ISSN : 0974 - 7508 Volume 9 Issue 10

Natural Products

An Indian Journal

Full Paper

NPAIJ, 9(10), 2013 [379-385]

Physicochemical and functional properties of full-fat, defatted and protein isolate flours from black crowder cowpea *(Vigna unguiculata)*

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ABSTRACT

This research work evaluated the physical, proximate composition and functional properties of dehulled black Crowder Cowpea (BCC) seed flours. The dry seeds were dehulled and processed into full fat, defatted and protein isolate flours. Significant differences ($P<0.05$) existed between the proximate compositions of the samples (full fat flour, defatted flour and protein isolate). The full fat and defatted flours contained 25.99 ±0.07% and 27.42 ±0.17% protein, 47.71 ±0.36% and 50.89 ±0.24% carbohydrate, 7.48 ±0.03% and 10.09 ±0.15% crude fibre, 8.96 ±0.21% and 6.43 ±0.01% moisture, 3.81 ±0.12% and 4.67 ±0.01% on dry matter basis respectively. The protein isolate showed protein content of 87.07 ±0.19%; carbohydrate 9.13 ±0.19%; and no crude fibre (0.00 ±0.00%). Hence, the protein content of the full fat, defatted and protein isolate flours varied in the order: protein isolate > defatted flour > full fat flour. Significant differences ($P<0.05$) also existed in the Water Absorption Capacity and Emulsion Capacity of the samples treated with temperatures ranging from 40°C to 100°C, while the same samples given the same temperature treatment were insignificantly different in their Swelling Index and Foaming Capacity. In general, the BCC seeds were found to compare favourably with other tropical legumes hence, it can find useful application in the food industry especially in food supplementation and in bakery and confectionary products as well as in other possible applications.

KEYWORDS

Black crowder cowpea;
Proximate composition;
Physical properties;
Functional properties.

INTRODUCTION

Black Crowder Cowpea (BCC) is one of the many tropical species of theses tropical legumes or cowpeas that are used less. It is hardly ever known or utilized a good deal, except among localized communities in the Eastern part of Nigeria where it is known as "Akidi ojii", "Akidienu" or "Akidiani" and eaten in different combinations and forms with other staple foods.

It is prepared in this area as porridge with yam or maize. It is also cooked dehulled or undehulled and made into a spiced paste used to eat Tapioka. Further inquiries would identify other parts of Nigeria where it is consumed. With the knowledge of the profound nutritional benefits and significant functional potentials of these vegetable protein sources, speculations are that consistent research on other tropical legumes like Black Crowder Cowpea could come out with tremendous results that could greatly provide laudable choices for food supplementation and diversification[4].

For the purpose of this work, investigations are directed to the highly variable local cowpea species of

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the tropics that are barely recognized or used, using Black Crowder Cowpea (*Vigna unguiculata*) as a case study. This is aimed at providing basic information on the proximate and functional compositions and properties of the full-fat flour, defatted flour and protein isolate made from the Crowder Cowpea.

MATERIALS AND METHODS

Dry seeds of Black Crowder Cowpea (BCC) used in this study were sourced from Akwata market in Enugu, Enugu state, Nigeria. Laboratory equipments and other facilities used in the analyses were obtained from Central Laboratory Service unit of the National Root Crops Research Institute (NRCRI), Umudike, Abia state, Nigeria.

Chemicals reagents

Chemicals and reagents used in the course of this work were of analytical grade (Analar). They included sodium hydroxide (NaOH), sulphuric acid (H_2SO_4), hydrochloric acid (HCl), boric acid (H_3BO_3), ethanol (C_2H_5OH), hexane (C_6H_{14}), selenium crystals, methyl red, bromocresol green, refined olive oil, etc.

Equipments

The equipments and apparatus used in this research work included the Cabolite electric oven, Authur Thomas laboratory mill, Satarious digital analytical balance, general laboratory glass wares (beakers, conical flasks, crucibles, petri dishes, desiccators, etc.), Gallen Camp electric muffle furnace, Excello Kjedahl apparatus, Colab fume chamber, retort stand, stop watch, thermometer, Satarious digital pH meter, Colab electric centrifuge, manual sieve, etc.

Preparation of samples

The Black Crowder Cowpea (BCC) seeds were first processed into full fat flour and defatted flour before the protein was isolated to get the Protein Isolate. The method described by Okezie and Bello was employed. The dry bean seeds were manually sorted to remove stones, residual vegetative components, insect-perforated and shriveled seeds, and other extraneous materials. The wholesome seeds were used.

Production of full fat BCC flour

The dry seeds of the BCC were first washed in clean water to dislodge adhering dusts and other possible surface contaminants. The wash water was discarded after selectively separating the seeds from it. The seeds were then soaked in another clean water at 1:5 (w/v i.e. bean weight per water volume) ratio for 24hours. The seed coat of the BCC was found to be as thick as bread fruit's and so was difficult to remove manually. It was therefore removed from the soaking water and slightly dried before it was dehulled using a loosely set manual mill as employed in local bread fruit dehulling process. The hulls were thus more easily separated. The dehulled seeds were dried in the oven at 30°C for about 48hours and milled thereafter into flour with the laboratory mill. The flour was then sieved through a 0.5mm sieve to obtain the full fat BCC flour.

Production of defatted BCC flour

The full fat flour sample was soaked overnight (about 18hours) in ethanol at 1:5 (w/v) ratio at room temperature. Thereafter, the extracted flour was filtered from the solvent-oil medium. The defatted flour was then air-dried for about 6hours and pulverized before it was sieved. Part of the defatted flour was then set aside for analysis while the rest were used for the production of Protein Isolate.

Production of the protein isolate

The method described by Okezie and Bello (1988) was used in the protein isolation. About 70g of the defatted flour was mixed with 1400ml of water to form a 1:20 (w/v) ratio of slurry. The pH of the solution was brought to 6.37 and the solution was then allowed to settle for 3hours. The spent residue was separated from the dissolved protein extract by decanting, after which the dissolved protein was centrifuged. The pH of the extracted protein was then adjusted with dilute HCl to its isoelectric point between 4.0 – 4.3. The precipitate formed was afterwards recovered by centrifugation at room temperature and discarding the whey. The resulting curd (Protein Isolate) was then dried under air and cooled in desiccators before it was powdered and sieved.

Methods

The features of the dry seeds were determined by the method employed by Fashakin and Fasanya[2]. The raw seeds were selected at random and examined by

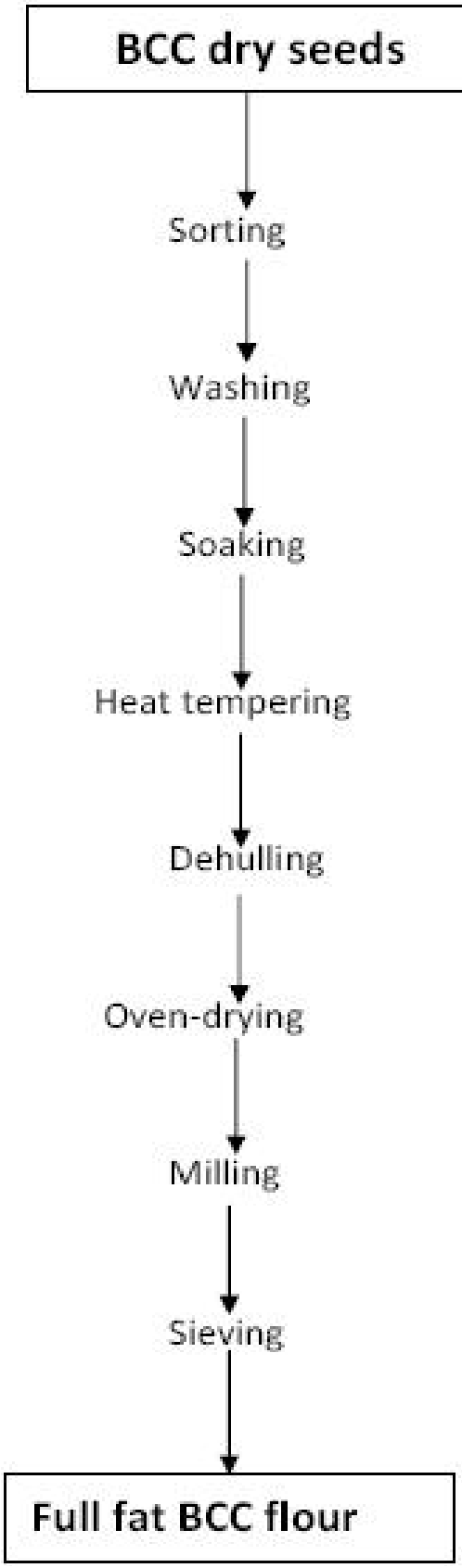

Figure 1 : Flow chart for the production of dehulled full fat BCC flour

subjective methods for shape, seed coat texture, seed colour and eye colour. The testa was described as smooth or rough, depending on the appearance to the eye. The degree of attachment of the testa (seed coat) to the cotyledon was described as tough or loose, depending on the ease of separation.

Seed weight

Weight of randomly selected hundred (100) seeds of BCC was determined by weighing on the analytical balance. The average weight per seed was evaluated by dividing the net weight by the number of seeds weighed (100).

Proximate composition

The chemical analyses for the fat, moisture, ash, protein, crude fibre, and carbohydrate contents were performed using the procedure as outlined by the Standard Official Methods of Analysis of the Association of Official Analytical Chemists, AOAC[1]. The analyses were carried out on the full fat flour, defatted flour and Protein Isolate from BCC seeds and triplicate results were obtained.

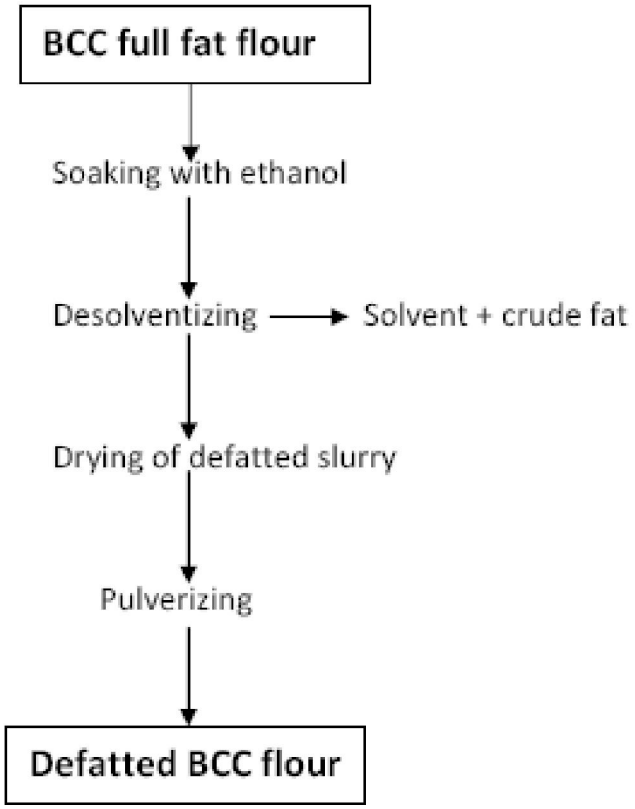

Figure 2 : Flow chart for the processing of full fat BCC flour into defatted BCC flour

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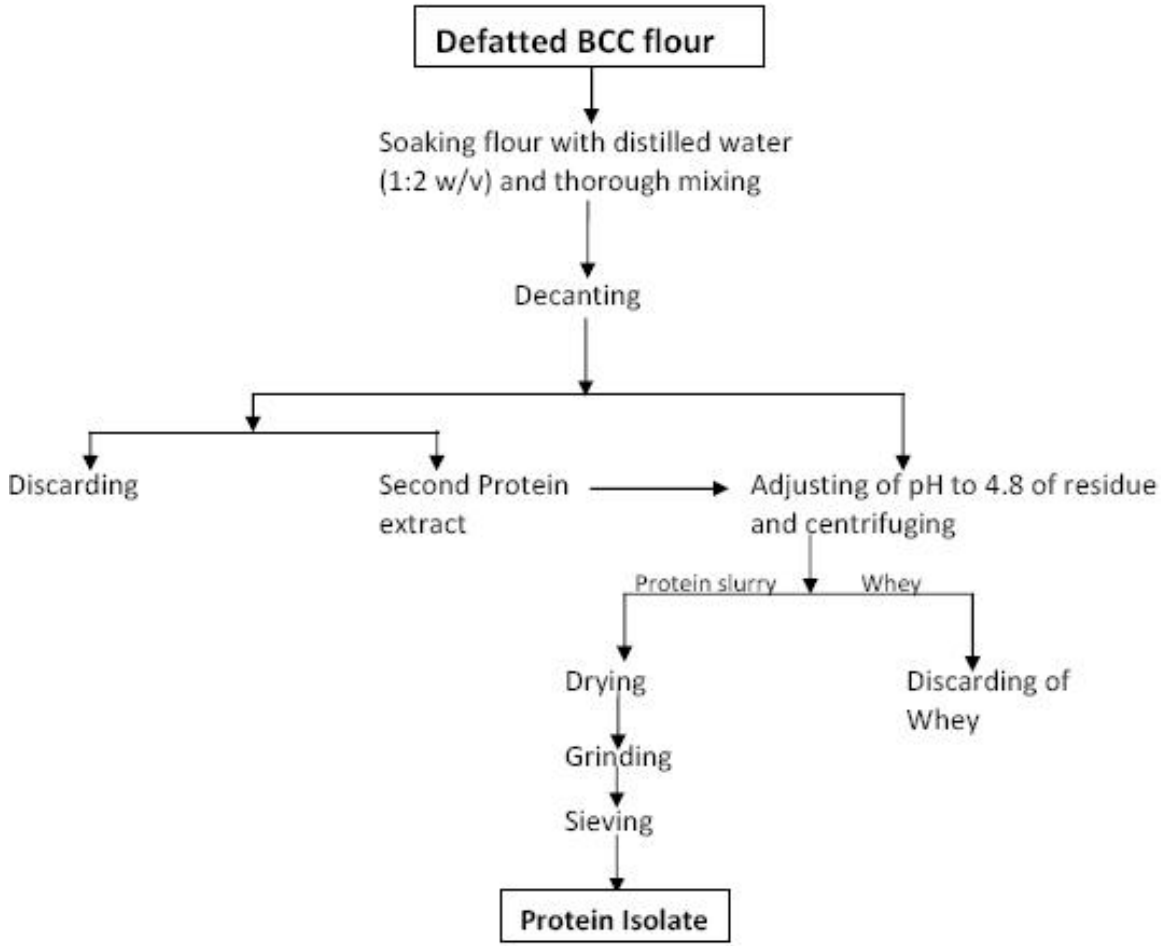

Figure 3 : Flow chart showing the production of Protein Isolate from defatted BCC flour

Functional properties of flour samples

Bulk density

The bulk density of the flour samples was determined mimicking the method described by James[3]. Separate 10 ml graduated measuring cylinders were gently filled with 5g (W) of each of the samples ensuring that the particles settled at the bottom. Thereafter, the volume (V) occupied by the sample of known weight is recorded.

Bulk density = W/V

Where: W = weight of sample in gram (g); V = Volume of sample in millilitre (ml) Measurements were reported as means of triplicate determinations.

Emulsion capacity

The method of Okezie and Bello[7] was used. 1g of the flour samples were blended with 10ml distilled water at room temperature for 30 seconds. After complete dispersion, 10ml of refined vegetable oil was added and blended for another 30 s. The mixture was later transferred into a centrifuge tube and centrifuged at 1,600 rpm for 5 min. Emulsion capacity was calculated as:

Emulsion capacity = (EH/WH) X 100

Where: EH = Emulsion Height (Volume of whole solution in the centrifuge tube) WH = Water Height

Swelling index

The swelling index was determined using the method of Okezie and Bello[7]. It was determined as the ratio of height of a unit weight of the sample to the height of the swollen sample left in contact with excess water for 1hour. 1g of the sample was weighed and dispensed into a test tube, leveled, and the height was noted. 10ml of distilled water was added to the sample and the test tube was left to stand for 1hour. The height which the sample then occupied was recorded and the swelling capacity was calculated as:

Swelling capacity = H2/H1

Where: H2 = Height occupied by the sample after swelling; H1 = Initial height occupied by sample

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Water/Oil absorption capacity (WAC/OAC)

The method of determination of water/oil absorption capacity described by Okezie and Bello[7] was used. 1g of sample was weighed and dispensed into a test tube and 10ml of distilled water/refined vegetable oil was added. The sample was later mixed thoroughly and allowed to stand for 30 min at room temperature. The mixture was centrifuged at 1,500 rpm for 30 min. The volume of free water or oil (the supernatant) was decanted and measured. Water/oil absorption capacity was determined thus:

WAC = (Vw1-Vw2)/mass of sample used

OAC = (Vo1-Vo2)/mass of sample used

Where: WAC = Water absorption capacity; Vw1 = Initial volume of water (10ml); Vw2 = Final volume of water decanted; OAC = Oil absorption capacity Vo1 = Initial volume of oil (10ml); Vo2 = Final volume of oil decanted

Triplicate results were obtained for each sample and their mean values reported.

Wettability

This was determined as the time (in seconds) taken by a unit weight (1g) of the sample to get completely wetted on a sample of distilled water in a beaker under laboratory conditions. This method was described by Okezie and Bello[7].

A 600ml capacity clean beaker was used to measure and retain about 500ml of water. With the aid of a retort stand, a set-up was arranged such that a clean (dry) test tube was clamped in an inverted position over the water in the beaker. The clamped position was adjusted such that the distance from the mouth of the test tube to the surface of the water in the beaker was exactly 10cm. Both the water in the beaker and the clamped position on the test tube were marked with masking tape.

Thereafter, the marked test tube was detached and 1g of the sample was weighed into it and its mouth covered with a (dry) thumb. It was carefully inverted over the water and clamped with the retort stand at the marked spot without removing the thumb. With the stop watch set to read the time, the thumb was removed and the sample allowed to fall onto the water surface as the stop watch was put on simultaneously.

The samples were observed closely and the stop watch stopped just as the last few sample particles go wet. The time (in seconds) was read from the stop watch and recorded as the wetting time. This experiment was repeated three times for each sample and the mean values obtained.

Gelatinization Temperature

5g of the sample was suspended in a beaker containing 20ml of water and heated while continuously stirring it. The temperature at which the suspension gels was recorded as the gelatinization temperature.

Foaming capacity

The method described by Onwuka[8], was adopted. One gram (1g) of the sample was blended with 10ml of distilled water in a warring blender for 5 minutes at room temperature. The mixture was quickly but carefully transferred to measuring cylinder and the foam volume was measured. The volume of foam formed was then recorded and used to calculate the foam capacity in percentage.

% Foaming Capacity (FC) =[(Va – Vb) / Vb]x 100

Where: Va = volume after whipping; Vb = volume before whipping; A final observation is made after 15, 30, 60, and 120 s to obtain the foam stability (in %).

Statistical analysis

The data obtained from the experiment were analyzed using Analysis of Variance (ANOVA) and the data were evaluated for significant differences ($P<0.05$) in their means. Differences between means were separated using the Fisher's Least Significant Difference (LSD) test procedure.

RESULT AND DISCUSSION

Seed characteristics

After the examination of the dry seeds, the results of the features were collected as shown in TABLE 1. The shape was oblong and its testa (seed coat) was black in colour, white eye-coloured, and smooth in appearance. The seed coat was also hard in texture and tough in its attachment to the cotyledon. The weight per 100 seeds was estimated at 14.23 ±0.26 g, and the average seed weight was 0.15 ±0.02 g.

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Proximate composition

The results of the proximate composition of the samples are shown in TABLE 2. The protein isolate flour had the highest protein content (87.07 ±0.19%) while the full fat flour had the lowest (25.99 ±0.07%). This protein value of the isolate compared favourably with those of other legumes. Potter and Hotchkiss recorded 86.6%, 74.6% and 64.3% total protein for Mung bean, Chickpea and Lima bean protein isolates respectively. There was significant difference ($p<0.05$) in protein content among the samples. The high protein content of the protein isolate is due to the hydrolysis and removal of the water-insoluble polysaccharides as well as the residual water-soluble sugar and minor constituents thus reducing these constituents in the isolate on dry matter basis and hence increasing the percentage protein content. Similarly, the protein content (25.99 ±0.07%) of the full fat flour of the BCC seeds was also found to be greater than some and in close proximity with many other legumes of its kind. Oguniji *et al.*[6], reported protein content of 28.17% for Limabean, 19.94% for Bambara, and 32.24% for Jackbean-W, and ash content of 3.06% for Jackbean-R, 3.26% for the Bambara and 3.58% for Mucuna BI. These protein and ash contents of tropical legumes were so similar to that obtained for the Black Crowder Cowpea (25.99 ±0.07%).

TABLE 1 : Seed characteristics of black crowder cowpea (BCC)

Average seed weight	Seed colour	Testa characteristics	Testa attachment to cotyledon
1.5 ±0.02g	Black	Oblong shape, white eye, mostly smooth n appearance	hard in texture and tough in its attachment to the cotyledon

TABLE 2 : Proximate composition of dehulled BCC full fat flour, defatted flour and protein isolate

Samples	Protein	Carbo hydrate	Fat	Moisture	Crude fibre	Ash
Full fat flour	25.99^{c} ±0.07	47.71^{b} ±0.36	6.21^{a} ±0.06	8.96^{a} ±0.21	7.49^{b} ±0.03	3.81^{a} ±0.12
Defatted flour	27.42^{b} ±0.17	50.89^{a} ±0.24	6.21^{a} ±0.03	8.96^{a} ±0.01	7.49^{b} ±0.15	3.81^{a} ±0.01
Protein Isolate	87.07^{a} ±0.19	9.13^{c} ±0.19	0.00^{c} ±0.00	3.57^{c} ±0.05	0.00^{c} ±0.00	0.23^{c} ±0.05

***All values are expressed as mean ± SD of their evaluation. ;The mean values along columns with different superscripts are significantly different at p <0.05.**

TABLE 3 : Functional properties of full fat flour, defatted flour and protein isolate made from black crowder cowpea

Samples	BD	WAC	OAC	EC	SWI	GT	FC	W
Full fat flour	1.68 ±0.02	2.43 ±0.05	1.89 ±0.04	62.06 ±0.94	1.51 ±0.02	78.50 ±0.41	20.50 ±0.35	30.67 ±1.25
Defatted flour	1.36 ±0.02	2.53 ±0.05	1.49 ±0.04	67.00 ±0.82	1.71 ±0.03	76.50 ±0.41	2.55 ±0.47	63.67 ±3.30
Protein Isolate	0.08 ±0.00	3.26 ±0.05	2.62 ±0.04	62.06 ±0.47	1.61 ±0.12	76.70 ±0.47	47.68 ±1.02	40.00 ±1.63

***All values are expressed as mean ± SD of their evaluations; Where:BD = Bulk density (g/cm^3); WAC = Water absorption capacity (ml/g); OAC = Oil absorption capacity (ml/g); EC = Emulsion capacity (%); SWI = Swelling Index (g/cm); GT = Gelling temperature (°C); FC = Foaming capacity (%); Wettability = (sec)**

Legumes and cereals are the best sources of the water-soluble fibres. Fibres (Pectin, hemicellulose, lignin, etc.) help to lower serum cholesterol levels in humans. The crude fibre content of full fat BCC flour was analysed as 7.49 ±0.03%, while the defatted flour gave a fibre content of 10.09 ±0.15%. These values suggest high fibre content in the flours from the dehulled seeds.

In addition, the ash contents of the flours and protein isolate was found to be in the order: defatted flour > full fat flour > protein isolate (see TABLE 2). Moisture contents of 8.96 ±0.21%, 6.43 ±0.01% and 3.57 ±0.05% were also gotten for the full fat, defatted and protein isolate flours respectively. Carbohydrate content of the samples ranges from 9.13% to about 50.89% with the defatted flour having the highest value, while the value for the protein isolate (9.13 ±0.19%) was the least. Black Crowder Cowpea is not an oil-bearing seed. As a result, it contains quite little proportion of fat. The fat content was 6.21 ±0.06% for the full fat and 0.50 ±0.03% for the defatted flour. About no fat was recorded in the protein isolate. These values were significantly different ($P < 0.05$) from each other.

Functional properties of flour

TABLE 3 shows results of the functional properties of the full fat flour, defatted flour and protein isolate. Taha and Ibrahim[9] stated that bulk density, BD (stated as g/ml or g/cm^3) is an important factor since it helps in choosing the appropriate packaging units. Bulk density of the BCC protein isolate (0.08 g/cm^3) was observed to be lower than those of the isolate of other legumes like African Yam Bean isolate (BD = 0.62 g/cm^3) and soy bean isolate (0.43 g/cm^3).

CONCLUSION

From the above results obtained from the analyses, it was concluded that full fat and defatted flours as well as the protein isolate of dehulled Black Crowder Cowpea have significant proximate and functional potentials that are better or favourably comparable with those of some other legumes that are more frequently recognized and utilized. The high protein content and foaming capacity suggests that it can find application in the food industry especially in food supplementation and as a functional ingredient in some food products.

REFERENCES

[1] Association of Analytic Chemists; Standard Official Methods of Analysis of the Association of Analytical Chemists. 14th Edition, S.W.Williams, (Ed); Washington DC, **(1984)**.

[2] K.B.Fashakin, J.I.Fasanya; Chemical Composition and Nutritive Changes of some improved variety of Cowpea (Vigna unguiculata L. Walp.). J.Tropical Sci., **28**, 117-118 **(1988)**.

[3] C.S.James; Experimental Methods in Analytical Chemistry of Foods. Chapman and Hall. New York, 28 **(1995)**.

[4] J.E.Kinsella; Functional Properties of Protein in Foods. A survey CRC Critical Review. Food Sci. and Nutr., **7**, 219–280 **(1979)**.

[5] A.Moure, J.Sineiro, Domínguez, J.C.Parajó; Functionality of oilseed protein products: A review. Food Research International, **39**, 945-963 **(2006)**.

[6] J.O.Ogunji, M.Wirth, D.I.Osuigwe; Nutrient Composition of some Tropical Legumes Capable of Substituting Fish Meal in Fish Diets. Journal of Agriculture and Rural Development in the Tropics and Subtropics, **104(2)**, 143–148 **(2003)**.

[7] B.O.Okezie, A.B.Bello; Physico–chemical and Functional Properties of Winged Bean Flour and Isolate, Compared with Soy Isolate. Journal of Food Science, **53**, 450–454 **(1988)**.

[8] G.I.Onwuka; Food Analysis and Instrumentation. Theory and Practice. Naphtali Prints, Lagos, Nigeria., 133–137 **(2005)**.

[9] F.S.Taha, M.A.Ibrahim; Effect of Degree of Hydrolysis on the Functional Properties of some Oilseed Proteins: Fats and Oils Department, National Research Center, Cairo, Egypt. Grasas y Aceites 273, Fasc. 3, **53**, 273–281 **(2002)**.