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Original Article

Protein fractions, amino acid composition and antinutritional constituents of high-yielding cowpea cultivars

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ABSTRACT

High-yielding cowpea (*Vigna unguiculata*) cultivars were analysed for major changes in seed protein types, amino acid profiles and antinutritional factors content. As usual, the globulins constitute the major seed proteins (493.2–573.3 g kg^{-1} total seed protein), followed by albumins (201.0–248.0 g kg^{-1}), basic glutelins (119.1–154.3 g kg^{-1}), acid glutelins (82.4–92.3 g kg^{-1}) and prolamins (13.2–20.2 g kg^{-1}). The electrophoretic patterns of seeds and protein fractions for all cowpea cultivars resembled to each other both qualitatively and quantitatively. However, they showed slight differences in the amino acid composition with common prevalence of glutamine/glutamic acid, asparagine/aspartic acid and phenylalanine + tyrosine. The methionine + cysteine contents were low for all cultivars and their protein fractions. Trypsin inhibitory activity varied among the cultivars and was much higher in the albumins (198.67–393.43 g kg^{-1} protein). Haemagglutinating activity was also higher in the albumin fraction and varied from 30,900 to 444,400 HU kg^{-1} flour. In conclusion, all cultivars showed the usual compositional characteristics of *V. unguiculata*, but the content of antinutritional factors differed among the cultivars although they remained concentrated in albumin and globulin fractions.

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is the most popular grain legume in Brazil, particularly for people living in the Northeast region, where it constitutes the principal source of protein and carbohydrate. Although it provides a good source of dietary protein and lysine (Juliano, 1999; Uwaegbute et al., 2000), cowpea seed is primarily deficient in methionine and cysteine, like other food legumes (Saikia et al., 1999; Mensa-Wilmot et al., 2001). In addition, it contains antinutritional factors such as protease inhibitors, lectin, phytic acid, tannin, among others, which can cause adverse physiological effects when ingested by humans and domestic animals (Maia et al., 2000; Preet and Punia, 2000).

The composition of various chemical substances may vary as a result of plant nutrition conditions, cultural practices and genetic manipulation (Vasconcelos et al., 1997). As a matter of fact, the concern with the adverse effects of genetic modification of foods on human health should be directed not only to the foods produced by rDNA technology, but also to all products including those produced by conventional breeding methods as well, since these also carry the potential for introducing unintended compositional changes that may have adverse effects on human health (Atherton, 2002).

Breeding efforts involving cowpea in Brazil have been directed towards the selection of high-yielding varieties associated with traits of resistance to drought, salt stress, pests and pathogens (Ehlers and Hall, 1997; Lopes et al., 2001). The chemical composition and nutritional properties of cowpeas vary considerably according to cultivar (Rangel et al., 2004; Giami, 2005). For effective utilization of newly developed cowpea cultivars for human and/or animal nutrition, removal or reduction of antinutrients and evaluation of their nutritional properties are necessary (Giami, 2005). However, little attention has been paid to the possible quantitative and qualitative alterations of the essential nutrients such as protein and amino acids and of antinutritional compounds (Akinyele and Abudu, 1990). The efforts put in by plant breeders in developing a high-yielding variety may be of little significance unless the varieties are evaluated nutritionally (Preet and Punia, 2000). Thus, this work aims to analyse three Brazilian high-yielding cowpea cultivars

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with respect to protein contents, protein fractionation in globulins, albumins, acid and basic glutelins and prolamins, the amino acid profile and antinutritional factors content of the whole seeds and their protein fractions.

2. Materials and methods

2.1. Materials

Mature seeds of cowpea cv. EPACE-10 were obtained from the Agronomy School at Universidade Federal do Ceará, Fortaleza, Brazil, and cv. IPA-206 and Olho de Ovelha from Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA semi-árido), Petrolina, Brazil. Human erythrocytes were obtained from Centro de Hemoterapia do Ceará, Fortaleza, Brazil. Rabbit blood was obtained by puncturing the marginal ear vein of healthy animals. Cow and pig blood cells were collected from healthy animals at the Agronomy School of Universidade Federal do Ceará, Fortaleza, Brazil. Bovine serum albumin (96%), Coomassie Brilliant Blue G and R, Kunitz-type soybean trypsin inhibitor (type I-S), *N*-α-benzoyl-L-arginine-*p*-nitroanilide (L-BAPNA), dimethyl sulphoxide (99.9%), bromelain (5–10 units mg^{-1}), papain (10–20 units mg^{-1}), subtilisin (7–15 units mg^{-1}), trypsin (type I) and molecular weight markers were purchased from Sigma Chemical Co, St Louis, MO, USA.

2.2. Extraction and preparation of albumins, globulins, glutelins and prolamins

The whole seeds were ground in a coffee grinder (Moulinex, Super Junior 'S', Dublin, Ireland) to a fine powder. To establish the best NaCl concentration to solubilise proteins, the seed flours were suspended in 0.15 M, 0.3 M, 0.5 M, 0.7 M and 1.0 M NaCl, pH 6.8, in the proportion of 1.0 g of meal to 10.0 mL of solution. Once the best salt concentration had been determined (0.5 M NaCl), it was buffered with glycine–HCl, pH 2.6, sodium phosphate, pH 7.0, sodium borate, pH 8.0, and glycine–NaOH, pH 9.0, all at 0.05 M to determine the optimum pH for extraction. The suspensions were stirred (400 rev min^{-1}, Stuart Scientific, UK, magnetic stirrer) for 4 h at 4 °C, centrifuged at 16,000 × *g* for 20 min and filtered in filter paper to obtain the crude extracts. After establishment of the most suitable extracting conditions, the various protein fractions in the cowpea seeds were obtained as shown in Fig. 1.

2.3. Protein determination

The protein content in the crude extracts and in each protein fraction was determined by the method described by Bradford (1976), using bovine serum albumin as standard or by calculating the nitrogen concentration × 6.25 (Baethgen and Alley, 1989).

2.4. Electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970). SDS-PAGE was carried out in a 2-mm vertical slab gel (10 cm × 8 cm) consisting of stacking gel mix, 5% total acrylamide, and main running gel mix, 17.5% acrylamide, prepared in 3.0 M Tris–HCl, pH 8.8. Samples (30 μg) were dissolved in Tris–HCl 0.0625 M, pH 6.8, containing 1% SDS and 1% 2-mercaptoethanol and incubated at 100 °C for 10 min. Electrophoresis was carried out

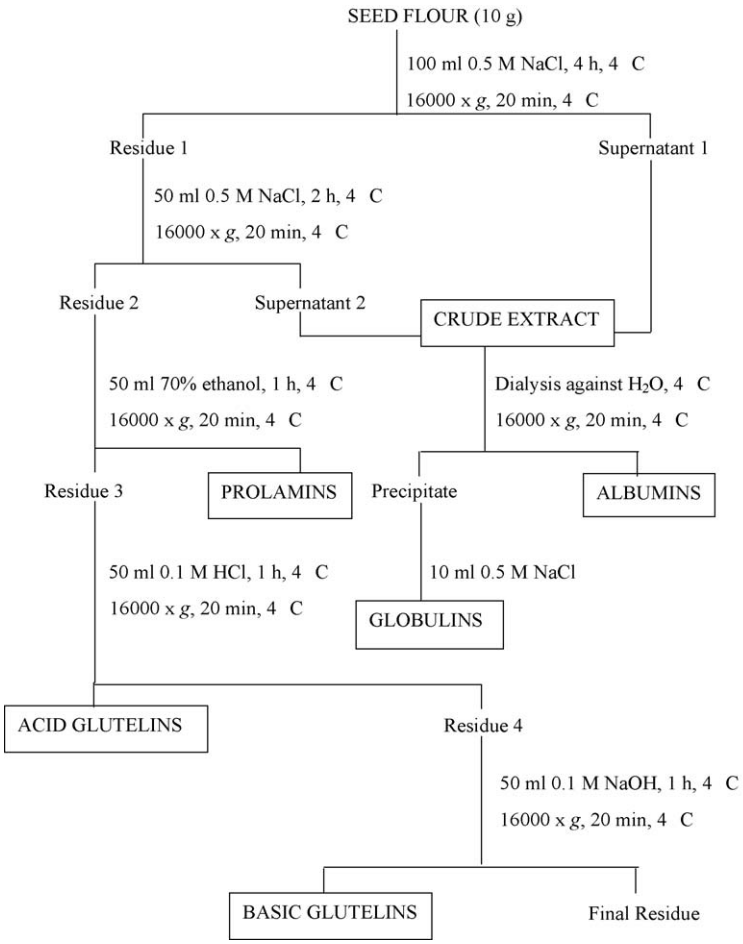

Fig. 1. Fractionation steps of cowpea seed proteins.

at 15 mA for 4 h. Protein bands were visualized by staining with 0.05% Coomassie Brilliant Blue R-250. Protein markers employed were bovine serum albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.2 kDa).

2.5. Amino acid composition

Amino acid analyses were performed after hydrolysis of the samples with 6 M HCl plus 10 g L^{-1} phenol at 110 °C for 22 h, in sealed glass tubes under N_2 atmosphere. HCl and phenol were removed by evaporation and the amino acid compositions were determined after chromatography on a Biochrom 20 system (Pharmacia). Tryptophan content was measured colorimetrically (Pintér-Szakács and Molnár-Perl, 1990). Chemical score was calculated by expressing the limiting essential amino acid (EAA) of seed proteins as a percentage of the same EAA in the hen egg protein.

2.6. Agglutination assay

Haemagglutinating activity present in the samples was assessed by serial twofold dilution of the protein fractions (Moreira and Perrone, 1977). The extracts and protein fractions were diluted with 0.15 M NaCl in glass tubes and mixed with erythrocytes (20 mg mL^{-1} suspension prepared in 0.15 M NaCl) from human (ABO system), rabbit, cow and pig. The degree of agglutination was monitored visually after the tubes had been left to stand at 37 °C for 30 min and at room temperature (22 ± 3 °C) for an additional 30 min. The results are reported as haemagglutination titre (HU), which is the reciprocal of the highest dilution giving visible agglutination. The red cell suspensions were enzyme-treated (10 mg L^{-1} solution prepared in 0.15 M NaCl) with trypsin, subtilisin, bromelain or papain for 1 h at room temperature (Lis and Sharon, 1972). Cells were washed 4 times with 0.15 M NaCl and the suspension final volume adjusted to attain the original concentration. To verify the effect of heat on the haemagglutinating activity, cooked bean samples were boiled for 30 min at 98 °C.

2.7. Trypsin inhibitor assay

Trypsin inhibitor activity was determined by a slight modification of the method originally described by Kakade et al. (1974) using trypsin and L-BAPNA (substrate) (Hamerstrand et al., 1981). Activity was expressed as the amount of trypsin inhibited, calculated from a calibration curve using soybean trypsin inhibitor.

2.8. Statistical analysis

The results were subjected to a one-way analysis of variance and the significance among means determined by Tukey's honest test, using the MSTATC program.

3. Results and discussion

3.1. Seed protein solubility

The highest solubilisation of seed proteins of the three studied cowpea cultivars was obtained with 0.5 M NaCl solution (123.7–163.6 g kg^{-1} flour), among the saline extracting solution tested (Table 1). When 0.5 M NaCl solution was buffered at pH 2.6, 7.0, 8.0 and 9.0, it was verified that – except for EPACE-10 cultivar – the seed protein solubility augmented with increasing pH, and that the best buffered solution for cowpea proteins extraction was 0.05 M glycine–NaOH, pH 9.0, containing 0.5 M NaCl (133.1–163.6 g kg^{-1} flour) (Table 1). As the amount of proteins solubilised with 0.5 M NaCl solution was not statistically different from that of the 0.5 M NaCl buffered solution at pH 9.0, the former was the extracting solution of choice (Fig. 1). Mahajan et al. (1988) and Rodriguez and Mendoza (1991) also employed 0.5 M NaCl to isolate and fractionate the soluble proteins from *V. umbellata* and *V. mungo*, respectively.

Table 1
Seed protein solubility (g kg^{-1} flour)* of three Brazilian cowpea cultivars at different NaCl concentration and pH.

Extracting condition	EPACE-10	Olho de Ovelha	IPA-206
NaCl [M]			
0.15	90.2 ± 3.4b	106.8 ± 6.9c	93.4 ± 5.5b
0.30	102.6 ± 4.5c	100.8 ± 3.7b	98.0 ± 4.6c
0.50	123.7 ± 11.0d	163.6 ± 4.0f	138.6 ± 7.4f
0.70	107.0 ± 2.6c	141.7 ± 4.1e	122.8 ± 9.3e
1.00	115.1 ± 5.1d	126.6 ± 6.5d	127.5 ± 7.7e
0.05 M *buffer* + 0.5 M *NaCl*			
Glycine–HCl, pH 2.6	22.5 ± 2.1a	19.2 ± 1.0a	12.1 ± 4.9a
PBS, pH 7.0	136.6 ± 9.9d	122.7 ± 7.2d	114.5 ± 1.3d
Borate, pH 8.0	124.2 ± 8.0d	133.1 ± 9.1e	129.0 ± 8.2e
Glycine–NaOH, pH 9.0	133.1 ± 5.1d	163.6 ± 1.3f	138.9 ± 0.7f

* Values are means ± standard deviation of three analyses, each in triplicate. Different letters in the same column differ significantly ($P < 0.05$).

3.2. Seed protein fractions

The seed protein contents of EPACE-10, Olho de Ovelha and IPA-206 were 237.1, 250.2 and 261.2 g kg^{-1} dry matter, respectively (Table 2), which are in agreement with other data for cowpea (Onwuliri and Obu, 2002; Ragab et al., 2004; Giami, 2005; Rivas-Vega et al., 2006). Upon protein fractionation (Fig. 1; Table 2) it was verified that the salt-soluble fraction (globulins), which ranged from 493.2 to 573.3 g kg^{-1} total seed protein, was the major protein constituent. Although the globulin content of the three cowpea cultivars differed significantly from each other, the data are in agreement with those for most legumes (Gopinathan et al., 1987). Nugdallah and El Tinay (1997), studying nine other cowpea cultivars, found out that the globulin fraction represented 656.0–797.0 g kg^{-1} of total seed protein. According to Mahajan et al. (1988), who studied the globulins from *V. mungo* in more detail, the legumin content (50.3%) predominates in relation to the vicilins (12.3%), which accounts for approximately 60% of globulins. The second most abundant seed protein for the studied cowpea cultivars was the water-soluble fraction (albumins), which varied from 201.0 to 248.0 g kg^{-1} total seed protein. The albumin contents of EPACE-10 and IPA-206 were very similar but 20% higher than that of Olho de Ovelha. In other *Vigna* species the globulin and albumin fractions predominate with contents varying from 380.0 to 596.0 g kg^{-1} and 120.0 to 269.0 g kg^{-1} total seed

Table 2
Protein fractions* of three Brazilian cowpea cultivars.

	EPACE-10	Olho de Ovelha	IPA-206
Seed protein[a] (g kg^{-1} dry matter)	237.1 ± 16.1a	250.2 ± 12.1b	261.2 ± 9.2b
Fractions (g kg^{-1} *total seed protein*)			
Globulins	530.1 ± 16.2b	573.3 ± 5.2c	493.2 ± 15.1a
Albumins	245.5 ± 13.1b	201.6 ± 9.2a	248.3 ± 13.3b
Prolamins	13.2 ± 1.1a	18.2 ± 1.2b	20.2 ± 1.3c
Acid glutelins	82.4 ± 4.1a	92.3 ± 4.2b	86.2 ± 5.1a
Basic glutelins	131.3 ± 7.1b	119.1 ± 4.2a	154.3 ± 12.1c

[a] $N \times 6.25$.
* Values represent means ± standard deviation for three analyses, each in triplicate. Different letters in the same row differ significantly ($P < 0.05$).

protein, respectively (Mahajan et al., 1988; Mohan and Janardhanan, 1993; Gopinathan et al., 1987). The acid and basic glutelins of the three studied cowpea cultivars taken together accounted for 211.4–240.5 g kg^{-1} total seed protein, whereas the prolamin type proteins were significantly less abundant, 13.2–20.2 g kg^{-1} total seed protein. Mohan and Janardhanan (1993) found only 90.0 g glutelins kg^{-1} total seed protein in *Vigna sinensis*, but the authors did not specify if they were basic- or acid-extracted. The very low values for the prolamin fraction observed in this study are typical for *Vigna* species (Mohan and Janardhanan, 1993; Gopinathan et al., 1987) and insignificant when compared to the values in cereals, which range from 30.0 to 60.0 g kg^{-1} total seed protein (Larkins, 1981). Overall it seems that the discrepancies among the contents of the different protein types within the *Vigna* genus depend on the extracting method employed, the species, cultivar and also on genetic and environmental variability (Adsule et al., 1986).

3.3. SDS-PAGE

The electrophoretic patterns of the seed proteins from the three studied Brazilian cowpea cultivar treated with SDS and 2-mercaptoethanol are shown in Fig. 2. In this procedure the same amount of protein (80 μg) from each sample was loaded into the gel wells to allow a better comparison. The protein patterns obtained resembled to each other both qualitatively and quantitatively. The relative molecular masses varied from about 12 to 125 kDa. Likewise, when the various protein fractions (crude extract, globulins, albumins, glutelin and prolamin type proteins) of these cultivars were submitted to electrophoresis under the same conditions, similar mobilities among the corresponding protein bands were observed regardless the studied cowpea cultivar. Therefore only the electrophoretic patterns related to EPACE-10 (Fig. 3) are shown in this present study.

Conformably, the representative electrophoretic patterns (Fig. 3) showed that the globulins are composed of at least 16 protein bands, and the most prominent ones have a molecular mass ranging from 44 to 63 kDa. These results are in agreement with the findings of Pedalino et al. (1990) and Araújo et al. (2002), who found similar band patterns for globulin fractions obtained from other cowpea cultivars, which presented prominent protein bands with 49, 52, 58, and 63 kDa.

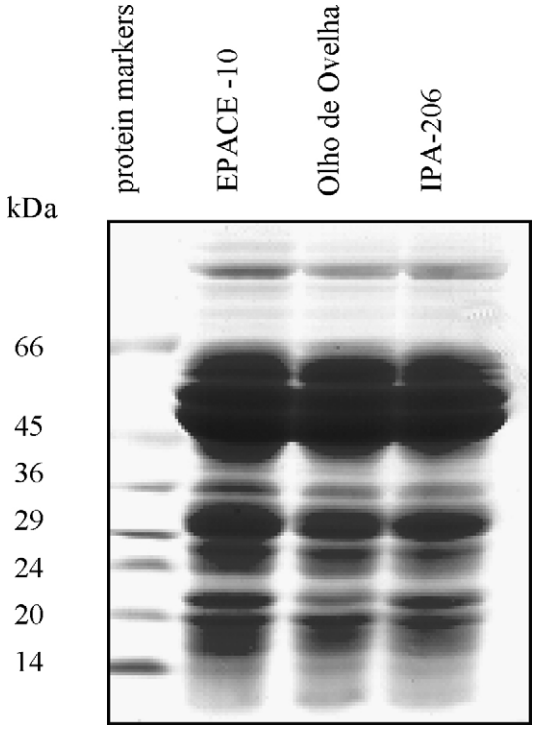

Fig. 2. SDS-PAGE of seed flours from three Brazilian cowpea cultivars.

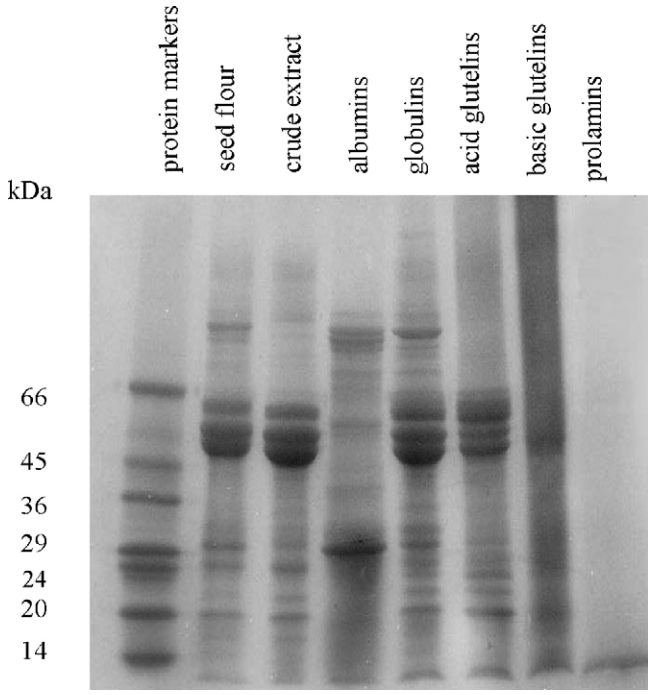

Fig. 3. SDS-PAGE of the seed flour, crude extract and protein fractions of EPACE-10 cowpea cultivar.

The albumin fractions of the cowpea cultivars examined in the present study seem to comprise at least 20 protein bands, with a prevalence of three distinct groups with molecular masses in the range of 81–93, 27–30, and 16–19 kDa. Protein bands of 95, 63, and 32 kDa were found for albumin fractions of other cowpea cultivars (Pedalino et al., 1990). Rao et al. (2000) reported that the 41 and 55 kDa protein components from the albumin fraction of *V. sinensis* were the major allergens and that the allergenicity was resistant to heat and proteolytic enzyme digestion.

This group of allergenic proteins in the Brazilian cultivars considered in the present study is less abundant than the other three prominent protein groups referred to. Regarding to acid glutelins they showed at least 16 protein bands with predominance of those with molecular mass from 45 to 63 kDa and other bands of 29 and 20 kDa. The basic glutelins showed about six poor-staining protein bands, with those of 57, 25 and 13 kDa prevailing. On the other hand, the prolamin type proteins presented only one scarcely visible protein band of approximately 13 kDa.

3.4. Amino acid composition

The whole-seed amino acid compositions of the three studied cowpea cultivars are shown in Table 3. Overall the data are very similar to those reported for other cowpea cultivars (Onwuliri and Obu, 2002) for which there is a prevalence of glutamine/glutamic acid, asparagine/aspartic acid and phenylalanine + tyrosine, as well as a secondary prevalence of other essential amino acids such as arginine, leucine and lysine. Both glutamine and asparagine, which are formed from glutamic acid and aspartic acid, respectively, constitute important reservoirs of amino groups for the body. Furthermore, glutamine has received attention as a primary fuel source for the intestinal tract, especially controlling glycogen

Table 3
Amino acid composition (g kg^{-1} protein) of the whole seeds of three Brazilian cowpea cultivars compared with hen egg protein.

Amino acid	EPACE-10	Olho de Ovelha	IPA-206	Hen egg
Essential				
Thr	43.3 (92.1)	38.9 (82.8)	39.6 (84.2)	47
Val	41.6 (63.0)	43.4 (65.7)	44.0 (66.7)	66
Ile	48.1 (89.1)	45.1 (83.5)	45.8 (84.8)	54
Leu	71.8 (83.5)	73.0 (84.9)	72.7 (84.5)	86
Lys	66.4 (94.8)	70.2 (100.3)	69.1 (98.7)	70
Phe + Tyr	111.1 (119.3)	105.0 (112.9)	105.0 (112.9)	93
Met + Cys	23.6 (41.4)	20.1 (35.3)	20.3 (35.6)	57
Trp	13.6 (80.0)	12.6 (74.1)	13.6 (80.0)	17
His	38.8 (176.4)	36.7 (166.8)	37.2 (169.1)	22
Arg	85.0 (141.7)	85.2 (142.0)	82.0 (136.7)	60
Nonessential				
Asx	108.2	107.2	108.6	
Glx	168.6	196.7	196.1	
Ser	44.6	41.8	41.0	
Gly	39.4	35.6	39.6	
Ala	46.0	41.8	42.4	
Pro	49.9	47.1	46.1	

Values are means of three analyses. The standard deviation values were all less than 5% of means. Figures within parentheses represent the chemical scores of essential amino acids calculated as a percentage of the corresponding amino acid in the hen egg protein.

synthesis and protein degradation (Mahan and Escott-Stump, 1996).

The nutritive value of a protein depends primarily on its capacity to satisfy the needs for nitrogen and essential amino acids and precise knowledge about these requirements is basic for the evaluation of the nutritional significance of dietary protein quality. Analysis of essential amino acid chemical scores (CS) showed (Table 3) that, except for phenylalanine + tyrosine and histidine, all other essential amino acids have contents lower than those of hen egg protein (FAO/WHO/UNU, 1985). The first limiting amino acids are methionine + cysteine with CS varying from 35.3 to 41.4. When compared to FAO/WHO/UNU (1985) scoring patterns of amino acid requirements for 2–5-year-old children (25.0 g kg^{-1} dietary protein), the three studied cowpea cultivars proved deficient only in methionine + cysteine (20.1–23.6 g kg^{-1} protein). For 10–12-year-old children (22.0 g kg^{-1} protein), however, EPACE-10 met the methionine + cysteine requirement.

Deficiency of sulphur-containing amino acids is common among cowpea cultivars (Nti and Plahar, 1995; Plahar et al., 1997; Onwuliri and Obu, 2002) and in the great majority of leguminous seeds (Laurena et al., 1991). Tryptophan contents of the studied cowpea seeds were slightly greater than 11 g kg^{-1} protein, which is considered ideal according to FAO/WHO/UNU (1985) scoring patterns of amino acid requirements for children (2–5 and 10–12 years of age).

Table 4 shows the amino acid composition of the crude extract, globulins, albumins, acid and basic glutelins and prolamins from EPACE-10, Olho de Ovelha and IPA-206 cultivars. Regardless of the protein fraction analysed, there was a prevalence of glutamine/glutamic acid, asparagine/aspartic acid and phenylalanine + tyrosine as in the whole seed of each cultivar. Similarly, the contents of methionine + cysteine of either the crude extract or the protein fractions of the three cultivars remained as low as the values detected for their whole seeds, and did not meet the requirements of 2–5-year-old children. Nevertheless, the crude extract of Olho de Ovelha and the basic glutelins of IPA-206 showed a slight increase in the content of these amino acids and approached the requirements of 10–12-year-old children (FAO/WHO/UNU, 1985).

Comparing the other essential amino acid profiles of the protein fractions with the FAO/WHO/UNU (1985) reference pattern (Table 4), some differences were observed among the three studied cowpea cultivars. The threonine content of the globulin fraction from Olho de Ovelha seems to be slightly deficient. Leucine contents are lower in the prolamins for all three cowpea cultivars studied. Lysine contents are lower in the acid and basic glutelins and prolamins for EPACE-10, whereas IPA-206 cultivar is lysine deficient in the crude extract, basic glutelins and prolamins. As to Olho de Ovelha cultivar only the crude extract and the prolamins showed deficiency of lysine. It is also noteworthy the reduction of tryptophan values, which turned out to be lower than the requirements for 2–5-year-old children. The exceptions were the crude extract, albumins, and globulins of Olho de Ovelha, albumins and basic glutelins of EPACE-10 and IPA-206. Thus, no one protein fraction can be considered a well-balanced protein, which could by itself meet the essential amino acid requirements

Table 4
Amino acid composition (g kg^{-1} protein) of cowpea seed crude extract and protein fractions from EPACE-10, Olho de Ovelha and IPA-206 cultivars.

Amino acid	Cultivars																	
	EPACE-10						Olho de Ovelha						IPA-206					
	CE	Gl	Al	AG	BG	Pr	CE	Gl	Al	AG	BG	Pr	CE	Gl	Al	AG	BG	Pr
Essential																		
Thr	38.0	33.8	59.0	39.8	44.3	54.2	36.8	32.0	59.0	37.0	43.9	56.8	40.2	36.4	49.9	39.1	43.3	47.8
Val	41.2	43.1	48.3	51.6	49.2	58.1	45.2	45.0	48.3	41.5	47.3	48.1	45.5	41.9	45.0	39.8	50.6	62.8
Ile	37.5	38.2	43.3	44.0	41.9	54.2	39.6	41.6	43.3	39.8	42.4	46.6	41.8	46.7	45.7	39.7	45.0	53.9
Leu	72.4	80.4	67.5	77.8	81.4	60.8	74.7	82.6	67.5	73.9	81.6	63.2	76.7	80.0	72.9	74.0	80.7	56.6
Lys	73.0	64.8	80.7	51.4	45.6	38.8	51.8	65.9	80.7	76.6	67.5	56.0	45.9	65.5	74.8	74.5	48.7	39.8
Phe + Tyr	95.1	110.8	99.4	107.2	107.7	113.7	103.7	109.5	99.4	99.4	109.1	102.4	110.0	119.6	106.7	102.6	107.5	115.9
Met + Cys	12.4	8.9	21.5	10.0	15.6	11.2	21.1	19.7	18.9	15.0	15.0	11.9	16.6	15.6	17.6	9.3	24.0	11.2
Trp	10.2	9.5	12.3	7.4	16.8	7.6	12.6	12.8	16.4	8.8	10.1	7.9	10.2	9.5	12.3	7.4	16.8	7.9
His	38.1	36.9	30.4	31.3	34.4	19.9	32.2	34.1	30.4	38.4	31.7	28.6	30.1	38.9	32.6	45.1	37.7	20.3
Arg	84.3	71.8	66.8	72.4	71.0	63.7	84.0	76.3	66.8	89.3	71.9	70.0	80.4	87.2	76.7	99.3	73.0	63.5
Nonessential																		
Asx	122.3	128.2	118.1	128.4	121.0	125.5	126.5	115.4	118.1	108.4	110.2	120.0	121.5	108.1	109.7	104.2	110.2	113.3
Glx	211.1	222.4	156.7	216.6	191.3	117.8	214.0	212.8	156.7	208.8	189.2	141.2	213.7	182.3	167.7	184.4	170.8	124.6
Ser	56.5	55.2	53.8	51.2	57.7	88.8	49.9	51.6	53.8	52.2	55.1	74.6	52.9	54.0	53.5	55.4	53.9	90.6
Gly	33.1	27.4	44.7	34.5	44.0	69.2	31.6	27.1	44.7	32.1	37.2	57.9	34.0	30.4	39.7	35.6	48.5	74.1
Ala	36.6	31.9	54.3	38.9	41.0	30.9	36.8	32.6	54.3	36.1	43.7	42.5	38.9	36.3	49.9	39.1	43.7	29.2
Pro	41.1	40.5	41.8	39.3	43.0	86.4	39.7	41.4	41.8	42.7	44.2	72.6	42.1	47.6	45.3	51.5	45.7	89.2

Values are means of three analyses. The standard deviation values were all less than 5% of means. CE: Crude Extract; Gl: globulins; Al: albumins; AG: acid glutelins; BG: basic glutelins; Pr: prolamins.

Table 5
Lectin activity of the seed crude extract and main protein fractions from three Brazilian cowpea cultivars against enzyme-treated rabbit erythrocytes.

Cultivar	Enzyme	Haemagglutinating activity (HU kg^{-1} flour)[a]		
		Crude extract	Globulins	Albumins
EPACE-10	Trypsin	240,000	111,000	114,500
	Subtilisin	190,200	76,200	114,800
	Bromelain	190,200	55,500	57,200
	Papain	179,500	76,200	86,000
Olho de Ovelha	Trypsin	540,300	35,600	444,400
	Subtilisin	259,500	35,600	111,100
	Bromelain	193,300	18,200	111,100
	Papain	179,700	72,000	111,100
IPA-206	Trypsin	180,300	28,200	123,600
	Subtilisin	99,600	40,200	30,900
	Bromelain	129,900	28,200	61,900
	Papain	99,600	20,500	46,300

[a] Activity is expressed as haemagglutination titre (HU), which is the reciprocal of the highest dilution giving visible agglutination.

Table 6
Trypsin inhibitory activity[a] of seed crude extract and main protein fractions from three Brazilian cowpea cultivars.

Trypsin inibitory activity	Cultivar		
	EPACE-10	Olho de Ovelha	IPA-206
Seed			
g kg^{-1} flour[b]	15.68 ± 0.64b	12.00 ± 1.12a	16.67 ± 1.42b
g kg^{-1} protein[c]	67.08 ± 2.91b	45.59 ± 2.28a	66.09 ± 2.24b
Globulins			
g kg^{-1} flour	2.12 ± 0.01a	2.56 ± 0.10b	2.67 ± 0.06b
g kg^{-1} protein	35.69 ± 0.38a	36.44 ± 0.93a	35.97 ± 0.59[a]
Albumins			
g kg^{-1} flour	3.44 ± 0.01a	3.93 ± 0.02b	4.20 ± 0.04c
g kg^{-1} protein	198.67 ± 8.75a	307.60 ± 6.78b	393.43 ± 16.7c

[a] Values represent means ± standard deviation of six analyses. Different letters in the same row differ significantly ($P < 0.05$).
[b] g trypsin inhibited per kilogram seed flour.
[c] g trypsin inhibited per kilogram protein.

for 2–5-year-old children. Rather, they are complementary to each other and must be provided together as they naturally occur in cowpea seeds.

3.5. Analysis of antinutritional compounds

The presence of lectin is shown in Table 5. Haemagglutinating activity in the seed crude extracts and main protein fractions was detected only against enzymatic-treated rabbit erythrocytes. Enzyme-treated cells were sensitive to agglutination, probably owing to a greater exposure of the carbohydrate moieties present in the cell membrane for which the lectin has higher affinity. Untreated or enzyme-treated erythrocytes of the ABO system and from cow and pig were not agglutinated. Nevertheless, even using rabbit erythrocytes the values obtained were very low when compared to other leguminous seeds (Vasconcelos et al., 1997). The highest haemagglutinating activity against enzyme-treated rabbit erythrocytes was found in the crude extract from Olho de Ovelha cultivar (540,300 HU kg^{-1} flour), which was about 2–3 times higher than those found for EPACE-10 (240,000 HU kg^{-1} flour) and IPA-206 (180,300 HU kg^{-1} flour).

The haemagglutinating activity present in the crude extract was distributed after fractionation both in the globulin and albumin type proteins. Overall, the latter presented the most noticeable haemagglutinating activity. None of the other protein fractions (acid and basic glutelins and prolamins) were able to induce haemagglutination when assayed under the conditions described above. Variations and low levels of activity have been reported for other cowpea cultivars (Marconi et al., 1993). Heat-treatment of cowpea seeds at 98 °C for 30 min completely abolished their haemagglutinating activity (data not shown), suggesting that this biological property was exclusively owing to the presence of lectin and not promoted by polyphenols, tannins or lipids. It has long been known that some lectins are highly toxic when ingested, leading to stunted growth, damage and enlargement of the small intestine, pancreatic hypertrophy and interference with the general metabolism of experimental animals (Lajolo and Genovese, 2002; Vasconcelos and Oliveira, 2004).

The trypsin inhibitor activity is shown in Table 6. All the three cultivar seed crude extracts were able to inhibit trypsin. The amounts of trypsin inhibited varied from 12.00 to 16.67 g kg^{-1} flour. Specific activities of EPACE-10 (67.08 g kg^{-1} protein) and IPA-206 (66.09 g kg^{-1} protein) were similar to each other, but significantly higher than that of Olho de Ovelha (45.59 g kg^{-1} protein). Variation in the protease inhibitor contents had been observed in several cultivated species of *Vigna* (Marconi et al., 1993, 1997). Apparently, domestication decreases the protease inhibitor contents of cowpea, which is nutritionally advantageous. However, from the point of view of the plant, it could enhance its susceptibility to insect and pathogen attack since proteinase inhibitors are supposed to play a defensive role (Casaretto and Corcuera, 1998; Carlini and Grossi-de-Sá, 2002).

Upon fractionation of cowpea seed proteins, the bulk of trypsin inhibitor activity was present in the albumin and globulin fractions (Table 6) and negligible for glutelin and prolamin type proteins (data not shown). The specific activity was more evident in the water-soluble proteins (albumins). However, there were significant variations in the trypsin inhibitor contents within each protein fraction examined among the cultivars. Furthermore, it was noticed that during the crude extract protein fractionation steps there was a huge loss of trypsin inhibitory activity as expressed per kg flour. It has been suggested that trypsin inhibitors are responsible for decreasing dietary protein digestibility, damaging of pancreatic metabolism and depressing growth rates in animals (Grant et al., 1995). As globulin and albumin are the major seed proteins of cowpea, their digestibility by gut proteases might be impaired owing to the presence of these inhibitors.

4. Conclusions

All cultivars showed the usual compositional characteristics of *V. unguiculata*, such as high protein content, methionine as the limiting amino acid, predominance of acidic amino acids and phenylalanine plus tyrosine. However, the content of antinutritional factors differed among the cultivars, but they were, as usual, concentrated in the albumin and globulin fractions. None of cultivars or their respective protein fractions proved to be a better protein source when considering together amino acid profile and antinutritional factors content. Thus nutritional assessment of new cowpea varieties produced conventionally should be a normal practice for food safety since compositional changes may occur and these changes may cause adverse effects to human health.

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