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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

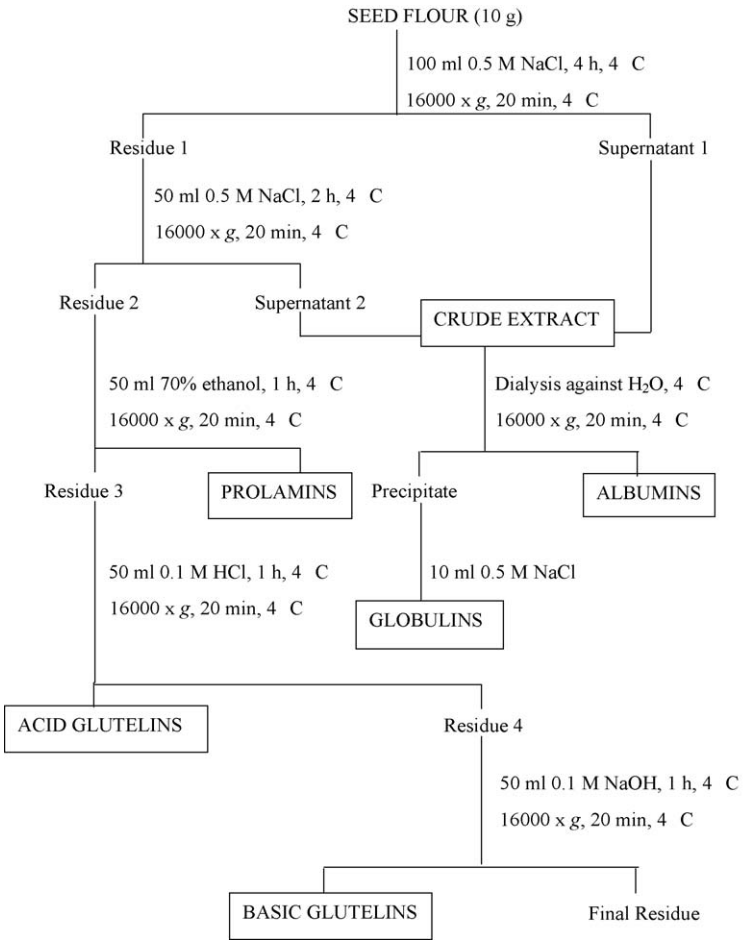

Fig. 1. Fractionation steps of cowpea seed proteins.