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SPECTROPHOTOMETRIC DETERMINATION OF PROXIMATE, VITAMIN B COMPLEX AND ELEMENTAL CONTENTS OF PIGEON PEAS (*CAJANUS CAJAN*)

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ABSTRACT

Pigeon pea is a typical food for every household in Nigeria. This study determined pigeon peas' proximate, vitamin B complex, and elemental contents using standard analytical methods, UV-visible and AAS spectrophotometers. The results of the analysis indicated the ash, moisture, fat, fibre, protein and carbohydrate contents were 3.9015% ± 0.02, 1.577 % ± 0.01, 1.885 % ± 0.03, 6.5595 % ± 0.05, 12.755 % ± 0.03 and 73.3045 % ± 0.01 respectively. Vitamin B9 exhibited the highest mean content at 6.944 mg/kg ±0.037, followed by vitamin B7, 5.942 mg/kg ± 0.036, and vitamin B5, 2.473 mg/kg ±0.270. The elemental composition was K (1.757 ppm ± 0.05), followed by Na (1.2545 ppm ± 0.02), Fe (0.829 ppm ± 0.01), Ca (0.521 ppm ± 0.07), and the least was Se (0.3325 ± 0.01). The findings affirmed pigeon peas as a valuable dietary source of carbohydrates, protein, and vitamins B9, B7, K, Na and Fe, contributing significantly to human health and nutrition. The analysis implies that pigeon peas should be encouraged for consumption.

Keywords: Elemental composition, Pigeon pea, Proximate, Spectrometry, Vitamin B complex

INTRODUCTION

Legumes are known to be the third largest family among flowering plants, consisting of approximately 650 genera and 20,000 species (Nwanekezi *et al.*, 2017). Legumes have a low glycemic index value and bioactive compounds with antioxidant properties, and therefore, can be an alternative to meet nutritional needs and fight several diseases, especially in developing countries (A'yuni *et al.*, 2022; Aruna and Devindra, 2018). Various countries have used legumes as their primary food sources, such as pigeon peas, chickpeas, lentils in South Asia, kidney beans in Latin America, etc. Kumar *et al.*, (2017) reported that India is the center of origin for cultivated pigeon peas. Pigeon pea (fio-fio) is an essential crop of the legume group, which belongs to the family Fabaceae and is affected by excess soil moisture (Meena *et*

al., 2016). The fruit of pigeon peas is a flat, straight, pubescent, 5-9 cm long x 12-13 mm wide pod. It contains 2-9 brown, red or black seeds, small and sometimes hard coated (Sarkar *et al.*, 2018). In southeastern Nigeria, it is usually consumed in cooked form, like cooked beans, due to its long softening period (Okafor *et al.*, 2017). Pigeon peas are processed and eaten as a stew and with plantain balls (Ngozi *et al*., 2015). It is a good source of crude protein, fibre, and vitamins, especially thiamine, riboflavin, niacin, choline, and antioxidants. It also contains anti-nutrients such as tannin, cyanogenic glycosides, alkaloids, etc., which inhibit the bioavailability of nutrients like proteins (Gomezulua and Mongi, 2022). Pigeon peas are important legumes that can be used to check the diverse problems of protein-calorie malnutrition and micronutrient deficiencies in Nigeria (Arukwe and Nwanekezi, 2022). It significantly contributes to meeting the nutritional needs of smallholders in terms of fiber, ash, fat, and minerals (Abebe, 2022). Anemone (2020), reported that the crude protein, crude fat, crude fibre and carbohydrate contents of the pigeon peas varieties were 20.95-22.61%, 1.43-3.35%, 2.10-3.90% and 59.72-64.69%, respectively. Vitamins are essential for humans. They are classified into fat-soluble (vitamins A, D, E and K) and water-soluble (vitamins B complex and C) (Clifford and Curely, 2020; Emily, 2023). Vitamin B complex is vital for normal body growth and development, healthy skin and heart functioning and red blood cell formation (Jacqueline and Sandeep, 2023; Akram *et al.*, 2020). It helps prevent anaemia onset because of the high folate content in the legumes (Kristina, 2018). One of the key minerals found in pigeon peas is potassium. It is best known as a vasodilator, which can reduce the constriction of blood vessels and thereby lower blood pressure (John, 2021). Pigeon peas are a good source of vitamin B. Pigeon peas improve energy levels without causing any weight gain or fat development (Amritha, 2018).

EXPERIMENTAL

Collection of Samples: Samples (2kg) of pigeon peas were purchased at Eke Oko main market, Orumba North L.G.A, Anambra State, Nigeria.

Proximate Composition Determinations (AOAC, 2006)

Ash: An empty platinum crucible was washed and dried, and its weight was noted.

Exactly 2g of wet sample was weighed into the platinum crucible and placed in a muffle furnace at 500℃ for 3 hours. After burning, the sample was cooled in a desiccator and weighed.

% Ash content = $\frac{W3-W1}{W2-W1} \times \frac{100}{1}$, where

W1 = weight of empty platinum crucible, W2 = weight of platinum crucible and sample before burning, W3 = weight of platinum and ash.

Moisture: Exactly 2g of the sample was weighed into the petri dish, and the weight of the petri dish and sample was noted before drying. The petri dish and sample were put in the oven and heated at 100℃ for 1 hour. The result was reported, and the oven was heated for another 1 hour until a steady result was obtained and the weight was noted. The drying procedure was continued until a constant weight was obtained.

% Moisture content = $\frac{W1-W2}{Weight\ of\ sample} \times \frac{100}{1}$, where

W1= weight of petri-dish and sample before drying, W2= weight of petri-dish and sample after drying.

Crude Fat: Exactly 5g of the sample was dried in 250 mL clean boiling flasks in an oven at 105-110℃ for about 30 minutes, transferred into a desiccator and allowed to cool. The cooled boiling flask was also weighed and labelled. The boiling flask was filled with 300 mL petroleum ether (boiling point 40-60℃). The extraction thimble was lightly plugged with cotton wool and soxhlet apparatus was assembled and refluxed for about six hours. The thimble was carefully removed, and petroleum ether at the top of the setup was allowed to drain into a container for reuse. When the flask was almost free of petroleum ether, the sample was removed and dried at 105-110℃ for 1 hour. It was transferred from the oven into a desiccator, cooled, and weighed.

Crude Fiber: Exactly 2g of the defatted sample from the crude fat analysis was boiled under reflux for 30 minutes with 200 mL of a solution containing 1.25g of H_2SO_4 per 100 mL solution. The solution was filtered through linen on a fluted funnel and further washed with boiling water in a beaker until the washings were no longer acidic which was confirmed by testing with litmus paper. The residue was transferred to another beaker and boiled for 30 minutes with 200 mL of a solution containing 1,25g of carbonate-free NaOH per 100 mL. The final residue was filtered through a thin, closed pad of washed and ignited asbestos in a Gooch crucible. It was finally incinerated, cooled and weighed.

The loss in weight after incineration × 100 is the percentage of crude fibre. %Crude fibre = $\frac{Weight\ of\ fibre}{Weight\ of\ Sample}$ X 100

Vitamins Analysis

Vitamin B_1 and B_2: One (1g) of the sample was weighed into a conical flask, and the sample was dissolved with 100 mL of deionized water; it was shaken thoroughly, heated for 5 minutes, and allowed to cool and filter. The filtrate was poured into a cuvette, and the respective wavelengths for the vitamins were set to read the absorbance using a spectrophotometer. Vitamin B_1 = 261nm, Vitamin B_2 = 242nm.

Calculations: Concentration (mg%) = $\frac{A \times DF \times Volume\ of\ curvette}{5}$. Where A = Absorbance, E = extinction coefficient = 25 for B_1 and B_2, DF = dilution factor

Vitamin B_3: Five **(**5g) of the sample was dissolved in 20 mL of anhydrous glacial acetic acid and warned slightly. 5mL of acetic anhydride was added and mixed. 2–3 drops of crystal violet solution were added as an indicator, and 0.1M perchloric acid was added to titrate to a greenish-blue colour.

Calculation: Vitamin B_3 = $\frac{Titre\ value \times 0.0122}{0.1}$

Vitamin B_5: Standard Preparation: 0.25 mL of vitamin B_5 working standard was taken into 25 volumetric flasks with a solution mixture (chloroform and methanol in the ratio of 1:9). It was gently shaken to mix thoroughly and was made up to the mark. 0.25mL of sample was measured into a 25 volumetric flask containing a solution mixture (chloroform and methanol in a ratio of 1:9). It was gently shaken to mix thoroughly, and absorbances were recorded at 246nm against the blank.

Vitamin B_6: Five (5g) samples were dissolved in a mixture of 5 mL of anhydrous glacial acetic acid and 6 mL of mercury (II) acetate solution. Two drops of crystal violet were added as an indicator and 0.1M perchloric acid was used to titrate to the green color endpoint.

Calculation: Each mL of 0.1M perchloric acid equals 0.02056g of $C_8H_{11}NO_3$ HCl.

Vitamin B_7 (Biotin): Sample Preparation: The sample (0.1mL) was taken into a separator. In the separator, 5mL of water was added, mixed well and added 5mL chloroform. The chloroform layer was discarded, and the water layer was taken into a 50mL volumetric flask by passing it through anhydrous sodium sulphate and made up to 50mL with water. Sample and blank solutions (2mL) were taken into test tubes. In each test tube, 2mL of 0.2% solution

of phenylhydrazine (in hydrochloric acid and alcohol in ratio of 1:5v/v) was added and mixed well. After, it was heated in a water bath until it almost dried and cooled at room temperature. Two millilitres of the solution mixture (ammonia and alcohol in a ratio of 1:1) was added to each test tube, and 1mL pyridine was added. Absorbance was recorded at 548nm against the blank. Standard was also treated and analyzed in the same way as the sample.

Vitamin B_9 (Folic Acid): The sample (0.2 millilitres) was weighed and taken into the separator. Five millilitres of water were added and mixed well, and 5mL of chloroform was used to extract the sample. The water was discarded, and chloroform was taken in a dry 50ml volumetric flask by passing it through anhydrous sodium sulphate, which was made up to 50mL with chloroform. The sample and the blank solutions were each put into separate test tubes. Two millilitres of 0.2% solution of phenylhydrazine (in hydrochloric acid and alcohol in a ratio of 1:5v/v) was added to each test tube and were well mixed. They were then each heated in a water bath until almost dry and cooled at room temperature. Fifteen millilitres of solution mixture (ammonia and alcohol in a ratio of 1:1) was added to each test tube. Its absorbances were recorded at 635nm against blank.

Vitamin B_{12}: Twenty-five (25mg) of the sample was dissolved in 250 mL of deionized water.

The absorbance was read at 361nm.

Calculation: Concentration (mg%) = $\frac{A \times DF \times Volume\ of\ curvette}{E}$

Where A =Absorbance, E = Extinction coefficient =25, DF = dilution factor =5

According to AOAC (2003), some Elemental Components were determined using an Atomic absorption spectrophotometer.

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RESULTS

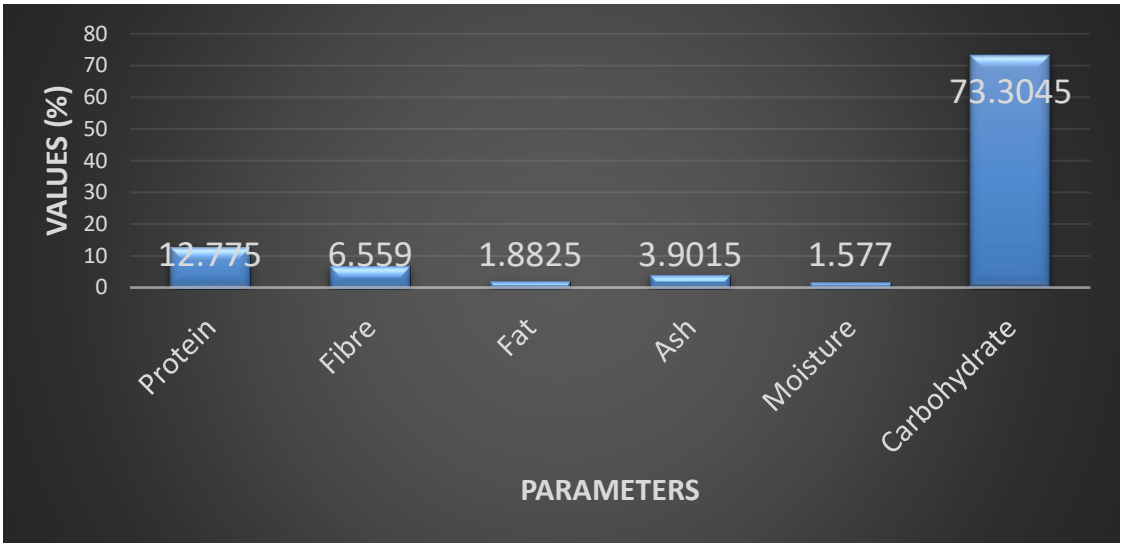

Figure 1: The Results of the Proximate Content of Pigeon pea

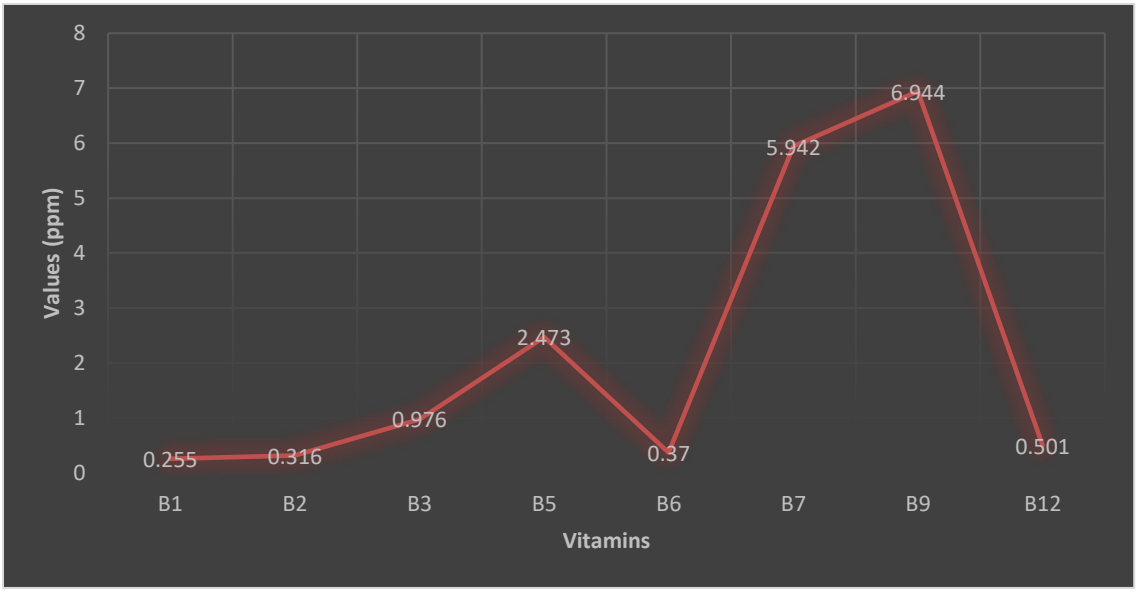

Figure 2: Vitamin B Complex Contents of Pigeon Pea

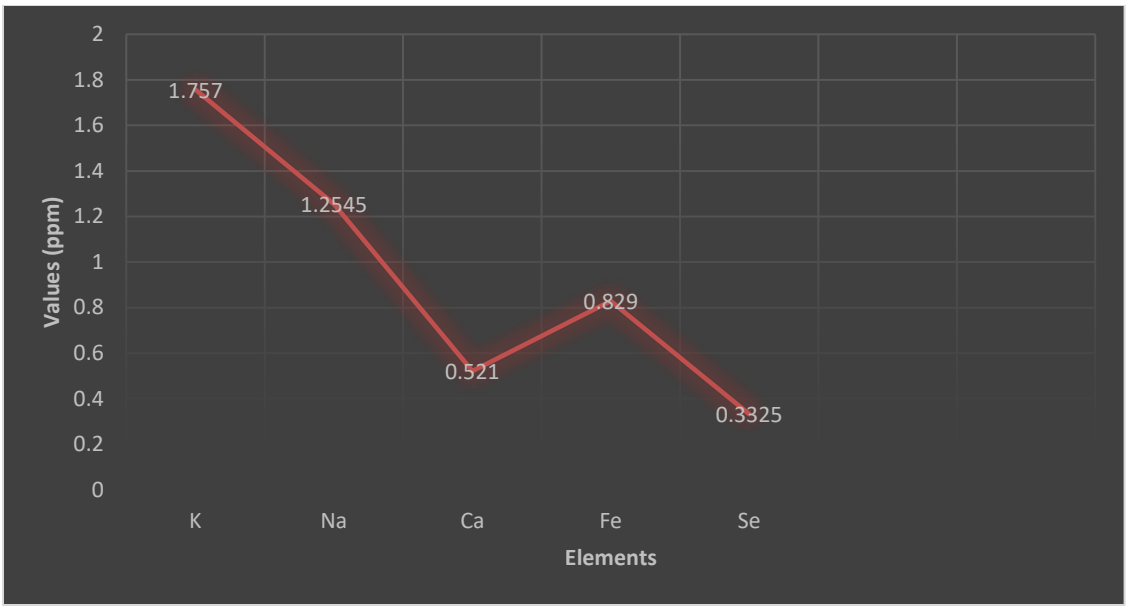

Figure 3: The Results of the Elemental Content of Pigeon Pea

DISCUSSION

Pigeon peas contain many bioactive constituents with therapeutic properties for diabetes, hepatitis, malaria, cancer, and other treatments (Orni *et al.,* 2018). The results of the proximate analysis of pigeon peas found in this analysis as shown in Figure 1, indicated that it contains 3.9015% ± 0.02 of ash. This value was in line with 3.21% ± 0.01, 4.35% ± 0.01 and 2.56% ± 0.05 obtained by Oyarekua *et al.,* (2022); A'yuni *et al.,* (2022); and Ayodele, (2017). Ash contents in food refer to the mineral and inorganic substances left after the food sample has been heated to a very high temperature thereby removing moisture, volatile and organic materials. Examples of the most common minerals and inorganic materials include calcium, magnesium, sodium and potassium, but smaller quantities of manganese, zinc, iron, etc., are also found in food materials (Petal *et al.,* 2020). The moisture content was 1.577% ± 0.01. Oke (2014) obtained 11.20% of the proximate analysis of *Cajanus cajan* leaves. Ayodele, (2017) pointed out that the moisture content of *cajanus cajan* seed native protein isolate was 3.69%. Moisture contents describe how dried a food substance has become, and that exposes the substance to bacterial spoilage if it is not well dried. However, the results obtained from this analysis indicated that the pigeon peas were dried and could last for a long time without spoilage. The fat content shown in Figure 1, was 1.8825% ± 0.03. Anemone (2020) obtained 1.43% ± 0.01 fat content in the

Isiyin PP species of pigeon peas. The amount of fat obtained in this analysis aligns with what Anaemene (2020), obtained. Fat is very essential for the proper functioning of the human body. Fat regulates cholesterol and blood clotting. It controls inflammation in the joints, tissues and blood. Fibre content was 6.5595% ± 0.05. The fibre content was higher than 3.20% ± 0.02 obtained by Anaemene, (2020). It was higher than 4.41% ± 0.06 obtained by A'yuni *et al.,* (2022). Fibre is important for the proper maintenance of a healthy weight and for lowering the risk of diabetes, heart disease and some types of cancer etc, (Mayo Clinic, 2023). Also, in Figure 1, the protein content of the pigeon peas was 12.775% ± 0.03, while 24.10% ± 0.03 was obtained by A'yuni *et al.,* (2022). Protein is essential in the repair and building of body tissues. The carbohydrate content was 73.3045% ± 0.01. This value was close to 69.48% ± 0.13 and 64.69% ± 0.02 obtained by A'yuni *et al.,* (2022) and Anaemene (2020) respectively. Carbohydrates are starches content of food that the body breaks down into glucose to obtain energy. The results of the vitamin B contents of pigeon peas presented in Figure 2, showed that vitamin B1 was 0.255 mg/kg ± 0.015. The results were in tandem with what Arukwe and Nwanekezi, (2022) obtained; they reported that the thiamine (vitamin B1) content of the blends of 50:50 ratio of sorghum and pigeon peas was 0.43mg/100 ± 0.02. Vitamin B1 (thiamin) is essential for energy metabolism and nerve function (Kumar *et al.,* 2017). Vitamin B2 exhibited a mean content of 0.316 mg/kg ± 0.006. The result aligns with 0.13 ± 0.04mg/100g of vitamin B2 content of a 50:50 ratio between sorghum and pigeon peas reported by Arukwe and Nwanekezi (2022). Vitamin B2 (riboflavin) is involved in the body's energy production and antioxidant defence mechanisms. The vitamin B3 content was 0.976 mg/kg ± 0.049, below 2.920mg/100 ± 0.03 of the vitamin contents of pigeon peas samples processed by different methods reported by Nwanekezi *et al.,* (2017). The disparity could be attributed to variations in soil conditions, preservation or processing methods, or pigeon peas varieties. Vitamin B3 (niacin) contributes to cellular metabolism and skin health, (Murray *et al.,* 2018). Vitamin B5 content was 2.473 mg/kg ± 0.270. Vitamin B5 (pantothenic acid) is necessary for coenzyme A synthesis and various metabolic processes, (Trumbo *et al.,* 2019). The vitamins B6, B7, and B9 were 0.370 mg/kg ± 0.033, 5.942 mg/kg ± 0.036 and 6.944 mg/kg ± 0.037, respectively.

Vitamin B6 (pyridoxine) is involved in neurotransmitter synthesis and red blood cell production (Gropper *et al.,* 2018), while vitamin B7 is important for energy metabolism and the formation of fatty acids and also plays a role in carbohydrate, fat, and protein metabolism, (Combs, 2019). Vitamin B9 (folate) is critical for DNA synthesis and cell division, (Bailey *et al.,* 2015). Lastly, in Figure 2, the vitamin B12 content of pigeon peas was 0.561 mg/kg ± 0.010. Vitamin B12 (cobalamin) is necessary for red blood cell formation and neurological function, (Combs, 2019). The elemental contents of the pigeon peas shown in Figure 3 indicated that it has reasonable amounts of K (1.757ppm ± 0.58), followed by Na (1.2545ppm ± 0.02), Fe (0.829ppm ± 0.11), Ca (0.52ppm ± 0.07), and Se (0.3325% ± 0.013). In a work by Anaemene (2020), on the comparative evaluation of the nutrient and anti-nutrient compositions of four pigeon peas varieties obtained 2.12mg/100g ± 0.03 of Fe and 94.43mg/100g of Ca, These elements play various roles in the proper functioning of the body Sodium plays a role in transmitting of nerve signals, muscle contraction, fluid balance and other chemical reactions in the body. Calcium is needed for bone formation. Selenium is a powerful antioxidant, while the iron is one of the most important minerals for the body needed for heamoglobin production, (Mayo Clinic, 2023).

CONCLUSION

This study has shown that pigeon peas contain reasonable quantities of carbohydrates, protein, fibre, vitamin B9, B7, potassium, and sodium, and these nutritional contents are crucial for harnessing their potential as a valuable dietary resource for the body.

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