Chemical and Biological Properties of Local Cowpea Seed Protein Grown in Gizan Region

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Abstract—The aim of the present study was to investigate the chemical and biological properties of local cowpea seed protein cultivated in Gizan region. The results showed that the cowpea and its products contain high level of protein (22.9-77.6%), high carbohydrates (9.4-64.3%) and low fats (0.1-0.3%). The trypsin and chymotrypsin activities were found to be 32.2 and 15.2 units, respectively. These activities were not affected in both defatted and protein concentrate whereas they were significantly reduced in isolated protein and cooked samples. The phytate content of cooked and concentrated cowpea samples varied from 0.25% -0.32%, respectively. Tannin content was found to be 0.4% and 0.23% for cooked and raw samples, respectively. The in vitro protein digestibility was very high in cowpea seeds (75.04-78.76%). The biological evaluation using rats showed that the group fed with animal feed containing casein gain more weight than those fed with that containing cowpea. However, the group fed with cooked cowpea gain more weight than those fed with uncooked cowpea. On the other hand, in vivo digestion showed high value (98.33%) among the group consumed casein compared to other groups those consumed cowpea contains feed. This could be attributed to low antinutritional factors in casein contains feed compared to those of cowpea contains feed because cooking significantly increased the digestion rate (80.8% to 83.5%) of cowpea contains feed. Furthermore, the biological evaluation was high (91.67%) of casein containing feed compared to that of cowpea containing feed (80.83%-87.5%). The net protein utilization (NPU) was higher (89.67%) in the group fed with casein containing feed than that of cowpea containing feed (56.33%-69.67%).

Keywords—Biological properties, Cowpea seed protein, Antinutritional factors, In vitro digestibility

I. INTRODUCTION

LARGE segments of the population in developing countries suffer from protein malnutrition. Projections based on current trends indicate a widening gap between human population and protein supply. Hence, intense research efforts are currently directed toward identification and evaluation of underexploited sources, such as alternative protein crops for the world of tomorrow [1]. In this regard, various studies are being carried out to assess the potential of legumes that are still not widely used as dietary sources of protein, as well as a genetic resource for the improvement of traditional legume crops [1]. Food legumes are an important constituent of daily diet in many countries. They are a very good source of protein and carbohydrates; however their protein digestibility is limited due to protein structure and also some antinutritional factors. Among the latter, tannins, phytate, trypsin inhibitors, and polyphenols play the key role. Legume starch has been believed to be less digestible in comparison to cereal starch

[2]. The cell wall structure of cotyledons, the presence of amylase inhibitors and the above-mentioned phytate and tannins have been discussed as the causes of the diminished digestibility [3]. Over the past 30 years, the use of concentrated proteins from plant seeds has increased tremendously because of greater knowledge of their functional properties, processing and nutritive value. While, historically, soybeans had a competitive advantage over other legume seeds, there is a need to develop other sources of concentrated plant proteins [4] which ideally should be crops that are widely grown in tropical countries. The cowpea (*Vigna unguiculata*) is a widely grown legume food crop of the tropics. Like other legumes, cowpea seeds contribute to the level of dietary protein in starchy tuber-based diets through their relatively high protein content (25%), and their quality, by forming complementary mixtures with cereals [5]. Many of the antinutritional factors in legumes can be eliminated or inactivated, to a large extent, by appropriate heating and processing during food preparation. Wet-milling and processing techniques, employed during protein concentration and isolation, are known to be effective in the detoxification of seed materials [6]. Cowpeas are already widely used in diets for humans and other mammals, and this suggests that the seeds do not have major adverse nutritional effects. Cowpea proteins are gaining interest as ingredients in food systems throughout many parts of the world; the final success of utilizing plant proteins as additives depends greatly upon the favorable characteristics that they impart to foods. Therefore, the relationship of protein quality with processing parameters that affect the functional performance of protein products is worthy of extensive investigation [7]. Solubility of a protein is one of the critical functional attributes required for its use as a food ingredient, because solubility greatly influences other properties, such as emulsification, gelation and foaming [8]. Thus it determines the behavior of a protein food product. For plant proteins to be useful and successful in food application they should ideally possess several desirable characteristics, referred to as functional properties, as well as providing essential amino acids [8]. These properties are intrinsic physicochemical characteristics, which affect the behavior of proteins in food system during processing, manufacturing, storage and preparation [9]. However, In Gizan region of western Saudi Arabia, the local variety (cultivor) of cowpea has been cultivated for long time; there is no study on its protein biological properties. Therefore, the present study was conducted to evaluate the chemical and biological properties of the seed protein of this local variety of cowpea cultivated in Gizan region.

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II. MATERIALS AND METHODS

A. Materials

The seeds of local variety (Cultivor) of cowpea were obtained from the local market in Gizan in western Saudi Arabia. The seeds were cleaned, grinded by electric grinder and filtered through 60 mesh to get fine cowpea flour. The flour was kept at 4°C in the refrigerator until used. The entire chemicals used were of analytical grade.

B. Defatting of cowpea flour

The oil was extracted from the flour of cowpea according to the method described by El Tinay [10] using the hexane in the range of 1 g sample to 5 mL solvent. After drying at room temperature for 24 h, the defatted flour was again ground and filtered to pass 60 mesh and kept at 4°C until used for the analysis.

C. Preparation of protein concentrate and protein isolate

The protein concentrate of the defatted cowpea seed flour was prepared following the method described by Mattil [11]. About 1g of the defatted flour was mixed with 10 mL of 70% ethanol, stirred for 2h and kept overnight. Then filtered and dried at room temperature, and thereafter ground to pass 60 mesh and kept at 4°C until used for the analysis.

To prepare the protein isolate of defatted cowpea seed flour about 1 g of the sample was mixed with 10 mL distilled water and the pH was adjusted to 9.0 with 1 N NaOH and stirred for 2 h at room temperature. Then centrifuged at 5000 rpm for 20 min and the soluble protein was precipitated by lowering the pH to 4.0 with 1N HCl. After centrifugation again at 5000 rpm for 20 min the precipitate was collected, dried at room temperature (24 h), grinded, and sieved using 60 mesh and kept at 4°C for further analysis.

D. Cooking of cowpea seeds

The seeds were cooked following the method used in Gizan region, in which the seeds were soaked in water for 24 h. Thereafter, the sample was cooked in low fire for about 3 h and then the water was decanted and the cooked sample was dried in oven at 60°C for 24h. The dried sample was grinded, sieved and kept at 4°C.

E. Chemical composition

The chemical composition of the samples of raw and cooked cowpea seed, defatted flour, protein concentrate, and protein isolate was determined according to the Standard Official Methods of Analysis [12]. Total carbohydrate of the samples was calculated by subtracting the value of protein, oil, fiber, ash and moisture content from 100.

F. Determination of Tannin content

Tannin content (TC) of pearl millet samples was estimated using modified Vanillin-HCl in methanol as described by Price et al. [13]. About 0.2g of ground sample was placed in 100 ml conical flask. Ten milliliters 1% HCl in methanol (v/v.) was added. The contents were mechanically shaken for 20 minutes and centrifuged at 2500 rpm for 5 minutes. One milliliter of supernatant was pipetted into a test tube and 5 milliliters of vanillin-HCl regent (mixing equal volume of 8% concentrated HCl in methanol and 1% vanillin in methanol) were added. The optical density was read using a colorimeter (Lab System Analyzer- 9filters, J, Mitra and Bros. Pvt. Ltd). At 500nm after 20 minutes incubation at 30˚C, a blank sample was carried out with each run of samples. A standard curve was prepared expressing the result as catechin equivalents, i.e. amount of catechin (mg per ml) which gives color intensity equivalent to that given by tannin after correcting for blank. Calculation:

$$\text{Tannin content } (\%) = \frac{C \times 10}{200} \times 100 \tag{1}$$

Where:

C = concentration corresponding to optical density.
10 = volume of extract in ml.
200 = sample weight in mg.

G. Determination of phytate content

Phytate content of the samples was determined according to the modified method of Wheeler and Ferrel [14]. One gram of finely ground sample was weighed into a 100 mL conical flask, and then 50 mL of 3% TCA solution (W/V); containing 10% (W/V) sodium sulfate; was added. After shaking for an hour, the slurry obtained was centrifuged at 3000 rpm for 15 minutes. Ten milliliters of the supernatant were transferred into 50 mL boiling tubes. Then, 4 mL of FeCl3 solution (2 mg Fe3+/mL 3% TCA), centrifuged at 3000 rpm for 15 minutes and the clear supernatant was carefully decanted. The precipitate was then washed twice by dispersing well into 25 mL 3% TCA, heating in a boiling water bath (10 minutes) and centrifuged. Washing was repeated once with water. The precipitate was dispersed in a few milliliters distilled water enriched with 3 mL 1.5 N NaOH with mixing. The volume was made approximately 30 mL with distilled water and heated in the water bath for 30 minutes. The contents of the tube were filtered hot through Whatman No. 1 filter paper and the filtrate was discarded. The precipitate from the paper was dissolved with 40 mL hot 3.2 N HNO3 into a 100 mL volumetric flask. The paper was washed with several portions of distilled water. The contents in the flask were cooled and diluted to volume with distilled water. Five milliliters aliquots were transferred into another 100 mL volumetric flask and diluted to approximately 70 mL with distilled water. Then, 20 mL of 1.5 M KSCN (Potassium thiocyanate) were added; completing the volume up to mark. The intensity of color was immediately read at 480 nm (Corning, 259). A blank probe was run with each set of sample. The iron content was calculated from prepared standard curve of Fe (NO3)3. The phytate was estimated from the assumption that it contains 28.2% P and phytate phosphorous from a molar ratio of 4:6 Fe:P.

The standard curve of phytate was obtained by weighing 0.4321g ferric nitrate {Fe (NO3)3} and then dissolved in distilled water in 1 L volumetric flask up to mark. This prepared stock solution of 100 μg/mL Fe3+ ions. Concentrations of 0, 5, 10, 15, 20, and 25 μg/mL were prepared by pepetting 0, 5, 10, 15, 20 and 25 mL of the stock solution into a series of 100 mL volumetric flasks. Then the density of the color was read after addition of 1.5 M KSCN as previously described. A standard curve was obtained by plotting concentrations against corresponding readings of absorbance giving a linear relationship.

H. Trypsin inhibitor activity assay

Trypsin inhibitor activity was assayed in 0.05M sodium citrate sample extracts following the method of Kakade et al. [15] using BAPA (N-benzoyl-DL-arginine-P-nitroanilide hydrochloride (Sigma Chemical Comp., St. Louis, MO) as substrate. Trypsin type III from bovine pancreas (Sigma Chemical Co.) was used for the assay. One trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm in 20 min for 10 ml of reaction mixture, under the conditions described in this method, and the trypsin inhibitor activity as the number of trypsin units inhibited (TUI).

I. Alpha-chymotrypsin inhibitor activity assay

The method of Kakade et al. [16] was employed for determining chymotrypsin inhibitor activity in 0.05M sodium citrate sample extract using bovine pancreas, type II chymotrypsin (Sigma Chemical Co.) and 1 casein (BDH Chemicals, Pools, England) as substrate. One chymotrypsin unit (CU) was arbitrarily defined as an increase of 0.01 absorbance unit at 275 nm in 10 min for 10 ml of reaction mixture under the conditions described in this method and the chymotrypsin inhibitor activity as the number of chymotrypsin units inhibited (CUI)

J. In vitro digestibility

To determine in vitro digestibility (IVPD) the procedure of Hsu et al. [17] as modified by Satterlee et al. [18] was used. The drop of pH of casein (control) and the samples after 20 minutes hydrolysis by proteolytic enzymes was measured using an Orion research digital ion analyzer/501 (USA). The enzymes used were trypsin type IX from porcine pancreas, chymotrypsin type II from bovine pancreas, peptidase type III from porcine intestine and protease type VI from streptomyces griseus. All enzymes were supplied by Sigma Chemical Company (St. Louis, MO. USA). Percent in vitro digestibility was calculated from the pH drop using the following equation [18].

$$IVPD\,(\%) = 234.84 - 22.56\,(X) \qquad (2)$$

Where X = the pH after 20 min of hydrolysis.

K. In vivo digestion

The in vivo digestion experiment was done using Wister strains of male rats of 4 weeks old following the method of Eggum [19]. First the rats we fed with a reference feed that contain casein for four days, weighted and divided to six groups. These groups were fed with each specific feed and each one rat was fed with 15 g/day for about 9 days. The rats were weighted before the beginning of the treatment and at the end of the treatments. Also the consumed feed of each rat was calculated as well as the urine and fecal of each rat was analyzed. After the analysis the biological value, true digestibility and net protein digestibility was calculated according to Eggum [19] equations.

L. Statistical Analysis

Each determination was carried out on three separate samples and was analyzed in triplicate and the tables were then averaged. Data were assessed using SAS [20]. Mean comparisons for the treatments were made using the Duncan multiple range tests with a probability $p \leq 0.05$.

III. RESULTS AND DISCUSSION

A. Chemical composition of cowpea seeds and products

Table 1. shows the approximate composition of cowpea seeds and its products. The results indicated low fat content in raw cowpea seed (1.3%) and it decreased significantly from 1.1% in the cooked seeds to 0.01% in the concentrated protein. The low content of fat in the protein concentrate could be attributed to the uses of the defatted flour as well as ethanol in the preparation of the protein concentrate. The protein content in the raw cowpea seeds was high (22.9%) and it significantly increased in the other products with the highest being in protein isolate (77.6%). Also the carbohydrate content in all cowpea products was high (60%-64%) except in the protein isolate which was very low (9.4%). These results of low fat and high protein content of cowpea seeds are similar to those reported for other legumes [21]. Furthermore, the results of fat, protein and carbohydrate content in the present study are in a good agreement with those reported previously [22], [23]. The results also indicated that soaking cowpea seeds in water for 24 h followed by cooking for 3h influenced the Ash, moisture, protein, fiber and carbohydrate content compared to that of raw cowpea seeds (Table 1). These results are in contrast with those reported by Giami [24] who showed that heat treatment of cowpea seeds flour at 121C for 15 min has no effect on the Ash, fat, carbohydrate and fiber. Moreover, Edijala [25] showed that soaking, cooking and wet milling treatments of cowpea seeds flour has no effect on the protein content of cowpea seeds.

B. Protein Yield

Table 2. shows the amount of the protein (g) and the protein yield of raw cowpea seeds as well as defatted flour, concentrated protein and protein isolate. The results showed

that each 100 g of cowpea seeds gave 96 g, 83 g, and 15 g of defatted flour, protein concentrate, and protein isolate, respectively. The percentage of the protein of cowpea products was found to be 22.9% of raw seeds, 23.5% of defatted flour, 21.2% for protein concentrate and 11.6 for protein isolate. Accordingly, the protein yield of defatted flour, protein concentrate and protein isolate was found to be 98%, 92% and 51%, respectively. These results were higher than those reported by Bryant et al. [26]for the protein yield of okra seeds.

C.Antinutrional factors in the raw seeds and products of cowpea

The results of antinutrional factors such trypsin inhibitor, chymotrypsin inhibitor, phytate and tannin in the cowpea seeds and its products are presented in Table 3. The results showed that the values of trypsin inhibitor and chymotrypsin inhibitor in the defatted cowpea flour and the protein concentrate have no significant differences than their values in the raw cowpea seeds. Whereas, there is significant difference in the values of both enzymes inhibitors in the protein isolate and the raw seeds. However, the cooked seeds showed significant differences in the values of these inhibitors compared to those of raw seeds. Heat treatment during cooking reduced the values of trypsin and chymotrypsin inhibitors by 72.3 % and 82% respectively. The results also showed that the value of trypsin inhibitor is 32.2 U/mg protein, which was found to higher than values (19.6-28.2 U/mg protein) reported for raw cowpea seeds [27]. Later, Obong [28] reported lower values (9.8-20.5 U/mg protein) of the trypsin inhibitor in various varieties of cowpea seeds. It is general known that the values of the trypsin inhibitor in cowpea seeds are low compared to that of other legumes such as faba bean, soybean [22]. Our results also demonstrated higher value of the chymotrypsin inhibitor (15.02 U/mg protein) in local variety of raw cowpea seed compared to those reported by Sumathi and Pattabiraman [29] for cowpea seed (7.2 U/mg protein) and soybean (6.6 U/mg protein). The reasons for higher values of these enzyme inhibitors in local cowpea seeds could be attributed to the environmental conditions as well as varietal differences.

The phytic acid content of the raw cowpea seeds differed significantly than that of cooked cowpea seeds. Moreover, phytic acid content of defatted seed flour, protein concentrate and protein isolate showed slight differences than that of raw seeds. The phytic acid content in the raw cowpea seed was 0.32% that is in a good agreement with the results obtained by Ologhobo and Fetuga [27] in many varieties of cowpea seeds (0.28%-0.33%). By contrast, Obong [28] and Preet and Punia [30] reported high content (0.72%, 0.99%, 0.82%, and 0.93%) of phytic acid in different varieties of cowpea seeds. Our results also demonstrated that soaking of the seeds for 24 h before cooking greatly reduced the phytic acid content of the cooked seeds by 24% compared to the raw seeds. The results is similar to the observations of Ologhobo and Fetuga [27] that cooking only reduced the phytic acid content of raw

cowpea seeds by 7.7% and 11.4%, while soaking only reduced the phytic acid content of some variety of cowpea seeds by 19.4% and 26.7%. It is worth to note that soaking of cowpea seeds before cooking is a common practice in preparing food in Gizan region.

The tannin content of the raw cowpea seeds differed significantly than that of cooked cowpea seeds, protein concentrate and protein isolate, whereas, there no significant difference of its content between the raw seed and defatted seed flour. The tannin content (0.23%) of the raw cowpea seeds in this study was lower than 0.42% and 0.78% reported for various varieties of cowpea seeds [27]. Lower content of tannin in cowpea seeds was also reported [28]. Interestingly, soaking and cooking significantly reduced the tannin content of the cowpea seeds with overall reduction of 83% in the cooked seeds compared to raw seeds. This observation is in good agreement with those reported by Nwokolo and Ilecukwu [22] who found that boiling cowpea seeds in water reduced the tannin content by 61%-80%. Ologhobo and Fetuga [27] also reported that cooking without soaking reduced the tannin content by 31% and 47.3% in some varieties of cowpea. It is worth to note that the way of cooking used in this study (common practice by people in Gizan region) is very effective in reduce the antinutrional factors in cowpea seeds and hence improve the nutritional quality of this meal.

D.In vitro protein digestibility

As could be seen in Table 4. that the in vitro protein digestibility of raw cowpea seeds has no significant differences from that of defatted seeds flour, cooked seeds and protein concentrate. The in vitro digestibility of protein isolate of cowpea seed is similar to that of casein. The digestibility of raw cowpea seeds of this study was 75.04% which is similar to 75.3%, 77.2% and 77.8% reported by Wolzak et al. [31], Obong [28], and Phillips and Baker [5] for several varieties of cowpea seeds. Whereas it was slightly lower than 82% reported by Ahmed and Nour [32] for cowpea seeds. The results of the present study also demonstrated that no effect of the processing on the in vitro protein digestibility, except for protein isolate in which the in vitro protein digestibility was increased and this could be attributed to alkali treatment during protein isolation as well as free enzymes [33]. By contrast it has been reported that heat treatment of cowpea seeds increased the in vitro protein digestibility [34].

E.Biological evaluation of cowpea seeds and its products

Table 5. represent the biological evaluation of cowpea seeds and its products by feeding rats with feed containing each one of the following; casein, raw cowpea seeds, defatted cowpea seeds flour, protein concentrate of cowpea seeds and protein isolate of cowpea seeds. The uptake of nitrogen (N) of the groups fed with casein was significantly differed than those fed with cowpea seeds and product except for those fed with protein isolate. It is noticeable, low uptake of N for the groups that fed with cowpea containing feeds compared to

those fed with casein containing feed. Jenkins and Mitchell [35] explained the reduced uptake of N by of non-casein feed to differences of the amino acids and palatability which reduced the ability to eat such feed. The results in Table 5 also showed significant enhancement of weight gain of the casein fed rats compared to those fed with cowpea containing feeds. The reason for such low weight for the rats that fed with cowpea containing feeds may be because of low protein uptake as well as low or deficient in some essential amino acids in these feeds. Interestingly the results demonstrated increased gained weight of the group fed with cooked cowpea seeds compare to those fed with other cowpea containing feeds. This could be attributed to the fact that cooking reduced the antinutrional factors and hence enhanced the biological values of the uptake food. The in vivo digestibility of the casein fed group was higher than those of cowpea fed groups. The low digestibility of legumes protein might be due to the presence of antinutritional factors, protein type, and protein interaction with starch and cellulose [36]. In the present study the true digestibility of raw cowpea seeds was 80.83% which is lower than those reported by Khan et al. [37] who found that the true digestibility of rats fed with different varieties of raw cowpea seeds was between 87% and 92% whereas, lower true digestibility (59% and 79%) was reported for some varieties of cowpea [38]. Our results (Table 5) also showed that the true digestibility was increased from 80.8% to 83.5% in the cooked seeds. This might be due to destroying the trypsin and chymotrypsin inhibitors by heat as observed by Liener [39] who found that heat treatment increased the digestibility from 79% to 83% in cowpea seeds. The true digestibility of cowpea seeds protein concentrate and protein isolate in this study were 87.5% and 86.67%, respectively, which were lower than 98% and 95% reported for soybean isolate and lentil protein concentrate, respectively [40]. The biological values of rats that fed with casein containing feeds were significantly higher than those fed with cowpea seeds and its products. This could be due to the absence of one or more essential amino acids in cowpea seeds protein [39]. The biological value of rat fed with cowpea protein isolate was significantly higher than those fed with raw cowpea seeds. It is well known that the biological value is main indicator of the nitrogen balance in the biological evaluation of the protein and gives signs for the percentage of the protein used for the growth and maintenance [13]. The biological value of the raw cowpea seeds was found to be 70.33% which within the range (45%-72%) reported for several varieties of cowpea seeds [38], whereas, it was higher than 55% and 59% reported for others varieties of cowpea seeds [37]. On the other hand, higher biological values (86.5%) of cowpea seeds grown in Nigeria has been reported [41]. The latter author found that germination and cooking of cowpea seeds improved the nutritional quality of protein. It should be noted that in the present study cooking does not significantly increase the biological value for the rats fed with cooked cowpea seeds compared to those fed with raw seeds. The results also showed that the net protein digestibility of the rats fed with

casein containing feeds was higher than those of fed with cowpea containing feeds. The net protein digestibility of the groups fed with cowpea seeds containing feeds was found to be 56.33%, 60.67%, 64.83%, and 69.71% for raw seeds, cooked seeds, protein concentrate and protein isolate, respectively. These results were higher than those reported by Khan et al. [37] who found that the net protein digestibility of cowpea seeds ranged from 50% to 55%. Obizoba [41] reported that the net protein digestibility of mixture of 30% sorghum and 70% cowpea was found to be 59% and 65%. This author also found that germination significantly enhanced the net protein digestibility (82%) of cowpea seeds. These indicate that germination improved the nutritional quality of cowpea seeds. Our results (Table 5) also showed that cooking had no effect of the net protein digestibility for cowpea seeds. However, the slight increased in the net protein digestibility of cowpea seeds concentrate and isolate could be due to the high values of true digestibility and biological value of these products. The reason may be because of the low antinutritional factors in these products.

IV. CONCLUSION

The local variety of cowpea seeds cultivated in Gizan region has high protein and carbohydrate and low fats which is a good characteristic of nutritional quality of this variety. The traditional practices of soaking and cooking decreased the antinational factors and improved the protein digestibility of cowpea seeds, while, they has no effect on the biological value and net protein digestibility. To further improve the nutritional quality of cowpea seeds germination followed by soaking and cooking should be used. Moreover, this study should be continued to check the amino acids content and calculate the protein efficiency ratio to better understand the nutritional quality of this local variety of cowpea.

TABLE I
APPROXIMATE COMPOSITION OF COWPEA SEEDS AND PRODUCTS

Sample	Fat (%)	Ash (%)	Moisture (%)	Protein (%)	Fiber (%)	Carbohydrate (%)
Raw	1.3 ±0.1[a]	3.17±0.06[b]	7.13±0.31[b]	22.93± 0.45[d]	4.83± 0.31 [b]	60.63± 1.03[b]
Defatted	0.37 ±0.06[c]	3.13±0.06[b]	6.77±0.46[b]	23.5± 0.36[c]	5.9± 0.2 [a]	60.33±0.21[b]
Cooked	1.1 ±0.1[b]	2.73±0.06[d]	3.1±0.44 [b]	23.7± 0.17 [c]	5.1± 0.1 [b]	64.27±0.32[a]
Concentrated	0.01 ±0 [d]	2.9±0 [c]	6.8±0.46 [b]	25.53± 0.21 [b]	4.63± 0.55 [b]	60.03±1.17[b]
Isolated	0.17 ±0.06 [d]	4.13±0.06[a]	8.5±0.3 [a]	77.6± 0.53 [a]	1.0± 0.1 [c]	9.37±0.3 [c]

Mean ± standard deviation (N = 3). a, b, c, Duncan´s groupings referring to significant differences at (p ≤ 0.05) among means in a raw.

TABLE II
THE PRODUCTIVITY AND YIELD OF THE PROTEIN FROM COWPEA SEEDS AND PRODUCTS

Product	Productivity(g)	Protein (%)	Protein (g)	Protein Yield (%)
Raw	100	22.9	22.9	100
Defatted	96	23.5	22.6	98
Concentrated	83	25.5	21.2	92
Isolated	15	77.6	11.6	51

Mean ± standard deviation (N = 3).

TABLE III
ANTINUTRITIONAL FACTORS OF COWPEA SEEDS AND ITS PRODUCTS

Sample	Trypsin Inhibitor (U/mg protein)	Chymotrypsin Inhibitor (U/mg protein)	Phytate (%)	Tannin (%)
Raw	32.2±0.0 [A]	15.07±0.35 [a]	0.32±0.07 [a]	0.23±0.06 [a]
Defatted	31.67±0.32 [a]	14.47±1.86 [a]	0.27±0.03 [ab]	0.25±0.09 [a]
Cooked	8.9±0.72 [c]	2.73±0.29 [c]	0.24±0.01 [b]	0.04±0.0 [b]
Concentrated	31.6±0.1 [a]	14.6±0.8 [a]	0.25±0.01 [ab]	0.04±0.0 [b]
Isolated	29.23±0.05 [b]	10.47±0.38 [b]	0.27±0.03 [ab]	0.05±0.0 [b]

Mean ± standard deviation (N = 3). a, b, c, Duncan´s groupings referring to significant differences at (p ≤ 0.05) among means in a raw

TABLE IV
IN VITRO PROTEIN DIGESTIBILITY (IVPD) OF COWPEA SEEDS AND ITS PRODUCTS

Sample	IVPD (%)
Casein	94.3±0.23 [a]
Raw	75.04±3.07 [B]
Defatted	75.43±0.55 [b]
Cooked	76.69±2.6 [b]
Concentrated	78.64±1.04 [b]
Isolated	91.43±0.85 [a]

Mean ± standard deviation (N = 3). a, b, c, Duncan´s groupings referring to significant differences at (p ≤ 0.05) among means in a raw

TABLE V
BIOLOGICAL PROPERTIES OF COW PEA SEEDS AND ITS PRODUCTS

Sample	Nitrogen content in the feed (mg)	Weight change (g)	Actual in vitro digestibility (%)	Biological Value (%)	Net protein digestibility (%)
Casein	846.83± 121.51 [a]	38.08±4.64[a]	98.33±1.03[a]	91.67±0.81[a]	89.67±1.03[a]
Raw	661.4± 92.67 [c]	12.12±2.98[b]	80.83±2.71[d]	70.33±2.42[c]	56.33±3.61[d]
Cooked	629.17± 40.5 [c]	16.22±6.01[b]	83.5±1.97[c]	72.67±3.93[c]	60.67±3.5[cd]
Concentrated	648.27± 69.9 [c]	5.47±1.18[c]	86.67±2.06[b]	75.17±6.32[bc]	64.83±7.13[bc]
Isolated	755.5 ± 63.9 [ab]	12.53±2.38[b]	87.5±78.88[b]	80.33±3.07[b]	69.17±6.64[b]

Mean ± standard deviation (N = 3). a, b, c, Duncan´s groupings referring to significant differences at (p ≤ 0.05) among means in a raw

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