African Journal of Agriculture and Food Science
ISSN: 2689-5331
Volume 7, Issue 3, 2024 (pp. 265-283)
www.abjournals.org

QUALITY EVALUATION OF COMPOSITE FLOUR FROM WHEAT, SOYBEAN AND SUNFLOWER SEEDS FOR BREAD MAKING

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Cite this article:

Msugh C. T., Adah C. A., Asemave K. (2024), Quality Evaluation of Composite Flour From Wheat, Soybean and Sunflower Seeds for Bread Making. African Journal of Agriculture and Food Science 7(3), 265-283. DOI: 10.52589/AJAFS-C84BU7GC

Manuscript History

Received: 22 Jun 2024

Accepted: 27 Aug 2024

Published: 9 Sep 2024

ABSTRACT: *Composite flours with good functional properties and high nutrient content was developed by substituting wheat with soybeans and sunflower seed flour. Wheat flour was supplemented with soybeans and sunflower seeds flour at a ratio of 100:0:0, 90:10:10, 80:10:10, 70:20:10 and 60:30:10, respectively. Standard analytical procedures were used for all the analysis on the samples. The composite flours obtained were evaluated for their functional properties, proximate composition and antinutritional properties. The results for the functional properties showed values which ranged from 1.76 to 2.38 mL/g and 1.85 to 2.45 mL/g for water absorption capacity, 1.02 to 1.10 mL/g and 1.10 to 1.25 mL/g for oil absorption capacity, 0.40 to 0.78 g/mL and 0.52 to 0.78 g/mL for bulk density, 8.33 to 12.10% and 8.66 to 12.10% for swelling capacity and 4.00 to 10.00% and 4.00 to 9.00% for least gelation concentration, respectively for the flours and flour blends. Addition of soybeans and sunflower seeds flour led to significant (p<0.05) increase in the protein content (15.22 to 28.90%), ash (1.01 to 3.31%), crude fibre (1.34 to 2.97%), fat (5.39 to 16.37%), energy value (388.05 to 434.67%) and a decrease in carbohydrate (69.68 to 42.94%) and moisture content (7.38 to 5.3%) for the flour blends. Processing methods affected the antinutritional factors as there was no significant increase in the tannin content (0.43 to 0.65 mg/100g), saponins (0.36 to 0.43 mg/ 100g), oxalates (1.24 to 2.40 mg/100g) and phytates content (1.22 to 2.12 mg/100g) concentration of the composite flour. The results reveal that the wheat, soybeans and sunflower seeds flours had good functional and nutritional density.*

KEYWORDS: Soybeans, Sunflower seeds, Antinutrients.

Article DOI: 10.52589/AJAFS-C84BU7GC
DOI URL: https://doi.org/10.52589/AJAFS-C84BU7GC

INTRODUCTION

Composite flour is defined as a mixture of several flours obtained from roots and tubers, cereals, legumes with or without the addition of wheat flour that is created to satisfy specific functional characteristics and nutrient composition (Bolarinwa et al., 2015). The use of composite flour to produce baked goods, if feasible, would help to lessen total dependence on imported wheat (Emmanuel et al., 2019).Wheat flour, which is widely used to make bread and other baked goods like cakes, biscuits, and doughnuts, is nutritionally poor and its low protein content has been a major source of concern when it comes to its usage. For this reason substituting wheat with other flours from legumes, oil seeds, tubers and other cereals (rich in proteins and other nutrients) will improve its usage.

Soybean (*Glycine max* L.) is a leguminous plant widely grown for its edible bean which has numerous uses. It is a good source of vitamins and minerals and an excellent source of protein (about 35 – 45%) with all essential amino acids required for proper growth and maintenance of the body (Ojinnaka&Nnorom, 2015). Soybean is usually processed and used as an essential part of functional foods and for enrichment of product quality (Admad et al., 2014). The addition of soybean flour to wheat may improve the protein quality of the flour and make it useful in the production of products such as bread with improved nutritional content.

Sunflower (*Helianthus annuus*) is one of the most important oilseeds crops in the world (Stefansson, 2007). The seeds of sunflowers contain healthy unsaturated fats, protein, fibre, selenium, copper, zinc, folate, iron, and phytochemicals (FAO-STAT, 2008). The daily consumption of complex plant foods like seeds, nuts, and whole grains is currently the focus of nutrition scientist research. Sunflower seeds can be added to baked foods like bread and are a good source of vitamin E (Sabitha & Puraikalan, 2014). The substitution of wheat with soybeans and sunflower seed flour will balance the amino acids, vitamins and minerals hence improve the nutritive value of cereal-based food products. Therefore, flours from legumes and other cereals can be successfully used in bread and other baked products to obtain a protein-enriched food with improved nutrient balance. The study therefore seeks to evaluate the quality of composite flour produced from wheat, soybeans and sunflower seeds for bread making.

LITERATURE REVIEW

Use of Composite Flour in Bread Making

Composite flours are either binary or ternary mixes of flours derived from different crops, either containing or excluding wheat flour (Shittu et al., 2007; Bolarinwa et al., 2015). For developing nations like Nigeria, using composite flours offered a number of benefits, including: saving hard currency; encouraging the growth of native plant species with high yields; improving the supply of protein for human nutrition; and improving the overall utilization of domestic agricultural production (Bugusu et al., 2001). Since composite flour encourages the use of locally grown crops as flour and lessens the importation of wheat flour, it is thought to be beneficial in developing nations (Hugo et al., 2000; Hasmadi et al., 2014).

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According to a report by the FAO, the demand for bread and pastry products could be met by using domestically grown crops rather than wheat, and the application of composite flour in various food products would be economically advantageous if wheat imports could be decreased or even eliminated (Jisha et al., 2008). The bakery products made with composite flour are known to be of high quality and shared certain attributes with bread made with wheat flour; however, they have a different texture and set of characteristics from wheat flour-made products, including a higher nutritional value and a different look. Wheat is regarded as nutritionally poor in addition to being a good source of calories and other nutrients since cereal proteins lack important amino acids like lysine and threonine (Dhingra & Jood, 2001). Thus, supplementation of wheat flour with cheap and available crops, such as legumes, and other oil seeds will help improve the nutritional quality of wheat products thus reducing malnutrition (Sharma et al., 1999).

MATERIALS AND METHODS

Soybeans (variety TGX 1957-3F) was obtained at the Benue State Agency for Agricultural and Rural Development Authority (BNARDA) Makurdi. The wheat flour, sunflower seeds used in this study were obtained from Wadata Market, Makurdi, Benue State, Nigeria. Processing of these materials was done at the Centre for Food Technology and Research (CEFTER) food laboratory, department of Chemistry, Benue State University, Makurdi.

Preparation of Composite flours

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

Similarly, 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 sprayed on oven trays and transferred into an oven heated at 55 ^{0}C for 8 h to enable it to dry. Thereafter the seeds were grinded into flour using a laboratory grinder. The flowchart for the preparation of the wheat, soybeans and sunflower seeds composite flour is shown in Figure 1 and the blend formulation is presented in Table 1 respectively.

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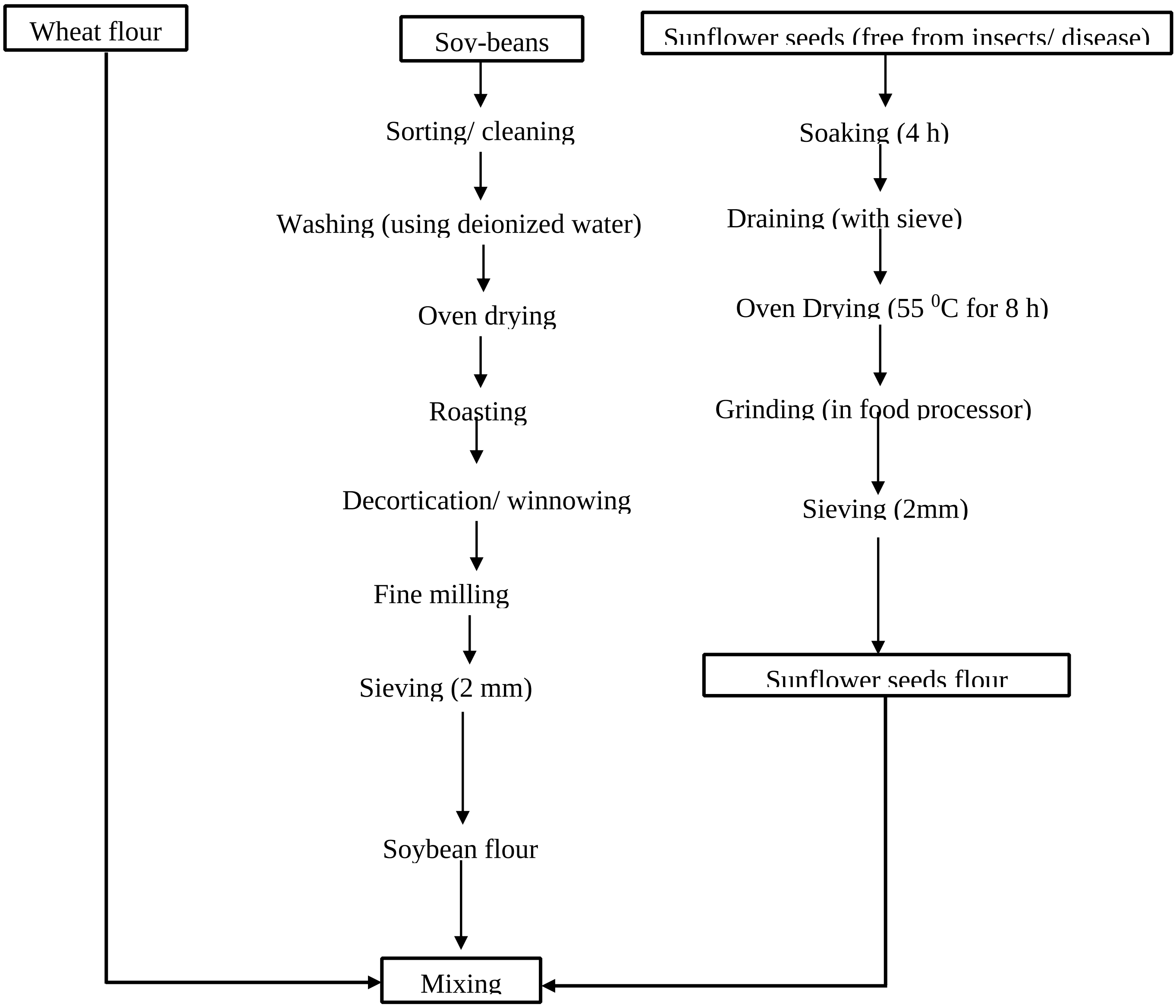

Figure 1: Flow chart for the preparation of wheat, soybean and sunflower seeds composite flour

Source: Ndife et al. (2011) with slight modification

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Table 1: Formulation of Wheat, Soybean and Sunflower Seeds Composite Flour Blends

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

ANALYTICAL METHODS

Functional Properties of Wheat, Soybean and Sunflower Seeds Composite Flour Blends

Determination of Bulk Density

This was determined using the method described by AOAC (2012). 5 g of sample was filled in a 20 mL graduated cylinder and its bottom tapped on the laboratory bench until there was no decrease in volume of the sample. The volume was recorded: Bulk density can be expressed mathematically as described in equation (1):

$$Bulk\ density = \frac{Weight\ of\ sample\ (g)}{Volume\ of\ sample (mL)} \quad (1)$$

Determination of Swelling Capacity

Swelling capacity was determined according to the method of Onwuka, (2005). About 100 mg of the sample was mixed with 10 mL of distilled water in a calibrated cylinder at ambient temperature. After equilibration for 18 h, the bulk volume was recorded and swelling capacity expressed as volume occupied by sample per gram of original sample dry weight. Swelling capacity can be expressed mathematically as described in the equation 2:

$$Swelling\ capacity\ (\%) = \frac{change\ in\ volume\ of\ sample}{Original\ weight\ of\ sample} \quad (2)$$

Water Absorption Capacity

Water absorption capacity was determined following the methodology of Robertson et al. (2000). About 0.5 g of sample was transferred to a test tube and deionized water (10 mL) was added. The mixture was agitated and left to hydrate for 30 min. After centrifugation at 1650 r/min for 10 min the mixture was left to settle and to separate the supernatant. Finally, the sediment was weighed. The water absorption capacity (WAC) was calculated as described by equation (3) below:

$$Water\ Absorption\ Capacity\ (\frac{mL}{g}) = \frac{Water\ absorbed}{Weight\ of\ sample} \quad (3)$$

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Where Water absorbed (mL) = (Volume of water added - Volume of water obtained after centrifugation).

Oil Absorption Capacity

The method described by Omowaye-Taiwo et al. (2015)was employed to determine the oil absorption capacity (OAC) of the flours. The density of the oil used was 0.92 g/mL. About 1 g the sample was mixed with oil (10 mL) and stirred thoroughly for 5 min. The volume of the decanted supernatant was measured and used to calculate the Oil Absorption Capacity as shown in equation 4

$$OAC = \frac{\text{initial volume oil} - \text{final volume of oil}}{\text{initial volume of oil}} X\ 100 \quad (4)$$

Least Gelation Concentration

Least gelation concentration was determined by the method described by Adeleke and Odedeji (2010). Test tubes containing suspensions of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20% (w/v) of flours in 5 mL deionized water were heated for 1 h in a boiling water bath. This was followed by rapid cooling under cold running tap water. The least gelation concentration (LGC) was taken as that concentration at which the sample in the inverted test tube did not fall down or slip.

Proximate Analysis

Determination of 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 ^{0}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 5:

$$Percentage\ Moisture\ content\ (\%\ MC) = \frac{W_1 - W_2}{W_1 - W_0} X100 \quad (5)$$

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 neutralised with alkali and distilled into a boric acid solution. The borate anions formed are titrated with standardised 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 6.

$$\text{Percentage Nitrogen} = \frac{\text{Titre} \times \text{N} \times \text{DF} \times 0{,}0140067}{\text{Weight of sample}} X100 \quad (6)$$

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Crude protein = % total Nitrogen × 6.25

Titre = Final burette reading - Initial burette reading

N = Normality of acid

DF = 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 ^{0}C for 30 min then cooled and weighed (W_1). Percentage of fat content was calculated as described in equation 7:

$$crude\ fat(\%) = \frac{Weight\ of\ fat\ in\ sample}{Weight\ of\ dry\ sample}\ X\ 100 \quad (7)$$

$$= \frac{W_0 - W_1}{Weight\ of\ dry\ sample} X 100$$

Determination of Crude Fibre

Crude fibre was determined using a method described by Nwafor et al. (2020). Exactly 2 g of samples were digested in a conical flask with 100 mL of Trichloroacetic acid (TCA). This solution was boiled and refluxed 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 ^{0}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 ^{0}C for 6 h, cooled and its weight recorded. The crude fibre was calculated using equation 8:

$$\%\ crude\ fibre\ = \frac{(final\ weight\ of\ crucible - initial\ weight\ of\ crucible)}{(weight\ of\ sample)}\ X\ 100 \quad (8)$$

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, carbonised on a hot plate and heated on a muffle furnace at 600 ^{0}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 9:

$$Ash\ (\%) = \frac{Weight\ of\ crucible + Ash - weight\ of\ empty\ crucible}{Weight\ of\ sample} X\ 100 \quad (9)$$

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Determination of Total Carbohydrates

The total carbohydrate content was estimated by difference as previously reported by Nwafor et al. (2020) using equation 10:

$$Carbohydrate\ (\%) = 100 - (moisture + fat + \ protein + \ ash + \ crude\ fibre)\% \quad (10)$$

Determination of Total Energy

The total energy value was calculated according to the method described by Oluyemisi et al. (2016) expressed in equation 11:

$$Total\ Energy\ (Kcal/100g) = [(\%\, carbohydrates\ \times 4) + (\%\, proteins\ \times 4) + (\%\, fat\ \times 9)] \quad (11)$$

Determination of Antinutrients

Determination of Tannins

Tannins were determined using the method described by Patchimapornet 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, and100 mL of isobutyl alcohol was added. The mixture waskept on a shaker for 5 h, and then passed through the No. 1 Whatmanfilter paper into a beaker containing 40% saturated $MgCO_3$ (20 mL),and filtered again to obtain a clear colourless solution. To 1 mL of the colourless filtrate, 2 mL of 5% $FeCl_3$ solution was added and incubatedfor 30 min. The absorbance of the colour formed was measured at 380nm.

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

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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.×250mm length). The mobile phase was 0.25 g/100 mL dehydrogenase phosphate and 0.0025 mol/L tetrabutylammonium 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 the pink colour of Wade reagent, which is formed upon the reaction of the ferric ion and sulfosalicylic acid. One millilitre of Wade reagent (0.03% solution of $FeCl_3 \cdot 6H_2O$ 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.

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 to 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 ± standard deviation.

RESULTS AND DISCUSSION

Functional Properties of Wheat, Soybeans and Sunflower Seeds Flours and Flour Blends

Functional properties are properties that determine the suitability of the food material for specific purposes. The result of the functional properties of wheat, soybean and sunflower seeds flour is presented in Table 1. Water absorption capacity is the ability of a food product to incorporate water. In this study, the highest values for water absorption were 2.38 mL/g and 2.45 mL/g while the least values were 1.76 mL/g and 2.03 mL/g for the flours and flour blends, respectively. The water absorption capacity increased from 2.02 mL/g (sample B) to

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2.45 mL/g (sample E) for the flour blends. This trend is in agreement with the study reported by Nwachukwu et al. (2020). The increase in water absorption is due to the high protein content of the soybean flour because proteins are capable of binding large quantities of water because they form hydrogen bonds between molecules and polar groups on the polypeptide chain (Akubor & Onimawo, 2003). The increase in WAC of the composite flour could therefore be attributed to the soybean flour and its protein content. High water absorption capacity indicates that such flour will be useful in the formulation of some foods such as bread and other bakery products (Chandra et al., 2015). There was a significant difference ($P< 0.05$) among all the samples.

The oil absorption capacity (OAC) is an essential functional property that contributes to enhancing the mouth feel while retaining the food products flavour (Iwe et al., 2016). The oil absorption capacity increased from 1.13 to 1.25 mL/g in the flour blends as the substitution with soybean flour increased. This trend is in agreement with the work of camel et al. (2019). Just like the water absorption capacity, the oil absorption capacity increased due to the high protein content in the soybean flour (Samsher, 2013). The bulk density is important for the dietary bulk and gives an indication of the relative volume of packaging material required for a food product (Oppong et al., 2015). Nutritionally, a loose bulk density not only promotes easy digestibility of food but also enhances nutrient and calories density and this offers an extra advantage in formulating complementary foods (Osundahunsi & Awoh, 2002). In this study, sample A (control) had the highest value (0.78 g/mL) for bulk density while sample E had the lowest value (0.52 g/mL). A decreasing trend in values for bulk density was observed from sample A (0.78 g/mL) to sample E (0.52 g/mL). This decreasing trend is in agreement with the study reported by (Akubor & Onimawo, 2003). This may be due to increase in substitution of soybean as both soybean flour and sunflower seeds flour have low bulk densities (Akubor & Onimawo, 2003). Flour with high bulk densities are used as thickeners in food products while those with low bulk density can be useful in formulation of complementary foods (Patience & Miracle, 2023). All the samples evaluated for bulk density were significantly different at 5% probability level.

Swelling capacity or index is a measure of the starch ability to absorb water and swell. It is considered a quality measure in some food products such as bakery products (bread) (Iwe et al., 2016). Sample A (Wheat flour) (12.10 g/mL) had the highest mean value and sample E (8.66 g/mL) had the lowest mean value for the flour blends respectively. The decreasing trend observed in this study is similar to that reported by Julianti et al. (2017). The decrease in swelling index may be as a result of the high lipid (fats) in the soybeans and sunflower seeds which acted as amylase swelling inhibitor, starch and protein interaction and attraction of their opposite charges to form inclusion complexes during gelatinization which restrict swelling (Shimelis et al., 2006).

The least gelation concentration (LGC) is defined as the lowest protein concentration at which gel remains in an inverted tube. Least gelation concentration is used to measure the ability of the protein to form a gel, as such; a lower least gelation concentration suggests a better gelling capacity (Abu et al., 2005). There was a significant difference in all the samples (at $p<0.05$) except for sample D and E which showed no significant difference. Sample A (100% wheat flour) and sample B (90% wheat, 10% soybean) formed gel at relatively low concentration (4.00% and 5.00%) respectively. while Sample C,D, E formed gel at higher concentration (6.00, 9.00, 9.00%) respectively. This is an indication that the gelling capacity of Wheat reduces as the level of other flour addition increases. The variation in the gelling

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properties may be as a result of the different constituents such as protein, carbohydrates and lipids (fats) present in the different flours (Soybeans and sunflower seeds flour) suggesting that interaction between such components may also have a significant role in the functional properties (Aremu et al., 2007). The lower the least gelation concentration, the better the gelling ability of the protein ingredient in the food formation (Patience & Miracle, 2023).

Table 1: Functional Properties of Wheat, Soybeans and Sunflower Seeds Flour

Sample	WAC(mL/g)	OAC (mL/g)	BD (g/mL)	SC (%)	LGC (%)
A	$1.85^{b}\pm0.01$	$1.10^{c}\pm0.01$	$0.78^{f}\pm0.01$	$12.10^{g}\pm0.00$	$4.00^{a}\pm0.00$
B	$2.03^{c}\pm0.00$	$1.13^{d}\pm0.00$	$0.67^{e}\pm0.03$	$10.53^{f}\pm0.01$	$5.00^{b}\pm0.00$
C	$2.12^{d}\pm0.01$	$1.15^{e}\pm0.00$	$0.58^{d}\pm0.00$	$10.15^{e}\pm0.00$	$6.00^{c}\pm0.00$
D	$2.28^{e}\pm0.00$	$1.19^{f}\pm0.01$	$0.56^{c}\pm0.01$	$9.65^{d}\pm0.00$	$9.00^{e}\pm0.00$
E	$2.45^{g}\pm0.01$	$1.25^{g}\pm0.01$	$0.52^{b}\pm0.01$	$8.66^{b}\pm0.00$	$9.00^{e}\pm0.00$
SBF	$2.38^{f}\pm0.02$	$1.05^{b}\pm0.02$	$0.51^{b}\pm0.01$	$9.52^{c}\pm0.01$	$8.00^{d}\pm0.00$
SSF	$1.76^{a}\pm0.03$	$1.02^{a}\pm0.02$	$0.40^{a}\pm0.02$	$8.33^{a}\pm0.02$	$10.00^{f}\pm0.00$

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= 100% wheat: 0% soybean: 0% sunflower seeds
B=90% wheat: 10% soybean: 0% sunflower seeds
C= 80% wheat: 10% soybean: 10% sunflower seeds
D= 70% wheat: 20% soybean: 10% sunflower seeds
E= 60% wheat: 30% soybean: 10% sunflower seeds
SBF= Soybean flour
SSF= Sunflower seeds flour
WAC = Water absorption capacity
OAC= Oil absorption capacity
BD = Bulk density
SC = Swelling Capacity
LGC = Least Gelation Concentration

Proximate Composition of Wheat, Soybeans and Sunflower Seeds Composite Flour

Table 2 shows the result for the proximate composition and energy value of wheat, soybeans and sunflower seeds composite flour. Sample A had the highest mean value (8.04%) and sample SBF had the lowest mean value (1.32%) for the flours while sample B had the highest value (7.38%) and sample E had the lowest value (5.53%) for the flour blends. The moisture content obtained from this study is below the maximum of 15.5% recommended by CODEX (CS 152-1985), and is an advantage as low moisture content will enhance the shelf life of the flour. The moisture content decreased with increase in substitution with soybean flour and 10% sunflower seeds flour from sample B to E. This decreasing trend is invariant with the study by Verem et al. (2021) who reported an increase in moisture content as substitution increased. The decrease may be as a result of the high total dry solids with high emulsifying properties contained in the soybean flour compared to other flours. The moisture content was significantly different across all the samples at 5% probability level.

Article DOI: 10.52589/AJAFS-C84BU7GC
DOI URL: https://doi.org/10.52589/AJAFS-C84BU7GC

The ash content varied from 0.59 to 5.02% for the flours and 1.01 to 3.31% for the flour blends. The ash content increased with increase in soybean flour and 10% sunflower seeds flour substitution. Ash content is a measure of mineral content of the food and the high values of the ash in the blends could imply more mineral content in the composite flour. The increase was significant at 5% probability level. This increasing trend of ash content with increase in substitution with soybean is in agreement with other studies (Ayo et al., 2014; Ndife et al., 2011). The fat content ranged from 3.21 to 33.77% for the flour and 5.39 to 11.37% for the flour blends. The highest fat content in the flour blends was recorded in sample E (16.37%) and the lowest in Sample A (control) 3.21%. The increase in fat content of the sample could be an added advantage as fats supply essential fatty acids and are known to enhance flavour. This increasing trend in fat content is in agreement with other studies (Ayo et al., 2014; Banureka & Mahendran, 2009), on soy flour supplementation for the production of biscuits and could be explained as soybean is the world's leading source of edible oils as well as sunflower seeds. Soybeans contain 20-24% fat, most of which are unsaturated in nature (Reddy, 2004). Sunflower seeds are also a good source of unsaturated fat (Sabitha & Puraikalan, 2014).

The fibre content ranged from 0.09 to 31.89% for the flours and 0.09 to 2.97% for the flour blends. Sample A had the lowest fibre content (0.09%) and sample SSF had the highest for the flowers while sample E had the highest for the flour blends. An increasing trend in fibre content was observed. A similar increase in fibre content was also reported by Ayo et al. (2014), and Farzana and Mohajan (2015) on the effect of supplementation of soybean flour on the production of biscuits. Consumption of food rich in dietary fibre has been reported to reduce the risk of diabetes mellitus, cancer, cardiovascular diseases, constipation, colon etc. (McRae, 2018; Masrul & Nindrea, 2019).

The protein content in this study was found to vary from 10.36 to 35.36% for the flours and 10.36 to 28.90% for the flour blends. The highest protein content (28.90%) was found in sample E and the lowest (10.32%) in sample A (control). An increasing trend in protein content was observed in this study and this was supported by other studies (Banureka & Mahendran, 2009; Ayo et al., 2014). This may be due to the fact that soybean is known to be a high protein legume crop and an excellent complement to lysine-limited cereal protein (Gars et al., 2014). Soybean flour contains a high protein as such could be used as an economical protein supplement in bread, biscuit, pasta and other cereal products. Another important point in this study was that the increase in protein was maintained even with the addition of sunflower seeds flour (10%). There was a significant difference at 5% probability level across all the samples.

Carbohydrate content was gradually decreasing with increasing supplementation of soybean flour and 10% sunflower seeds flour. The carbohydrate value ranged from 13.73 to 77.73% for the flours and 42.94 to 77.73% for the flour blends. the highest carbohydrate content (77.73%) was observed in the control (sample A) while the lowest (42.94%) was recorded in sample E. similar decreasing trend in carbohydrate content was also reported by other studies (Banureka & Mahendran, 2009; Ayo et al., 2014) and may be due to the low carbohydrate content of the added soybean flour (31.49%) and sunflower seeds flour (13.08%).

In this study, the calorie content (energy value) of the composite flours increased from 381.21 (sample A) to 434.69 Kcal/100g (sample E) with the addition of soybean and sunflower seeds

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(10%) flours. The increase in the energy value may be attributed to the increase in protein and fat content of the soybean and sunflower seeds flours (oil seeds) used for the substitution.

Table 2: Proximate Composition of Wheat, Soybeans and Sunflower Seeds Flour and their Blends

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= 100% wheat: 0% soybean: 0% sunflower seeds
B=90% wheat: 10% soybean: 0% sunflower seeds
C= 80% wheat: 10% soybean: 10% sunflower seeds
D= 70% wheat: 20% soybean: 10% sunflower seeds
E= 60% wheat: 30% soybean: 10% sunflower seeds
SBF= Soybean flour
SSF= Sunflower seeds flour

Sample	Moisture (%)	Ash (%)	Fat (%)	Fibre (%)	Protein (%)	Carbohydrate (%)	Energy (Kcal)
A	8.04^{g}±0.03	0.59^{a}±0.03	3.21^{a}±0.03	0.09^{a}±0.03	10.36^{a}±0.40	77.73^{g}±0.30	381.21^{a}±0.18
B	7.38^{f}±0.03	1.01^{b}±0.04	5.39^{b}±0.03	1.34^{b}±0.03	15.22^{c}±0.10	69.68^{f}±0.22	388.05^{b}±0.24
C	6.83^{e}±0.03	1.32^{c}±0.03	9.72^{c}±0.03	2.42^{d}±0.03	26.34^{d}±0.10	53.37^{e}±0.18	406.26^{c}±0.08
D	6.01^{d}±0.01	2.08^{d}±0.01	12.01^{d}±0.03	2.80^{e}±0.03	26.97^{e}±0.04	50.16^{d}±0.13	416.55^{d}±0.12
E	5.53^{c}±0.03	3.31^{e}±0.24	16.37^{e}±0.03	2.97^{f}±0.03	28.90^{f}±0.32	42.94^{c}±0.13	434.67^{e}±1.05
SBF	1.32^{a}±0.03	5.02^{f}±0.03	25.43^{f}±0.03	1.41^{c}±0.03	35.36^{g}±0.45	31.49^{b}±0.35	496.19^{f}±0.18
SSF	4.33^{b}±0.03	3.23^{e}±0.22	33.77^{g}±0.03	31.89^{g}±0.03	13.73^{b}±0.41	13.08^{a}±0.70	411.09^{g}±0.94

Anti-Nutritional Content of Wheat, Soybeans and Sunflower Seeds Flour

The anti-nutritional factors in the flour and flour blends are shown in Table 3. Anti-nutrients are secondary metabolites produced through various metabolic pathways. They function by providing a defense mechanism for the plant against predators to ensure the survival of the plant. When consumed as food most impair the digestion and absorption of vital nutrients into the body. In this study, the highest tannin content (for flour blends) was recorded in sample E (0.69 mg/100g) and the lowest in sample A (0.34 mg/100g) which was the control. The increasing trend observed could be as a result of the high tannin content in the soybeans (1.12 mg/100g) and sunflower seeds flour (1.69 mg/100g). The findings of this study are in agreement with the study carried out by Samuel et al. (2012) who reported an increase in tannin content with increase in soybean flour addition. Tannins cause decrease iron absorption, alter excretion of cations, increase excretion of proteins and essential amino acids, and consequently damage the intestinal tract, suppress growth, and enhance carcinogenesis (Anuonye et al., 2010). There was a significant difference (p<0.05) across all the samples.

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The highest mean value for saponin concentration was recorded in sample E (0.43 mg/ 100g) and lowest value was in sample A (0.29 mg/100g). An increasing trend in saponin content was observed in the flour blends with increase in the substitution of soybean and 10% sunflower seeds flours. This trend is in agreement with the study reported by Obasi and Askepnde (2023). Saponins are naturally occurring surface-active glycosides found in plants, including oil seeds such as soybean, groundnut, and sunflower. When saponin is ingested, it produces some toxic effect, perhaps due to its capacity to alter cell wall permeability. Its centre of action is the small intestine, where it binds to the intestinal wall, thus limiting nutrient absorption across the wall (Akande et al., 2010).

The Oxalates content in this study ranged from 1.16 to 3.56 mg/100g for the flours and 1.16 to 2.40 mg/100g for the flour blends. The oxalate content for all samples was significantly different at 5% probability level. Nwosu (2011) reported a range of 2–5 mg/g oxalate as dangerous and unfit in flours for consumption. The oxalate content in this study was low because of the processing method in which the soybean and sunflower seeds were exposed since they are heat labile. The soybeans were roasted while the sunflower seeds were soaked for 8 hrs and oven dried. Oxalates chelate calcium hence making it unavailable in the body (Barasi, 2003). Oxalates bind to calcium to form crystals. Sources include seeds, nuts, and most vegetables (Makinde et al., 2016).

Phytates have excess negatively charged phosphate groups, which can form stable complexes that normally makes minerals non available for intestinal absorption. An increasing trend in phytate content was observed in the values obtained; this could be as a result of the high phytate concentration in the soybean and sunflower seeds flour. Tajoddin et al. (2011)also reported that phytate content is high in legumes and it decreases the bioavailability of essential minerals and protein by forming insoluble phytate-mineral and phytate-protein complexes. The average daily intake of phytate was estimated to be 2,000-2,600 mg for vegetarian diets as well as diets of inhabitants of rural areas of developing countries and 150-1,400mg for mixed diets (Reddy, 2002). However, the value of phytate obtained in this study is lower (0.98- 2.12 mg/100g) compared to the acceptable concentrations. This might be due to processing conditions followed during sample preparation (e.g., soaking and roasting).

Table 3: Anti-Nutritional Factors Wheat, Soybean and Sunflower Seeds Flour and their Blends

		mg/100g		
	Tannins	**Saponins**	**Oxalates**	**Phytates**
A	$0.34^{a}\pm0.01$	$0.29^{a}\pm0.01$	$1.16^{a}\pm0.02$	$0.98^{a}\pm0.02$
B	$0.43^{b}\pm0.02$	$0.36^{b}\pm0.01$	$1.24^{b}\pm0.02$	$1.22^{b}\pm0.01$
C	$0.58^{c}\pm0.02$	$0.43^{c}\pm0.01$	$1.32^{c}\pm0.02$	$1.89^{c}\pm0.01$
D	$0.61^{d}\pm0.01$	$0.53^{d}\pm0.01$	$1.71^{d}\pm0.01$	$2.00^{d}\pm0.01$
E	$0.65^{e}\pm0.02$	$0.43^{c}\pm0.01$	$2.40^{e}\pm0.02$	$2.12^{e}\pm0.01$
SBF	$1.12^{f}\pm0.02$	$0.62^{d}\pm0.01$	$2.47^{f}\pm0.03$	$2.74^{f}\pm0.01$
SSF	$1.69^{g}\pm0.01$	$0.95^{e}\pm0.01$	$3.53^{g}\pm0.03$	$4.07^{g}\pm0.03$

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

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SBF= Soybean flour
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IMPLICATION TO RESEARCH AND PRACTICE

When compared to other interventions, the inclusion of legumes in composite flour preparations has shown to be a practical and long-term strategy for addressing all forms of malnutrition and fostering food security. Rich in protein and minerals, soybeans and sunflower seeds are easily accessible for preparing composite flour for bread making. When whole wheat flour is used to make bread, malnutrition is frequently a concern. This study has shown the possibility of preparing composite flour from wheat, soybean and sunflower seeds flour for bread making.

CONCLUSION AND RECOMMENDATION

Wheat flour was used in the preparation of composite flour substituted with soybean and sunflower seeds flour. The functional properties showed that a shelf stable and nutritious bread could be made from the composite flour. The approximate composition of the composite flour showed that the raw material (soybeans and sunflower seeds) had lots of proteins, fibre, minerals and other essential nutrients which showed that bread made from this flour will be nutritious. The nutritional content increased with substitution with soybeans and 10% sunflower seeds. The concentration of antinutrient was within safe limits due to the processing method used for both the soybean and sunflower seeds. Sample E had more nutritional density than all the other samples. Therefore, this composite formulation if adopted will go a long way in reducing malnutrition and fostering food security. Further studies should be carried out on vitamin content, rheological properties, storage stability of the composite flour.

Author Contributions

Msugh performed the laboratory analysis while Adah and Asemave performed supervisory roles. All authors have read and agreed to the published version of this manuscript.

Acknowledgement

Article DOI: 10.52589/AJAFS-C84BU7GC
DOI URL: https://doi.org/10.52589/AJAFS-C84BU7GC

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

Conflict of Interest

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

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