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Combined Effects of Different Processing Methods on Vitamins and Antinutrients Contents of Pigeon Pea (Cajanus Cajan)Flour

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Abstract *Combined effects of different processing methods on the vitamins and anti-nutrients contents of pigeon pea were investigated. The pigeon pea seeds were soaked (S_oPPF) i.e., control, soaked and boiled (S_oBPPF), soaked and fermented (S_oFPPF), soaked, boiled and fermented (S_oBFPPF), soaked and sprouted (S_oS_pPPF), soaked, sprouted and boiled (S_oS_pBPPF), soaked, sprouted and fermented (S_oS_pFPPF) and soaked, sprouted, boiled and fermented (S_oS_pBFPPF). The vitamins analyzed significantly ($p<0.05$) increased for sprouted and fermented samples and increased tremendously for combined sprouted/fermented samples with values for vitamins A 0.038 – 0.077ug/100g, C 2.515 – 13.820mg/100g, $B_1$0.730 – 1.165mg/100g, $B_2$0.145 – 0.665mg/100g and $B_3$2.920 – 6.510mg/100g respectively. The boiled processes recorded decreased vitamin contents. The results equally showed that the oligosaccharides (raffinose and stachyose) contents were reduced by the processing methods with the combined sprouting/fermentation processes recording the highest reduction. The anti-nutrients analyzed were saponins, phytic acids, oxalates, alkaloids, cyanides, trypsin inhibitors, haemagglutinins and tannins and they were reduced by all the processing methods used with the combined sprouting/fermentation (S_oS_pBFPPF and S_oS_pFPPF) effecting the highest reduction of the anti-nutrients.*
Keywords: *Combined effects, different processing methods, vitamins, oligosaccharides, anti-nutrients.*

I. Introduction

Legume is a term applied broadly to all plants of pea and bean family, botanically referred to as leguminosae or fabaceae (Elegbede, 1998). Legumes are known to be the third largest family among the flowering plants consisting of approximately 650 genera and 20,000 species (Doyle, 1994).Pigeon pea (*Cajanus cajan*) is a perennial legume grown for food. It is cultivated at subsistence level in Nigeria. Pigeon pea is commonly called fio-fio in Anambra State (Igbo speaking) part of Nigeria (Enwere, 1998). Vitamins are organic substances that can be found in plants or chemically synthesized in animals, which are required in trace amounts for health, growth and reproduction. When ingested by animals, most of them as the active vitamins or pro-vitamins are modified into co-enzymes that act in concert with enzymes to catalyze biochemical reactions (Zubay, 1993). The scientific evidence supporting the important role of vitamins in promoting health and preventing non-communicable diseases, independent of other nutritional constituents, has been stressed (Blunberg, 1995). Vitamins essential for human are classified into fat-soluble (Vitamins A, D, E and K), and water-soluble (Vitamins of the B group and vitamin C).

Anti-nutritional factors in food legumes are chemical substances present in products, although non-toxic but generate adverse physiological responses in animals or humans that consume them. In most cases, anti-nutritional factors interfere with the utilization of nutrients in legume products (Nwokolo, 1996) and their presence could cause flatulence. Anti-nutritional factors such as proteases (trypsin and chymotrypsin) inhibitors, amylase inhibitors and polyphenols which are a known problem in most legumes are less problematic in pigeon pea than soybean, peas and field beans (Farris and Singh, 1990). Within pigeon pea cultivars, anti-nutritional factors are mainly found among dark-seeded genotypes (Farris and Singh, 1990) that are typically grown in Asia. The native African pigeon pea types are largely cream or white seeded with relatively less anti-nutritional factors.

The anti-nutritional factors found in pigeon pea include trypsin inhibitors, chymotrypsin inhibitors, amylase inhibitors, hemagglutinins (lectins), tannins (polyphenols), saponins, cyanide, phytic acid, oxalate (Farris and Singh, 1990; Nwosu., 2013). Trypsin and chymotrysin inhibitors affect the digestibility of legume proteins while other anti-nutritional factors like tannins, phytates, cyanide and hemagglutinins impart bitter or unacceptable taste to the legumes, causing decreased protein digestibility and absorption of divalent metal ions such as Fe^{2+}, Zn^{2+} in the intestine (Abdu *et al.*,2008). These anti-nutritional factors can be removed by different processing methods like cooking, soaking, sprouting, fermentation, etc. (Adeparasi, 1994).

Some researchers (Henry and Massey, 2001; Oluwole and Taiwo, 2009) have studied the effects of processing methods like soaking, boiling, fermentation, sprouting or germination or maltingon the toxic factors and anti-

nutrients in legumes. But information on combined effects of different processing methods on the vitamins and anti-nutrients composition of pigeon pea has not been reported hence this study.

II. Materials And Methods

MATERIALS COLLECTIION

The pigeon pea (*Cajanus cajan*) seeds for this research were purchased from a market (Ahia ohuu) in Aba, Abia State. The chemicals used were obtained from Food Science and Technology laboratory, Imo State University, Owerri and National Root Crops Research Institute, Umudike, Umuahia.

SAMPLE PREPARATION

Sixteen kilograms of pigeon pea seeds were sorted to remove dirt and other foreign particles after which they were washed. The grains were then soaked in water for 3 hours using a large container and the water drained.

Production of Soaked pigeon pea flour (control): Two (2) kilograms of the soaked grains were dehulled and dried in an oven at 60˚C for 7 hours. The dried seeds were milled into flour using disc attrition mill (Asiko All, Addis Nigeria). The flour was then sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

Production of Soaked and Boiled pigeon pea flour: Two (2) kilograms of the soaked grains were dehulled and boiled in water for 1hour at 100˚C. The water was drained and the seeds dried in an oven at 60˚C for 7 hours. The dried seeds were milled into flour using disc attrition mill (Asiko All, Addis Nigeria). The flour was then sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

Production of Soaked and Fermented pigeon pea flour: Two (2) kilograms of the soaked grains were dehulled, crushed, wrapped in plantain leaves and allowed to ferment for 4 days. After fermentation, the grains were dried in an oven at 60˚ C for 7 hours. The fermented dried cotyledons were milled into flour with disc attrition mill (Asiko All, Addis Nigeria) and standard 1.0mm mesh sieved before packaging in polyethylene bag for further studies.

Production of Soaked, Boiled and Fermented pigeon pea flour: Two (2) kilograms of the soaked grains were boiled with water for 1 hour and the water drained. The grains were wrapped in plantain leaves and allowed to ferment for 4 days as described by Ikemefuna (1998). After fermentation, the seeds were dehulled and dried in an oven at 60C for 7 hours. The dried seeds were milled into flour using disc attrition mill (Asiko All, Addis Nigeria). The legume flour were sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

Production of Soaked and Sprouted pigeon pea flour: Sprouting was carried out according to the method described by Ariahu *et al.* (1999). Eight (8) kilograms of the soaked grains were spread in a single layer on a moistened jute bag and allowed to germinate (sprout) at room temperature for 3 days. During this time, the grains were sprayed with water at intervals of 12 hours until the last day of sprouting. After sprouting, the seeds were dehulled and rootlets removed. Then the cotyledons were divided into four portions of 2kg each. Then the portion (2kg) for the production of soaked and sprouted pigeon pea flour were dried in an oven at 60˚C for 7 hours and milled into flour using a disc attrition mill (Asiko All, Addis Nigeria) and 1.0 mm mesh sieved before packaging into polyethylene bag for further studies.

Production of Soaked, Sprouted and Boiled Pigeon Pea Flour: Two (2) kilograms of the sprouted pigeon pea seeds were boiled for 1 hour with water at 100˚C, drained and dried in an oven at 60˚ C for 7 hours. The dried sprouted boiled cotyledons were milled with disc attrition mill (Asiko All, Addis Nigeria), sieved with standard sieve (1.0mm mesh) and packaged in polyethylene bag for further studies.

Production of Soaked, Sprouted and Fermented Pigeon Pea Flour: Two (2) kilograms of the sprouted dehulled grains were wrapped in plantain leaves and allowed to ferment for 4 days as described by Ikemefuna (1998). After fermentation, the cotyledons were dried in an oven at 60C for 7 hours and milled into flour with disc attrition mill (Asiko A11, Addis Nigeria) and standard 1.0mm mesh sieved before packaging in polyethylene bag for further studies.

Production of Soaked, Sprouted, Boiled and Fermented Pigeon Pea Flour: Two (2) kilograms of the sprouted dehulled grains were boiled in water for 1 hour at 100˚ C and the water drained. The sprouted, dehulled and boiled cotyledons were wrapped in plantain leaves and allowed to ferment for 4 days as described by Ikemefuna (1998). After fermentation, the cotyledons were dried in an oven at 60˚ C for 7 hours. The dried sprouted-boiled-fermented grains were milled into flour with disc attrition mill (Asiko All, Addis Nigeria) and standard 1.0mm mesh sieved before packaging in polyethylene bag for further studies.

DETERMINATION OF ANTI-NUTRITIONAL FACTORS.

Tannins: This was determined by Folin Denis colometric method. Five grams (5g) of each sample was put inside a volumetric flask and 50 ml of distilled water dispensed inside the volumetric flask. The mixture was shaken for 30 minutes at room temperature and filtered to obtain the extract. Two millilitres (2ml) of standard tannic acid solution and equal volume of distilled water was dispensed into a separate 50 ml volumetric flask to

serve as a standard and reagent blank respectively. Then 2 ml of each sample extract was put in their respective labelled flasks. The content of each flask was mixed with 35 ml distilled water and 1 ml of the Folin Denis reagent was added to each. This was followed by 2.5 ml of saturated Na_2CO_3 solution. Then each flask was diluted to the 50ml mark with distilled water and incubated for 90 min at room temperature. Their absorbances were measured at 760mm in a spectrophotometer (Atomic Absorption Spectrophotometer – AAS Model SP9) with the reagent blank at zero.

The tannin content was calculated as:

% Tannin = $\frac{100}{W}\ x\ \frac{au}{as} x\ c\ x\ \frac{vt}{va}$

Where: W = weight of sample, au = absorbance of test sample, as = absorbance of standard tannin solution, c = concentration of standard tannin solution, vt = total volume of extract, va = volume of extract analysed

Saponins: This was done by the double solvent extraction gravimetric method (Harborne, 1973). Five (5) grams of each sample was mixed with 50 ml of 20% aqueous ethanol solution and incubated for 12 hours at 55°C with constant agitation. After that, the mixture was filtered through Whatman No. 42 grades of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 min and the extracts combined and weighed together. The combined extract was reduced to about 40 ml by evaporation and then transferred to a separating funnel and equal volume (40 ml) of diethyl ether was added to it. After mixing well, there was a partition and the upper layer of the partition was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH reduced to 4.5 with drop-wise addition of dilute NaOH solution. Saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of normal butanol. The combine extracts was washed with 5% NaCl solution and evaporated to dryness in a previously weighed evaporating dish. The saponin was then dried in the oven at 60°C (to remove any residual solvent), cooled in a desiccator and reweighed. The saponin was determined and calculated as percentage of the original samples.

% Saponin = $\frac{W2-W1}{W} x\ 100$

Where: W = weight of sample used

W_1 = weight of empty evaporating dish

W_2 = weight of dish + saponin extract

Alkaloids: The alkaline precipitation gravimetric method (Harborne, 1973) was used. Five (5) grams of each sample was dispersed in 100ml of 10% acetic acid in ethanol solution. The mixture was well shaken and allowed to stand for 4 hours at room temperature and shaken every 30 minutes. At the end of this period, the mixture was filtered through Whatman No. 42 filter paper. The filtrate (extract) was concentrated by evaporation to a quarter of its original volume. The extract was treated with drop-wise addition of concentrated NH_3 solution to precipitate the alkaloid. The dilution was done until the NH_3 was in excess. The alkaloid precipitate was removed by filtration using weighed Whatman No. 42 filter paper. The paper was dried at 60°C and re-weighed after cooling in a desiccator. The weight of alkaloid was determined and expressed as a percentage of the sample.

% Alkaloid = $\frac{W2-W1}{Weight\ of\ sample} x\ 100$

Where: W_1 = weight of empty filter paper

W_2 = weight of filter paper + alkaloid precipitate

Cyanide: The cyanide contents of the samples were determined using the method described by Bradbury *et al.* (1985). Five (5) grams of each sample was dissolved in 50ml distilled water in a corked conical flask to extract cyanide. The cyanide extraction was allowed to run overnight. The extract was filtered through a filter paper. Alkaline picrate solution was prepared by dissolving 1g of picric acid and 5g of sodium carbonate in warm water in a volumetric flask and the volume made up to 200ml with distilled water. To 1ml of the sample filtrate was added 4ml alkaline picrate and this was incubated in water bath for 5 minutes for colour development. After the development of the reddish brown colour, the absorbance of the solution was read at 490nm on a spectrophotometer. The absorbance of a blank containing 1ml distilled water and 4ml alkaline picrate solution was also read. Standard cyanide solution was prepared from different concentrations of potassium cyanide solution containing 5 to 50 µg cyanide in a 500ml conical flask, 25ml of 1N HCl was added. The experiment was carried out in triplicates. Cyanide was expressed as mg per kilogram of the sample.

HCN (mg/kg) = $1000 \times 0.05 \times W \times \frac{au}{as}$

Where: W = weight of sample

au = absorbance of the test sample

as = absorbance of standard solution

Phytate: Phytate content was determined using the method described by Haugh and Lantzsch (1993). Two (2) grams of each sample was extracted with 20ml of 2% HCl for 3 hours. To 1ml of the extract was added 1ml of 0.3% ferric ammonium sulphate solution in a test tube and stoppered. The mixture was then boiled for 30 min in

water bath. The tube was cooled in ice for 15 min and allowed to adjust to room temperature. The contents of the test tubes were then centrifuged at 3000rpm for 30 min. One millilitre (1.0ml) of the supernatant was mixed with 1.5ml of 0.02M 2, 2 dipyridine solution and the absorbance was read at 519nm against a blank (distilled water) in a spectrophotometer (Atomic Absorption Spectrophotometer –AAS Model SP9). Phytate will be expressed in percentage and calculated as shown below.

% Phytate = $\frac{au}{as} x \frac{C}{W} x \frac{Vf}{Va} x \frac{100}{1}$

Where: au = Absorbance of test sample, as = Absorbance of standard solution, C = Concentration of standard solution, W = Weight of sample used, Vf = Total volume of extract, Va = Volume of extract used

Oxalate: This was carried out according to AOAC (2005). Two (2) grams of each sample was extracted thrice at 50°C, stirred for 1 hour with 20ml of 0.3N HCl.The combined extract was diluted (made up) to 100ml with distilled water and used for total oxalate estimation. The oxalate was estimated by pipetting about 5ml of the extract which was made alkaline with 1ml of 5N ammonium hydroxide. About 3 drops of phenolphthalein was added to the extract and acetic acid was added in drops. Also about 1ml of 5% aqueous calcium chloride was then added to the mixture and allowed to stand for 2 hours after which it was centrifuged at 3000 rpm for 15 minutes. The supernatants were discarded and the precipitates washed three times with hot water, thoroughly mixed and centrifuged each time. In the test tube, 2ml of 3N H_2SO_4 was added and the precipitate was dissolved by warming in water bath at 75^{o}C. The content of the test tube was then titrated with freshly prepared 0.01N $KMnO_4$ at room temperature until the first pink colour appeared throughout the solution. This was then warmed at 75°C and the titration continued until the pink colour persisted. This experiment was carried out in triplicates.

% Oxalate = $\frac{Vt}{W} x$ *Vme x Titre x 100*

Where: Vt = total volume of titrate = 100

Ws = weight of the sample = 2g

Vme = volume – mass equivalent (i.e.1cm^3 of 0.05M $kMn0_4$ is equivalent to 0.00225g anhydrous oxalic acid)

Haemagglutinin: This was determined using the spectrophotometric method of Arntfield *et al*. (1985). Half of a gram of each sample was mixed with phosphate buffer solution (50ml) and filtered to obtain the extract used in the analysis. An aliquot (1ml) of the extract from each sample was added to a test tube containing 9ml of suspended trypsinated rabbit red blood cells. A control test tube contained the suspended cells but without extract. The tubes were allowed to stand at room temperature for 10mins before their respective absorbances were measured in a spectrophotometer (Atomic Absorption Spectrophotometer – AAS Model SP9) of 510nm wavelength. The experiment was carried out in triplicates. The amount of agglutinin in the test sample was expressed as the number of units of agglutinin observed per gram of the test sample.

HUI/g = $\frac{1}{W} x (au - as) x \frac{Vf}{Va}$

Where: HUI/g = Haemagglutinin units per gram, W = Weight of sample used, au = Absorbance of test sample, as = Absorbance of standard sample,Vf = Total extract volume, Va = Volume of extract used

Trypsin Inhibitor: This was done using the spectrophotometric method described by Armtfield *et al.* (1985). Five (5) grams of each sample was dispersed in 50ml of 0.5M NaCl solution and stirred for 30 minutes at room temperature. It was centrifuged and the supernatant filtered through Whatman No. 42 filter paper. The filtrate was used for the assay. Standard trypsin wasprepared and used to treat the substrate solution (N-benzol-D1-arginine-p-anilide, BAPA). The extent of inhibition was used as a standard for measuring the trypsin. In the tube containing2ml of extract, 10ml of the substrate (BAPA) was added. Also, the second part of the standard trypsin solution was added in another test tube containing only 10ml of the substrate. The latter served as the blank. The content of the tubes were allowed to stand for 30 minutes and then the absorbances of the solution were measured spectrophotometrically (Atomic Absorption Spectrophotometer – AAS Model SP9) at 410nm wavelength. One trypsin activity unit inhibited is given by an increase on 0.01 absorbance unit at 410nm. The experiment was carried out in triplicates.

Trypsin unit inhibited/100g = $\frac{au}{as}$ *x 0.01 x F*

Where: au = Absorbance of test sample

as = Absorbance of standard sample

F = Experimental factor is given as $\frac{Vf}{Va} x \frac{1}{w}$

Where: Vf = Total Volume of extract

Va = Volume extract analyzed

W = Weight of sample analyzed

VITAMIN ANALYSIS

The vitamin (Vitamin A, Vitamin C (Ascorbic acid, Vitamin B_1 (Thiamine), Vitamin B_2 (Riboflavin), Vitamin B_3 (Niacin)) contents of the samples was carried out by the method as described by AOAC (2005)

III. Results And Discussion

Vitamin Contents of Pigeon Pea Flour Samples Processed By Different Methods

The results of the vitamins contents of the pigeon pea flour samples are shown in Table 1. The vitamin A contents ranged from 0.011 – 0.077ug/100g. There were significant differences ($p<0.05$) in the vitamin A contents of the pigeon pea flours, with the sprouted/fermented sample (S_oS_pFPPF) having the highest vitamin A content (0.077 µg/100g) and this was significantly higher than those of samples S_oS_pPPF (0.051 µg/100g) and S_oFPPF (0.045ug/100g) at 5% level of significance. The lowest vitamin A content (0.011 µg/100g) was recorded for sample S_oBPPF and this value was significantly ($p<0.05$) lower than those of the control (S_oPPF) and the other boiled samples.

The result showed that sprouting, fermentation and combined sprouting/fermentation processes led to increase in the vitamin A content of the pigeon pea flours. This increase in might be due to synthesis of vitamin A by increased activities of hydrolytic enzymes and fermenting microorganisms. Boiling of the pigeon pea before conversion to flour decreased the vitamin A content of the flour. This decrease may be due to leaching of vitamin A into the boiling water. The result is in agreement with the report of Walters *et al*. (1993) and Fadahunsi (2009) who stated that boiling leads to decrease in vitamins while fermentation leads to increase in vitamin of bambara groundnut. Shah *et al*. (2011) reported that sprouting increases vitamin A content of legumes. Olanipekun *et al.* (2015) equally observed that boiling reduced the vitamin content of kidney bean flour.

There were significant differences ($p<0.05$) in the vitamin C (ascorbic acid) contents of the pigeon pea flour samples. The vitamin C contents of the flour samples ranged from 0.945 – 13.82mg/100g. The highest vitamin C value (13.820mg/100g) was observed in sample S_oS_pFPPF, followed by sample S_oS_pPPF (12.820 mg/100g) and S_oFPPF (8.630 mg/100g). The lowest vitamin C content (0.945 mg/100g) was recorded in sample S_oBPPF followed by the control sample (S_oPPF) and the other boiled samples. This increase could be due to synthesis of vitamin C by the hydrolytic activities of enzymes and activities of microorganisms during sprouting and fermentation respectively. And the decrease may be due to leaching of the vitamin into the boiling water. Fadahunsi (2009) reported that fermentation led to increase of vitamin in bambara groundnut. Shah et al. (2011) observed that sprouting increased vitamin C (antioxidant vitamin) of legumes. Uherova et al. (1993) reported that boiling caused a high loss of ascorbic acid in vegetables. Sangronis and Machado (2005) stated that germination

Table 3: Vitamin Contents of Pigeon Pea Flour Samples Processed By Different Methods

Flour sample	Vitamin A µg/100g	Vitamin C (Ascorbic acid) mg/100g	Vitamin B_1 (Thiamine) mg/100g	Vitamin B_2 (Riboflavin) mg/100g	VitaminB_3 (Niacin) mg/100g
S_oPPF	0.038 ± 0.001^{e}	2.515 ± 0.02^{g}	0.730 ± 0.01^{d}	0.145 ± 0.01^{d}	2.920 ± 0.03^{d}
S_oBPPF	0.011 ± 0.001^{h}	0.945 ± 0.02^{h}	0.270 ± 0.01^{h}	0.045 ± 0.04^{f}	1.615 ± 0.02^{h}
S_oFPPF	0.045 ± 0.001^{c}	8.630 ± 0.04^{c}	0.920 ± 0.01^{c}	0.505 ± 0.02^{c}	4.915 ± 0.03^{c}
S_oBFPPF	0.022 ± 0.001^{g}	3.115 ± 0.02^{f}	0.335 ± 0.02^{g}	0.115 ± 0.02^{e}	2.325 ± 0.04^{g}
S_oS_pPPF	0.051 ± 0.002^{b}	12.820 ± 0.03^{b}	0.965 ± 0.02^{b}	0.565 ± 0.01^{b}	5.795 ± 0.02^{b}
S_oS_pBPPF	0.031 ± 0.0^{f}	4.710 ± 0.01^{e}	0.380 ± 0.0^{f}	0.135 ± 0.02^{de}	2.510 ± 0.01^{f}
S_oS_pFPPF	0.077 ± 0.002^{a}	13.820 ± 0.03^{a}	1.165 ± 0.02^{a}	0.665 ± 0.02^{a}	6.510 ± 0.01^{a}
S_oS_pBFPPF	0.043 ± 0.002^{d}	4.820 ± 0.03^{d}	0.420 ± 0.03^{e}	0.135 ± 0.02^{de}	2.640 ± 0.01^{e}
LSD ($p<0.05$)	0.0013	0.027	0.019	0.0245	0.023

Values are means $\pm$ Standard deviations from the means. Means with different letter within a column are significantly different ($p<0.05$). LSD= Least significant difference

Key:

S_oPPF = soaked pigeon pea flour (control)

S_oBPPF = soaked and boiled pigeon pea flour

S_oFPPF = soaked and fermented pigeon pea flour

S_oBFPPF = soaked, boiled and fermented pigeon pea flour

S_oS_pPPF = soaked and sprouted pigeon pea flour

S_oS_pBPPF = soaked, sprouted and boiled pigeon pea flour

S_oS_pFPPF= soaked, sprouted and fermented pigeon pea flour

S_oS_pBFPPF = soaked, sprouted, boiled and fermented pigeon pea flour

Increased ascorbic acid of pigeon pea by 208.4%. Satya et al. (2010) explained that during sprouting process, biosynthesis of vitamin C takes place. Vitamin C (Ascorbic acid) is an antioxidant vitamin which helps to scavenge free radicals in the cells. Vitamin C is very vital in iron metabolism and subsequent fight against iron deficiency anaemia (Manoranja and Sood, 2010). Olanipekun *et al*. (2011) also reported that vitamins A and C being antioxidants help to fight diseases like cancer and diabetes and also help to slow down the natural

aging process. Therefore combined use of sprouting/fermentation in the processing of pigeon pea flour raised the vitamin C content of the flour from 2.515 to 13.820mg/100g which is 449.50% increase.

There were significant differences ($p<0.05$) in the vitamin B_1 (thiamine) contents of the pigeon pea flours and these ranged from 0.270 – 1.165mg/100g. The highest vitamin B_1 value (1.165 mg/100g) was recorded in sample S_oS_pFPPF followed by samples S_oS_pPPF (0.965 mg/100g) and S_oFPPF (0.920 mg/100g) and these were significantly different ($p<0.05$) from one another in vitamin B_1 content. The control sample recorded vitamin B_1 value of 0.730 mg/100g, while the lowest value (0.270mg/100g) was observed in sample S_oBPPF. There were significant differences ($p<0.05$) in vitamin B_1 content of samples S_oS_pBFPPF (0.420 mg/100g), S_oS_pBPPF (0.380 mg/100g), S_oBFPPF (0.335 mg/100g) and S_oBPPF (0.270 mg/100g). This result is in agreement with the report of Shah *et al*. (2011) who stated that vitamin B_1 (thiamine) is increased by sprouting.
There were significant differences ($p<0.05$) in the vitamin B_2 (riboflavin) contents of pigeon pea flour samples. The highest vitamin B_2 content (0.665 mg/100g) was recorded in sample S_oS_pFPPF followed by samples S_oS_pPPF (0.565 mg/100g) and S_oFPPF (0.505 mg/100g) and these were significantly different ($p<0.05$) from each other. The control sample had a vitamin B_2 value of 0.145 mg/100g. The least vitamin B_2 content (0.045 mg/100g) was observed in sample S_oBPPF and this was also significantly different ($p<0.05$) from samples S_oBFPPF (0.045mg/100g), S_oS_pBPPF (0.135mg/100g) and S_oS_pBFPPF (0.135mg/100g).Uherova *et al.* (1993) maintained that conventional cooking caused a high loss of thiamine, riboflavin and ascorbic acid in vegetables as they are highly soluble in water. Riboflavin (vitamin B_2) plays a critical role in the body energy production.

There were significant differences ($p<0.05$) in the vitamin B_3 (niacin) contents of pigeon pea flour samples. The highest vitamin B_3 content (6.510 mg/100g) was recorded in sample S_oS_pFPPF followed by sample S_oS_pPPF (5.795 mg/100g) and S_oFPPF (4.915 mg/100g). The lowest vitamin B_3 content (1.615 mg/100g) was observed in sample S_oBPPF. The control sample had a vitamin B_3 content of 2.920 mg/100g. There were significant differences ($p<0.05$) in the vitamin B_3 contents of samples S_oBFPPF (2.325 mg/100g), S_oS_pBPPF (2.510 mg/100g) and S_oS_pBFPPF (2.640 mg/100g).The results are in agreement with the reports that boiling leads to decrease in the vitamins while fermentation leads to increase in vitamin of Bambara groundnut (Walters *et al.*, 1993; Fadahunsi, 2009). Amany *et al.* (2014) reported that germination of chickpea caused an increase in vitamin B_3 content at the level of 36.50%. Niacin (vitamin B_3) (nicotinic acid and nicotinamide) facilitates lipid catabolism and plays a key role in tricarboxylic acid cycle.

Anti-nutrients Content of Pigeon Pea Flour Samples Processed By Different Methods

The anti-nutrient contents of the pigeon pea flour samples processed with different methods are shown in Table 2. The saponin contents of the pigeon pea flour samples were reduced by each of the processing methods (boiling, fermentation, sprouting) and were reduced too much lower levels by the combination of boiling, sprouting and fermentation processes (Table 2). The control sample (S_oPPF) had the highest saponin value (2.97mg/100g) followed by the soaked/boiled sample, S_oBPPF which hada value of 2.15mg/100g. There were significant differences ($p<0.05$) among the saponin contents of almost all the flour samples with the exception of the soaked/fermented, the soaked/sprouted, the soaked/boiled/fermented and soaked/sprouted/boiled pigeon pea flour samples which had similar values. The reduction in saponin due to sprouting would probably have been caused by enzymatic hydrolysis while that due to fermentation was due to microbial degradation. And that due to boiling would have been caused by leaching of the saponins into the boiling water.Processing techniques such as boiling, sprouting and fermentation had been noted to reduce anti-nutritional contents of legume flours (Trugo *et al.* 1990; Henry and Massey, 2001; Edema and Sanni, 2006).

There were also significant differences ($p<0.05$) in the phytic acid contents of the pigeon pea floursamples with the exception of the samples S_oBFPPF (1.10mg/100g), S_oS_pBPPF (1.04mg/100g), S_oFPPF (1.90mg/100g) and S_oS_pPPF (1.82mg/100g) which had no significant differences ($p<0.05$). The control sample (SoPPF) had the highest value of phytic acid (5.76mg/100g). Boiling of the soaked pigeon pea reduced the phytic acid content of the flour by more than 50% (2.76mg/100g). The combined soaking, sprouting, boiling and fermentation processes reduced the phytic acid to the lowest value of 0.22mg/100g, and this value was significantly ($p<0.05$) lower than the phytic acid content (0.53mg/100g) obtained for the soaked/sprouted/fermented flour sample (S_oS_pFPPF).Boiling also contributed in the reduction due to degradation of the phytic acid by the heat of the boiling water. Reduction in phytic acid has also been reported by Adeniran *et al*. (2013) in lima bean seeds and locust bean seeds after fermentation. Esonu *et al.* (1998) and Egli *et al.* (2002) also noted that phytic acid was hydrolysed during sprouting of legume seeds. Reduction of phytic acid during sprouting and fermentation could be due to enzymatic action which increased the level of phytase to cause a decrease in phytic acid.

Table 2: Anti- nutrients Content of Pigeon Pea Flour Samples Processed By Different Methods

Flour sample	Saponin mg/100g	Phytic acid mg/100g	Oxalate mg/100g	Alkaloids mg/100g	Cyanide mg/100g	Trypsin Inhibitor TIU/100g	Haemagglutinin mg/100g	Tannin mg/100g
S_oPPF	2.97±0.02^a	5.76±0.02^a	3.44±0.02^a	6.97±0.02^a	2.30±0.01^a	10.27±0.02^a	8.22±0.03^a	1.03±0.02^a
S_oBPPF	2.15±0.02^b	2.79±0.02^b	1.98±0.01^b	2.92±0.03^b	0.93±0.01^b	4.77±0.04^b	3.10±0.01^b	0.83±0.01^b
S_oFPPF	1.17±0.01^c	1.90±0.01^c	1.22±0.01^d	1.92±0.01^d	0.32±0.03^c	3.59±0.03^c	2.41±0.01^c	0.74±0.02^d
S_oBFPPF	0.82±0.01^d	1.10±0.01^d	0.87±0.01^e	1.26±0.02^e	0.10±0.01^e	1.27±0.01^e	1.13±0.03^f	0.34±0.01^f
S_oS_pPPF	1.14±0.02^c	1.82±0.01^c	1.53±0.02^c	2.09±0.02^c	0.17±0.01^d	2.08±0.02^d	2.11±0.02^d	0.78±0.01^c
S_oS_pBPPF	0.85±0.0^d	1.04±0.01^d	0.92±0.02^e	0.92±0.01^f	0.08±0.01^f	1.10±0.01^f	1.25±0.0^e	0.41±0.01^e
S_oS_pFPPF	0.58±0.02^e	0.53±0.0^e	0.22±0.02^f	0.22±0.01^g	0.03±0.002^g	0.67±0.02^g	0.92±0.01^g	0.30±0.01^g
S_oS_pBFPPF	0.39±0.01^f	0.22±0.02^f	0.04±0.002^g	0.05±0.0^h	0.01±0.001^h	0.14±0.0^h	0.45±0.03^h	0.17±0.0^h
LSD ($p<0.05$)	0.173	0.164	0.017	0.018	0.015	0.023	0.021	0.015

Values are means. ± Standard deviations from the means. Means with different letter within a column are significantly different ($P<0.05$). LSD= Least significant difference

Key:S_oPPF = soaked pigeon pea flour (control), S_oBPPF= soaked and boiled pigeon pea flour, S_oFPPF = soaked and fermented pigeon pea flour, S_oBFPPF = soaked, boiled and fermented pigeon pea flour, S_oS_pPPF = soaked and sprouted pigeon pea flour, S_oS_pBPPF = soaked, sprouted and boiled pigeon pea flour, S_oS_pFPPF = soaked, sprouted and fermented pigeon pea flour, S_oS_pBFPPF = soaked, sprouted, boiled and fermented pigeon pea flour

There were significant differences ($p<0.05$) in the oxalate contents of the pigeon pea flour samples with the exception of the samples S_oBFPPF (0.87mg/100g) and S_oS_pBPPF (0.92mg/100g) where there was no significant difference ($p<0.05$). The highest oxalate content (3.44mg/100g) was recorded in the control sample (S_oPPF) followed by sample S_oBPPF witha value of 1.98mg/100g. Samples S_oFPPF and S_oS_pPPF had oxalate contents of 1.22mg/100g and 1.53mg/100g respectively. The lowest oxalate content (0.04mg/100g) was recorded in the flour sample that was soaked/sprouted/boiled and fermented (S_oS_pBFPPF) and the second lowest oxalate value (0.22mg/100g) was recorded in the flour sample S_oS_pFPPF.This result is in agreement with the report of El-Moneim *et al.* (2012) who stated that soaking, boiling, sprouting and fermentation reduced levels of oxalate in sorghum. This result corroborates with the findings of Henry and Massey (2001) and Nwosu *et al.* (2013) who indicated a decrease in oxalate content of pigeon pea seed as a result of malting treatment.

There were significant differences ($p<0.05$) in the alkaloid contents of the pigeon pea flour samples. The control sample (S_oPPF) had the highest alkaloid content (6.97mg/100g) followed by sample S_oBPPF (1.98mg/100g). Sample S_oFPPF had an alkaloid value of 1.92mg/100g, which was significantly different ($p<0.05$) from those of samples S_oBFPPF (1.26mg/100g) and S_oS_pPPF (2.09mg/100g). The reduction of alkaloids due to boiling, sprouting and fermentation had been reported respectively by Farris and Singh (1990), Livernais-Saettel (2002) and Edema and Sanni (2006). Udedibie and Nwaiwu (1988) had earlier reported that cooking for 60 minutes eliminated alkaloid in jack beans

There were significant differences ($p<0.05$) in the cyanide contents of some of the pigeon pea flour samples. The highest cyanide content (2.30mg/100g) was recorded in the control sample (S_oPPF) and that was significantly higher ($p<0.05$) than the values of samples S_oBPPF (0.93mg/100g), S_oFPPF (0.32mg/100g), S_oS_pPPF (0.17mg/100g) and the other samples.It has been reported that boiling (Farris and Singh, 1990; Carlini and Udedibie, 1997; Balogun *et al.*, 2001), sprouting (Henry and Massey, 2001; Livernaise-Seattel, 2002) and fermentation (Barimalaa *et al.*, 1994; Edema and Sanni, 2006) processes reduce cyanide content of legumes.

There were significant differences ($p<0.05$) in the trypsin inhibitor contents of the pigeon pea floursamples. The control flour sample (S_oPPF) had the highest trypsin inhibitor content (10.27TIU/100g) followed by the soaked/boiled flour sample(4.77TIU/100g) and soaked/fermented flour sample (3.59TIU/100g). The reduction was highest in the flour sample which was processed by the combined processing methods (sprouting/boiling/fermentation, S_oS_pBFPPF) (0.14TIU/100g), followed by the soaked/sprouted/fermented flour sample, S_oS_pFPPF (0.67TIU/100g). The reduction in the levels of trypsin inhibitor are in agreement with the results of other workers who reported that boiling (Farris and Singh, 1990; Carlini and Udedibie, 1997; Balogun *et al.*, 2001), sprouting (Khan *et al.*, 1991; Henry and Massey, 2001) and fermentation (Edema and Sanni, 2006; El-Moneim *et al.*, 2012) are able to decrease trypsin inhibitor activity.

There were significant differences ($p<0.05$) in the haemagglutinin contents of the pigeon pea flour samples. The highest haemagglutinin content (8.22mg/100g) was recorded for the control sample (S_oPPF), followed by sample S_oBPPF (3.10mg/100g) and sample S_oFPPF (2.41mg/100g). The lowest haemagglutinin content (0.45mg/100g) was recorded for sample S_oS_pBFPPF followed by sample S_oS_pFPPF (0.92mg/100g) and sample S_oS_pBPPF (1.25mg/100g). There were significant differences ($p<0.05$) in the tannin contents of all the samples of pigeon pea flourstudied. The highest tannin content (1.03mg/100g) was recorded for the control flour

sample (S_oPPF) and that was significantly higher ($p<0.05$) than that of sample S_oBPPF (0.83mg/100g). The decrease in tannin content is in agreement with the findings of Obizoba and Atti (1991) and Adeparasi (1994).

IV. Conclusion

The increments in vitamins have shown the quality that can be obtained from combined sprouted/fermented pigeon pea flour. This was shown in the increased vitamin A, C, B_1, B_2, and B_3. The decreased anti-nutrients by combined sprouting/fermentation processing method might have contributed to the increments in vitamins. It is therefore recommended as the best method for eliminating anti-nutritional factors in pigeon pea.

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