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A comparative evaluation of the nutrient and anti-nutrient compositions of four pigeon pea (*Cajanus cajan*) varieties

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Conflict of Interest:

The author hereby declares that there is no conflict of interest.

ABSTRACT

Background: Pigeon pea is a rich source of protein, fibre, minerals and vitamins but wide variations exist in the nutrient and anti-nutrient contents. Identification of good quality pigeon pea is paramount to its increased utilization and improved child nutrition.
Objectives: This study evaluated and compared the nutrient and anti-nutrient compositions of four Pigeon pea (*Cajanus cajan*) varieties.
Methods: Five hundred grams of pigeon pea seeds [Iseyin pea (dark brown), NSWCC 34A (red-brown), NSWCC 34 (light red-brown) and 7D (cream)] were cleaned and analysed for proximate, mineral and anti-nutrient composition using standard procedures. Significant differences were detected using ANOVA and means were separated using Least Significant Differences at $p<0.05$.
Results: The ranges of crude protein, crude fat, crude fibre and carbohydrate contents of the pigeon pea varieties were 20.95-22.61%, 1.43-3.35%, 2.10-3.90% and 59.72-64.69%, respectively. The protein (22.61%) and ash (5.41%) contents of NSWCC 34A were significantly higher compared to others. The four pigeon pea varieties differed significantly in fat, fibre and carbohydrate contents at $p<0.001$. Significantly higher calcium (121.40mg/100g), iron (5.20mg/100g) and zinc (4.70%) contents were found in 7D compared to the other varieties. 7D variety had the lowest values of tannin (61.5mg/100g), phytate (802.5mg/100g), oxalate (659mg/100g), saponin (271mg/100g) and polyphenol (389.5 mg/100g). Iseyin pigeon pea had the highest tannin (303.5mg/100g) and polyphenol (4635mg/100g) contents ($p<0.001$) while NSWCC 34A variety had the highest phytate (2257.5mg/100g), oxalate (1191.5mg/100g) and saponin (2299.5mg/100g) contents.
Conclusion: 7D variety of pigeon pea was the best among the four varieties and recommended for use in complementary food production.

Keywords: Pigeon pea, nutrient, anti-nutrients, underutilized legume

1.0 INTRODUCTION

The nutritional and economic importance of pigeon pea has been widely documented. Pigeon pea is a perennial crop that originated from India about 3500 years ago (Van der Maeson, 1995). It belongs to the family of leguminosae. The main colours of the seeds are cream, brown, red and black with various shades of these colours. The common names of pigeon pea include 'red gram' or '*dhal*' in India (Mula and Saxena 2010), '*gungo peas*' (Jamaica), '*mbaazi*' (Kenya), '*otili*' (Yoruba), '*fiofio*' or '*agbugbu*' (Igbo) and '*Aduwa*' in some parts of Northern Nigeria. This legume is grown in the six geopolitical zones of Nigeria for multiple purposes which include food for humans and animals, fuel and fodder (Egbe and Kalu, 2006). The great potentials of this pea are yet untapped because of its hard-to-cook nature and anti-nutrient contents (Emefiene *et al*., 2013). Variations in nutrient and anti-nutrient contents of pigeon pea varieties are wide. Identification of good quality pigeon pea is paramount to its increased utilization and improved child nutrition.

It is a rich source of protein, fibre, minerals and vitamins (Fasoyiro and Arowora, 2013). It contains 20-26% protein, 1-2% fat, 53-65% carbohydrate, and 3.8-8.1% ash (Ajayi *et al*., 2010; Saxena and Kumar, 2010). It is a good source of water-soluble vitamins and carotenoids (Ellong *et al*., 2015). Pigeon pea has high lysine content but low in cysteine and methionine (Akande *et al*., 2010). Linoleic acid was found to be the most abundant polyunsaturated fatty acid (PUFA) (Ade-Omowaye *et al*., 2015). The presence of polyphenols and flavonoids bestows anti-oxidant properties on pigeon pea (Rani *et al*., 2014; Aruna and Devindra, 2018). Devindra *et al*. (2017) reported the

prebiotic potential of pigeon pea due to its raffinose (oligosaccharide) content. Other health benefits include glucose-lowering (Devindra *et al*., 2016), weight reduction and colon health due to its high fibre content (Fasoyiro *et al*., 2019). Pigeon pea possesses anti-nutrients such as tannin, phytate, trypsin inhibitors, hemagglutinin and saponin among others (Francis *et al*., 2001; Onwuka *et al*., 2006; Pele *et al*., 2016). The presence of these anti-nutritional factors negatively affects the bioavailability of protein, calcium, iron and zinc (Kaushik *et al*., 2018). Several research studies have reported the reduction of anti-nutrients using various processing methods such as fermentation, germination, dehulling, soaking, boiling (Duhan *et al*., 2004; Onwuka *et al*., 2006).

Among the Yorubas, pigeon pea is commonly consumed as cooked beans with stew. The Southeasterners prefer to serve it with yam or corn. It can also be taken as fresh or immature seeds (Kabuo *et al*., 2015) and taken with rice or maize. Pigeon pea, although takes a long time to cook is preferred to cowpea by rural people because it tastes better and fills more (Fasoyiro *et al*., 2010). Other reasons why it is consumed include its sweetness and long conservation period (Esan and Ojemola, 2018). The nutrition of the rural populace could be improved through increased utilization of pigeon pea.

Fortification of cereals with pigeon pea at about 70:30 improves protein quantity and quality (Faris and Singh, 1990; Fasoyiro *et al*., 2013). Several products including complementary foods have been developed from pigeon pea flour blends. Pigeon pea compares with soybean (Akporhonor *et al*., 2006) and therefore could be added to cereal based complementary food with other ingredients to produce good quality complementary food. There is need for continued research on pigeon pea to determine varieties with desirable qualities. There are still variants of pigeon pea unaccounted for. Therefore this study was undertaken to evaluate as well as compare the nutrient and anti-nutrient composition of pigeon pea seeds bought from Bodija Market, Ibadan, Oyo State and three variants from Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. This research was a preliminary study in the development and evaluation of pigeon pea fortified complementary food using animal models.

2.0 MATERIALS AND METHODS

Source and identification of materials: Pigeon pea seeds (grown at Iseyin, Oyo State, Nigeria) were purchased from Bodija market, Ibadan. This was denoted as Iseyin pea (dark brown). Three pigeon pea varieties identified as NSWCC 34A (red-brown), NSWCC 34 (light red-brown) and 7D (cream or white) were collected from the Institute of Agriculture, Research and Training Centre (IAR&T), Ibadan, Oyo State.

Sample preparation: Five hundred grams of the seeds were sorted to remove stones and unwholesome seeds. The clean seeds were washed, drained and dried in the oven (50^0C). The dried seeds were milled and packaged separately in small tightly covered plastic cups for chemical analysis.

2.1 Proximate analysis

The moisture, fat, protein (N x 6.25) and ash contents of the pigeon pea seeds were determined in triplicates according to the approved procedures of the Association of Official Analytical Chemists (2005).

2.2 Mineral analysis

2.2.1 Determination of calcium (AOAC (2005))

Five millilitres of 10% perchloric acid ($HCI0_3$) was used to digest ashed sample and heat was applied to the mixture. The concentration of calcium in the filtrate was read on the Jenway Digital Flame Photometer (PFP7, Bibby Scientific Limited, United Kingdom). The concentration of calcium was determined using the formula: element. The concentration of calcium was determined using the formula:

$$\%\ calcium\ content = \frac{meter\ reading\ x\ slope\ x\ dilution factor}{10000}$$

2.2.2 Determination of iron and zinc (AOAC (2005))

The iron and zinc contents were determined using the atomic absorption spectrophotometer (AAS) (Buck 200, Buck Scientific, USA). Percentage values of iron and zinc were determined as follows:

$$\%\ iron/zinc\ contents = \frac{meter\ reading \times slope \times dilution\ factor}{10000}$$

$$Dilution\ Factor = \frac{Volume\ of\ Solvent}{Sample\ weight}$$

2.3 Evaluation of selected anti-nutritional factors

2.3.1 Determination of oxalate

The method of Onwuka (2005) was employed in the determination of oxalate. After

determined as follows:

$$\%\, iron/zinc\ contents = \frac{meter\ reading \times slope \times dilution\ factor}{10000}$$

$$Dilution\ Factor = \frac{Volume\ of\ Solvent}{Sample\ weight}$$

After cooling, the solution was centrifuged at 832 g (2500 rpm) for 5 minutes and filtered through Whatman No. 42 filter paper. The precipitate formed was dissolved in 10 mL of 20% H_2SO_4. Distilled water was poured into the solution to reach the 300 mL mark and ammonium hydroxide (NH_4OH) was added to reprecipitate oxalic acid. The contents were boiled and allowed to settle overnight. The quantity of oxalic acid was determined by titrating the mixture with 0.05N $KMnO_4$ as indicated below:

$$1\ ml\ of\ 0.05NKMnO_4 = 0.00225\ anhydrous\ oxalic\ acid$$

$$\%\ oxalicacid = \frac{Titrevaluex\ 0.00225}{2} x\ 100$$

2.3.2 Determination of tannin

Tannin was determined by the method described by Joslyn (1970). About 20 mL of 50% methanol was added to 0.2 g of the sample and placed in a water bath at 77-80^{o}C for 1 hour with constant agitation. After filtration, about 20 mL distilled water, 2.5 mL Folin-Denis reagent and 10 mL of 17% Na_2CO_3 were added and the solution mixed properly. The absorbance of the solution and prepared tannic acid standard solutions were read after colour development on a UV-Vis spectrophotometer (V6300, Venway, UK) at a wavelength of 760 nm.

% Tannin was calculated using the formula:

$$\%Tannin = \frac{Absorbance\ of\ sample \times Average\ Gradient \times Dilution\ Factor}{Weight\ of\ Sample \times 10000}$$

2.3.3 Determination of Phytate

The method of Maga (1983) was employed. One hundred millitres of 2% Hydrochloric acid was added to 2 g of each sample in 250 mL conical flask. Then 50 mL of the filtrate was diluted with 107 mL distilled water and 10 mL of 0.3% Ammonium Thiocyanate (NH_4SCM) solution was added into each solution. The resulting solution was titrated with standard iron (III) chloride solution having 0.00195 g iron per ml. A slightly brownish-yellow colour which persisted for 5 minutes was obtained. The % phytic acid was determined using the formula:

$$\%\ PhyticAcid = \frac{Titrevalue \times 0.00195 \times 1.19 \times 100 \times 3.55}{weightofsample}$$

2.3.4 Determination of Saponin

The spectrophotometric method of Brunner (1984) was used to determine the saponin content of the samples. One hundred millilitres of isobutyl alcohol was added into a 250 ml beaker containing 1 g flour sample. One millilitre of filtrate was then pipetted into 50 mL volumetric flask into which 2 mL of 5% $FeCl_3$ solution was added and distilled water added to make up to the mark. The mixture was left for about 30 minutes till blood-red colour appeared. The absorbance of the solution and prepared standard saponin solutions were read after colour development in a spectrophotometer (V6300, Jenway, UK) at a wavelength of 380 nm.

$$\%\ Saponin = \frac{Absorbanceofsample \times gradient\ factor \times dilution\ factor}{Weightofsample \times 10000}$$

2.3.5 Statistical analyses

The data from proximate, mineral and anti-nutritional determination were analysed using SPSS version 20. The means were subjected to analysis of variance (ANOVA) and Least Significant Difference (LSD) used to separate means when the difference was significant.

3.0 RESULTS

3.1 Proximate composition of the four pigeon pea varieties

The proximate composition of the different varieties of pigeon pea is displayed in Table 1. The crude protein values ranged from 20.95-22.61% with NSWCC 34A being significantly the highest in protein content. The ranges of crude fat, crude fibre and carbohydrate contents of the pigeon pea varieties were 1.43-3.35%, 2.10-3.90% and 59.72-64.69%, respectively. The four pigeon pea varieties differed significantly in fat, fibre and carbohydrate contents at $p<0.001$. The 7D variety had the highest fat content (3.35%) while NSWCC 34 had the lowest fat content (2.06%) which was similar to that of NSWCC 34A (2.31%) at $p>0.092$. Among all the pigeon pea varieties, NSWCC 34A contained the highest fibre (3.90%) followed by Iseyin pigeon pea (3.20%). The ash contents of 7D (3.90%), NSWCC 34 (3.92%) and Iseyin (4.20%) pigeon pea varieties did not differ significantly ($p> 0.815$) but were significantly lower at $p<0.001$ than that of NSWCC 34A (5.41%).

Table 1: Proximate composition of the four pigeon pea varieties (dry weight)

	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Carb (%)
IseyinPP	$8.53^{c} \pm 0.01$	$21.90^{c} \pm 0.04$	$1.43^{c} \pm 0.01$	$3.20^{b} \pm 0.02$	$4.20^{b} \pm 0.01$	$64.69^{a} \pm 0.02$
NSWCC 34	$9.42^{b} \pm 0.04$	$20.95^{c} \pm 0.08$	$2.06^{b} \pm 0.03$	$2.71^{c} \pm 0.06$	$3.92^{b} \pm 0.05$	$63.66^{c} \pm 0.05$
NSWCC 34A	$9.96^{a} \pm 0.09$	$22.61^{a} \pm 0.07$	$2.31^{b} \pm 0.03$	$3.90^{a} \pm 0.01$	$5.41^{a} \pm 0.02$	$59.72^{d} \pm 0.02$
7D	$6.85^{d} \pm 0.05$	$21.87^{b} \pm 0.17$	$3.35^{a} \pm 0.02$	$2.10^{d} \pm 0.10$	$3.90^{b} \pm 0.10$	$64.05^{b} \pm 0.05$

Values are expressed as mean ±SEM (n=3). Means in the same column with different superscripts are significantly different from each other at $P< 0.05$

Table 2: Mineral composition of the four pigeon pea varieties (dry weight)

	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
IseyinPP	$94.43^{b} \pm 0.58$	$2.12^{c} \pm 0.03$	$3.16^{c} \pm 0.15$
NSWCC 34	$78.07^{d} \pm 0.06$	$4.03^{b} \pm 0.11$	$4.00^{b} \pm 0.09$
NSWCC 34A	$91.28^{c} \pm 0.07$	$3.92^{b} \pm 0.10$	$2.00^{d} \pm 0.07$
7D	$121.40^{a} \pm 0.11$	$5.20^{a} \pm 0.11$	$4.70^{a} \pm 0.10$

Values are expressed as mean ±SEM (n=2). Means in the same column with different superscripts are significantly different from each other at $P< 0.05$.

Table 3: Anti-nutrient contents of the four varieties of pigeon pea (mg/100g dry weight)

Parameters	Tannin (mg/100g)	Phytate (mg/100g)	Oxalate (mg/100g)	Saponin (mg/100g)	Polyphenol (mg/100g)
IseyinPP	$303.50^{a} \pm 6.50$	$1235.00^{c} \pm 35.36$	$967.50^{c} \pm 2.56$	$2225.00^{a} \pm 55.00$	$4635.00^{a} \pm 56.57$
NSWCC 34	$62.50^{b} \pm 0.50$	$2149.65^{b} \pm 25.12$	$1122.61^{b} \pm 11.46$	$2084.00^{b} \pm 5.35$	$1644.50^{b} \pm 9.56$
NSWCC 34A	$74.25^{b} \pm 0.25$	$2257.50^{a} \pm 3.50$	$1191.50^{a} \pm 11.50$	$2299.50^{a} \pm 6.50$	$1729.50^{b} \pm 6.36$
7D	$61.5^{b} \pm 2.50$	$802.50^{d} \pm 3.50$	$659.00^{d} \pm 2.83$	$271.00^{c} \pm 6.00$	$389.50^{c} \pm 7.50$

Values are expressed as mean ±SEM (n=2). Means in the same column with different superscripts are significantly different from each other at $P< 0.05$.

3.2 Mineral composition of the four pigeon pea varieties

There were statistically significant differences in the calcium contents of the four pigeon pea varieties (Table 2). The result revealed that 7D had the highest calcium content (121.40 mg/100g) followed by Iseyin pigeon pea (94.43 mg/100g) while variety NSWCC 34 had the lowest calcium content (78.07 mg/100g). The mineral contents data showed significantly higher iron (5.20 mg/100g) and zinc (4.70%) contents in 7D compared to the other varieties. The lowest iron value was found in Iseyin pigeon pea (2.12 mg/100g). However, there was no significant difference between the iron contents of NSWCC 34 (4.03 mg/100g) and NSWCC 34A (3.92 mg/100g).

3.3 Anti-nutrient contents of the four varieties of pigeon pea

Table 3 displays selected anti-nutritional components of the different pigeon pea varieties. It was observed that the 7D variety had the lowest values of all the measured anti-nutrients (tannin (61.5mg/100g, phytate (802.5mg/100g), oxalate (659 mg/100g), saponin (271 mg/100g) and polyphenol (389.5 mg/100g). Iseyin pigeon pea had the highest tannin (303.5 mg/100g) and polyphenol (4635 mg/100g) contents ($p<0.001$). The tannin content of Iseyin pigeon pea was more than four times the tannin values of the other varieties. NSWCC 34A variety had the highest phytate (2257.5 mg/100g), oxalate (1191.5 mg/100g) and saponin (2299.5 mg/100g) contents. (tannin, phytate, oxalate, saponin and polyphenol).

4.0 DISCUSSION

The pigeon pea varieties had above 20% protein contents. This finding agrees with 21.03% and 22.6% protein values reported by Akande *et al*. (2010) and Solomon *et al*. (2017) respectively but lower than 23.33 to 25.21% found by Fasoyiro *et al*. (2010). Pigeon pea is a rich source of

protein (Odeny, 2007; Aruna and Devindra, 2018). High protein content is a desirable quality for good complementary food. It is very essential for the growth and development of infants. Although pigeon pea contains lower protein content than cowpea and soybean, combined with cereals, it provides a well balanced meal comparable to soybean (Akporhonor *et al*., 2006). Pigeon pea was used to replace soybean without adversely affecting the performance of experimental animals (Adamu and Oyetude, 2013).

It is noteworthy that NSWCC 34A variety with the highest ash contents (Table 1) had lower mineral content compared to 7D which had the lowest ash content (Table 2). This finding could be attributed to the probable effect of high fibre (Table 1) and phytate (Table 3) contents of NSWCC 34A. High fibre rich foods are known to have high phytate contents which form complexes with minerals making them unavailable (Bora, 2014). The 7D variety had the lowest fibre content (Table 1) and was the best in terms of calcium, iron and zinc values (Table 2). The iron and zinc values were comparable to 5.6 mg/100g and 2.67 mg/100g, respectively, reported by Kunyanga *et al*. (2013) and higher than the values (iron-1.03 mg/100g; zinc-0.48 mg/100g) reported by Abiola *et al*. (2019). Calcium is needed for optimal dental and bone development of growing children. Iron and zinc are essential micronutrients responsible for cognitive development and efficient metabolism. Therefore, foods rich in these minerals are preferred in the formulation of complementary food. Among the pigeon pea varieties, 7D was found to possess the lowest contents of all the measured anti-nutrients. This discrepancy could be related to the colour of the seed coat. It has been reported that the cream or white seeded varieties have relatively less anti-nutritional factors and higher nutritional value than the dark coloured ones (Odeny, 2007). Among all the anti-nutrients, phytate is of major concern in human nutrition (Kumar *et al*., 2010), particularly in infant nutrition. This is related to its high chelating nature that affects protein and mineral bioavailability (Bora, 2014; Pele *et al*., 2016). However, the anti-nutritional factors in pigeon pea are less than those in soybean, pea and common beans (Singh and Diwakar, 1993). The NSWCC 34A variety had higher phytate, oxalate and saponin contents compared to the other pigeon pea varieties. High variability existed in the tannin composition of the pigeon pea varieties assessed in this study. Nwosu *et al*. (2006) and Harris (2014) reported tannin contents of 222 and 230 mg/100g in pigeon pea. These values are within the range obtained in this study. Phytate contents in the range of 3780-5650 mg/100g were reported by Fasoyiro et al. (2010). Solomon et al., (2017) also noted that the tannin and phytate contents of pigeon pea were 6880 and 5010 mg/100g, respectively. A range of 50-230 mg/100g saponin contents of pigeon pea was found by Jain *et al*. (2009). Kaur et al. (2014) also reported a range of 929-3514 mg/100g of saponin in chickpea. The differences observed could be due to variations in seed varieties, growth conditions, duration and conditions of storage (Amaefule and Onwudike, 2000; Saxena *et al*., 2010).

5.0 CONCLUSION

This study has contributed additional information on the nutritional quality of pigeon pea varieties. The four pigeon pea varieties had good macronutrient properties and appreciable mineral contents. Anti-nutrient contents were higher in Iseyin pigeon pea, NSWCC 34 and NSWCC 34A varieties compared to 7D. 7D pigeon pea variety was also rated best in terms of calcium, iron and zinc values. Therefore, 7D variety of pigeon pea is considered the best among the four varieties for use in complementary food production. Further investigation should be undertaken on the effect of processing methods on the cooking time and nutritional quality of these four varieties.

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Pigeon pea (NSWCC 34)

Pigeon pea (NSWCC 34A)

Market pigeon pea (Bought from Iseyin, Oyo State, Nigeria)