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Nutritional and Antinutritional Composition of Defatted Flours, Protein Concentrates and Protein Isolates of Honey Bean (*Vigna unguilculata* L Kalp) and Pinto Black (*Phaseolus vulgaris*)

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Abstract: Nutritional and antinutritional composition of honey bean (*Vigna unguiculata* L. Kalp) and pinto black bean (*Phaseolus vulgaris*), along with their defatted flour (DF), protein concentrate (PC), and protein isolate (PI), were studied. Protein concentrates and isolates were prepared from defatted seeds using the isoelectric precipitation method, followed by proximate and antinutritional analyses. The results showed carbohydrate compositions of 56.24 & 61.45% in DF, 16.26 & 19.43% in PC, and 3.36 & 1.35% in PI for honey bean (HB) and pinto black bean (PB), respectively. The protein values of the samples differed significantly ($p < 0.05$), showing a progressive increase from DF (33.35 & 27.92%) to PC (75.16 & 72.28%) and PI (88.17 & 92.02%), respectively. The percentage of ash, fiber, fat, and moisture in the defatted flours was 4.11 & 4.11%, 2.52 & 2.22%, 1.55 & 2.01%, and 2.23 & 2.97% for honey bean and pinto black bean, respectively. Only trace amounts of fat were detected in PC and PI. The antinutritional factors studied included phytate (3.42 & 3.40%), saponins (0.70 & 0.82%), tannins (0.20 & 12.62 mg/100g), alkaloids (8.33 & 8.05%), oxalates (1.27 & 0.23%), flavonoids (3.73 & 2.08%), cyanide (0.52 & 0.24 mg/100g), and total phenols (0.87 & 0.58%) in DF. The levels of these antinutritional factors in PC and PI were significantly lower and posed no nutritional concerns. The amino acid profile indicated that both samples contained substantial amounts of essential amino acids. The most abundant essential amino acid was leucine, with values of 4.55 & 4.81 g/100 g crude protein in DF, 5.90 & 5.17 g/100g crude protein in PC, and 10.53 & 9.19 g/100g crude protein in PI. Glutamic acid was the most abundant amino acid across all samples, with the highest concentration observed in PI (17.76 & 19.00 g/100g crude protein). The amino acid analysis demonstrated that the PI samples were superior compared to the FAO/WHO provisional reference pattern. However, supplementation may be necessary for DF samples. The results also suggest that the isolates can be used to supplement cereal-based diet.
Keywords: Nutritional, antinutritional, defatted flours, protein concentrates, protein isolates

Introduction

In the northern part of Nigeria, cowpeas are considered one of the most popular grain legumes, playing a vital role in human nutrition as a source of protein, carbohydrates, vitamins, and minerals [1]. Although they provide a good source of dietary protein, cowpea seeds are primarily deficient in methionine and cysteine, like other food legumes [2, 3]. In addition, cowpeas contain antinutritional factors such as protease inhibitors, lectins, phytic acid, and tannins, among others, which can cause adverse physiological effects when ingested by humans and domestic animals [4]. The composition of various chemical substances may vary due to plant nutrition conditions. Legumes also serve as essential food sources in both tropical and subtropical countries [5]. Protein malnutrition is a major nutritional problem in developing and underdeveloped countries and is a leading cause of illnesses like kwashiorkor and marasmus among children and the elderly [6].

The existing problems of food insecurity and malnutrition, coupled with an increasing population, uncertain crop yields, and the high cost of animal-based food supplies in Nigeria and other developing or underdeveloped countries, have urged contemporary researchers to identify and incorporate alternative, affordable sources of protein to enrich traditional food formulations. Cowpea protein provides an excellent solution to Protein-Energy Malnutrition (PEM). Furthermore, all parts of the plant used as food are nutritious, providing proteins, carbohydrates, vitamins, and other essential nutrients 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 proteins" because they contain all essential amino acids, and plant protein (soybean, peanut, cowpea, etc.), which are considered "second-class proteins" because they lack one or two essential amino acids [7]. Protein concentrates and isolates from the flour of different foods have been produced to decrease the non-protein components, thereby yielding a final product with high protein content [8].

Depending on the protein concentration on a dry basis, they are classified as protein concentrates, with maximum values of 65-79%, or protein isolates, which can reach 80-95% [9]. However, in some cases, these products may have low solubility or allergenicity [10]. Protein concentrates are obtained by eliminating non-protein components such as carbohydrates, soluble minerals, antinutritional factors, and some low molecular weight nitrogenous compounds, using aqueous-alcoholic solutions (e.g., ethanol, 1-butanol, isopropyl alcohol), or acidic or basic solutions. To obtain protein isolates, proteins are solubilized in aqueous media by adjusting the pH with sodium

hydroxide [11]. The application of any protein in food, whether as a supplement or nutritional enhancer, depends largely on its chemical composition. Understanding the nutritional and antinutritional components of the various processed flours is essential in determining their potential uses and incorporation into different product formulations. Therefore, the present study aims to determine the nutritional and antinutritional composition, of defatted flour (DF), protein concentrates (PC), and protein isolates (PI) from two varieties of cowpea commonly found in the northern part of Nigeria: Honey Bean and Pinto Black. This would expand their utilization in food formulation and new product development.

Materials and Methods

Collection, identification and preparation of samples

Honey bean (*Vigna unguilculata* L Kalp), Pinto black bean (*Phaseolus vulgaris*), were obtained from a local trader in Rukuba market Jos North, Plateau State, Nigeria and identified at International Institute of Tropical Agriculture (IITA) Ibadan. They flour were prepared according to the method described by Audu & Aremu [12].

Production of protein concentrate

Seed protein concentrate (PC) was prepared by a method modified by Gbadamosi *et al.* [13]. A known weight (100 g) of defatted flour was dispersed in 1 L distilled water to give final flour to water ratio of 1:10. The dispersion was then gently stirred on a magnetic stirrer for 10 min to form a suspension, after which the pH of the resultant slurry was adjusted with 1.0 M HCl to the point at which the protein was least soluble (pH 4; a value obtained from preliminary solubility results of the defatted flour) to precipitate the proteins. The precipitation process was allowed to proceed with gentle stirring for 4 h, keeping the pH constant. Soluble carbohydrates (oligosaccharides) and minerals were removed by centrifugation at 3500×g for 30 min using a centrifuge (Bosch, TDL-5, United Kingdom). The precipitate (concentrate) was afterward washed twice with distilled water to remove the residual minerals and soluble carbohydrates and the pH was adjusted with 1.0 M NaOH to 7.0 for neutralization and then centrifuged at 3500×g for 10 min. The resultant precipitate (concentrate) was collected and dried in an oven at 45°C for 8 h (Uniscope SM9053 Laboratory Oven, Singerfriend, England) and kept for further analysis.

Reparation of seed protein isolate

Seed Protein Isolate (PI) was prepared by a method described by [13]. A known weight (200 g) of the seed defatted flour was dispersed in 2 L of distilled water to give final flour to liquid ratio of 1:10. The suspension was gently stirred on a magnetic stirrer for 10 min. The pH of the resultant slurry was adjusted by drop-wise addition of 1.0 M NaOH with constant stirring until the pH was adjusted to the point at which the protein was most soluble (pH 10.0) and the extraction was allowed to proceed with gentle stirring for 4 h keeping the pH constant to solubilize the proteins. The mixture was centrifuged (Harrier 15/80 MSE) at 3500×g for 10 min to remove the non-soluble materials (residue). The proteins were precipitated from the supernatant by adjusting the pH to the point of least soluble (pH 4.0, 1.0 M HCl) and the soluble proteins was recovered by centrifugation (3500×g for 10 min). After separation of proteins by centrifugation, the precipitate was washed twice with distilled water to remove the excess salt formed during the pH adjustment. The precipitated protein was re-suspended in distilled water and the pH was adjusted to 7.0 with 1.0 M NaOH prior to freeze-drying using a freeze-dryer (Laboao LFD 10 A Vacuum freeze dryer, Zhengzhou Labooo instrument equipment, Co Ltd., China). The freeze-dried protein was stored in airtight plastic container at room temperature for further use.

Proximate composition

The ash, moisture, crude protein (N x 6.25), crude fat, crude fibre and carbohydrate (by difference) were determined in accordance with the standard methods [14]. All proximate analyses of the sample flours were carried out in triplicate and reported in %. All chemicals were of Analar grade. All results were on dry weight basis.

Antinutrients analysis

The contents of oxalate, saponins, alkaloids, flavonoids, tannins, cyanide, phytate, and total phenols were determined on each of the sample flours by methods described by some workers [15].

Amino acid analysis

The amino acid analysis was by Ion Exchange Chromatography (IEC) [16], using the Technicon Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 mL min^{-1} at 60^{o}C with reproducibility consistent within ± 3%. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. Amino acid values reported were the averages of two determinations. Nor–leucine was the internal standard. Tryptophan was determined after alkali (NaOH) hydrolysis by the colorimetric method.

Determination of Amino Acid Scores (AAS)

This index is one of the parameters for assessing the nutritional value of a food sample. Amino acid scores were determined based on whole hen's egg [17]. In this method, essential amino acids was scored, Met+Cys and Phe+Tyr was taken as a unit while Amino Acid Score (AAS) was calculated using the following formula [18]:

$$AAS = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in 1 g of reference protein}} + \frac{100}{1} \quad (1)$$

Determination of the predicted protein efficiency ratio

The predicted protein efficiency ratio (P-PER) of differently processed samples was calculated from their amino acid composition [19]:

$$P\text{-}PER = -0.468 + 0.454\,(Leu) - 0.105\,(Tyr) \quad (2)$$

Statistical analysis

Every measurement was conducted three times. The data generated were analyzed by one way analysis of variance (ANOVA) and the differences between the treatment means were separated using Duncan's multiple range tests. A level of P value less than 0.05 was considered to be significant.

Results and Discussion

Table 1 presents the proximate analysis of the defatted flours (DF), protein concentrates (PC), and protein isolates (PI) of honey bean (HB) and pinto black bean (PB). There was a significant difference ($p<0.05$) in the proximate composition of all the products, except for ash and fiber contents. The presence of carbohydrates indicates that these plants are good sources of energy, while the protein content suggests that they can contribute to physical and mental growth and development [20]. The crude protein content of DF, PC, and PI ranged from 27.92 to 33.35%, 72.28 to 75.16%, and 88.17 to 92.02%, respectively. Other researchers [21, 22, 23, 12] obtained a similar range of protein content (12.1 to 31.7%) when compared to DF. These values highlight the significant protein contribution of these legumes, particularly in the PC and PI samples.

There was a significant difference in the fat content across the samples ($p<0.05$). The concentration of crude fat in the PC and PI samples was negligible, which aligns with the findings of Mune *et al.* [24] and Chandra *et al.* [25] on cowpea protein concentrates. The low ash and fat content can be attributed to the extraction process. This low-fat composition underscores their suitability for low-fat dietary formulations, especially for health-conscious consumers [26]. Additionally, the poor fiber content in protein isolates has been previously reported [27]. Moisture content plays a crucial role in determining shelf life and controlling the rate of deterioration and infestation of grains during storage [28]. The moisture content of the DF samples aligns with most literature on cowpeas [29] and varies across all samples, as expected: from 2.23 ± 0.15b to 2.97 ± 0.18a in DF, 2.02 ± 0.12a to 2.35 ± 0.14a in PC, and 1.31 ± 0.10b to 1.61 ± 0.11a in PI. The DF samples (56.24 ± 1.01b and 61.45 ± 1.20a) had the highest carbohydrate content ($p<0.05$) compared to PC (16.26 ± 0.95b and 19.43 ± 1.10a) and PI (3.36 ± 0.15b and 1.35 ± 0.11a). It is not surprising that the carbohydrate content in DF was higher than in PC and PI, as the acid-based precipitation process removes soluble carbohydrates and fats. This finding is consistent with the work of Samaila *et al.* [30].

Generally, water-soluble sugars and minerals are significantly eliminated during the protein concentration and isolation processes [24]. The metabolizable energy in this study indicates that the calculated fatty acid levels were higher in DF (1.24 ± 0.08a to 1.61 ± 0.10b) compared to PC (0.55 ± 0.05b to 0.17 ± 0.03a) and PI (0.24 ± 0.02b to 0.12 ± 0.01a). In terms of caloric value, the present study showed that both samples have energy concentrations comparable to cereals. Overall, the high protein content, low fat, and moderate energy levels of PC and PI underscore their potential as affordable, sustainable, and nutritionally dense ingredients for functional food products and therapeutic diets.

Table 1: Proximate composition of DF, PC and PI samples

Parameters	HB DF	PB DF	HB PC	PB PC	HB PI	PB PI
Crude Protein	33.35 ± 1.23b	27.92 ± 1.05a	75.16 ± 1.12b	72.28 ± 1.05a	88.17 ± 1.10a	92.02 ± 1.20b
Fat	1.55 ± 0.11a	2.01 ± 0.12b	0.69 ± 0.08b	0.21 ±0.05a	0.30 ± 0.05b	0.15 ± 0.03a
Ash	4.11 ± 0.12a	4.11 ± 0.14a	4.23±0.11a	4.06 ± 0.13a	5.51 ± 0.10a	5.08 ± 0.08b
Crude Fiber	2.52 ± 0.10a	2.22 ± 0.08a	1.64±0.10a	1.67 ± 0.12a	1.05 ± 0.12a	0.09 ±0.01b
Moisture	2.23 ± 0.15b	2.97 ± 0.18a	2.02±0.12a	2.35±0.14a	1.61 ± 0.11b	1.31 ± 0.10a
NFE	56.24 ± 1.01b	61.45 ± 1.20a	16.26±0.95b	19.43 ± 1.10a	3.36 ± 0.15b	1.35 ± 0.11a
Fatty Acid	1.24 ± 0.08a	1.61 ± 0.10b	0.55±0.05b	0.17 ± 0.03a	0.24 ± 0.02b	0.12 ± 0.01a
Energy	1580.38 ± 12.35b	1593.66 ± 13.45a	1579.67±11.45a	1566.84 ± 12.30b	1567.11 ± 8.23b	1592.84 ± 9.12a

Means in the same raw with different letters are significantly different ($p < 0.05$). Means ± Standard deviation of replicate analysis

Table 2: Antinutrients composition ofDF, PC and PI samples

Parameters	HB DF	PB DF	HB PC	PB PC	HB PI	PB PI
Phytate	3.42 ± 0.10b	3.40 ± 0.12b	2.48 ± 0.15a	2.43 ± 0.13a	1.42 ± 0.20b	2.07 ± 0.25a
Saponin (%)	0.70 ±0.15a	0.82 ± 0.18a	0.19 ± 0.05a	0.16 ± 0.04a	0.05 ± 0.01a	0.05 ± 0.01a
Tannin (mg/100g)	0.20 ± 0.02b	12.62 ± 1.10a	0.04 ± 0.01b	4.43 ± 0.50a	0.02 ± 0.01b	0.03 ± 0.01a
Alkaloids (%)	8.33 ± 0.25a	8.05 ± 0.22a	7.10 ± 0.30a	6.81 ± 0.28a	6.05 ± 0.30a	5.63 ± 0.28a
Oxalate (%)	1.27 ± 0.18a	0.23 ± 0.05b	0.68 ± 0.12a	0.11 ± 0.03b	0.23 ± 0.05a	0.08 ± 0.02b
Flavonoids (%)	3.73 ± 0.20a	2.08 ± 0.15b	0.18 ± 0.08a	0.85 ± 0.15b	0.08 ± 0.02a	0.30 ± 0.05a
HCN (mg/100g)	0.52 ± 0.05b	0.24 ± 0.03a	0.32 ± 0.05a	0.12 ± 0.03b	0.24 ± 0.05a	0.09 ± 0.02b
Total phenols (%)	0.87 ± 0.10b	0.58 ± 0.08a	0.56 ± 0.10b	0.24 ± 0.05a	0.55 ± 0.10b	0.11 ± 0.03a

Means in the same raw with different letters are significantly different ($p < 0.05$). Means ± Standard deviation of replicate analysis

The results of the antinutrients analysis are presented in Table 2. PI has a significantly lower ($p < 0.05$) composition of DF than the PC and DF samples. Generally, the DF samples were found to contain significantly higher ($p < 0.05$) phytochemicals than both the PC and PI samples (Table 3). However, PB samples had significantly higher ($p < 0.05$) values in DF, PC, and PI compared to HB samples. Total phenolic content is an index of the antioxidant power/activity of foods and provides an estimate of phenolics such as cinnamic acid, gallic acid, coumaric acid, catechin, ferulic acid, and resveratrol [31]. The variation in total phenolic content across varietal boundaries aligns with the reports of [32, 33]. Dehulling reduced the total phenolic concentration in both varieties, similar to findings reported by Badifu [34] and Adebowale *et al*. [35].

Alkaloids were the most abundant antinutrients, with values in HB and PB depending on treatment levels: 8.33 ± 0.25a & 8.05 ± 0.22a mg/100 g in HBDF and PBDF, while HBPC and PBPC had 7.10 ± 0.30a & 6.81 ± 0.28a mg/100 g. Finally, HBPI and PBPI had 6.05 ± 0.30a & 5.63 ± 0.28a mg/100 g. These contents were much lower compared to reported values of 8.6% (scarlet runner bean), 9.6% (lima bean), and 5.0% (black turtle bean) [36]. High levels of tropane alkaloids can cause rapid heartbeat, paralysis, and, in fatal cases, death if consumed [29]. The DF, PC, and PI samples contained oxalate levels of 1.27 ± 0.18a & 0.23 ± 0.05b mg/100 g, 0.68 ± 0.12a & 0.11 ± 0.03b mg/100 g, and 0.23 ± 0.05a & 0.08 ± 0.02b mg/100 g, respectively. There was a significant difference ($p < 0.05$) across all samples. These values are lower than the 4.37 mg/100 g and 3.77 mg/100 g found in raw cowpea seeds [29, 37]. The low level of oxalates contributes to the local utilization of these samples, as oxalates can cause irritation and swelling in the mouth and throat [38].

Flavonoid content in DF (3.73 ± 0.20 & 2.08 ± 0.15b) was drastically reduced to negligible levels in PC and PI due to the treatment process. While DF samples contain higher antinutrient values, their levels remain within manageable limits. The low antinutrient levels observed in concentrates and isolates are desirable from both a functional and nutritional perspective, particularly for the preparation of high-quality food products [39]. It was demonstrated that the antinutritional factors in cowpeas can be reduced while improving nutritional quality through dehulling, defatting, and extraction processes. Additionally, the presence of beneficial bioactive compounds, such as flavonoids and phenols, suggests potential health-promoting properties, making these flours valuable for functional food applications.

The results of the amino acid analysis are presented in Table 3. The protein quality or nutrient value of food depends on its amino acid content and the physiological utilization of specific amino acids after digestion, absorption, and metabolism. The two prepared samples (PC and PI) are rich in isoleucine, leucine, lysine, total aromatic amino acids (tyrosine and phenylalanine), and total acidic amino acids. Similar observations have been reported [40]. The amino acid composition of DF, PC, and PI is reported as g/100 g crude protein (cp). The protein isolates are rich in leucine, with values of 10.53 ± 0.20a and 9.19 ± 0.15b g/100 g cp for HBPI and PBPI, respectively. Similar results were reported by Fernandez-Quintela [41] for pea and faba bean protein isolates and by Elhardallou *et al.* [42] for cowpea protein isolates (CPII and CPIM). The least abundant essential amino acid in PC and PI samples is tryptophan (0.64 ± 0.05b and 0.76 ± 0.08a in PC, and 0.87 ± 0.08a and 0.83 ± 0.05a in PI).

From the data obtained, there was a significant difference ($p < 0.05$) in the tryptophan values between DF and PC. Aremu *et al.* [43], in their study on the crude protein and amino acid composition of leguminous seeds grown in Nigeria, found that tryptophan is the least abundant essential amino acid in most leguminous plants and is sometimes not determined. Frota *et al.* [44] and Rangel *et al.* [45] found similar results regarding the amino acid composition of PI in cowpea. The most abundant non-essential amino acids are glutamic acid (6.22 ± 0.50b & 8.51 ± 0.25a, 6.75 ± 0.50a & 9.67 ± 0.30b, and 17.76 ± 0.50b & 19.00 ± 0.60a g/100 g cp) and aspartic acid (6.42 ± 0.25b & 6.88 ± 0.20a, 7.65 ± 0.22a & 7.05 ± 0.18b and 15.32 ± 0.25a & 14.12 ± 0.20b g/100 g cp) in DF, PC, and PI, respectively. Other scholars [45, 38, 30 and 42] have also reported high concentrations of glutamic acid and aspartic acid as the most abundant non-essential amino acids. The protein concentrates and isolates exhibited higher total essential and non-essential amino acid levels than their original seeds (Table 3).

As shown in Table 4, the TEAA contents (%) of DF, PC, and PI are well above the 39% required for an ideal protein source for infants, 26% for children, and 11% for adults [18]. The values of essential aromatic amino acids (EArAA) are 3.23 ± 0.20b & 3.23 ± 0.20b g/100 g cp, 3.37 ± 0.20b & 3.96 ± 0.30b g/100 g cp, and 9.79 ± 0.50a & 8.05 ± 0.40b g/100 g cp for DF, PC, and PI, respectively. These values align with the findings of Aremu et al. [46] but are generally lower than the ideal range of 6.8 – 11.8 g/100 g cp for infant protein, as prescribed by FAO/WHO/UNU [18]. The results clearly show that the TAAA and %TAAA values are higher than the TBAA and %TBAA values, indicating that the protein is likely acidic in nature. This finding is consistent with previous reports [38, 47]. Cystine is the most limiting sulfur-containing amino acid (Table 5), which is a common nutritional issue in most legume seeds. However, both protein concentrates and isolates contained higher levels of total sulfur and aromatic amino acids compared to the original seeds [42].

Table 3: Amino Acids Composition ofDF, PC and PI Samples

Amino Acid	HB DF	PB DF	HB PC	PB PC	HB PI	PB PI	FAO/WHO Reference[c]
Leucine	4.55 ± 0.15a	4.81 ± 0.20b	5.90 ± 0.20a	5.17 ± 0.10b	10.53 ± 0.20a	9.19 ± 0.15b	6.6 (1.9)
Lysine	4.35 ± 0.25a	3.45 ± 0.30b	4.54 ± 0.18a	3.63 ± 0.15b	7.19 ± 0.30a	6.62 ± 0.25b	5.8 (1.6)
Isoleucine	3.11 ± 0.18a	4.22 ± 0.20a	3.50 ± 0.12b	4.26 ± 0.15a	3.89 ± 0.25b	5.01 ± 0.30a	2.8 (1.3)
Phenylalanine	3.23 ± 0.14b	4.30 ± 0.21a	3.96 ± 0.20b	4.33 ± 0.18a	8.05 ± 0.40b	9.04 ± 0.35a	6.3 (1.9)
Tryptophan	0.56 ± 0.05b	0.72 ± 0.06a	0.64 ± 0.05b	0.76 ± 0.08a	0.87 ± 0.08a	0.83 ± 0.05a	1.1
Valine	3.08 ± 0.18b	4.29 ± 0.30a	3.45 ± 0.15b	4.67 ± 0.20a	5.18 ± 0.20b	6.72 ± 0.30a	3.5 (1.3)
Methionine	0.08 ± 0.01a	0.64 ± 0.05b	0.98 ± 0.08a	0.71 ± 0.05b	3.15 ± 0.10b	2.85 ± 0.08a	2.5 (1.7)
Arginine	4.90 ± 0.35a	4.92 ± 0.20a	4.99 ± 0.30a	5.33 ± 0.22a	5.76 ± 0.12a	6.19 ± 0.15a	
Histidine	2.09 ± 0.12a	2.24 ± 0.10a	2.17 ± 0.12b	2.46 ± 0.10a	4.16 ± 0.15a	3.83 ± 0.12a	1.9 (1.6)
Threonine	2.31 ± 0.10b	3.75 ± 0.20a	2.56 ± 0.20b	3.88 ± 0.25a	4.11 ± 0.18b	5.48 ± 0.30a	3.4 (0.9)
Proline	3.16 ± 0.20a	2.10 ± 0.15b	3.03 ± 0.18a	2.54 ± 0.12b	17.44 ± 0.40a	17.01 ± 0.35a	
Tyrosine	2.91 ± 0.20a	1.91 ± 0.12b	3.02 ± 0.20a	2.04 ± 0.15b	10.08 ± 0.50b	11.21 ± 0.60a	6.3 (1.9)
Cystine	0.08 ± 0.01a	0.44 ± 0.05b	0.77 ± 0.10a	0.56 ± 0.05b	1.04 ± 0.08a	0.62 ± 0.05b	
Alanine	3.22 ± 0.25a	3.12 ± 0.20a	3.43 ± 0.22a	3.87 ± 0.25a	4.54 ± 0.18a	4.40 ± 0.15a	
Glutamic acid	6.22 ± 0.50b	8.51 ± 0.25a	6.75 ± 0.50a	9.67 ± 0.30b	17.76 ± 0.50b	19.00 ± 0.60a	
Glycine	2.06 ± 0.10a	2.08 ± 0.10a	2.26 ± 0.15a	2.22 ± 0.10a	3.32 ± 0.10a	2.83 ± 0.12a	
Serine	2.01 ± 0.08a	2.08 ± 0.12b	2.44 ± 0.12a	2.25 ± 0.10a	4.11 ± 0.15a	3.75 ± 0.12a	
Aspartic acid	6.42 ± 0.25b	6.88 ± 0.20a	7.65 ± 0.22a	7.05 ± 0.18b	15.32 ± 0.25a	14.12 ± 0.20b	
Pi	3.29 ± 0.30a	3.52 ± 0.15a	3.67 ± 0.25b	3.80 ± 0.20a	7.13 ± 0.08a	7.25 ± 0.05a	
P-PER	1.29 ± 0.15a	1.51 ± 0.12b	1.89 ± 0.15a	1.66 ± 0.12b	3.25 ± 0.12b	2.52 ± 0.15a	
Leu/Ile	1.46 ± 0.12a	1.13 ± 0.08a	1.68 ± 0.10b	1.21 ± 0.08a	2.70 ± 0.20b	1.83 ± 0.12a	

Means in the same raw with different letters are significantly different ($p < 0.05$). Means ± Standard deviation of replicate analysis, * All amino acid (AA) values are expressed as grams per 100 g of protein. [c] Numbers in parentheses of FAO/WHO recommended content[29] represent essential amino acid for adults and numbers outsidethe parentheses represent essential amino acid for pre-school children(2~5 years).

Table 4: Concentrations of essential, non-essential, neutral, sulphur, aromatics, etc of DF, PC and PI samples

Amino Acids	HB DF	PB DF	HB PC	PB PC	HB PI	PB PI
TAA	49.67 ± 0.50b	54.34 ± 0.80a	56.62 ± 0.80b	62.04 ± 1.00a	128.33 ± 0.90a	126.50 ± 0.80a
TNEAA	24.51 ± 0.30b	26.08 ± 0.40a	30.08 ± 0.70b	29.35 ± 0.60b	76.98 ± 0.60b	77.77 ± 0.70b
%TNEAA	49.34 ± 1.20b	47.99 ± 1.10b	53.12 ± 1.50a	47.30 ± 1.20b	59.98 ± 0.90b	61.47 ± 1.00a
TEAA with His	25.16 ± 0.70b	28.26 ± 0.90a	26.54 ± 0.90b	32.69 ± 1.20a	49.38 ± 1.00a	47.13 ± 0.90b
TEAA without His	23.10 ± 0.60b	26.17 ± 0.80a	24.23 ± 0.80b	30.52 ± 1.10a	45.76 ± 0.90a	42.97 ± 0.80b
%TEAA with His	50.65 ± 1.20b	52.00 ± 1.30b	46.87 ± 1.30b	52.69 ± 1.50a	38.47 ± 0.80a	37.25 ± 0.70b
%TEAA without His	46.50 ± 1.10b	48.15 ± 1.20b	42.79 ± 1.20b	49.19 ± 1.50a	35.65 ± 0.90a	33.96 ± 0.80b
EAAA	11.17 ± 0.50c	13.05 ± 0.70b	11.60 ± 0.70c	15.41 ± 0.90b	24.83 ± 0.90a	23.71 ± 0.80b
EArAA	3.23 ± 0.20b	3.23 ± 0.20b	3.37 ± 0.20b	3.96 ± 0.30b	9.79 ± 0.50a	8.05 ± 0.40b
TNAA	25.29 ± 0.80b	30.36 ± 1.00a	29.19 ± 0.90b	35.94 ± 1.20a	80.98 ± 0.60b	80.47 ± 0.50b
%TNAA	50.91 ± 1.30b	55.87 ± 1.50a	51.55 ± 1.40b	57.93 ± 1.60a	63.10 ± 0.80b	63.61 ± 0.80b
TAAA	15.06 ± 0.60b	12.64 ± 0.50c	17.44 ± 0.80b	14.40 ± 0.70c	34.89 ± 0.80a	33.08 ± 0.70b
%TAAA	30.32 ± 1.00a	23.26 ± 0.80c	30.80 ± 1.10a	23.21 ± 0.90c	27.18 ± 0.50a	26.15 ± 0.40b
TBAA	9.32 ± 0.50b	11.34 ± 0.70a	9.99 ± 0.60b	11.70 ± 0.70a	16.08 ± 0.60a	17.11 ± 0.70a
%TBAA	18.76 ± 0.80b	20.86 ± 1.00a	17.64 ± 0.80b	18.85 ± 0.90a	12.53 ± 0.50a	13.52 ± 0.60a
TSAA	1.16 ± 0.10b	0.16 ± 0.05c	1.45 ± 0.10b	1.75 ± 0.12a	4.07 ± 0.30b	4.19 ± 0.40b
%Cys in TSAA	34.48 ± 2.00b	50.00 ± 2.50a	43.44 ± 2.10b	44.00 ± 2.20b	22.11 ± 1.50b	24.82 ± 1.70a

Total amino acids (**TAA**), Total non–essential amino acids (**TNEAA**), Total essential amino acids (**TEAA**), Essential aliphatic amino acids (**EAAA**), Essential aromatic amino acids (**EArAA**), Total neutral amino acids (**TNAA**), Total acidic amino acids (**TAAA**), Total basic amino acids (**TBAA**), Total sulphur amino acids (**TSAA**). Means in the same raw with different letters are significantly different ($p < 0.05$). Means ± Standard deviation of replicate analysis

Table 5: Amino acid scores of DF, PC and PI samples

EAA	PAAESP g/100g protein	HB DF EAAC	HB DF AAS	PB DF EAAC	PB DF AAS	HB PC EAAC	HB PC AAS	PB PC EAAC	PB PC AAS	HB PIEAAC	HB PIAAS	PB PI EAAC	PB PI AAS
Ile	4	3.27 ± 0.20a	0.81 ± 0.05b	3.11 ± 0.18a	0.77 ± 0.04b	3.46 ± 0.10b	0.86 ± 0.03b	3.50 ± 0.11b	0.87 ± 0.03b	4.45 ± 0.15a	1.11 ± 0.04a	3.89 ± 0.13b	0.97 ± 0.03b
Leu	7	5.08 ± 0.28a	0.72 ± 0.04b	4.55 ± 0.25b	0.65 ± 0.03b	5.11 ± 0.15b	0.73 ± 0.02c	5.90 ± 0.18a	0.84 ± 0.03b	10.62 ± 0.35a	1.51 ± 0.05a	10.53 ± 0.34a	1.50 ± 0.05a
Lys	5.5	3.23 ± 0.20a	0.58 ± 0.03b	4.35 ± 0.25a	0.79 ± 0.05a	3.38 ± 0.10b	0.61 ± 0.02b	4.54 ± 0.13a	0.82 ± 0.03a	6.87 ± 0.24a	1.24 ± 0.05a	7.19 ± 0.25a	1.30 ± 0.05a
Met + Cys (TSAA)	3.5	1.16 ± 0.10b	0.33 ± 0.03b	0.16 ± 0.01c	0.04 ± 0.01c	1.45 ± 0.06b	0.41 ± 0.02b	1.75 ± 0.07a	0.50 ± 0.02a	4.07 ± 0.18b	1.16 ± 0.05b	5.15 ± 0.22a	1.47 ± 0.06a
Phe + Tyr	6	4.31 ± 0.30b	0.71 ± 0.05b	6.14 ± 0.40a	1.02 ± 0.06a	5.20 ± 0.18b	0.86 ± 0.03b	6.98 ± 0.23a	1.16 ± 0.04a	18.43 ± 0.63b	3.07 ± 0.11b	18.13 ± 0.62b	3.02 ± 0.10b
Thr	4	2.04 ± 0.13b	0.51 ± 0.04b	2.31 ± 0.16b	0.57 ± 0.04b	2.12 ± 0.08b	0.53 ± 0.02b	a2.56 ± 0.10a	0.64 ± 0.03a	4.03 ± 0.16b	1.00 ± 0.04b	4.11 ± 0.16b	1.02 ± 0.04b
Try	1	0.68 ± 0.04a	0.68 ± 0.04a	0.56 ± 0.03b	0.56 ± 0.03b	0.76 ± 0.03a	0.76 ± 0.03a	0.64 ± 0.02b	0.64 ± 0.02b	1.10 ± 0.03a	1.10 ± 0.03a	0.87 ± 0.03b	0.87 ± 0.03b
Val	5	0.77 ± 0.06c	0.15 ± 0.01c	3.08 ± 0.20b	0.61 ± 0.04b	0.91 ± 0.05c	0.18 ± 0.01c	3.64 ± 0.15b	0.72 ± 0.03b	5.73 ± 0.23a	1.15 ± 0.05a	5.18 ± 0.21b	1.03 ± 0.04b
Total	36	20.54 ± 1.30b	4.52 ± 0.30b	24.26 ± 1.50a	5.04 ± 0.40a	22.39 ± 0.80b	4.96 ± 0.18b	29.51 ± 1.02a	6.21 ± 0.24a	55.30 ± 1.83b	11.36 ± 0.46b	55.05 ± 1.82b	11.21 ± 0.45b

Essential amino acids (**EAA**); Provisional amino acids, (Egg) scoring pattern (**PAAESP**); Essential amino acid composition (**EAAC**); Amino acids score (**AAS**), Means in the same raw with different letters are significantly different ($p < 0.05$). Means ± Standard deviation of replicate analysis

The relatively low values of sulfur-containing amino acids, such as methionine and cysteine, in the samples could be attributed to the high loss of albumins during the extraction process, as albumins are rich in sulfur-containing amino acids like lysine, methionine, and cysteine [48]. The essential/non-essential amino acid ratio showed a significant increase in the protein concentrates and isolates compared to the defatted flour (DF). Based on chemical scores, both concentrates and isolates exhibited higher scores than the defatted flour (Table 5). The low leucine-to-isoleucine (Leu:Ile) ratio in DF, PC, and PI is desirable, as it helps maintain amino acid balance in cereals, which are typically high in leucine but low in tryptophan and isoleucine. Overall, the essential amino acid score indicates that the PI samples are a good source of several essential amino acids, including isoleucine (Ile), leucine (Leu), methionine + cysteine (Met + Cys), phenylalanine + tyrosine (Phe + Tyr), threonine (Thr), tryptophan (Trp), and valine (Val). However, based on dietary recommendations, DF and some PC samples may require supplementation with these essential amino acids to achieve optimal nutritional value.

Conclusion

The defatted flour (DF), protein concentrate flour (PC), and protein isolate (PI) exhibited changes in their proximate composition, highlighting PC and PI as good and ideal sources of functional protein. The essential amino acids in PC and PI were found at acceptable levels compared to reference proteins. The chemical scores for they isolates exceeded the reference pattern. Combining legumes and cereals in the diet can help compensate for deficiencies or low levels of lysine in cereals and sulfur-containing amino acids in grain legumes. In the future, it will be important to examine their functionality.

Conflict of interest: The authors declare that there is no conflict of interest.

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