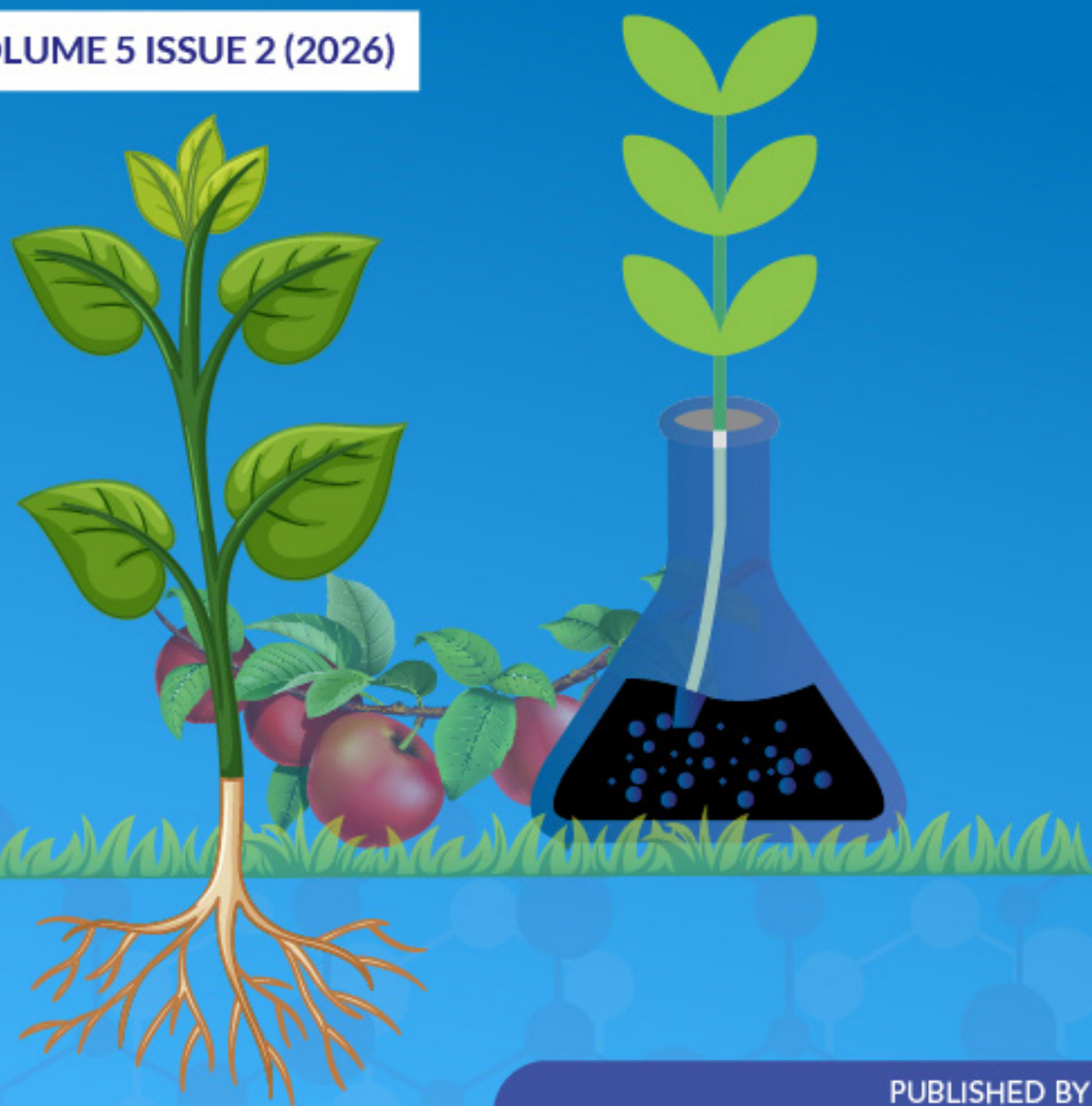




American Journal of Food Science and Technology (AJFST)

ISSN: 2834-0086 (ONLINE)

VOLUME 5 ISSUE 2 (2026)



PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

Nutritional Profile and Sensory Properties of Sourdough Bread from Composite Flour of Wheat, Soybeans and Sunflower Seeds

Msugh C. T^{1*}, Adah C. A², Asemave K², Demkiir T. T¹, Mtindian S.U¹, Hough A. S³

Article Information

Received: July 20, 2026

Accepted: September 25, 2026

Published: October 15, 2026

Keywords

Amino Acids, Antinutrients, Bread, Sunflower Seeds, Sourdough

ABSTRACT

This study focused on improving the nutritional quality of bread through the use of composite flours and sourdough technology. The research aimed to evaluate the nutritional profile and sensory properties of sourdough bread produced from composite flour of wheat, soybeans, and sunflower seeds. Wheat flour was partially substituted with soybean flour at varying ratios (0-30%), while sunflower seed flour was kept constant at 10%, and a sourdough starter culture was incorporated. The bread samples produced were analyzed for physicochemical properties, proximate composition, essential amino acids, antinutritional factors, mineral content, and sensory attributes using standard laboratory and statistical methods. The findings showed that increasing the inclusion of soybean and sunflower seed flour improved protein (9.13 – 18.92%), fibre, ash, fat, essential amino acids, and mineral contents (such as calcium, iron, and zinc), while reducing carbohydrate content. Processing kept antinutritional factors at low, safe levels. However, loaf volume and sensory acceptability decreased with higher substitution levels, with commercial bread recording the highest sensory scores. Composite sourdough bread enriched with soybean and sunflower seed flour significantly improved the nutritional quality and demonstrated the potential to combat malnutrition.

INTRODUCTION

Bread can be described as a fermented, confectionary product made mainly from wheat flour, water, yeast and salt by a series of processes involving mixing, kneading, proofing, shaping and baking (Suchintita *et al.*, 2023). Although wheat flour is widely used in the production of bread and other baked products, its relatively low protein content and poor essential amino acid balance have remained major nutritional concerns (Lancelot *et al.*, 2021). This limitation is particularly critical in developing countries where bread constitutes a substantial part of the daily diet but contributes inadequately to addressing protein-energy malnutrition and micronutrient deficiencies. In addition, the increasing dependence on imported wheat places economic pressure on such countries, thereby necessitating the exploration of alternative, locally available nutrient-rich raw materials. Legumes such as soybean (*Glycine max* L.) and oilseeds like sunflower (*Helianthus annuus*) have been recognized as valuable sources of high-quality protein, essential amino acids, minerals, and vitamins (Islam *et al.*, 2007; Jahreis *et al.*, 2016; Arshad *et al.*, 2020). Soybean, in particular, contains about 30–45% protein and has been described as a “protein hope of the future” due to its superior nutritional profile (Hossain *et al.*, 2020), while sunflower seeds are rich in unsaturated fats, vitamin E, and essential minerals (Sabitha & Puraikalan, 2014). Incorporation of these ingredients into wheat-based products has the

potential to significantly enhance their nutritional value. However, the use of composite flours in bread making is often limited by technological and sensory challenges such as gluten dilution, reduced loaf volume, and the development of undesirable beany flavour associated with soybean (Banwo *et al.*, 2020), which negatively affect consumer acceptability.

Sourdough fermentation, one of the oldest biotechnological processes in food production, has recently gained renewed interest due to its functional and nutritional benefits (Couch, 2016). It involves the activity of lactic acid bacteria which produce organic acids that improve flavour, extend shelf life, enhance mineral bioavailability, and reduce antinutritional factors (Ma *et al.*, 2020). Furthermore, sourdough fermentation has been reported to mask off-flavours in legume-enriched products, thereby improving their sensory quality (Banwo *et al.*, 2020). It also offers health benefits such as reduced risk of obesity, diabetes, cardiovascular diseases, and improved gut health due to the presence of beneficial microorganisms (Ribet *et al.*, 2023).

Despite these advantages, most existing studies have focused either on the use of composite flours or sourdough fermentation independently, with limited research on their combined application in bread production. In particular, information remains limited on how sourdough fermentation can be used to improve the nutritional quality and sensory acceptability of bread

¹ Centre for Food Technology and Research, Department of Chemistry, Rev. Fr. Moses Orshio Adasu University, Makurdi, Benue State, Nigeria

² Department of Chemistry, Rev. Fr. Moses Orshio Adasu University, Makurdi, Benue State, Nigeria

³ Department of Chemical Sciences, University of Mkar, Mkar, Benue state, Nigeria

* Corresponding author's e-mail: tersoocephas@gmail.com

made from composite flour blends of wheat, soybean, and sunflower seeds. This gap highlights the need for a comprehensive evaluation of such formulations.

Therefore, this study was designed to produce sourdough bread from composite flour of wheat, soybeans, and sunflower seeds, and to evaluate its physicochemical properties, nutritional profile, antinutritional content, and sensory attributes, with the aim of developing a more nutritious and acceptable alternative to conventional wheat bread.

MATERIALS AND METHODS

Materials

Soybeans (variety TGX 1957-3F) was obtained from the Benue State Agency for Agricultural and Rural Development Authority (BNARDA) Makurdi. The wheat flour, sunflower seeds, and other ingredients (for bread making) used in this study were obtained from Wadata market, Makurdi, Benue state, Nigeria. Processing of these materials was done at CEFTER food laboratory,

department of Chemistry, Benue State University, Makurdi.

Preparation of Composite Flours

The method described by Ndife *et al.* (2011) was used in the preparation of the soybean flour. The soy beans were cleaned from dirt by sorting out contaminants such as sands, sticks and leaves, and were later washed and oven dried. The soy beans were roasted and winnowed. The soy beans were later milled using a laboratory grinder and sieved into fine flour of uniform particle size, by passing them through a 2 mm mesh sieve.

The method of Srivasava and Verma, (2014) was used in the preparation of the sunflower seeds flour. Insects/ disease free sunflower seeds were collected and soaked for 4 h then drained using a sieve. The seeds were later spray on oven trays and transferred into an oven heated at 55°C for 8 h to enable it dry. Thereafter the seeds were grinded into flour using a laboratory grinder. In preparing the composite flour, refined wheat flour, soybeans and

Table 1: Formulation of Wheat, Soybean and Sunflower seeds composite flour blends

Samples	Wheat (%)	Soybeans (%)	Sunflower (%)
A	Commercial bread		
B	100	0	0
C	90	10	0
D	80	10	10
E	70	20	10
F	60	30	10

sunflower seeds flour were mixed as shown in Table 1.

Preparation of Sourdough (starter culture)

The method described by Ogunsakin *et al.* (2015) was used (with slight modification) to prepare the starter culture.

Sourdough starter culture was prepared by mixing 150 g of wheat flour with proportional luke warm de-ionized water in a container with lid and left for 72 h to ferment (anaerobic) then it became ready for use as a starter.

Table 2: Recipe for wheat, soybean and sunflower seeds flour composite dough

	Samples					
Ingredients	A	B	C	D	E	F
Wheat flour (g)	Commercial bread	100	90	80	70	60
soybean flour (g)		0	10	10	20	30
Sunflower seeds flour (g)		0	0	10	10	10
Salt (g)		2	2	2	2	2
Sourdough (g)		25	25	25	25	25
Yeast(g)		2	2	2	2	2
Sugar (g)		6	6	6	6	6
Water (mL)		65	65	65	65	65
Total dough weight (g)		200	200	200	200	200

Sourdough Bread Making

The composite sourdough bread was made using the method described by Ogunsakin *et al.* (2015) with slight modification. The batter method was used in the bread making. First, the wheat, soybean flour was mixed with

constant 10% inclusion of sunflower seeds flour. Then water, salt, sugar, yeast and sourdough were added and thoroughly mixed. The resulting mixture was gently kneaded and the batter scaled (batter weight: 200 g) into greased baking pans (9cmx12cmx6cm) and allowed to

proof for 24 h at ambient temperature. The pans were carefully transferred into an electric oven to prevent collapse and baked at 180-200 °C for 45 min then cooled at ambient temperature (as shown in figure 1) after which they were ready for sensory and proximate analysis.

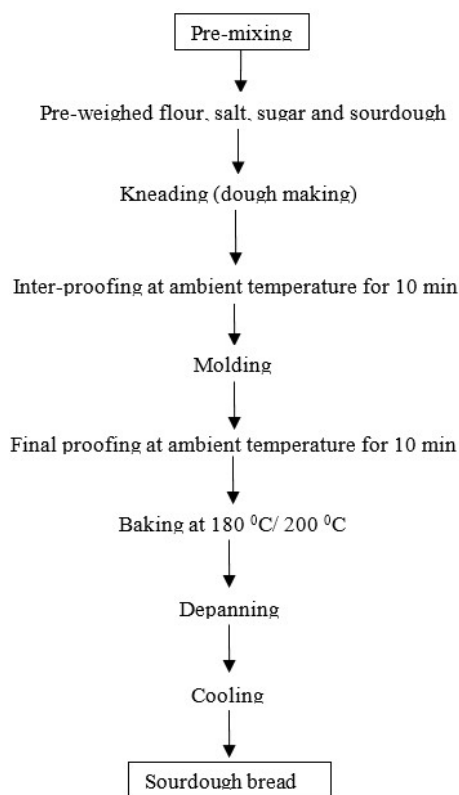


Figure 1: Flow chart for the production of sourdough bread

Methods of Analysis

Determination Moisture Content

Moisture content was determined using the oven drying method as outlined by Nwafor *et al.* (2020). Exactly 5 g of sample was weighed into pre-weighed dry crucibles and heated in an oven at 105 °C for 3 h Cooling and weighing of dried samples were done at intervals of 30 min until there was no weight change after three successive weight measurements. The final weight of dried samples was noted and the moisture content calculated as described by equation 1:

$$\text{Percentage Moisture content (\% MC)} = \frac{m(W_1 - W_2)}{(W_1 - W_0)} \times 100 \quad (1)$$

where, W_0 = weight of dish only, W_1 = weight of dish and sample after drying, W_2 = weight of dish and sample before drying, $W_1 - W_0$ = weight of sample originally before drying,

Determination of Protein

Protein content was determined using the method described by Yusufu and Ejeh (2018). Nitrogen content was determined using the micro-Kjeldahl procedure. 0.5 g of the sample was weighed, heated and digested using conc. Sulphuric acid with the aid of a catalyst mixture. The digest was neutralized with alkali and distilled into a boric acid solution. The borate anions formed are titrated

with standardized acid, which is converted to nitrogen in the sample. The protein content was calculated using the formula. The percentage Nitrogen was calculated as shown in equation 2.

$$\text{Percentage Nitrogen} = \frac{(\text{Titre} \times N \times DF \times 0.0140067)}{(\text{Weight of sample}) \times 100} \quad (2)$$

$$\text{Crude protein} = \% \text{ total Nitrogen} \times 6.25$$

$$\text{Titre} = \text{Final burette reading} - \text{Initial burette reading}$$

$$N = \text{Normality of acid}$$

$$DF = \text{Dilution factor}$$

Determination of Crude Fat

Crude fat was determined by the soxhlet extraction method as outlined by Nwafor *et al.* (2020). 5 g of dried samples was weighed into a preconditioned and weighed (W_0) extraction thimble and placed in the soxhlet extraction apparatus. Fat content of the samples was extracted using petroleum ether and boiled under reflux for 6 h. The extraction thimbles were then removed and dried in an oven at 105 °C for 30 min then cooled and weighed (W_1). Percentage of fat content was calculated as described in equation 3:

$$\begin{aligned} \text{crude fat(\%)} &= \frac{(\text{Weight of fat in sample})}{(\text{Weight of dry sample})} \times 100 \\ &= \frac{(W_0 - W_1)}{(\text{Weight of dry sample})} \times 100 \quad (3) \end{aligned}$$

Determination of Crude Fibre

Crude fibre was determined using method described by Nwafor *et al.* (2020). Exactly 2 g of samples was digested in a conical flask with 100 mL of Trichloroacetic acid (TCA). This solution was boiled and reflux for 40 min. After digestion, the flask was removed from the heater, cooled and the solution filtered with (Whatman number 1) filter paper. 1 g of the residues was washed with distilled water several times and once with methylated spirit. The residue was transferred to a porcelain (Evaporating dish) dish and heated for 24 h at 500 °C. The porcelain dishes were later taken to the desiccator to cool and their weight taken. The digested residue after drying was ashed at 600 °C for 6 h, cooled and its weight recorded. The crude fibre was calculated using equation (4)

$$\% \text{ crude fibre} = \frac{((\text{final weight of crucible} - \text{initial weight of crucible}))}{((\text{weight of sample}))} \times 100 \quad (4)$$

Determination of Ash Content

Total ash content was determined by dry ashing according to Nwafor *et al.* (2020). 5 g of the sample was weighed in dry crucibles, carbonized on a hot plate and heated on a muffle furnace at 600 °C for 8 h after which they were cooled in a desiccator and weighed. Ash content was determined by the difference in weight after cooling the samples in desiccators at ambient temperatures. Percentage Ash was calculated as described by equation 5:

$$\text{Ash (\%)} = \frac{(\text{Weight of crucible} + \text{Ash-weight of empty crucible})}{(\text{Weight of sample})} \times 100 \quad (5)$$

Determination of Total Carbohydrates

The total carbohydrate content was estimated by

difference as previously reported by Nwafor *et al.* (2020) using equation 6:

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{fat} + \text{protein} + \text{ash} + \text{crude fibre})\% \quad (6)$$

Determination of Total Energy

The total energy value was calculated according to the method described by Oluyemisi *et al.* (2016) expressed in equation 7:

$$\text{Total Energy (Kcal/100g)} = [(\% \text{ carbohydrates} \times 4) + (\% \text{ proteins} \times 4) + (\% \text{ fat} \times 9)] \quad (7)$$

Determination of Antinutrients

Determination of Tannins

Tannins were determined using the method described by Patchimapon et al (2019). 1 g of the sample was weighed and then 5 mL of 1% (v/v) of HCl added in methanol. The sample was allowed to stand at ambient temperature for 15 min before vortex mixing and centrifugation at 3000 rpm for 10 min. The supernatant of 2.5 mL was transferred to a 10 mL flask containing 7.5 mL of water, then 0.5 mL of Folin-Denis reagent and 1 mL of sodium carbonate was added. The final volume was adjusted to 10 mL with water, and the absorbance was determined after 30 min of incubation at ambient temperature using a UV/Vis spectrophotometer (model Genesys G10S, USA) at 760 nm. The amount of tannin was calculated from the standard curve of tannic acid solution.

Determination of Saponins

The saponins were measured spectrophotometrically using the method described by Mir, (2016). 2 g of the sample was transferred into a beaker, and 100 mL of isobutyl alcohol was added. The mixture was kept on a shaker for 5 h, and then passed through the No. 1 Whatman filter paper into a beaker containing 40% saturated MgCO_3 (20 mL), and filtered again to obtain a clear colorless solution. To 1 mL of the colorless filtrate, 2 mL of 5% FeCl_3 solution was added and incubated for 30 min. The absorbance of the colour formed was measured at 380 nm.

Determination of Oxalates

The oxalates were determined using the method described by Savage et al (2018) with slight modification. Oxalates were extracted from 0.5 g of composite flour samples with 50 mL of 0.2M HCl and then incubated in a water bath at 80 °C for 15 min. The extracts were allowed to cool to ambient temperature before being transferred to a 100 mL volumetric flask; the final volume was adjusted with water. Subsequently, the extracted samples were centrifuged at 2800 rpm for 15 min. The supernatant was filtered through a 0.45 μm cellulose acetate filter (Sartorius, Gottingen, Germany) prior to analysis with High-Performance Liquid Chromatography (HPLC). The HPLC system used consisted of a binary pump (model ProStar 210, Varian, USA), auto-sampler (model Triathlon, Spark, Holland) with a UV detector

set at 210 nm (model Waters 2487 Dual λ , absorbance detector, Waters Corporation, USA). Separation was conducted in a Supelco reversed phase column (4.6mm I.D. $\times$ 250mm length). The mobile phase was 0.25 g/100 mL dehydrogenate phosphate and 0.0025 mol/L tetrabutyl ammonium hydrogen sulphate buffered at pH 2.0 with ortho-phosphoric acid. The column temperature was set at ambient temperature and the total running time was 20 min at a flow rate of 1 mL/min. The injection volume was 5 μL . Identification of oxalic acid was carried out by comparing the HPLC retention time of unknown peaks to the chromatogram with added standard.

Determination of Phytate

The method of Abera *et al.* (2023) was modified to determine the phytate content. Phytate was extracted from 1 g of the sample with 20 mL of 2.4% (v/v) hydrochloric acid (HCl) by shaking at room temperature for 2 h, followed by high speed centrifugation of the suspension for 15 min. The supernatant decanted and filtered through Whatman No. 1 filter paper. A 3 mL aliquot of filtrate was diluted to 18 mL with distilled water and the diluted sample was passed through a 200–400 mesh AG1-X8 chloride anion exchange resin (Bio-Rad Laboratories GmbH, Munchen, Germany). Inorganic phosphorus was eluted with 0.07M sodium chloride (NaCl) followed by elution of phytate with 0.7M NaCl. Phytate was determined colorimetrically based on pink color of Wade reagent, which is formed upon the reaction of the ferric ion and sulfosalicylic acid. One milliliter of Wade reagent (0.03% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ containing 0.3% (v/v) sulfosalicylic acid in water) was added to 3 mL of the clear supernatant sample and then centrifuged at 2000 rpm for 15 min. The absorbance was measured by a spectrophotometer (model Genesys G10S, USA) at 500 nm. The phytate content was calculated using a standard phytic acid curve and results were expressed as mg/100 g.

Physical Characteristics of Sourdough Bread

Determination of Loaf Height

Loaf height was measured with a meter rule in centimeters (cm). Three different places were measured on the loaves and the mean values of the measurements were recorded as the heights of the loaves (Ogunsakin *et al.*, 2015).

Determination of Loaf Weight

Loaf weight was measured 30 min after the loaves were removed from the oven using a laboratory scale (CE-410I, Camry Emperors, China) and the readings recorded in grams (Ogunsakin *et al.*, 2015).

Determination of Loaf Volume

The loaf volume was determined by using rape seed displacement method as described by Yusufu and Ejeh (2018). This was done by loading 3000 mL of sorghum grains into a calibrated container, mark until it reached the marked level and unloaded back, the bread sample was put into the container and the measured sorghum

was loaded back again, the remaining sorghum gains left outside the container was measured using measuring cylinder and recorded as loaf volume in cm^3 .

Specific Loaf Volume

The specific volume (volume to mass ratio) (cm^3/g) was calculated as described by Yusufu and Ejeh (2018).

Specific loaf volume was calculated using equation 8

$$\text{Specific loaf volume} = (\text{Loaf volume})/(\text{Loaf mass}) \quad (8)$$

Determination of the Amino Acid

Amino acid content was determined using Pico-Tag method described by Prasad, (2017). 2.0 g of the sample was hydrolysed, evaporated in a rotary evaporator, and loaded into Technicon Sequential Multi-Sample Amino Acid Analyser (TSM-1) (Technicon Instruments Corporation, New York, USA). A 10 μL of each hydrolysate was dispensed into the cartridge of the analyzer. The analyzer was separated and analyzed free acidic, neutral, and basic amines, which will last for 76 h. Norleucine was employed as the internal standard. A 10 μL of the standard solution mixture of the amino acid was also loaded into the analyzer. Values of both the standard and samples were recorded and printed out as chromatogram peaks by the chart recorder.

Calculation from the peaks: The net height of each peak produced on the chromatogram (each representing amino acid) was measured. The half-height of each peak was located, and the width of the peak at half-height was accurately measured. Approximate area of each peak was then obtained by multiplying the height with the width of the half height. All measurement was in millimeter (mm). The Norleucine equivalent (NE) for each amino acid in the standard mixture was calculated as described by equation 9

$$\text{NE} = (\text{Area of neulocine peak})/(\text{area of amino acid in the standard mixture}) \quad (9)$$

Mineral Analysis

The method described by AOAC, (2005) with slight modification was used in analyzing minerals. The analysis involved two main steps: Digestion and instrumental determination. 1 g of the bread sample was digested by adding concentrated nitric acid and heating at 150°C until a pale yellow solution formed, after which perchloric acid was added and heating continued at 200°C until the solution became clear, indicating complete digestion. The digest was then cooled, diluted with deionize water, boiled, filtered, and made up to a fixed volume in a volumetric flask. The resulting solution was analyzed using an Atomic Absorption Spectrophotometer (ASS) to determine the concentration of minerals such as calcium, iron, zinc, magnesium, and phosphorus, with results expressed in $\text{mg}/100\text{ g}$ of sample.

Sensory Evaluation

Sensory evaluation was done using the 9-point hedonic scale as described by Ihekoronye and Ngoddy, (1985). A

twenty-member panelist was drawn from a cross section of adult population (students and staff) of Benue State University Makurdi and given the coded samples to evaluate. The quality attributes assessed were appearance (colour), flavour (aroma and taste), texture and overall acceptability.

Statistical Analysis

The results generated were subjected to statistical analysis using SPSS version 26 (SPSS Inc., Chicago, IL) and MS-Excel (Microsoft Inc., USA) at 95% confidence level. The analysis of variance (ANOVA) was used for replicate determinations to detect the existence (or otherwise) of significant differences amongst the results. For replicate determinations, the mean and standard deviation from the mean for each measured parameter were calculated. Duncan's new multiple range test was used to separate the means at 5% level of significance. Where applicable, the results were presented as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

Physicochemical Properties of Wheat, Soybeans and Sunflower Seeds Sourdough Bread

The physical and chemical properties of the sourdough bread are presented in Tables 3 and 4. The loaf weight of composite bread increased significantly ($p < 0.05$) with increasing levels of soybean and 10% sunflower seeds flour substitution. Sample F had the highest weight value of 238.05 g while the lowest value was found on sample B at 233.50 g. The increase in loaf weight could be attributed to increased moisture absorption and decreased air entrapment, resulting in heavy dough and heavy loaves (Horsfall *et al.*, 2007). The height of the loaves significantly ($p < 0.05$) decreased with sample A having the highest (8.25 cm) while sample F had the lowest height (3.40 cm) respectively. This decrease in height of the sourdough bread loaves could be as a result of decrease in gluten content of the composite flour which resulted in low carbon dioxide retention of the dough (Adebiyi *et al.*, 2017; Makinde and Akinoso, 2014; Onyango, 2010). The loaf volume and specific volume of the bread decreased significantly ($p < 0.05$) with increase in substitution with soybean and 10% sunflower seed flour. Sample A (commercial bread) had the highest value for loaf volume and specific volume, 1156.45 cm^3 and $4.94\text{ cm}^3/\text{g}$ respectively. Sample F had the lowest value of loaf volume (646.50 cm^3) and specific volume ($2.71\text{ cm}^3/\text{g}$), respectively. The low loaf and specific volumes may be attributed to low levels of gluten in the dough because of the decrease in structure forming proteins in the composite flour which resulted into flour retaining less carbon dioxide gas and a dense texture. Makinde & Akinoso (2014) demonstrated that the protein content of wheat flour was diluted when partially replaced with black sesame flour and interfered with the optimal formation of gluten matrix during mixing of dough, fermentation and baking process. Therefore, dilution of gluten in the

flour blends significantly decreased the specific volume of composite bread. In addition, different physicochemical changes in the flour blends which have positive effect on the rheological properties of the dough might subsequently decrease the loaf volume of bread (Sibanda *et al.*, 2015). The inability of dough to rise during proofing is due to decrease in structure forming protein which leads to low bread volume (Bibiana *et al.*, 2014). A similar decreasing trend in loaf volume and specific volume was also reported by Amandikwa *et al.* (2015) for wheat-yam flour composite bread. The composite bread did not show any crack formation and similar results were reported by Mudau *et al.* (2021) on the physicochemical characteristics of bread partially substituted with finger millet flour.

The proximate composition of the composite sourdough bread is shown in Table 4. The moisture content of the sourdough bread increased significantly ($p < 0.05$) from 14.02 to 25.67% with sample F having the highest value and sample A having the lowest value. These values were lower than those reported by Ndife *et al.* (2011) whose values ranged from 28.50 to 39.50% for wheat, soybean composite bread. The increasing trend in moisture in this study is been associated with increase in fibre content (Maneju *et al.*, 2011). High moisture content has been associated with short shelf life of composite breads as they encourage microbial proliferation that lead to spoilage (Ezeama, 2007).

The ash content showed a percentage increase from 2.66 (sample A) to 4.63% (sample F) as wheat flour was substituted with soybeans and 10% sunflower seeds flour. However the values were higher than those obtained for different sourdough breads reported by Jemima *et al.* (2023). The ash content of food products is an indicator of their mineral composition. Hence, it can be deduced that all the produced sourdough breads were generally better sources of minerals for the body compared to commercial bread.

The fat content also increased from 2.26 to 3.24% in the composite breads. Soybean and sunflower seeds from which the flour was produced are oil seeds, and must have contributed most of the oil content to the product. The fat content of the produced sourdough breads (sample B to F) were higher than the commercial bread (sample A). The documented high fat content in the sourdough breads has the potential of increasing energy and as well as boosting immunity (Yao *et al.*, 2022).

The crude fibre of the sourdough bread varied significantly from 0.62 to 1.33 % with sample F having the highest value (1.33%) and sample A (control) having the lowest (0.62%). However the fibre content values in this study were lower than the composite bread produced from wheat and soybean flour reported by Ndife *et al.* (2011). An increasing trend was observed in the fibre content and this could be attributed to the use of undehulled sunflower seeds flour which had high fibre content.

The increased fibre and lower carbohydrate content of composite breads have several health benefits, as it will aid in the digestion of the bread in the colon and reduce constipation often associated with bread produced from refined wheat flour (Elleuch *et al.*, 2011). According to well documented studies, it is now accepted that dietary fibre plays a significant role in the prevention of several diseases such as; cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer and diabetes (Elleuch *et al.*, 2011). The crude fibre contents of the composite breads, was within the recommended range of not more than 6 g dietary fibre and other nonabsorbable carbohydrates per 100 g dry matter (FAO/WHO, 1994). There was also an increase in the protein content of the composite sourdough breads with increase in soybeans and 10% sunflower seeds flour substitution in the range of 9.13 to 18.95%. This increase could be as a result of the addition of soybeans and sunflower seed flours. Mashayekh *et al.* (2008) also reported an increase in protein content of the bread as a result of the addition of soybean flour. Other studies have also reported a similar increase in protein content in sorghum-soybean composite flours (Singh *et al.*, 2000; Awadelkareem *et al.*, 2008).

The carbohydrate content and energy values decreased from 71.23% and 342.65 Kcal in sample A to 46.73% and 287.20 Kcal in sample F respectively. The decreasing trends in the carbohydrate and energy values indicates that the sourdough introduced in the dough during mixing aided in the breakdown of carbohydrates during the fermentation which also resulted in the possible decrease in energy value. Similar trends were reported by Jemima *et al.* (2023), Serrem *et al.* (2011) and Islam *et al.* (2007) in the production of gluten free composite bread from underutilized African cereals; fortification of wheat flours with defatted and non-defatted soy flour, respectively. Although carbohydrate is known to provide energy that fuel all needed anabolic and catabolic activities in the body however, sourdough addition helps in slowing starch digestibility in wheat flour based products such as bread. Interestingly, the slowing down of starch digestibility constitutes a major nutritional problem, particularly with people on a diet based on wheat bread produced with baker's yeast, because its starch content is rapidly digested and absorbed, leading to hyperglycemia in people with diabetes (Eleazu, 2016). However, the use of sourdough delays the digestion of starch, because the organic acids produced during fermentation reduce the postprandial glycemic response in human blood. For instance, acetic acid reduces gastric emptying, while lactic acid induces interactions between starch and gluten, thereby reducing starch availability (Zuhal *et al.*, 2020). The composite breads in this study contained energy values in the range of 287.20 to 342.65 Kcal, and hence conformed to the (FAO/WHO, 1994) recommended minimum energy content of 1674 kJ/ 100 g.

Table 3: Physical properties of sourdough bread

Sample	Loaf Weight (g)	Loaf Height (cm)	Loaf Volume (cm ³)	Specific Volume (cm ³ /g)	Crack formation	Colour
A	234.30 ^b ±0.20	8.25 ^f ±0.25	1156.45 ^f ±1.35	4.94 ^f ±0.02	No cracks	Yellowish brown
B	233.50 ^a ±0.50	6.80 ^e ±0.00	801.15 ^e ±0.65	3.43 ^e ±0.01	No cracks	Yellowish brown
C	234.65 ^b ±0.15	6.50 ^d ±0.00	764.90 ^d ±2.40	3.26 ^d ±0.01	No cracks	Brown
D	235.25 ^c ±0.05	5.30 ^c ±0.00	710.45 ^c ±0.45	3.02 ^c ±0.00	No cracks	Dark brown
E	237.80 ^d ±0.20	4.60 ^b ±0.10	701.60 ^b ±0.40	2.95 ^b ±0.01	No cracks	Dark brown
F	238.05 ^d ±0.05	3.40 ^a ±0.10	646.50 ^a ±2.00	2.71 ^a ±0.01	No cracks	Dark brown

Values are means ± standard deviation of triplicate determinations. Values in the same column with different superscripts are significantly different at $p < 0.05$

Key: A= Commercial bread; B=100% wheat: 0% soybean: 0% sunflower seeds; C=90% wheat: 10% soybean: 0% sunflower seeds; D= 80% wheat: 10% soybean: 10% sunflower seeds; E= 70% wheat: 20% soybean: 10% sunflower seeds; F= 60% wheat: 30% soybean: 10% sunflower seeds

Table 4: Proximate composition of wheat, soybeans and sunflower seeds flour sourdough bread

Sample	Moisture (%)	Ash (%)	Fat (%)	Fibre (%)	Protein (%)	CHO (%)	Energy (Kcal)
A	14.02 ^a ±0.02	2.66 ^a ±0.02	2.36 ^a ±0.02	0.62 ^a ±0.01	9.13 ^a ±0.01	71.23 ^f ±0.07	342.65 ^f ±0.05
B	16.97 ^b ±0.00	3.37 ^b ±0.02	2.78 ^b ±0.02	0.85 ^b ±0.02	13.34 ^b ±0.01	62.71 ^e ±0.06	329.16 ^e ±0.05
C	18.35 ^c ±0.01	3.78 ^c ±0.01	2.86 ^c ±0.00	0.93 ^c ±0.00	14.80 ^c ±0.02	59.29 ^d ±0.03	322.10 ^d ±0.04
D	21.49 ^d ±0.01	3.89 ^d ±0.00	3.19 ^d ±0.01	0.98 ^c ±0.01	16.88 ^d ±0.01	53.59 ^c ±0.02	310.53 ^c ±0.02
E	21.54 ^c ±0.01	4.31 ^e ±0.00	3.21 ^d ±0.01	1.11 ^d ±0.01	18.65 ^e ±0.01	51.20 ^b ±0.01	308.21 ^b ±0.05
F	25.67 ^f ±0.02	4.63 ^f ±0.01	3.24 ^e ±0.02	1.33 ^e ±0.10	18.92 ^f ±0.02	46.73 ^a ±0.54	287.20 ^a ±2.15

Values are means ± standard deviation of triplicate determinations. Values in the same column with different superscripts are significantly different at $p < 0.05$

Key: A= Commercial bread; B=100% wheat: 0% soybean: 0% sunflower seeds; C=90% wheat: 10% soybean: 0% sunflower seeds; D= 80% wheat: 10% soybean: 10% sunflower seeds; E= 70% wheat: 20% soybean: 10% sunflower seeds; F= 60% wheat: 30% soybean: 10% sunflower seeds

Anti-Nutrient Content of the Wheat, Soybean and Sunflower Seeds Sourdough Bread

The anti-nutritional content of the composite sourdough bread is shown in Table 5 The tannin, saponin, oxalates and phytate content of the sourdough bread varied significantly ($P < 0.05$) from 0.17 to 0.73 mg/ 100g; 0.15 to 0.41 mg/ 100g; 0.67 to 1.96 mg/ 100g; 0.39 to 2.76 mg/ 100g respectively. It was observed that the values for anti-nutrient concentration were low, this could be due to fermentation and heating (during baking) in the course of production of the bread. According to WHO, tannin level in foods below 5 mg/100g is safe for human consumption. Tannins have been reported to form complexes with proteins and reduce their digestibility and palatability (Eka, 1998). Saponins are not destroyed during cooking (Nwosu *et al.*, 2012). However, Onimawo and Akubor (2012) reported that trace amounts of saponins are nutritionally beneficial because of their hypocholesterolemic activity

(cholesterol lowering). Saponin is another anti-nutritional factor whose toxicological effects should be balanced with its benefits. Oxalic acid and oxalate occur naturally in plants but they have little or no useful effect on human health as high levels in diets lead to irritation of the tissues, the digestion system, particularly the stomach and kidney (Ogbuagu, 2008). The use of sourdough as a fermentation agent during bread making has been known to be effective in reducing the phytic acid content (Zuhal *et al.*, 2020). Knowing that its presence interferes with the absorption of minerals via the complexation mechanism with divalent minerals (Zn^{2+} ; Ca^{2+} ; Mg^{2+} ; Fe^{2+}), leading to the formation of phytates, and thus disrupting their homeostasis in the human body, hence the emergence of mineral deficiencies (Bora, 2014; Grases *et al.*, 2017). Ingestion of 2.5 g or more of phytic acid per day has been reported to cause reduction in bioavailability of calcium, iron and zinc (Onimawo and Akubor, 2012).

Table 5: Anti-nutritional factors in wheat, soybean, and sunflower seeds sourdough bread

Sample	Tannins	Mg/100g Saponins	Oxalates	Phytates
A	0.17 ^a ±0.02	0.15 ^a ±0.01	0.67 ^a ±0.01	0.39 ^a ±0.02
B	0.27 ^b ±0.02	0.23 ^b ±0.01	0.87 ^b ±0.02	0.46 ^b ±0.01
C	0.26 ^b ±0.03	0.26 ^c ±0.00	0.94 ^c ±0.01	0.83 ^c ±0.02

D	0.33 ^c ±0.01	0.29 ^d ±0.01	1.57 ^d ±0.01	1.36 ^d ±0.01
E	0.64 ^d ±0.02	0.36 ^e ±0.02	1.78 ^e ±0.01	1.22 ^e ±0.02
F	0.73 ^e ±0.01	0.41 ^f ±0.01	1.96 ^f ±0.01	2.76 ^f ±0.02

Values are means ± standard deviation of triplicate determinations. Values in the same column with different superscripts are significantly different at $p < 0.05$

Key: A= Commercial bread; B=100% wheat: 0% soybean: 0% sunflower seeds; C=90% wheat: 10% soybean: 0% sunflower seeds; D= 80% wheat: 10% soybean: 10% sunflower seeds; E= 70% wheat: 20% soybean: 10% sunflower seeds; F= 60% wheat: 30% soybean: 10% sunflower seeds

Essential Amino Acid Profile of Wheat, Soybeans and Sunflower Seeds Sourdough Bread

The results for the essential amino acid composition of the composite bread is presented in Table 6 Generally the Total essential amino acids of the composite sourdough bread samples in this study ranged from 21.68 to 42.47 mg/100g protein. The increase in the amino acid composition of the composite sourdough bread showed the protein quality of the bread produced. The values for Lysine, Tryptophan, Histidine, Phenylalanine, Leucine, Isoleucine, Threonine, Methionine, and Valine, of the wheat, soybean and sunflower seeds sourdough bread were higher than that of the commercial bread. The results further revealed that lysine and sulphur

containing amino acids (methionine) and tryptophan, which are known to be limiting in cereals and legumes, respectively were significantly improved in the blends. This showed that the complementary effect of cereal / legume mixture improved both the protein quality and the levels of the limiting amino acids. A similar trend was observed by Okoye (2016) who prepared biscuits from the blend of wheat, soybean and bambara groundnut flour. The same trend was also reported by Emelike *et al.* (2020) who produced bread from a blend of wheat and cashew kernel flours. The essential amino acids are vital from the nutritional point of view since the body cannot synthesize them and should therefore be supplemented in the diet.

Table 6: Essential amino acid profile of sourdough bread

Sample	Lys	Try	His	Phy	mg/100g Leu	Iso	Thr	Met	Val	TEAA
A	3.85 ^a ±0.00	1.06 ^a ±0.01	0.79 ^a ±0.01	5.89 ^a ±0.01	1.55 ^a ±0.00	1.65 ^a ±0.00	1.51 ^a ±0.01	2.66 ^a ±0.01	2.72 ^a ±0.01	21.68
B	3.87 ^b ±0.01	1.54 ^b ±0.01	0.86 ^b ±0.01	6.25 ^b ±0.01	1.75 ^b ±0.01	2.01 ^b ±0.01	1.54 ^b ±0.02	2.72 ^b ±0.01	2.75 ^b ±0.01	23.29
C	4.01 ^c ±0.01	2.62 ^c ±0.01	1.13 ^c ±0.01	7.34 ^c ±0.01	2.25 ^c ±0.00	2.85 ^c ±0.01	1.87 ^c ±0.01	3.78 ^c ±0.01	3.84 ^c ±0.01	29.69
D	4.17 ^d ±0.01	3.16 ^d ±0.01	1.23 ^d ±0.01	8.13 ^d ±0.01	3.41 ^d ±0.01	2.97 ^d ±0.01	2.45 ^d ±0.00	4.11 ^d ±0.01	4.34 ^d ±0.01	33.97
E	4.26 ^e ±0.01	4.17 ^e ±0.01	1.32 ^e ±0.01	8.93 ^e ±0.01	3.66 ^e ±0.01	4.04 ^e ±0.01	3.86 ^e ±0.01	5.26 ^e ±0.01	3.87 ^e ±0.01	39.37
F	4.66 ^f ±0.01	4.73 ^f ±0.51	1.48 ^f ±0.01	8.94 ^f ±0.01	4.66 ^f ±0.01	4.04 ^f ±0.01	3.93 ^f ±0.01	5.86 ^f ±0.01	4.17 ^f ±0.01	42.47

Values are means ± standard deviation of triplicate determinations. Values in the same column with different superscripts are significantly different at $p < 0.05$

Key: A= Commercial bread; B=100% wheat: 0% soybean: 0% sunflower seeds; C=90% wheat: 10% soybean: 0% sunflower seeds; D= 80% wheat: 10% soybean: 10% sunflower seeds; E= 70% wheat: 20% soybean: 10% sunflower seeds; F= 60% wheat: 30% soybean: 10% sunflower seeds; Lys= lysine, Try= Typtophane, His= Histidine, Phy = Phynylalanine, Leu= Lencine, Iso= Isoleucine, Thr= Threonine, Met= Methionine, Val= Valine, TEAA= Total essential amino acids

Mineral Content of Wheat, Soybeans and Sunflower Seeds Sourdough Bread

The mineral content for the composite sourdough bread is shown in Table 7. The mineral content of the sourdough bread increased from 42.56 to 88.12 mg/100g; 85.15 to 151.36 mg/100g; 175.34 to 260.00 mg/100g; 4.36 to 12.92 mg/100g; 0.86 to 6.89 mg/100g for magnesium, potassium, calcium, iron and zinc respectively. It was observed that the study samples (sample B, C, D, E, and F) had higher mineral content (especially calcium) than sample A (commercial bread). The values obtained for magnesium, potassium, calcium,

were higher but comparable to those reported by Henry-Unaeze *et al.* (2022) for bread produced from wheat, African yam beans and corn composite flour blends. The high mineral content in the sourdough bread could be due to the fact that soybean and sunflower seeds (used in preparing the composite flour) are good sources of essential minerals. The importance of minerals in human health has already been underscored (Emelike *et al.*, 2020; Bibiana *et al.*, 2014). Potassium is important in the metabolism of carbohydrates and protein, the regulation of heartbeat, and the reduction in risk of hypertension and stroke (Bibiana *et al.*, 2014).

Table 7: Mineral content of wheat, soybeans and sunflower seeds sourdough bread

Sample	Mg	P	mg/100g Ca	Fe	Zn
A	42.56 ^a ±0.01	85.15 ^a ±0.01	175.34 ^a ±0.01	4.36 ^a ±0.01	0.86 ^a ±0.01
B	47.70 ^b ±0.02	115.39 ^c ±0.01	232.03 ^c ±0.01	8.57 ^b ±0.01	1.38 ^b ±0.01
C	55.65 ^c ±0.01	137.34 ^c ±0.02	235.85 ^d ±0.00	9.35 ^c ±0.01	2.55 ^c ±0.00
D	74.60 ^d ±0.03	94.32 ^b ±0.02	260.00 ^f ±0.00	11.26 ^d ±0.01	3.65 ^d ±0.01
E	67.34 ^c ±0.01	125.63 ^d ±0.01	241.22 ^e ±0.01	12.65 ^e ±0.00	4.86 ^e ±0.01
F	88.12 ^f ±0.01	151.36 ^f ±0.01	224.63 ^b ±0.01	12.92 ^f ±0.01	6.89 ^f ±0.01

Values are means ± standard deviation of triplicate determinations. Values in the same column with different superscripts are significantly different at $p < 0.05$

Key: A= Commercial bread; B=100% wheat: 0% soybean: 0% sunflower seeds; C=90% wheat: 10% soybean: 0% sunflower seeds; D= 80% wheat: 10% soybean: 10% sunflower seeds; E= 70% wheat: 20% soybean: 10% sunflower seeds; F= 60% wheat: 30% soybean: 10% sunflower seeds

Sensory Properties of Wheat, Soybeans and Sunflower Seeds Sourdough Bread

Sensory properties are important measures for assessing quality in the development of new products and for meeting consumer requirements. The sensory scores are shown in Table 8 The commercial bread was rated higher in all the sensory attributes assessed. The scores for all attributes decreased with increase in soybeans and 10% sunflower seeds flours. Sample A (commercial bread) had the highest scores for appearance while sample F (60% wheat flour, 30% soybean and 10% sunflower seeds) had the lowest and there was a significant difference at 5% probability level. The low scores in appearance of the composite bread could be attributed to the added dehulled sunflower seeds which imparted a dark brown colour to the sourdough bread samples. There was no significant difference ($P < 0.05$) in the taste of the composite sourdough bread samples. However, the control (sample A) tasted differently from the other bread samples. Interestingly, the soybean off flavour (which is common when soybean is used in composite flour formulation) was not reported by the panelist. This could be as a result of the sour taste brought about by

the lactic acid (from sourdough) produced by the lactic acid bacteria during fermentation of the bread (Espinosa and Rosa-angulo, 2011). In terms of mouth feel, sample A felt different in the mouth when compared to the other sourdough bread samples. However there was no significant difference ($p < 0.05$) in mouth feel among the sourdough bread samples. The baking conditions (temperature and time variables); the state of the bread components, such as fibre, starch, protein (gluten) weather damaged or undamaged and the amounts of absorbed water during dough mixing, all contribute to the final mouth feel of the bread (Serrem *et al.*, 2011). From the results, the mean score for aroma of the breads ranged from 5.67 to 7.93. Sample A (Commercial bread) had the highest mean scores (7.93) followed by sample B (100% sourdough) (6.87). The results showed that the levels of supplementation significantly affected the overall acceptability of the bread samples. As such, the values ranged from 6.00 to 8.47 with sample A having the highest score of 8.47 followed by sample B (7.73). In general, the baking properties of composite flours are often impaired as well as the organoleptic attributes of the products, due to the dilution of the gluten content (Jideani and Onwubali, 2009).

Table 8: Sensory properties of wheat, soybeans and sunflower seeds sourdough bread

Sample	Appearance	Taste	Mouthfeel	Aroma	General acceptability
A	8.47 ^c ±0.52	8.53 ^b ±0.52	8.00 ^c ±0.76	7.93 ^c ±0.96	8.47 ^b ±0.74
B	7.73 ^d ±0.80	6.93 ^a ±1.22	7.33 ^{bc} ±1.23	6.87 ^b ±1.41	7.73 ^b ±0.96
C	7.00 ^c ±1.07	6.93 ^a ±1.75	6.80 ^{ab} ±1.52	6.67 ^{ab} ±1.63	6.67 ^a ±1.35

D	6.40 ^{bc} ±0.99	6.33 ^a ±1.35	6.00 ^a ±1.46	6.20 ^{ab} ±1.66	6.40 ^a ±1.50
E	6.13 ^{ab} ±0.99	5.93 ^a ±1.16	6.07 ^a ±1.49	6.20 ^{ab} ±1.32	6.20 ^a ±1.08
F	5.47 ^a ±0.22	6.00 ^a ±1.31	5.80 ^a ±1.21	5.67 ^a ±1.63	6.00 ^a ±1.46

Values are means ± standard deviation of triplicate determinations. Values in the same column with different superscripts are significantly different at $p < 0.05$

Key: A= Commercial bread; B=100% wheat: 0% soybean: 0% sunflower seeds; C=90% wheat: 10% soybean: 0% sunflower seeds; D= 80% wheat: 10% soybean: 10% sunflower seeds; E= 70% wheat: 20% soybean: 10% sunflower seeds; F= 60% wheat: 30% soybean: 10% sunflower seeds

CONCLUSION

The study evaluated the nutritional profile and sensory properties of sourdough bread from composite flour of wheat, soybean and sunflower seeds. It was discovered that addition of 10, 20 and 30% soybean and 10% sunflower seeds flour improved the nutritional content of the bread. This study further demonstrated that soybean and 10% sunflower seeds flour addition to sourdough bread had considerable effect on the nutritional and sensory properties of the bread. With percentage increase in soybeans and 10% sunflower seeds flour, the nutritional properties, protein, fiber, ash and fat contents of the bread increased. The study also established that the protein quality of the product improved with increasing amounts of all the essential amino acids and minerals as such had the potentials of preventing micronutrients malnutrition. Similarly, the sensory properties showed that the commercial bread had the highest scores and was preferred in terms of all the sensory attributes evaluated. However, sample B (100% wheat sourdough bread) followed closely in terms of preference. Based on these results, it can be concluded that sourdough bread produced from this composite flour has more nutrient density than the commercial bread as such will go a long way to prevent malnutrition. Also the use of sourdough in bread making is encouraged especially for composite flour preparation involving soybeans since it is effective in silencing the beany off flavours associated with it.

Acknowledgement

The authors appreciate the leadership of the Benue State University Center for Food Technology and Research (CEFTR), Makurdi for the financial support received during the laboratory analysis of this research work.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

Authors declared that they have no conflict of interest regarding the work detailed in this manuscript.

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