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In vitro digestibility of globulins from cowpea (*Vigna unguiculata*) and xerophitic algaroba (*Prosopis juliflora*) seeds by mammalian digestive proteinases: a comparative study

Ana H. Araüjo[a], Patrícia C.B. Cardoso[a], Railene A. Pereira[a], Liziane M. Lima[a], Adeliana S. Oliveira[a], Maria Raquel A. Miranda[b], José Xavier-Filho[c], Maurício P. Sales[a,*]

[a]*Laboratório de Química e Função de Proteínas, Departamento de Bioquímica, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Campus Universitário, Av Salgado Filho s/n, 59072-970 Natal, RN, Brazil*
[b]*Departamento de Fitotecnia, Centro de Ciências Agrárias, Universidade Federal do Ceará, Fortaleza, CE, Brazil*
[c]*Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego 2000, 28015-620 Campos dos Goitacazes, RJ, Brazil*

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Abstract

Globulins were purified from mature and immature cowpea and algaroba seeds by gel filtration (S-200), anion exchange (DEAE-Sepharose) and other chromatographic methods. These globulins (native and heated) were submitted to hydrolysis by pepsin, chymotrypsin and trypsin and their digestion products were analysed by SDS-PAGE. Results showed that native globulins of both legumes were weakly digested by pepsin and were not digested by serine proteases. Heated proteins from cowpea were digested more rapidly by pepsin and chymotrypsin than was algaroba globulin. Trypsin rapidly digested proteins from both cowpea and algaroba. Comparing the hydrolysis of bean globulins to the algaroba globulins by mammalian enzymes, the globulins from immature cowpea showed better digestibility than mature cowpea; globulins from algaroba pod, which is used as an alternative food, were difficult to digest. © 2002 Published by Elsevier Science Ltd.

Keywords: *Prosopis*; *Vigna*; Legume; Globulins; Digestibility

1. Introduction

During seed development, proteins, carbohydrates, lipids, vitamins, minerals, and non-nutrients are laid down, leading to the unique chemical composition of plant seeds that determines their nutritional and functional properties (Lumen, 1990). Plant seeds contribute significantly to human and animal diet and cereal/legumes seeds are a major part of the human diet. Cereals support 50% of the daily per capita protein supply in the world. Although the production of grain legumes is relatively small when compared to cereals, it has a greater relative contribution to human nutrition (FAO, 1980). Legumes are major crops in many tropical countries and serve as important sources of carbohydrates and proteins to poor populations in these regions (Singh & Rachie, 1985). Cowpea and algaroba are two important legume crops of the northeast region of Brazil. This region has long dry seasons and, sometimes, prolonged droughts. During period of intense droughts, cowpea (*Vigna unguiculata*) yields are greatly reduced due to lack of rain. These seeds constitute a primary source of protein (25%) and carbohydrate (63%) for the population. The cowpea proteins are deficient in methionine, cisteine and tryptophan and rich in lysine, leucine and phenylalanine (Kay, 1979). Algaroba seeds are also used as an alternative food source for poor human populations and production is not very sensitive to dry weather conditions. Protein content of algaroba pods ranges from 10 to 14%, and carbohydrate around 49%; these proteins also have low levels of sulphur-containing amino acids (methionine and cysteine), but high levels of hydrophobic amino acids (leucine, lysine and valine;

* Corresponding author. Fax: +55-84-2119208.
E-mail address: msales@cb.ufrn.br (M.P. Sales).

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Silva et al., 1990). The use of algaroba as food is still limited, due the lack of information on its nutritive value. Most studies designed to answer questions related to nutritional quality of legume proteins have focused on proteins in the globulin fraction. Globulin (7S storage proteins) fractions are present in the seeds in high amounts and is in fractions of antinutritional factors (Shewry, 1995).

The objective of this work was to study the action of mammalian proteinases, trypsin, chymotrypsin and pepsin, on globulins from mature cowpea seeds, immature cowpea seeds and algaroba seeds, in an attempt to relate these in vitro results to nutritional evaluation of these proteins.

2. Material and methods

2.1. Seeds

Green and dry Cowpea (*V. unguiculata*) seeds were acquired from a local market. Algaroba pods and seeds were supplied by Instituto Brasileiro do Meio Ambiente (IBAMA), Natal, Rio Grande do Norte, Brazil.

2.2. Enzymes

Commercially-available trypsin and chymotrypsin were obtained from Sigma Co. (St. Louis, USA). Pepsin was a product of Nutritional Biochemical Co.

2.3. Protein determination

Protein content was measured by the procedure developed of Bradford (1976) with bovine serum albumin (BSA) as the protein standard.

2.4. Purification of globulins

Globulins were prepared from cowpea and algaroba seeds by the procedure of Macedo, Fernandes, Sales, and Xavier-Filho (1995). Ground meals, extracted with 50 mM borate buffer pH 8.0 for 30 min at room temperature, were centrifuged (30 min at 8000 × ***g***, 5 °C) and supernatant proteins were fractionated by ammonium sulphate precipitation. The 70–90% saturation fraction was dialysed against water, freeze-dried and applied to a Sephacryl S-200 column (3 × 40 cm) equilibrated and eluted with the same buffer as used for extraction. The globulin-rich fractions were recovered after an ion-exchange chromatography on a DEAE-Sepharose column (2 × 20 cm), equilibrated with 50 mM Tris–HCl pH 8.0 and eluted with a NaCl gradient (0–1 M) in the same buffer. The recovered globulin-rich fractions were submitted to chromatography on a Sephacryl S-400 column (2.5 × 70 cm) in 0.1 M Tris–HCl, 0.25 M NaCl, pH 8.0, for further purification. Globulins were dialysed against water and freeze-dried.

2.5. In vitro digestibility of globulin

Globulins were dissolved in 0.01M phosphate buffer, pH 6.0 at 0.5 mg/ml concentration. Globulins (aliquots of 250 μl) were assayed for digestion by 10 μl pepsin (25 μg/ml in 50mM HCl); or trypsin (25 μg/ml in 0.01M phosphate buffer, pH 7.0) or chymotrypsin (25 μg/ml in 0.01M phosphate buffer, pH 7.0), at 37 °C for periods of 15 min, 30 min, 1 h, 2 h and 4 h. The ratio of substrate to proteinase was 20:1. Adding a 10% SDS solution stopped the digestion. The enzyme activities used were as the in vivo digestion process.

2.6. SDS-PAGE-Polyacrylamide gel electrophoresis

This method was carried out using a Laemmli (1970) system. Proteins used as molecular mass standards were: bovine serum albumin (66k Da), ovalbumin (45k Da), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α-lactoglobulin (14.2 kDa).

3. Results and discussion

Mature and immature cowpea and algaroba globulins were purified by gel filtration and ion-exchange chromatography according to Macedo et al. (1995) (data not shown). Globulins from algaroba had two subunits with relative molecular masses of 23 and 15 kDa and globulins from mature and immature cowpea seeds had relative molecular masses of about 66–45 kDa, as could be observed by SDS-PAGE. These correspond to the typical molecular mass of 7S storage proteins, in agreement with data previously reported by several research groups (Casey, Domeney, & Ellis, 1986; Khan, Gatehouse, & Boulter, 1980; Macedo, Fernandes, Sales, & Xavier-Filho, 1995; Pedalino, Paino-d'Urzo, Dello Done, Grillo, & Rao, 1992; Fig. 1).

Most of the literature on the degradation of legume proteins concerns the major storage protein (globulins), which constitutes 50–75% total seed proteins (Khan et al., 1980; Shewry, 1995). This protein fraction is free of antinutritional factors, such as proteinase inhibitors and lectins, and this is why it is often used in studies designed to answer questions related to the nutritional value of proteins.

Digestion of native globulins and heated cowpea globulins by mammalian digestive proteinases was carried out as a comparative model for the action of pepsin, trypsin and chymotrypsin. The main reason is the fact that cowpea is most frequently used as a conventional food source by local populations. On the other hand, algaroba was studied because it is an alternative source

of food, used during intense droughts by poor local populations and also because there is almost no information on its digestibility, in current literature.

Incubation of native globulins from algaroba and mature and immature cowpea seeds with pepsin, for up to 4 h at 37 °C showed weak signs of hydrolysis by SDS-PAGE (Figs. 2A, 3A, and 4A). However, incubation of heated globulins from three seeds with pepsin showed signs of hydrolysis on SDS-PAGE. Globulins from mature and immature cowpea showed signs of hydrolysis after 30 min; globulins from algaroba were degraded after 4 h (Figs. 2D, 3D, and 4D). These results were in agreement with previous findings that globulins are resistant to hydrolysis by pepsin (Vaintraub, Seliger, &

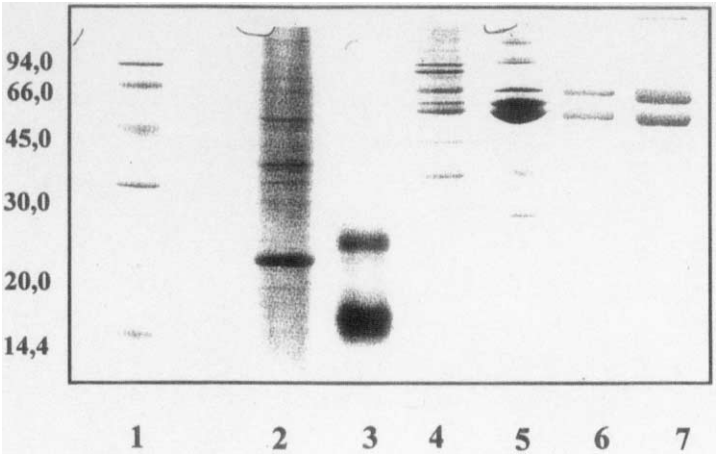

Fig. 1. SDS-PAGE patterns of, globulin and albumin seeds. (A) Albumins from *Prosopis juliflora* seeds; (B) globulins from *Prosopis juliflora* seeds; (C) albumins from dry *Vigna unguiculata* seeds; (D) globulins from dry *Vigna unguiculata* seeds; (E) albumins from green *Vigna unguiculata* seeds; (F) globulins from green *Vigna unguiculata* seeds. Vertical numbers indicate molecular weight markers in kDa.

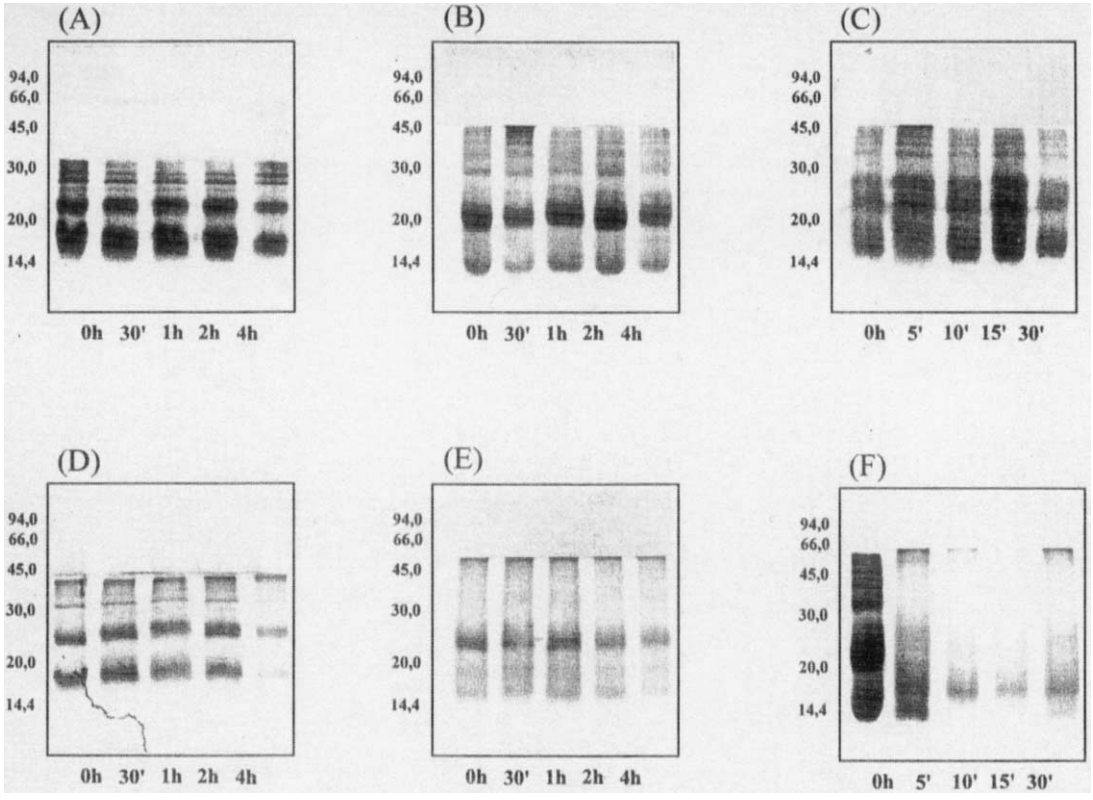

Fig. 2. SDS-PAGE patterns of *Prosopis juliflora* seed globulins digestion by pepsin, chymotrypsin and trypsin. (A) Native globulin digestion by pepsin; (B) native globulin digestion by chymotrypsin; (C) native globulin digestion by trypsin; (D) heated globulin digestion by pepsin; (E) heated globulin digestion by chymotrypsin; (F) heated globulin digestion by trypsin. Vertical numbers indicate molecular weight markers in kDa. Horizontal numbers refer time of digestion.

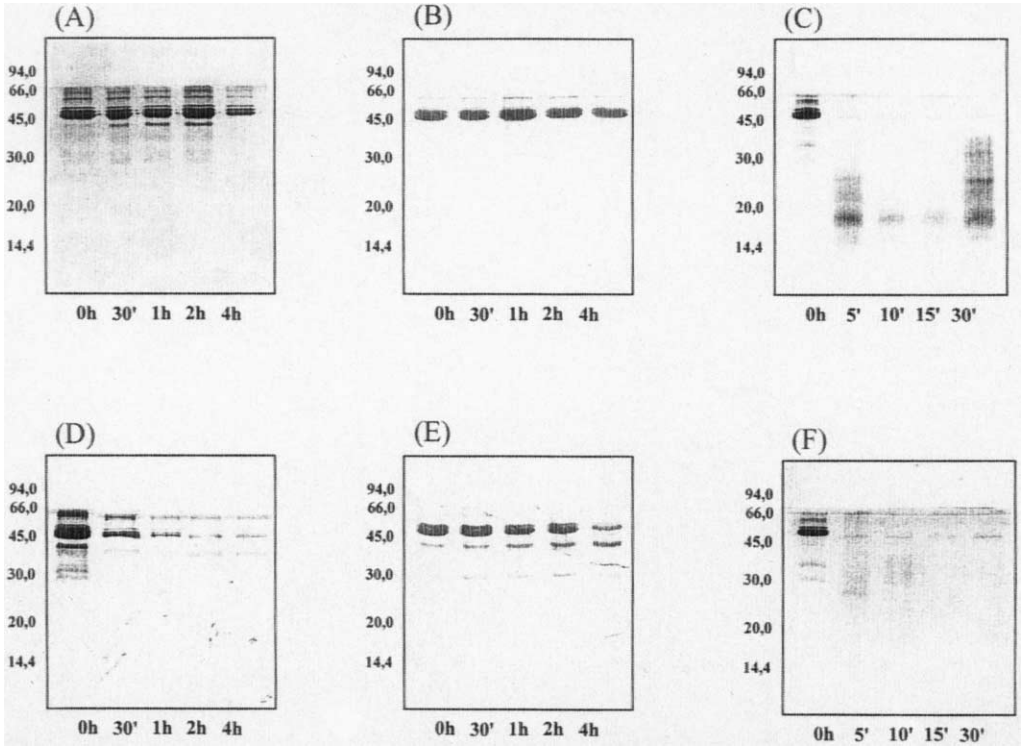

Fig. 3. SDS-PAGE patterns of dry *Vigna unguiculata* seeds globulins digestion by pepsin, chymotrypsin and trypsin. (A) native globulin digestion by pepsin; (B) native globulin digestion by chymotrypsin; (C) native globulin digestion by trypsin; (D) heated globulin digestion by pepsin; (E) heated globulin digestion by chymotrypsin; (F) heated globulin digestion by trypsin. Vertical numbers indicate molecular weight markers in kDa. Horizontal numbers refer to time of digestion.

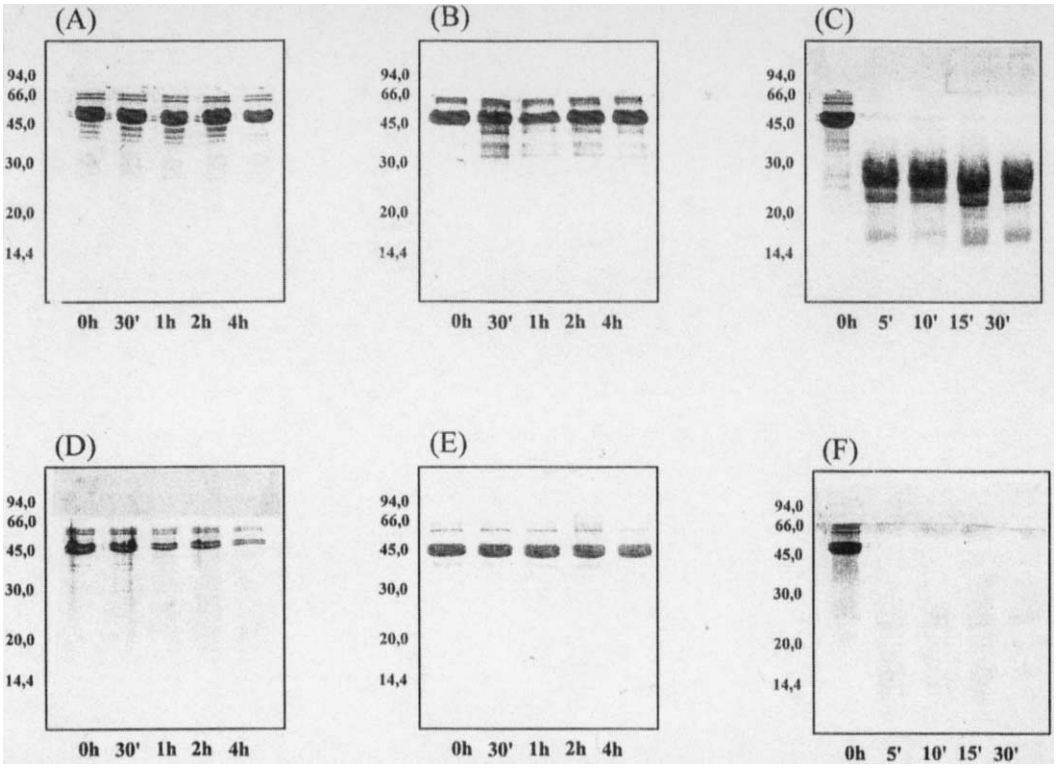

Fig. 4. SDS-PAGE patterns of green *Vigna unguiculata* seeds globulins digestion by pepsin, chymotrypsin and trypsin. (A) native globulin digestion by pepsin; (B) native globulin digestion by chymotrypsin; (C) native globulin digestion by trypsin; (D) heated globulin digestion by pepsin; (E) heated globulin digestion by chymotrypsin; (F) heated globulin digestion by trypsin. Vertical numbers indicate molecular weight markers in kDa. Horizontal numbers refer to time of digestion.

Shutov, 1979; Bradbear & Boulter, 1984; Deshpande & Nielsen, 1987; Shashikala & Prakash, 1995). In vitro hydrolysis with chymotrypsin, for 4 h, showed that native globulins from cowpea and algaroba were resistant to hydrolysis, while heated globulins from cowpea were slowly degraded by this enzyme (Figs. 2B, 3B, and 4B). These results on chymotrypsin digestion suggest that native globulins are cleaved in such way that the main part of the protein remains intact, as is observed with phaseolin from *Phaseolus vulgaris* seeds (Nielsen, 1988). Cooking globulins apparently does not open up proteins to allow access to chymotrypsin cleavage sites (Figs. 2E, 3E, and 4E). Trypsin is a unique enzyme, since it cleaves both native mature and immature cowpea globulins, and its degradation products range from 30 to 18 kDa at 5 min digestion time. On the other hand, algaroba globulins were not hydrolysed by trypsin (Figs. 2C, 3C, and 4C). After heat-treatment, hydrolysis was increased, both mature and immature cowpea globulins were completely digested by trypsin and algaroba globulins proved much more susceptible to hydrolysis (Figs. 2F, 3F, and 4F). Native globulins are resistant to digestion by isolated enzymes, but hydrolysis is greatly increased by cooking. Similar results were observed in various storage proteins submitted to in vitro hydrolysis studies (Agudelo, Alarcon, & Flidel, 1998; Deshpande & Nielsen, 1987; Liener & Thompson, 1980; Petzke, Ezeagu, Proll, Akinsoyinu, & Metges, 1997; Romero & Ryan, 1978; Sales, Macedo, & Xavier-Filho, 1992; Shashikala & Prakash, 1995). In previous works, done by the authors above, the results were similar to those found here with the combined action of the digestive mammalian enzymes (data not shown) and with the fact that algaroba globulins were more resistant to hydrolysis than both, mature and immature, cowpea globulins. These results are suggestive of the presence of more refractory structures in the globulin of the algaroba (*Prosopis juliflora*) that contributes to its low nutritional value and poor digestibility. Studies comparing the breakdown of these legume proteins by digestive mammalian proteinases lead to a better understanding of protein structures, digestibility and nutritive values.

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