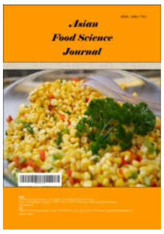

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Effects of Pawpaw (*Carica papaya*) Seed Flour Addition on the Quality of Wheat Bread

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAK designed the study, performed the statistical analysis. Author EDI wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Author NSD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The effects of pawpaw (*Carica papaya*) seed flour addition to wheat based bread was studied. Matured, ripen pawpaw fruits were washed, and the seeds were collected, extracted, dried and milled. Different proportions of wheat and pawpaw seed flour with increasing level of pawpaw seed flour at 0, 2.5, 5, 7.5, 10 and 12.5% addition in wheat were prepared. Control sample was 100% wheat flour and its bread. The functional properties of the flours of wheat and pawpaw seed were determined. The proximate composition (breads and pawpaw seed flour), vitamins content and sensory attributes of the bread samples were determined using standard procedures. The GENSTAT Statistical Software (version 17.0) was used for data analyses. The Data obtained showed an increased oil absorption capacity (0.62-1.23 g/g), foaming capacity (14.54-19.88%) and a decreased water absorption capacity (1.88-1.35 g/g) and bulk density (0.78-0.41 g/cm^3) with increased pawpaw seed flour addition. The proximate composition of the bread samples showed significant ($p<0.05$) increase in moisture (23.80-30.83%), ash (0.78-3.00%), crude fibre (0.58-1.48%), crude fat (2.20-9.68%) and crude protein (11.46-17.71%) but decrease in carbohydrate (61.19-37.31%) contents with increased pawpaw seed flour addition. The pawpaw seed flour showed proximate composition of 8.15% moisture, 7.46% ash, 5.44% crude fibre, 25.41% crude

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fat, 28.18% crude protein and 25.38% carbohydrate. Vitamin C (0.57-12.95 mg/100 g) and pro-vitamin A (0.26-7.37 µg/100 g) significantly ($p<0.05$) increased with increased pawpaw seed flour percentage while Vitamin B_3 decreased with increased addition. Appearance and taste panel scores indicated that up to 5% addition of pawpaw seed flour was acceptable in bread formulation.

Keywords: bulk density; crude protein; ash, pro-vitamin A, sensory attributes.

1. INTRODUCTION

Bread is an important staple food in both developing and developed countries and constitutes one of the most important sources of nutrients such as carbohydrate, protein, fibre, vitamins and minerals in the diets of many people worldwide [1]. The consumption of bread in Nigeria is on a steady increase because it is a convenient and ready to eat food normally consumed at breakfast, lunch and sometimes dinner [2].

Pawpaw (*Carica papaya*) is among the currently most important tropical fruits grown in Brazil and in the world. The fruit is mainly consumed fresh although it offers many industrial products. The processing of this fruit, as well as its fresh consumption, results in large amounts of waste, such as peels and seeds [3]. Pawpaw consumption is one of the causes of significant loss of food value; therefore, new aspects on the use of its waste as by-products, or in the production of food additives, or even the incorporation of its flour in food have aroused great interest because these are products of high nutritional value and their use may be economically viable [4].

Wasted food is a considerable component of the world's food system challenges. Food waste can be described as all edible food materials produced for human consumption but left uneaten, either lost or discarded throughout the food supply chain, from farm to fork. It is organic waste discharged from various sources including food processing plants and domestic/ commercial kitchens, cafeterias and restaurants [4].

Wheat is the most important stable food crop for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crops. It is nutritious, easy to store and transport and can be processed into various types of food. Wheat flour is used to prepare bread, produce biscuits, confectionary products, noodles and vital wheat gluten or seitan [5].

Composite flour can be defined as a mixture of several flours obtained from roots and tubers, cereal, legumes, etc., with or without the addition of wheat flour [6]. Usually, the aim of producing composite flour is to get a product that is better than the individual components. Thus, several developing countries have encouraged the initiation of programmed to evaluate the feasibility of alternative locally available flours as a substitute for wheat flour [7]. Therefore, the objective of this research was to evaluate the quality of bread produced from wheat substituted with pawpaw (*Carica papaya*) seed flour.

2. MATERIALS AND METHODS

2.1 Materials Procurement

Wheat flour, butter, sugar, salt, yeast and vanilla flavour were purchased from Makurdi Northbank market, Benue State Nigeria. Pawpaw (*Carica papaya*) fruits also were purchased from fruit market, Makurdi Benue State Nigeria and were taking to the Department of Food Science and Technology, Federal University of Agriculture Makurdi for further processing.

2.2 Preparation of Pawpaw (*Carica papaya*) Seed Flour

The Pawpaw seed flour was prepared according to the method of Cláudia et al. [3]. Pawpaw fruits were washed thoroughly with water. They were manually peeled using knife, cut, the seeds were extracted and washed with running water to remove mucilage. Subsequently, the seeds were spread on trays and dried (oven drying at 50ºC for 72 h) (Uniscope Surgifriends Medicals, England). After drying, the samples were milled using a TECNAL mill (TE-631) and then sieved through 0.50 mm aperture sieve. Fine flour was then obtained and packaged in sealed containers (Rubbermaid) for further use.

2.3 Formulation for Bread

Presented in Table 1. is the formulation for pawpaw (*Carica papaya*) seed flour addition to wheat for bread production.

Table 1. Formulation for Pawpaw (*Carica papaya*) Seed Flour Addition to Wheat for Bread Production

Wheat Flour (%)	Pawpaw Seed Flour (%)
100	0
97.5	2.5
95	5
92.5	7.5
90	10
87.5	12.5

3.4 Preparation of Bread from Wheat and Pawpaw (*Carica papaya*) Seed Flour

Breads were produced from the flours of wheat and pawpaw (*Carica papaya*) seed based on the recipe formulation shown in Table 2.

The bread was produced in accordance with the modified method of Joseph et al. [8]. Flour, butter, sugar, yeast, salt and other baking ingredients with water after scaling (weigh balance model: Metler Toledo, made in Switzerland) were manually mixed together in a bowl. The mixture was kneaded using the kneading machine (mod. CG 38 Italy) until the dough was developed (5 mins)then moulded and allowed to proof in pans for 1 h. The dough were baked (180ºC for 1 h, 30 mins) in the oven (Uniscope Surgifriends Medicals, England) and allowed to cool then packaged(Low Density Polyethylene) for further analysis.

2.5 Determination of the Functional Properties of Flours of Wheat and Pawpaw (*Carica papaya*) Seed

2.5.1 Water absorption capacity

The method described by Onwuka [9] was used. One gram (1 g) of the flour sample was weighed into a 15 mL centrifuge tube and suspended in 10 mL of water. It was shaken on a platform tube rocker for 1 minute at room temperature. The sample was allowed to stand for 30 min and centrifuged (SM 800B Uniscope Surgifriends Medicals, England) at 500 rpm for 30 min. The volume of free water was read directly from the centrifuge tube. Density of water was taken to be 1 g/cm^3 as expressed in equation 1.

$$WaterAbsorptionCapacity(\%) = \frac{Amountofwateradded - Freewater}{Weightofsample} \times densityofwater \times 100 \qquad (1)$$

2.5.2 Oil absorption capacity

The method as described by Onwuka [9] was used. One gram (1 g) of the flour was mixed with 10 mL refined oil in a centrifuge tube and allowed to stand at room temperature (30 ± 2°C) for 1 h. It was centrifuged (SM 800 B Uniscope Surgifriends Medicals, England) at 500 rpm for 30 min. The volume of free oil was recorded and decanted. Oil absorption capacity was expressed as mL of oil bound by 100 g dried flour**.** Density of oil was taken to be 0.98 g/cm^{3}as expressed in equation 2.

$$OilAbsorptionCapacity(\%) = \frac{Amountofoiladded - Freeoil}{Weightofsample} \times densityofoil \times 100 \qquad (2)$$

2.5.3 Bulk density

The method described by Onwuka [9] was adopted for determination of bulk density. A graduated cylinder 10 mL was weighed and gently filled with the flour sample up to the 10 mL mark. The bottom of the cylinder was then tapped gently on a laboratory bench several times. This continued until no further diminution of the test flour sample in the cylinder after filling to mark was observed. Weight of cylinder plus flour was measured and recorded. Bulk density was calculated according to equation 3.

$$BulkDensity\ (g/\text{cm}^3) = \frac{weightofsample\ (g)}{volumeofsample\ (cm^3)} \qquad (3)$$

2.5.4 Foaming capacity

Foaming capacity was determined according to the method described by Onwuka [9]. Two grams (2 g) of flour sample was weighed and added to 50 mL distilled water in a 100 mL measuring cylinder. The suspension was mixed and properly shaken to foam and the total volume after 30 sec. was recorded. The percentage increase in volume after 30 seconds is expressed as foaming capacity(equation 4).

$$FoamingCapacity(\%) = \frac{Volumeafterwhipping - Volumebeforewhipping}{Volumebeforewhipping} \times 100 \quad (4)$$

2.6 Determination of the Proximate Composition of Pawpaw (*Carica papaya*) Seed Flour and Bread from Wheat and Pawpaw Seed

The proximate composition of pawpaw seed flour and bread from wheat and pawpaw seed flours were determined according to the methods of AOAC [10] and Carbohydrate content was determined by difference as described by Ihekoronye and Ngoddi [11].

2.6.1 Moisture content

Moisture content was determined using the oven dry method. A clean dish with a lid was dried in an oven (Uniscope Surgifriend Medicals, England) at 105ºC for 30 min. It was cooled in desiccators and weighed. Two grams (2 g) of sample was then weighed into the dish. The dish with its content was then put in the oven at 105ºC and dried to a fairly constant weight. The loss in weight from the original sample (before heating) was reported as percentage moisture (Equation 5).

$$\%\,Moisture = \frac{WeightLoss\ (W_2 - W_3)}{WeightofSample\ (W_2 - W_1)} x\ 100 \quad (5)$$

$where$:
$W_1 = Weightofdish,$
$W_2 = Weightofdish + samplebeforedrying,$
$W_3 = Weightofdish + samplebeforedrying.$

2.6.2 Ash content

Two grams (2 g) of sample was weighed into a crucible which had been pre-heated, cooled in a desiccator and weighed soon after reaching room temperature. The crucible and content was then heated in a muffle furnace at 550ºC for 6 h. The crucible was cooled in a desiccator and weighed soon after reaching room temperature. The total ash was calculated as percentage of the original sample weight(Equation 6).

$$\%\,Ash = \frac{(W_3 - W_1)}{(W_2 - W_1)} x\ 100 \quad (6)$$

$where$:
$W_1 = weightofemptycrucible, W_2 = weightofcrucible + samplebeforeashing,$
$W_3 = weightofcrucible + contentafterashing$

2.6.3 Crude fibre content

Two grams (2 g) of the sample was defatted using Diethyl ether. This was digested and filtered through the California Buchner system. The resulting residue was dried at 130ºC for 2 h, cooled in a dessicator and weighed. The residue was then transferred into a muffle furnace (Uniscope Surgifriend Medicals, England) and ignited at 550 ^{0}C for 30 min, cooled and weighed. The percentage crude fibre content was calculated (equation 7) as:

$$\%\,CrudeFibre = \frac{Lossinweightafterincineration}{Weightoforiginalfood} \times 100 \quad (7)$$

2.6.4 Crude fat content

Crude fat content was determined using Soxhlet method. Samples were weighed into a thimble and loose plug fat free cotton wool was fitted into the top of the thimble with its content inserted into the bottom extractor of the Soxhlet apparatus. Flat bottom flask (250 mL) of known weight containing 200 mL of hexane was fitted to the Soxhlet apparatus. The apparatus was heated and fat extracted for 8 hrs. The solvent was recovered and the flask (containing oil and solvent mixture) was transferred into a hot air oven (Uniscope Surgifriend Medicals, England) at 105ºC for 1 h to remove the residual moisture and to evaporate the solvent. It was later transferred into desiccator to cool for 15 min before weighing. Percentage fat content was calculated as equation 8.

$$\%\,CrudeFat = \frac{weightofextractedfat}{WeightofSample} x\ 100 \quad (8)$$

2.6.5 Crude protein content

The Kjeldahl method was used to determine the percentage crude protein. Two grams (2 g) of sample was weighed into a Kjeldahl digestion flask using a digital weighing balance (Uniscope Surgifriend Medicals, England: Max. 180 g). A catalyst mixture weighing 0.88 g (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) was added.

Concentrated sulphuric acid (7 mL) was added and swirled to mix content. The Kjeldahl flask was heated gently in an inclined position in the fume chamber until no particles of the sample was adhered to the side of flask. The solution was heated more strongly to make the liquid boil with intermittent shaking of the flask until clear solution was obtained. The solution was allowed to cool and diluted to 25 mL with distilled water in a volumetric flask. Ten (10) mL of diluted digest was transferred into a steam distillation apparatus. The digest was made alkaline with 8 mL of 40% NaOH. To the receiving flask, 5 mL of 2% boric acid solution was added and 3 drops of mixed indicator (protein indicator) was dropped. The distillation apparatus was connected to the receiving flask with the delivery tube dipped into the 100 mL conical flask and titrated with 0.01 M HCl. A blank titration was done. The percentage nitrogen was calculated (equation 9) from the formula:

$$\% \, Nitrogen = \frac{(S-B)\times 0.0014\times 100\times D}{SampleWeight} \quad (9)$$

$where$

$S = sampletitre, B = blanktitre, S - B = correctedtitre, D = dilutedfactor$

$$\% \, CrudeProtein = \% \, Nitrogen \times 6.25 \, (correctionfactor) \quad (10)$$

2.6.6 Carbohydrate content

Carbohydrate content was determined by difference according to Ihekoronye and Ngoddy [11] as shown in equation 11.

$$\% \, Carbohydrate = 100 - (\% \, Moisture + \% \, Ash + \% \, Fibre + \% \, Fat + \% \, Protein) \quad (11)$$

2.7 Determination of Vitamin Content (mg/100 g) of Bread

2.7.1 Determination of ascorbic acid (vitamin C)

The method of AOAC [10] was used. The standard solution was prepared by dissolving 50 mg standard ascorbic acid tablet in 100 mL of water. The solution was filtered to get clear solution. Ten millilitre of 4% oxalic acid (4 g oxalic acid in 100 mL water) was added to 5 mL of the working standard solution and was titrated against the dye 2, 6 – dichloroindophenol dye (V_1 mL). The end point was the appearance of pink colour which persisted for a few minutes. The amount of dye consumed was equivalent to the amount of ascorbic acid. The test sample (5 g) was extracted with 4% oxalic acid and filtered and made up to 100 mL volume. This was finally centrifuged and 10 mL of 4% oxalic acid was added to 5 mL of the supernatant. This was titrated against the dye (V_2mL) (equation 12).

Calculation

$$AscorbicAcid \, (mg/100 \, g) = \frac{0.5mg\times V_2\times 100}{V_1 ml\times 5 \, ml\times Sampleweight} \quad (12)$$

$where$

$Sampleweight = 5 \, g$

$Volumeofextract = 100 \, mL$

$Volumeofextractused = 5 \, mL$

$V_1 = Dyetitreagainststandar \, (12.40 \, mL)$

$V_2 = Dyetitrevalueagainstsample$

2.7.2 Determination of pro-vitamin A (µg/100g)

The method of AOAC [10] was used for the determination. Pyrogallol (antioxidant) was added to 2 g sample prior to saponification with 200 mL alcohol KOH. The saponification took place in water bath for 30 min. The sample solution was transferred to a separatory funnel where water was added. The sample solution was extracted with 1.5 mL of hexane. The extract was washed with equal volume of H_2O. The extract was filtered through filter paper containing 5 g anhydrous Na_2SO_4 into a volumetric flask. The filter paper was rinsed with hexane and made up to volume. The hexane was evaporated from the solution and blank. One millilitre chloroform and $SbCl_3$ solution was added to the extract and blank. The reading of the solution and blank were taken from the colourimeter adjusted to zero absorbance. Standard curve was prepared using USP vitamin A reference standard ranging from 0.07 to 0.7.This is as shown in equation 13.

Calculation

$$VitaminA \, (\mu g/100g) = A_{620}nm \times SL \times (V/wt) \quad (13)$$

$A620 \, nm = Absorbanceat \, 620 \, nm$

$SL = Slopeofstandardcurve \, (vitAconcentration \div A620reading)$

$V = Finalvolumeincolorimetertube$

$Wt = Weightofsample$

Table 2. Recipe Formulation for Bread

Component	Quantity (g)
Flour*	100
Butter	3.0
Sugar	5
Salt	0.5
Yeast	2.5
Vanilla flavour	1
Water	65 (mL)

**Wheat and Pawpaw Seed Flour, adapted from Joseph et al. [8] with modification*

2.7.3 Determination of niacin (vitamin B$_3$)

This was determined using the method described by AOAC [10]. Five grams (5 g) of homogenized sample was weighed into 100 mL volumetric flask. 0.1 N hydrochloric acid was added and mixed, then autoclaved for 30 min. at 121ºC. The samples were allowed to cool. Interfering substances were precipitated by adjusting the pH to 6.0 followed immediately by readjusting the pH to 4.5. This was then diluted to volume with water and filtered and 5 mL of 6% enzyme (mylase 100) was added and incubated for 3 h at 45-50ºC. This was then cooled and pH adjusted to 3.5 and diluted with water to volume, mixed and filtered. 10 mL of diluted extract was oxidized by passing through a sepak C18 cartridge followed by 5 mL 0.01 M phosphate buffer at pH 7.0. The vitamin was separated by high performance liquid chromatography (HPLC) (Model: BLC-10/11, Buck scientific, USA) using a 4.6 mm x 25 cm ultrasphere ODS (operational data store), 5 column or equivalent and detected by florescence at 360 nm/415 nmex/em. The vitamin content was measured by the calculation (equation 14) below.

$$mg/100g = C \times V(D_f \times W_t) \quad (14)$$

$where:$

$C = Conc. of vitamin in \mu \frac{g}{ml} obtained from height or area of sample and standard$

$V = Sample volume\ (ml)$

$D_f = Dilution factor$

$W_t = Weight of sample\ (g)$

Table 3. Proximate Composition (%) of Pawpaw (*Carica papaya*) Seed Flour

Nutrient	Composition (%)
Moisture	8.15±0.07
Ash	7.46±0.01
Crude fibre	5.44±0.03
Crude fat	25.41±0.06
Crude protein	28.18±0.01
Carbohydrates	25.38±0.02

Table 4. Effects of Pawpaw (*Carica papaya*) Seed Flour Addition on the Functional Properties of Wheat Flour

Sample Wheat: Pawpaw Seed	Water Absorption Capacity (g/g)	Oil Absorption Capacity (g/g)	Bulk Density (g/cm^3)	Foam Capacity (%)
100:0	1.88^a±0.01	0.62^a±0.01	0.78^f±0.02	14.54^a±0.02
97.5:2.5	1.75^b±0.00	0.69^b±0.00	0.64^e±0.01	16.68^b±0.01
95:5	1.65^c±0.01	0.75^c±0.01	0.57^d±0.01	17.46^c±0.01
92.5:7.5	1.57^d±0.01	0.85^d±0.01	0.51^c±0.01	18.27^d±0.01
90:10	1.44^e±0.01	0.95^e±0.01	0.44^b±0.00	19.06^e±0.01
87.5:12.5	1.35^f±0.01	1.23^f±0.01	0.41^a±0.00	19.88^f±0.01

Values are means ± standard deviations of triplicate determinations. Means with different superscript in the same column are significantly (p<0.05) different

2.8 Sensory Evaluation of Bread

Sensory evaluation of the bread samples was carried out according to the method described by Retapol and Hooker [12]. A panel of twenty (20) members consisting of students and members of staff from Food Science and Technology Department, Federal University of Agriculture, Makurdi, Nigeria were chosen based on their familiarity and experience with wheat-based breads for sensory evaluation. Breads produced from each flour blend, along with the reference sample were presented in coded form (100:0 – 87.5:12.5 %) and were randomly presented to the panelists. The panelists were provided with portable water to rinse their mouth between evaluations. However, a questionnaire describing the quality attributes (crumb appearance, taste, aroma, crumb texture and overall acceptability) of the bread samples was given to each panelist. Each sensory attribute was rated on a 9-point hedonic scale (1 = dislike extremely and 9 = like extremely). Bread was produced from wheat flour (100 %) as control.

2.9 Statistical Analysis

The GENSTAT Statistical Software (version 17.0) was used for data analyses. Data were subjected to analysis of variance (ANOVA) and the separation of means was done using Fisher's Least Significant Difference (LSD) at ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Functional Properties of Flours of Wheat and Pawpaw (*Carica papaya*) Seed

Functional properties of a food material are parameters that determine its application and end use [13]. Water absorption capacity is the ability of product to incorporate water and water inhibition is an important functional trait in food such as sausages, custard and dough [14]. The reduction in water absorption capacities of the flours with increased pawpaw seed substitution, was due to the low protein and high carbohydrate contents recorded in 100% wheat bread, as carbohydrates have been reported to greatly influence the water absorption capacity of foods [15]. Also, wheat flour may have contained more hydrophilic constituents than pawpaw seed flour, which gave rise to higher water absorption capacity [16]. The lower moisture content of wheat bread also enhanced its flour water absorption capacity [16]. It has been suggested that flours with high water absorption capacity as seen in this study will be very valuable in bakery products and this could thwart staling by reducing moisture loss [17] and also, help maintain the freshness of bread, cakes and sausages and could favour their use as soup thickener [18].

Oil absorption capacity is the flavour retaining capacity of flour which is very important in food formulations [19]. The results of the oil absorption capacity of flour samples showed that the ability of all the samples to absorb oil varied significantly ($p<0.05$). The oil absorption capacity increased with increased level of pawpaw seed flour substitution. The oil absorption capacities of the flours of wheat and pawpaw seed tended to increase with increase in protein content since the protein in foods influences fat absorption [20]. The flours of wheat and pawpaw seed have higher oil absorption capacity as a result of the hydrophobic character of protein in the flour. The presence of protein exposes more non-polar amino acids to the fat and enhances hydrophobicity as a result of which the flour absorbs more oil [21]. Also, the high oil absorption capacity observed in the flours suggested the lipophilic nature of the constituents of the pawpaw seed flour [22], and this also suggest that the flours will potentially be useful in structural interaction in food especially in flavour retention, improvement of palatability and extension of shelf life of bakery or meat products, doughnuts, baked goods, pancakes and soup mixes where fat absorption is desired [23]. Mepba et al. [24] and Dogo et al. [25], reported similar increase of oil absorption capacity for wheat-plantain and wheat-defatted cashew (Anacardium occidentale L.) kernel composite flours respectively.

The result of bulk density is used to evaluate the flour heaviness, handling requirement and the type of packaging materials suitable for storage and transportation of food materials [26]. The bulk density of the flours decreased as the incorporation level of pawpaw seed flour increased. Ufot et al. [27] and Raihan and Saini [28], observed similar decreased bulk density for wheat-unripe plantain and wheat-oats, sorghum, amaranth composite flours respectively. It has been reported that bulk density is influenced by the structure of the starch polymers and loose structure of the starch polymers could result in low bulk density [29]. The observed decrease in bulk density with increased pawpaw seed flour addition could be due to decrease in carbohydrate in the flours of wheat and pawpaw seed. Pawpaw (*Carica papaya*) seed being proteinous in nature, does not significantly contribute to bulk density. Low bulk density of flour is a good physical attribute when determining transportation and storability [30]. Low bulk density is also important in infant feeding where less bulk is desirable. The low bulk density of the flours with increasing level of pawpaw seed flour would be an advantage in the use of the flour for preparation of complementary foods [16].

Foaming capacity is used to determine the ability of flour to foam which is dependent on the presence of the flexible protein molecules which decrease the surface tension of water [31]. The values for foaming capacity increased as the percentage inclusion of pawpaw seed flour increased. The increased foaming capacity of the flours with increased pawpaw seed flour substitution was as a result of high protein content of pawpaw seed. Proteins have been reported to enhance foam formation [32]. The higher foaming capacity in the flours of wheat and pawpaw seed shows that pawpaw seed flour is a good foaming agent. Protein foams are important in many processes in the beverage and food industries and this has stimulated interest in their formulation and stability. Foams are used to improve texture, consistency and appearance of foods [33]. Good foam capacity is a desirable attribute for flours in the food system due to its high percentage of porosity intended for the production of a variety of baked products such as ice cream, cakes, muffins, *akara*, and also act as functional agents in other food formulations.

3.2 Proximate Composition (%) of Pawpaw (*Carica papaya*) Seed Flour

The results of the proximate composition of pawpaw seed flour recorded substantial values of ash, crude fibre, crude fat and crude protein contents. These are similar with the findings of Adesuyi and Ipinmoroti [34], Oyeleke et al. [35], Kadiri [36] and Makanjuola and Makanjuola [37] who revealed significant presence of nutrients in pawpaw seeds.

3.3 Effects of Pawpaw (*Carica papaya*) Seed Flour Addition on the Proximate Composition (%) of Wheat Bread

The observed value of increased ash, fat and protein contents with increased pawpaw seed substitution in the wheat and pawpaw seed breads was an evidence of the high content of ash, fat and protein found in the pawpaw seed flour [36].

The increase in the moisture content in the bread could be attributed to the greater water holding capacity of wheat flour than the pawpaw seed flour as seen from the water absorption capacity of flour samples. This trend is similar to the findings reported by Fot and Inemesit [27,38] and [39] for wheat-unripe plantain, wheat-yam flour and wheat-taro flour composite breads respectively.

The ash contents of the bread samples increased significantly ($p<0.05$) with increased levels of pawpaw seed flour substitution. Ash content gives an insight to the mineral content of food hence, pawpaw seed can be described as a good source of minerals hence the relatively high-value of minerals recorded in the wheat and pawpaw seed breads. Samia et al. [40], Oyeleke et al. [35], Cláudia et al. [3] and Makanjuola, and Makanjuola [37]reported high pawpaw seed ash contents. The results obtained in this study are in agreement with similar reports byBhosale and Udachan [41]; Olorode et al. [42] for wheat-*Papaya* seed composite cookies respectively.

The crude fibre contents of the products increased progressively as the level of pawpaw seed supplementation increased and the differences in the crude fibre contents are significant ($p<0.05$). The increased was as the result of high fibre content of pawpaw seed [35]; [3] and [34]. Fibre consumption has been linked to decreased incidence of heart disease, various types of cancer and diverticulosis [43]. Also, high levels of fibre in foods help in digestion of foods and also contribute to the health of the gastrointestinal tract and system in man by aiding normal bowel movement thereby reducing constipation problems which can lead to colon cancer [44]. The high fibre contents of the wheat and pawpaw seed breads suggest that they would be ideal food for people suffering from obesity, diabetes, cancer and gastrointestinal disorders Ufotand Inemesit [27]; Olorode et al. [42] and Bhosaleand Udachan [41] reported high fibre contents of wheat-*Papaya* composite cookies.

Fat acts as lubricating agent which improves the quality of the bread in terms of texture and flavour. Also, fat provides energy and is essential as it carries along fat soluble vitamins A, D, E and K [45]. Proteins are major constituents of body enzymes, antibodies, many hormones as well as body fluids such as blood and milk. They are essential to all life and help to form structural, supporting and protective tissues such as muscles, cartilages, skin, hairs and nails [46]. The crude fat and protein contents of the breads increased significantly (p<0.05) with increased levels of pawpaw seed flour. The increased crude fat and crude protein contents of the wheat and pawpaw seed breads could be attributed to the high fat and protein contents in pawpaw seed [34,35] and [37].

Carbohydrate provides heat and energy for all forms of body activities and as such its inadequacy can cause the body to divert proteins and body fat to produce needed energy and this might lead to depletion of body tissues [47]. Carbohydrate content decreased with increased substitution of wheat flour with pawpaw seed flour. Increased and decreased carbohydrate contents of wheat and wheat and pawpaw breads could be due to the high and low carbohydrate contents of wheat and pawpaw seed flours respectively.

Table 5. Effects of Pawpaw (*Carica papaya*) Seed Flour Addition on the Proximate Composition (%) of Wheat Bread

Sample Wheat: Pawpaw Seed	Moisture	Ash	Crude fibre	Crude fat	Crude Protein	Carbohydrate
100:0	23.80^{a}±0.14	0.78^{a}±0.04	0.58^{a}±0.01	2.20^{a}±0.03	11.46^{a}±0.06	61.19^{f}±0.01
97.5:2.5	25.16^{b}±0.08	1.59^{b}±0.12	1.17^{b}±0.01	5.47^{b}±0.02	13.54^{b}±0.09	53.09^{e}±0.30
95:5	26.75^{c}±0.01	1.80^{c}±0.03	1.25^{c}±0.01	6.67^{c}±0.01	14.73^{c}±0.06	48.80^{d}±0.13
92.5:7.5	27.40^{d}±0.14	2.49^{d}±0.04	1.33^{d}±0.01	7.91^{d}±0.07	15.59^{d}±0.04	45.29^{c}±0.01
90:10	29.22^{e}±0.25	2.96^{e}±0.03	1.42^{e}±0.01	8.53^{e}±0.05	16.40^{e}±0.06	41.48^{b}±0.26
87.5:12.5	30.83^{f}±0.26	3.00^{e}±0.01	1.48^{f}±0.01	9.68^{f}±0.30	17.71^{f}±0.22	37.31^{a}±0.24

Values are means ± standard deviations of triplicate determinations. Means with different superscript in the same column are significantly (p<0.05) different

Table 6. Effects of *Pawpaw* (*Carica papaya*) Seed Flour Addition on the Vitamin Composition (mg/100 g)of Wheat Bread

Sample Wheat: Pawpaw Seed	Ascorbic Acid (Vitamin C)	β-Carotene (Pro-Vitamin A) (µg/100g)	Niacin (Vitamin B_3)
100:0	0.57^{a}±0.02	0.26^{a}±0.04	0.69^{a}±0.00
97.5:2.5	9.39^{b}±0.06	5.47^{b}±0.16	0.45^{b}±0.00
95:5	9.73^{c}±0.04	5.91^{c}±0.00	0.44^{c}±0.01
92.5:7.5	10.31^{d}±0.02	6.34^{d}±0.25	0.43^{d}±0.00
90:10	11.91^{e}±0.04	6.49^{d}±0.03	0.39^{e}±0.01
87.5:12.5	12.95^{f}±0.01	7.37^{e}±0.04	0.38^{f}±0.00

Values are means ± standard deviations of triplicate determinations. Means with different superscript in the same column are significantly (p<0.05) different

Table 7. Effects of Pawpaw (*Carica papaya*) Seed Flour Addition on the Sensory Attributes of Wheat Bread

Sample Wheat: Pawpaw Seed	Crumb Appearance	Taste	Aroma	Crumb Texture	Overall Acceptability
100:0	8.40^{e}±0.68	8.40^{c}±0.60	8.55^{d}±0.51	8.10^{d}±0.72	8.25^{d}±0.72
97.5:2.5	7.85^{d}±0.81	8.00^{c}±0.65	7.70^{c}±0.80	7.10^{c}±0.72	7.60^{c}±0.94
95:5	7.45^{d}±0.60	7.90^{c}±0.72	6.80^{b}±1.11	6.85^{bc}±0.93	7.25^{c}±0.97
92.5:7.5	6.75^{c}±0.79	6.85^{b}±0.93	5.95^{a}±1.05	6.40^{ab}±1.39	6.05^{b}±1.10
90:10	5.15^{b}±0.75	6.30^{b}±1.22	5.55^{a}±1.28	6.35^{ab}±1.31	5.90^{b}±1.07
87.5:12.5	4.60^{a}±0.82	4.35^{a}±1.14	5.35^{a}±1.23	5.75^{a}±1.25	5.10^{a}±0.97

Means with different superscript in the same column are significantly (p<0.05) different

3.4 Effects of Pawpaw (*Carica papaya*) Seed Flour Addition on the Vitamin Composition (mg/100g)of Wheat Bread

Vitamins are organic substances required only in small amounts in the body for metabolism. Their requirements are in milligrams (mg), micrograms (µg), millie quivalents (mEq) and international units (IU) [46].

The observed increase in vitamin C and pro-vitamin A in wheat and pawpaw seed breads was due to the high levels of both vitamins in pawpaw seed [36]. Niacin (vitamins B_3) decreased in the breads due to low values of B_3 in both wheat and pawpaw seed. Chukwuka et al. [48] reported low values of niacin (vitamin B_3) in pawpaw seed.

Vitamin A is an essential nutrient required for maintaining immune function. It is often known as retinol because it produces the pigment in the retina of the eye [49]. Vitamin A refers to pro-vitamin A carotenoids and the preformed retinols, plus their metabolites. A deficiency of vitamin A constitutes one of the major public health problems in developing countries. Vitamin A deficiency is the leading cause of non-accidental blindness. Children from impoverished Nations are especially susceptible because their inadequate intake and diminished stores of vitamin A fail to meet the increased needs associated with rapid growth [50]. Vitamin C is needed in the body for the synthesis of collagen, an intracellular cement substance which binds animal cells together. The presence of vitamin C is required to maintain the structure and integrity of bone matrix, cartilage, connective tissues, blood vessels, gums and skin. Its deficiency results in scurvy and also characterized by fragile, easily ruptured capillaries with consequent tissue bleeding and it is needed for wound healing [46].

3.5 Effects of Pawpaw (*Carica papaya*) Seed Flour Addition on the Sensory Attributes of Wheat Bread

Sensory evaluation is usually carried out towards the end of product development or formulation cycle and this is done to assess the reactions of consumers/Panelists about the product to determine the acceptability of such product. It is an important criterion for assessing quality in the development of new products and for meeting consumer requirements [25].

Appearance is an important sensory attribute of any food because of its influence on acceptability. The brown colour resulting from Maillard reaction is always associated with baked goods. Table 6 shows significant decrease ($p<0.05$)in the crumb appearance of the bread samples with increased pawpaw seed substitution. This is due to the darkish nature of pawpaw seed. According to Sudha et al. [51], progressive increase in supplementation with non-wheat flour, appearance turns towards darker leading to lower acceptability. Appearance change also, might be due to caramelization, dextrinization of starch or Maillard reaction involving the interaction of reducing sugar or proteins [52]. Ubbor and Akobundu [22] and Sengev et al. [53] reported similar darkish nature of bread on supplementation of wheat flour with watermelon seed-cassava composite flour and *Moringa oleifera* leaf powder respectively. Taste is a sensory parameter that affects the quality and acceptability of food products. No matter how rich or nutritious a food is, if it tastes bad, such food would not be accepted by people. Taste is the primary factor determining the acceptability of any product and has the highest impact in determining the market success of product. The taste scores showed decreased values with increased pawpaw seed addition. This might be due to the peperish nature of pawpaw seed. Substitution of wheat flour with pawpaw seed at 97.5:2.5% and 95:5% did not significantly ($p>0.05$) affect the taste of the breads with the control sample. Aroma is another attribute that influences the acceptability of baked food products even before they are tasted. From the result of the sensory attributes of breads, there is decreasing trend in the mean score of aroma as the level of supplementation increased downwards. The results are in consistent with those observed in earlier studies by Mian et al. [54] and Mepba et al. [24] in which wheat flour was supplemented with defatted rice bran and plantain respectively in bread production. Similar, decreased trend was observed for crumb texture as the level of pawpaw seed addition increased. Overall acceptability was determined on the basis of quality scores obtained from evaluation of crumb appearance, taste, aroma and crumb texture. Mepba et al. [24]; Amandikwa et al. [38] and Joseph et al. [8] reported similar decreased values of overall acceptability for wheat-plantain, wheat-yam and wheat-ripe banana composite breads respectively.

4. CONCLUSION

The study showed an increased oil absorption capacity and foaming capacity while the water absorption capacity and bulk density decreased with increased addition of pawpaw seed flour. The addition of pawpaw seed to wheat in bread formulation significantly increased the moisture, ash, crude fibre, crude fat and protein contents of wheat based bread but with a decreased carbohydrate content as the level of pawpaw seed flour addition increased. The vitamins content such as ascorbic acid (vitamin C) and β-carotene (pro-vitamin A) increased while the niacin (vitamin $B_{3)}$ decreased as the level of pawpaw seed flour addition increased. Sensory results showed that the pawpaw seed flour could be incorporated up to the levels of 2.5 to 5% in the wheat flour without greatly influencing the sensory characteristics of the breads.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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