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Chemical Composition and Functional Properties of Protein Concentrate from Selected Cowpea Seeds in Nigeria

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Abstract

Cowpea protein concentrates were extracted by isoelectric precipitation from four local cowpea seeds (*Achishuru, Dan-Borno, Dan-Kurmi* and *Danila*) in Nigeria and designated as samples A, B, C and D respectively. The samples were evaluated for their chemical composition and functional characteristics. Samples had significantly ($p < 0.05$) high crude protein (75.50 to 86.00%) and low fat content (0.01 to 0.07%). Samples A, C and D compared favorably in proximate parameters, sodium, potassium and iron contents. The essential amino acids of sample A (30.26g/16g) was significantly ($p < 0.05$) high, followed by samples B (28.11g/16g) and C (27.22g/16g) while sample D (24.90g/16g) had the lowest value. Samples were not significantly ($p > 0.05$) different in functional properties measured except in nitrogen solubility where sample D was significantly ($p < 0.05$) high. High nutrient composition of sample A suggest its application in food formulation requiring high nutrient density.

Keywords: *Cowpea seeds; Protein Concentrate; Chemical Composition; Functional Properties*

Introduction

Legumes play a vital role in human nutritionas source of protein, carbohydrate, some vitamins and minerals [1]. Cowpea (*Vigna unguiculata* (L) Walp) is a dicotyledonous plant belonging to the family *Fabaceae* and Sub-family *Fabiodeae*. Like other grains, legumes serve as vital food stuff in both tropical and sub-tropical countries [2]. Protein malnutrition is one of the major nutritional problems in the developing and under developed countries and the major cause of illnesses like kwashiorkor and marasmus among children and the elderly [3]. The existing problems of food insecurity and malnutrition coupled with increasing population, tentative crop yield and high cost of animal based food supply in Nigeria and other developing and under- developed countries have urged contemporary researchers to identify and incorporate other cheap source of proteins to enrich our traditional food formulation. Cowpea protein provide an excellent solution to Protein-Energy-Malnutrition (PEM). Furthermore, all part of the plant used as food are nutritious providing proteins, carbohydrate, vitamins and other essential nutrient to the body.

Generally, there are two main sources of protein: animal protein (meat, fish, eggs, poultry, and milk) which are referred to as "first class protein" because they contain all essential amino acids and plant protein (soybean, peanut, cowpea etc.) considered to be "second class protein" because they lack one or two essential amino acids [4].

Protein concentrate refers to protein product that contains 70-80% protein with other nutrient present in small quantity and is used as a supplement to human and animal diet. Protein concentrate differs from protein isolates in that protein isolates are the most refined form of protein containing about 90-95% protein with no fiber [4]. Umar., *et al.* [5] reported that, the need for relatively cheap sources

of protein that can be incorporated to value added food products is increasing worldwide and numerous researches are still on-going on various sources of plant proteins that may help to improve the nutritional value of food products at a very low cost.

The application of any protein in food either as supplement, nutritional enhancer, functional inducer etc. largely depends on the chemical composition and functional behavior of such proteins. Understanding the functional characteristics of various refined flours is essential in determining their potential uses and incorporation into different product formulation. Therefore, the present study is to determine chemical composition and functional properties of protein concentrate from four (4) local varieties of cowpea in Nigeria namely *Achishuru, Dan-Borno, Dan-Kurmi*, and *Danila*. This would expand their utilization in food formulation and new product development.

Materials and Methods

Source of material

Four (4) varieties of cowpea seeds namely, *Achishuru, Dan-Borno, Danila*, and *Dan-Kurmi* were purchased from Kure Ultra-Modern Market Minna, Niger State Nigeria.

Raw material preparation

Cowpea flour was prepared as described by [2]. The seeds of each varieties were separately cleaned of extraneous material such as stones, sand, chaff, metals, unhealthy seeds, and insect infested seeds prior to milling. The seeds were separately milled using an attrition miller and sieved to a fine flour of 1 mm particle size. The flour was defatted following the method described by [5].

Protein concentrate extraction

Protein concentrate was extracted from each varieties using the method described by [6] with some modification. The flour was dissolved in distilled water at ratio 1:5 (w/v) and the pH of the suspension was adjusted to pH 8.0 with 4N NaOH. The mixture was stirred at room temperature for 60 min and centrifuged at 4000 rpm for 20 min. The insoluble portion was discarded and the supernatant was adjusted to pH 4.5 with 4N HCl and stirred at room temperature for 20 min. Protein concentrate was re-centrifuged at 4000 rpm for 20 min. The residue was washed by re-dissolving the precipitate using distilled water and then neutralized to pH 7.0 with 4N NaOH prior to drying. The protein precipitate was air dried and then followed by oven drying at 400C for 50 min.

Proximate determination

Determination of moisture content

The procedure of [7] was used. A porcelain crucible was washed and dried in a hot air oven for 30 min. at 1050C. It was then cooled in a desiccator for another 30 min. The crucible was then weighed and 2g of the sample was poured into the crucible dish and recorded as W1 and W2 respectively. The crucible and the content were placed in an oven at 1050C for 3 hours. It was then removed, cooled in the desiccator for 30 minute and weighed recorded as W_3.

$$\% \ Moisture \ Content = \frac{W_3 - W_1}{W_2} \times 100$$

Crude protein determination

Method as described by [7] was used. 0.5 g of sample was weighed into 500 ml Kjeldahl flask. One tablet of catalyst (Selenium) and 20 ml of 25% concentration of sulphuric acid (H2SO2) was added and the flask was fixed into Kjeldahl digestion plate. Digestion lasted for 6 hours and the liquid was clear and free from brown or black coloration. The digested mixture was allowed to cool and made up to 100 ml in a conical flash. 2 drops of indicator (2% methyl red) was added and placed under the collection spigot of the distillation apparatus. 10 ml of the digester was pipetted into stopper portion of the condenser and 10 ml of 40% sodium hydroxide solution was added the solution was allowed to distill for 15 minutes or when the volume of ammonia collected in basic acid in the receiver flask was 50 ml and when the red solution had turned blue, the distillate was then titrated against 0.1 M hydrochloric acid (HCl) to a pinkish colour. The protein was calculated as:

$$\%\ gram\ Nitrogen = \frac{T \times 0.014 \times Molarity\ of\ HCl \times Dilution\ factor}{Weight\ of\ sample} \times 100$$

% crude protein = %gram Nitrogen X 6.25

Where; T = Titre value.

Determination of crude fiber

The crude fiber was determined using the [7] method. 2g of the sample was weighed into 500 ml beaker and boiled in 200 ml HCl (10% V/V) for 30 minutes. The suspension was filtered and the residue was washed vigorously with distilled water until it was no longer acidic. It was then boiled in 200 ml 1.25 M NaOH for 30 minute filtered through Whattman filter paper (No. 1) and then washed with distilled water. The residue obtained was transferred into a pre weighed crucible in hot air oven for 30 minutes, then cooled in desiccator and reweighed.

$$\%\ crude\ fiber = \frac{W_2 - W_3}{W_1} \times 100$$

Where; W_1 = weight of sample used; W_2 = weight of crucible + sample; W_3 = weight of sample in crucible + ash.

Determination of ash

[7] method was adopted for ash determination. The weight of crucible dish was taken and 2 g of the sample was added to the crucible and place in a muffle furnace rack and the temperature was set to 5000C for 16 h until there was complete ash. The ash in the crucible dish was removed and kept in desiccator to cool before it was weighed and the percentage ash calculated as:

$$\%\ Ash = \frac{Total\ weight\ of\ extracted\ ash}{Weight\ of\ sample} \times 100$$

Fat determination

The fat was determined as described by [7]. 2 g of the sample was weighed and the weight of the flat bottom flask was also taken with the extractor in it. The weighed sample was carefully transferred into the thimble. Extraction was carried out using petroleum ether (boiling point 60^0C), the thimble was blocked with cotton wool and the extraction carried out continuously for 8 h. The solvent was evaporated using water bath and the remaining sample dried at 105^0C for 60 min in an oven after which it was placed in desiccator to cool. The flask was weighed again and % fat calculated as follows:

$$\%\ fat = \frac{Weight\ of\ extracted\ fat}{Weight\ of\ sample} \times 100$$

Carbohydrate determination

Carbohydrate content of the protein concentrate was determined by subtracting from 100% the addition of percentage values of moisture, ash, crude protein, crude fiber, and fat content.

% Carbohydrate = 100 – (% moisture + % ash + % protein + % crude fiber + % fat).

Determination of minerals

Preparation of solution

This was prepared by the procedure outlined by [7]. 2 g of the sample was transferred into beaker and 30 ml of digestion acid (Hydrochloric acid) was added. The beaker was covered with a watch glass and allowed to stand overnight. The covered beaker was placed on a thermostatically controlled hot plate maintained at approximately 1000C. When the initial reaction has subsided, the temperature of the hot plate was increased sufficiently to maintain the oxidation but with evaporation of nitric acid or noticeable reduction in the volume of

beaker contents. The samples were heated continuously for 2 h until oxidation was apparently completed. The beaker content was clear red brown liquid when oxidation appeared to be completed the temperature of the hot plate was increased to 180-2000C. The watch glass was sided and excess nitric acid was allowed to evaporate from the beaker reducing the content to approximately 5 ml until white fumes appeared. The temperature of hot plate was increased to 2400C and heating was continued until the per chloric acid volatilized and the beaker contained a dry residue. The beaker was removed from the hot plate and cooled. 10 ml of approximately 2M hydrochloric acid was added and brought to boil and gently simmered for approximately 5 min. Watch glass was removed and rinsed. The washing was collected in a beaker without delay. The content of the beaker was transferred to a 50 ml volumetric flask and allowed to cool and diluted to 50 ml. It was then filtered through 90 mm Whattman number 541 filter paper, the first few ml was rejected and the remainder was retained and a blank determination was carried out.

Quantitative analysis of mineral

The samples were analyzed with Buck Scientific Atomic Absorption Spectrophotometer (AAS) as described by [7]. The samples were digested and filtered with Whattman number 1 quantitative circle 125 filter paper. The filtrate was placed in different cuvettes and labeled accordingly. Since each metal has a characteristic wavelength that will be absorbed, the Specific Hitachi Hollow Cathode Lamps were selected accordingly. The slit width for each element was also identified. Each sample of interest was aspirated into the flame. The metal present in the sample absorbed the same light, thus reducing the intensity of the light. The computer data system converted the change in intensity of light into an absorbance which was directly proportional to the concentration of the metal ions present in the sample. The concentrations of the metals present were determined from the working curve after calibrating the instrument with standards known concentration.

Amino acid determination

The Amino Acid profile in the known sample was determined using methods described by [9]. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM).

Defatting of sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus [9].

Functional properties

Bulk density

The bulk density was determined as described by Kaur (2005). 2g of flour sample was put gently into 10ml graduated cylinder and the bottom of the cylinder was tapped several times on a laboratory bench until there is no further diminution of the sample level. The ratio of mass of the samples to their volume was recorded.

$$Bulk\ density\ \left(g/ml\right)=\frac{Weight\ of\ sample(g)}{Volume\ of\ sample\left(ml\right)}$$

Protein solubility index

The method described by Yu., *et al.* was used to determine the solubility index. The concentrate was mixed with water at a ratio of 1/20 (w/v) and the suspension was stirred at room temperature for 1 hour and then centrifuged at 3000rpm for 20 minutes. The protein concentration in each supernatant was determined by Kjeldahl method using digestion block and 6.25 as the conversion factor.

Protein solubility was calculated as percentage solubility = W_1/ W_0

Where; W_1= weight of amount of the protein in the supernatant (g); W_0= weight of amount of protein in the sample (g).

Foam capacity (FC)

The capacity of foam was determined by blending 2g of sample with distilled water in a warring blender and whipping the suspension at 1600rpm for 5 minutes and immediately transferring into 250ml graduated cylinder. The volume was then recorded before and after whipping. The foaming capacity was expressed as the percentage volume induced by whipping.

$$FC = \frac{Volume\ after\ whipping\ -\ Volume\ before\ whipping}{Volume\ before\ whipping} \times 100$$

Water/oil absorption capacity

Water/Oil absorption were determined by mixing 1g of sample with 10ml distilled water/vegetable oil for 30 seconds using a warring whirl mixer. The sample was allowed to stand for 30 minutes at room temperature and then centrifuged at 3000rpm for 30 minutes. The volume of the supernatant was read directly from the graduated centrifuge tube. Water absorption was expressed as the amount of grams of water absorbed per gram of sample [10] while the oil absorption was expressed as gram of oil absorbed per gram of protein isolate [10]. Water and oil have densities of 1.0 and 0.92g/ml respectively.

Water and oil absorption capacity were calculated as follows:

$$FC = \frac{Volume\ of\ total\ water\ or\ oil\ added\ to\ the\ sample\ -\ Volume\ of\ free\ water\ or\ oil}{Weight\ of\ the\ sample\ taken}$$

Emulsifying capacity

[12] was adopted for the determination of emulsion capacity. 2g protein concentrate was homogenized for 30 seconds with 25ml distilled water using warring blender. After complete dispersion, 25ml of vegetable oil was gradually added and continued with the blending for another 30 seconds. The mass was transferred into centrifuge tube and centrifuged at 1,600rpm for 5 minutes. The volume of oil separated from the sample after centrifuge is read directly from the tube. The emulsion capacity is expressed as the amount of oil emulsified and held per gram of sample

Emulsion Capacity = X x Y/100

Where X = height of emulsified layer, Y = height of whole solution in the centrifuge tube

Gelation capacity

Sample suspensions of 2 – 20% (W/V) in 5ml distilled water was prepared in test tubes and the sample test tubes were heated for 1hr in a boiling water bath followed by rapid cooling under running cold tap water and the test tubes were further cooled for 2h at 40C [12].

Statistical analysis

The data obtained was subjected to analysis of variance (ANOVA) and separation of the mean values was carried out using Duncan Multiple Range Test at ($p < 0.05$) level.

Result and Discussion

Proximate composition

The overall assessment of the composition and nutritional status of any ingredient intended for food use depend on the chemical composition. The result for the proximate composition (Table 1) of the protein concentrate shows that cowpea varieties used in this study showed no variability in protein except sample B (*Dan-Borno*) which showed significantly ($p < 0.05$) low content. The protein content obtained in this study (75.50 - 86.00%) agrees with the findings of [13] who reported 85.82% protein for Bambara bean protein concentrate and [14] who reported 85.97% protein in protein isolate extracted from Bambaranut. The value also compared favorably with 82.95%, 89.25%, 85.46% and 83.61% reported by [1] for protein isolates from pigeon pea, cowpea, mung bean and pea respectively. The high protein content obtained in this study implies that, the concentrates can be utilized in cereal-base-foods to improve their protein content thereby alleviating protein - energy malnutrition.

Parameter (%)	A	B	C	D
Crude protein	$86.00^{a} \pm 2.88$	$75.50^{b} \pm 2.12$	$81.65^{a} \pm 0.92$	$84.00^{a} \pm 0.00$
Moisture	$1.25^{a} \pm 0.35$	$1.60^{a} \pm 0.14$	$1.61^{a} \pm 0.13$	$1.51^{a} \pm 0.01$
Fat	$0.07^{a} \pm 0.00$	$0.03^{a} \pm 0.00$	$0.01^{b} \pm 0.00$	$0.04^{b} \pm 0.00$
Ash	$2.55^{a} \pm 0.07$	$1.00^{b} \pm 0.00$	$2.50^{a} \pm 0.00$	$2.50^{a} \pm 0.00$
Crude fibre	$2.67^{a} \pm 0.01$	$0.67^{c} \pm 0.00$	$0.67^{c} \pm 0.01$	$1.33^{b} \pm 0.01$
Carbohydrate	$7.50^{c} \pm 3.11$	$21.50^{a} \pm 2.12$	$13.50^{b} \pm 0.70$	$10.80^{b} \pm 0.28$

Table 1: *Proximate composition of the protein concentrate Values are means±standard deviation of duplicate determination. Means in the same row not followed by the same super scripts are significantly (P < 0.05) different.*

Key: A = Achishuru (black bean); B = Dan-Borno (large size brown bean); C = Dan-Kurmi (large size white bean); and D = Danila (medium size black eye white bean).

The moisture content of the concentrate flour in this study 1.25-1.61% indicates that, the concentrates can be stored for longer period of time without spoilage due to low water activity. The results obtained in this study were low compared to 8.88% and 8.92% moisture content for cowpea and Bambara nut protein concentrates reported by [13,15].

The lipid content 0.01-0.07% in this study were similar to the finding of [14] for Bambara nut protein isolate and low compared with 0.27% for walnut protein concentrate [16]. The low lipid content obtained in this study was as a result of defatting process prior to concentration. However, the results obtained in this study was low compared to 2.38% lipid for cowpea protein concentrate and 13.15% lipid content for Bambara nut flour [15]. Samples A, B and D showed significantly ($p < 0.05$) high fat. The low lipid content observed in this study implies that, the concentrates would have little or no lipid peroxidation.

The ash content of sample B (1.00%) was significantly ($p < 0.05$) low compared with 2.55% and 2.20% for samples A, C and D respectively. The ash content obtained in this study 1.00 to 2.55% agrees with 2.67% and 2.55% reported by [17] and [16] for cowpea protein isolate and walnut protein concentrate respectively. However, the value is fairly low compared to 3.37%, 3.96%, 4.36% and 13.96% for pigeon pea, cowpea, mung bean and peas protein isolates respectively [3,14].

Sample A (2.67%) was significantly ($p < 0.05$) high in fiber while samples B and C had the least fiber content. The results obtained in this study 0.67-2.67% is in line with earlier findings such as 1.82% fiber for Bambara bean protein concentrate [15] and 1.25-2.83%fiber for pigeon pea and 1.54-1.81% fiber for cowpea [18].

The result of this study shows that sample B showed significantly ($p < 0.05$) high carbohydrate content (21.50%) while sample A had the least value (7.50%). This results shows that seed varieties account for variation in carbohydrate content. The carbohydrate yield of sample B was significantly ($p < 0.05$) high compared with 8.8% carbohydrate in cowpea protein concentrate [15] and 13.00% carbohydrate in Bambara protein concentrate [15,17] further reported that flatus causing oligosaccharide such as starchyose, raffinose etc., are eliminated during concentrate preparation. The variations in the chemical composition of the protein concentrate in this study could be attributed to species, genetic factors, environmental condition and soil type.

Mineral composition

The result for the mineral composition of the flour concentrate is shown on (Table 2). Samples A and C were significantly ($p < 0.05$) high in sodium while sample B had the least value (29.95mg/100g). The value obtained in this study 29.95-43.90mg/100g was low compared to 72mg/100g for whole sesame seeds [19]. Samples A and D showed significantly ($p < 0.05$) high potassium content while sample B had the lowest content (14.50mg/100g). Calcium was not detected in the samples. This implies that cowpea concentrates constitute poor source of calcium most especially the ones used in this study. Concentrate flours showed no variation in iron content except sample C (*Dan-Kurmi*) that had the lowest content (1.79mg/100g). Cowpea samples used in this study can serve as sources of iron. Their increase

consumption will help in addressing problems of anemia. However, the potassium content 14.50 - 172.50mg/100g and iron content 1.79 - 2.70mg/100g were low compared with 580mg/100g potassium and 10.6mg/100g iron in sesame seed flour [19]. Also, the iron content was also low compared to 20.85mg/100g in Bambara protein concentrate [15].

Parameter (mg/100g)	A	B	C	D
Sodium	$43.90^{a} \pm 0.14$	$29.95^{c} \pm 1.44$	$42.99^{a} \pm 0.01$	$36.99^{b} \pm 1.41$
Potassium	$172.50^{a} \pm 1.56$	$14.50^{c} \pm 0.71$	$99.95^{b} \pm 1.35$	$149.97^{a} \pm 0.52$
Calcium	ND	ND	ND	ND
Iron	$2.55^{a} \pm 0.49$	$2.70^{a} \pm 0.28$	$1.79^{b} \pm 0.02$	$2.15^{a} \pm 0.44$
Manganese	$0.78^{a} \pm 0.04$	ND	ND	ND

Table 2: *Mineral composition of the cowpea protein concentrate Values are means±standard deviation of duplicate determination. Means in the same row not followed by the same super scripts are significantly (P < 0.05) different.*

Key: A = Achishuru (black bean); B = Dan-Borno (large size brown bean); C = Dan-Kurmi (large size white bean); and D = Danila (medium size black eye white bean); ND = Not Detected.

Amino acids composition

(Table 3) shows the amino acid composition of the flour concentrate. The result of the essential amino acid shows that, samples A and B had significantly ($p < 0.05$) high histidine, isoleucine, leucine, lysine, threonine, and phenyl alanine while tryptophan and valine were not detected in the samples. Leucine (6.23-7.25g/16g) and lysine (5.20-5.71g/16g) contents obtained in this study favorably compares with 9.45g/16g leucine and 6.50g/16g lysine for Bambara bean protein concentrate [15]. Samples showed no variation in cereal limiting amino acid (lysine). The result of the total essential amino acid reveals that sample A was significantly ($p < 0.05$) high (30.26g/16g) followed by samples B and C while sample D had the lowest value (24.90g/16g). This result implies that local cowpea varieties are good sources of essential amino acids. The trend of essential amino acids distribution in the samples was similar with non-essential amino acids. Samples A and B were significantly ($p<0.05$) high in alanine, arginine, aspartic acid, glutamic acid, glycine, proline, serine and tyrosine. The high in arginine 6.19-8.79g/16g, aspartic acid 7.94-8.79g/16g and glutamic acid 11.39-12.79g/16g reported in this study agree with earlier report by [20]. The fairly low value of Sulphur containing amino acids such as methionine, cysteinein the samples could be attributed to the high loss of albumins during extraction process which are rich in Sulphur amino acid such as lysine, methionine and cysteine [21].

Amino acids (g/16g)	A	B	C	D
Essential amino acids				
Histidine	$3.14^{a} \pm 0.04$	$3.08^{a} \pm 0.03$	$2.79^{b} \pm 0.12$	$2.32^{b} \pm 0.17$
Isoleucine	$3.99^{a} \pm 0.01$	$3.47^{a} \pm 0.11$	$3.07^{c} \pm 0.09$	$3.01^{c} \pm 0.04$
Leucine	$7.25^{a} \pm 0.04$	$6.57^{ab} \pm 0.30$	$6.75^{b} \pm 0.12$	$6.23^{c} \pm 0.16$
Lysine	$5.71^{a} \pm 0.02$	$5.41^{ab} \pm 0.11$	$5.20^{b} \pm 0.01$	$5.20^{b} \pm 0.28$
Methionine	$1.42^{a} \pm 0.04$	$1.21^{ab} \pm 0.01$	$1.41^{ab} \pm 0.10$	$1.06^{b} \pm 0.21$
Threonine	$3.43^{a} \pm 0.05$	$3.40^{a} \pm 0.11$	$3.18^{b} \pm 0.06$	$2.99^{c} \pm 0.19$
Tryptophan	ND	ND	ND	ND
Valine	ND	ND	ND	ND
Phenylalanine	$5.32^{a} \pm 0.18$	$4.97^{b} \pm 0.05$	$4.82^{b} \pm 0.09$	$4.09^{c} \pm 0.05$
Total	$30.26^{a} \pm 0.34$	$28.11^{b} \pm 0.72$	$27.22^{b} \pm 0.59$	$24.90^{c} \pm 1.10$

Non-essential amino acids				
Alanine	$3.93^{a} \pm 0.06$	$3.93^{a} \pm 0.06$	$3.41^{b} \pm 0.03$	$3.09^{b} \pm 0.05$
Arginine	$7.09^{b} \pm 0.04$	$8.79^{a} \pm 0.15$	$6.48^{c} \pm 0.02$	$6.19^{c} \pm 0.27$
Aspartic acid	$8.79^{a} \pm 0.15$	$8.50^{a} \pm 0.16$	$8.03^{b} \pm 0.08$	$7.94^{c} \pm 0.07$
Cystine	$1.30^{b} \pm 0.11$	$1.24^{b} \pm 0.01$	$2.04^{a} \pm 0.05$	$1.14^{b} \pm 0.04$
Glutamic acid	$12.79^{a} \pm 0.04$	$12.64^{ab} \pm 0.50$	$11.97^{ab} \pm 0.04$	$11.39^{c} \pm 0.07$
Glycine	$3.98^{a} \pm 0.05$	$3.64^{b} \pm 0.19$	$3.28^{c} \pm 0.01$	$3.05^{c} \pm 0.08$
Proline	$2.78^{a} \pm 0.00$	$2.84^{a} \pm 0.08$	$2.60^{b} \pm 0.07$	$2.37^{c} \pm 0.07$
Serine	$3.29^{a} \pm 0.01$	$3.16^{ab} \pm 0.02$	$3.03^{bc} \pm 0.10$	$2.89^{c} \pm 0.13$
Tyrosine	$2.94^{a} \pm 0.06$	$2.86^{a} \pm 0.07$	$2.36^{b} \pm 0.06$	$2.19^{b} \pm 0.05$
Total	$46.89^{a} \pm 0.52$	$47.60^{a} \pm 1.24$	$43.20^{b} \pm 0.46$	$40.25^{c} \pm 0.83$

Table 3: *Amino acids composition of the cowpea protein concentrate. Values are means± standard deviation of duplicate determination. Means in the same row not followed by the same super scripts are significantly (P < 0.05) different.*

Key: A = Achishuru (black bean); B = Dan-Borno (large size brown bean); C = Dan-Kurmi (large size white bean); and D = Danila (medium size black eye white bean); ND = Not Detected.

The essential amino acids of the concentrate most especially histidine, isoleucine, leucine, and lysine met the daily requirement (mg/day/Kg body weight) for infants (3 to 4 months), children (2 years) and adult [22]. For example, I gramme of the concentrate supplies 356.88mg of lysine. This is capable of providing more than 3 times, 8 times and 7 times lysine requirement per day per Kg body weight for infants, children and adults respectively.

Functional properties of cowpea protein concentrate

The result of the functional properties of the flour concentrates is shown in (Table 4). Bulk density indicates the porosity of a product that affects package design and could be used to determine the type of packaging material required [11,23] reported that the high bulk densities of flours suggest their suitability for use in various food preparations and it is desirable for greater ease of dispersibility of flours. In this study, the result obtained (0.925g/ml to 1.09g/ml) was high compared to 0.80g/ml for Tona and 0.69g/ml for Nhyira cowpea varieties in Ghana [24]. High bulk density reduces paste thickness which is an important factor in complementary food. In contrast, low bulk density is important in infant feeding and the formulation of complementary foods [25]. High bulk density also implies that the required packaging material for the product will be denser than other packaging material [23]. The result in this study implied that, different varieties of cowpea used did not in any way influence the bulk density of their concentrate.

Emulsifying capacity is the protein present in the sample acting as an oil/water interface to form a stabilized fat emulsion and it is related to high solubility and protein content [26]. The result obtained in this study (73.93% to 83.16%) agrees with [27] who reported 80.25% emulsion capacity for protein concentrate from black cowpea stating that emulsifying capacity depends on the pH and hydrophilic-lipophilic balance of soluble protein. The result is however higher than those reported for defatted yam bean flour (35.7% - 36.0%) [28] and 47.35% for chickpea, 54.65% for pea flour and 38.81% for lentil whole flour [29]. Varieties of cowpea used in this study had no effect on emulsion capacity.

The water absorption capacity in this study (2.96 to 3.46ml/g) was high compared to 1.89 to 2.15ml/g, 1.30ml/g, 2.10ml/g and 1.80ml/g for cowpea flour, DE hulled and defatted cowpea flour, cowpea protein isolate and soybean flour respectively [24,30,31]. High water absorption capacity is an index of intact starch granules. The result in this study implies that, extraction method adopted in this study did not significantly affect the integrity of the macromolecules of the concentrate. [23] report high water absorption capacity

4.37ml/g for whole cowpea flour while DE hulled cowpea flour had low value (2.56ml/g). This suggests that processing method affect water absorption capacity. However, [32], reported 24% water absorption capacity for soybean flour while soybean concentrate had 360%; sunflower flour having 180%while sunflower concentrate had 390%. The variation in water absorption may be due to different degree of interaction with water, protein concentrate, conformational characteristics and the quantity of damaged and undamaged starch present with the flour sample [3,23]. Carbohydrate also influences water absorption capacity of foods and the ability for protein to bind water depends on its water absorption. In order to have high energy density food, weaning food should have low water absorption capacity which is desirable for making thinner gruels with high caloric density per unit volume.

Parameter	A	B	C	D
BD (g/ml)	1.00 ± 0.01	0.93 ± 0.06	1.09 ± 0.16	1.05 ± 0.10
EC (%)	73.93 ± 5.82	77.10 ± 6.13	82.20 ± 1.14	83.10 ± 0.24
WAC (ml/g)	2.95 ± 0.01	2.96 ± 0.03	3.05 ± 0.13	3.46 ± 0.65
OAC (ml/g)	1.79 ± 0.04	1.79 ± 0.01	1.95 ± 0.21	2.06 ± 0.40
FC (%)	1.75 ± 1.06	2.15 ± 0.22	1.93 ± 0.12	2.30 ± 0.43
NSI (%)	0.17^{b} ± 0.06	0.19^{b} ± 0.01	0.09^{b} ± 0.02	0.39^{a} ± 0.92

Table 4: *Functional properties of the protein concentrate. Values are means ± standard deviation of duplicate determination. Means in the same row not followed by the same super scripts are significantly (P < 0.05) different.*

Key: A = Achishuru (black bean); B = Dan-Borno (large size brown bean); C = Dan-Kurmi (large size white bean); and D = Danila (medium size black eye white bean); ND = Not Detected; BD = Bulk Density; EC = Emulsion Capacity; WAC = Water Absorption Capacity; OAC= Oil Absorption Capacity; FC = Foam Capacity; NSI = Nitrogen Solubility Index.

The oil absorption capacity result showed no significant difference ($p > 0.05$) between the samples analyzed. The result obtained in this study (1.79 – 2.06ml/g) favorably compared to the result reported by [24] on varieties of cowpea flour (1.95 to 2.3ml/g). The oil absorption capacity is high compared to the result by [30] for DE hulled defatted cowpea flour (1.04ml/g) and cowpea protein isolate (1.93ml/g). The ability of cowpea protein concentrate to considerably bind oil makes it useful in food systems where oil absorption is required. High oil absorption capacity makes the protein concentrate suitable in enhancing flavor and mouth feel when used in food preparations such as sausage production.

Foaming capacity indicates the presence of protein in samples acting as at air or water interface to form stable layer of entrapped air bubbles [18]. The value obtained in this study 1.75 to 2.30% was low compared to 87.66% report by [27] for cowpea protein concentrate from black cowpea affected by pH adjustment and protein extraction methods. Low foaming capacity can be attributed to inadequate electrostatic repulsions, lesser solubility or protein denaturation and excessive protein to protein interactions [33]. Since the concentrate was extracted at the product isoelectric point, its solubility will be low. Flour concentrate obtained in this study would be useful in product formulation requiring low product foaming.

The solubility of a protein is an important functional property since protein needs to be soluble in order to be applicable in food systems. Other functional properties like emulsification, foaming and gelation depends on the solubility of protein [34]. Low percentage solubility (0.17 to 0.89%) was obtained in this study. In some legumes flours such as winged bean flour, lablab flour solubility value of 25-28% was reported [18]. Also, cowpea protein isolate showed lower protein solubility at low pH and high NaCl concentration but increases with increase in pH. Solubility of protein is influenced by temperature, ionic strength and the pH [30]. Decreased protein solubility in this study could be as result of protein denaturation during fat removal, chemical treatments during protein extraction and thermal treatment. However, sample D showed significantly ($p < 0.05$) high values (0.39%).

Least gelation capacity

Gelation is an aggregation of denatured molecules which is the minimum protein concentrate at which the gel does not slide along the test tube walls in inverted position [18]. The lower the least gelation concentration the better the gelling ability of proteins [35] because protein gels are aggregates of denatured molecules. The result (Table 5) showed that least gelation concentration for the samples was observed at 14% concentration for samples A, C and D while for sample B it was at 12% concentration. [18] reported 14% least gelation concentration for pigeon pea and cowpea protein isolate, 14% for black cowpea protein concentrate [27]. Similarly, [12] reported 12% least gelation concentration for cowpea protein isolates. The least gelation capacity of a product depends on the ability of protein dissociated by heat present in the flour [3]. The gel forming ability is reported to be influenced by the nature of the flour, starch, protein in the samples as well as their interaction during processing treatment [35].

Gelatin	A	B	C	D
2	-	-	-	-
4	-	-	-	-
6	-	-	-	-
8	-	-	-	-
10	-	-	-	-
12	-	+	-	-
14	+	++	+	+
16	++	++	++	++
18	++	++	++	++
20	++	++	++	++

Table 5: *Least gelation capacity of the concentrates.*

Key: ++ = Gel much; + = Gel less; - = No gel; A = Achishuru (black bean); B = Dan-Borno (large size brown bean); C = Dan-Kurmi (large size white bean); D = Danila (medium size black eye white bean).

Starch and protein concentration does not only affect gelation but also the type of protein and starch and the presence of non-protein components such as minerals and fibers [29]. In addition, changes in physicochemical conditions such as pH and ionic strength and manufacturing processes used influence gelling properties [29].

Conclusion

The results of this study indicate that samples A and B showed high chemical composition most especially the mineral composition and amino acids profile. This implies that they can be incorporated in to various products for enhanced nutritional composition. However, all samples were not significantly ($p > 0.05$) different in all the functional properties measured except in nitrogen solubility where sample D had significantly ($p < 0.05$) high value. This implies that sample D would be useful in product formulation requiring high solubility.

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