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Effects of Different Processing Methods on the Chemical Composition and Antioxidant Properties of Pigeon Pea (*Cajanus cajan*) Seed

Rebecca Olajumoke Oloniyo[1]*; Favour Ojonugwa Caleb[1]; Abimbola Beatrice Adeko[1]; Isah Laisi Rashidi[1]; Netalla Jibril[1]; Wisdom Otaru Oniwon[2]

[1]Department of Food, Nutrition and Home Science, Faculty of Agriculture, Prince Abubakar Audu University, Nigeria.

[2]Department of Biochemistry, Faculty of Natural Sciences, Prince Abubakar Audu University, Nigeria.

***Corresponding Author(s): Rebecca Olajumoke Oloniyo**

Department of Food, Nutrition and Home Science, Faculty of Agriculture, Prince Abubakar Audu University, P.M.B 1008, Anyigba, Kogi State, Nigeria.

Email: oloniyo.ro@ksu.edu.ng

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Abstract

This study was conducted to determine the effects of different processing methods on pigeon pea seeds chemical composition and antioxidant properties. Pigeon pea seed was cleaned and subjected to various processing methods (drying, germination, fermentation, and toasting). Each processed pigeon pea seed was milled separately and analyzed for proximate, mineral, anti-nutrient, and antioxidant properties. The results obtained show that ash content ranged from 2.42 to 2.62%, crude protein ranged from 27.78 to 36.27%, potassium content ranged from 611.01 to 617.40 mg/kg, calcium ranged from 138.13 to 171.40 mg/kg, iron content ranged from 18.43 to 23.40 mg/kg, and zinc content ranged from 2.67 to 3.17 mg/100g, phytates ranged from 14.48 to 17.58 mg/100g, tannins ranged from 23.79 to 28.42 mg/100g, saponin ranged from 12.25 to 19.72 mg/100g and trypsin inhibitor ranged from 1.48 to 3.33 mg/100g, FRAP ranged from 14.70 to 33.20 mmol/100g, total-phenol ranged from 98.4 and 132.6 mg GAE/g, total-flavonoid ranged from 13.5 and 42.1 mg QE/g, alpha - amylase ranged from 75.57 to 84.44 µg/mL, alpha – glycosidase ranged from 33.22 to 48.48 µg/mL, iron chelation ranged from 0.03 to 0.04 mg/mL and DPPH properties ranged from 0.11 to 0.25 mg/g respectively. Hence, the processing methods of the pigeon pea flour revealed significant ($p<0.05$) differences in the results of tested parameters. Therefore fermented-toasted pigeon pea flour was considered the best processing method because it enhances the nutritional quality and significantly reduces the antinutritional properties of the flour compared to other processing methods examined.

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Introduction

Legumes are nutritious foods and a substitute for animal protein arises from the knowledge of the nutritional properties of seed meal and other products [1,2]. Botanically, legumes belong to the Fabaceae family of angiosperms and are considered rich in high-quality proteins with significant impacts on the nutrition, diet, and health of many people worldwide [3]. Legumes are good sources of protein, its deficiency leads to malnutrition known as kwashiorkor, which prevails due to lack of sufficient animal protein in the human diet, hence the search for alternative protein sources from lesser-known legumes instead of expensive and scarce animal protein is needed [4]. Some plants, especially legumes, have been reported to be good sources of proteins that provide all essential amino acids [1]. Examples of legumes include pigeon pea (*Cajanus cajan*), soybean (*Glycine max*), Bambara groundnut (*Vigna subterranean*), African yam bean (*Sphenostylis stenocarpa*), kidney beans (*Phaselous vulgaris*), mung bean (*Vigna radiata*), groundnut (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), pea (*Pisum sativum*), chick pea (*Cicer arietinum*), guar legume (*Cyamopis tetragonoloba*) lentils (*Lens culinaris*) and winged bean (*Psophocarpus tetragonoloba*).

Pigeon pea (*Cajanus cajan L.*), a legume, belongs to the tribe Phaseoleae of the Fabaceae family [5]. It is an underutilized crop, grown annually or perennially [6]. It can be consumed as a green seeded or peeled vegetable [7]. It is a good source of minerals such as phosphorus, magnesium, iron, calcium, sulfur, and potassium, but is deficient in sodium [8]. It is abundant in antioxidant, hypocholesterolemic, antimicrobial, anti-inflammatory, and hepatoprotective properties [9]. Pigeon pea is an important legume among the poor rural population and despite its nutritional importance; It is an excellent source of protein, energy, and fiber [8]. It is cultivated in some tropical and subtropical countries of the world such as India, Myanmar, Nepal, Nigeria, Kenya, Malawi, Uganda, Mozambique, and Tanzania [10]. It ranks fifth in importance among the world's edible legumes after soybeans, beans, peanuts, and chickpeas [11]. India is the major producer of pigeon peas, and interest in pigeon peas in other parts of the world including Nigeria is a result of their nutritional, medicinal, economic, and agronomic usefulness [12].

Pigeon pea is a food crop rich in proteins and carbohydrates [8] therefore; could be a good food source to alleviate Protein-Energy Malnutrition (PEM). Despite all these potentials, it remains underutilized in West Africa, especially in Nigeria, where its cultivation is gradually becoming extinct due to its hard and firm seed coat, which contributes to its long hydration and cooking time [13,14]. Like other legumes, pigeon peas also contain some anti-nutritional factors that are harmful to human health, these include hemagglutinins, trypsin and chymotrypsin inhibitors, cyanoglycosides, alkaloids, oxalate, hydrogen cyanide, saponins, phytates, urease, and tannins [13]. Antinutritional Factors (ANFs) are biological components present in foods that can reduce nutrient utilization or food uptake, thereby leading to impaired gastrointestinal functions, reduced protein digestibility, reduced absorption of minerals and vitamins even resulting in direct detrimental effects on the body system [14]. ANFs play a vital role in determining the use of plants for both human and animal foods. Anti-nutrients are one of the key factors, which reduce the bioavailability of various components of cereals and legumes when consumed. These factors can cause micronutrient malnutrition and mineral deficiencies. Antinutrients such as trypsin inhibitors and phytates, which are present in legumes and cereals, reduce the digestibility of proteins and mineral absorption in the body [14].

The presence of all these anti-nutritional factors has a great influence on the absorption of nutrients in the body. Researchers have worked immensely on tactics to reduce these anti-nutritional factors present in pigeon peas through different processing methods such as cooking, extrusion, boiling, fermentation, roasting, germination, autoclaving, and microwave oven roasting [9,15-19]. Despite all the processes adopted by early researcher, pigeon pea remains underutilized and there is a paucity of information on the effects of processing on the chemical composition and antioxidant properties of germinated–toasted and fermented–toasted pigeon pea seed. Therefore, this study aimed to determine the effects of different processing methods (germinated–toasted and fermented–toasted pigeon pea seed) on pigeon pea seeds chemical composition and antioxidant properties.

Material and method

Source of materials

White Pigeon pea (*Cajanus cajan*) seeds were purchased from Anyigba Market, Kogi State Nigeria.

Sample preparation

Preparation of raw pigeon pea seed flour:

The white pigeon pea seeds were processed into flour as described by Akubor [12]. The white pigeon pea seed was cleaned of foreign materials and hydrated in cold water (30 ± 2°C) for 60 min, dried in a glass house for 3 h, and ground to a fine particle using an attrition mill. Then, the ground pigeon pea flour was sieved through a 0.1 mm sieve and packed in airtight bags before use.

Preparation of germinated pigeon pea seed:

White pigeon pea seed was germinated using the methods described by Ukom [9] with slight modifications. Pigeon pea seeds were surface sterilized with 1.5% sodium hypochlorite solution and then soaked in 70% ethanol (0.7 mL per 100 L) for 20 min using a seed-to-liquid ratio of 1:10. The seeds were then immersed in water for 11 h 30 min, using a seed to distilled water ratio of 1:10. The seeds were drained and spread on a tray containing a cloth moistened with sodium hypochlorite for 3 days in a dark environment. After 3 days, the ungerminated seeds were separated from the germinated seeds. The germinated seeds were rinsed with tap water, drained, and dried in a greenhouse for 6 h. The dried germinated pigeon pea seed was kept for further analysis.

Preparation of fermented pigeon pea seed:

White pigeon pea seed was fermented using the methods described by Akubor [12]. The pigeon pea seed was mixed with water in a ratio of 3:2 (3 water: 2 pigeon pea seeds) in a covered plastic container. The seed was fermented for 5 days. After fermentation, the water was drained, and the seeds were washed, drained, and dried in a glass house for 3 h. The fermented and dried pigeon pea seed was preserved for further analysis.

Preparation of germinated-toasted and fermented-toasted pigeon pea flour:

Both germinated and fermented pigeon pea seeds were toasted separately using the methods described by Akubor [12]. Both the germinated and the fermented pigeon pea seeds were spread separately on different trays and toasted at 120°C for 30 min in an air convection oven with intermittent mixing. The toasted pigeon pea seeds were ground using an attrition mill, sieved through a 0.1 mm sieve, and preserved before use. The process flow chart is shown in Figure 1.

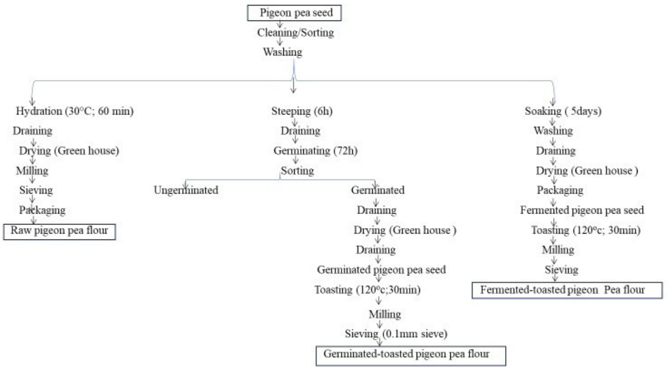

Figure 1: Processing flow chat of raw pigeon pea flour, germinated–toasted and fermented–toasted pigeon pea (*Cajanus cajan*) flour.
Source: Ukom, [9], Akubor [12].

Analytical methods

Determination of the proximate composition of pigeon pea flour:

The proximate composition (moisture, crude protein, crude fat, crude fibers and crude ash) of pigeon pea flour was determined according to the method described by the Association of Official Analytical Chemists [20].

The total carbohydrate was determined by difference and the caloric value was calculated using the Atwater factor the method described by Osborne and Voogt [21].

Thus: Total carbohydrate = 100 – (% fat + % protein + % crude fiber + % moisture content + % ash)

Caloric value = [(4 x protein content) + (4 x carbohydrate content) + (9 x fat content)]

Determination of some mineral elements of pigeon pea flour:

The contents of Calcium (Ca), Iron (Fe), Sodium (Na), Magnesium (Mg), Zinc (Zn), Potassium (K}, and Phosphorus (P) were determined as described by the Association of Official Analytical Chemists-AOAC [20]. The AOAC single official multiple element method (AOAC 968.08) and the atomic absorption spectroscopy method were used to determine the content of Ca, Fe, and Zn, and the AOAC official method was used to determine the "P" content. 964.04. The content of "Na" and "K" was determined using a flame-photometer.

Determination of antinutritional factors of pigeon pea flour:

The phytate content of pigeon pea flour was determined using the indirect colorimetric method described by Wheeler and Ferrel [22]. About 4 g of the sample was soaked in 100 mL of 2% HCl for 3 h and then filtered through a No 1 Whatman filtered-paper. About 25 mL was taken out of the filtrate and placed inside a conical flask, thereafter, 5 mL of 0.3% ammonium thiocyanate solution was added as indicator. In addition, 53.5 mL of distilled water was added to the prepared solution to make it acidic, then, the acidic solution was titrated against 0.00566 g per milliliter of standard iron (III) chloride solution that contained about 0.00195 g of iron per milliliter until a brownish color which persisted for 5 min was obtained.

The oxalate content of pigeon pea flour was determined as described by the Association of Official Analytical Chemists [20]. About 1 g of the sample was soaked in 75 mL of 1.5 H_2SO_4 for 1 h, it was then filtered through a No 1 Whatman filtered-paper. About 25 mL was taken out of the filtrate and placed inside a conical flask and was titrated hot about (80–90°C) against 0.1 M of $KMnO_4$ until a pink color that persists for 15s.

The tannin content of pigeon pea flour was determined as described by Makkar and Goodchild [23]. About 0.2 g of finely grounded sample was weighed into a 50 mL sample bottle. 10 mL of 70% aqueous acetone was added and properly covered. The bottles were inside an ice-bath shaker and it was shaken for 2 h at 30°C. Each solution was then centrifuged and the supernatant was stored at a very low temperature of -4°C. 0.2 mL of each solution was pipetted into the test tube and 0.8 mL of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/mL of the stock and the solutions made up to 1 mL with distilled water. 0.5 mL of Folin-Ciocalteu reagent was added to both sample and standard followed by 2.5 mL of 20% Na_2CO_3. The solutions were then vortexed and allowed to incubate for 40 min at room temperature, the absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic curve was prepared.

Determination of phytate mineral molar ratio:

The moles of phytate and minerals were obtained by dividing the weight of phytate with minerals of each mineral molecular weight as described by Oloniyo [24]. The molar proportion of the mineral phytate was obtained by dividing the moles of phytate by the moles of each mineral as shown in the following mathematical expression:

$$Molar\ ratio\ (Mr) = \frac{P\,a/M\,wPa}{Mi\,n/M\,win} \quad (1)$$

Equation (1) was used to calculate the molar ratio.

Where:

Pa= Calculated phytate content

Mwpa= Molecular weight of Pa = 660

Min= Mineral content obtained for each mineral (i.e Zn, Ca, and Fe)

Mwin= mineral molecular weight (Zn = 65, Fe = 56 & Ca = 40 g/mol).

Some antioxidant properties of defatted and un-defatted cashew kernel nut flour:

Determination of total phenol content: The total phenol content of germinated-toasted and fermented-toasted pigeon pea flour was determined as suggested by Pereira [25]. Briefly, appropriate dilutions of the extracts were oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm using a Visible Spectrophotometer (Model 721 Visible Spectrophotometer, Axiom Mediral LMD, UK). The total phenol content of the sample was calculated as mg Gallic Acid Equivalent (GAE)/g.

Determination of total flavonoid content: The total flavonoid content of germinated–toasted and fermented–toasted pigeon pea flour was determined using the Folin Ciocalteu reagent as described by Singleton [26]. About 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 µL 10% AlCl3, 50 µL 1M potassium acetate, and 1.4 mL water, and incubated at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm using a Visible Spectrophotometer (Model 721 Visible Spectrophotometer); the total flavonoid content was then calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content and its content was calculated and recorded as mg Quercetin Equivalents per gram (QE*)/*g.

Determination of ferric reducing antioxidant property (FRAP): FRAP of germinated–toasted and fermented-toasted pigeon pea flour was determined as described by Oyaizu [27]. About 2.5 mL aliquot was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min and 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a Visible Spectrophotometer (Model 721 Visible Spectrophotometer), and ferric reducing antioxidant property was subsequently calculated and recorded mmol/100g.

Determination of 1,1-diphenyl–2 picrylhydrazyl (DPPH) free radical scavenging ability: The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Gyamfi [28]. Briefly, appropriate dilution of the extracts (1 mL) was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Determination of Fe^{2+} Chelation assay: The Fe^{2+} chelating ability of both extracts were determined using a modified method of Minotti, & Aust, [29]. Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL 0.1 M Tris–HCl at pH 7.4, 218 µL saline and the extracts (0–25 µL). The reaction mixture was incubated for 15 min at 37°C, thereafter, 13 µL of 0.25% Otho-phenanthroline (w/v) was added. The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Iron (II) chelating ability was subsequently calculated.

Statistical analysis

The data were generated in triplicates; it was analyzed using Statistical Package for Social Sciences (SPSS) Version 21. The significant differences between the means were separated using Duncan Multiple Range Test (DMRT) at 95% confidence level (P<0.05). The proximate and mineral values were also compared with the international permissible limits of the United States Department of Agriculture USDA [24,30].

Results and discussions

Proximate composition and energy value of raw, germinated–toasted and fermented–toasted pigeon pea flour

The proximate composition (%) and energy value (kcal/100g) of raw, germinated-toasted, and fermented-toasted pigeon pea flour are presented in Table 1. The moisture content of pigeon pea flour ranged between 10.34 and 11.80% (Table 1). Sample C (fermented-toasted pigeon pea flour) had the lowest moisture content at 10.34%, while sample A (raw pigeon pea flour) had the highest moisture content at 11.80%. There was a significant difference in moisture content. The low moisture content observed in sample C (fermented-toasted pigeon pea flour) could be attributed to the fermentation process to which the pigeon pea was subjected. This result agrees with the finding of Adebowale [32] who reported that the moisture content of fermented pigeon pea flour ranged between 10.20 and 13.24%. The United States Department of Agriculture [30] recommends a moisture content between 1 and 15% as a safe moisture content for long storage of leguminous products. Moisture content is an indicator of longer shelf life. Moisture content less than 14% is recommended for long storage periods, so there is good potential during storage [32].

The ash content of pigeon pea flour ranged between 2.42% and 2.62% (Table 1). Sample A (raw pigeon pea flour) had the lowest ash content with 2.42%, while sample C (fermented-toasted pigeon pea flour) had the highest ash content at 2.62%. The values obtained in this study were lower compared to greater than three percentages (>3%) recommended by the United States Department of Agriculture [30]. The result of this study agrees with the report of Anthony [33]. That the ash content for different processing methods ranged between 1.69 and 5.19%. Ash content is a measure of the mineral and other inorganic matter content in biomass that remains after combustion or complete acid-facilitated oxidation of organic matter in foods and is used to determine the authenticity and purity of samples. [34].

The crude fiber content of pigeon pea flour ranged between 3.73% and 4.22% (Table 1). Sample C (fermented-toasted pigeon pea flour) had the lowest crude fiber content at 3.73%, while the highest crude fiber was observed in sample A (raw pigeon pea flour) at 4.22%. The low crude fiber content observed in sample C (fermented-toasted pigeon pea flour) could be due to hydrolysis or enzymatic degradation during the fermentation process to which it was subjected. The result of this study agrees with the report of Anthony [33] that crude fiber for different processing methods ranged from 1.30 to 5.31%. Crude fiber is the residue of a plant food that helps stimulate digestion and also promotes the production of intestinal bacteria [35].

The fat content of pigeon pea flour ranged between 1.23% and 1.87% (Table 1). sample C (fermented-toasted pigeon pea flour) had the lowest fat content at 1.23%, while sample A (raw pigeon pea flour) had the highest fat content at 1.87%. The fat values obtained in this study were lower than the 10-15% recommended by the United States Department of Agriculture [30]. This could be because pigeon pea is not an oily seed. Fat is present in foods and provides energy, it helps to protect organs, promotes cell growth, keeps cholesterol and blood pressure under control, and helps the body absorb vital nutrients [36].

The crude protein of pigeon pea flour ranged between 27.78% and 36.27% (Table 1). Sample A (raw pigeon pea flour) had the lowest crude protein content at 27.78% while sample C (fermented-toasted pigeon pea flour) with 36.27% had the highest protein content. The protein values obtained in this research agree with the value (between 15 and 28%) recommended by the United States Department of Agriculture [30]. The high crude protein content observed in sample C(fermented-toasted pigeon pea flour) could be attributed to the microbial activities occurring during the fermentation process that could have increased the amino acid content of the pigeon pea flour. The protein value obtained in these studies was higher than the values (between 23.0 and 24.9%) obtained by Akubor [12] for different pigeon pea seeds using different processing methods. Protein is the measure of nitrogen in a food that promotes the growth and development of the body [37].

The carbohydrate content of pigeon pea flour ranged between 45.82% and 51.94% (Table 1). sample C (fermented-toasted pigeon pea flour) had the lowest carbohydrate value at 45.82%, while sample A had the highest carbohydrate content at 51.94%. The low carbohydrate content observed in sample C (fermented-toasted pigeon pea flour) could be due to the decomposition of sugar during fermentation or germination by microbes. The carbohydrate values obtained in this study were lower compared to the findings of Anthony [33] that the carbohydrate content of different processing methods ranged from 55.88 to 60.09%. Carbohydrates are sugar molecules that provide the body with glucose that is converted into energy used to support bodily functions and physical activity [38].

The energy value of pigeon pea flour ranged between 335.71 and 339.43 kcal/100g (Table 1). Sample A (raw pigeon pea flour) had the lowest energy value while sample C (fermented-toasted pigeon pea flour) had the highest value with 339.43 kcal/100g. The values obtained were lower compared to the findings of Akubor [12] for different pigeon pea seed processing methods that ranged between 367.8 and 372.4 kcal/100g.

Table 1: Proximate composition (%) and energy value (kcal/100g) of raw, germinated–toasted and fermented-toasted pigeon pea flour.

Sample	Moisture content	Crude Ash	Crude Fibre	Crude Fat	Crude Protein	Carbohydrate	Energy value
A	11.80±1.0[a]	2.42±1.0[c]	4.22±1.0[a]	1.87±1.0[a]	27.78±1.0[c]	51.94±1.0[a]	335.71
B	11.58±1.0[b]	2.53±1.0[b]	3.95±1.0[b]	1.65±1.0[b]	30.81±1.0[b]	49.49±1.0[b]	336.05
C	10.34±1.0[c]	2.62±1.0[a]	3.73±1.0[c]	1.23±1.0[c]	36.27±1.0[a]	45.82±1.0[c]	339.43
Standard	<15	>3	<3	10-15	15-28	>64	>344

Values represent mean ± SD. Means with the same superscript are not significantly different from each other (P>0.05).
A- Raw pigeon pea seed flour
B- Germinated-toasted pigeon pea seed flour
C- Fermented-toasted pigeon pea seed flour

Mineral composition of raw germinated–toasted and fermented–toasted pigeon pea flour

The mineral composition of the germinated–toasted and fermented–toasted pigeon pea flour is presented in Table 2. The potassium (K) content of the pigeon pea flour varied from 611.01 to 617.40 mg/kg (Table 2); sample A (raw pigeon pea flour) had the lowest potassium content at 611.01 mg/kg while sample B (germinated-toasted pigeon pea flour) had the highest potassium content at 617.40 mg/kg. There is a significant difference in the potassium content of different processed pigeon pea flours. The result of this study is consistent with the report of Mbaeyi and Onweluzo [39] that the potassium content of a sprouted and pregelatinized pigeon pea sorghum mixture ranged from 104.0 to 660.0 mg/kg. Potassium is known as an electrolyte because it carries a small electrical charge that activates various cellular and nervous functions. It is an essential mineral needed to help maintain normal fluid levels in cells, regulate heartbeat, and ensure the proper functioning of muscles and nerves [40].

The Magnesium (Mg) content ranged between 143.47 and 146.10 mg/kg (Table 2); sample A (raw pigeon pea flour) had the lowest value of 143.47 mg/kg while sample C (fermented-toasted pigeon pea flour) had the highest value of 146.10 mg/kg. There were significant differences in the magnesium contents of the different processed pigeon peas. The obtained result is consistent with Akubor [12], who reported that the magnesium content of different pigeon pea processing methods ranged between 132 and 148 mg/kg. Magnesium is an essential macronutrient that plays a key role in many bodily processes, including the health and mood of muscles, nerves, and bones [41].

The Calcium (Ca) content ranged between 138.13 and 171.40 mg/kg (Table 2). Sample A (raw pigeon pea flour) had the lowest value of 138.13 mg/kg while sample C (fermented-toasted pigeon pea flour) had the highest value of 171.40 mg/kg. It was observed that the value obtained in this study was higher compared to the findings of Akubor [12], who reported that the calcium value of different processing methods ranged between 92 and 112 mg/kg. The increase observed in calcium content could be attributed to the degradation of anti-nutrients such as phytates and oxalates which bound the minerals during the fermentation process. Calcium is the most abundant mineral in the body and is associated with healthy bones and teeth, which helps circulate blood, move muscles, and release hormones and also helps transport messages from the brain to other parts of the body [42].

The iron (Fe) content ranged between 18.43 and 23.40 mg/kg (Table 2). Sample A (raw pigeon pea flour) had the lowest value while sample C (fermented-toasted pigeon pea flour) had the highest value. Significant differences were found in the iron contents of the different processed pigeon peas. The greater variation observed in this study with the pigeon pea flour produced shows that fermented-toasted pigeon pea is a rich source of iron compared to others. This result is not in agreement with the findings of Akubor [12] for different pigeon pea seed processing methods which ranged from 3.0 to 5.5 mg/kg. Iron is an important component of hemoglobin, a type of protein in red blood cells that transports oxygen from the lungs to all parts of the body, necessary for growth and development [43].

The Phosphorus (P) content ranged between 404.53 and 423.17 mg/kg (Table 2). Sample A had the lowest value while sample C (fermented-toasted pigeon pea flour) had the highest value. There were no significant differences between samples B and C (germinated-toasted and fermented - toasted pigeon pea flour) but there was a significant difference between sample A (raw pigeon pea flour) in the phosphorus contents of the different processed pigeon peas. The variation in this result shows that both sample B and C are a good source of phosphorus. The result of this study is consistent with the report of Mbaeyi and Onweluzo [39] that the potassium content for germination and pre-gelatinization effect of sorghum and pigeon pea mixtures ranged from 108.1 to 959.9 mg/kg. Phosphorus is an essential mineral naturally present in many foods and available as a dietary supplement necessary for the growth, maintenance, and repair of all tissues and cells and also for the production of genetic components [44].

The Zinc (Zn) content ranged between 2.67 and 3.17 mg/kg (Table 2). Sample A (raw pigeon pea flour) had the lowest value while sample C (fermented-toasted pigeon pea flour) had the highest value. Significant differences were found in the zinc contents of the different processed pigeon peas. The variation observed in this study shows that fermented-toasted pigeon pea flour is a good source of zinc compared to others. This result is consistent with the findings of Akubor [12] for different pigeon pea seed processing methods that ranged between 2.0 and 4.0 mg/kg. Zinc is a trace mineral that supports immune function and can help treat diarrhea, promote wound healing and growth [45].

Table 2: Mineral Composition (mg/kg) of germinated–toasted and fermented-toasted pigeon pea flour.

Sample	K	Mg	Ca	Fe	P	Zn
A	611.01±1.00[c]	143.47±1.00[c]	138.13±1.00[c]	18.43±1.00[c]	404.53±1.00[b]	2.67±1.00[c]
B	617.40±1.00[a]	145.13±1.00[b]	157.47±1.00[b]	20.07±1.00[b]	418.33±0.08[a]	2.98±1.00[b]
C	613.57±1.00[b]	146.10±1.00[a]	171.40±1.00[a]	23.40±1.00[a]	423.17±0.08[a]	3.17±1.00[a]

Values represent mean ± SD. Means with the same superscript are not significantly different from each other (P>0.05).
Key
A- Raw pigeon pea seed flour
B- Germinated-toasted pigeon pea seed flour
C- Fermented-toasted pigeon pea seed flour
K – Potassium
Mg – Magnesium
Ca – Calcium
Fe – Iron
P – Phosphorus
Zn – Zinc

Antinutritional properties and molar ratios of germinated–toasted and fermented–toasted pigeon pea flour

The antinutritional properties and molar proportions of germinated–toasted and fermented–toasted pigeon pea flour is presented in Table 3. The phytate content of pigeon pea flour ranged between 14.48 and 17.58 mg/100g (Table 3). Sample C (fermented-toasted pigeon pea flour) had the lowest value at 14.48 mg/100g, while sample A (raw pigeon pea flour) had the highest value at 17.58 mg/100g. There were significant differences between the different processing methods. The variation in the low phytate content in sample C (fermented-toasted pigeon pea flour) could be due to microbial activities during fermentation. The values obtained in this study is higher compared to the findings of Anthony [33] that the phytate content for different processing methods ranged from 0.13 to 2.55 mg/100g. Phytate or phytic acid is the main storage form of phosphorus in many plant tissues, especially bran and seeds, which help the body calcify and form kidney stones and reduce blood glucose and lipids. It forms complexes with metals or proteins therefore reduce their bioavailability in the gastrointestinal tract [46].

The oxalate content of pigeon pea flour ranged between 12.78 and 17.24 mg/100g (Table 3). Sample C (fermented-toasted pigeon pea flour) had the lowest value with 12.78 mg/100g and sample A (raw pigeon pea flour) had the highest value with 17.24 mg/100g. There were significant differences between the different processing methods. The variation observed in this study could be attributed to fermentation that reduces the antinutritional factors of pigeon pea flour. This result is consistent with the findings of Akusu [47], who reported that the oxalate content of raw and processed sesame seed flour ranged from 1.54 to 24.74 mg/100g. Oxalates are not so essential for our body, so they bind to other waste products from food and reach the kidneys to be excreted. Oxalates often bind to minerals such as calcium and are excreted from the body through urine and feces [48]. Oxalate is a potent dietary inhibitor of calcium absorption because it interferes with absorption of calcium depending on the calcium-oxalate ratio in the food that is, the source of the oxalic acid.

The tannin content of pigeon pea flour ranged between 23.79 and 28.42 mg/100g (Table 3). The lowest value was obtained from sample C (fermented-toasted pigeon pea flour) with 23.79 mg/100g while the highest value was obtained from sample A (raw pigeon pea flour) with 28.42 mg/100g. There were significant differences between the different processing methods. The variation observed in this study could be attributed to roasting because roasting reduces the antinutrient in pigeon pea flour. This result is higher compared to the findings of Anthony [33], who reported that the tannin content for different processing methods ranged from 0.42 to 0.91 mg/100g. Tannins are one of the essential physicochemicals that help accelerate blood coagulation, reduce blood pressure, lower serum lipid levels, produce liver necrosis, and modulate immune responses [49].

The saponin content of pigeon pea flour ranged between 12.25 and 19.72 mg/100g (Table 3), sample C (fermented–toasted pigeon pea flour) had the lowest value of 12.25 mg/100g while sample A had the highest value of 19.72 mg/100g. There were significant differences between the different processing methods. This result is consistent with the findings of Okoye [50] that the saponin content of different processing methods ranged from 1.09 to 20.43 mg/100g. Saponin lowers blood lipids, reduces cancer risks, and reduces blood glucose response; Saponins affect the immune system in ways that help protect the human body against cancer and also reduce cholesterol levels [51].

The trypsin inhibitor content of pigeon pea flour ranged between 1.48 and 3.33 mg/100g (Table 3). The lowest value was observed in sample C (fermented–toasted pigeon pea flour) with 1.48 mg/100g, while the highest value was observed in sample A (raw pigeon pea flour) with 3.33 mg/100g. There were significant differences between the different samples. The result of this study is consistent with the findings of Akubor [12], who reported that the trypsin inhibitory effect of different pigeon pea flour processing methods ranged from 0.08 to 4.5 mg/100g. Trypsin is an enzyme involved in the breakdown of many different proteins, mainly as part of digestion in humans and other animals such as monogastric and young ruminants [52].

It was observed that phytate: zinc (Phy:Zn) ranged between 0.45 and 0.65 (Table 3), phytate: iron (Phy:Fe) ranged between 0.05 and 0.08 (Table 3) and phytate: calcium (Phy:Ca) was 0.01 (Table 3). Researchers have previously reported that the molar ratios of a food sample are considered safe when phytate: calcium >1.56, phytate: iron >14, and phytate: zinc >10 [24,53]. This implies that the bioavailability of Ca, Fe and Zn are not inhibited by the phytate concentration in the processed pigeon pea flour examined. This was an indication that all the minerals (Ca, Fe, and Zn) in the processed pigeon pea flour would be adequately absorbed by the body when consumed. The molar ratio between phytate and divalent cations (Ca, Fe, and Zn) indicates the impact of phytate on the bioavailability (the ability of the body to absorb and digest minerals in food after consumption) of dietary minerals and the absorption of these cations was not negatively affected by the amount of phytate in the processed pigeon pea flour examined [54].

Table 3: Anti-nutrient Properties (mg/100g) and molar ratios of germinated–toasted and fermented–toasted pigeon pea flour.

Sample	Phytate	Oxalate	Tannins	Saponin	Trypsin Inhibitor	Phy:Zn	Phy: Fe	Phy: Ca
A	17.58±1.0[a]	17.24±1.0[a]	28.42±1.0[a]	19.72±1.0[a]	3.33±1.0[a]	0.65	0.08	0.01
B	16.81± 1.0[b]	16.35±1.0[b]	27.14±1.0[b]	16.52±1.0[b]	2.22±1.0[b]	0.56	0.07	0.01
C	14.48±1.0[c]	12.78±1.0[c]	23.79±1.0[c]	12.25±1.0[c]	1.48±1.0[c]	0.45	0.05	0.01
Standard	<450	<50				>10	>14	>1.56

Values represent mean ± SD. Means with the same superscript are not significantly different from each other (P>0.05)
A - Raw pigeon pea seed flour
B -Germinated-toasted pigeon pea seed flour
C- Fermented-toasted pigeon pea seed flour
Phytate: Zinc (Phy: Zn)
Phytate: Iron (Phy: Fe)
Phytate: Calcium (Phy: Ca)

Antioxidant Properties of germinated-toasted and fermented-toasted pigeon pea flour

The antioxidant compositions of germinated–toasted and fermented–toasted pigeon pea flour is shown in Figure 2. The FRAP content of pigeon pea flour ranged between 14.70 and 33.20 mmol/100g as shown in Figure 2. Sample A (raw pigeon pea flour) had the lowest value with 14.70 mmol/100g while sample C (fermented-toasted pigeon pea flour) had the highest value of FRAP content with 33.20 mmol/100g. The result in the table shows that there was no significant difference (P<0.05) in the reduction rate of both sample A and sample B, while sample C had the highest significant value. The high FRAP composition of sample C (fermented-toasted pigeon pea flour) can be attributed to the fermentation activity. The result of this study was higher compared to the findings of Ukom [9] who reported that the FRAP content of sprouted white and sprouted brown pigeon peas was 2.51 and 2.26 mmol/100g respectively.

Total phenol ranged between 98.4 and 132.6 mg GAE/g as shown in Figure 2. Sample B (germinated-toasted pigeon pea flour) had the lowest value with 98.4 mg GAE/g while sample A (raw pigeon pea flour) had the highest value. value with 132.6 mg GAE/g. There were significant differences in all samples. The result of this study agrees with the findings of Ukom [9] who reported that the total phenol of sprouted white and sprouted brown pigeon peas are 93.58 and 97.15 mg GAE/g respectively. Phenol derivatives have been found to possess antimicrobial, analgesic, anti-inflammatory, antioxidant, anticonvulsant, anticancer, anesthetic, antiseptic and disinfectant, antituberculosis, and antiparkinsonian activities [9].

The total flavonoid ranged between 13.5 and 42.1 mg QE/g as shown in Figure 2. Sample C (fermented–toasted pigeon pea flour) had the lowest flavonoid value with 13.5 mg QE/ g, while sample A (raw pigeon pea flour) had the highest value of flavonoids with 42.1 mg QE/g. the highest flavonoid with 42.1 mg QE/g. The lowest value of flavonoid obtained in sample C might be attributed to the toasting condition (120°C for 30 min) to which the pigeon pea was subjected to after the fermentation process. There were significant differences in all samples. The result of this study was higher compared to the findings of Ukom [9] who reported that the total flavonoid of sprouted white and sprouted brown pigeon peas was 0.44 to 0.55 mg QE/g respectively. Flavonoids are also powerful antioxidant agents that help regulate cellular activity and fight free radicals that cause oxidative stress in the body [56].

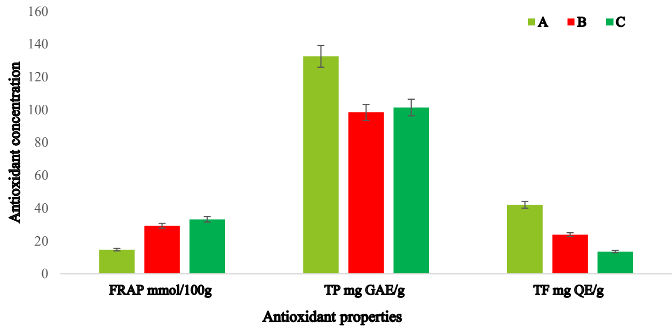

Figure 2: Antioxidant properties of germinated-toasted and fermented-toasted pigeon pea flour.

A - Raw pigeon pea seed flour
B -Germinated-toasted pigeon pea seed flour
C- Fermented-toasted pigeon pea seed flour
FRAP - Ferric Reducing Antioxidant Power
TP- Total phenol
TF -Total flavonoid

Inhibitory activities of germinated–toasted and fermented–toasted pigeon pea flour

The alpha-amylase and alpha glycosidase of germinated–toasted and fermented–toasted pigeon pea flour is shown in Figure 3. The alpha-amylase ranged between 75.57 and 84.44 µg/mL as shown in Figure 3; the lowest value was observed in sample A (raw pigeon pea flour) with 75.57 µg/mL while the highest value was observed in sample C (fermented–toasted pigeon pea flour) with 84.44 µg/mL. All samples showed significant differences. Alpha amylase helps break down carbohydrates so they are easily absorbed by the body. This is why it is often recommended to chew food thoroughly before swallowing, as amylase enzymes in saliva help break down carbohydrates to facilitate digestion and absorption [58].

Alpha- glycosidase ranged from 33.22 to 48.48 µg/mL, as shown in Figure 3; Sample A (raw pigeon pea flour) had the lowest value with 33.22 µg/mL, while sample C (fermented – toasted pigeon pea flour) had the highest value with 48.48 µg/mL. There were significant differences in all samples. Acid alpha-glucosidase is the enzyme responsible for the degradation of glycogen polymers to glucose in the acidic environment of lysosomes. α-Glucosidases play key roles in clinical detection, disease prevention, and treatment, body metabolic mechanism and alcohol fermentation, and sugar hydrolysis, widely used in chemical fields such as chemical synthesis [57].

Alpha (α)-amylase and alpha (α) -glucosidase are enzymes that digest carbohydrates and increase the postprandial glucose (blood glucose test that determines the amount of glucose in the plasma after a meal) level in the body. Inhibiting the activity of these two enzymes can help to control postprandial hyperglycemia thereby reducing the risk of developing diabetes [58].

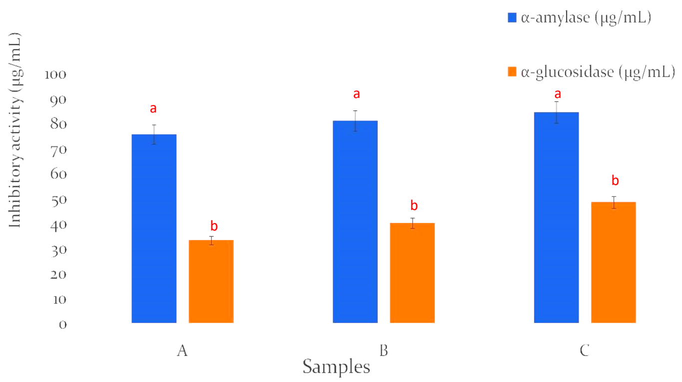

Figure 3: Inhibitory Activities of Germinated-toasted and Fermented-toasted Pigeon Pea Flour.

A- Raw pigeon pea seed flour
B- Germinated-toasted pigeon pea seed flour
C- Fermented-toasted pigeon pea seed flour

The Inhibitory concentration - Ic_{50} of germinated – toasted and fermented – toasted pigeon pea flour

The inhibitory concentration - Ic_{50} of germinated–toasted and fermented–toasted pigeon pea flour was presented in Table 4. Iron chelation ranged between 0.3 and 0.4 mg/g (Table 4). Sample A (raw pigeon pea flour) had the lowest value with 0.3 mg/g while sample C (fermented–toasted pigeon pea flour) had the highest value with 0.4 mg/g. There were significant differences in all samples. Iron chelating agents reduce iron overload in different organs, such as the liver and heart, and prevent complications, such as endocrinological, hepatic, and cardiac dysfunctions [59].

The concentration of DPPH ranged between 0.11 and 0.25 mg/g (Table 4). Sample C (fermented–toasted pigeon pea flour) had the lowest value at 0.11 mg/g while sample B (germinated –toasted pigeon pea flour) had the highest value at 0.25 mg/g. The concentration of pigeon pea flour required for a 50% inhibition of cell growth is normally measured in molar concentrations and is used as a measure of antagonist potency; the lower the Ic_{50} values, the greater the antioxidant [60].

Conclusion

It was observed that fermented–toasted pigeon pea flour compared to germinated-toasted pigeon pea flour has reduced anti-nutritional properties in terms of phytate, oxalate, tannin, saponin, and trypsin inhibitors. It has a high content of proteins, ash, and some minerals (magnesium, phosphorus, calcium, iron, and zinc values, but low in potassium). It is high in FRAP but germinated-toasted pigeon pea flour is rich in potassium. Therefore, the fermented-toasted process of pigeon pea flour is considered the best processing technique among the three (3) processing methods considered because the process helps to enhance and retain some nutritional health benefits in the flour for a better bioavailability of micronutrients when consumed. Furthermore, in this study it was concluded that processing reduces the antioxidant and antinutritional properties of processed pigeon pea flour compared to the raw sample.

Table 4: The inhibitory concentration - IC50 (mg/g) of germinated–toasted and fermented-toasted pigeon pea flour.

SAMPLE	Fe^{2+} chelation	DPPH
A	0.03	0.20
B	0.04	0.25
C	0.04	0.11

A - Raw pigeon pea seed flour
B -Germinated-toasted pigeon pea seed flour
C- Fermented-toasted pigeon pea seed flour
DPPH – 1,1 – Diphenyl – 2 Picryhydrazyl
Fe^{2+} - Iron II

Author Statements

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Rebecca Olajumoke Oloniyo, Abimbola Beatrice Adeko and Wisdom Otaru Oniwon conceived and designed the research, analyzed the data and wrote the paper. Favour Ojonugwa Caleb performed the experiments. Rebecca Olajumoke Oloniyo, Isah Laisi Rashidi and Netalla Jibril supervised the research. All of the authors have read and approved the final manuscript. This manuscript has not been submitted to, nor is under review at another journal or other publishing venue.

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