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Nutritional quality of the protein of *Vigna unguiculata* L. Walp and its protein isolate[1]

Qualidade nutricional da proteína de *Vigna unguiculata* L. Walp. e de seu isolado proteico

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ABSTRACT - A protein's true digestibility and amino acid composition are important characteristics for its nutritional characterisation. This study determined the true digestibility of the protein from cowpea (*Vigna unguiculata* L. Walp.) and of its protein isolate, and their nutritional values were estimated after correcting for the amino acid score. The protein was isolated from the defatted whole bean by its alkaline solubilisation and isoelectric precipitation. The amino acid composition of the protein from the whole bean and from the isolate was determined by ion-exchange chromatography. Protein digestibility was assessed by the nitrogen balance method. The amino acid score is the lowest value obtained from the ratio between the individual amount of each essential amino acid present within the protein and the recommendation of each one for preschool infants. Methionine was the limiting amino acid in both the whole bean and the isolate. The true digestibility of the protein corrected by the amino acid score is an estimate of its nutritional value. The amino acid score for the whole bean was 0.44, and protein digestibility was 86.7%. For the isolate, these values were 0.60 and 96.7%, respectively. Correcting each score by the digestibility resulted in an estimated nutritive value of 38% and 58% for the whole bean and the isolate, respectively. Therefore, by having higher digestibility and increasing the bioavailability of essential amino acids, cowpea protein isolate is of interest for inclusion in food products, especially cereal-based products, which contain lysine as a limiting amino acid and are rich in methionine.

Key words: Legume. Amino acid composition. Proteins. Digestibility.

RESUMO - A digestibilidade verdadeira de uma proteína e sua composição de aminoácidos são importantes características para sua caracterização nutricional. Neste trabalho foram determinadas a digestibilidade verdadeira da proteína do feijão-caupi integral (*Vigna unguiculata* L. Walp.) e do seu isolado proteico e estimados seus valores nutritivos, após correção pelo escore de aminoácidos. O isolado proteico foi obtido por precipitação isoelétrica da proteína de feijão integral desengordurada. A composição em aminoácidos do feijão integral e do isolado foi determinada por cromatografia de troca iônica. A digestibilidade da proteína foi determinada pelo método do balanço nitrogenado. O escore de aminoácidos é o menor valor obtido a partir da razão entre a quantidade de cada aminoácido essencial presente na proteína e a recomendação destes para pré-escolares. Metionina foi o aminoácido limitante tanto no feijão integral como no isolado. A digestibilidade verdadeira da proteína corrigida pelo escore de aminácidos estima o seu valor nutritivo. O escore de aminoácidos para o feijão integral foi de 0,44 e a digestibilidade de sua proteína foi de 86,7%. Para o isolado, estes valores foram de 0,60 e 96,7%, respectivamente. Corrigindo cada escore pela digestibilidade obtiveram-se 38 e 58% de valor nutritivo estimado para o feijão integral e o isolado, respectivamente. Portanto, por ter maior digestibilidade e aumentar a biodisponibilidade de aminoácidos essenciais, o isolado proteico é interessante para incorporação em produtos alimentícios, especialmente naqueles à base de cereais, os quais têm lisina como aminoácido limitante e excesso de metionine.

Palavras-chave: Leguminosa. Composição de aminoácidos. Proteínas. Digestibilidade.

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INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a legume of African origin with high protein content. It is cultivated in tropical and subtropical regions and widely distributed throughout the world, occupying a global area of about 12.5 million hectares, with 8 million (64% of the world's area) in western and central Africa and the rest in South and Central America and Asia (FERY; SINGH, 1997). The main world producers are Nigeria, Niger, and Brazil (ELHARDALLOU *et al*., 2015; FROTA *et al*., 2008; MARQUES *et al*., 2015).

Legumes are an important source of protein for a large part of the world's population, especially in countries with the poorest population and with high rates of protein-energy malnutrition. However, the presence in these seeds of limiting amino acids and anti-nutritional factors such as trypsin inhibitors, tannins, saponins, and phytates may influence the protein quality of leguminous plants such as cowpea (KALATAN; MOHAN, 2013; KHATTAB; ARNTFIELD, 2009).

The nutritional quality of the protein can be defined as the protein ability of a food to meet the metabolic needs of amino acids and nitrogen. Their evaluation is determined by the amount of total nitrogen (N), essential amino acid compositions, and digestibility of this protein (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012). Protein isolation is an alternative for the minimisation of anti-nutritional factors, improved digestibility, and bioavailability of leguminous amino acids (SARWAR; XIAO; COCKELL, 2012).

A high-protein food does not necessarily have good digestibility or the ability to meet the individual amino acid needs of the body, especially in relation to the essential amino acids. Knowledge of protein quality is a key criterion for the nutritional adequacy of the population (SHAHEEN *et al*., 2016). To supplement the nutritional information of a protein, FAO/WHO (1991) recommends the true digestibility (TD) method of protein corrected by the amino acid score.

The development of cultivars with high protein content is one of the main objectives of the genetic improvement of legumes. Several studies have been conducted to evaluate different genotypes of cowpea in relation to their nutritional characteristics, especially in relation to proteins (ANDRADE, 2010; FREIRE FILHO *et al*., 2011). The objective of this study was to evaluate the nutritional quality of whole cowpea protein and its protein isolate (PI) by the TD of these proteins, corrected by the amino acid score.

MATERIAL AND METHODS

Cowpea (*Vigna unguiculata* L. Walp), cultivar BRS-Milênio, was supplied by Embrapa Meio-Norte, Teresina, PI, Brazil, already clean. Part of the bean (1 kg) was autoclaved for 30 min and, after cooling, the bean was lyophilised (freezer dryer model FDB-5503 Operon, Japan) for 24 h, crushed and dried in a ventilated oven at 60 ºC, and ground again, originating a flour of cooked whole bean. Another part of the bean (3 kg) was ground in a hammer mill (model MML-100 Astecma, Brazil), with a medium internal sieve of 1.0 mm in diameter (Tyler 16), resulting in a flour of raw whole bean. This flour was defatted with hexane in the ratio 1:6 (m/v), reducing the lipids to approximately 27% of the initial value, resulting in a flour with less than 1% (0.61 g/100 g) fat. This flour remained for 24 h at room temperature and was then dried in an oven with air circulation for 2 h at 50 ºC and sieved in a 0.42 mm (Tyler 35) sieve.

The method of protein isolation was based on the methodology suggested by Wright and Bumstead (1984), with modifications. The defatted whole bean flour was dispersed with low ionic-strength buffer (50 mM Tris-HCl and 200 mM NaCl, pH 8.5) in the ratio 1:10 (m/v) under horizontal stirring. The mixture was then maintained for 2 h at room temperature, filtered, and the residue discarded. The filtrate was centrifuged at 10,000 × g for 20 min; after centrifugation, the precipitate was removed. The supernatant had the pH adjusted to 4.5, corresponding to the isoelectric point of the vicillin (precipitation step), by the addition of 1M HCl. The mixture was then stored under refrigeration (4 °C) for 12 h for flocculation. After this step, the mixture was centrifuged at 10,000 × g for 20 min. The insoluble fraction (precipitate) represented the cowpea PI that was collected and then lyophilised.

The determination of the amino acid profile of the isolated protein and of the Fcr was based upon the hydrolysis of the proteins (25 mg) in an acidic medium (10 mL of 6 N HCl under vacuum at 110 °C for 22 h). A 25 mL aliquot was injected into an analyser (Dionex DX 300) to separate the amino acids in a ion-exchange column followed by post-column reaction with ninhydrin. The colour developed by reaction was recorded in a colorimeter, obtaining peak areas that were used to quantify each amino acid. The areas of the peaks obtained from the investigated samples were compared to a standard mixture of amino acids, Pierce (SPACKMAN; STEIN; MOORE, 1958). The tryptophan concentration was determined by spectrophotometry after protein enzymatic hydrolysis with pronase at 40 °C for 24 h, according to the method described by Spies (1967). The hydrolysed sample was submitted to the colorimetric reaction with p-dimethylamine benzaldehyde (DAB) and

subsequent spectrophotometer reading at 590 nm. The tryptophan composition was calculated by comparison with a standard curve.

The biological assay was developed at the animal experiment laboratory of the Institute of Tropical Medicine of the University of São Paulo (IMT-USP). The experimental protocol follows the Canadian Council on Animal Care (OLFERT; CROSS; MCWILLIAM, 1993). The procedures were approved by the Animal Experimentation Ethics Committee of FCF/USP (CEEA protocol no. 89) and by the Research Ethics Committee of the Institute of Tropical Medicine.

We used Golden Syrian hamsters (*Mesocricetus auratus*; *Cricetinae*) (males, freshly weaned, with conventional sanitary standard) from the Central Biotherm of the Faculty of Medicine of USP. The animals were housed in individual cages with a metallic background adapted for the collection of faeces. After a five-day adaptation period, the animals were divided among three groups (n = 10). The first group received a diet whose only source of protein was cooked bean flour; the second group received a diet whose only protein source was the PI; and the third group received a diet similar to the other groups, except for the absence of protein (Table 1).

The faeces of the animals were collected each day from the 5th to the 10th experimental day and then frozen and lyophilised for further analysis. At the end of the experiment, the animals were sacrificed by cardiac exsanguination using ketamine (85 mg/kg body weight) and xylazine (8.3 mg/kg body weight) as anaesthetics.

Nitrogen from the diet and all faeces samples was determined by the micro-Kjeldahl method (AOAC, 2007). The calculation of TD of the proteins was performed according to the FAO/WHO (1991) method, subtracting from the amount of nitrogen ingested in the diet the amount excreted via the faeces minus the metabolic loss in the faecal material. The latter is estimated by the amount of nitrogen excreted by the hamsters fed the non-protein diet.

$$TD = \frac{I - (F - Fk)}{I} X100 \quad (1)$$

Where TD: true digestibility; I: g of nitrogen ingested; F: g of nitrogen excreted via the faeces of the experimental diet; and Fk: g of nitrogen excreted in faeces of the animal with the non-protein diet.

The amino acid score was obtained by the ratio between the individual amount of each essential amino acid present within the protein and the recommended value of that amino acid for pre-school children (1 to 3 years) (FAO/WHO 1991):Minor value from the ratio:

$$\frac{Amounof\ the\ essential\ a\ \min\ o\ acid\ in\ the\ protein}{Recommendedamount\ of\ the\ essentil\ a\ \min\ o\ acid} = Score\ of\ amino\ acid\ in\ the\ protein \quad (2)$$

Correction of the TD by the amino acid score was obtained by multiplying the TD value by the amino acid score, being:

TDCEA = (True digestibility) X (Score of amino acid)

RESULTS AND DISCUSSION

The amino acid profile of cowpea and PI as well as the recommendations of FAO/WHO (1991) are presented in Table 2.

Table 1 - Composition of the experimental diets

Ingredients (g/kg)	Cowpea	Protein isolate	Non-protein
Cowpea	865.8	-	-
Cowpea protein isolate	-	232.2	-
L-methionine	2	2	-
Sucrose	50	50	50
Corn starch	-	431.6	651
Cellulose	-	100	100
Soy oil	-	14.8	15.7
Coconut oil	134.3	134.3	134.3
Cholesterol	1	1	1
Choline chloride	3	3	3
Mineral mix[(1)]	35	35	35
Vitamin mix[(1)]	10	10	10

[(1)] Reeves, Nielsen, and Tahey (1993)

Table 2 - Amino acid profile of cowpea (*Vigna unguiculata* L. Walp) and of its protein isolate (g/100 g) as compared to the essential amino acid recommendation according to FAO/WHO (1991)

Amino acid	Cowpea	Protein isolate	FAO/WHO recommendation
Arginine	7.0	6.8	-
Cysteine	0.3	0.4	2.5*
Methionine †	0.8	1.1	
Histidine †	3.1	3.0	1.9
Isoleucine †	4.3	4.3	2.8
Leucine †	7.9	8.1	6.6
Lysine †	6.8	6.7	5.8
Tyrosine	1.6	2.0	6.3**
Phenylalanine†	5.4	5.4	
Threonine †	4.0	3.7	3.4
Tryptophan †	4.4	1.0	1.1
Valine	4.9	6.0	3.5
Glycine	4.0	3.1	-

Recommendation for pre-schoolers: *(Met+Cys) ** (Phe+Tyr); † Essential amino acids

Amino acids are classified as those that cannot be synthesised by the body and therefore must be obtained by the diet, called 'essential' (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val), and those that the body can produce, the so-called 'non-essential' amino acids (Asp, Asn, Glu, Ala, Ser, Cys, Tyr, Gly, Arg, Gln, and Pro). A protein has a good amino acid profile when it presents all the essential amino acids in significant quantity (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012).

The quality of a food protein as a source of amino acids can be evaluated in comparison with the recommendation of the amino acid standard of the FAO/WHO (1991) for children (2–5 years). Cowpea presented cysteine and methionine as limiting amino acids, whereas the other essential amino acids met the recommendations. Similar results were found by Vasconcelos *et al*. (2010). However, other authors have found values of all the essential amino acids below the recommendation in different cowpea cultivars (ANJOS *et al*., 2016; ELHARDALLOU *et al*., 2015); this difference is probably derived from genetic and agronomic factors that may influence the amino acid profile (PANDURANGAN *et al*., 2015).

In the PI, methionine, cysteine, and tryptophan appeared in amounts lower than those recommended, although the latter was in very close concentrations (1.0 versus 1.1). Rangel *et al*. (2004) found similar results as in this study regarding the amino acid composition of the PI of cowpea. Elhardallou *et al*. (2015) observed a lack of valine, in addition to methionine, cysteine, and tryptophan. These authors also found an increase in the concentration of essential amino acids in the isolate in relation to the cowpea flour that was not observed in the present study, which was probably caused by the selection of p fractions due to methodological differences during protein isolation.

The low content of methionine and cysteine and high content of lysine observed in whole cowpea and its PI in this study can be complemented by their inclusion in cereal preparations. The reason is that since cereals have lysine as a limiting amino acid and an excess of methionine, this combination would provide a complete protein in all essential amino acids (CERVANTES-PAHM; LIU; STEIN, 2014; FAO/WHO 1991; IQBAL *et al*., 2006; RUTHERFURD; BAINS; MOUGHAN, 2012).

The results of TD, amino acid score, and TD corrected for the amino acid score of cowpea flour and its PI are shown in Table 3.

In addition to the amino acid profile, digestibility is another important factor for a protein to be considered of high nutritional value. The maximum value for the digestibility corrected by the amino acid score is 100%, since this value indicates that the protein will be fully utilised. If a protein has a limiting amino acid (amino acid score less than 1), even if it has a maximum TD of 100%, this indicates that the supply of that amino acid will be below the recommendation, impairing its nutritional value. On the other hand, another protein may have an amino acid score of more than 1 — that is, it does not have a limiting amino acid — but if its digestibility is low, only part of the amount of these amino acids will actually be used, since

Table 3 - True digestibility, amino acid score, and true digestibility corrected by the amino acid score of cowpea and its protein isolate

True digestibility (%)	Cowpea whole bean	Protein isolate
	86.7 ± 2.5b	96.7 ± 2.6a
Least amino acid score*	0.44	0.60
True digestibility corrected by the amino acid score	38.4 ± 1.1b	57.6 ±1.6a

* Cysteine + methionine score (lower score based on recommendation); Student's t test, $p < 0.05$. Different letters on the same line mean there is a statistical difference

they will not be totally digested and absorbed, impairing their nutritional value (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; SILVA; FLEA; ARÊAS, 2012).

Table 3 shows that the PI presented a higher TD value (96.7%) than the whole cowpea (86.7%). This improvement in digestibility is expected, since the isolation provides the exclusion of components such as inhibitors of trypsin, chymotrypsin, lectins, phenols, tannins, and dietary fibres, which may interfere with the absorption and utilisation of the protein and, therefore, hinder the complete hydrolysis of proteins by pancreatic proteases (DAMADORAN, 2010; SARWAR; XIAO; COCKELL, 2012). The same can be observed in relation to the digestibility corrected by the amino acid score (TDCEA), which provides an estimate of the nutritive value of the protein, with values of 38.4% and 57.6% for the whole bean and the isolate, respectively. Rangel *et al*. (2004) observed TD of 86.9% in PI of cowpea, a result inferior to that found in this study.

Other works with common bean (*Phaseolus vulgaris* L.) presented similar results to this study. Cruz *et al*. (2005) observed TD values varying between 76.77% and 86.65% in different common bean varieties. Rutherfurd *et al*. (2014) reported 80.4% of TD and 62.4% of TDCEA. Pires et al. (2006) found 78.7% and 62% TD and TDCEA, respectively. Pereira and Costa (2002) observed TD values of albumin and globulin isolated from common bean of 82.62% and 68.53%, respectively, and TDCEA of 61% and 51%, respectively.

Taking into account the TD of foods traditionally designated as sources of proteins of high biological value, such as beef (TD = 98%) and casein (TD = 99%) (FAO/WHO, 1991), the protein of cowpea whole bean (TD = 86.7%) and its PI (TD = 96.7%) are quite digestible by the organism. However, the presence of limiting amino acids caused low levels of TDCEA.

Cereals have adequate amounts of methionine and cysteine, but, like legumes, also have good TD and low TDCEA due to the presence of lysine as a limiting amino acid (CERVANTES-PAHM; LIU; STEIN, 2014; RUTHERFURD *et al*., 2014; SHENHEIM *et al*., 2016). The inclusion of legumes and cereals in the same preparation promotes a better TDCEA in relation to these grains alone (ANYANGO; KOCK; TAYLOR, 2011; VILLARINO *et al*., 2014). Cowpea could be added in preparations with cereals such as wheat and corn, which, despite high TD (89.44% and 82.38%, respectively), present a low TDCEA of 40% and 37%, respectively (PIRES *et al*. 2006).

CONCLUSIONS

This study demonstrated that the nutritional quality of the protein isolate of cowpea is higher than that of the whole cowpea bean, due to its higher true digestibility. After correcting the protein digestibility by the amino acid score, the protein isolate remained in the highest quality, and the methionine remained as a limiting amino acid after protein isolation, which characterised a true disgestibility corrected by the amino acid score lower than that of a high biological value protein. This suggests that cowpea protein isolate is an alternative for incorporation into food products, especially cereal-based foods, which have lysine as a limiting amino acid, which would promote an increase in their nutritional value.

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