

Quality properties of bread from blends of wheat flour and sweet orange seed protein concentrate

Peter Isah AKUBOR^{1,2}, Saa-Aondo Patrick SHABU¹, Goodluck Obioma OKEREKE^{2,3*} and Aaron Mutlong Amak DAMAK^{1,2}

¹Department of Food Science and Technology, Federal University, Wukari, PMB 1020 Wukari, Taraba State, Nigeria.

²Department of Chemistry, Food Science and Technology, Centre for Food Technology and Research, Benue State University, Makurdi, PMB 102119 Makurdi, Benue State, Nigeria.

³Coca-Cola Hellenic Bottling Company, Owerri, Imo State, Nigeria.

Corresponding author. Email: gocoke79@gmail.com; Tel: +2348064282105.

Copyright © 2023 Akubor et al. This article remains permanently open access under the terms of the [Creative Commons Attribution License 4.0](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

Received 21st November 2023; Accepted 27th December 2023

ABSTRACT: The study investigated the effect of substitution of wheat flour with sweet orange seed protein concentrate on the quality of bread. Protein concentrate, obtained from sweet orange (*Citrus sinensis*) seeds was used to substitute 5, 10, 15, 20 and 30% of wheat flour in breads. The flours were assessed for functional and pasting properties while the breads were evaluated for proximate composition, physical and sensory properties. Sweet orange seed protein concentrates and wheat flour had bulk densities of 0.45 g/cm³ and 0.72 g/cm³, respectively while those of the flour blends ranged from 0.61 - 0.70 g/cm³. Wheat flour had lower contents of moisture, crude fiber, ash, protein, and fat than the protein concentrate, which had lower carbohydrate content. Sweet orange seed protein concentrate had highest foaming capacity, water and oil absorption capacities but lowest least gelation concentration among all the flours. The peak viscosities of protein concentrate and wheat flour were 886.21 RVU and 675.20 RVU, respectively. The peak, trough, breakdown, final and setback viscosities of the flour blends decreased while peak time and pasting temperature increased with increase in the substitution of wheat flour with protein concentrate. In length, loaf weight, height, oven spring, loaf volume, specific volume and weight loss, the 100% wheat flour breads had 12.03 cm, 172.60 g, 5.35 cm, 0.62 cm, 471.50 cm³, 2.73 cm³/g and 10.42%, respectively while breads containing 5% sweet orange seed protein concentrate had 12.02 cm, 171.40 g, 4.60 cm, 0.61 cm, 350.50 cm³, 2.04 cm³/g and 9.28 %, respectively. The proximate composition of the breads containing 5 and 10% sweet orange seed protein concentrate were improved over that of wheat flour bread. The sensory attributes of the breads decreased with increased level of sweet orange seed protein concentrate in the breads. However, the breads containing 5 and 10% sweet orange seed protein concentrate were not significantly ($p > 0.05$) different from the 100% wheat bread in colour. Thus, the incorporation of up to 10% sweet orange seed protein concentrate in wheat flour improved the functional properties of the flour blends and the qualities of the bread. Adoption of this approach will address malnutrition, wastages and environmental pollution (caused by the discard of orange seeds).

Keywords: Bread and quality properties, functional and pasting properties, protein concentrate, sweet orange seeds, wheat flour.

INTRODUCTION

For many years, now, bread has been a major staple wheat-based food product with rapid increasing consumption worldwide, especially, in Nigeria and other developing countries (Adeyeye *et al.*, 2019; Akubor and Gambo, 2020; Wang and Jian, 2022; Moreno-Araiza *et al.*,

2023). It is prepared from hard wheat flour, yeast, fat, sugar, salt and water. Predominantly rich in carbohydrates, bread is limited in lysine (an essential amino acid) and thus, can be an appropriate vehicle for food fortification with essential nutrients such as protein, vitamins and

minerals (Chikpah *et al.*, 2023). Indeed, there is a growing interest in using composite flour for bread making owing to some economic, social, and health reasons (Dhingra *et al.*, 2011; Olapade *et al.*, 2011; Menon *et al.*, 2015; Okereke *et al.*, 2021a; Coțovanu *et al.*, 2023). Unhappily, the partial substitution of wheat flour by other flour types presents considerable technological difficulties because their proteins lack the ability to form the necessary gluten network for holding the gas produced during the fermentation (Okereke *et al.*, 2021a). Wheat is the choice cereal for manufacture of bread because it contains a large amount of gluten, which produces raised loaves (Badifu *et al.*, 2005; Janssen *et al.*, 2022). More so, the rising demand for bread, which is the most popular wheat-based product, continues to transmit research-pressure for suitable replacements (partial or total) of the key raw material (wheat flour) with local and nutritionally healthy raw materials. For example, in addition to the exorbitant cost of wheat importation, the conventional wheat bread is deficient of some key nutrients such as protein for healthy living (Okereke *et al.*, 2021b; Nakakana *et al.*, 2023).

According to Okereke *et al.* (2021a), though, the deployment of the composite technology in bread making has recorded some successes but it is still confronted with the challenges of poor baking properties, thereby pushing food scientists further towards incorporation of high-nutrient-dense materials (like protein concentrates) in wheat-based products like bread. Protein concentrates are animal or plant based dietary supplements that have high protein content (25-90%) with fat and carbohydrate taking up the remaining percentage (Mao and Hua, 2012; Lee *et al.*, 2016; McSweeney *et al.*, 2020; Britannica, 2023). Supplementation of proteins in diets is indispensable for a healthy lifestyle (Meganaharshini *et al.*, 2023). Proteins play vital roles in biological systems in the synthesis of enzymes, muscles, hormones, organs, tissues and immune-regulating bodies. Protein deficiency symptoms include increased infections and illnesses, reduced muscle mass (sarcopenia), swollen limbs, slow wound-healing and high blood pressure (preeclampsia) (John and Gosh, 2021; Leal, 2022). Huge nutritional gains have been achieved in feeding programmes through application of protein concentrates on foods such as confectioneries, cereals, nutrition bars, processed cheeses, sports beverages and muscle gain formulations, spaghetti, macaroni, pasta, meat analogs, baby food formulations, vegetarian foods, bakery products and meat extenders (Stokes *et al.*, 2018; Shevkani *et al.*, 2019; Adhikari *et al.*, 2022; Qin *et al.*, 2022). There is no doubt that this approach can help curb the menace of protein malnutrition in developing countries like Nigeria where meat and egg supply is far below the dietary demand of the population. Though, a lot of studies have investigated the suitability of cereal, pseudocereals, root and tuber crops, as a partial or total replacement to wheat flour in production of wheat-based products but little or none has been done on sweet orange seeds and their derivatives.

Worldwide, sweet orange (*Citrus sinensis*) is the most important citrus specie in terms of production volume and cultivation area (Seminara *et al.*, 2023). Besides, their awesome organoleptic properties and high nutraceutical value are exploitable. Sweet orange is mainly consumed or utilized in the form of fresh fruit, juice concentrates and thin dried slices by consumers and orange juice processing companies, giving rise to generation of large quantities of wastes (peel, pulp and seeds) that cause environmental pollution (Emorjoho and Akubor, 2016). For instance, the environmental nuisance caused by discarding the seeds results in the release of greenhouse gases and also, huge economic losses to the food industries (Papargyropoulou *et al.*, 2014; Adubofuor *et al.*, 2021). The seeds (constituting about 38% of the weight of the fruit) contain crude fat (6.37%), ash (4.31%), crude protein (2.36%), crude fibre (1.67%), moisture (10.92%) and carbohydrate (74.37%) (Uzama *et al.*, 2021). Contrary to the popular perception that orange seeds are of low economic value, published literatures have enumerated their rich bioactive and nutritional properties such as essential fatty acids, phytosterols, flavonoids, phenols, carotenoids, tannins, saponins, tocopherols, fibers, minerals, and vitamins (Malacrida *et al.*, 2012; El-Safy *et al.*, 2012; Reazai *et al.*, 2014; Farag *et al.*, 2020; Adubofuor *et al.*, 2021; Uzama *et al.*, 2021; Zayed *et al.*, 2021; Moser *et al.*, 2023). These compounds are treasured for their health-promoting potentials in food applications. Epidemiological surveys have shown an inverse relationship between dietary flavonoid intake from citrus and cardiovascular disease (Di Majo *et al.*, 2005; Emorjoho and Akubor, 2016). Regrettably, in spite of these highly beneficial quality attributes, the use of the seeds in foods is limited by the presence of limonoids that causes bitterness. Thus, protein concentrate and protein isolates could be prepared from the seeds and used in various food applications.

The incorporation of sweet orange seed protein concentrates in bread will not only help reduce environmental pollution due to the discarded seeds by consumers and orange fruit juice processing companies but will also increase protein content of breads. This strategy will help address protein malnutrition, especially, in developing countries, in addition to diversifying the use of citrus seeds for sustainable production, and increasing the revenue of citrus farmers.

In this study, bread prepared from flour blends of wheat flour and sweet orange seed protein concentrate at 5, 10, 15, 20 and 30%, were assessed of their quality properties after investigating the functional and pasting properties of the flour blends.

MATERIALS AND METHODS

Sweet orange fruits (*Citrus sinensis*) were purchased from Zaki Biam market, Benue State, Nigeria. Wheat flour,

sugar, yeast, salt and margarine were purchased from a confectionary store at New Market, Wukari Township, Taraba State, Nigeria. These raw materials were packed in polyethylene bags and stored (at dry condition and ambient room temperature of 27°C) in the Laboratory of Food Science and Technology Department, Federal University, Wukari, Taraba State, Nigeria.

Preparation of wheat flour

The commercial wheat flour was sieved through a 150 µm mesh screen, packaged in high density polythene bags and stored in airy clean dry place at ambient temperature (27°C) prior to use.

Preparation of sweet orange seed powder

The sweet orange seed powder was produced as described by Emorjho and Akubor (2016) as shown in Figure 1. The harvested sweet orange fruits were washed with tap water, cut into halves with a clean sharp knife and the seeds were removed, and sundried at 30± 2°C, for 8 hours to a constant weight. The sweet orange seeds (20 kg) were manually dehulled, milled in an attrition mill (9FC-36, China); and sieved through a 150 µm mesh sieve. The powder was packaged in high density polyethylene bags (Ziplock, China) and stored in a dry place at ambient temperature (27°C) prior to use.

Defatting of the sweet orange seed flour

Sweet orange seed powder was defatted by cold solvent extraction method using n-hexane as described by Navarro Del Hierro *et al.* (2021) with slight modifications on temperature, type of drier and use of attrition mill. The sweet orange seed powder was submerged in 95% hexane at a ratio of 1:5 (w/v) in an Ultra-Turrax (11000 rpm) for 5 minutes. The mixture was centrifuged at 4500 rpm for 10 minutes at 27°C before removing the supernatant. The resulting powder was extracted of the remaining fat by repeating the process before it was strained with a 150 µm muslin cloth to obtain the defatted meal. The defatted powder was dried in a fume cupboard at 30 ± 2°C for 2 hours until no hexane odor was discerned. The dried powder was milled using an attrition mill (9FC-36, China); sieved through a 150 µm sieve and then stored in air-tight high density polythene bag (Ziplock, China) as defatted sweet orange seed flour, until needed.

Preparation of sweet orange seed protein concentrate

Sweet orange seed protein concentrate was prepared using the procedure reported by Ocheme *et al.* (2018).

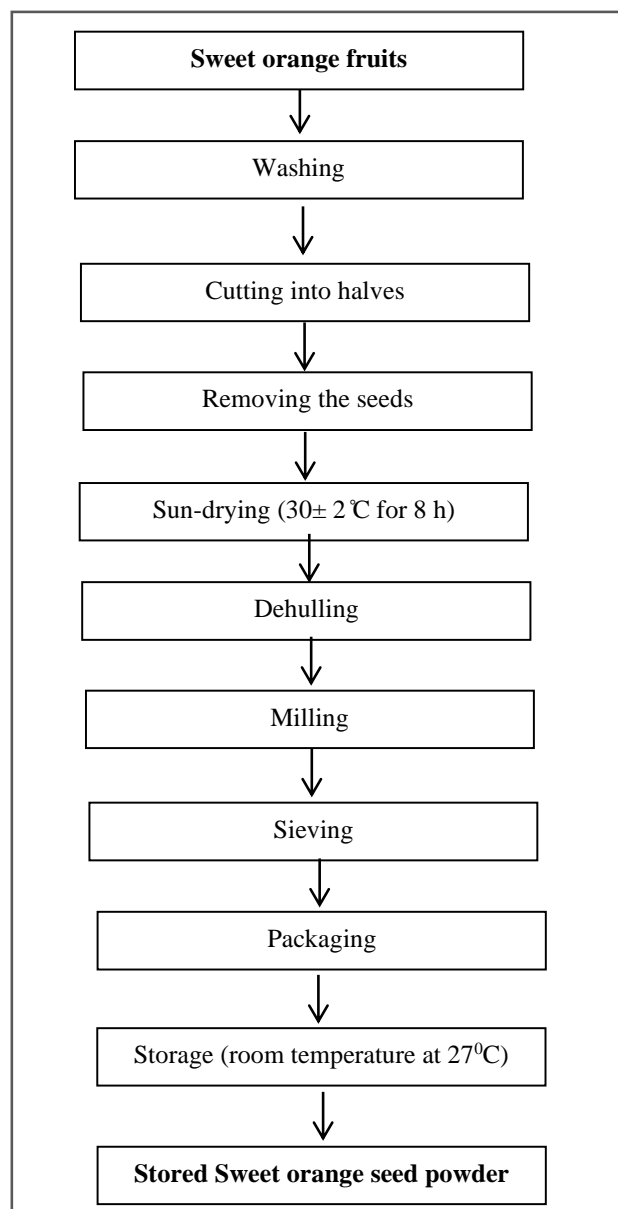


Figure 1. Flow chart for production of sweet orange seed powder (Source: Emorjho and Akubor (2016)).

Defatted sweet orange seed flour was mixed with water at a ratio of 1:10 (w/v). The mixture was shaken at ambient temperature (28°C) for an hour, and the pH was adjusted to 4.5 with 4 mol/L concentration of HCl. The suspension was centrifuged at 959 × g for 20 minutes. The supernatant was discarded, and the precipitate resuspended in water at ratio 1:10 (w/v) and stirred at ambient temperature for 1 hour so as to clear the acid. Thereafter, it was centrifuged at 3,500 rpm for 30 minutes. The supernatant was discarded, and the precipitate removed from the tube with a spatula, dried in an oven at 40°C, packaged, and stored at 4 ± 2°C until required for use.

Table 1. Formulation of flour blends from sweet orange seed protein concentrate and wheat flour

Flour Blend	Wheat flour (g)	Sweet orange seed protein concentrate (g)
PC	0.00	100.00
WFPC ₀	100.00	0.00
WFPC ₁	95.00	5.00
WFPC ₂	90.00	10.00
WFPC ₃	85.00	15.00
WFPC ₄	80.00	20.00
WFPC ₅	70.00	30.00

WFPC₀ = 100% Wheat flour; WFPC₁ = Flour blend (95% wheat flour: 5% Sweet orange seed protein concentrate); WFPC₂ = Flour blend (90% wheat flour: 10% Sweet orange seed protein concentrate); WFPC₃ = Flour blend (85% wheat flour: 15% Sweet orange seed protein concentrate); WFPC₄ = Flour blend (80% wheat flour: 20% Sweet orange seed protein concentrate); WFPC₅ = Flour blend (70% wheat flour: 30% Sweet orange seed protein concentrate).

Table 2. Recipe formulation for production of bread sample.

Bread Sample	Wheat flour (g)	Sweet orange seed protein concentrate (g)	Salt (g)	Yeast (g)	Margarine (g)	Sugar (g)	Water (mL)
WPB ₀	100	0	1.5	5	10	8	55
WPB ₁	95	5	1.5	5	10	8	55
WPB ₂	90	10	1.5	5	10	8	55
WPB ₃	85	15	1.5	5	10	8	55
WPB ₄	80	20	1.5	5	10	8	55
WPB ₅	70	30	1.5	5	10	8	55

WPB₀ = Bread of 100% Wheat flour; WPB₁ = Bread of Flour blend (95% wheat flour: 5% Sweet orange seed protein concentrate); WPB₂ = Bread of Flour blend (90% wheat flour: 10% Sweet orange seed protein concentrate); WPB₃ = Bread of blend (85% wheat flour: 15% Sweet orange seed protein concentrate); WPB₄ = Bread of Flour blend (80% wheat flour: 20% Sweet orange seed protein concentrate); WPB₅ = Bread of Flour blend (70% wheat flour: 30% Sweet orange seed protein concentrate) (Source: Ceserani *et al.*, 1995).

Formulation of the flour blends

The sweet orange seed protein concentrate was used to substitute 100, 0, 5, 10, 15, 20 and 30% of wheat flour in a food blender (Philip HR2811 Model) that was operated at full speed for 10 minutes to obtain different flour blends labeled samples WFPC₀, WFPC₁, WFPC₂, WFPC₃, WFPC₄ and WFPC₅ as shown in Table 1. The blends were packaged in labeled polyethylene bags (Ziplock, China) prior to use.

Production of the bread samples

The recipe for the production of the bread samples is shown in Table 2 (Ceserani *et al.*, 1995). The straight dough method described by Okereke *et al.* (2021a) was used to produce the eight bread samples labeled WPB₀, WPB₁, WPB₂, WPB₃, WPB₄ and WPB₅ (Ceserani *et al.*, 1995). All the ingredients (flour blend, salt, margarine,

sugar, yeast and water) were weighed, added together, thoroughly mixed and kneaded to obtain the dough. The different dough samples were placed in baking pans that were smeared with vegetable oil, covered with baking pan cover and allowed to stay for one hour for fermentation to take place (proofing). The dough samples were then, baked in the oven at 200°C for 20 minutes. The baked loaves were carefully de-panned, cooled to ambient temperature and packaged in polyethylene bags for analyses. The procedure is shown in Figure 2.

Evaluation of the functional properties of the flour samples

The water absorption capacity, oil absorption capacity, bulk density, foaming capacity, foaming stability, least gelation concentration (LGC) of wheat flour, sweet orange seed protein concentrates and their blends were evaluated.

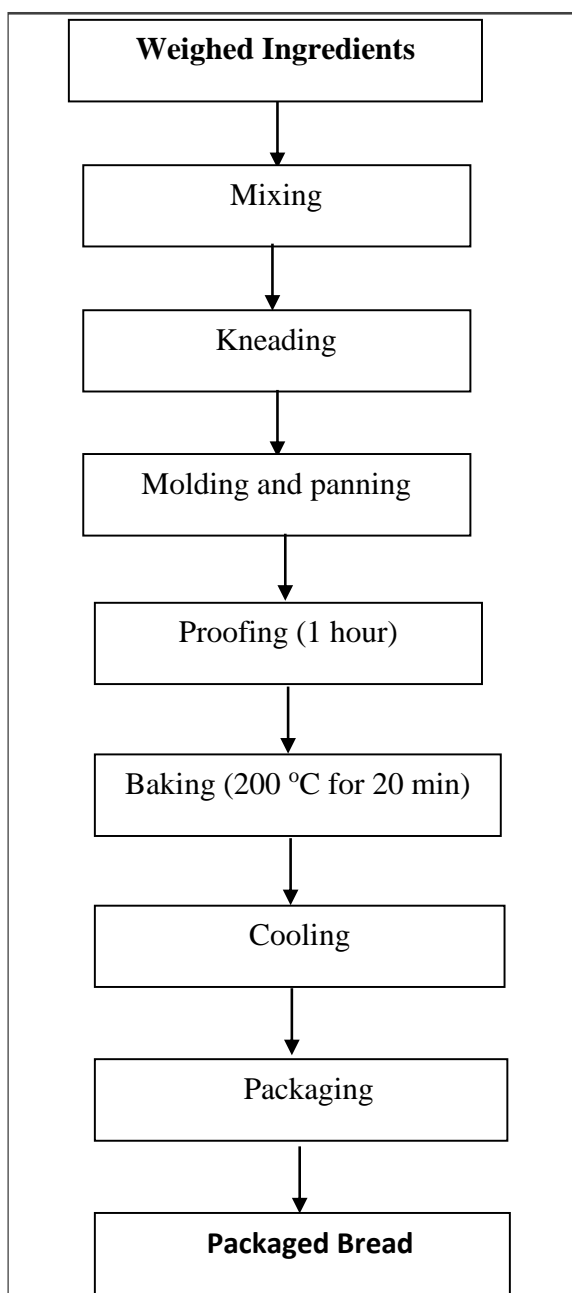


Figure 2. Flow chart for production of bread (Source: Modified Damak *et al.*, 2022b).

Water absorption capacity

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

$$WAC (g/g) = \left(\frac{WSC - WT}{WA} \right)$$

Where: WAC = Water Absorption Capacity, WSC = Weight of sample after centrifuge, WT = Weight of tube, WA = Weight of sample.

Oil absorption capacity

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

$$OAC (g/g) = \left(\frac{WSC - WT}{WA} \right)$$

Where: OAC = Oil Absorption Capacity, WSC = Weight of sample after centrifuge, WT = Weight of tube, WA = Weight of sample.

Bulk density

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

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

Foam capacity (FC) and foam stability (FS)

The method of Onwuka (2005) was used for the determination of foam capacity and foam stability of the flour samples. Sample (2 g) was blended with 100 mL of distilled water in a warring blender and the suspension was whipped at 1600 rpm for 5 minutes. The mixture was poured into a 250 mL measuring cylinder and the volume was recorded after 30 seconds. The foam capacity was expressed as percent increase in volume as:

$$FC (\%) = \frac{V2 - V1}{V1} \times 100$$

Where: FC = Foam Capacity, V2 = Volume after whipping, V1 = Volume before whipping

Then foam volumes at 15, 30, 60 and 120 minutes after whipping were recorded to determine the foam stability as follows:

$$FS (\%) = \frac{\text{Foam volume after 2 hours.}}{\text{Initial foam volume}} \times 100$$

Where: FS = Foam Stability

Least gelation capacity

Gelation capacity was determined according to the method of Coffmann and Garciaj (1977) as described by Okereke *et al.* (2022a). Suspensions of 2 - 18 g sample/100 mL in distilled water were prepared. A 10 mL each of dispersion was transferred into a test tube. The test tube was heated in a boiling water bath for 1 hour and followed by rapid cooling in a cold-water bath. The tubes were further cooled at 4°C for 2 hours. The Least gelation concentration (LGC) was determined as the concentration when the sample from the inverted test tube did not slip or fall.

Pasting properties of the flour samples

The pasting characteristics were determined with a Rapid Visco Analyzer (RVA), (Model RVA3Dt, Network Scientific, and Australia). The flour sample (2.5 g) was weighed into dried empty canisters. Then, 25 l of distilled water was dispensed into the canister containing the sample. The solution was thoroughly mixed and the canisters was fitted into a Rapid Visco Analyzer (RVA) as recommended. The slurry was then heated from 50 to 60°C with holding time of two minutes followed by cooling to 50°C with 2 minutes holding time. The rate of heating and cooling was at a constant rate of 11.25°C per min. The pasting temperature, viscosity at 95°C, stability, cooking time and setback viscosities were read off the amylograph.

Determination of the proximate composition of the wheat flour, sweet orange seed concentrate, and bread samples from their blends

Proximate analysis was carried out on the samples of wheat flour, sweet orange seed protein concentrate, and bread from the blends for the moisture, ash, crude fibre, fat, protein and carbohydrate contents using the method outlined by the Association of Official Analytical Chemists (2010).

Moisture content

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

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

W₁= weight of cooled empty dish; W₂ = weight of empty dish + undried sample; W₃= weight of dish + dried sample

Ash content

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

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

W₁= weight of cooled empty crucible; W₂ = weight of empty crucible + undried sample; W₃= weight of crucible + dried sample.

Crude fibre content

Crude fibre contents of the samples were determined using the method of AOAC (2010). The sample (5 g) was weighed into a 500 ml Erlenmeyer flask and 100 ml of TCA digestion reagent was added. It was then brought to boiling and refluxed for exactly 40 minutes counting from the start of boiling. The flask was removed from the heater, cooled a little then filtered through a 15.0 cm number 4 Whatman paper. The residue was washed with hot water, stirred once with a spatula and transferred to a porcelain dish. The sample was dried overnight at 105°C. After drying, it was transferred to a desiccator and weighed as W₁. It was then burnt in a muffle furnace at 500°C for 6 hours, allowed to cool, and reweighed as W₂. The crude fiber content was calculated as:

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

W₁ = Weight of crucible + fiber + ash; W₂ = Weight of crucible + ash; W₀ = Dry weight of food sample.

Crude fat content

The Soxhlet extraction method described by AOAC (2010) was used in determining fat contents of the samples. Two grams (2 g) of the flour/biscuit sample were weighed into a weighed flat bottom flask, with the extractor mounted on it. The thimble was held half way into the extractor and the

weighed sample. Extraction was carried out using boiling point of hexane (40 - 60°C). The thimble was plugged with cotton wool. At completion of extraction which lasted for 8 hours, the solvent was removed by evaporation on a water bath and the remaining part in the flask was dried at 80°C for 30 minutes in the air oven to dry the fat and then cooled in a desiccator. The flask was reweighed and percentage fat content calculated as follows:

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

Protein content

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

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

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

Carbohydrate content

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

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

Evaluation of the physical properties of bread samples

All the various bread samples were analyzed for length, height, weight, volume, density, oven spring and specific volume.

Length

The lengths of the bread samples were measured with a meter rule.

Height

Loaf height was measured using a meter rule (Okereke, 2023). Three loaves of bread were placed on a level laboratory bench and the heights of their middle sections were taken with a meter rule. The average height of the three loaves of the bread was calculated and taken as the height of the bread sample. This procedure of measuring height of bread was done for all the six bread samples.

Oven spring

Oven spring of the bread sample was determined from the differences in height of dough before and after baking.

Weight

The weights of the bread samples were determined by weighing them with an electronic balance.

Weight loss

The weight loss of the bread sample was determined from the difference in the weight of the dough before and after baking (Kim *et al.*, 2003), and calculated as percentage weight loss as:

$$\% \text{ WL} = \left(\frac{\text{Weight of dough} - \text{Weight of bread}}{\text{Weight of dough}} \right) \times 100$$

Where: WL = Weight Loss of Bread Sample.

Volume

The volume of the bread sample was determined by seed displacement method as described by Hallén *et al.* (2004). A medium sized transparent bucket was filled with rice grains and then poured into a bowl. A bread sample was put into the transparent bucket; the rice grains were poured into the bucket and levelled. The remaining rice grains were poured into a measuring cylinder and the volume was obtained.

Specific volume

Specific volume was obtained by dividing the loaf volume of the bread sample by its corresponding volume

(Okereke, 2023) as shown:

$$\text{Specific Volume} = \left(\frac{\text{Loaf Volume}}{\text{Loaf Weight}} \right)$$

Sensory evaluation of the bread samples

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

Experimental design

The experiments were based on completely randomized design. Seven (7) samples of wheat flour, sweet orange seed concentrate and their blends were generated in triplicates for each experiment on the functional and pasting properties, yielding twenty-one (21) samples/experiment analyzed; and two (2) samples of wheat flour and sweet orange seed protein concentrate were generated in triplicates for each experiment on the proximate composition, yielding six samples/experiment analyzed; while six (6) samples of bread samples were generated in triplicates for each experiment on the proximate composition, physical and sensory properties, yielding eighteen (18) samples per experiment analyzed.

Statistical analysis

Data obtained were analyzed as means of triplicate values by one way analysis of variance (ANOVA) in completely randomized design using Statistical Package for Social Science (SPSS) Version 20.00. The statistically significant differences were separated using the Duncans Multiple Range Test (DMRT) at $p < 0.05$

RESULTS AND DISCUSSION

Proximate composition of wheat flour and sweet orange seed protein concentrate

Table 3 shows the proximate composition of wheat flour and sweet orange protein concentrate. The crude fibre,

crude protein, ash and crude fat contents were significantly ($p < 0.05$) higher in the sweet orange seed protein concentrate than in wheat flour, but there was no significant ($p < 0.05$) difference in the moisture contents. However, carbohydrate content in wheat flour (26.38%) was significantly ($p < 0.05$) higher than in sweet orange seed protein concentrate (10.40%). The protein content of PC was lower than the reported values of 75.56% by Mao and Hua (2012) and 72.3 – 77.3% by Lee *et al.* (2016). The results have shown that sweet orange seed protein concentrate is a high nutrient-dense derivative of sweet orange seeds, that could be utilized in supplementation or fortification of food products like bread (Mao and Hua, 2012; Lee *et al.*, 2016).

Functional properties of the flours

Table 4 shows the functional properties of sweet orange seed protein concentrate, wheat flour and the flour blends. Functional properties are characteristics of flour that affect its behavior and that of the products to which it is added during food processing (Akubor *et al.*, 2023a). They determine the suitability of a flour or flour blend for a particular purpose (Damak *et al.*, 2022a). The values for bulk density ranged from 0.45 to 0.72 g/cm³, with sweet orange seed protein concentrate having the least value while 100% wheat flour had the highest value. The bulk density of the blends decreased with the increase in the level of incorporation of sweet orange seed protein concentrate, which was similar to the report of Ocheme *et al.* (2018). The lower bulk density of the flour blends (i.e. compared to 100% wheat flour) could be as a result of reduction in carbohydrate content, which has been reported to have high bulk density (Gernah *et al.*, 2011; Ocheme *et al.*, 2018). Bulk density is generally affected by the particle size and density of the flour and it is very important in determining the packaging requirement, material handling and application in wet processing in the food industry (Adeleke and Odedeji, 2010; Damak *et al.*, 2022a; Akubor *et al.*, 2023). High bulk density is desirable for greater ease of dispersibility and reduction of paste thickness (Akubor *et al.*, 2023). However, low bulk density of flour is a good physical attribute in transportation and storability since the products could be easily transported and distributed to the required locations. Low bulk density increases appetite for food, which translates to consumption of more nutrients. This property is an asset in formulation of complementary foods (Akpata and Akubor, 1999; Chandra *et al.*, 2015; Akubor *et al.*, 2023b; Akubor *et al.*, 2023). The foam capacities (amount of interfacial area created by protein) of sweet orange seed protein concentrate, wheat flour and the blends ranged from 4.55 to 6.60%. The highest foaming capacity was observed for sweet orange seed protein concentrate and the lowest for 100% wheat flour. The foaming capacity of

Table 3. Proximate composition of wheat flour and sweet orange seed protein concentrate.

Samples	Crude fiber	Crude protein	Moisture	Ash	Crude fat	Carbohydrate
WFPC ₀	0.34 ^b ±0.01	12.96 ^b ±0.01	11.37 ^a ±0.01	0.85 ^b ±0.01	1.82 ^b ±0.01	26.38 ^a ±0.01
PC	5.93 ^a ±0.28	59.05 ^a ±0.21	11.51 ^a ±0.99	2.63 ^a ±0.14	10.49 ^a ±0.49	10.40 ^b ±0.28

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different ($p < 0.05$). WFPC₀ = 100% Sweet orange seed protein concentrate; PC = 100% Sweet orange seed protein concentrate.

Table 4. Functional properties of wheat flour, sweet orange seed protein concentrate and the flour blends.

Sample	Bulk density (g/cm ³)	Foaming capacity (%)	Least gelation concentration (%)	Water absorption capacity (g/g)	Oil absorption capacity (g/g)
PC	0.45 ^c ±0.01	6.60 ^a ±0.14	4.00	3.15 ^a ±0.07	1.25 ^a ±0.07
WFPC ₀	0.72 ^a ±0.03	4.55 ^e ±0.07	8.00	0.55 ^e ±0.07	1.02 ^d ±0.02
WFPC ₁	0.71 ^a ±0.02	4.75 ^d ±0.07	8.00	0.65 ^{de} ±0.07	1.12 ^c ±0.03
WFPC ₂	0.69 ^a ±0.04	4.96 ^c ±0.08	8.00	0.75 ^{cd} ±0.07	1.15 ^{bc} ±0.01
WFPC ₃	0.67 ^{ab} ±0.01	5.01 ^c ±0.02	6.00	0.85 ^c ±0.07	1.16 ^{bc} ±0.02
WFPC ₄	0.65 ^{ab} ±0.01	5.28 ^{bc} ±0.04	6.00	0.85 ^c ±0.07	1.18 ^{abc} ±0.02
WFPC ₅	0.61 ^b ±0.05	5.38 ^b ±0.04	6.00	1.11 ^b ±0.02	1.21 ^{ab} ±0.01

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different ($p < 0.05$). PC = 100% Sweet orange seed protein concentrate; WFPC₀ = 100% Wheat flour; WFPC₁ = Flour blend (95% wheat flour: 5% Sweet orange seed protein concentrate); WFPC₂ = Flour blend (90% wheat flour: 10% Sweet orange seed protein concentrate); WFPC₃ = Flour blend (85% wheat flour: 15% Sweet orange seed protein concentrate); WFPC₄ = Flour blend (80% wheat flour: 20% Sweet orange seed protein concentrate); WFPC₅ = Flour blend (70% wheat flour: 30% Sweet orange seed protein concentrate).

composite flours increased with an increase in the level of incorporation of sweet orange seed protein concentrate probably due to the additional effect of protein, which is in agreement with the reports of Chandra *et al.* (2015) and Ocheme *et al.* (2018). Flours with high foaming ability could form large air bubbles (easier to collapse) surrounded by thinner or a less flexible protein film (Chandra *et al.*, 2015). The values for the least gelation concentration (the lowest protein concentration at which gel remained in the inverted tube) varied from 4.00 to 8.00% with sweet orange seed protein concentrate having the lowest value. The decrease in the least gelation concentration of composite flours as compared to 100% wheat flour could be attributed to the increased level of incorporation of sweet orange seed protein concentrate and the variations in the gelling properties of the flours, which may be due to variations in the ratio of different constituents such as carbohydrates, lipids and proteins that make up the flours (Adeleke and Odedeji, 2010; Chandra *et al.*, 2015). These values (4-8%) are lower than the reported values of various legume flours (10 -16%) (Hasmadi *et al.*, 2020). The lower the least gelation concentration, the better the gelation capacity of the ingredient as a small amount is needed to form gel in a measured volume of water (Okereke, 2023). The water absorption capacities varied from 0.55 to 3.15 g/g. The incorporation of 5% sweet orange seed protein

concentrate in wheat flour did not cause any significant ($p < 0.05$) difference in water absorption capacity. However, there was a significant difference ($p > 0.05$) in the water absorption capacity of the blends with 10% sweet orange seed protein concentrate and above. The increase in water absorption capacity could be attributed to the high water absorption capacity of sweet orange seed protein concentrate, which probably improved the structural matrix for holding water, sugars and other components (Jideani, 2011). Flour blends of high-water absorption capacities are utilized in formulation of foods such as sausage, dough, processed cheese and bakery products (Chandra *et al.*, 2015; Okereke *et al.*, 2022a). The oil absorption capacities of sweet orange protein concentrate and the flour blends ranged from 1.02 to 1.25g/g with sweet orange seed protein concentrate having the highest value while 100% wheat flour had the least value. There was slight increase in oil absorption capacities of the flour blends with increasing levels of incorporation of sweet orange seed protein concentrate. Oil absorption capacity is useful in structure interaction in food, especially, in flavor retention, mouth-feel, improved palatability and extension of shelf life particularly in soups, bakery and meat products (Okereke *et al.*, 2022a; Okereke, 2023). The mechanism of oil absorption is attributed mainly to the physical entrapment of oil and the binding of fat to the apolar chain of protein (Adeleke and Odedeji, 2010).

Table 5. Pasting properties of wheat flour, sweet orange seed protein concentrate and the flour blends.

Sample	Peak viscosity (RVU)	Trough viscosity (RVU)	Break down (RVU)	Final viscosity (RVU)	Setback viscosity (RVU)	Peak time (min)	Pasting temperature (°C)
PC	886.21 ^a ±0.30	789.13 ^a ±0.18	88.06 ^a ±0.08	1310.00 ^a ±0.14	502.07 ^a ±0.09	5.70 ^f ±0.04	88.32 ^f ±0.03
WFPC ₀	675.20 ^b ±0.28	632.17 ^b ±0.24	43.11 ^b ±0.16	984.27 ^b ±0.38	352.09 ^b ±0.13	6.03 ^e ±0.04	88.71 ^f ±0.01
WFPC ₁	548.08 ^c ±0.11	527.06 ^c ±0.08	21.22 ^c ±0.31	830.31 ^c ±0.44	303.25 ^c ±0.35	6.34 ^d ±0.01	89.61 ^e ±0.01
WFPC ₂	412.27 ^d ±0.38	401.20 ^d ±0.28	21.09 ^c ±0.01	626.16 ^d ±0.25	225.20 ^d ±0.28	6.81 ^e ±0.01	90.81 ^d ±0.0c1
WFPC ₃	323.17 ^e ±0.23	308.14 ^e ±0.20	18.16 ^d ±0.23	497.09 ^e ±0.13	189.16 ^e ±0.23	6.88 ^b ±0.01	92.01 ^c ±0.01
WFPC ₄	327.14 ^f ±0.20	219.18 ^g ±0.25	15.20 ^e ±0.28	375.08 ^g ±0.11	156.11 ^f ±0.16	6.88 ^b ±0.01	93.21 ^b ±0.01
WFPC ₅	352.15 ^e ±0.21	238.13 ^f ±0.	11.14 ^f ±0.20	401.06 ^f ±0.08	151.10 ^g ±0.14	6.91 ^a ±0.01	94.31 ^a ±0.01

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different ($p < 0.05$). PC = 100% Sweet orange seed protein concentrate; WFPC₀ = 100% Wheat flour; WFPC₁ = Flour blend (95% wheat flour: 5% Sweet orange seed protein concentrate); WFPC₂ = Flour blend (90% wheat flour: 10% Sweet orange seed protein concentrate); WFPC₃ = Flour blend (85% wheat flour: 15% Sweet orange seed protein concentrate); WFPC₄ = Flour blend (80% wheat flour: 20% Sweet orange seed protein concentrate); WFPC₅ = Flour blend (70% wheat flour: 30% Sweet orange seed protein concentrate).

Pasting properties of wheat flour, sweet orange seed protein concentrate and the blends

The pasting properties of wheat flour, sweet orange seed protein concentrate and the blends are presented in Table 5. The peak viscosities ranged from 323.17 to 886.21 RVU, with sweet orange seed protein concentrate having the highest value while the lowest value was observed in the blend containing 15% protein concentrate. The peak viscosity is indicative of the strength of paste, which are formed during gelatinization in food applications. Peak viscosity, also linked to water absorption capacity and the finished product quality, demonstrates the starch's capacity to swell freely before starch's physical and structural breakdown (Onimawo and Akubor, 2015). Peak viscosity also indicates the viscous loads likely to be encountered during mixing (Okereke *et al.*, 2021b). The trough viscosities ranged from 219.18 to 789.13 RVU with PC (sweet orange seed protein concentrate) having the highest value while WFPC₄ (blend containing 20% sweet orange seed protein concentrate) had the least value. There was a significant difference ($p < 0.05$) between the values of the trough viscosities of the flours. There was decrease in trough viscosity with the increase in the level of incorporation of sweet orange seed protein concentrate. Trough viscosity is a measure of the least viscosity that a paste can have while still remaining stable upon cooling. The breakdown viscosities of the flours ranged from 11.14 to 88.06 RVU with sweet orange seed protein concentrate having the highest value while WFPC₅ (the blend containing 30% concentrate) had the least value. Breakdown viscosity reflects the stability of the paste during processing (Okereke *et al.*, 2022b). The higher the breakdown viscosity, the lower the ability of the starch in the flour sample to withstand heating and shear stress during processing (Ofia-olua, 2014; Okereke *et al.*, 2022b). The high breakdown viscosity shows low level of

stability of the paste. The values for final viscosity of the flours ranged from 375.08 to 1310.00 RVU, with sample PC (sweet orange seed protein concentrate) having the highest value while WFPC₄ (the blend containing 20% sweet orange seed protein concentrate) had the lowest value. There was significant difference ($p < 0.05$) among the flour samples. Final viscosity, which reflects flour's capacity to transform into viscous paste upon cooking and cooling, is used to describe flour quality. The setback viscosity of the flours ranged from 151.10 to 502.07 RVU. The highest value was observed in PC (sweet orange seed protein concentrate) while the WFPC₅ (blend containing 30% sweet orange seed protein concentrate) had the lowest value with a significant difference among flours. Setback viscosity indicates gel stability and potential for retrogradation (Okereke, 2023). High setback values are indication of the propensity of the starch molecules to disperse in hot paste and re-associate readily during cooling (Chinma *et al.*, 2010). The peak time values ranged from 5.70 to 6.91 min. The cooking time of paste is judged by the peak time. The attainment of the pasting temperature is essential in ensuring swelling, gelatinization and subsequent gel formation during processing. The pasting temperature (i.e the temperature at which the viscosity starts to rise) ranged from 88.32 to 94.31°C, with sample PC having the lowest pasting temperature while sample WFPC₅ had the highest value. The decrease in peak viscosity, trough viscosity, breakdown and final viscosity with increasing levels of substitution of wheat flour in composite flours was similar to the results of (Ofia-olua, 2014; Ocheme *et al.*, 2016; Imoisi *et al.*, 2020).

Proximate composition of the bread samples

The proximate composition of the bread samples from the

Table 6. Proximate composition (%) of breads made from 100% wheat flour, and blends of wheat flour and sweet orange seed protein concentrate.

Sample	Crude fibre	Crude protein	Moisture	Ash	Crude fat	Carbohydrate
WPB ₀	3.90 ^d ±0.06	13.07 ^f ±0.04	15.98 ^c ±0.05	1.03 ^c ±0.02	4.15 ^d ±0.03	61.88 ^a ±0.02
WPB ₁	3.94 ^c ±0.02	16.19 ^e ±0.08	15.86 ^d ±0.02	1.06 ^c ±0.02	4.18 ^{cd} ±0.02	58.79 ^b ±0.12
WPB ₂	4.04 ^c ±0.02	19.28 ^d ±0.04	16.05 ^c ±0.04	1.09 ^c ±0.01	4.27 ^c ±0.04	55.29 ^c ±0.07
WPB ₃	4.49 ^b ±0.04	22.32 ^c ±0.05	17.06 ^b ±0.03	2.03 ^b ±0.02	4.70 ^b ±0.04	49.42 ^d ±0.01
WPB ₄	4.86 ^a ±0.05	26.06 ^b ±0.07	17.07 ^b ±0.06	2.35 ^a ±0.12	4.91 ^a ±0.06	44.77 ^e ±0.12
WPB ₅	4.89 ^a ±0.01	29.10 ^a ±0.09	17.38 ^a ±0.04	2.41 ^a ±0.11	5.00 ^a ±0.04	41.23 ^f ±0.21

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different ($p < 0.05$). WPB₀ = Bread made of 100% Wheat flour; WPB₁ = Bread from Flour blend (95% wheat flour: 5% Sweet orange seed protein concentrate); WPB₂ = Flour blend (90% wheat flour: 10% Sweet orange seed protein concentrate); WPB₃ = Flour blend (85% wheat flour: 15% Sweet orange seed protein concentrate); WPB₄ = Flour blend (80% wheat flour: 20% Sweet orange seed protein concentrate); WPB₅ = Flour blend (70% wheat flour: 30% Sweet orange seed protein concentrate).

%100 wheat flour, and blends of wheat flour and sweet orange protein concentrate is presented in Table 6. The crude fibre content, which increased significantly ($p < 0.05$) with increasing levels of sweet orange seed protein concentrate substitution ranged from 3.90 to 4.88%. The crude fiber contents of the breads containing sweet orange seed protein concentrate were slightly higher than that of the 100% wheat flour bread. However, the value for 100% wheat flour bread was not significantly different ($p < 0.05$) from bread containing 5% sweet orange seed protein concentrate. Studies have suggested relationship between decrease in fiber consumption and increase in certain disease such as diverticulosis and colonic cancer (Kamaljit *et al.*, 2011; Okereke, 2023). Dietary fiber acts as bulking agent, increasing intestinal motility and wet faecal mass of faeces (Kamaljit *et al.*, 2011; Damak *et al.*, 2022b). These effects are important in reducing diseases of the colon. Some reports showed that some plant fibers can lower serum cholesterol (Kamaljit *et al.*, 2011). The crude protein contents of the breads ranged from 13.07 to 29.10%. The increase in crude protein was due to the increase in the incorporation of sweet orange seed protein concentrate. Protein is essential for body building and the repair of worn-out tissues by formation of new cells (Akubor *et al.*, 2023b). The moisture contents of the breads varied from 15.97 to 17.38%. The increase in moisture content of composite bread could be attributed to the higher water absorption capacity of the composite flours. Moisture is necessary for the keeping quality of bread but high moisture has negative effect on storage stability of bread. The ash contents of the breads ranged from 1.03 to 2.40%, increasing significantly ($p < 0.05$) with increasing levels of sweet orange seed protein concentrate incorporation. Adeleke and Odedeji (2010) reported similar results on bread made from wheat and sweet potato flour blends. The higher ash contents of composite flour breads indicated increase in minerals, which was contributed by sweet orange seed protein concentrate. The fat contents of the breads increased significantly ($p < 0.05$) with increasing

level of sweet orange seed protein concentrate due to the addition of sweet orange seed protein concentrate. Functionality of fat such as emulsifying capacity will also affect bread texture and bubble formation. Composite bread samples with significantly ($p < 0.05$) high fat content will be more palatable since fat increases food palatability (Bolarinwa *et al.*, 2019). The carbohydrate contents of the breads ranged from 41.22 to 61.87%. The decrease in carbohydrate contents of the composite flour breads could be ascribed to the increase in the contents of other components such as protein, fat, crude fibre and ash.

Physical properties of the bread samples

The physical properties of bread samples prepared from 100% wheat flour, and blends of wheat flour and sweet orange seed protein concentrate are presented in Table 7. The length of breads decreased with the level of the incorporation of sweet orange seed protein concentrate, which ranged from 11.99 to 12.03 cm. The lengths were not significantly ($p < 0.05$) different among the composite flour bread samples. The weights of the bread samples ranged from 163.80 to 172.60 cm. The weight of 100% wheat flour bread was not significantly different ($p > 0.05$) from wheat bread incorporated with 5% sweet orange seed protein concentrate. The result is in disagreement with the reports of Nwosu *et al.* (2014) and Kiin-Kabari (2015) who reported increased weights of composite breads due to substitution effects, but in agreement with the report of Okereke *et al.* (2021a) that had a contrary observation. The height of the bread samples, which decreased with the increase in the level of incorporation of sweet orange seed protein concentrate ranged from 3.68 to 5.35 cm. The loaf volumes decreased with increase in the level of incorporation of sweet orange seed protein concentrate. The height of 100% wheat flour bread was significantly different ($p > 0.05$) from the heights of the composite flour breads. The oven spring of breads, which

Table 7. Physical properties of breads made from 100% wheat flour, and blends of wheat flour and sweet orange seed protein concentrate.

Sample	Length (cm)	Loaf weight (g)	Height (cm)	Oven Spring (cm)	Loaf volume (cm ³)	Specific volume (cm ³ /g)	Weight loss (%)
WPB ₀	12.03 ^a ±0.01	172.60 ^a ±0.99	5.35 ^a ±0.07	0.62 ^a ±0.03	471.50 ^a ±2.12	2.73 ^a ±0.01	10.42 ^a ±0.03
WPB ₁	12.02 ^a ±0.01	171.38 ^a ±0.03	4.59 ^b ±0.02	0.61 ^a ±0.01	350.50 ^b ±2.12	2.04 ^b ±0.02	9.29 ^b ±0.02
WPB ₂	12.01 ^a ±0.02	166.50 ^b ±0.71	4.42 ^c ±0.02	0.41 ^b ±0.02	278.50 ^c ±2.12	1.67 ^c ±0.01	8.88 ^c ±0.03
WPB ₃	12.01 ^a ±0.01	166.05 ^b ±0.50	4.22 ^d ±0.03	0.41 ^b ±0.01	277.50 ^c ±3.54	1.67 ^c ±0.02	8.87 ^c ±0.04
WPB ₄	11.99 ^a ±0.02	163.80 ^c ±0.28	4.05 ^e ±0.071	0.31 ^c ±0.02	239.00 ^d ±1.41	1.46 ^d ±0.01	8.78 ^d ±0.04
WPB ₅	11.99 ^a ±0.03	166.50 ^b ±0.71	3.68 ^f ±0.03	0.22 ^d ±0.02	202.50 ^e ±3.54	1.21 ^e ±0.01	8.59 ^e ±0.02

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different ($p < 0.05$). WPB₀ = Bread made of 100% Wheat flour; WPB₁ = Bread from Flour blend (95% wheat flour: 5% Sweet orange seed protein concentrate); WPB₂ = Flour blend (90% wheat flour: 10% Sweet orange seed protein concentrate); WPB₃ = Flour blend (85% wheat flour: 15% Sweet orange seed protein concentrate); WPB₄ = Flour blend (80% wheat flour: 20% Sweet orange seed protein concentrate); WPB₅ = Flour blend (70% wheat flour: 30% Sweet orange seed protein concentrate).

is the difference between the height of the baked bread and the height of the dough before baking, ranged from 0.22 to 0.62 cm. The oven spring values showed that sample WPB₀ (100% wheat flour bread) had the highest value while sample WPB₅ had the least value. However, there was no significant difference ($p < 0.05$) between WPB₀ (100% wheat flour bread) and WPB₁ (bread containing 5% sweet orange seed protein concentrate). The loaf volumes of breads which also decreased with the increase in the level of incorporation of sweet orange seed protein concentrate varied from 202.50 g/mL (WPB₅) to 471.50 g/mL (WPB₀) The values for loaf volume of 100% wheat flour bread was significantly different ($p > 0.05$) from breads incorporated with sweet orange seed protein concentrate. The decrease in loaf volume may be due to dilution of wheat flour, which resulted in low gluten content (Ameh *et al.*, 2013; Okereke *et al.*, 2021a). Flours with high gluten content have ability to extend and trap CO₂ produced during fermentation (Onimawo and Akubor, 2012); which beneficially increases loaf volume of bread. The decrease in height and length of the breads may be due to high water absorption capacity of sweet orange seed protein concentrate, similar to the result obtained by Akubor and Eze (2012). Ingredient which absorbs water during mixing reduces height of the product after baking. Another reason for the reduced height could be due to the reduced gluten contents of the blends (Akubor and Eze, 2012). The specific volumes of the bread samples decreased with the increase in the level of incorporation of sweet orange seed protein concentrate with values that ranged from 1.21 to 2.73 m³/g, with 100% wheat flour bread (WPB₀) having the highest values while bread substituted with 30% sweet orange seed protein concentrate (WPB₅) had the least score. The low specific volume of the composite breads may be attributed to the reduced gluten content for gluten is responsible for enhancing dough rise in leavened wheat flour bread

(Amandikwa *et al.*, 2015). The weight loss of breads ranged from 8.59% in WPB₅ to 10.42% in WPB₀, values decreased with increase in the level of incorporation of sweet orange seed protein concentrate. The weight loss of 100% wheat bread was significantly different from composite flour breads. The weight loss of bread is determined by the amount of moisture and carbon dioxide diffused out of the loaf during baking (Shittu *et al.*, 2007).

Sensory properties of the bread samples

The sensory properties of breads prepared from blends of wheat flour and sweet orange seeds protein concentrate are presented on Table 8. Sensory properties are significant in acceptance of food products by consumers. The scores for the colour of the breads ranged from 5.90 - 8.30. The breads containing 5%, 10% and 15% sweet orange seed protein concentrate were not significantly different ($p < 0.05$) from the 100% wheat flour bread for colour. The scores for the flavour of the breads ranged from 5.65 - 7.80. The effect of addition of sweet orange seed protein concentrate had a significant difference ($p > 0.05$) on the flavour. The scores for the taste of the breads ranged from 3.50 - 7.80 where the addition of sweet orange seed protein concentrate caused significant difference ($p > 0.05$) in the taste of the breads. The scores for mouth feel of the breads varied from 3.85- 7.90. The scores for the texture (softness and chewiness) ranged from 5.15 - 8.00. The baking conditions (temperature and time), state of the bread components such as fibers, protein (gluten), starch (whether damaged or undamaged) and the amount of water absorbed during dough mixing, contribute to final texture of bread (Amandikwa *et al.*, 2015). The scores for general acceptability of breads ranged from 4.25 - 8.45. The incorporation of sweet orange seed protein concentrate had a significant effect ($p > 0.05$)

Table 8. Sensory properties of the bread samples.

Samples	Color	Flavour	Taste	Mouthfeel	Texture	General acceptability
WPB ₀	8.30 ^a ±1.17	7.80 ^a ±1.11	7.60 ^a ±1.85	7.90 ^a ±1.21	8.00 ^a ±0.79	8.45 ^a ±0.69
WPB ₁	7.50 ^a ±1.32	6.40 ^b ±1.85	4.70 ^b ±2.03	5.10 ^b ±1.89	6.30 ^b ±1.69	5.65 ^b ±2.30
WPB ₂	7.30 ^a ±1.49	6.15 ^b ±1.81	4.10 ^b ±1.89	4.10 ^b ±1.97	6.25 ^b ±1.86	5.05 ^b ±2.28
WPB ₃	7.15 ^a ±1.39	6.65 ^b ±1.63	4.50 ^b ±2.37	3.90 ^b ±2.07	6.25 ^b ±1.86	4.95 ^b ±2.70
WPB ₄	5.55 ^b ±2.54	5.85 ^b ±2.25	3.55 ^b ±2.26	4.00 ^b ±2.64	5.90 ^b ±2.38	4.35 ^b ±2.54
WPB ₅	5.90 ^b ±2.25	5.65 ^b ±1.73	3.50 ^b ±2.14	3.85 ^b ±2.56	5.15 ^b ±2.52	4.25 ^b ±2.67

Values are means ± standard deviation of 3 replications. Means within a column not followed by the same superscript are significantly different ($p < 0.05$). WPB₀ = Bread made of 100% Wheat flour; WPB₁ = Bread from Flour blend (95% wheat flour: 5% Sweet orange seed protein concentrate); WPB₂ = Flour blend (90% wheat flour: 10% Sweet orange seed protein concentrate); WPB₃ = Flour blend (85% wheat flour: 15% Sweet orange seed protein concentrate); WPB₄ = Flour blend (80% wheat flour: 20% Sweet orange seed protein concentrate); WPB₅ = Flour blend (70% wheat flour: 30% Sweet orange seed protein concentrate).

on the general acceptability of breads. 100% wheat flour bread was rated higher in all the sensory attributes assessed. The scores for all attributes decreased with the increase in the level of incorporation of sweet orange seed protein concentrate.

Conclusion

The results of the study showed that the addition of sweet orange seed protein concentrate in wheat flour improved the functional and pasting properties of the blends, and the qualities of the breads from the blends. By this approach, malnutrition, wastages and environmental pollution caused by discard of orange seeds could be addressed in Nigeria and other developing countries.

Recommendations

The functional and pasting properties of the various blends of wheat flour and orange seed protein concentrate, reveals their potentials for utilizations in the formulations of complementary foods, sauces, confectioneries, thickeners, texturizers, energy-giving foods, cheese, gravies and beverage products. The flours and their blends would be useful in energy and time savings (cost effectiveness) at commercial applications. Incorporation of 10% sweet orange seed protein concentrate in wheat flour bread should be encouraged in a commercial scale to combat protein deficiencies in our diet and also control environmental pollution by orange seeds. Further studies should be carried out using animal model to verify the result while storage studies on the stability of bread containing 10% sweet orange seed protein, should be researched.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

- Adeleke, R. O., & Odedeji, J. O. (2010). Functional properties of wheat and sweet potato flour blends. *Pakistan journal of Nutrition*, 9(6), 535-538.
- Adeyeye, S. A. O., Bolaji, O. T., Abegunde, T. A., Adebayo-Oyetoro, A. O., Tihamiyu, H. K., & Idowu-Adebayo, F. (2019). Quality characteristics and consumer acceptance of bread from wheat and rice composite flour. *Current Research in Nutrition and Food Science*, 7(2), 488-495.
- Adhikari, S., Schop, M., de Boer, I. J. M., & Huppertz, T. (2022). Protein quality in perspective: A review of protein quality metrics and their applications. *Nutrients*, 14(5), 947.
- Adubofuor, J., Akyereko, Y. G., Batsa, V., Apeku, O. J. D., Amoah, I., & Diako, C. (2021). Nutrient composition and physical properties of two orange seed varieties. *International Journal of Food Science*, Volume 2021, Article ID 6415620, 11 pages.
- Akubor, P. I., & Eze, J. I. (2012). Quality evaluation and cake making potential of sun and oven dried carrot fruit. *International Journal of Biosciences*, 10(2), 19-27.
- Akubor, P. I., & Gambo, J. (2020). Production and quality evaluation of bread from wheat and pregelatinized cassava flour blends incorporated with carboxyl methylcellulose. *South Asian Journal of Food Technology and Environment*, 6(2), 943-953.
- Akubor, P. I., Agada, T. O., & Okereke, G. O. (2023a). Quality evaluation of noodles produced from blends of wheat, unripe banana and cowpea flours. *Journal of Nutrition Food Science and Technology*, 4(2), 1-13.
- Akubor, P. I., Onogwu, O. C., Okereke, G. O., & Damak, A. M. A. (2023b). Production and quality evaluation of gluten free biscuits from maize and soybean flour blends. *European Journal of Nutrition & Food Safety*, 15(8), 59-79.
- Akubor, P. I., Owolagba, D., & Ikram, A. (2023). Quality evaluation of *Akamu* powder incorporated with fermented sweet orange pulp flour. *Journal of Culinary Science & Technology*, 1-15.
- Amandikwa, C., Iwe, M. O., Uzoma, A., & Olawani, A. I. (2015). Physico-chemical properties of wheat-yam flour composite bread. *Nigerian food Journal*, 10(1), 115-125.
- Ameh, M. O., Gernah, D. I., & Igbabul, B. D. (2013). Physico-chemical and sensory evaluation of wheat bread supplemented with stabilized undefatted rice bran. *Food and Nutrition Sciences*, 4(9), 43-48.

- AOAC (2010). Official Methods of Analysis 18th edition, Revision 3, Association of Official Analytical Chemists. Washington DC.
- Badifu, I. O., Chima, C. E., Ajayi, Y. I., & Ogoro, A. F. (2005). Influence of mango mesocarp flour Supplementation on micronutrient, physical and organoleptic qualities of wheat based bread. *Nigerian Food Journal*, 23, 59-68.
- Bolarinwa, I. F., Aruna, T. E., & Raji, A. O. (2019). Nutritive value and acceptability of bread fortified with moringa seed powder. *Journal of the Saudi Society of agricultural Sciences*, 18(2), 195-200.
- Britannica, T. (2023). Editors of Encyclopaedia. *Protein Concentrate*. *Encyclopedia Britannica*. Retrieved from <https://www.britannica.com/topic/protein-concentrate>.
- Ceserani, V., Kinkton, R., & Fosket, D. (1995). *Practical cookery*, 8th Edition. Hodder and stronghton.
- Chandra, S., Singh, S. & Kumari, D. (2015). Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of Food Science and Technology*, 52(6), 3681-3688.
- Chikpah, S. K., Korese, J. K., Hensel, O., Sturm, B., & Pawelzik, E. (2023). Influence of blend proportion and baking conditions on the quality attributes of wheat, orange-fleshed sweet potato and pumpkin composite flour dough and bread: optimization of processing factors. *Discover Food*, 3, Article number 2.
- Chinma, C. E., Abu, J. O., & Ojo, J. A. (2010). Chemical, functional and pasting properties of starches from brown and yellow tigernuts (*Cyperus esculentus*). *Nigerian Food Journal*, 28(2), 1-13.
- Coffmann, C. W., & Garciaj, V. V. (1977). Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science & Technology*, 12(5), 473-484.
- Coțovanu, I., Mironeasa, C., & Mironeasa, S. (2023). Nutritionally improved wheat bread supplemented with quinoa flour of large, medium and small particle sizes at typical doses. *Plants*, 12(4), 698.
- Damak, A. M. A., Akubor, P. I., Ariahu, C. C., & Okereke, G. O. (2022a). Functional and nutritional properties of various flour blends of arrowroot starch and wheat flour. *Asian Food Science Journal*, 21(6), 12-25.
- Damak, A. M., Akubor, P. I., Ariahu, C. C., & Okereke, G. O. (2022b). Evaluation of bread made from various blends of arrowroot starch and wheat flour. *Journal of Food Chemistry & Nanotechnology*, 8(3), 108-118.
- Dhingra, D., Michael, M., Rajput, H., & Patil, R. T. (2012). Dietary fibre in foods: A review. *Journal of Food Science and Technology*, 49, 255-266.
- Di Majo, D., Giammanco, M., La Guardia, M., Tripoli, E., Giammanco, S., & Finotti, E. (2005). Flavanones in Citrus fruit: Structure–antioxidant activity relationships. *Food Research International*, 38(10), 1161-1166.
- El-Safy, F. S., Salem, R. H., & Abd El-Ghany, M. E. (2012). Chemical and nutritional evaluation of different seed flours as novel sources of protein. *World Journal of Dairy & Food Sciences*, 7(1), 59-65.
- Emojorho, E. E., & Akubor, P. I. (2016). Effect of debittering methods on the minerals, and phytochemical properties of orange (*Citrus Sinensis*) seeds flour. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 10(9), 34-139.
- Farag, M. A., Abib, B., Ayad, L., & Khattab, A. R. (2020). Sweet and bitter oranges: An updated comparative review of their bioactives, nutrition, food quality, therapeutic merits and biowaste valorization practices. *Food Chemistry*, 331, 127306.
- Gernah, D. I., Ariahu, C. C., & Ingbian, E. K. (2011). Effects of malting and lactic fermentation on some chemical and functional properties of maize (*Zea mays*). *American Journal of Food Technology*, 6(5), 404-412.
- Hallén, E. İbanođlu, Ş., & Ainsworth, P. (2004). Effect of flour addition on the rheological and baking properties of wheat flour. *Journal of Food Engineering*, 63(2), 177-184.
- Hasmadi, M., Noorfarahzilah, M., Noraidah, H., Zainol, M. K., & Jahurul, M. H. A. (2020). Functional properties of composite flour: A review. *Food Research*, 4(6), 1820-1831.
- Ihekoronye, A. I., & Ngoddy, P. O. (1985). *Integrated food science and technology for the tropics*. Macmillian Publishers Ltd, London. Pp. 10-22, 270-288.
- Imoisi, C., Iyasele, J. U., Imhontu, E. E., Ikpahwore, D. O., & Okpebho, A. O. (2020). Pasting properties of Composite cassava and wheat flours. *Journal of Chemical Society of Nigeria*, 45(6), 1157-1163.
- Janssen, F., Wouters, A. G., & Delcour, J. A. (2021). Gas cell stabilization by aqueous-phase constituents during bread production from wheat and rye dough and oat batter: dough or batter liquor as model system. *Comprehensive Reviews in Food Science and Food Safety*, 20, 3881-3917.
- Jideani, V. A. (2011). Functional properties of soybean food ingredients in food systems. In: Tzbun N. G. (ed.). *Soybean biochemistry, chemistry and physiology* (pp. 345-366). Rijeka, Croatia InTech.
- John, J. A., & Ghosh, B. C. (2021). Production of whey protein hydrolyzates and its incorporation into milk. *Food Production, Processing and Nutrition*, 3, Article number 9.
- Kamaljit, K., Amarjeet, K., & Pal, S. T. (2011). Analysis of ingredients, functionality, formulation optimization and shelf life evaluation of high fiber bread. *American Journal of Food Technology*, 6(4), 306-313.
- Kiin-Kabari, D. B., Eke-Ejiofor, J., & Giami, S. Y. (2015). Functional and pasting properties of wheat/plantain flours enriched with Bambara groundnut protein concentrate. *International Journal of Food Science and Nutrition Engineering*, 5(2), 75-81.
- Kim, H. J., Morita, N., Lee, S. H., & Moon, K. D. (2003). Scanning electron microscopic observations of dough and bread supplemented with *Gastrodia elata* Blume powder. *Food Research International*, 36(4), 387-397.
- Larmond, E. (1991). *Laboratory methods for sensory evaluation of food*. 2nd Edition, Canadian Department of Agriculture Publication, Ottawa. p. 4
- Leal, D. (2022). The effects of protein deficiency. *Nutrition Basics*. Retrieved from <https://www.verywellfit.com/what-are-the-effects-of-protein-deficiency-4160404>.
- Lee, H. J., Park, S. H., Yoon, I. S., Lee, G. W., Kim, Y. J., Kim, J. S., & Heu, M. S. (2016). Chemical composition of protein concentrate prepared from Yellowfin tuna *Thunnus albacares* roe by cook-dried process. *Fisheries and Aquatic Sciences*, 19, Article number 12.
- Malacrida, C. R., Kimura, M., & Jorge, N. (2012). Phytochemicals and antioxidant activity of citrus seed oils. *Food Science and Technology Research*, 18(3), 399-404.
- Mao, X., & Hua, Y. (2012) Composition, structure and functional properties of protein concentrates and isolates produced from walnut (*Juglans regia* L.). *International Journal of Molecular Sciences*, 13(2), 1561-1581.

- McSweeney, D. J., Maidannyk, V., Montgomery, S., O'Mahony, J. A., & McCarthy, N. A. (2020). The influence of composition and manufacturing approach on the physical and rehydration properties of milk protein concentrate powders. *Foods*, 9(2), 236.
- Meganaharshini, M., Sudhakar, V., Dhivya Bharathi, N., & Deepak, S. (2023). Review on recent trends in the application of protein concentrates and isolates – A food industry perspective. *Food and Humanity*, 1, 308-325.
- Menon, L., Majumdar, S. D., & Ravi, U. (2015). Development and analysis of composite flour bread. *Journal of Food Science and Technology*, 52(7), 4156-4165.
- Moreno-Araiza, O., Boukid, F., Suo, X., Wang, S., & Vittadini, E. (2023). Pretreated Green Pea Flour as Wheat Flour Substitutes in Composite Bread Making. *Foods*, 12(12), 2284.
- Moser, B. R., Dorado, C., Bantchey, G. B., Winkler-Moser, J. K., & Doll, K. M. (2023). Production and evaluation of biodiesel from sweet orange (*Citrus sinensis*) lipids extracted from waste seeds from the commercial orange juicing process. *Fuel*, 342, 127727.
- Nakakana, H., Sherifat, A. M., Hassan, S. M., & Abdullahi, H. (2023). Production and quality evaluation of enriched bread from flour blends of whole wheat, bambara nut, soybeans and cashew nut seed. *FUDMA Journal of Sciences*, 7(5), 34-38.
- Navarro Del Hierro, J., Cantero-Bahillo, E., Fornari, T. & Martin, D. (2021). Effect of defatting and extraction solvent on the antioxidant and pancreatic lipase inhibitory activities of extracts from *Hermetia illucens* and *Tenebrio molitor*. *Insects*, 12(9), 789.
- Nwosu, J. N., Owuamanam, C. I., Omeire, G. C., & Eke, C. C. (2014). Quality parameters of bread produced from substitution of wheat flour with cassava flour using soybean as an improver. *American Journal of Research Communication*, 2(3), 99-118.
- Ocheme, O. B., Adedeji, O. E., Chinma, C. E., Yakubu, C. M., & Ajibo, U. H. (2018). Proximate composition, functional, and pasting properties of wheat and groundnut protein concentrate flour blends. *Food Science and Nutrition*, 6(5), 1173-1178.
- Ofia-olua, B. I. (2014). Chemical, functional and pasting properties of wheat (*Triticum spp*)-walnut (*Juglans regia*) flour. *Food and Nutrition Sciences*, 5, 1591-1604.
- Okereke, G. O. (2023). Quality evaluation of modified starches from white yam, trifoliate yam, sweet potato and utilization in wheat-*Moringa oleifera* seed-based bakery products. Ph.D Thesis. Benue State University, Makurdi, Nigeria.
- Okereke, G. O., Igbabul, B. D., & Ikya, J. K. (2021a). Evaluation of breads made from wheat flour, modified white yam/trifoliate yam/sweet potato starches and *Moringa oleifera* seed flour blends. *International Journal of Food Science and Nutrition*, 6(2), 107-119.
- Okereke, G. O., Igbabul, B. D., Ikya, J. K., & Araka, O. (2022a). Physicochemical and functional properties of modified starches of white yam, trifoliate yam and sweet potato. *Journal of Food Chemistry and Nanotechnology*, 8(2), 50-60.
- Okereke, G. O., Igbabul, B. D., Ikya, J. K., & Kanu, A. N. (2021b). Utilization of moringa oleifera seeds flour and starches of white yam, trifoliate yam and sweet potato in cookies. *International Journal of Food Science and Nutrition*, 6(4), 39-50.
- Okereke, G. O., Igbabul, B. D., Ikya, J. K., Kanu, N. A. & Damak, A. M. A. (2022b). Proximate composition and pasting properties of modified starches of white yam, trifoliate yam and sweet potato. *World Journal of Food Science and Technology*, 6(3), 58-68.
- Olapade, A. A., Aworh, O. C., & Oluwole, O. B. (2011). Quality attributes of biscuit from acha (*Digitaria exilis*) flour supplemented with cowpea (*Vigna unguiculata*) flour. *African Journal of Food Science and Technology*, 2(9), 198-203.
- Onimawo, I. A., & Akubor, P. I. (2012). *Food Chemistry (Integrated Approach with biochemical background)*. 2nd edition. Joytal printing press, Agbowo, Ibadan, Nigeria. Pp. 158-298.
- Onwuka, G. I. (2005). *Food analysis and instrumentation: Theory and practices*. Naphthali Prints, Surulere, Lagos. Pp. 104-128.
- Papargyropoulou, E., Lozano, R., Steinberger, J. K., Wright, N., & bin Ujang, Z. (2014). The food waste hierarchy as a framework for the management of food surplus and food waste. *Journal of Cleaner Production*, 76(1), 106-115.
- Qin, P., Wang, T., & Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. *Journal of Agriculture and Food Research*, 7, 100265.
- Reazai, M., Mohammadpourfard, I., Nazmara, S., Jahanbakhsh, M., & Shiri, L. (2014). Physicochemical characteristics of citrus seed oils from Kerman, Iran. *Journal of Lipids*, Volume 2014, Article ID 174954, 3 pages.
- Seminara, S., Bennici, S., Di Guardo, M., Caruso, M., Gentile, A., La Malfa, S., & Distefano, G. (2023). Sweet Orange: Evolution, Characterization, Varieties, and Breeding Perspectives. *Agriculture*, 13(2), 264.
- Shevkani, K., Singh, N., Chen, Y., Kaur, A., & Yu, L. (2019). Pulse proteins: Secondary structure, functionality and applications. *Journal of Food Science and Technology*, 56(6), 2787-2798.
- Shittu, T. A., Raji, A. O., & Sanni, L.O. (2007). Bread from composite cassava-wheat flour. I. effect of baking time and temperature on some physical properties of bread loaf. *Food Research International*, 40, 280-290.
- Stokes, T., Hector, A. J., Morton, R. W., McGlory, C., & Phillips, S. M. (2018). Recent perspectives regarding the role of dietary protein for the promotion of muscle hypertrophy with resistance exercise training. *Nutrients*, 10(2), 180.
- Uzama, D., Okolo, S. C., Okoh-esene, R. U., & Orishadipe, A. T. (2021). The study of phytochemical and mineral contents sweet orange seed and peels. *Nigerian Journal of Chemical Research*, 26(1), 25-36.
- Wang, Y., & Jian, C. (2022) Sustainable plant-based ingredients as wheat flour substitutes in bread making. *Science of Food*, 6, Article number, 49
- Zayed, A., Badawy, M. T., & Farag, M. A. (2021). Valorization and extraction optimization of Citrus seeds for food and functional food applications. *Food Chemistry*, 355, 129609.