

PHYSICO-CHEMICAL, ALVEOGRAPH AND ANTI-NUTRITIONAL PROPERTIES OF BREADS FORMULATED FROM WHEAT AND PAWPAW (CARICAPAPAYA)SEED FLOUR BLENDS

Abstract

The feasibility of partially substituting wheat flour with pawpaw seed flour in bread formulation was investigated. Matured, ripped 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 physical properties of breads and alveograph properties of dough blends were determined. Also, the mineral and anti-nutritional contents of the bread samples were determined using standard procedures. The GENSTAT Statistical Software (version 17.0) was used for data analyses. Physical properties of the bread samples significantly ($p < 0.05$) decreased in oven spring (5.49-2.39 cm), loaf volume (1022.50-901.60 cm³) and specific volume (4.09-3.14 cm³/g) but increased in loaf weight (250.20-288.50 g) with increased pawpaw seed flour addition. Values for dough maximum pressure (91.00-109.50 mm), extensibility (80.50-54.50 mm) and baking strength (255.00-237.50 $\times 10^{-4}$ joules/g) significantly ($p < 0.05$) decreased while the ratio of resistance to extensibility increased (1.36-1.66) with increased seed flour addition. The result of the mineral analysis of the breads showed significant ($p < 0.05$) increase in all the mineral parameters determined. High values were recorded in magnesium (143.00-182.50 mg/100g), calcium (252.60-342.60 mg/100g) and phosphorus (73.50-127.30 mg/100g). The anti-nutritional contents of the breads significantly ($p < 0.05$) increased as the level of pawpaw seed flour substitution increased. Tannins, oxalate, Phytate, and Trypsin inhibitor ranged from 1.76-2.68 mg/100g, 0.06-0.28 mg/100g, 0.06-0.29 mg/100g and 1.28-9.71 TIU/100g respectively.

Keywords: bread, pawpaw seed, physical, alveograph, minerals, anti-nutrients.

1. Introduction

Bread is a staple food that closely related to people's daily life. It is prepared by baking dough which consists of flour, leavening agents and water. Bread is popular around the world and one of the oldest foods. Bread is usually known as important source of carbohydrates in the food pyramid to ensure that a person can get enough nutrition needed by the body. The way of thinking about healthy baking is to focus on their formulation ingredients. Bread and bakery products are widely consumed throughout the world and bread has been an important food and energy source throughout human history [1].

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 [2]. It is nutritious, easy to store and transport and can be processed into various types of food. Wheat is considered a good source of protein, minerals, B-group vitamins and dietary fibre [3] although the environmental conditions can affect nutritional composition of wheat grains with its essential coating of bran, vitamins and minerals; it is an excellent health-building food.

Pawpaw 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. 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]. Pawpaw seed has a considerable amount of protein content. Protein composition of 28.55 % was estimated in flour produced from the seeds [4]. The protein content of some

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commonly consumed oil seed in Nigeria namely *Colocynthiscitrullus*, peanut flour, rapeseed and sunflower (28.44, 24.3, 25 and 28.7 % respectively) had been reported to be relatively lower to that of pawpaw seed flour. This makes it a likely ingredient and additives in foods such as meat products, doughnuts pancakes and in the stabilisation of colloidal food systems where water and oil binding properties are of prime importance [5].

Wheat, being the major ingredient of bread, is grown in temperate region and Nigeria climate does not favour its large scale production. Wheat is also low in protein, fat and minerals. In order to reduce wheat importation and make its bread affordable by low-income earners who constitute the larger population of consumers, there is need to use novel sources of crops such as pawpaw seeds as flour substitute for the wheat to boost its nutrient contents. Agricultural commodities value chains generate tremendous amounts of wastes as by-products such as pawpaw seeds which innovatively could be exploited for wealth generation. Supplementation of wheat with pawpaw(*Carica papaya*) seed flour in bread production will enhance the nutritional composition of wheat based bread. Similarly, utilization of food waste materials in food formulation will be encouraged. The aim of this study was therefore to provide information on the chemical, alveograph properties of dough blends and anti-nutritional compositions of breads produced from wheat and pawpaw seed composite flour.

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2. Materials and Methods

2.1 Materials Procurement

Wheat flour, butter, sugar, salt, yeast and vanilla flavour were purchased from Makurdi North bank 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 [4]. Pawpawfruits were washed thoroughly with water. They were manually peeled using knife, cut and the seeds extracted and washed with running water to remove mucilage. Subsequently, the seeds were spread on trays and were dried (oven drying at 50 °C for 72 h). 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 for further use as shown in Figure 1.

2.3 Formulation for Bread

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

2.4 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 [7]. 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 until the dough was developed then moulded and allowed to proof in pans for 1 h. The doughs were baked (180 °C for 1 h, 30 mins) in the oven (Model: UniscopeSurgifriends Medicals, England) and allowed to cool then packaged for further analysis.

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

| Wheat Flour (%) | PawpawSeed Flour (%) |
|-----------------|----------------------|
| 100 | 0 |
| 97.5 | 2.5 |
| 95 | 5 |

| | |
|------|------|
| 92.5 | 7.5 |
| 90 | 10 |
| 87.5 | 12.5 |

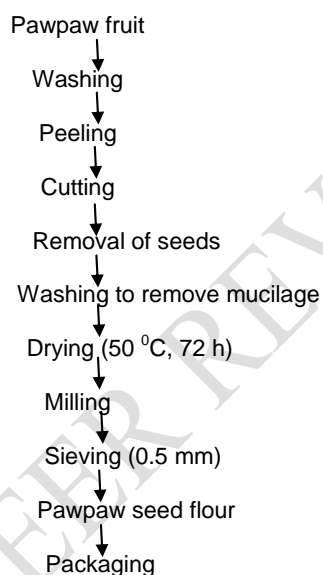


Figure 1: Flow Chart Showing the Production of PawpawSeed Flour
Source: [4].

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

Source: Igbabule *et al.* [6] with modification

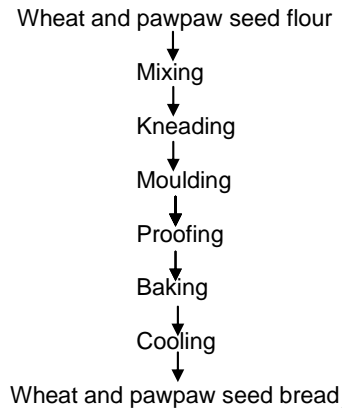


Figure 2: Flow Chart Showing the Production of Wheat and Pawpaw Seed Bread

Source: Joseph *et al.* [4] with modification

2.5 Determination of the Physical Characteristics of Bread

2.5.1 Determination of oven spring

Oven spring of the loaves was determined using the method of [8]. The dough height was measured before baking using a straight edge metric rule and height was measured again after baking. The difference in height equal the oven spring.

$$\text{Oven Spring (cm)} = \text{Height after baking} - \text{Height before baking} \quad (1)$$

2.5.2 Determination of loaf volume

Determination of loaf volume was by seed displacement method modified by Giami *et al.* [9]. A container (126.44 g) of fixed dimensions (18 cm³×12.7 cm³×12.4 cm³) of internal volume 2834 cm³ was filled with rice grain; a straight edge ruler was used to cut off all grains above the container rim. The grains were poured out and weighed (W_1). A weighed loaf was placed in the container and the weighed seeds were used to fill the container and leveled off as before. The overspill was weighed (W_2) and from the weight obtained, volume of rice displaced by the loaf was calculated using the following equation.

$$\text{Loaf Volume (cm}^3\text{)} = \frac{W_2 \times \text{actual volume of container}}{W_1} \quad (2)$$

where

W_1 = Weight of rice grains that filled the container

W_2 = Weight of rice grains displaced by the loaf samples

2.5.3 Determination of loaf weight

The bread samples were weighed (g) using a weigh balance (model: Sliding Harvard Trip balance, 2 kg-51 b capacity) as described by Mepbaet *et al.* [8].

2.5.4 Determination of specific volume

The specific loaf volume was determined by dividing the loaf volume by its corresponding loaf weight (cm³/g) as described by Araki *et al.* [10]. The specific volume is the ratio between loaf volume and loaf weight expressed in cubic centimetre per gram.

$$\text{Specific Volume (cm}^3\text{/g)} = \frac{\text{Loaf Volume}}{\text{Loaf Weight}} \quad (3)$$

2.6 Determination of the Alveograph Properties of Wheat and Pawpaw (*Carica papaya*) Seed Dough Samples

The alveograph properties of wheat and pawpawseed doughs samples were determined using an alveograph (Chopin, Model MA 82, France) using standard recommended alveograph procedures as

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described by Bordes *et al.* [11]. Flour (250 g) was kneaded with water (500 mL) containing 25 % NaCl in the alveograph mixer. A mixing time of 8 min. at 29 °C and 20 min rest period were the condition used. From alveograms obtained, the following rheological parameters of dough were calculated;

- (i) The height of curve, P (mm) which measured the pressure applied during inflation and indicated the resistance of dough to deformation.
- (ii) The length of the curve, L (mm), which measured the extensibility of the dough.
- (iii) Mechanical work for deformation, (W), (10^{-4} joules/g) which measured the overall strength of gluten height/length rate of the curve.
- (iv) Height/length ratio of curve, P/L.

2.7 Determination of the Mineral Content (mg/100g) of Bread

2.7.1 Determination of magnesium

Magnesium was determined according to the method described by [12]. 1 g of magnesium ribbon was accurately weighed and dissolved in 10 mL concentrated HCl. The solution was then boiled and evaporated almost to dryness on a water bath. De-ionized water was added and the solution was transferred into a 100 mL volumetric flask. The solution was made to mark with de-ionized water. From this stock solution which contains 1000 mg/mL of Mg^{2+} ions, four standard solutions of concentrations 0.0, 0.5, 1.0 and 1.5 ppm were prepared. Strontium chloride solution was added to magnesium solutions such that there is 1500 /mg of strontium ions in the final solution. The concentration was determined using calibration curves.

2.7.2 Determination of calcium

Calcium was determined using the atomic absorption spectrophotometer [12]. Calcium carbonate (2.495 g) was dissolved and diluted to 100 mL with de-ionized water. This solution contains 1000 mg Ca^{2+} ions and from this stock solution, calcium standard of the following concentration levels 0.0, 3.0, 6.0, 9.0 were prepared. The absorbance of both the sample and the standard working aliquot were determined in the atomic absorption spectrophotometer (Uniscopesurgifriends, England) at 239.9 nm. The concentration of the test mineral in the sample was calculated with reference to the graph (standard curve) and obtained as follows:

$$\text{Calcium (mg/100g)} = \frac{100 \times Y \times Vf \times D}{W \times 100 \times Va} \quad (4)$$

where

W = weight of the sample analyzed,

Y = Concentration of Calcium obtained from the standard curve,

Vf = Total volume of extract

Va = volume of extract used

D = Dilution factor

2.7.3 Determination of phosphorus

Phosphorus was determined using spectrophotometer [12]. Phosphorus in the sample was determined by the molybdate method using hydroquinone as a reducing agent. Sodium sulphate (1.0 mL), 1.0 mL of ammonium molybdate and 1 mL of hydroquinone were added to 1 mL of the sample digest. The mixture was agitated and allowed to stand for 30 minutes for the blue colour to develop. The absorbance of the sample was determined using the spectrophotometer at 600 nm. The phosphorus standard was prepared by dissolving 1.1 g of monobasic potassium phosphorus (KA_2PO_4) into a 500 mL volumetric flask containing 500 mL of distilled water. Five drops of toluene were added to diminish microbial activity. Twenty millilitre of the Standard stock was collected and made up to 100 mL. This contained 100 ppm. Standard stock (0.1 mL) = 0.2 ppm. Zero to one millilitre of the 100 ppm phosphorus stock solution was poured into 100 mL volumetric flask separately and treated the same way as the sample. The reading of the standard was taken at 600 nm in UV/VIS spectrophotometer (Uniscopesurgifriend Medicals, England) and a standard curve was plotted.

$$P \text{ (mg/100g)} = \frac{100 \times A_u \times C \times Vf}{W \times A_s \times Va} \quad (5)$$

where

W = Weight of sample analyzed

A_u = Absorbance of test sample

A_s = Absorbance of standard phosphorus solution

C = Concentration (in mg/mL) of sample

V_f = Total volume of extract

V_a = Volume of extract analyzed

2.7.4 Determination of potassium

Potassium determination was by Flame Photometry [12]. One gram (1 g) of sample was dissolved in 20 mL of acid mixture (650 mL of concentrated HNO₃; 80 mL PCA; 20 mL conc. H₂SO₄) and aliquots of the diluted clear digest were taken for photometry using Flame analyzer.

2.7.5 Determination of iron

Standard solution containing 100 mg/mL of Fe³⁺ ions was prepared from 1 g pure iron wire. The wire was dissolved in 20 mL concentrated HNO₃, boiled in water bath and diluted to 1000 mL with distilled water. Standard solutions with concentrations 0, 0.5, 1.0, 2.0 and 4.0 ppm was prepared. Two milliliter of sample aliquot was diluted to 100 mL and was used to determine the absorbance of the sample using an atomic absorption spectrophotometer (Uniscopie Surgifriends Medicals, England) at 510 nm. The standard and samples absorbance were noted and concentration of iron in the sample was determined from the standard curve (AOAC, 2012).

2.7.6 Determination of sodium

Weight of 0.2542 g of NaCl was dissolved in 1 litre of distilled water to give 100 ppm Na. This working standard solution was diluted to produce a range containing 0-10 ppm sodium and made up to 100 mL mark and 2 mL sample aliquot (sample stock solution) was read using a flame photometer [12]. The concentration of the test mineral in the sample was calculated with reference to the graph (standard curve) and obtained as follows:

$$\text{Sodium (mg/100g)} = \frac{100 \times Y \times V_f \times D}{W \times 100 \times V_a} \quad (6)$$

where:

W = Weight of the sample analyzed

Y = Concentration of Na obtained from the standard curve

V_f = Total volume of digest/extract (100 mL)

V_a = Volume of extract used

D = Dilution factor

2.8 Determination of Anti-Nutritional Factors of Breads

2.8.1 Determination of tannins

Tannin was determined using the Folin-Denis spectrophotometer method described by Pearson [13]. The sample (0.5 g) was weighed into a conical flask and 100 mL of distilled water was added into it. This was gently boiled for one hour and then filtered using Whatman filter paper into a 100 mL capacity volumetric flask. The filter paper was re-washed with distilled water and the filtrate was diluted to 100 mL mark and then cooled. Fifty millilitre aliquot was put into each flask for the development of greenish-blue colour. Five millilitre of Folin-Denis reagent (100 g sodium tungstate, 20 g phosphomolybdic acid, 50 mL of 85 % phosphoric acid and 750 mL of water) and 10 mL of saturated sodium carbonate solution was added into it. This was diluted to 100 mL mark with distilled water after a thorough mixing. The flask was allowed to stand in a water bath at 25 °C for one-half hour and the absorbance was measured in the UV/VIS spectrophotometer (Uniscopie Surgifriend Medicals, England) at 760 nm. Distilled water was used as blank for the calibration curve. A standard curve was plotted and concentration of each sample was obtained and used for the tannin calculation.

$$\text{Tannin mg/100g} = \frac{A_n \times C \times 100 \times V_f}{A_s \times W \times V_a} \quad (7)$$

where

A_n = Absorbance of test sample

A_s = Absorbance of standard solution

C = Concentration of standard

W = Weight of sample used

V_f = Total volume of extract

V_a = Volume of extract analyzed

2.8.2 Determination of oxalate

Oxalate was determined by the method described by Onwuka [14]. This determination involved three major steps: digestion, oxalate precipitation and permanganate titration

Digestion

Two grams (2 g) of sample flour was suspended in 190 mL of distilled water in a 250 mL volumetric flask. Ten millilitres of 6 M HCL was added and the suspension was digested at 100 °C for 1 h. It was cooled and made up to 250 mL before filtration.

Oxalate precipitation

Duplicate portions of 125 mL of the filtrate were measured into beakers and four drops of methyl red indicator was added. This was followed by the addition of concentrated NH₄OH solution (drop wise) until the test solution changed from pink to a faint yellow colour. Each portion was then heated to 90 °C, cooled and filtered to remove precipitate containing ferrousion. The filtrate was again heated to 90 °C and 10 mL of 5 % CaCl₂ solution was added while stirred constantly. After heating, it was cooled and left overnight at 5 °C. The solution was then centrifuged at 2500 rpm for 5 mins. The supernatant was decanted and the precipitate was completely dissolved in 10 mL of 20 % (v/v) H₂SO₄ solution and then filtered for titration.

Permanganate titration

The filtrate was made up to 300 mL. Aliquot of 125 mL from the filtrate was heated to near boiling and then titrated against 0.05 M standardized KMnO₄ solution to a faint pink colour which persisted for 30 seconds. The calcium oxalate content was calculated using the formula below.

$$\text{Oxalate (mg/100g)} = \frac{T \times (V_{me})(D_f) \times 10^5}{M_E \times M_F} \quad (8)$$

where

T = Titre of KMnO₄

V_{me} = Volume mass equivalent

D_f = Dilution factor

M_E = Molar equivalent of KMnO₄

M_F = Mass of sample used

2.8.3 Determination of phytate

Phytate was determined using the method described by Oberleas [15]. The sample was first extracted with 0.2 HCl. One millilitre of the extract was poured into a test tube fitted with a ground glass stopper together with 1 mL of ferric solution. The ferric solution was prepared by dissolving 0.2 g ammonium iron (III) sulphate in 10 mL of 2 NHCl. The solution was then made up to 100 mL with distilled water. The tube was heated in a boiling water bath for 30 minutes, cooled in ice for 15 minutes and then allowed to reach ambient temperature. The content of the tube was centrifuged for 30 minutes (300 rpm). After centrifugation the supernatant (1 mL) was mixed with 1.5 mL of 2,2 bipyridne solution and the absorbance measured at 519 nm against distilled water using UV/VIS spectrophotometer (Uniscope Surgifriend Medicals, England). Thus, the phytic acid content is calculated as shown below.

$$\text{Phytic Acid } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{100 \times V_f \times C}{W \times V_a \times 100} \quad (9)$$

where

C = Concentration of curve ms/mole

V_a = Total volume of extract analysed

V_f = Total volume of extract

W = Weight of sample

2.8.4 Determination of trypsin inhibitor

Trypsin inhibitor was determined by spectrophotometry method described by Arntfield *et al.* [16]. A standard trypsin was prepared and used to treat the substrate solution. The standard trypsin was prepared using N. Bensoyl – DL arginine – P-nitroanilide (BAPA). The extent of inhibitor of trypsin hydrolysis of the substrate was used as a standard to measure the trypsin inhibition activities of the test sample extract.

Extraction of sample

One gram (1 g) of the test sample was dispersed in 50 mL of 0.5 M NaCl solution. The mixture was stirred for 30 minutes at room temperature and centrifuged. The supernatant was filtered and the filtrate (extract) was used for the assay. Two millilitre of the trypsin solution was added to 10 mL of the extract in a test

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tube. Ten millilitre of the blank of the same substrate (trypsin) was prepared with no extract (test sample) added. The test sample and the blank were allowed to stand for at least 5 minutes and then measured with UV/VIS spectrophotometer (Uniscope Surgifriend Medicals, England) spectrophotometer at 420 nm.

Calculation

$$\text{Trypsin Inhibitor Unit } \left(\frac{\text{TIU}}{\text{mg}} \right) = \frac{b-a \times F}{0.01} \quad (10)$$

where

b = Absorbance of test sample solution

a = Absorbance of the blank (control)

$$F = \frac{l \times V_f \times D}{w \times V_a} \quad (11)$$

where

V_f = Total volume of extract

V_a = Volume of extract used in the assay

D = Dilution factor

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 Alveograph Properties of Wheat and Pawpaw(*Carica papaya*) Seed Dough Blends

Rheology is the science of the deformation and flow of matter. It is the study of the manner in which materials respond to applied stress or strain [17]. The alveograph is important dough testing instrument use to evaluate the quality of wheat flours for bread and biscuit and cookie making [18]. The alveograph test provides results that are common specifications used by flour millers and processors to ensure a more consistent process and product. It measures the resistance to expansion and the extensibility of dough by providing the measurement for maximum over pressure, average abscissa at rupture, index of swelling, and deformation energy of dough [19]. The Peak height (P) also referred to as the maximum pressure or tenacity indicated the resistance that the dough offered to deformation and it is related to the tensile strength or stability that the dough exhibited during the proofing stage of bread making [8]. The differences in the strength parameters of the dough samples are significant ($p < 0.05$). The addition of pawpawseed flour to wheat significantly decreased the maximum pressure (P) of the dough samples. This is due to the dilution of the gluten network of the wheat dough by the pawpawseed flour. The values for extensibility (L) of the dough decreased with increased level of pawpawseed substitution. The inclusion of pawpawseed flour decreased the carbohydrate content which decreased the quantity and quality of protein needed to sustain the viscoelastic behaviour of their dough. The low values of maximum pressure (P) and extensibility (L) observed with increased pawpaw seed addition indicated weak gluten of the wheat and pawpaw seed dough samples and will be useful in cakes and other confectionery products and not in breads. The mechanical work (W), which is the measure of energy for dough deformation decreased with increased level of blending with pawpawseed flour. The high baking strength value of wheat flour dough indicated the presence of strong gluten, which appeared to get weakened and destabilized by incorporation of pawpawseed flour. This explains the drastic reductions in baking strength value of the doughs with increased dilutions of wheat flour with pawpawseed flour. The ratio of resistance to extensibility also known as curve configuration ratio (P/L) is an index of gluten behaviour and this showed an increased value with increased pawpawseed flour addition. The results of the alveograph properties observed in this study are in agreement with the findings of [8; 20; 21] and [22] for wheat-plantain, wheat- glucose oxidase, wheat-sorghum and wheat-flaxseed composite flours respectively.

3.2 Physical Properties of Bread Produced from Wheat and Pawpaw(*Carica papaya*) Seed Flour Blends

Physical properties are properties that can be measured or observed without changing the chemical nature of the substance. The dilution effect on gluten was the reason for the observed decreased oven

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spring, loaf volume and specific volume with increased pawpawseed flour addition. The gluten fraction is responsible for the elasticity of the dough by causing it to extend and trap the carbon dioxide generated by yeast during fermentation. When gluten coagulates under the influence of heat during baking, it serves as the framework of the loaf, which becomes relatively rigid and does not collapse [22]. The decrease is also attributed to the lower levels of gluten network in the doughs and consequently, less ability of the dough to rise due to weaker cell wall structure [24]. Studies show that loaf volume is affected by the quantity and quality of protein in the flour used for baking [25]. According to Yusnita and Wong [26], addition of dietary fibre rich substances in baking products reduce loaf volume. It is ultimate that bread samples with reduced volume will also have reduced specific volume [27]. The observed increase in weight of wheat and pawpaw seed bread samples with increased pawpaw seed flour addition is as a result of less retention of carbon-dioxide gas in the blended dough, hence providing dense bread texture. Also, the higher moisture contents of the flours could have contributed to the higher loaf weight relative to 100 % wheat bread [28]. The results of this study are in-line with the findings of [29; 30; 31; 32; 33; 6] and [28] who reported increased bread loaf weight but decreased oven spring, loaf volume and specific volume with the increased substitution of wheat flour with debittered *Moringa oleifera* seed, cocoyam, sweet potato, *Moringa oleifera* leaf powder, cocoa pod husk powder, maize/orange fleshed sweet potato and unripe plantain respectively.

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Table 3. Alveograph Properties of Wheat and Pawpaw (*Carica papaya*) Seed Dough Blends

| Sample | Maximum Pressure P (mm) | Extensibility L (mm) | Baking Strength W ($\times 10^{-4}$ joules/g) | Ratio of Resistance to Extensibility (P/L) |
|-----------|----------------------------|-------------------------|---|--|
| 100:0 | 109.50 \pm 0.71 | 280.50 \pm 0.71 | 255.00 \pm 0.00 | 1.36 \pm 0.01 |
| 97.5:2.5 | 105.50 \pm 0.71 | 70.50 \pm 0.71 | 253.50 \pm 0.71 | 1.48 \pm 0.00 |
| 95:5 | 102.50 \pm 0.71 | 68.00 \pm 0.00 | 252.50 \pm 0.71 | 1.50 \pm 0.01 |
| 92.5:7.5 | 97.50 \pm 0.71 | 64.50 \pm 0.71 | 251.00 \pm 0.00 | 1.51 \pm 0.01 |
| 90:10 | 94.50 \pm 0.71 | 59.00 \pm 0.00 | 247.50 \pm 0.71 | 1.60 \pm 0.01 |
| 87.5:12.5 | 91.00 \pm 0.00 | 54.50 \pm 0.71 | 237.50 \pm 0.71 | 1.66 \pm 0.04 |
| LSD | 1.58 | 1.41 | 1.41 | 0.04 |

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

Key: LSD = Least Significant Different.

Table 4: Physical Properties of Breads Produced from Wheat and Pawpaw (*Carica papaya*) Seed Flour Blends

| Sample | Oven Spring | Loaf Volume | Loaf Weight | Specific Volume |
|--------|-------------|-------------|-------------|-----------------|
|--------|-------------|-------------|-------------|-----------------|

| Wheat: Pawpaw Seed | (cm) | (cm ³) | (g) | (cm ³ /g) |
|--------------------|-------------------------|----------------------------|---------------------------|-------------------------|
| 100:0 | 5.49 ^f ±0.19 | 1022.50 ^f ±0.62 | 250.20 ^a ±1.24 | 4.09 ^f ±0.01 |
| 97.5:2.5 | 4.33 ^e ±0.13 | 996.60 ^e ±0.33 | 263.50 ^b ±0.06 | 3.78 ^e ±0.00 |
| 95:5 | 3.64 ^d ±0.02 | 970.00 ^d ±0.25 | 270.20 ^c ±0.42 | 3.58 ^d ±0.01 |
| 92.5:7.5 | 2.82 ^c ±0.08 | 948.40 ^c ±0.55 | 275.60 ^d ±0.54 | 3.44 ^c ±0.00 |
| 90:10 | 2.51 ^b ±0.04 | 922.00 ^b ±0.05 | 281.40 ^e ±0.92 | 3.28 ^b ±0.01 |
| 87.5:12.5 | 2.39 ^a ±0.02 | 901.60 ^a ±0.59 | 288.50 ^f ±0.37 | 3.14 ^a ±0.01 |
| LSD | 0.25 | 1.10 | 1.73 | 0.02 |

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

Key: LSD = Least Significant Different.

3.3 Mineral Composition (mg/100g) of Breads Produced from Wheat and Pawpaw(*Carica papaya*) Seed Flour Blends

Minerals are inorganic elements which are essential for the normal functioning of the body. They are required in smaller quantities in addition to proteins, carbohydrates, fats and vitamins, they are inorganic or “ash constituents” of foods which cannot be destroyed by heating [34]. Although they yield no energy, they have important roles to play in many activities in the body [35]. As ash content gives an insight to the mineral content of the food, hence, bread produced from wheat and pawpawseed flour can be described as a rich source of minerals as seen from the significant (p<0.05) increase in the mineral contents of breads with increased levels of pawpawseed addition. All the bread samples appeared to be good source of magnesium, calcium, phosphorus, potassium, iron and sodium.

The increase in magnesium content of wheat and pawpaw seed breads may largely be a pointer to the fact that pawpawseed is a rich source of magnesium. Magnesium in the diet and cell catalyzes hundreds of metabolic reactions resulting in changes in energy status, catalyzes the oxidative phosphorylation on adenosine diphosphate (ADP), and adenosine triphosphate (ATP). Also, the release of energy which results when ATP is converted to ADP requires magnesium [34]. Calcium is a mineral required by the body for a variety of physiological functions and the maintenance of bone tissues throughout life [36]. Calcium is necessary for supporting bone formation and growth; it also helps in the maintenance of healthy teeth, skeletal and soft tissue, mucous membranes and skin. Phosphorus works closely with calcium to build strong bones and teeth [34]. As an essential nutrient for human health, Iron also plays an important role in normal growth and development [37]. Potassium activates several enzyme reactions and helps in the release of energy from carbohydrates, fats and proteins. It also functions with sodium and calcium to regulate neuromuscular excitability [34]. Though the results showed relatively low contents of sodium which makes the wheat and pawpawseed breads good products for people with high blood pressure and heart disease problems [38]. [39; 5; 40] and [41] revealed significant presence of mineral elements such as calcium, potassium, sodium and phosphorus in pawpaw seeds.

3.4 Ant-nutritional Contents of Breads Produced from Wheat and Pawpaw(*Carica papaya*) Seed Flour Blends

The anti-nutritional contents of the bread in Table 6 showed low levels of tannins, oxalate, Phytate and Trypsin inhibitor. Similar low levels of tannins, oxalate and Phytate had been reported by Adenijiet *al.* [42] and Oyeleke *et al.* [43] for plantain/banana cultivars and pawpawseeds respectively.

Tannin is an anti-nutrient that inhibits activity of digestive enzymes [43]. Tannins are known for their ability to precipitate with iron and other metals, thereby reducing their absorption [39]. The lower values

Comment [MM20]: Nutritionally, the fact that the new bread contains a significant difference in the content of a certain nutrient with respect to the control bread does not mean that it is considered a rich source of that nutrient. To be considered this way, the portion of it must cover a certain percentage of the requirement of the population to which it is intended. Data that are not shown in this study. If it can be said that it increases the mineral content

Comment [MM21]: It would be important that the centesimal composition of papaya seed flour be included in the work.

Comment [MM22]: This paragraph corresponds to the introduction, not to the discussion of the results.

obtained for tannin is very important because tannic acid above 10 % of total dry weight affects overall nutritional potential of food material. Importantly, tannin can be used in treatment of skin eruption due to their astringent properties [43].

Oxalates affect the metabolism of magnesium and calcium. It also reacts with proteins to form complexes which have an inhibitory effect in the digestion of peptic. High oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stone. Oxalate content of the bread samples showed increased value with increased level of pawpawseed addition. Generally, small amounts of oxalate may occur in many vegetables and fruits but do not pose nutritional problems [43].

Phytate has strong binding capacity and forms insoluble complexes with multivalent cations, including Ca, Mg, Fe and Zn, and render them biologically unavailable [43].

Trypsin inhibitor has been reported to increase with increased level of ripening [39] but the reduced levels observed here could be as a result of its thermolability which may be destroyed with the application of heat[44]. The levels of these anti-nutrients in all the samples were relatively low, below toxic levels and may not hinder the bioavailability of essential nutrients in the breads. Also, the levels observed here are not in considerable levels of inhibitors that may inhibit the absorption of minerals [39]. The results are similar to the findings of Samia *et al.* [5] but lower than the ones discovered in foods by Ross and Martha [45].

Table 5: Mineral Composition (mg/100g) of Breads Produced from Wheat and Pawpaw(*Carica papaya*) Seed Flour Blends

| Sample | Magnesium (Mg) | Calcium (Ca) | Phosphorus (P) | Potassium (K) | Iron (Fe) | Sodium (Na) |
|--------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------------------|
| Wheat: Pawpaw Seed | | | | | | |
| 100:0 | 143.00 ^a ±0.85 | 252.60 ^a ±0.28 | 73.50 ^a ±0.23 | 26.55 ^a ±0.21 | 21.55 ^a ±0.21 | 46.80 ^a ±0.14 |
| 97.5:2.5 | 157.50 ^b ±0.42 | 269.40 ^b ±0.35 | 98.50 ^b ±0.25 | 28.10 ^b ±0.00 | 33.48 ^b ±0.33 | 49.35 ^b ±0.35 |
| 95:5 | 161.60 ^c ±0.21 | 273.50 ^c ±0.50 | 104.90 ^c ±0.18 | 28.55 ^{bc} ±0.07 | 35.66 ^c ±0.06 | 50.60 ^c ±0.28 |
| 92.5:7.5 | 173.30 ^d ±0.28 | 275.50 ^d ±0.50 | 109.50 ^d ±0.58 | 28.95 ^c ±0.07 | 39.50 ^d ±0.42 | 51.45 ^d ±0.50 |
| 90:10 | 178.70 ^e ±0.35 | 290.40 ^e ±0.21 | 121.40 ^e ±0.26 | 30.45 ^d ±0.21 | 42.07 ^e ±0.06 | 63.30 ^e ±0.28 |
| 87.5:12.5 | 182.50 ^f ±0.28 | 342.60 ^f ±0.35 | 127.30 ^f ±0.18 | 36.25 ^e ±0.35 | 48.36 ^f ±0.08 | 65.45 ^f ±0.21 |
| LSD | 1.11 | 0.93 | 0.76 | 0.47 | 0.58 | 0.77 |

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

Key: LSD = Least Significant Different.

Table 6: Anti-nutritional Contents of Breads Produced from Wheat and Pawpaw(*Carica papaya*) Seed Flour Blends

| Sample | Tannins (mg/100g) | Oxalate (mg/100g) | Phytate (mg/100g) | Trypsin Inhibitor (TIU/100g) |
|------------------|-------------------------|-------------------------|-------------------------|---------------------------------|
| Wheat:PawpawSeed | | | | |
| 100:0 | 1.76 ^a ±0.02 | 0.06 ^a ±0.00 | 0.06 ^a ±0.01 | 1.28 ^a ±0.61 |
| 97.5:2.5 | 1.87 ^b ±0.01 | 0.10 ^b ±0.01 | 0.11 ^b ±0.01 | 2.74 ^b ±0.34 |
| 95:5 | 1.99 ^c ±0.04 | 0.13 ^c ±0.01 | 0.12 ^b ±0.00 | 3.39 ^b ±0.53 |

| | | | | |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|
| 92.5:7.5 | 2.19 ^d ±0.04 | 0.15 ^d ±0.01 | 0.20 ^c ±0.01 | 4.54 ^c ±0.01 |
| 90:10 | 2.35 ^e ±0.03 | 0.26 ^e ±0.01 | 0.27 ^d ±0.01 | 7.27 ^d ±0.58 |
| 87.5:12.5 | 2.68 ^f ±0.04 | 0.28 ^f ±0.01 | 0.29 ^e ±0.01 | 9.71 ^e ±0.39 |
| LSD | 0.07 | 0.02 | 0.02 | 1.12 |

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

Key: LSD = Least Significant Different.

4. CONCLUSION

The oven spring, loaf volume and the specific volume of the bread samples showed a decreased trend but an increased trend of the loaf weight as the level of addition of pawpaw seed increased. The study also showed an increased ratio of resistance to extensibility but decrease maximum pressure, extensibility and baking strength of the dough samples with increased level of *Papaya* seed addition. The minerals and vitamins content such as magnesium, calcium, phosphorus, potassium, iron, sodium, increased as the level of pawpawseed flour addition increased. There was an increased trend in the anti-nutritional contents of the bread samples with increased level of pawpawseed flour addition.

REFERENCES

- [1] Nwanekezi, E. C. Composite Flours for Baked Products and Possible Challenges: A Review. Official Journal of Nigerian Institute of Food Science and Technology. 2013;31(2): 8-17.
- [2] Shewry, P. R. The Healthgrain Programme Opens New Opportunities for Improving Wheat for Nutrition and Health. Nutrition Bulletin. 2009;34(2): 225-231.
- [3] Shewry, P. R. Improving the Protein Content and Composition of Cereal Grain. Journal of Cereal Science. 2007;46: 239-250.
- [4] Cláudia, M. S., Celeste, M. P. A., Juliana, M. F., Estela, D. R. Q., and Marcelle, M. M. Chemical Characterization of the Flour of Peel and Seed from two *Papaya* Cultivars. Journal of Food Science and Technology. 2014;34(2): 353-357.
- [5] Samia, F. E., Rabab, H. S. and Abd-El-Ghany, M. E. Chemical and Nutritional Evaluation of Different Seed Flours as Novel Sources of Protein. World Journal of Dairy and Food Sciences. 2012;7(1): 59-65.
- [6] Igbabul, B., Num, G. and Amove, J. Quality Evaluation of Composite Bread Produced from Wheat, Maize and Orange Fleshed Sweet Potato Flours. American Journal of Food Science and Technology. 2014;2(4): 109-115.
- [7] Joseph, A., Isaac, A., Vida, B., Pearl, B. A. and Josephine, A. B. Nutrient Composition and Sensory Evaluation of Ripe Banana Slices and Bread Prepared from Ripe Banana and Wheat Composite Flours. American Journal of Food and Nutrition. 2016;4(4): 103-111.
- [8] Mepba, H., Eboh, L. and Nwaojigwa, S. U. Chemical Composition Functional and Baking Properties of Wheat-Plantain Composite Flours. African Journal of Food, Agriculture, Nutrition and Development. 2007;7: 1-22.
- [9] Giami, S. Y., Adindu, M. N. and Akusu, M. O. Effect of Processing Methods on Fluted Pumpkin (*Telfairia occidentalis*) and other Chemical Constituents. Proceedings of 23rd Annual NIFST Conference Abuja. 1999; pp. 127-129.

Comment [MM23]: Correct summary of results, missing a conclusion that highlights the importance of the study. In your criteria and according to the rheological results, mineral content and antinutrients present, which supplementation you consider best.

- [10] Araki, E., Ikeda, M. T., Ashida, K., Tanaka, K., Yanaka, M. and Iida, S. Effects of Rice Flour Properties on Specific Loaf Volume of one-loaf Bread made from Rice Flour with Wheat Vital Gluten. *Journal of Food Science and Technology Research*. 2009;15(4): 439-448.
- [11] Bordes, J., Branlard, G., Oury, F. X., Charmet, G. and Balfourier, F. Agronomic Characteristics, Grain Quality and Flour Rheology of 372 Bread Wheats in a Worldwide Core Collection. *Journal of Cereal Science*. 2008;48(3): 569-579.
- [12] Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis of AOAC International*. 19th edition. AOAC International, Gaithersburg, Maryland, USA. Pearson, D. (1976). *The Chemical Analysis of Food*. Seventh edition. Churchill, London. 2012; Pp.353-354.
- [13] Pearson, D. *The Chemical Analysis of Food*. Seventh edition. Churchill, London. 1976;Pp.353-354.
- [14] Onwuka, G. I. *Food Analysis and Instrumentation*. Surulere, Lagos, Naphthal print. Pp. 133-139. Oberleas, D. (1973). Phytates, in: *Toxicants Occurring Naturally in Foods*, 2nd ed., Washington, D.C., National Academy of Sciences. 2005; p. 363.
- [15] Oberleas, D. Phytates, in: *Toxicants Occurring Naturally in Foods*, 2nd ed., Washington, D.C., National Academy of Sciences. 1973; p. 363.
- [16] Arnfield, S. D., Ismond, M. A. H. and Murray, E. D. The Fate of Anti-Nutritional Factors During the Preparation of Faba Bean Protein Isolate using Micellization Technique. *Canadian Institute of Food Science and Technology Journal*. 1985;18: 137-143.
- [17] Mirsaeedghazi, H., Zahra, E. D. and Sayed, M. A. M. Rheometric Measurement of Dough Rheological Characteristics and Factors Affecting It. *International Journal of Agriculture and Biology*, 2008; 10(1): 112-119.
- [18] Janssen, A. M., Van, T. V. and Vereijken, J. M. Fundamental and Empirical Rheological Behaviour of Wheat Flour Doughs and Comparison with Bread Making Performance. *Journal of Cereal Science*, 1996; 23: 43-54.
- [19] Indrani, D. and Rao, G. V. Rheological Characteristics of Wheat Flour Dough as Influenced by Ingredients of Parotta. *Journal of Food Engineering*. 2007;79:100-105.
- [20] Milan, V., Miroslav, H. and Jelena, T. J. Alveograph and Bread Making Quality of Wheat Dough as Affected by added Glucose Oxidase. *Quality of Life*. 2013;4(3-4): 49-54.
- [21] Thulani, S., Thembekile, N. and Nyasha, N. Rheological Properties and Bread Making Quality of White Grain Sorghum-Wheat Flour Composites. *International Journal of Food Science and Nutrition Engineering*. 2015;5(4): 176-182.
- [22] Georgiana, G. C. and Silvia, M. Bread Quality and Alveograph Rheological Properties of Composite Flour Made from Flaxseed and 650 Type Wheat of Strong Quality for Bread Making. *International Journal of Food Engineering*, 2018;4(2):117-121.
- [23] Makinde, F. M. and Akinoso, R. Physical, Nutritional and Sensory Qualities of Bread Samples made with Wheat and Black Sesame (*Sesamum indicum* Linn) Flours. *International Food Research Journal*. 2014;21(4): 1635-1640.
- [24] Abdelghafor, R. F., Mustafa, A. I., Ibrahim, A. M. H. and Krishnan, P. G. Quality of Bread from Composite Flour of Sorghum and Hard White Winter Wheat. *Advance Journal of Food Science and Technology*. 2011;3: 9-15.

- [25] Shittu, T., Raji, A. O. and Sanni, L. O. Bread from Composite Cassava-Wheat Flour: I. Effect of Baking Time and Temperature on some Physical Properties of Bread Loaf. *Food Research International*, 2007;40: 280-290.
- [26] Yusnita, H. and Wong, F. L. Physicochemical Properties and Acceptance of High Fiber Bread Incorporated with Corn Cob Flour. In: *The 12th ASEAN Food Conference*, BITEC Bangna, Bangkok, Thailand, 2011;16-18; pp. 647-651.
- [27] Iftikhar, A. S., Nida, S., Asadullah, M., Aijaz, H. S. and Shahzor, G. K. Loaf Characteristics and Sensory Properties of whole Wheat Bread Fortified with Sorghum and Rice Flour. *Journal of Basic and Applied Sciences*. 2017;13: 606-610.
- [28] Ufot, E. I. and Inemesit, E. A. Physico-chemical and Sensory Qualities of Functional Bread Produced from Wholemeal Wheat and Unripe Plantain Composite Flours. *Malaysian Orthopaedic Journal of Food Processing and Technology*. 2016;2(2): 48-53.
- [29] Ogunsina, B. S., Radha, C. and Indrani, D. Quality Characteristics of Bread and Cookies Enriched with Debittered Moringa Oleifera Seed Flour. *International Journal of Food Science and Nutrition*. 2011;62(2): 185-194.
- [30] Mongi, R. J., Ndabikunze, B. K. and Chove, B. E. Proximate Composition, Bread Characteristics and Sensory Evaluation of Cocoyam-Wheat Composite Breads. *African Journal of Food, Agriculture, Nutrition and Development*. 2011;11(7): 5586-5599.
- [31] Babatunde, I. O., Sunday, A. M. and Ezekiel, O. O. Quality Assessment of Flour and Bread from Sweet Potato-Wheat Composite Flour Blends. *International Journal of Biological and Chemical Sciences*. 2012;6(1): 65-76.
- [32] Sengeev, A. I., Joseph, O. A., and Gernah, D. I. Effect of *Moringa oleifera* Leaf Powder Supplementation on Some Quality Characteristics of Wheat Bread. *Food and Nutrition Sciences*. 2013;4: 270-275.
- [33] Amir, I. Z., Hanida, H. S. and Syafiq, A. Development and Physical Analysis of High Fibre Bread Incorporated with Cocoa (*Theobroma cacao*) Pod Husk Powder. *International Food Research Journal*. 2013;20(3): 1301-1305.
- [34] Chidinma, W. A., Jiddari, W. U. and Hassan, S. C. A Student Handbook on Food and Nutrition. 1st edition, Kaduna, De-New Creation Prints Ltd Publishers. 2010; 222pp.
- [35] Ihekoronye, A. I. and Ngoddy, P. O. *Integrated Food Science and Technology for the Tropics*. London: Macmillan Publ. Ltd. 1985; Pp. 265.
- [36] Grosvernor, M. B. and Smolin, L. A. *Nutrition: From Science to Life*. Harcourt College Publishers, New York, USA, 2002; pp. 288-371.
- [37] Inyang, U. E. and Ekop, V. O. Physico-Chemical Properties and Anti-Nutrient Contents of Unripe Banana and African Yam Bean Flour Blends. *International Journal of Nutrition and Food Sciences*. 2015;4(5): 549-554.
- [38] Ramulu, P. and Udayaekhara, R. P. Effect of Processing on Dietary Fibre Content of Cereals and Pulses. *Plant Foods for Human Nutrition Journal*. 1997;50: 249-257.
- [39] Adesuyi, A. O. and Ipinmoroti, K. O. The Nutritional and Functional Properties of the Seed Flours of three Varieties of *Caricapapaya*. *Current Research in Chemistry*. 2011;3: 70-75.

- [40] Chukwuka, K. S., Iwuagwu, M. and Uka, U. N. Evaluation of Nutritional Components of *Carica papaya* L. At Different Stages of Ripening. *Journal of Pharmacy and Biological Science*.2013;6(4): 13-16.
- [41] Kadiri, O. P. Studies on the Chemical Composition, Functional and Antioxidant Properties of *Carica papaya* (Pawpaw) Seed Flour, Protein Concentrate and Protein Isolate. M.Sc. Thesis. Department of Food Science and Technology. Obafemi Awolowo University, Ile-ife, Nigeria. 2017; 157pp.
- [42] Adeniji, T. A., Sanni, L. O., Barimalaa, I. S. and Hart, A. D. Anti-nutrients and Heavy Metals in some New Plantain and Banana Cultivars. *Nigeria Food Journal*. 2007;25(2):165-170.
- [43]Oyeleke, G. O., Isola, A. D., Salam, M. A. and Ajao, F. D. Evaluation of Some Chemical Composition of Pawpaw (*Carica papaya*) Seeds under Normal Storage Ripening.*IOSR Journal of Environmental Science, Toxicology and Food Technology*,2013;4(6): 18-21.
- [44]Ohizua, E. R.,Abiodun, A. A.,Micheal, A. I.,Olajide, P. S.,Adeniyi, T. A.,Raphael, O. I.,Simeon, O. A., Tolulope, O. O. andAyorinde, F.Nutrient Composition, Functional, and Pasting Properties of Unripe Cooking Banana, Pigeon Pea, and Sweet Potato Flour Blends. *Journal of Food Science and Nutrition*. 2016;5(3): 750-762.
- [45]Ross, P. H. and Martha, K. Estimation of the Oxalate Content of Foods and Daily Oxalate Intake. *Journal of Kidney International*. 2000;57: 1662-1667.