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Nutritional Profile, Protein Quality and the Biological Value of Raw and Cooked Pigeon Pea (*Cajanus cajan*) Seeds

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Abstract

Pigeon pea (*Cajanus cajan*) of the family Fabaceae, is highly appreciated for its health benefits and nutritive value. This present study attempted to investigate the nutritional profile, protein quality and the biological value of raw and cooked pigeon pea seeds. Proximate compositions of the raw and cooked pigeon pea were determined using standard methods. The following minerals (Cr, Mg, Fe, Cu, Zn and Mn) were determined spectrophotometrically. Amino acids composition was determined using amino acid analyzer while the contents of vitamins A and C composition were determined using titrimetric method. Sixteen weaning albino rats were divided into four groups of four rats in each. Rats in group1 were fed on casein diet, group 2 were fed on basal diet, group 3 and 4 were fed on the raw and cooked pigeon pea seeds respectively for fourteen days with the weights of the rats and the amount consumed monitored daily. The protein efficiency ratio (PER) was determined and afterwards, the rats were sacrificed and digested for further analysis. The net protein utilization (NPU) and the true digestibility (TD) were determined and used to calculate the biological value.

Results of proximate analysis showed that the raw seeds contain a significantly ($p < 0.05$) higher contents of carbohydrates, ash and crude fiber; while the cooked peas contain a significantly ($p < 0.05$) higher amount of protein and lipid with no significant difference in the moisture content. There was no significant ($p < 0.05$) difference in the contents of copper, zinc, manganese and chromium in both the raw and cooked peas. However, the cooked pea contain a significantly ($p < 0.05$) higher amount of magnesium while the raw contains a significantly ($p < 0.05$) higher amount of iron. There were no significant differences in most of the amino acids contents of the raw pea as compared to the cooked pea. However, serine was significantly ($p < 0.05$) higher in the cooked pea as compared to the raw while arginine was significantly ($p < 0.05$) higher in the raw as compared to the cooked. There was no significant difference in the contents of vitamin C present in both samples, but the cooked sample contained a significantly ($p < 0.05$) lower amounts of vitamin A. There was a significant ($p < 0.05$) increase in the body weight and PER of the animals fed on the cooked seeds as compared to those fed on raw. The TD, NPU and BV values obtained for the cooked pigeon pea seed was significantly ($p < 0.05$) higher than that of the raw. The cooked pigeon pea has a higher biological value with considerable nutritional value and can therefore be a viable protein supplement.

Keywords: *Protein Quality; Biological Value; Pigeon Pea (Cajanus cajan)*

Introduction

Plants have been utilized by men for ages as various parts of plants have been utilised as food and for therapeutic purposes due to the nutrients and phytochemicals embedded in them [1]. Nutrients are chemicals obtained from food that are essential for the maintenance of life and health [2]. Among all nutrients, proteins play a very crucial role as they are needed for the growth and repair of body tissues, perform different roles as enzymes, transport, contractile, structural etc. in the body [3]. Due to the high cost of animal based proteins and the prevalence of protein deficiencies among Nigerians, more attention is shifted to alternative plant sources. A common index for determining the quality of the protein present in a particular food sample is the biological value. Biological value is a measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism's body. It captures how readily the digested protein can be used in protein synthesis in the cells of the organism [4].

Leguminous plants are consumed as a major source of plant proteins. The pigeon pea is one of the major pulse crops of the tropics and a perennial legume from the family *Fabaceae* [5,6]. The pods are 5 - 10 cm long and usually constricted obliquely between the seeds [6].

The colour of seeds ranges from cream to black through different shades of yellow, red and brown. Various parts of the pigeon pea have been utilized in tradomedical practice for treatment of a wide range of ailments of the skin, liver, lungs, and kidney [7]. The roots are used to treat febrile diseases and relieve fever, constrict tissue for controlling bleeding, and destroy internal worms. The leaves can be used to treat jaundice, trauma, cough, burn infection, and bedsores [8].

The use of pigeon peas (*Cajanus cajan*) seeds as a source of food proteins in human nutrition have of recent become popular due to their widespread range of uses [9]. However, little report exists on the nutritional content of the seeds and its biological value. Most plant products are subjected to different processing techniques in order to improve its palatability and to reduce the level of some of the anti-nutrients present in these plants [10]. This study will provide more insight on the effect of processing on the nutritional value of pigeon pea seeds and its potential as a viable protein supplement. This study was aimed at determining the effect of processing on the nutritional value and protein quality of raw and cooked pigeon pea seeds.

Methodology

Collection and processing of pigeon pea seed samples

Pigeon pea was obtained from a local market in Ilorin, Kwara state, Nigeria. The peas were thoroughly hand- picked to remove the bad ones and stones. The peas were then divided into two groups; raw and cooked. The raw samples were milled into pelletized form. 700g of the peas were cooked for about ten hours and was sun dried afterwards for forty-eight hours. The dried raw seed samples and cooked seed samples were milled separately into powdery form and then stored in sealed cellophane bags and kept in a freezer at 20°C until required.

Proximate composition analysis

Proximate analysis of the raw and cooked pigeon pea seed powder samples was carried out to determine crude protein, crude fibre, total ash, crude lipid and moisture content in accordance to the methods described by AOAC (1995). Carbohydrate content was calculated by deducting the sum of other proximate chemical components from 100 as shown in equation below:

Carbohydrates = 100- (protein + crude fat+ ash+ fiber + moisture) (AOAC, 2010).

Minerals analysis

1g of the powdered sample was taken in a 100 ml digestion flask. 10 ml of nitric acid (HNO_3) was added to it and the flask was placed in the dark overnight. On the next day, 5 ml of perchloric acid ($HClO_4$) was added to it. The mixture was then placed on a hot plate at 50°C for 15 minutes and then the temperature was raised slowly up to 200°C. Heating was continued till the white dense fumes of perchloric acid disappeared. After digestion, the contents were cooled and filtered through Whatman filter paper. Then it was transferred to a 50 ml volumetric flask and diluted with deionized water up to the mark. An Analyst 200 Perkin Elmer Atomic Absorption Spectrophotometer equipped with hollow cathode lamp was used for the analysis of calcium, magnesium, iron, copper, zinc, manganese and lead. Phosphorous content in the seed samples was determined using a spectrophotometer while content of sodium and potassium were determined using flame photometer [11].

Determination of vitamin contents

The contents of vitamin A and C present in both the raw and cooked pigeon pea seed powder were determined following the procedures of association of official analytical chemist [12].

Amino acid profile analysis

Extraction and analysis were carried out by following the modified method of AOAC [13] and Danka., *et al.* [14] in the simultaneous identification and determination of total content of amino acids in food substances

0.5g of the samples was weighed into 250 ml conical flask. The sample was defatted by extracting the fat content of the sample with 30 ml of the petroleum spirit three times with Soxhlet extractor. The defatted sample was then soaked in 30 ml of the 1M potassium hydroxide solution and was incubated for 48 hours at 110°C in hydrolysis. In order to ensure total amino acid recovery, the sample was hydrolyzed three times. The hydrolysate was neutralized to get pH in the range of 2.5 - 5.0 and the solution was purified by cation exchange solid phase extraction. The amino acids in purified solution were derivatized with ethyl chloroformate by the established mechanism.

Determination of protein quality and biological value

Experimental animals

A total of sixteen male littermate rats weighing between 40 - 50g were used for this study. They were allowed to acclimatize under the same condition for two weeks. The animals were kept in clean cage and maintained under the standard laboratory conditions for

temperature (26 ± 6ºC) humidity (60 ± 5%) and controlled environment and were allowed free access to standard pellet and water. All experimental procedures were carried out in accordance to guidance of the animal commission ethics committee.

Composition of the diet

The raw and cooked powdered seed samples of pigeon pea was allocated to the rats on raw diet and cooked diet respectively with 20g of the allocated feeds. The basal and the reference protein diet were compounded with the mixture of vitamins, starch, minerals as shown in table 1.

Ingredients	Basal diet (g)	Reference protein diet (g)	Raw guinea pea seed powder (g)	Cooked guinea seed powder (g)
Maize starch	80	70	-	-
Cotton seed oil	10	10	-	-
Milled seeds	-	-	20	20
Mineral mixture	4	4	-	-
Casein	-	10	-	-
Vitamin mixture	1	1	-	-
Cellulose powder	5	5	-	-

Table 1: *Composition of diet.*

Experimental design

The rats were then randomly allocated into four groups with four rats in each after acclimatization as follows:

- Rats in group1 served as the control and were placed on casein diet (the reference protein diet),
- Rats in group 2 were given the nitrogen-free diet (basal diet).
- Rats in group 3 and 4 were fed on the raw and cooked pigeon pea seed powder respectively.

The experiment spanned for fourteen days during which daily records of food consumption were kept and the faeces were also collected. At the end of the experiment, animals were fasted for 12 hours and sacrificed under light ether anaesthesia by cervical dislocation.

Pre-digestion and determination of total nitrogen content

Pre-digestion was done by placing the dead rat in a beaker containing 20 ml of sulphuric acid and the resulting solution was made up to 50 ml. For the determination of digestibility; the total nitrogen content in the carcass of the rat and the total faeces was determined using the Kjeldahl method [12]. Samples were weighed into a Kjeldahl flask. 10ml of concentrated sulphuric acid was added followed by one Kjeltec tablet (Kjeltec-Auto 1030 Analyzer, USA). The mixture was digested on heating racket to obtain a clear solution and afterwards cooled, and made up to 50 ml with distilled water and transferred onto Kjeldahl distillation set up followed by 50 ml of 40% sodium hydroxide solution, the ammonia formed in the mixture was subsequently distilled into 25 ml, 2% boric acid solution containing 0.5 ml of the mixture of 100 ml of bromocresol green solution (prepared by dissolving 100 mg of bromocresol green in 100 ml of methanol) and 70 ml of methyl red solution (prepared by dissolving 100 mg of methyl red in 100 ml methanol) indicators. The distillate collected was then titrated with 0.05M HCl. Blank determination was carried out by excluding the sample from the above procedure

$$N = \frac{1.401 \times M \times (ml\ titrant - ml\ blank)}{sample\ weight}$$

where,

N= nitrogen content (%)

$$M = Molarity\ of\ acid\ used = 0.05\ (\frac{mol}{dm})$$

The balance sheet method of Mitchell (1923) was used to determine the true digestibility (TD).

The nitrogen retained in the experimental animal was calculated as the algebraic difference between the food and the sum of both the faecal nitrogen for the collection period.

NR = N1- (FN) where; NR= Nitrogen retained; N1= Nitrogen in food; FN = Faecal Nitrogen

Protein efficiency ratio (PER), net protein utilisation (NPU) and biological value (BV) determination

For the determination of protein efficiency ratio (PER), net protein utilisation (NPU) and the biological value (BV), the food intake by the rats in the last fourteen days period was measured. The determined crude protein content of the diet was used to calculate the amount of protein consumed during the test. The PER was then calculated according to Osborne., *et al.* [15] using the formula below:

$$PER = \frac{\text{Weight gain or loss}}{\text{Protein consumed}}$$

The NPU values were calculated using the methodology of Mitchell [16]-4a and the BV was calculated by dividing NPU by TD:

$$NPU = \frac{\text{Body nitrogen (N) of Test Group - Body N of non- protein diet group + Food consumed by non-protein diet}}{\text{N Consumed by test Group}}$$

The true digestibility (TD) of the dietary nitrogen was determined by the original balance-sheet method of Mitchell [16] and calculated as:

True digestibility (%) = Nitrogen intake - (faecal nitrogen - metabolic nitrogen)/nitrogen intake

Metabolic nitrogen is the total faecal nitrogen excreted by the animals on the basal protein-free diet during the experimental period.

The biological value was then determined using the formula below:

$$BV = \frac{NPU}{T.D} \text{ X } 100$$

Statistical analysis

Data reported were averages of three determinations. Analysis of variance (ANOVA) was performed on each of the variables and the least significant difference (LSD) test at a significant level ($p < 0.05$) was performed using SPSS 16 software to compare the differences between treatment means. Results were expressed as the means ± standard deviation of three separate determinations.

Results

Proximate composition

The result of the proximate composition of the raw and cooked samples of pigeon pea as shown in table 2 reveals that the raw pigeon pea contained significantly ($p < 0.05$) higher content of carbohydrates (CHO) and crude fiber as compared to the cooked sample. However there was no significant ($p < 0.05$) difference in the moisture and ash content of the raw pea as compared to the cooked. There was a significant ($p < 0.05$) increase in the protein and lipid content of the cooked pea as compared to the raw pea.

Proximate composition (%)	Raw	Cooked
CHO	46.94 ± 0.34[a]	41.21 ± 0.02[b]
Protein	19.05 ± 0.09[b]	25.64 ± 0.13[a]
Lipid	3.81 ± 0.04[b]	6.41 ± 0.09 [a]
Moisture	12.17 ± 0.13	12.68 ± 0.07
Ash	5.86 ± 0.23	4.18 ± 0.06
Crude fibre	12.18 ± 0.04[a]	9.9 ± 0.18 [b]

Table 2: *Proximate composition of the raw and cooked pigeon pea seed powder.*

Values are means ± SD for triplicate determinations.

Means on the same row with different superscripts are significantly different ($p < 0.05$).

Amino acid composition of raw and cooked pigeon pea

The result of the amino acid composition of the raw and cooked pigeon pea as shown in table 3 below reveals that the cooked pigeon pea contained significantly ($p < 0.05$) higher content of serine and tyrosine as compared to the raw sample. However, there was no significant ($p < 0.05$) difference in the amounts of glycine, alanine, valine, threonine, isoleucine, leucine, aspartate, lysine, methionine, glutamate, phenylalanine, histidine, tryptophan and cysteine in the raw in comparison to the cooked sample.

Amino acid (g/100g)	Raw pigeon pea	Cooked
Glycine	5.48 ± 0.00	5.95 ± 0.01
Alanine	4.1 ± 0.01	4.35 ± 0.25
Serine	5.03 ± 0.62[b]	6.1 ± 0.50[a]
Proline	4.43 ± 0.01	3.63 ± 0.12
Valine	5.42 ± 0.38	5.58 ± 0.02
Threonine	4.87 ± 0.05	4.94 ± 0.75
Isoleucine	5.21 ± 0.10	5.23 ± 0.20
Leucine	7.41 ± 0.04	7.76 ± 0.30
Aspartate	9.32 ± 0.32	9.82 ± 0.13
Lysine	6.02 ± .51	6.28 ± .46
Methionine	0.85 ± 0.01	0.88 ± 0.02
Glutamate	12.02 ± 0.90	12.79 ± 0.88
Phenylalanine	6.49 ± 1.00	6.95 ± 0.65
Histidine	3.69 ± 0.02	3.71 ± 0.11
Arginine	7.46 ± 0.65	6.90 ± 0.32
Tyrosine	4.37 ± 0.50[a]	4.76 ± 0.66[b]
Tryptophan	0.42 ± 0.01	0.45 ± 0.01
Cysteine	1.10 ± 0.03	1.15 ± 0.02

Table 3: *Amino acid composition of raw and cooked pigeon pea seed powder.*

Values are means ± SD of triplicate determination.

Means on the same row with different superscripts are significantly different ($p \leq 0.05$).

Mineral composition of raw and cooked pigeon pea

The result of the mineral composition of the raw and the cooked samples of the pigeon pea as shown in the table 4 below reveals that the raw pigeon pea contains significantly ($p < 0.05$) higher content of iron (Fe) as compared to the cooked sample. However, there was no significant ($p < 0.05$) difference in the amounts of copper, zinc, manganese and chromium in both the raw and cooked samples. However, there was a significant increase ($p < 0.05$) in the content of magnesium in the cooked sample as compared to the raw.

	Raw	Cooked
Cu	0.3116 ± 0.0002	0.1853 ± 0.0030
Zn	0.4801 ± 0.0040	0.4359 ± 0.0002
Mg	4.8203 ± 0.5320[b]	12.9206 ± 2.5330[a]
Mn	0.1386 ± 0.0001	0.1858 ± 0.0000
Cr	0.3188 ± 0.0053	0.2649 ± 0.00050
Fe	1.0261 ± 0.0030[a]	0.7946 ± 0.0237[b]

Table 4: *Mineral composition of the raw and cooked pigeon seed powder.*

Values are means ± SD for triplicate determination.

Means on the same row with different superscripts are significantly different ($p < 0.05$).

Vitamin composition of the raw and cooked pigeon pea seeds powder

Table 5 shows the result of the vitamin composition of the raw and cooked pigeon pea seed powder samples. The raw sample contained a significantly ($p < 0.05$) higher content of vitamin A. The raw pigeon pea sample contained higher amounts of vitamin C than the cooked though not significant at ($p < 0.05$).

Result of PER, TD, NPU AND BV

An increase in the body weight of the animals fed on the cooked pigeon pea seed as compared to those fed on the raw pigeon pea seed sample was observed. Table 6 revealed the results obtained for the PER, TD, NPU and BV from the animals fed on the raw, cooked and the casein diet respectively. The PER,NPU, TD and BV values obtained from the animals fed on the cooked pigeon pea seed was significantly ($p < 0.05$) higher as compared to those fed on the raw.

Vitamin	Raw pigeon pea	Cooked pigeon pea
VIT C (mg/100g)	5.04 ± 0.55	4.41 ± 0.20
VIT A (mg/100g)	9.66 ± 0.95[a]	9.29 ± 1.25[b]

Table 5: *Vitamin composition of the raw and cooked pigeon pea seed samples.*

Values are Means ±SD for triplicate determination.

Means on the same row with different superscripts are significantly different (p < 0.05).

Group	PER	NPU	TD	BV
Casein (Control diet)	0.93 ± 0.041[a]	63.0 ± 0.10[a]	70.0 ± 0.21[a]	90.0 ± 0.34[a]
Raw guinea pea seed	0.42 ± 0.006[c]	21.49 ± 0.02[c]	53.15 ± 0.10[c]	40.15 ± 0.91[c]
Cooked guinea pea seed	0.87 ± 0.001[b]	42.48 ± 0.20[b]	70.80 ± 0.34[b]	60.00 ± 0.01[b]

Table 6: *The PER, NPU, TD and BV obtained from animals fed on the control, raw and cooked pigeon pea seed diets.*

Each figure represents the mean ± SD values; means on the same row with different superscripts are significantly different (p < 0.05).

PER: Protein Efficiency Ratio; TD: Total Digestibility; BV: Biological Value; NPU: Net Protein Utilization.

Discussion

The proximate composition of the raw and cooked samples of pigeon pea as presented in table 2 showed that both the raw and cooked contained the different nutrients in various proportions. Saxena., *et al.* [17] reported that dry pigeon pea seeds contain protein (20 - 22%), carbohydrate (62.78%), fat (1.5%) and ash (8.1%). There are slight variations in comparison to our own results as they recorded a much higher content of carbohydrate. The variations might be due to climatic and soil factors.

The raw sample contained a significantly ($p < 0.05$) higher content of carbohydrate than the cooked. This might be adduced to the fact that some of the carbohydrates had been lost during cooking due to the application of heat. It might also be as a result of the shooting up of the crude-protein content in the cooked pea. Carbohydrates are very important in the production of energy and maintenance of other metabolic processes in the body [18]. However, the raw sample had a significantly ($p < 0.05$) lower protein content as compared to the cooked. This is due to the fact that boiling as a processing method helps to reduce the anti-nutrients present in the seed, thereby enhancing the availability of the protein content accumulated in the seed [19]. Proteins are vital in the body for tissue development and cell regeneration [20] and also perform catalytic and structural roles in the body system [3].

The moisture content of both raw and cooked pigeon pea was found to be high though there was no significant difference ($p < 0.05$). High moisture content results in low shelf life and speedy deterioration of food products [19].

The crude fiber content in the raw sample is similar to that recorded by Saxena., *et al* [21]. There was a significant ($p < 0.05$) reduction in the fiber content of the cooked which might be due to leaching. Fiber helps in food digestion thereby enhancing optimal growth. It also purifies the digestive tract by removing likely toxins from the body and inhibits the absorption of excess cholesterol [22]. Ash content which indicates the level of minerals present in the sample is present in guinea pea in minute amounts, with the cooked pigeon pea containing lower ash content (4.18%) than the raw (5.86%) due to leaching of some of the minerals during cooking. This result is close to the ash content reported by Adamu., *et al* [23].

However, both the raw and cooked pigeon pea contained small amounts of lipid, though the cooked sample contained a higher amount. Dietary fat releases energy when oxidized and also enhances the delectableness of food [24].

The mineral contents of the raw and cooked samples of pigeon pea as presented in table 4 indicates that both samples contain minute amounts of minerals. This low content might have been due to the fact that ripe seeds were used in this present research, because it had been reported that in Africa, pigeon pea seeds are mainly eaten green unlike in India where dry dehulled split-pea is most popular and such green seeds are a richer source of iron, copper and zinc than the mature seed [25]. Adamu., *et al.* [23] also reported that pigeon pea contains small amounts of copper, manganese and zinc in addition to high concentration of magnesium. Copper is a trace essential element that plays a role in the formation of connective tissue and in the normal functioning of muscles, immune and nervous system. Copper, together with iron plays a cogent role in the formation of red blood cells [26]. The trace mineral zinc plays a role in immune function [27,28], wound healing [27], protein synthesis, DNA synthesis and cell division [29]. Manganese is a trace element that helps to maintain healthy nerves, important for energy production and essential for proper iron metabolism [30]. Pigeon pea seed contains minute amounts of chromium which is required in very small amounts in the body for the regulation of blood sugar and transport of glucose into the cells [31].

As seen from the results obtained, the raw sample contained a significantly ($p < 0.05$) higher amount of vitamin A as compared to the cooked. This might be due to leaching during cooking. Vitamin A (beta carotene) is important for healthy vision, skin, bones and other tissues of the body. The raw sample contained a slightly higher amount of vitamin C compared to the cooked though not significant different ($p < 0.05$). Vitamin C is a water soluble vitamin often called ascorbic acid which is responsible for the growth and repair of tissues in all parts of the body. Vitamin C strengthens the body immunity against infections, helps in collagen and thyroxin synthesis and enhances iron absorption [32].

The result of the amino acid obtained from this research is within the range of values recorded by Bogoro., *et al* [33]. Results obtained in this study on the amino acid composition of pigeon pea showed that the cooked samples contained higher content of some of the amino acids as compared to the raw; especially essential amino acids like threonine, isoleucine, lysine, phenylalanine, tryptophan. Amino acids are the monomeric units of proteins. Some amino acids are non-essential while some are essential which cannot be synthesized in the body and hence must be obtained from the diet [4].

As obtained from the results, the cooked sample had a significantly ($p < 0.05$) higher biological value, true digestibility and net protein utilization as compared to the raw. The lower biological value of the raw pigeon seed might be due to the higher loss of endogenous nitrogen that occurs through the peeling of the intestinal mucosa when raw legume seed proteins are consumed [34,35]. Additionally, the presence of various anti-nutritional substances in the raw sample which hamper the complete digestion of protein and increases the endogenous faecal excretion of nitrogen [36] might also be responsible for the decrease in the true digestibility value of the raw pigeon pea seeds. Cooking as one of the food processing methods reduces the anti-nutritional factors thereby increasing the availability of the protein content and its true digestibility which is responsible for the higher biological value of the cooked sample. The higher biological value of the cooked sample as a result of reduced anti-nutritional factors might also be responsible for the increase in the weight of the rats fed on the cooked pigeon pea as compared to those fed on the raw.

Conclusion

Cooked pigeon pea seeds have excellent nutritional properties and a higher protein quality than the raw and hence can be incorporated into the diet of humans as a protein supplement. However, the effect of other processing methods on the nutritional properties of pigeon pea seeds should also be further investigated.

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