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Effects of added fluted pumpkin seed flour on the chemical composition, physical and sensory quality of acha-wheat bread

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Abstract—This study investigated the effect of incorporating fluted pumpkin seed flour (FPSF) into acha-wheat bread on its chemical composition, physical attributes, and sensory qualities. Acha-wheat bread samples were prepared by substituting wheat flour with varying percentages (5%, 10%, 15%and 20%) of FPSF. Proximate analysis revealed that the addition of FPSF significantly increased the protein and dietary fiber content of the bread from 10.79- 13.03 and 0.04-0.21%, while ash and fat content also showed slight increment from 1.40-2.50 and 5.47-6.71% respectively. The moisture content decreased marginally with increasing FPSF concentration. The physical characteristics: loaf volume decreased with higher FPSF levels which could be attributed to reduced gluten content and impaired gas retention. However, crust and crumb color improved, yielding a golden colour and appealing appearance. Generally, sensory evaluation showed that bread with up to 10% FPSF substitution was the most preferred in terms of taste, texture, and overall quality. Incorporating FPSF in acha-wheat bread enhances its nutritional profile and certain physical qualities up to a certain level, it may necessitate adjustments in formulation to optimize sensory attributes and maintain consumer acceptability.

***Keywords*—fluted pumpkin seed flour; chemical composition; physical and sensory quality of acha-wheat bread**

INTRODUCTION

Bread is the loaf that results from the baking of dough which is obtained from a mixture of flour, salt, sugar, yeast and water. However, other ingredients like milk, sugar and egg etc. may be added. Bread is the most common among all baked products of wheat. It is consumed and enjoyed by both children and adults from different socio-economic statuses in Nigeria, therefore leading to its high daily demand (Inyang and Asuquo, 2016). Water and flour are the most significant ingredients in a bread recipe, as they affect texture and crumb the most. Flour (14.5% moisture, 13% protein, 0.55% ash, pH 5.7–6.1, Reisman, 2021) is always 100%, and the rest of the ingredients are a percent of that amount by weight.

The productions of bread in Nigeria encounter several challenges that affect its quality, nutritional value, and shelf life. One common problem is staling, which refers to the firming of bread as it ages, leading to reduced consumer acceptability. Staling is influenced by factors such as moisture loss, retrogradation of starch, and changes in the bread's structure (Scanlon and Zghal, 2001). Another problem is the development of off-flavors and off-odors, which can arise due to improper fermentation, contamination, or the use of low-quality ingredients (Cauvain, 2013). Moreover, bread production may also face challenges related to ingredient availability, cost fluctuations, and the need for adapting to diverse consumer preferences.

While wheat is a primary ingredient in bread making, it also presents challenges that need to be addressed. One major challenge is the variability in wheat quality due to factors such as environmental conditions, growing practices, and storage conditions. This variability can affect dough properties, fermentation, and the overall quality of the bread (Shewry *et al*., 2020). Additionally, gluten-related disorders have led to an increased demand for gluten-free bread alternatives, requiring the development of suitable wheat substitutes or gluten-free grains for baking (Lionetti *et al.*, 2015).

Wheat is the most extensively cultivated cereal grain around the globe and holds a crucial place in agriculture (Anam *et al.*, 2022). Internationally, wheat supplies approximately 55% of the carbohydrates and 21% of food calories consumed worldwide (Riaz *et al.*, 2021). It beats every other single grain crop (including rice, maize, etc.) in production and acreage and is grown across a broad range of climatic situations (Erenstein *et al.*, 2022); it is therefore the most significant grain crop on the entire planet. Wheat is of supreme importance among cereals mainly because of its grains, which comprise protein with exclusive physical and chemical attributes. It also encompasses other useful components, such as minerals (Cu, Mg, Zn, Fe, and P), protein, and vitamins (riboflavin, thiamine, niacin, and alpha-tocopherol), and is also a valuable source of carbohydrates (Garg *et al.,* 2021). However, wheat proteins have been found to lack vital amino acids; for example, lysine and threonine (Siddiqi *et al*., 2022) hence call for enrichment such as pumpkin seeds.

Pumpkin belongs to the family *Cucurbitaceae* and is a widely grown vegetable all over the world. Based on the color of the seeds, the origin of pumpkin has been attributed to Guetmala, The chemical composition of pumpkin seeds includes carbohydrates(27.86%), proteins(28.90%), dietary fiber(4.59%), healthy fats (31.75% such as omega-3 and omega-6 fatty acids), vitamins (particularly vitamin E), minerals (such as magnesium, phosphorus, and zinc), and phytochemicals (including antioxidants like carotenoids and tocopherols) (Gossell-Williams *et al.,* 2011). While pumpkin seeds are largely regarded as agro-industrial waste; they serve as powerhouses of nutrients with interesting nutraceutical properties (Abdel-Aziz *et al.,* 2018). Pumpkin seeds are rich in functional components such as vitamin E (tocopherols), carotenoids, provitamins, pigments, pyrazine, squalene, saponins, phytosterols, triterpenoids, phenolic compounds and their derivatives, coumarins, unsaturated fatty acids, flavonoids and proteins (Aghaei *et al*., 2014). Moreover, pumpkin seeds are good source of magnesium, potassium, phosphorus, as well as other minor minerals such as zinc, manganese, iron, calcium, sodium, and copper Some of these bioactive and minerals act simultaneously at different or identical target sites with the potential to impart physiological benefits, promote well-being and reduce the risk of non-communicable disorders such as tumors microbial infections hyperglycemia and diabetes oxidative stress associated complications prostate disorders and urinary bladder complications (Chari *et al*., 2018).

Acha (*Digitaria spp*.) which is also known with other names such as fonio, iburu, findi, fundi, pom and kabug in different West African countries has been reported to be the oldest West African cereal (Ukim *et al*., 2021). The two main varieties majorly cultivated are *Digitaria exilis* or white acha and *Digitaria iburua* called black acha (Ukim *et al.,* 2021). The major constituents of cereals are carbohydrate and protein. It contains 7% crude protein which is high in leucine (9.8%), methionine (5.6%) and valine (5.5%). The protein in the crop is reported to be unique in that it has greater methionine and leucine content than other cereals, which implies that acha is a very good source of protein" Acha is the most nutritious cereals and a good source of cysteine and methionine, which are important to the health of humans and growth of poultry, which are not readily found in most cereals. In a study conducted by Mbaeyi-Nwaoha and Ofoegbu (2022) on the proximate and chemical composition of acha to determine the content amino acids and essential minerals, it was found that acha contained more methionine and some essential minerals and trace elements, like, calcium, magnesium, iron and copper than most cereals.

The tiny acha grains are gluten-free and when cooked is light and easy to digest and can be included in many recipes (Chinwe *et al*., 2015). Acha is an excellent source of protein that is rich in the sulfur-containing amino acids methionine and cysteine, which are deficient in rice, maize, and sorghum (Small, 2015), and their concentrations are slightly higher than those defined for the Food and Agriculture Organization (FAO) protein reference (Charlie *et al.*, 2020). Acha, contains high water absorption capacity that gives it capacity to be utilized in baked foods. It also contains pentosans (Ayo and Andrew 2016; Deriu *et al.,* 2022) which gives it the ability to form gel in the presence of oxidizing agents at room temperature with high residual protein coupled with high levels of sulphur and hydrophobic amino acid residues which makes it useful in baking (Ayo and Andrew 2016). Furthermore, fonio has a low glycemic index, and it may serve as an alternative grain for people with gluten intolerance (Small, 2015), can relatively evoke low sugar on consumption (Ayo *et al.,* 2018) an advantage for people with diabetes.

Most cereals are limited in some essential amino acids like threonine and tryptophan, though rich in lysine but cannot effectively provide nutrients required by the body (Emelike *et al.,* 2020). Since blending legumes and cereals provide high quality cheaper protein that contains all essential amino acids in proper proportion, because their amino acids complement each other (Emelike *et al.,* 2020). The research work is aimed at evaluating the quality of bread produces from acha, pumpkinseed cotyledon and wheat flour blends.

MATERIALS AND METHODS

A. Materials

The materials required for this study include acha, wheat flour, pumpkin seed,, yeast, salt, and sugar were locally purchase from Wukari market, Wukari, Nigeria.

B. Material Preparation

Production of Acha Flour

Acha flour was produced using the method described by Ayo (2007). Acha grains were winnowed to remove chaff and dust. Adhering dust and stones were removed by washing in water (sedimentation) using local calabashes. The washed and destoned grains were dried in a cabinet drier at 45°C. The dried grains were milled (attrition milling machine), sieved (0.4mm mesh size), packaged in air-tight containers and stored in refrigerator until usage (Ayo *et al.*, 2007).

Fluted Pumpkin Seed Flour

Fluted pumpkin (*Telfairiaocci dentalis* Hook) seeds were collected from the fruits, washed, sun-dried and manually decorticated.. The seeds were screened to remove all particles, oven dried (50℃), milled(electric grinder - Super Intermit lender S1-462 model), sieved(0.4mm mesh size), package 9air-tight contains) and kept in refrigerator (Okoro, 2021).

Formulation of Flour Blend and Production of Bread

A preliminary work was carried out to determine the acceptable acha: wheat ratio. Acha was substituted into wheat flour at 0,10,15,20,25 % , mixes with yeast(1.5%), salt(1.0%),baking fat(3.5%), sugar(7.0%) and water(52%) to produce dough, fermented and baked (Ayo *et al.*, 2022). The sensory quality of the acha-wheat flour blend bread was determined. The mix ratio of the most preferred acha-wheat bread (15:85) was used as baseline for production of acha-wheat flour blends (Ayo *et al.*, 2022, Ayo *et al.*, 2024). The pumpkin flour was substituted into the acha-wheat flour blends at 0, 5, 10, 15, 20, 25% and mixed with yeast (1.5%), salt (1.0%),baking fat (3.5%), sugar (7.0%) and water (52%) to produce dough, fermented and baked to produce bread.

C. Methods

Determination of Chemical Composition of Bread Produced from Acha-Wheat and Pumpkin Cotyledon Flour

Proximate Analysis of Acha, Wheat, and Fluted Pumpkin Seed Composite

The proximate composition and calorific content of the flour blend bread were determined using AOAC (2015) method.

Determination of Functional Properties of Composite Flour from Acha, Wheat, and Fluted Pumpkin Seed Composite

Foaming capacity (FC) was determined as described by Ocloo *et al.* (2010). The volume of foam at 30 sec of whipping were expressed as FC. The volume of foam was be recorded 1 h after whipping to determine FC as a percent composition of the initial foam volume. Bulk density was determined by the method of Subramanian and Viswanathan, (2007) and Atuonwu *et al*. (2010). Water and oil absorption capacities were determined following the methods of Akubor, (2003) and Ikpeme *et al.* (2010) respectively. Swelling index was determined by the method of (Buckman, 2018).

Foaming Capacity

This was determined by the method described by Ocloo *et al.* (2010). About 2 g of sample was blended with 100 mL distilled water in a Kenwood blender. The suspension was whipped in an ace homogenizer (NSEIAM-6) at 1600 rpm for 5 min. The mixture was poured into a 250 mL graduated cylinder and the volume was recorded after 30 sec. The foaming capacity was expressed as percentage increase in volume using the formula:

$$FC = \frac{(Volume\ After\ Whipping - Volume\ Before\ )}{Volume\ Before\ Whipping} \times 100$$

Bulk Density

Bulk density was determined by the method described by Atuonwu *et al*. (2010). Ten (10 mL) capacity graduated measuring cylinder was filled gently with each sample. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level. Bulk density was calculated as shown below.

$$Bulk\ Density = Final\ Weight - Initial\ Weight\ Volume$$

Water Absorption Capacity (WAC)

This was determined using the method of Akubor, (2003). One gram of the sample was dispensed into a weighed centrifuge tube with 10 ml of distilled water and mixed thoroughly. The mixture was allowed to stand for 1 hour before being centrifuged at 3500 rpm for 30 minutes. The excess water (unabsorbed) was decanted and the tube inverted over an adsorbent paper to drain dry. The weight of water absorbed was determined by difference. The water absorption capacity was also be calculated as:

$$WAC = \frac{(Volume\ of\ Water\ Used - Volume\ of\ Free\ Water)}{Weight} \times 100$$

Swelling Index

The flour for the bread samples were analyzed to obtain the value for the swelling index. The method described by Buckman, (2018) was used. Ten grams (10 g) of the sample were measured into a 300 mL measuring cylinder. Then 150 mL of distilled water was added to the sample and allowed to stand for four hours. The final volume after swelling were recorded. The percentage swelling was calculated as:

$$Swelling\ Index\ \% = \frac{Final\ Volume - Initial\ Volume}{Initial\ Volume} \times 100$$

Oil Absorption Capacity

The oil absorption capacity was performed in line with the method described by Ikpeme *et al.* (2010). 10 mL distilled oil was mixed with 1 g of the flour sample, the mixture was allowed to rest at 30˚C ± 2˚C for 30 min and then centrifuged at 200 xg for 30 min.

Determination of Mineral Content of Composite Flours produced from Acha, wheat and fluted pumpkin seed flour blends

Determination of Zinc

Zinc in the samples of composite four were determined according to Onwuka (2005) by moldy date method using hydroquinone as a reducing agent. Five milliliters (5 mL) of the test solution were pipette into 50 ml graduated flask. Then 10 mL of moldy date mixture was added and diluted to mark with water. It was allowed to stand for 30 minutes for color development. The absorbance was measured at 660 nm against a blank. A curve relating absorbance to mg zinc present was constructed. Using the zinc standard solution, and following the same procedure for the test sample, a standard curve was plotted to determine the concentration of zinc in the composite flour sample.

$$\%\ Zinc = Graph\ Reading \times\ Solution\ Volume\ \times 100$$

Determination of Iron

Iron was determined following the phenanthro line method of five milliliters of digested sample of composite four were placed in a 50 mL volumetric flask. Then 3 mL of phenanthroline solution, 2 mL of hydrochloric acid and 1mL of hydroxylamine solution were added to the sample in sequence. The sample solution was boiled for 2 minutes and 9 ml of ammonium acetate buffer solution was added to the solution. The solution was diluted with water to 50 mL volume. The absorbance was determined at 510 nm wavelength. Iron standard solution was prepared in order to plot a calibration curve to determine the concentration of the sample. Standard solution containing 100 mg/mL of ferric irons were prepared from 1g pure iron wires. The wires were dissolved in 100 mL concentrated nitric acid, boiled in a water bath and diluted to 100 mL with distilled water after cooling. Standard solutions of known concentrations were prepared by pupating 2, 4, 6, 8 and 10 mL standard iron solution into 100 mL volumetric flasks and made up to volume.

Determination of Potassium

Potassium was determined by a procedure described by Sadeghi *et al.* (2022) using a flame photometer. Potassium standard was prepared. The standard solution was used to calibrate the instrument read out of composite four. The meter reading was at 100% E (emission) to aspire the top concentration of the standards. The %E of all the intermediate standard curves was plotted on linear graph paper with these readings. The sample solution was aspired on the instrument and the readings (%E) were recorded. The concentration of the element in the sample solution was read from the standard curve.

$$\%\, Potassium = Ppm\ \times 100 \times DFI\ millions$$

Determination of Calcium

Calcium was determined using the method described by Bilge *et al.* (2016). Twenty-five milliliter (25 mL) of the digested samples of composite flour were pipetted into 250 mL conical flask and a pinch of Eriochrome Black-T-Indicator (EBT) was added. Thereafter, 2 mL of 0.1N NaOH solution was added and the mixture titrated with standard EDTA (0.01M EDTA) solution.

$$Ca\ (mg/L) = Titre\ Value\ \times Molarity\ of\ EDTA \times Equiv.Wt.of.calcium \times 1000\ Vol.of\ sample\ used$$

Determination of anti-nutritional factors

Tannin content was determined by the vanillin –HCl method as described by Enwere and Ukeaghu, (2009). Each sample (1g) was mixed vigorously with 3ml of methanol at room temperature (28± 2ºc) and allowed to extract for 1min. This was filtered, and 3ml of the extract reacted with 3ml of 0.1ml NH4Cl and 3ml of 0.08ml $K_3Fe(CN)_6$ in a test tube in triplicates. Absorbance of the color developed was read at 720mm, using catching as standard, and tannin content extrapolated from previously prepared standard graph.

Determination of Phytate Content

Phytate content was determined according to the method of AOAC (2012). Flour samples (4.0 g) were soaked in 100 ml of 95 % methanol solvent for 3 h and then filtered through what man N0. 2 filter paper. The filtrates (25 ml) were pipetted into 50 ml conical flasks, and 5ml of 3% ammonium thiocyanate solution added, after which 53.5 ml of distilled water was added and the mixture titrated against standard Iron (iii) Chloride solution containing 0.00195 g Fe^{3+} / ml until a brownish yellow color persists for 5min. The phytate content of the flours were expressed as percentage (%) phytate in the flour samples.

Determination of Total Phenol Contents

Total phenol content was determined using Folin-ciocalteau method as described by Roesler *et al.* (2006). Total phenol content was determined by mixing 0.5ml aliquot of freshly prepared spice extract with equal volume of water, 0.5 ml Folin-Ciocalteu's reagent and 2.5 ml of saturated solution of sodium carbonate (Na2C03). The absorbance was measured after 40 min at 725nm according to (Singleton *et al.,* 1999). Garlic acid was used at concentrations of 0.0, 3.0, 6.0, 12.0, 18.0, 24.0 and 30.0µg/ml to prepare a standard curve, and total phenol content extrapolated from the curve using the absorbance values and expressed as garlic acid equivalents (GAE/100g).

Determination of Oxalate Content

Oxalate content of samples were determined A blend of each ground spice sample (1.0 g) in 190 ml of 95% methanol solvent and 10 ml of 6M HCl in 250 ml volumetric flask were digested in a water bath at 90OC for 4 hours, and then centrifuged at 2000 rpm for 5 min. The supernatant was diluted to 250 ml with distilled water and titrated with concentrated ammonium hydroxide solution drop wise, using methyl orange as an indicator. The coloration changed to faint yellow at the endpoint of titration and the resulting solution heated at 90ºC for about 20 min. on a water bath and 10ml of 5 % Calcium Chloride ($CaCl_2$) solution was added to precipitate oxalate as Calcium oxalate. The resulting solution was determined rested overnight, then centrifuged and decanted to get the residue. The residue were oven-dried at 60ºC for 48 h, cooled and then weighed. This was repeated thrice and the mean weight determined and expressed as percentage oxalate content using the expression,

$$\%Oxalate\ Content = \frac{Weight\ of\ Oxalate}{Weight\ of\ Spice\ Sample} \times 100$$

Sensory Evaluation

The samples were analyzed based on appearance, color, flavor, taste, odor and general acceptability using a 9-point hedonic scale by 25 member panel who are untrained (native) but used to the product and randomly selected among the female and male students and staff from the Department of Food Science and Technology. The panellist were given invitation and information as to the evaluation exercise. The sliced samples at the room temperature (39 - 40oC), were coded and arranged randomly on a flat plat of the same size and colour background, arranged in the testing bot/cupboard under the bright illumination of light. Panellists are invited into the set cupboard and provided with score sheet for rating the samples. The rating of the samples ranged from 1 (extremely dislike) to 9 (like extremely) (Igbabul and Abu, 2016).

Statistical Analysis

The resulting data were analysed using Statistical Package for Social Sciences (SPSS, version 25.0). The means and standard deviations (SD) of the results was calculated. The data generated was subjected to a one-way analysis of Variance (ANOVA) and mean separation done using Duncan's New Multiple Range Test (DNMRT) at 5% level of probability ($p<0.05$).

RESULTS AND DISCUSSIONS

Proximate Composition of the Bread Produced from Acha, Wheat and Pumpkin Seed Flour

Proximate composition (moisture, ash, fat, crude fibre, protein and carbohydrate) of the flour blend bread is shown in Table 1. The protein, moisture, fat, ash, and fiber percentages of the flour blend bread increased from 6.95 to 13.03, 12.16 to 13.03, 6.39 to 6.71, 0.80 to 2.50 and 0.05 to 0.21%, respectively, while the carbohydrate content decreased from 73.83 to 64.21% . The general increase in the proximate agreed with the finding of Agu *et al* (2010) with addition of pumpkin seed flour

The increase in the moisture content in this present report aligns with the findings of by Kiin-Kabari *et al.* (2020), who reported increased moisture (13%), with higher fluted pumpkin seed flour content (25%). mg/100g. The increase in the fat and ash content could be due to the flour's inherent mineral richness, particularly magnesium (29.7 mg/100g), potassium (0.12 mg/100g),, and calcium (150.6 mg/ 100g), while the fat content is augmented by the seeds' natural oils, predominantly unsaturated fatty acids, could enhance the bread's nutritional profile as reported by Zlateva *et al.* (2022) and Kumari *et al*. (2021). The increase in the fiber content (0.8 t0 2.50%) could be attributed to the composition of the added pumpkin flour as observed by Kiin-Kabari *et al*. (2020).

Protein is vital for the growth and repair of tissues in the body. It is composed of amino acids, nine of which are essential and must be obtained from the diet. Proteins are crucial for building and repairing muscles, bones, skin, and hair. They are involved in the synthesis of hormones and enzymes that regulate various bodily functions. Proteins help form antibodies that protect against diseases (Tim, 2020).

Fats are essential in the diet to maintain bodily processes, such as hormone metabolism, reproductive functions and neurotransmitter functions (Tim, 2020). Fats are an important part of the diet they can be categorized into saturated, unsaturated, and Trans fats, with unsaturated fats being the healthiest choice. They can also provide the body with energy. Play a role in hormone production, cell growth, energy storage, and the absorption of important vitamins. Fats also are essential for lubricating joints reducing inflammation (Tim, 2020 and Ho, 2016).

Fibre a type of carbohydrate, is crucial for digestive health and is categorized into soluble and insoluble fibre, each serving different functions: Insoluble fibre adds bulk to stool and helps prevent constipation, while soluble fibre can help manage blood sugar levels and lower cholesterol. High-fibre foods promote a feeling of fullness, which can aid in weight management. (Threapleton, 2013).

Table 1: Proximate composition of the bread produced from acha, wheat and pumpkin seed flour

Sample	CHO (%)	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Fibre(%)
A	71.01b ±0.26	10.79b±0.10	11.34a±0.23	5.47b ±0.13	1.40d ±0.01	0.04c±0.04
B	73.83^{a} ±1.29	6.95^{c} ± 0.51	12.16^{a}±0.19	6.26^{ab}±0.97	0.80^{e} ± 0.00	0.09^{a}±0.07
C	66.64d±0.13	12.05a±0.21	12.57b ±0.16	6.39a ±0.28	2.35c ±0.05	0.11ab ±0.14
D	65.82cd ±0.39	12.50a ± 0.46	12.89b ± 0.47	6.42ab±0.29	2.37c ± 0.03	0.13ab ±0.09
E	65.15^{bc} ±0.91	12.74^{a} ± 0.71	13.15^{b} ± 0.72	6.57^{b} ±0.85	2.39^{bc}±0.00	0.15^{bc} ±0.04
F	64.76^{bc} ±0.42	12.90^{a} ± 0.45	13.39^{b} ± 0.27	6.65^{ab}±0.60	2.45^{ab}±0.02	0.18^{bc}±0.02
G	64.21^{cd} ±0.07	13.03^{a} ± 0.20	13.55^{b} ± 0.19	6.71^{ab}±0.35	2.50^{a} ±0.64	0.21^{abc}±0.00

The data are means ± standard deviation of duplicate scores. Mean within a row with different superscripts were slightly significantly different ($p < 0.05$). Key: A=100% WF, B=100% AF, C=95:5% AW and PSF, D=90:10% AW and PSF, E=85:15% AW and PSF, F=80:20% AW and PSF, G=75:25% AW and PSF. W= Wheat flour, A= Acha flour, AW= Acha and Wheat (15:85) and PS= Pumpkin seed flour.

Phytochemical Composition of the Bread Produced from Acha, Wheat and Pumpkin Seed Flour

The phytochemical composition of the flour blend bread is shown in Table 2. The carotenoid, flavonoid and phenol content increased from 0.01 to 0.54, 32.92 to 32.40 and 14.17 to 14.35mg/100g with increase in the added pumpkin seed flour. This observation agreed with findings of Jeevitha and Bhuvana (2019) and Zlateva *et al.* (2022) in their respective research works with wheat flour and pumpkin seed flour.

Carotenoids are lipophilic pigments that give fruits and vegetables their vibrant colors (They are the sources of the yellow, orange, and red colors of many plants) They are potent antioxidants that can neutralize free radicals and reactive oxygen species, protecting cells from oxidative damage (Higdon *et al*.,2021). Dietary carotenoid intake has been associated with reduced risk of chronic diseases like cardiovascular disease, type 2 diabetes, and certain cancers. The body cannot synthesize carotenoids, so they must be obtained from the diet. Major dietary carotenoids include β-carotene, lutein, zeaxanthin, and lycopene (Andarwulan *et al*., 2021)

Flavonoids are a large group of polyphenolic compounds with diverse structures and functions They exhibit strong antioxidant activity by scavenging free radicals, chelating metals, and modulating enzyme activity. Flavonoids have been shown to inhibit digestive enzymes and reduce the micellarization of carotenoids, potentially enhancing their bioavailability. (Escovar-Cevoli *et al*.,2017). Epidemiological studies suggest that habitual flavonoid consumption is associated with lower risks of cardiovascular disease, type 2 diabetes, and certain cancers .They have captivated considerable research interest due to their well-established biological properties, such as antioxidant, anti-inflammatory, and anti-carcinogenic effects data from observational and intervention studies summarized by Vogiatzoglou *et al*. (2015).

Phenols are aromatic compounds with one or more hydroxyl groups attached to a benzene ring. They can act as antioxidants by donating hydrogen atoms or electrons to free radicals, stabilizing them and preventing oxidative damage. Phenols may also exhibit anti-inflammatory, antimicrobial, and anticarcinogenic properties Dietary sources of phenols include fruits, vegetables, whole grains, tea, coffee, and cocoa. (Castañeta *et al*., 2024)

Table 2: Phytochemical composition of the bread produced from acha, wheat and pumpkin seed flour

Sample	Carotenoid(mg/100g)	Flavonoids(mg/100g)	Phenol(mg/100g)
A	$0.01^{c} \pm 0.00$	$32.22^{a} \pm 0.21$	$14.17^{ab} \pm 0.00$
B	$0.11^{c} \pm 0.00$	$32.25^{a} \pm 0.42$	$14.20^{f} \pm 0.16$
C	$0.43^{a} \pm 0.01$	$32.29^{bc} \pm 0.16$	$14.23^{cb} \pm 0.64$
D	$0.45^{ab} \pm 0.01$	32. $31^{c} \pm 0.10$	$14.26^{d} \pm 0.71$
E	$0.49^{b} \pm 0.21$	$32.33^{d} \pm 0.04$	$14.29^{e} \pm 0.77$
F	$0.52^{ab} \pm 0.05$	$32.38^{d} \pm 0.01$	$14.33^{cd} \pm 1.41$
G	$0.54^{ab} \pm 0.07$	$32.42^{e} \pm 0.03$	$14.35^{a} \pm 2.06$

The data are means ± standard deviation of duplicate scores. Mean within a row with different superscripts were slightly significantly different ($p < 0.05$). Key: A=100% WF, B=100% AF, C=95:5% AW and PSF, D=90:10% AW and PSF, E=85:15% AW and PSF, F=80:20% AW and PSF, G=75:25% AW and PSF. W= Wheat flour, A= Acha flour, AW= Acha and Wheat(15:85) and PS= Punkin seed flour.

Vitamin and Mineral Composition of the Bread Produced from Acha, Wheat and Pumpkin Seed Flour

The vitamin and mineral composition of the flour blend bread are presented in Table 3. The vitamin B_1 and vitamin B_2 content of the flour blend bread increased from 0.06 to 0.26 and 0.07 to 0.20mg/100g that of iron and potassium increased from 1.27 to 1.48 and 50.80 to 83.70mg/100g, respectively, with increase in the added pumpkin seed flour. The observation agreed with the findings of Zlateva *et al.* (2022) and Olagunju (2019) in their respective research on acha-wheat blend flour bread. Research has shown that vitamin B_1 is essential for energy metabolism.

Vitamin B1 (thiamine) is essential for producing various enzymes that help break down blood sugar. Thiamine is essential for the production of acetylcholine and myelin and the maintenance of glutamate, aspartate, and gamma-aminobutyric acid levels (Hanna *et al*., 2022).

Vitamin B2 riboflavin a water-soluble vitamin is essential for the growth and development of body cells and helps metabolize food Vitamin and also helps break down proteins, fats, and carbohydrates. It plays a vital role in maintaining the body's energy supply. (Yvette, 2023).

Iron is an essential mineral that plays a crucial role in maintaining healthy blood. It is a major component of hemoglobin, a protein in red blood cells that carries oxygen from the lungs to all parts of the body. Without enough iron, there aren't enough red blood cells to transport oxygen, leading to fatigue and anemia (National Institutes of Health Office of Dietary Supplements, 2019). Iron is also important for cell growth and development normal body functions healthy brain development and growth in children production and function of various cells and hormones. (Clifford *et al*., 2015)

Potassium is an essential mineral that plays a vital role in various physiological functions in the body. Potassium helps maintain normal fluid levels within cells and throughout the body. It is essential for proper muscle contraction, including the heart muscle. Adequate potassium levels support healthy heart rhythms and muscle performance. (National Institutes of Health, 2017). Potassium is critical for transmitting electrical signals in the nervous system, which is necessary for muscle movement and reflexes. Higher potassium intake is associated with lower blood pressure levels. It helps counteract the effects of sodium, reducing the risk of hypertension and related cardiovascular diseases. Some studies suggest that potassium may contribute to bone health by helping to maintain bone mineral density. This effect may be due to potassium's role in neutralizing dietary acids, which can otherwise lead to calcium loss from bones. Potassium aids in kidney function by helping to regulate the excretion of sodium and maintaining electrolyte balance. Adequate potassium levels may also reduce the risk of kidney stones. (Stone *et al*., 2016).

Table 3: Vitamin and Mineral composition of samples

Sample	B1(mg/100g)	B2(mg/100g)	IRON(mg/100g)	POTASSIUM(mg/100g)
A	$0.06^{e} \pm 0.00$	$0.07^{b} \pm 0.05$	$1.27^{c} \pm 0.35$	50.80 ± 1.51
B	$0.08^{f} \pm 0.00$	$0.08^{b} \pm 0.09$	$1.29^{d} \pm 0.06$	$56.26^{a} \pm 0.21$
C	$0.12^{b} \pm 0.01$	$0.10^{a} \pm 0.01$	$1.32^{a} \pm 0.17$	$61.70^{c} \pm 0.42$
D	$0.15^{c} \pm 0.01$	$0.12^{a} \pm 0.01$	$1.35^{ab} \pm 0.12$	$62.45^{d} \pm 0.21$
E	$0.17^{d} \pm 0.00$	$0.14^{a} \pm 0.01$	$1.37^{b} \pm 0.41$	$74.05^{g} \pm 0.00$
F	$0.20^{bc} \pm 0.00$	$0.18^{a} \pm 0.00$	$1.42^{c} \pm 0.18$	$78.11^{e} \pm 0.21$
G	$0.24^{a} \pm 0.00$	$0.20^{a} \pm 0.00$	$1.48^{d} \pm 0.06$	83 $.70^{d} \pm 0.42$

The data are means ± standard deviation of duplicate scores. Mean within a row with different superscripts were slightly significantly different ($p < 0.05$). Key: A=100% WF, B=100% AF, C=95:5% AW and PSF, D=90:10% AW and PSF, E=85:15% AW and PSF, F=80:20% AW and PSF, G=75:25% AW and PSF. W= Wheat flour, A= Acha flour, AW= Acha and Wheat(15:85) and PS= Punkin seed flour.

Physical properties of the bread samples

The physical properties of the flour blend bread is presented in Table 4. The loaf volume index, which is a crucial indicator of the bread's lightness, was highest in sample G at 4.75 and decreased with the addition of pumpkin seed flour to 1.11 in sample A. This reduction in loaf volume index with increased pumpkin seed flour addition could be attributed to the gluten-reducing effect of the pumpkin seed flour, as also noted in the research work of Zlateva *et al.* (2023), where the addition of 10% pumpkin seed flour resulted in a significant increase in mineral content and also affected the bread's structural properties. The sensory and textural quality of the bread is also impacted by these changes, as a study on the addition of pumpkin flour to rice bread found that while the bread crumb and crust characteristics were not significantly impacted, the taste and flavor decreased with higher amounts of added pumpkin flour, affecting overall acceptability. These studies highlight the importance of considering both the nutritional enhancements and the physical and sensory attributes when formulating bread with alternative flours.

Table 4: Physical properties of the bread samples

Sample	LOAF WEIGHT(g)	LOAF VOLUME(cm^3)	LOAF VOLUME INDEX
A	$230.05^{de} \pm 1.34$	$255.00^{a} \pm 7.07$	$1.13^{a} \pm 0.01$
B	$235.40^{e} \pm 0.42$	$270.00^{d} \pm 7.07$	$1.18^{b} \pm 0.04$
C	$239.65^{de} \pm 0.01$	$325.00^{a} \pm 0.00$	$2.59^{a} \pm 0.00$
D	$243.35^{bcd} \pm 0.63$	$395.00^{b} \pm 7.07$	$2.77^{a} \pm 0.04$
E	$245.30^{ab} \pm 7.35$	$485.00^{b} \pm 7.07$	$3.77^{ab} \pm 1.15$
F	$249.20^{bc} \pm 0.42$	$557.00^{c} \pm 7.07$	$4.39^{a} \pm 0.04$
G	$259.40^{a} \pm 0.42$	$665.00^{c} \pm 7.07$	$4.75^{a} \pm 0.04$

The data are means ± standard deviation of duplicate scores. Mean within a row with different superscripts were slightly significantly different ($p < 0.05$). Key: A=100% WF, B=100% AF, C=95:5% AW and PSF, D=90:10% AW and PSF, E=85:15% AW and PSF, F=80:20% AW and PSF, G=75:25% AW and PSF. W= Wheat flour, A= Acha flour, AW= Acha and Wheat(15:85) and PS= Punkin seed flour.

Sensory Quality of Bread produced from Pumpkin seed - acha-wheat flour blends

The sensory quality of the bread produced from pumpkin seed and acha-wheat flour blends is summarized in Table 5. The average means scores of the aroma, taste, texture and colour increased from 5.90 to 5.70, 6.10 to 6.25, 6.15 to 6.25 and 6.40 to 6.45 with increase in the added PSF, 5 to 10 % and further above 10% caused general decrease. The sensory evaluation result showed the general acceptability of the all the products with exception of 100% acha, and compared favorably with the control (100% wheat flour). However the most preferred product is that that contained 10% pumpkin seed flour with an average means (general acceptability) of 6.35.

Table 5: Sensory Quality of Bread produced from Pumpkin seed - acha-wheat flour blends

Samples	Aroma	Taste	Texture	Colour	Gen. Accept
A	8.40 ± 0.75^{a}	8.60 ± 0.60^{a}	8.30 ± 0.73^{a}	8.45 ± 0.56^{a}	8.40 ± 0.46^{a}
B	3.30 ± 0.92^{c}	2.85 ± 0.42^{c}	3.90 ± 0.15^{d}	3.50 ± 0.99^{c}	3.40 ± 0.39^{c}
C	5.90 ± 0.12^{b}	6.10 ± 0.33^{b}	6.15 ± 0.42^{b}	6.40 ± 0.10^{b}	6.30 ± 0.30^{b}
D	5.70 ± 0.52^{b}	6.25 ± 0.42^{b}	6.25 ± 0.83^{bc}	6.45 ± 0.40^{b}	6.35 ± 0.20^{b}
E	5.70 ± 0.69^{b}	5.90 ± 0.74^{b}	5.85 ± 0.95^{bc}	5.90 ± 0.62^{b}	5.80 ± 1.32^{b}
F	4.95 ± 0.85^{b}	5.35 ± 0.84^{b}	5.35 ± 0.87^{c}	5.50 ± 0.12^{b}	5.40 ± 0.12^{b}
G	4.50 ± 0.93^{b}	5.05 ± 0.34^{b}	5.00 ± 0.81^{bc}	5.05 ± 0.73^{b}	5.15 ± 0.73^{b}

Values are means ± standard deviation of duplicate determinations. Means in the same column with different superscripts are significantly ($p<0.05$) different. Key: A=100% WF, B=100% AF, C=95:5% AW and PSF, D=90:10% AW and PSF, E=85:15% AW and PSF, F=80:20% AW and PSF, G=75:25% AW and PSF. W= Wheat flour, A= Acha flour, AW= Acha and Wheat(15:85) and PS= Punkin seed flour.

CONCLUSIONS

The study has showed that composite bread with improved nutritional composition and acceptable sensory quality could be produced from blends of pumpkin seed, orange fleshed sweet potato and acha flours. Though the flour blend bread are generally acceptable, however the most preferred is that containing 10% pumpkin seed flour.

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