancing its techno-functional properties [13]. *Saccharomyces cerevisiae* produces a variety of enzymes such as β-glucosidases, carboxylesterases, and possibly feruloyl esterases, which decompose the complex cellulose in bran for better digestibility, reduced antinutrients, and improved texture of the bran [12]. This study investigates how *Saccharomyces cerevisiae* fermentation modifies the nutritional profile, chemical composition, and functional properties of cowpea bran.

The broad aim of the study is to assess the quality of bread produced from fermented cowpea bran and wheat flour blends.

2.0 MATERIALS AND METHODS

2.1 Materials

Cowpea, the microorganism (*Saccharomyces cerevisiae*), Wheat flour (Golden Penny Plc., Lagos, Nigeria), Margarine (Simas Limited, Kano, Nigeria), salt, and sugar, potable water, and instant dry yeast (STK Royals) were all procured at New Market, Wukari LGA, Taraba State, Nigeria.

2.1.1 Fermentation of cowpea bran with *saccharomyces cerevisiae*

Fermentation treatment with *saccharomyces cerevisae* was done by subjecting cowpea bran to solid-state fermentation as performed by Chen *et al.* [14].

Cowpea bran (250 g) was added to a beaker containing 500 mL of distilled water and mixed manually (2 min). *Saccharomyces cerevisiae* (12.5 g) was added and fermented at 30 ºC for 24 h. Fermentation was terminated using a microwave oven and the fermented cowpea bran was dried for 48 h at 32 ºC. The samples were milled and sieved with 250 µm sieve to get fermented cowpea bran flour.

2.1.2 Preparation of wheat flour and fermented cowpea bran flour blends

Fermented cowpea bran flour was substituted into wheat flour (0, 5, 10, 15, 20 and 25 %) to produce flour blends.

2.1.3 Production of bread

The straight-dough technique was employed for bread production (Method 10-10.3, [51]) with some modification in fermentation/proofing time. The bread ingredients to be used include sugar (21.0 g), fat (10.5 g), dry yeast (4.5 g), and salt (3.0 g). The dry ingredients were manually mixed. Water (180 ml) was added to wheat and fermented cowpea bran flour blends (300.0 g) and carefully mixed to form dough. The bread samples were left to rest for 30 min at 30 °C (first proofing). After first proofing, the dough was kneaded back to expel carbon dioxide and moulded into baking pans and allowed to rest for some time (30 min) at 30 °C (second proofing). The dough was baked at 150 °C for 45 min. The obtained samples were allowed to cool and stored properly prior to subsequent analysis.

2.2 Analytical Methods

2.2.1 Functional properties of fermented cowpea bran and wheat flour blends determination

Bulk density: The analysis was conducted according to the standardized protocol of Adebowale et al. [15]. Briefly, 10 g aliquots of flour blends were transferred to 25 mL graduated cylinders. For packed density measurements, cylinders were tapped ten times from a consistent 5-8 cm elevation to achieve compaction, while loose density measurements omitted this step. Volumetric readings were converted to g/mL, with final calculations performed using Equation 3.1."

$$pb = \frac{mass\ of\ flour}{volume\ of\ cylinder} \quad (3.1)$$

Water absorption capacity (WAC): Water absorption capacity was quantified through a modified Adebowale et al. procedure [15]. Sample-water mixtures (1:10 w/v) were vortexed (5 min), centrifuged (3,500 × g, 30 min), and the unabsorbed water fraction measured volumetrically. Absorption values were derived from volume differentials (Equation 3.2).

$$\text{WAC (mL/g)} = \frac{m2}{m1} \quad (3.2)$$

Oil absorption capacity (OAC): Oil binding capacity was quantified according to Adebowale et al. [16] with modifications. Sample-oil mixtures (1:10 w/v) were vortexed (5 min), centrifuged (3,500 × g, 30 min), and free oil volume determined gravimetrically. Absorption values were derived from volume differences (Equation 3.3).

$$\text{OAC (mL/g)} = \frac{m2}{m1} \quad (3.3)$$

Swelling capacity: Volumetric swelling capacity was assessed by modifying Chinma et al.'s protocol [17]. Pre-weighed samples (20 g) in graduated cylinders were

lightly compacted (3 taps), hydrated with distilled water (80 mL), and volumetrically measured after 60 min. The swelling index was expressed as the volume ratio (hydrated:dry).

Foam capacity (FC): The FC of the flour mixtures was assessed following the procedure outlined by Chinma et al. [17]. A quantity of three (3) grams of flour was placed into a 50 ml graduated test tube (Oven dried at 50 °C). The flour was evened out, and the initial volume (V_0) was logged. Then, 30 ml of distilled water was introduced to aid in the flour's dispersion within the test tube, and the value was documented as the volume before homogenization. The test tube containing the dispersion was mixed manually and the new volume was measured as volume after homogenization. The test tube was set aside until the foam settled, and at regular intervals (every 10 minutes), the volume of the foam was measured by subtracting the volume before homogenization from the volume after homogenization. The FC and SF of the flours were calculated using Equations 3.4 and 3.5, respectively.

$$FC\ (\%) = \frac{Vol\ after\ homogenization - Vol\ before\ homogenization}{Vol\ before\ homogenization} \text{ eqn (3.4)}$$

SF = eqn (3.5)

2.2.2 Determination of the chemical composition of bread produced fermented cowpea bran flour and wheat flour blends

2.2.2.1 The proximate composition, including moisture, crude fat, crude protein, ash, crude fiber, and carbohydrate content, was determined following the standard methods outlined by AOAC [18] method

2.2.2.2 Phytochemical composition determination

Total Phenolic Content (TPC) Analysis

Total phenolic content (TPC) was determined using a modified *Folin-Ciocalteu* method as described by Ali et al. [19]. Briefly, 1 g of the sample was homogenized with 1 mL of methanol. An aliquot of 0.5 mL from this extract was mixed with 0.5 mL of *Folin-Ciocalteu* reagent, followed by intermittent manual shaking at 15–20-second intervals. After 3 minutes, 1 mL of saturated sodium carbonate solution and 1 mL of distilled water were added to the reaction mixture. The samples were then incubated in the dark for 2 hours. Absorbance was recorded at 725 nm using a spectrophotometer, with deionized water serving as the blank. TPC was quantified using a gallic acid standard calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g), as outlined in Equation 3.6.

$$TPC = \frac{Volume\ of\ extract\ used \times Concentration\ of\ calibrated\ curve}{Mass\ of\ extract\ used} \text{ eqn (3.6)}$$

Determination of flavonoids

Total flavonoid content (TFC) was quantified using the aluminum chloride colorimetric method, following a modified protocol from Shoib and Shahid [20]. Briefly, 1 mL of ethanolic crude extract (1 mg/mL) was prepared in methanol and mixed with 4 mL of deionized water and 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution. After 5 minutes of reaction at room temperature (25 ± 2°C), 0.3 mL of 10% aluminum chloride ($AlCl_3$) solution was added, followed by a 6-minute incubation. Subsequently, 2 mL of 1 M sodium hydroxide (NaOH) solution was added, and the volume was adjusted to 10 mL with distilled water. Absorbance was measured at 510 nm using a UV-Vis spectrophotometer, with suitable blank corrections. Quantification was achieved by comparing against a standard calibration curve (Equation 3.7), with results expressed as milligram catechin equivalents per gram of sample (mg CE/g)

$X = (A/A_0) \times (m_0/m) \times$ conversion factor (3.7)

Where:

A = Measured absorbance of the plant extract solution

A_0 = Absorbance value of the quercetin standard solution

m = Mass (in mg) of the crude plant extract analyzed

m_0 = Mass (in mg) of the quercetin standard used

The conversion factor accounts for any necessary unit adjustments or dilution factors applied during sample preparation.

Total carotenoids: Total carotenoid was measured as done by Ali *et al.*, [19]. Acetone-water mixture (4:1 VV) was used as a solvent. The absorbance (UV/AVIS spectrometer T60U, Leicestershire, UK) maxima was read at 470m. The total carotenoid content was calculated using Equation 3.8.

$$Total\ carotenoid\ (ug/ml) = \frac{1000A_{470} - 2.27\ (chl\ a) - 81.4(chl\ b)}{227} \text{ (3.8)}$$

2.2.2.3 Determination of Antioxidant activity

FRAP determination: A 0.1 g sample was mixed with 1.0 mL of pre-cooled extraction solution and shaken for 30 minutes after thorough homogenization. The mixture was centrifuged at 10,000 × g for 5 minutes at 4°C, and the resulting supernatant was carefully collected. A 30 µL aliquot of the supernatant was diluted with 90 µL of distilled water and adjusted to a final volume of 900 µL using the assay buffer. Following a 10-minute incubation at room temperature (25 ± 1°C), absorbance was recorded at 593 nm using a microplate reader. A standard curve was prepared using freshly prepared $FeSO_4 \cdot 7H_2O$ solutions (0–1000 µM), as described by Cong-Cong et al. (2021). The ferric reducing antioxidant power (FRAP) was calculated and expressed as µmol Fe^{2+} equivalents per gram of sample (µmol/g), based on the linear regression equation (Eq. 3.9) from the standard curve.

$$FRAP\ (\mu mol/g) = X \times V_{total}\left({}^{V}/_{V}\ extract \times W\right) = 34 \times {}^{X}/_{W} \text{ (3.9)}$$

DPPH scavenging activity determination: A 0.1 g test portion was homogenized with 1.0 mL of extraction solvent [specify solvent if applicable] using vortex mixing. The homogenate underwent 30 min incubation at 40°C in a temperature-controlled water bath. Following incubation, samples were centrifuged at 10,000 × g for 10 min at 25°C to separate particulates. From the clarified supernatant, a 10 µL aliquot was reacted with 190 µL of 0.1 mM DPPH methanolic solution (final volume 200 µL) in amber microtubes. The reaction proceeded for 30 min under dark conditions at ambient temperature (25 ± 2°C). Absorbance was measured at 515 nm using a UV-Vis spectrophotometer, with methanol serving as blank. Radical scavenging activity (%) was calculated according to Equation 3.10 as described by Cong-Cong et al. (2021), with modifications for sample matrix.

$$DPPH\ scavenging\ activity\ (\%) = A\ Blank - \frac{A\ Supernatant - A\ Control}{A\ Blank} \times 100\ \%\ (3.10)$$

ABTS+ scavenging activity determination: A 0.1 g sample was mixed with 1.0 mL of extraction buffer and incubated at 40°C for 30 minutes. Following incubation, the mixture was centrifuged at 10,000 rpm for 10 minutes at 25°C. Next, 50 µL of the supernatant was added to 850 µL of working solution and 100 µL of buffer solution, followed by a 6-minute incubation in the dark at room temperature.

Absorbance was measured at 405 nm, and ABTS+ scavenging activity was calculated using Equation 3.11.

$$ABTS + \ scavenging\ activity\ = A\ Blank - \frac{A\ Supernatant - A\ Control}{A\ Blank} \times 100\ \% \quad (3.11)$$

2.2.2.4 Determination of Dietary fiber composition

Dietary fiber content was determined using the enzymatic–gravimetric method as outlined by Zheng et al. [21], employing the Total Dietary Fibre Assay Kit (Megazyme Ltd., Wicklow, Ireland). Briefly, 0.5 g of the sample was homogenized in 40 mL of MES-TRIS buffer. To initiate starch hydrolysis, 5 mL of α-amylase was added, and the mixture was incubated at 50°C for 15 minutes with continuous shaking at 30 rpm in a thermostatic water bath. Subsequently, 10 mL of protease was introduced, and digestion was continued at 30°C under the same agitation conditions.

After enzymatic digestion, the mixture was cooled to 10°C, and the pH was adjusted to 4.1–4.8 using 5 mL of 0.561 N HCl. This was followed by the addition of 20 mL of amyloglucosidase, with further incubation at 30°C for 15 minutes. The solution was then subjected to phase separation. Insoluble dietary fiber (IDF) was recovered through initial filtration. The resulting filtrate was mixed with 95% ethanol, heated to 65°C, and left to stand for 1 hour. A second filtration was performed, and the residue obtained was quantified as soluble dietary fiber (SDF).

2.2.3 Physical properties of bread produced from fermented cowpea bran and wheat flour blends

Determination of loaf weight: Thirty minutes after baking, the loaf weight was determined using an analytical balance (CE-410I, Camry Emperors, China), and the results were documented in grams [22].

Determination of loaf volume: This was measured using the rape seed displacement technique outlined by Ayo et al., [22]. This involved filling a calibrated container with 3000 ml of millet grains up to the designated level and then emptying it; the bread sample was subsequently placed in the pan, and the millet returned, with the excess millet grains values collected as loaf volume in cm^3.

Determination of loaf volume index: The volume-to-weight ratio of each loaf was determined using Equation 3.12, where the sample's volume (cm^3) was divided by its mass (g), as described in the protocol established by Ayo et al. [22].

$$volume\ index = \frac{volume\ of\ loaf\ sample}{weight\ of\ loaf\ sample} \quad (3.12)$$

2.2.4 Sensory evaluation of bread produced from wheat flour and fermented cowpea bran flour blends

Twenty semi-trained panelists (6 male, 14 female) aged 25–28 years were randomly selected from the Department of Food Science and Technology at Federal University Wukari, Taraba State, comprising both staff and students. Participants assessed multiple bread samples, including a control, to rate color, aroma, mouthfeel, and general preference, following the methodology of Olaoye and Obideqwe (2018). Evaluations used a 9-point Hedonic scale (1 = "dislike extremely," 9 = "like extremely"). Between samples, panelists cleansed their palates with drinking water. The study took place in a standardized sensory lab with optimal lighting and airflow to minimize external influences.

2.2.5 Statistical Analysis

All results are presented as mean ± standard deviation (SD) based on duplicate measurements. Statistical differences among groups were assessed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post-hoc comparisons. Differences were considered statistically significant at $p < 0.05$. All analyses were performed using SPSS software (Version 2.3).

3.0 RESULTS AND DISCUSSION

3.1 Functional Properties of Bread Produced from Fermented Cowpea Bran Flour and Wheat Flour Blends

Table 1 displays the functional characteristics of composite flour mixtures containing wheat flour supplemented with fermented cowpea bran. The OAC, WAC, swelling capacity and FC, increased from 1.25 to 1.75 g/ml, 1.75 to 2.00 g/ml, 1.20 to 1.25 g/ml and 0.11 to 0.15 g/ml, respectively while bulk density reduced from 0.75 to 0.62 g/ml with increasing FCBF inclusion level.

The BD, OAC, WAC, FC, and swelling capacity all differed significantly ($p<0.05$). A food material's application and final use are determined by its functional attributes [23]. The functional properties of food substances are influenced by the quality attributes of their macromolecules—such as proteins, starches, carbohydrates, sugars, fibers, and fats—which in turn affect their utilization and potential for various industrial applications [24]. Due to their influence on the product's textural property, Bhat and Yahya [25] assert that the functional qualities of legume and cereal flour are highly significant factors to take into account when developing food products [26].

The findings of the OAC revealed a significant increase with the increase in the substitution of WF with FCBF. The lowest OAC of (1.25 g/ml) was observed in sample B (95% WF and 5% FCBF) while the highest oil absorption capacity (1.75 g/ml) was recorded in sample F (25% FCBF). Statistical analysis revealed significant variations ($p < 0.05$) among the composite flour formulations. This result disagrees with data reported by Ubbor *et al*., **[27]** who examined the physical-chemical properties and sensory attributes of cookies formulated with blended flours (wheat, Bambara groundnut, and orange-fleshed sweet potato) was conducted. Findings from this present study suggests that fermentation of cowpea bran which was carried out significantly improved the OAC of the flour blends. The heightened oil absorption can be linked to the availability of more hydrophobic proteins, which demonstrate a greater lipid binding ability [28].

The WAC improved with an increase in FCBF inclusion, higher water absorption capacity was recorded in sample F (25% FCBF) as compared to the controls. This finding concurs with that of Ihembe *et al*. [29] where WAC increases significantly ($p<0.05$) with protein content. he water absorption capacity (WAC) of flour is significantly affected by the concentration and properties of water-soluble components, particularly proteins, as well as pH levels. This characteristic reflects the flour's ability to bind water in low-moisture environments, such as in dough or paste formulations. WAC is particularly important in ready-to-eat (RTE) food production, as enhanced water retention capacity improves product integrity and cohesion [29].

Additionally, it has been indicated that WAC is vital for the bulking density and texture of food products.

Foaming capacity measures the ability of flour to create foam, which relies on the flexible protein molecules that lower the surface tension of water [30]. Proteins have been reported to enhance foam formation [31][52] and hence, the highest foaming capacity recorded in sample F (25% FCBF) could be attributed to its high flour protein content. The foams play a crucial role in various processes within the food and beverage industries, leading to a growing interest in their studies and formulations. Foams enhance the sensory property of food products [32]. Flour with good foam capacity is a valued quality in the food system due to its high porosity, which is essential for creating a range of baked goods such as cakes, muffins, and akara, while also serving as functional agents in other food formulations [33].

The ability of flour granules to swell shows the strength of the associative forces within them and their capacity to absorb water [29]. An increase in the swelling index may result from strong forces between the wheat and fermented cowpea bran flour, along with a decrease in the carbohydrate composition of the composite flours [29]. The swelling capacity of flours is influenced by several factors such as particle size, variety type, and the processing methods or unit operations used. As the level of substitution increases, the swelling capacity of the composite flour also increases. Bulk density is influenced by the interplay of several factors, including the strength of attractive inter-particle forces, particle size, and the number of contact points between particles. The results regarding bulk density showed a gradual decline as the amount of wheat flour was substituted with fermented cowpea bran flour, with the lowest bulk density (0.44 g/ml) recorded in sample G (5% UFCBF). The findings related to the bulk density of wheat and fermented cowpea bran composite flours support the conclusions made by Zhang et al. [34], who stated that pre-treatment reduces the bulk density of foods. Assessing the bulk density of the blends is crucial for meeting consumer expectations regarding package fullness and shipping considerations [35].

Table 1: Functional properties of fermented cowpea bran flour and wheat flour blends

Samples	Oil absorption capacity(g/ml)	Water absorption capacity (g/ml)	Swelling capacity (g/ml)	Foam capacity (g/ml)	Bulk density (g/ml)
A	1.25^{g}±0.35	1.75^{g}±0.35	1.17^{g}±0.03	0.07^{g}±0.01	0.76^{g}±0.00
B	1.25^{g}±0.35	1.75^{g}±0.35	1.20^{gf}±0.00	0.11^{fe}±0.01	0.75^{g}±0.00
C	1.35^{g}±0.35	1.75^{g}±0.35	1.21^{fe}±0.00	0.11^{fe}±0.00	0.74^{g}±0.00
D	1.75^{g}±0.35	1.75^{g}±0.35	1.23 ^{e}±0.00	0.12^{e}±0.00	0.66^{g}±0.00
E	1.75^{g}±0.35	1.87^{gf}±0.17	1.23^{fe}±0.00	0.13^{d}±0.00	0.64^{g}±0.00
F	1.75^{g}±0.35	2.00^{gf}±0.00	1.25^{e}±001	0.15^{c}±0.00	0.62^{g}±0.00
G	1.75^{g}±0.36	2.50^{f}±0.00	1.29^{d}±0.03	0.09^{f}±0.00	0.44^{g}±0.05

**Data represent mean values ± standard deviation (n=2). Different superscript letters within columns indicate statistically significant differences (p < 0.05) as determined by Duncan's Multiple Range Test.*

**Key: A= 100% WF + 0% FCBF, B= 95% WF + 5% FCBF, C= 90% WF + 10% FCBF, D= 85% WF + 15% FCBF, E= 80% WAF + 20% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF*

**FCBF= Fermented Cow Pea Bran Flour, UFCBF=Unfermented Cow Pea Bran Flour, WF=Wheat Flour*

3.2 Chemical Composition of Bread Produced Fermented cowpea bran and Wheat Flour Blends

3.2.1 Proximate Composition of Bread Produced Fermented cowpea bran and Wheat Flour Blends

The findings of the proximate composition (bread) are shown in Table 2. The content of protein, fat, crude fiber, and ash is detailed as increasing from 11.34 to 13.06, 3.31 to 3.41, 2.56 to 2.71 % and 8.63 to 8.81 % respectively, while carbohydrate content reduced from 67.87 to 65.05 % and there were no significant changes in moisture content with increase in added content of fermented cowpea bran flour (FCBF). The consequence of added FCBF on the blends bread was significant ($p<0.05$).

The protein levels in the flour samples rose as the amount of fermented cowpea bran increased. This was anticipated because cowpeas are legumes, whereas wheat is a cereal grain, and legumes typically have higher protein content than cereals, even though the primary protein in wheat is gluten, which is essential for baking. Protein increased from 11.34 to 13.06 %, and the sample with 25% fermented cowpea bran had the highest content with the controls, 100% wheat and 95:5% (wheat to unfermented cowpea bran) having 9.49 and 11.02 % respectively. The Increase in protein was attributed to the release of proteins initially bound to the antinutritional factors which were released after fermentation [36]. Protein is crucial for the construction and repair of tissues and it also contributes to the making of hormones and enzymes.

Fat content increased from sample B (5 % FCBF) with 3.31 % to sample F (25 % FCBF) with 3.41% and samples A and G (100% and 95 %:5 % wheat to unfermented cowpea bran) had 2.53% and 3.26% respectively. The mechanisms underlying the rise in fat content could be attributed to the extensive breakdown of large fat molecules into simple fatty acids, the increased activity of lipolytic enzymes that may have produced more fatty acids during fermentation, the fat from dead microflora, and/or the presumption that the fermenting microflora did not use the fat as an energy source [37]. Fats are essential for cell structure and insulation, act as a store of energy, and aid in the body's absorption of fat-soluble vitamins. Additionally, they serve to enhance the sensory qualities of baked goods and maintain flavor. However, people who consume a lot of fat are more likely to develop coronary heart disease and obesity [38]. The highest crude fiber content in sample F (25% FCBF) with 2.71 % could be as a result of the high cowpea bran crude fiber content. The high crude fiber content will improve digestion in the body [39]. The increased fiber content was due to the increase in FCBF, confirming that cowpea bran is a good source of dietary fiber. The increase in fibre was observed as an improvement in the nutrient status since they are agents in food which aids in absorption during the digestion process [27].

The ash content of the flours, which reflects the mineral composition, increased steadily with higher levels of supplementation. An increase in ash content suggests that samples with a greater percentage of ash could serve as good mineral sources [40]. The carbohydrate content diminished from the 95:5% to 75:25% ratio (wheat to FCBF) as the level of substitution rose. This decline could be linked to the actions of fermenting microorganisms, which convert and utilize carbohydrates for energy to

support growth and other cellular functions. A statistically significant difference ($p<0.05$) was observed in the carbohydrate levels of the flour samples. A reported 3% decrease in carbohydrate content during the fermentation of red beans (Phaseolus angularis) was attributed to carbohydrates being utilized as an energy source for fungal growth [41]. Numerous authors, including Espinosa-Paez et al. [42], Difo et al. [43], Chinma et al. [44], and Asensio-Grau et al. [45], have similarly documented reductions in carbohydrate content during the fermentation processes of various legumes, such as African oil bean (7%), tempeh (0.7%), cowpea (3%), mahogany bean (up to 61%), kidney bean (17%), lentil (6%), African yam bean (4%), and Lyon bean (up to 26%). These reductions are attributed to the consumption of carbohydrate-related compounds for energy by fermenting microorganisms, along with the transformation of oligosaccharides into simpler sugars.

Significant increase in the moisture values of flour blends with the control (100% wheat) having 8.31 % and all other samples remaining 8.01 %) was not observed. This could be attributed to the fermentation of cowpea bran which alters dough hydration properties, leading to higher moisture loss during baking**.** The bread samples generally had relatively low moisture content, which suggest improved shelf life.

Table 2: Proximate composition of bread produced from fermented cowpea bran flour and wheat flour blends (%)

Samples	Moisture	Crude Protein	Crude-Fat	Crude Fibre	Ash	Carbohydrate
A	8.31^{f}±0.02	9.49^{g}±0.00	2.53^{g}±0.02	1.16^{g}±0.01	4.72^{g}±0.01	58.24^{g}±0.02
B	8.01^{g}±0.02	11.34^{e}±0.02	3.31^{f}±0.02	2.56^{f}±0.02	8.93^{c}±0.02	67.87^{b}±0.00
C	8.01^{g}±0.02	11.73^{d}±0.02	3.33^{fe}±0.02	2.59^{e}±0.01	8.78^{d}±0.02	67.06^{c}±0.00
D	8.01^{g}±0.02	12.13^{c}±0.02	3.36^{fe}±0.01	2.62^{e}±0.01	8.63^{f}±0.02	66.67^{d}±0.01
E	8.01^{g}±0.02	12.59^{b}±0.02	3.38^{ed}±0.02	2.66^{d}±0.02	8.72^{e}±0.02	65.26^{e}±0.55
F	8.01^{g}±0.02	13.06^{a}±0.01	3.41^{d}±0.02	2.71^{c}±0.02	8.81^{d}±0.02	65.05^{e}±0.00
G	8.01^{g}±0.00	11.02^{f}±0.02	3.26^{c}±0.02	2.56^{f}±0.02	8.63^{f}±0.02	63.49^{f}±0.00

**Data represent mean values ± standard deviation (n=2). Different superscript letters within columns indicate statistically significant differences (p < 0.05) as determined by Duncan's Multiple Range Test.*
**Key: A= 100% WF + 0% FCBF, B= 95% WF + 5% FCBF, C= 90% WF + 10% FCBF, D= 85% WF + 15% FCBF, E= 80% WAF + 20% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF*
**FCBF= Fermented Cow Pea Bran Flour, UFCBF=Unfermented Cow Pea Bran Flour, WF=Wheat Flour*

3.2.2 Phytochemical Composition of Bread Produced from Fermented Cowpea Bran Flour and Wheat Flour Blends

The phytochemical content of bread is shown in Table 3. Carotenoids, flavonoids and Phenols increased with the addition of FCBF from 6.17 to 8.20, 12.16 to 18.73 and 39.38 to 49.64 mg/100g respectively. Control samples (A and F) for carotenoids, flavonoids and phenols with 5.53 and 6.02; 10.13 and 10.97; 37.73 and 38.01 mg/100g respectively. This increase can be as a result of the ability of *S. cerevisiae* to produce enzymes such as β-glucosidase, cellulases, and esterases, which hydrolyze complex polyphenols into simpler, more bioavailable forms. This enzymatic action facilitates and improves the release of bound flavonoids alongside phenolic compounds from the cowpea bran matrix. Fermentation disrupts the plant cell wall structure, releasing more phenolic compounds, flavonoids, and carotenoids that were previously bound within the lignocellulosic matrix [44]. The carotenoid content TFC, and TPC of both the composite bread and the control samples were found to differ significantly ($p<0.05$). Phytochemicals contribute to human health by reducing the risk of several degenerative diseases; consequently, phenolics and flavonoids represent the primary categories of polyphenols present in plant-based foods.

Table 3: Phytochemicals Composition of Bread Produced from fermented cowpea bran flour and wheat flour blends (mg/100g)

Samples	Carotenoids	Flavonoid	Total-Phenols
A	5.53^{g}±0.02	10.13^{g}±0.02	37.73^{g}±0.03
B	6.17^{g}±0.02	12.16^{e}±0.02	39.38^{f}±0.09
C	6.68^{g}±0.01	12.83^{ed}±0.33	42.15^{e}±0.05
D	7.18^{g}±0.00	13.51^{d}±0.69	44.92^{d}±0.02
E	8.20^{g}±0.01	18.73^{c}±0.33	49.64^{c}±0.37
F	9.22^{g}±0.01	23.96^{b}±0.02	54.37^{b}±0.72
G	6.02^{g}±0.01	10.97^{f}±0.01	38.01^{g}±0.01

**Data represent mean values ± standard deviation (n=2). Different superscript letters within columns indicate statistically significant differences (p < 0.05) as determined by Duncan's Multiple Range Test.*
**Key: A= 100% WF + 0% FCBF, B= 95% WF + 5% FCBF, C= 90% WF + 10% FCBF, D= 85% WF + 15% FCBF, E= 80% WAF + 20% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF*
**FCBF= Fermented Cow Pea Bran Flour, UFCBF=Unfermented Cow Pea Bran Flour, WF=Wheat Flour.*

3.2.3 Antioxidant Activity of Bread Produced from Fermented Cowpea Bran Flour and Wheat Flour Blends

The antioxidant activity is presented in Table 4. The antioxidant capacities of the blends against three free radicals (DPPH, FRAP and ABTS+) increased from 9.47 to 12.77 %, 4.31 to 8.28 µmol/g) and 12.42 to 21.39 % respectively as FCBF levels increased. The lowest DPPH, FRAP and ABTS value was recorded in sample B (5% FCBF). An increase in the antioxidant activity of wheat and fermented cowpea bran could be due to lowered pH during fermentation which enhances the stability and solubility of antioxidant compounds. The redox environment of fermentation promotes the generation of electron-donating compounds, further improving the total antioxidant capacity also the combined effects of polyphenols, flavonoids, carotenoids, and yeast-derived antioxidants create a synergistic antioxidant response, significantly improving free radical scavenging capacity [46]. The increased antioxidant activity of food products is strongly correlated with polyphenol content. Antioxidants are essential to health as they improve digestion and brain health, reduced the risk of chronic disease and anti-inflammatory effects.

Table 4: Antioxidants Activities of Aqueous Extracts of Bread Produced from Fermented Cowpea Bran and Wheat Flour Blends

Samples	FRAP(µmol/g)	ABTS (%)	DPPH (%)
A	1.08^{g}±0.01	10.53^{g}±0.02	7.21^{g}±0.02
B	4.31^{e}±0.01	12.42^{e}±0.00	9.47^{e}±0.02
C	5.49^{d}±0.02	15.37^{d}±0.00	10.09^{d}±0.00
D	6.68^{c}±0.02	18.33^{c}±0.00	10.73^{c}±0.00
E	7.48^{b}±0.01	19.86^{b}±0.01	11.75^{b}±0.01
F	8.28^{a}±0.00	21.39^{a}±0.01	12.77^{a}±0.02
G	2.54^{f}±0.01	11.03^{f}±0.04	9.12^{f}±0.01

**Data represent mean values ± standard deviation (n=2). Different superscript letters within columns indicate statistically significant differences (p < 0.05) as determined by Duncan's Multiple Range Test. *Key: A= 100% WF + 0% FCBF, B= 95% WF + 5% FCBF, C= 90% WF + 10% FCBF, D= 85% WF + 15% FCBF, E= 80% WAF + 20% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF*

FCBF= Fermented Cow Pea Bran Flour, UFCBF=Unfermented Cow Pea Bran Flour, WF=Wheat Flour

3.2.4 Dietary Fibre Composition of Bread Produced from Fermented Cowpea Bran Flour and Wheat Flour Blends

The dietary fiber composition is presented in Table 5. Soluble dietary fibre (SDF) of composite flours increased from 6.56 to 9.68 with 4.39 and 11.36 for control samples. The present study displayed a significant increase ($p<0.05$) in SDF in all products compared to sample A (100 % WF) and a decrease compared to control sample G (95:5 % WF to UFCBF) this can be attributed to partial depolymerization of some soluble dietary fiber (SDF) components (e.g., arabinoxylans, β-glucans), making them more digestible or absorbable. This transformation can reduce the overall fiber content as certain fiber fractions become more bioavailable or fermentable. Research findings indicated a notable rise in both insoluble dietary fiber (IDF) and total dietary fiber (TDF) content within range of 21.38 to 23.42 and 27.95 to 33.10 % respectively with increased levels of added FCBF, 19.89 and 26.72 % for control samples of IDF also 24.28 and 38.08 % for control samples of TDF. The decrease in IDF and TDF composition of bread samples compared to sample G (control: 5% UFCBF) can be linked to the production of organic acids during fermentation which helps break down dietary fiber by weakening the fiber structure. The reduced pH enhances fiber hydrolysis, further contributing to the loss of fiber content. Also, while lignin is generally resistant to microbial degradation, some oxidative enzymes from *S. cerevisiae* may contribute to partial lignin breakdown, reducing total dietary fiber content. The overall reduction in total dietary fiber depends on fermentation conditions (time, temperature, pH, enzyme activity).

Table 5: Dietary Fibre Composition of Bread Produced from fermented cowpea bran flour and wheat flour blends (%)

Samples	SDF	IDF	TDF
A	4.39^{g}±0.10	19.89^{g}±0.01	24.28^{g}±0.12
B	6.56^{f}±0.02	21.38^{d}±0.12	27.95^{f}±0.14
C	7.65^{e}±0.02	21.01^{e}±0.06	28.68 ±0.08
D	8.75^{d}±0.02	20.65^{f}±0.00	29.41^{d}±0.02
E	9.21^{c}±0.02	22.03^{c}±0.02	31.25^{c}±0.03
F	9.68^{b}±0.02	23.42^{b}±0.02	33.10^{b}±0.04
G	11.36^{a}±0.01	26.72^{a}±0.02	38.08^{a}±0.04

Data represent mean values ± standard deviation (n=2). Different superscript letters within columns indicate statistically significant differences ($p < 0.05$) as determined by Duncan's Multiple Range Test.
Key: A= 100% WF + 0% FCBF, B= 95% WF + 5% FCBF, C= 90% WF + 10% FCBF, D= 85% WF + 15% FCBF, E= 80% WAF + 20% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF
FCBF= Fermented Cow Pea Bran Flour, UFCBF=Unfermented Cow Pea Bran Flour, WF=Wheat Flour
Abbreviations: SDF (soluble dietary fiber), IDF (insoluble dietary fiber), TDF (total dietary fiber).

3.3 Physical Properties of Bread Made from Blended Fermented Cowpea Bran and Wheat Flour

The physical attributes of the bread samples are presented in Table 6. and Plate 1. The loaf weight increased from 238.71 to 251.11g with 241.11 and 221.10 for the control samples (A and G) respectively. The greater loaf mass likely resulted from enhanced water retention and reduced CO_2 gas retention in the composite dough formulation, ultimately yielding denser baked products [47].

The loaf volume of the bread decreased from 632.50 to 425.00 cm^3 for composite bread samples, 505.00 and 625.50cm^3 respectively for the control samples (A and G). This trend is in line with the report of Nwosu [48] on the production of bread using wheat-cassava flour blends. The volume of the loaf is regarded as the key characteristic of bread because it offers a measurable indicator of baking quality [47].

Plate 1: physical state of the bread samples

A B D F G

A B D F G

Key: A = 100% WF + 0% FCBF, B = 95% WF + 5% FCBF, D= 85% WF + 15% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF
WF= Wheat four, FCBF=Fermented cowpea bran flour, UFCBF= Unfermented cowpea bran flour

The bread's loaf volume index decreased from 2.64 to 1.68cm^3/g and the control samples A and G with 2.08 and 2.49 cm^3/g respectively. This result is in line with Makinde and Akinoso, [49] during the production of bread from wheat and black sesame. The findings are contrary to the report of Ayo *et al*., [50] which showed an increase in the specific volume of bread produced from soya beans and acha composite flours. The observed reduction in loaf volume index may be explained by gluten network disruption caused by FCBF incorporation [49]. As FCBF substitution levels rose, baked loaves demonstrated higher mass but lower volume and volume-to-weight ratios.

Table 6: Physical properties of bread produced from fermented cowpea bran flour and wheat flour blends

Sample	Loaf weight (g)	Loaf volume (cm^3)	Loaf-volume index (cm^3/g)
A	241.11^{f}±0.02	505.00^{d}±7.07	2.08 ^{d}±0.01
B	238.71^{g}±0.69	632.50^{b}±3.53	2.64 ^{a}±0.01
C	240.82^{f}±0.02	557.50^{c}±3.53	2.31^{c}±0.02
D	242.41^{e}±0.01	482.50^{e}±3.53	1.98^{e}±0.00
E	246.76^{d}±0.01	452.50^{f}±3.53	1.83^{f}±0.01
F	251.11^{c}±0.02	425.00^{g}±7.07	1.68^{g}±0.01
G	260.71^{b}±0.02	625.50^{a}±3.53	2.49^{b}±0.00

Data represent mean values ± standard deviation (n=2). Different superscript letters within columns indicate statistically significant differences ($p < 0.05$) as determined by Duncan's Multiple Range Test.
Key: A= 100% WF + 0% FCBF, B= 95% WF + 5% FCBF, C= 90% WF + 10% FCBF, D= 85% WF + 15% FCBF, E= 80% WAF + 20% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF
FCBF= Fermented Cow Pea Bran Flour, UFCBF=Unfermented Cow Pea Bran Flour, WF=Wheat Flour

3.4 Sensory Quality Evaluation of Bread Produced from Fermented Cowpea Bran Flour and Wheat Flour Blends

The sensory studies result of bread made from the flour blends is displayed in Table 7. The average mean values of color, taste, aroma, texture and appearance ranged from 3.70 to 8.20, 4.80 to 7.90, 5.95 to 7.65, 3.95 to 7.90 and 2.75 to 7.85 respectively. Based on all parameters, the sample (A) with 100% wheat flour was the best.

Sample F(25% FCB) was rated the lowest in color, taste and texture, the aroma and taste of composite bread were most acceptable at sample B and G (5% FCB and 5% UFCB), however decreased with an increase in the addition of FCB. This could be attributed to the strong fermented flavour, high fiber texture and dark brown colour of FCB [47]. Sensory evaluation revealed an inverse relationship between FCB incorporation levels and color acceptability scores, with higher substitution ratios correlating with progressively lower visual appeal ratings. The sample labeled F showed the lowest color rating in the composite bread. The decrease in color rating of the bread as the amount of wheat flour replaced by FCB flour rose can be linked to the color contributed by FCB. The texture of the bread also deteriorated with a higher level of FCB substitution. Sample A with 100% wheat flour has the highest value 7.90 followed by sample B (5% FCB). There was a progressive significant decrease as FCB increased from 10-25%. Sensory evaluation results demonstrated that the control sample received significantly higher overall acceptability scores compared to test formulations sample. This was followed by sample B and G with 5% FCB and 5% UFCB respectively.

Table 7: Organoleptic Evaluation of Bread Made from Wheat-Fermented Cowpea Bran Composite Flours

Sample	Colour	Taste	Aroma	Texture	Appearance	Over all Acceptability
A	$8.20^{c}\pm0.76$	$7.90^{e}\pm0.78$	$7.65^{e}\pm0.87$	$7.90^{b}\pm0.78$	$7.85^{c}\pm0.93$	$8.25^{b}\pm0.71$
B	$6.10^{e}\pm0.96$	$7.70^{e}\pm1.12$	$6.80^{f}\pm1.00$	$6.90^{c}\pm0.91$	$6.75^{d}\pm0.85$	$6.75^{c}\pm0.71$
C	$5.30^{f}\pm1.03$	$6.45^{f}\pm0.99$	$6.75^{f}\pm1.16$	$6.25^{e}\pm0.71$	$5.25^{e}\pm0.85$	$6.10^{d}\pm0.71$
D	$4.90^{d}\pm1.07$	$5.35^{g}\pm1.03$	$5.95^{g}\pm1.14$	$5.75^{e}\pm0.78$	$4.15^{f}\pm0.74$	$5.30^{e}\pm0.73$
E	$3.95^{g}\pm0.88$	$5.20^{g}\pm0.76$	$6.10^{gf}\pm1.11$	$4.70^{f}\pm0.97$	$2.75^{g}\pm0.63$	$4.05^{f}\pm0.88$
F	$3.70^{g}\pm0.73$	$4.80^{g}\pm1.10$	$6.45^{gf}\pm0.94$	$3.95^{g}\pm1.19$	$2.85^{g}\pm0.87$	$3.45^{f}\pm0.88$
G	$6.95^{d}\pm1.09$	$7.50^{e}\pm0.82$	$6.45^{gf}\pm1.14$	$6.65^{dc}\pm0.67$	$6.85^{d}\pm0.93$	$6.95^{c}\pm0.82$

**Values are means ± standard deviation of duplicate determinations. This Means differently superscripted along the vertical columns are significantly ($p<0.05$) different from each other using the Duncan multiple range test.*
**Key: A= 100% WF + 0% FCBF, B= 95% WF + 5% FCBF, C= 90% WF + 10% FCBF, D= 85% WF + 15% FCBF, E= 80% WAF + 20% FCBF, F= 75% WF + 25% FCBF, G= 95% WF + 5% UFCBF*
**WF= Wheat four, FCBF=Fermented cowpea bran flour, UFCBF= Unfermented cowpea bran flour*

4.0 CONCLUSION

The findings from this research established that bread can be produced from wheat and complemented with fermented cowpea flour. According to the findings of this research, cowpea bran fermented with *Saccharomyces cerevisiae* notably ($p\leq0.05$) enhanced the functional characteristics, chemical composition, and nutritional profile of the bread made from it. Loaves prepared using the experimental flour blend demonstrated general acceptability but most preferred at 5% inclusion of fermented cowpea bran. Fermented cowpea flour could be substituted with wheat flour for bread production up to 5% for improved nutritional composition.

Based on this study, fermented cowpea bran should be added at 5% levels for bread production. Fermentation using *saccharomyces cerevisiae* is recommended in the food industry for the modification and nutritional improvement of cowpea bran. Substitution of fermented cowpea bran in other food products should be considered. Nevertheless, additional research needs to be conducted to assess the storage longevity of the composite breads for enhanced quality retention.

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