Original Article

COMPARATIVE STUDY ON THE PHYSICO-CHEMICAL PROPERTIES OF PIGEON PEA (Cajanus cajan) FLOUR AND PROTEIN ISOLATE

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Received 10 August 2012; accepted 05 October 2012

Abstract
Pigeon Pea seeds (Cajanus cajan) were produced into three different flour samples; full fat, defatted and protein isolate. They were subjected to proximate analysis and determination of some functional properties. Proximate composition was carried out on moisture content, protein content, fat content, carbohydrate, ash content and crude fibre content. The proximate analysis (%) showed that protein isolate had the lowest moisture content of 6.63 ± 0.025 compared to full fat and defatted flour having 6.85 ± 0.012 and 6.76 ± 0.016 respectively. Also, the result showed that protein isolate had the highest protein content of 90.65 ± 0.005% than that of full fat, and defatted flour having 24.02 ± 0.016%, and 26.30 ± 0.016%. The fat, carbohydrate, ash and fibre content (% for full fat, defatted and protein isolate were (2.017 ± 0.062, 0.113 ± 0.0094, 0.00 ± 0.00), (62.23 ± 0.029, 62.22 ± 0.016, 0.2 ± 0.016) (3.14 ± 0.016, 3.05 ± 0.033, 2.52 ± 0.016) and (1.24 ± 0.016, 1.56 ± 0.015, 0.00 ± 0.00) respectively. Generally, the result of the protein isolate showed no value for fibre and fat content and very low value for carbohydrate. The presence of fibre in full fat flour and defatted flour helps in the functioning of gastrointestinal tract and aid in digestion of food. Finally, the proximate composition of three samples showed that they are good sources of protein, fat and carbohydrate with functional properties that are favourable to human consumption and useful application in food systems.

INTRODUCTION
Pigeon pea (Cajanus cajan) is among the dry leguminous cultivated for food in Nigeria. It is also an important food legume in African and south – East Asia (Muhammed et al; 1979). Leguminous seeds are important source of proteins, energy and other nutrients in the diets of large population groups around the world, forming and excellent source of lysine, methionine and tryptophan and other water – soluble vitamins (riboflavin, niacin and folacin) and of the minerals phosphorus, iron and magnesium. (Ramcharan and Walker, 1985).

These seeds, therefore, have high food value and are second to cereals as a source of human and animal food leguminous seeds are especially significant in that they provide supplementary proteins to diet based on cereal grains such as rice, and starchy food e.g plantain and cassava (Doughty and Walker, 1982). Green pods of legumes such as winged bean and lima bean and some varieties of pigeon pea are used as vegetables (Nandanie, 2003). The high content of varieties of legumes make them important sources of protein in the diets of population groups of many tropical countries (Kon, 1979, Ekpeyoung and Borchers, 1980).

The common legumes cultivated in Nigeria include soybean, bambara groundnuts, Africa yam bean and pigeon pea. A mature grain legume seed has three major components; the seed coat (testa or hull), the cotyledons and the embryo axis (Okaka, 1997). Pigeon pea seeds have a growing season of 6 to 9 months and are either harvested dry and used mainly in dahl soap, or harvested earlier and eaten as a green vegetable. It is used as a food crop (dried peas, flour or green vegetable peas) and also as forage / cover crop. In combination with cereals, pigeon pea known as “Fiolio” in Eastern part of Nigeria, make a well balanced human food. It is an annual crop reaching 3 to 12ft (1 to 4 meters) in height. The leaves have three leaflets that are green and pubescent above and silvery grayish green with silvery grayish green with longer hairs on the underside. The flowers are yellow with red to reddish – brown lines or a red outside. Pigeon pea seed-lings
emerge 2-3 weeks after sowing. Vegetative growth begins slowly but accelerates at 2-3 months. Pigeon pea roots are thing with a taproot reaching up to 6ft (2m) in dept. This deep rooting system helps to improve water infiltration into the soil. Pigeon pea is not cultivated in large quantities because the use of this bean variety is limited. The objective of the study therefore was to determine the proximate and functional properties of full fat, defatted and protein isolate from pigeon pea so as to be able to ascertain its useful application in food systems.

MATERIAL AND METHOD

SOURCE OF MATERIAL

Pigeon pea seeds in this work were obtained from Akwata, a local market in Enugu, Enugu state, Nigeria. Laboratory and other facilities used in the practical were sourced from central laboratory service unit of National Root Crops Research Institute (NRCRI), Umudike, Umuahia Abia State.

EQUIPMENT

Equipment and instrument used in this study included the satorious digital weighing balance, cabolite electric oven, excello soxhlet apparatus, excello Kjeldahl apparatus, gallen lamp electric muffle furnance, colab fume cupboard, electric water bath, colab electric centrifuge, Author Thomas laboratory glass wares pH meter, thermometer, retort stand, and stop watch.

CHEMICAL REAGENTS

The chemicals and reagents used in the project practical were of analytical grade (Analar) and they include hydrochloric acid, ethanol, sodium hydroxide, selenium crystals, methyl red, buric acid, bromocressol green, sulphuric acid, hexane, oil etc.

SAMPLE PREPARATION

Prior to separation of protein isolate, pigeon pea seeds were processed. The method described by Aluko and Yada (1995) was employed. First the bean seeds were sorted manually to remove extraneous materials like residue, dirt’s, stones and diseased seeds. The healthy ones were used.

PRODUCTION OF FULL FAT PIGEON PEA FLOUR

In th production of full fat pigeon pea flour dry seeds were soaked overnight (24 hours) in water at 1:5 (W/v) ratio the next day, the seeds were manually dehulled to separate the seed coats from the cotyledon, the dehulled seeds were dried in the oven at temperature of 60°C for 48 hours before they were ground with a laboratory mill. The sample was sieved through a 0.5mm sieve to obtain flour sample for analysis.

PRODUCTION OF DEFATTED PIGEON PEA FLOUR

The full fat flour sample was soaked in the solvent at 1:5 (W/v) ratio and allowed to stand overnight at room temperature. The next day, the mixture was filtered with filtration apparatus. The defatted flour was air dried for 8 hours and pulverized in a motor. Parts of the defatted flour were set aside for analysis while the rest were used for the production of protein isolate.

PRODUCTION OF PROTEIN ISOLATE

The protein isolation was done following the method described by (Pomeranz, 1991). 70g of flour with 1400 ml of water to form a 1:20 (W/v) ratio of slurry. The solution at Fig1: Flow diagram for the production of dehulled fat pigeon pea flour.

6.37pH was allowed to settle for 3 hours. The spent residue was separated from the dissolved protein extract by decanting after which centrifugation took place. The pH of the extracted protein was adjusted with HCl to its iso-electric point between 4.0-4.3 the precipitate formed was subsequently removed by centrifugation at room temperature by removing the whey which contain soluble sugars, residual protein, peptides, salt, minor constituents. The resulted curd (protein isolated) was then dried under air using desiccators before Fig 2: Flow diagram for commercial processing of defatted pigeon pea flour.
grinding and sieving took place

**METHODS**

**PHYSICAL SEED CHARACTERISTICS**

The characteristics were determined following the procedure of Fashaken and Fasanya (1988) the raw seeds were randomly selected and then examined by subjective methods for shape, testa texture, seed colour, eye colour and testa attachment to the cotyledon, the degree of attachment was described as smooth or rough depending on how the seeds appear to the eye.

**SEED WEIGHT**

Weight of 100 seeds randomly selected was determined by weighing (AOAC, 1984). The average seed weight was calculated.

**PROXIMATE COMPOSITION**

The procedures for the chemical analysis for the moisture, crude protein, ash, crude fiber, carbohydrate and fat contents were as outlined by the Association of official analytical chemists (AOAC, 1984). The analysis was carried out in both full fat, defatted and protein isolate from pigeon pea seeds and results obtained in triplicates.

**DETERMINATION OF MOISTURE CONTENTS**

The gravimetric method was used as described by (James, 1995). A measured weight of the samples (full fat, defatted and protein isolate) was weighted into a previously weighed moisture can. It was cooled in a desiccator and weighed. It was returned to the oven for further drying. Drying, washing and weighing were done respectively hourly (1 hour) interval until there were no further diminution in the weight (i.e constant weight was obtained).

The weight of moisture lost was calculated and expressed as a percentage of the weight of sample analyzed. It was given by the expression below:

\[
\% \text{ moisture} = \frac{w_2 - w_3}{w_3 - w_1} \times 100 \\
\]

Where \( w = \) weight of empty moisture can \\
\( w_2 = \) weight of empty can \( \times \) sample before drying \\
\( w_3 = \) weight of can \( \times \) sample dried to constant weight.

**DETERMINATION OF PROTEIN**

This was done by Kjeldahl method described by (Muhammed et al; 1979). The total N\(_2\) was determined and multiplied with factor 6.25 to obtain the protein content. 1 gram of sample was mixed with 10mls of concentrated H\(_2\)SO\(_4\) in a digestion flask. A tablet of selenium catalyst was added to tit before it was heated under a fume cupboard until a clear solution was obtained (i.e the digest). The digest was diluted to 100mls in a volumetric flask and used for analysis. Then 10mls of the digest was mixed with equal volume of 45% NaOH solution in a Kjeldahl distillation apparatus. The mixture was distilled into 10ml of 4% buric acid containing 3 drops of mixed indicator 9bromocressol green and methyl red). A total of 50mls of distillate was collected and titrated against 0.02N EDTA from green to a deep red end point. A reagent lank was also digested, distilled and titrated. The N\(_2\) content and the protein content were calculated using the formula below.

\[
\% \text{ protein} = \frac{(100/N \times N \times 14 \times vt) \times 7-B}{100} \\
\]

Where

\( W = \) weight of sample (1g) \\
\( N = \) Normality of tritrunt (0.02N.H\(_2\)SO\(_4\)) \\
\( Vt = \) total digest volume (100mls) \\
\( V = \) Volume of digest analyzed (10ml) \\
\( Y = \) sample of titre value \\
\( B = \) Blank titre value

**ASH DETERMINATION**

This was done by the furnaces incineration gravimetric method (James, 1995). 5g of the processed samples was measured into a previously weighed porcelain crucible. The sample was burnt to ashes in the muffle furnace at 550\(^\circ\)c for 5 hours. When it has become completely ashed, it was cooled in the desiccators and weighed. The weight of ash obtained was calculated by difference and expressed as a percentage of the weight of sample analyzed as showed below:

\[
\% \text{ Ash} = \frac{w_3 - w_2 \times 100}{W_1} \\
\]

Where \( W_1 = \) weight of empty crucible \\
\( W_2 = \) weight of crucible \( \times \) Ash

**DETERMINATION OF CRUDE FIBRE**

The weende method (James, 1995) was employed 2g of the processed sample was boiled in 150mls of 1.25% H\(_2\)SO\(_4\) solution for 30 minutes under some conditions. After washing in several portion of hot water, the sample was allowed to drain dry before being transferred quantitatively to a weighed crucible where it was dried in the oven at 105\(^\circ\)c to a constant weight. It was thereafter taken to a muffle furnace in which it was burnt until only ash was left of it by difference; the weight of fiber was obtained and expressed as a percentage of weight of sample analyzed. It was given by the formula below:

\[
\% \text{ crude fiber} = \frac{100(W_2 - W_1)}{W_1} \\
\]

Where \( W_2 = \) weight of crucible \( \times \) sample after boiling, washing and drying \\
\( W_1 = \) weight of crucible \( \times \) sample and ash

**DETERMINATION OF FAT**

The solvent extraction gravimetric method (Gabriel et al; 1986) was used. 1gram of the sample was wrapped in a porous paper (Whiteman filter paper) and put in a thimble. The thimble was placed in a sox let reflux flask and mounted to a water condenser. The solvent extraction gravimetric method (Gabriel et al; 1986) was used. 1gram of the sample was wrapped in a porous paper (Whiteman filter paper) and put in a thimble. The thimble was placed in a sox let reflux flask and mounted to a water condenser. The solvent extraction gravimetric method (Gabriel et al; 1986) was used. 1gram of the sample was wrapped in a porous paper (Whiteman filter paper) and put in a thimble.

**DETERMINATION OF CARBOHYDRATES**

Carbohydrate content was by difference. It was calculated using the formula below as described by (James, 1995).

\[
\% \text{ carbohydrate} = 100 - \% (\text{moisture of crude protein + ash + crude fibre + crude fat}) \\
\]

**FUNCTIONAL PROPERTIES OF SEEDS**

**PERCENT SEED COAT**

Fifty seeds were weighed and soaked in 100mls of tap water over night (soaking for a shorter period did not facilitate the removal by hand, drained on filter paper, weighed wet and
are weighed after drying at constant weight by air Oven method (AOAC, 1984). The final weight was expressed as percent of total seeds weight.

**SEED DENSITY**

Hundred seeds of pigeon pea were weighed and then transferred into a 100ml measuring cylinder containing 50ml of tap water. The seeds were allowed to soak for 10 minutes for equibration and the volume of water displaced was recorded. (Akinyele et al; 1986). The density was calculated thus:

\[
\text{Density} = \frac{\text{mass}}{\text{volume}}
\]

**FUNCTIONAL PROPERTIES OF FLOUR SAMPLES**

The functional properties of pigeon pea flour (full fat, defatted, protein isolate) were determined using the method specified by Aluko and Yada (1995), and Navansiga Rao (1982).

**BULK DENSITY**

This method of Aluko and Yada (1995) was used. 2g of the processed flour samples was measured into calibrated measuring cylinder. The bottom of the cylinder was tapped repeatedly on a pad placed on a laboratory beach. Tapping was done until there was no further diminution (reduction) in the volume occupied by the sample. The bulk density was determined as the ratio of the weight of the sample to its volume calculated as shown below:

\[
\text{Density} = \frac{\text{weight}}{\text{volume}}
\]

**SWELLING INDEX**

Swelling index was calculated using the method of (Akinyele et al; 1986). 1 gram of the processed sample was weighed and dispersed into a test tube, leveled and the height noted. Distilled water (10mls) was added and allowed to stand for 1 hour. The height was then recorded and the swelling index calculated as the ratio of the final height to the initial height.

\[
S = \frac{H_2}{H_1}
\]

Where \( S \) = Swelling index
\( H_1 \) = initial height
\( H_2 \) = final height

**WATER ABSORPTION CAPACITY**

This was determined as the height of water absorbed and held by 1g of the sample (Ezeama, 1989). 1 gram of the sample was weighed and put into a test tube. 10mls of distilled water was added to the sample and mixed well. The mixture was allowed to stand for 30 minutes at room temperature. The mixture was centrifuged at 2500pm for 30 minutes. The supernatant was decanted and measured.

Therefore WAC = \( \frac{V_1 - V_2 \times 1.0}{100} \)

**OIL ABSORPTION CAPACITY**

This was determined in the same way as water absorption capacity. However, a refined vegetable oil was used in place of water and the time allowed for absorption was longer (1 hour at room temperature as against 30 minutes for water). The oil absorption capacity was determined by difference, as the volume of oil absorbed and held by 1v gram of the sample as shown below;

\[
\text{OAC} = \frac{V_1 - V_2 \times 1.0}{100}
\]

**GELATION CAPACITY**

5g of sample was weighed into a beaker with 20ml of water and heated with gelling point the temperature at which it gels was measured using a thermometer

**EMULSION CAPACITY**

This method was done by the method described by Aluko and Yada (1995). 1gram of sample was mixed with 10ml of distilled water in a test tube and shaken for 30 seconds. 10mls of refined oil was added and shaken continuously until properly mixed. The test tube was left to stand for 30 minutes. The height of oil separated from the sample was measured. The emulsion capacity was expressed as the amount of oil emulsified and hold per gram of the sample. It was calculated as shown below.

\[
\text{Emulsion capacity} = \left( \frac{V_1 - V_2}{V} \right) \times 100
\]

Where \( V_1 \) = total volume of oil added
\( V_2 \) = Volume of oil left after formation of emulsion

**FOAMING CAPACITY**

The method of Navanyawa and Navasinga Rao (1982) was used. 1gram of sample was mixed with 10mls of distilled water and blended for 5 minutes after the resulting mixture; the height of foam was recovered after 30 secs. The foaming capacity was expressed as = percentage of foam produced after whipping. It is calculated as

\[
\text{Foaming capacity} = \left( \frac{V_2 - V_1}{V_1} \right) \times 100
\]

Where \( V_1 \) = height after whipping
\( V_2 \) = height before whipping

**WETTABLEITY**

This was determined as the time (in seconds) taken by a unit weight (1g) of the flour sample to get completely wet on the surface of water under laboratory conditions. The method used was described by Aluko and Yada (1995). About 500ml of distilled water was measured into a clean glass beaker (600ml capacity). With the aid of a retort stand, it was arranged that a clean test tube was clamped in an inverted position over the water in the beaker. The clamped was adjusted such that the distance from the mouth of the test tube to the surface of water in the beaker was exactly 10cm.

Both water in the beaker and the clean position on the test tube were marked with masking tape. Subsequently, 1g of the sample was weighted into marked test tube and its mouth covered with a thumb. It was carefully converted over the water and clamped with the retort stand at the marked spot without removing the thumb. With the stop watch set to read, the thumb was removed and the sample allowed to fall into the water surface as the stop watch was put on simultaneously. The flour sample was observed and the stop watch stopped as soon as the last samples got wet. This

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International Journal of Agriculture and Food Science 2012, 2(4): 121-126
experiment was repeated three times for each sample and the mean value was taken.

**STATISTICAL ANALYSIS**
Experimental data were analyzed using analysis of variance (ANOVA) and Duncan’s multiple range test was used to determine significantly different means.

**RESULT AND DISCUSSION**

**CHARACTERISTICS OF PIGEON PEA SEEDS**
The results of the seed characteristics of pigeon pea (pp) seeds is presented in Table 1. The seeds were white in colour, had brown eye colour, firm attachment to the cotyledons, of smooth testa. The average seed weight was 0.012 ± 0.01g.

**PROXIMATE COMPOSITION OF PIGEON PEA FLOUR**
The proximate composition of the full fat flour, defatted flour and protein isolate of pigeon pea are shown in Table 11 below. Results showed that pigeon pea contains nutrients (protein, carbohydrate, fat, fibre, water, ash) at different composition. From the Table 11 below, it showed that the protein isolate has the highest protein content of 90.65 ± 0.025% which is high than that of full fat and defatted flour of pigeon pea having 24.02 ± 0.016% and 26.30 ± 0.016% respectively. The high content of protein isolate, showed that it could be incorporated into foods like ice cream, baked products and infant foods for enrichment purposes to increase the protein content of the food. The moisture content of the protein isolate is shown to be lower compared with defatted flour, and full fat flour which indicates that it possesses a long storage capability and there will be inhibition of microbes in the flour.

The moisture content of full fat, defatted and protein isolate flour from pigeon pea are 6.85 ± 0.012%, 6.76 ± 0.016% and 6.63 ± 0.015% respectively.

The result for the fat on pigeon pea from the table was 2.017 ± 0.062% indicating that pigeon pea is not an oil seed and has low fat content compared with soybean, African yam bean. Generally, the result of the protein isolate showed no values for fibre and fats content and very low value for carbohydrate. The presence of fibre in full fat flour (1.24 ± 0.016%) and defatted flour (1.56 × 0.015%) helps in the functioning of gastrointestinal tract and aid digestion of food. Finally, the proximate composition of the three samples differ significantly.

**FUNCTIONAL PROPERTIES OF PIGEON PEA FLOURS**
The functional properties of full fat flour, defatted flour, protein isolate from pigeon pea is shown in Table 11 below.

The oil absorption capacity of full fat flour and defatted is (1.62 ± 0.016ml/g) and (3.02 ± 0.016ml/g) is lower than that recorded for protein isolate which is 3.98 ±0.016ml/g. Due to the fat, the protein isolate has high oil absorption which is suitable in baked products (Natt and Narasima, 1981) and also desirable in products like meat extenders to help maintain juiciness and improvement of Mouth feel. (Lin et al; 1974). Results of oil absorption of the three samples compared with chick pea flour (1.10ml/g) and cowpea (0.89ml/g) showed that pigeon pea flour absorption capacity, (Kinsella, 1979). Also, the water absorption of full fat flour, defatted flour and protein isolate are 1.62 ± 0.016 ml/g, 3.02 ± 0.016ml/g and 4.3 ± 0.082 ml/g respectively. Swelling index of protein isolate showed a higher value of 2.63 compared with other samples and is in agreement with the range values of 2.0 – 3.0 (Nwoji, 2005). The emulsion capacity of pigeon pea flour is attributed to the protein in the flour, since the flour sample from pigeon pea was observed to exhibit high percentage of protein, more units of the protein may migrate to the interface and absorb more oil and water (Gabriel and Elizabeth, 1986). Results showed that defatted flour has higher emulsion capacity of 90.67ml/g.

The low gelling capacity obtained in the seed flour samples could be attributed to high protein content of the flour. The result of no gelling in protein isolate agrees with reports of Ragab et al; (2004). Due to gelling property of full fat flour and defatted flour. It is reported that it could be used for food systems which require thickening and gelling. (Sathe and Salunkhe, 1981). Result also showed that protein isolate foam less (4.93 ± 0.050ml) compared with defatted and full fat flour having 6.23 ± 0.025 and 11.27 ± 0.216 respectively which is attributed to increased net changes of the protein, which hasten the hydrophobic interactions but increase the flexibility of the protein. (Aluko and Yada, 1995).

Generally, the whole results showed that the flour samples obtained from pigeon pea were highly functional depending in the product to be produced.

**CONCLUSION**
In conclusion, physiochemical and functional characteristics of full fat, defatted and protein isolate of pigeon pea were investigated. Good functionality of the protein isolate suggests that they would find good application as additives for food products such as cakes, bread, and ice cream etc.

Equally, results showed that the three samples analyzed were significantly different at P = 0.05 both in proximate and functional properties showing that the three products are highly important in food industries and in the developments of new products.

**RECOMMENDATION**
Considering the important contribution of pigeon pea in the diet of food, it is therefore recommended that more research be carried out to improve the utilization of the seeds and encourage people to consume pigeon pea due to its high nutritional content.

**REFERENCES**


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Source of support: Nil; Conflict of interest: None declared