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PROXIMATE COMPOSITION, MINERAL, TRACE ELEMENTS AND AMINO ACID COMPOSITION OF SEEDCOAT OF BLACK SEEDED COWPEA.

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***Abstract**: The seed coat of black-seeded Vigna unguiculata is often discarded as an agricultural byproduct, despite its potential nutritional and functional benefits. This study aimed to examine the proximate composition, mineral and trace element content and amino acid profile of the seed coat to assess its potential for food and feed applications. Samples of black seeded cowpea were collected from the International Institution of Tropical Agriculture (IITA), Tarauni, Kano State, Nigeria. The proximate composition were determined using AOAC methods. Mineral and trace element concentrations were quantified using Atomic Absorption Spectroscopy, while Sodium and Potassium were using flame photometer Model. Amino acid composition was analyzed using automatic amino acid analyzer. Results indicated that the seed coat is rich in dietary fiber and carbohydrates, with moderate protein content. Mineral analysis identified essential minerals and trace elements, indicating the potential role of the seed coat in combating micronutrient deficiencies. Amino acid profiling revealed the presence of essential and non-essential amino acids, indicating its potential as a supplementary protein source and further emphasizing its nutritional value. These findings suggest that the black seeded cowpea seed coat is a valuable yet underutilized agricultural byproduct, could serve as a functional ingredient for fortification in human and animal nutrition.*

Keywords: *Amino acid profile, Black seeded cowpea, FAO references, Mineral, Proximate composition, Seed coat and Trace elements.*

1.0 Introduction

Cowpea (*Vigna unguiculata)* is an important legume of the tropics, with various uses, as grains in processed foods, as a vegetable (fresh leaves, peas and pods) and as dry haulms and fodder [1]. It is an inexpensive source of vegetables protein, and a handy crop well adapted to relatively dry environments. *Vigna unguiculata* is a dicotyledonous belonging to the kingdom plantae, division spermatophyte, class dycotyledonea, order fabales, family fabaceae, subfamily fabadeae, tribe phaseoleace, sub tribe phaseolinae, genus vigna, specie unguiculata and section catiang[2, 3]. Vigna is a pan-tropical genus with several species, whose exact number varies according to authors. Plants of *Vigna unguiculata* are herbaceous annuals with slender stems and branches. Most plant characteristics vary depending on the genotype. Plant growth habit ranges from erect, semi-erect and spreading to climbing and twining, plant may have branches (borne from present on the main stem) or they may not [4].

Cowpea, also known as black eye beans or southern peas are major food legume in Africa they are extensively grown in Central and South America, [5]. More than 70% of world cowpea production is concentrated in three countries. Nigeria, Brazil and Niger [1]. Nigeria has about 4.0 Million hectares in cowpea and produces an estimated 1million tons annually most of it in the three Northern States of Kano, Sokoto and Borno. Brazil produces about 0.4tons on 1.7 million hectares and Nigeria 0.27 million tons on about 1.1 million hectares [6]. Other important cowpea producers are Senegal, Ghana, Togo and Cameroon in West Africa, Tanzania, Kenya and Uganda. Cowpea is of major importance to the livehoods of millions of poor people in the less developed countries of the tropics. From the production of these crops, rural families variously derive food, animal feed and cash together with spillover benefits to their farmlands through, in situ decay of root residues, use of animal manures and ground cover from cowpea spreading and low growth habit. In addition, the grain is widely traded out of the major production areas; it provides a cheap and nutritious food for relatively poor urban communities [7].

In fresh farm, the young leaves, immature pods and peas are used as vegetables, while several snacks and main meal dishes are prepared from the grain. All the plant parts that are used for food are nutritious, providing protein, vitamins and minerals. Cowpea grains contain an average 23-25% protein and 50-67% starch. Petty trading of fresh produce and processed foods provides both rural and urban opportunities for earning cash, particularly by women [7]. The nutritional value of legumes leaves, such as cowpea has been largely discounted due to their high water content and the difficulty of documenting their production and consumption [8]. Nutritionally, cowpea leaves compared well with other tropical leaf vegetable and with cowpea seeds compared to cooked cowpea leaves contain seven times more calcium and three times more iron [7]. The phosphorus content of cooked cowpea leaves is 50% that of cooked seeds. The phosphorus in leaves is not present as phytic acid, a storage form of phosphorus that accumulates in legume seeds [9, 10]. The minerals of cowpea leaves are more bioavailable than those in sees, because phytic acid reduces bio-availability of minerals such as calcium and iron [9, 10].

Compared to raw cowpea sees, raw leaves cowpea have about 20% thiamine, twice the riboflavin and equal amount of niacin. Cowpea leaves are sources of β-carotene and ascorbic acid. Like cowpea seeds [11, 12]. Cowpea leaves are excellent sources of folacin (334µg and 2012µg of free and total folacin per 100g of solids in raw leaves. [13]. However, freshly harvested cowpea leaves when cooked by the traditional l method retain β-canotene, but losses vitamin C and the free and total folacin were 87%, 49% and 66% respectively [13]. Recoveries in the cooking water were 5.6%, 20% and 12%, respectively. Other reports indicate that β-carotene and ascorbic acid are well retained in fresh or dried cooked leaves [14, 15]. Cowpea leaves commercially canned as “spinach” area good source of the minerals phosphorus, zinc, iron and vitamin-ascorbic acid, β-Carotene and folic acid [13].

Protein output from cowpea leaves is about 15times that from cowpea sees, because the leaves are produced earlier and in much greater quantity than the seeds [8, 17]. Cowpea leaf protein contents range from 29% to 43% protein on a dry weight basis (dwb), but vary with leaf age [18, 19]. Leaf protein content is higher than cowpea seeds protein content 21-33% dwb [20, 21, 22, 23, 24]. However, these seed and leaf protein values are calculated from total N, as determined by the standard micro Kjedahl procedure. Part of the N in cowpea leaves is not protein N, because vegetative plant material is known to accumulate nitrate and other non-protein N [25]. Using procedures to differentiate total, protein, ad NO_3 nitrogen, testing of cowpea grown in a controlled environment chamber indicated that 50-67% of the N contained in cowpea leaves in protein N. Of the non-protein N, 25-50% was nitrate N [26]. Such tests of field and greenhouse-grown cowpea leaves are needed to determine what portion of the leaf is protein N.

Proximate Composition

Proximate composition analysis provides valuable information about the nutritional content and quality of foods and biological substances, aiding in dietary assessment, food labeling and formulation of balanced diets. The components are categorized into several major groups which play a crucial role in determining the overall nutritional value and suitability of food or substance for various dietary needs.

A study by [26, 27, and 28] revealed that proximate composition of cowpea leaves changes as they expand. Protein contents of the greenhouse-grown cowpea (calculated from N) was 43% (dwb) for 7- to 10 – day – old expanding leaves and 30.5% (dwb) for 22 – to 25- day old expand leaves [26, 27] reported that the protein content of greenhouse-grown cowpea leaves harvested at 5 or 7 weeks did not differ among fie cultivars tested, but the mean protein content of leave harvested at 5 weeks (40.2% dwb) was higher than that of leave harvested at 7 weeks (37.9% dwb).

Fat (4-5%, dwb) and ash (14-17% dwb) content of greenhouse – grown cowpea leaves remained constant as they aged [27, 28]. The fat content of cowpea leave is similar (2-5%, dwb) under field, greenhouse, and controlled environment growth conditions. However, the ash content of cowpea leaves from controlled environment growth chambers (18-25%, dwb) is considerably higher than for greenhouse (14-17%) or field (12-14%) conditions [13, 26, 27 and 28]. The total dietary fibre content of greenhouse –grown cowpea leaves has been shown to increase with time to harvest, from 19% (dwb) at 20 days after planting to 26% (dwb) at 50 days after planting [28].

Mineral and Trace Elements

Minerals and trace elements are essential nutrients required by the human body in small amounts for various physiological functions ensuring proper growth and development. They are essential nutrients that the body cannot produce on its own and must be obtain through the diet.

Metals are present in both plants and animals as component of simple salts and complexes performing various functions such as physiological processes. In plants potassium, magnesium, calcium and iron are indispensable for growth. Manganese, zinc, molybdenum are essential and are required in trace [29].

Iron is an essential element in the formation of red blood cells, liver and spleen [30].

Amino Acids

Amino Acids are organic molecules containing at least one carboxyl group (-COOH) and one amino group ($-NH_2$).they are the essential constituents of plant and animal tissues [31]. They occur in plant cells both as free acids or amides [32]. Over 200 different amino acids have been found in higher plants and 20 are known to be the building blocks of protein found in the cytoplasm]33]. About ten of these are referred to as essential amino acids, because, animals lack the ability to synthesize them and must therefore be supplied in their diets [34]. These include isoleucine, lysine, phenylalanine, tryptophan, threonine, valine, methionine, histidine, and cystine [35, 36]. However higher plants, microorganisms and ruminants have the ability to synthesize these amino acids. The amino acids in protein are all α- type with L-configuration resembling L-glyceraldehyde, whereas the synthetic ones occur as race mates [.31, 35 and 37]. Amino acids may be acidic, basic or neutral as zwitterions depending on the pH. AT low pH they exist as acids and at high pH they are bases, whereas at a particular intermediate pH, they carry no charge although they still ionize [38].

1.2 Statement of Problem

The seed coat of black-seeded cowpea (*Vigna unguiculata*) is often considered a byproduct of processing and is largely discarded as waste, despite its potential nutritional and functional properties. While significant attention has been given to the proximate composition and nutritional value of the cowpea seed itself, limited scientific data exist regarding the nutrient profile of the seed coat, which may contain valuable macro- and micronutrients, trace elements, and essential amino acids. This gap in knowledge limits its utilization in food systems and as a potential source of nutrients in addressing malnutrition and food insecurity. Understanding the proximate composition, mineral content, trace elements, and amino acid profile of the seed coat is critical for evaluating its nutritional potential and exploring its use in food and industrial applications. Without this information, a potentially rich and sustainable resource remains underutilized, contributing to waste and missed opportunities for promoting dietary diversity, reducing food waste, and improving nutritional outcomes in communities where cowpea is a staple crop. This research aims to address this gap by analyzing the seed coat of black-seeded cowpea, thereby unlocking it's potential as a valuable dietary or industrial resource.

1.3 Objectives of the study

The objective of the study is to examine the proximate composition, mineral, trace element and amino acid composition of the seed coat of black seeded cowpea (*Vigna unguiculata*).

The specific objectives of the study are:

i. To determine the proximate composition, mineral and trace element.

ii. To analyze the amino acid composition.

iii. To evaluate Nutritional Potential and Health Benefits.

2.0 Materials and Methods

Sampling:

Samples of dry black seeded cowpea with an ID number IT24K-1101-5 were collected from the International Institute of Tropical Agriculture (IITA), Tarauni, Kano State. Nigeria.

Sample Treatment:

The seed coats were removed from the seed using milling machine. It was then crushed to a fine powder in a steel – bladed electric mill (National Food Grinder, Model Mu 308, Japan) and was sieved through a 40micometer mesh sieve [39]. Samples were oven dried at 102°C to a constant weight. The samples were then packed in a double stacked water proof polythene bag and stored in screw capped bottle before analysis.

Chemicals:

Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Petroleum ether, Potassium Sulphate, (K_2SO_4) Copper Sulphate ($CuSO_4$), Sulphuric acid (H_2SO_4), Boric acid, Methyl orange indicator, Nitric acid (HNO_3), Acetate buffer.

Determination of Moisture Content

Moisture content was determined by drying in a thermostatically controlled oven at 105^{0}C to constant weight. The moisture content was calculated by the weight difference [40].

Determination of Ether Extract

The ether extract was determined using ether extraction by reflux soxhlet method. The experiment was repeated using different weights of sample and the fat content was calculated by difference [40].

Determination of Crude Protein

The Kjedahl method was used to determine the crude protein. From this percentage nitrogen in the sample was obtained and from which the percentage crude protein was calculated [40].

Determination of Crude Fibre

The crude fibre in the seed coat was determined using AOAC method [40].

Determination of Ash Content

The furnance method was used to determine the ash content at 600^{o}c for 6 hours. The experiment was repeated for different sample weights and average result was taken [41].

Determination of Mineral and Trace Elements

The determination of copper, calcium, lead, manganese, zinc, chromium, magnesium, iron and nickel were carried out after digestion of the ash content at their respective wavelengths using Alpha-4 Model AAS. Sodium and potassium were determined using flame photometer Model at their respective wavelengths.

Determination of Amino Acid Composition

The amino acids profiles of the seed coat were quantitatively analyzed [42] using automatic amino acid analyzer (Technicon (TSM) Sequential multisample amino acid analyzer). Samples were hydrolysed for determination of amino acids except for tryptophan in constant boiling of 6mol/dm^3 hydrochloric acid for 24 hours under nitrogen flush [43].

3.0 Results and Discussion

Seed coat of *vigna unguiculata* (cowpea) was analyzed for proximate composition, mineral, trace elements and amino acid composition. The results of the analyses are shown in Tables 1-5.

Table 1: Percent proximate composition of seed coat of *Vigna unguiculata* (cowpea).

Parameter	Percentage
Moisture	3.43 ± 0.47
Ash	3.29 ± 0.10
Crude protein	9.30 ± 0.77
Crude ether extract	9.32 ± 0.33
Crude fibre	28.3 ± 2.02
NFE	49.84 ± 1.17
Dry matter	96.49 ± 0.49

The proximate composition of the seed coat of *Vigna unguiculata* (cowpea) is as shown in Table 1. The moisture content of the dry sample is 3.43 ± 0.47% ad is within the accepted range for keeping [44]. The ash content was 3.29 ± 0.10%. The crude protein has a mean of 9.30 ± 0.07 %. The crude ether extract is 9.32 ± 0.33% whilst its nitrogen free extract is 49.84 ± 1.17%. The crude fibre content is 28.3 ± 2.02% while the dry matter has a mean value of 96.59 ± 0.49%. The high crude fibre content indicates the potential use of the seed coat as a dietary fibre source, while the moderate protein content suggests some nutritional value.

Table 2: Mean Trace Metal Concentration (mg/100g) in the seed coat of cowpea (*Vigna unguiculata*).

Element	Concentration (mg/100g)
Calcium	22.9 ± 2.99
Chromium	1.70 ± 0.00
Copper	2.75± 0.35
Iron	5.50 ± 0.71
Lead	2.50 ± 1.65
Magnesium	9.00 ± 1.13
Manganese	0.38 ± 0.18
Nickel	0.75 ± 0.35
Potassium	6.70 ± 0.00
Sodium	28.75 ± 8.84
Zinc	8.50 ± 0.71

The mean metal concentrations are shown in Table 2. This indicates high concentration of calcium and sodium. The high concentrations of this metal may be related to their physiological roles in the seed coat. The order of metal concentrations in seed coat is Na> Ca >Mg> Zn> K> Fe >Pb >Cr >Ni >Mn. The mineral analysis showed that the seed coat contained significant levels of essential minerals and trace metals highlighting the potential contribution of the seed coat to micronutrient intake.

Table 3: Amino Acid composition of seed coat of *Vigna unguiculata* (cowpea)

Amino Acid	g/100g Protein
Alanine	2.61 ± 0.13
Arginine	5.14 ± 0.18
Aspartic Acid	7.98 ± 0.05
Cystine	2.16 ± 0.20
Glutamic Acid	10.20 ± 0.21
Glycine	3.01 ± 0.04
Histidine	1.48 ± 0.03
Isoleucine	3.01 ± 0.04
Leucine	7.21 ± 0.11
Lysine	4.12 ± 0.12
Metheonine	1.00 ± 0.03
Phenylalanine	4.07 ± 0.10
Proline	2.92 ± 0.15
Serine	2.96 ± 0.06
Threonine	3.15 ± 0.20
Tryptophan	ND
Tyrosine	3.13 ± 0.10
Valine	4.06 ± 0.08

Table 4: Essential Amino Acid in Seed coat of *Vigna unguiculata* (cowpea) compared with FAO reference.

Essential Amino Acids	FAO Reference	Seed coat of *Vigna unguiculata* (cowpea)
Histidine	2.40	1.48
Isoleucine	4.20	3.14
Leucine	4.80	7.21
Lysine	4.20	4.12
Metheonine	2.20	1.00
Phenylalanine	2.80	4.07
Threonine	2.80	3.15
Tryptophan	1.40	ND
Valine	4.20	4.06

Quantitative chromatographic analysis of seed coat hydrolysate revealed seventeen amino acids (Table 3).

A comparative study of its hydrolysate and that of FAO reference revealed that the amino acid were in sufficient quantity and are essential for man and animal (Table 4). The seed coat was rich in essential amino acid except methionine. The non- essential amino acid such as glutamic acid and aspartic acid were predominant, contributing to the overall nutritional quality of the seed coat. The sulphur amino acid is significant in diet not only for growing children but nursing mothers and pregnant women as its absence has been reported to fetal resorption in pregnant rats [45].

Table 5: Chemical Score of Seed coat of *Vigna unguiculata* (cowpea) Amino acids relative to hen's egg [46].

Amino Acid	Seed coat	Hens egg	% Chemical Score
Alanine	2.16	5.87	44.46
Arginine	5.14	7.06	72.80
Aspartic acid	7.92	5.80	137.59
Cystine	2.16	0.64	337.50
Glutamic acid	10.30	3.36	306.55
Glycine	3.01	13.14	22.91
Histidine	1.48	2.96	50.00
Isoleucine	3.14	7.32	42.90
Leucine	7.21	9.58	75.26
Lysine	4.12	7.26	56.75
Methionine	1.00	3.52	28.41
Phenylalanine	4.07	6.58	61.85
Proline	2.92	4.43	65.91
Serine	2.92	7.45	39.73
Threonine	3.15	1.92	164.06
Tryptophan	-	4.96	-
Tyrosine	3.13	-	-
Valine	4.06	8.04	50.50

Comparing the amino acid of the seed coat with that of hen's egg (Table 5). However, it is deficient in glycine, metheonine and serine.

4.0 Conclusion

The proximate composition of black seeded cowpea seed coat highlights its substantial nutritional value. The analysis reveals a significant presence of dietary, carbohydrate, protein and essential minerals, along with trace elements which indicating the seed coat's contribution to dietary micronutrient intake and to the overall health. The amino acid profiles demonstrate that the seed coat contains a range of essential amino acids, making it a valuable component in dietary protein sources. These findings underscore the seed coat's potential as a functional ingredient in nutrition, suggesting its utility in enhancing dietary quality and promoting health benefits. The result also showed that these samples were of high nutritive values and can be used as supplement in diet.

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