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Interaction and digestibility of phaseolin/polyphenol in the common bean

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ABSTRACT

The polyphenols and phaseolin interaction in common bean varieties was studied. Raw beans of three different colours were analysed: black (BRS Supremo), brown (BRS Pontal) and white (WAF-75). Based on the phaseolin digestibility *in vitro* and phaseolin–polyphenol complexation obtained by SDS–PAGE on a 10% polyacrylamide gel, it was observed that the polyphenols interfere with the digestibility of beans by decreasing the hydrolysis of phaseolin, especially in the darker ones. Furthermore it was possible to verify a difference in the electrophoretic pattern of phaseolin, indicating an interaction between phaseolin and polyphenols.

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1. Introduction

Beans are a rich source of nutrients and are considered an important food in Brazil. Aside from being an excellent source of some vitamins and minerals, the common bean (*Phaseolus vulgaris* L.) is rich in nutrients and has significant amounts of protein, calories, unsaturated fatty acids (linoleic acid), and dietary fibre, particularly soluble fibre (Kutos, Golob, Kac, & Plestenjak, 2003; Villavicencio, Mancini-Filho, & Delinceé, 2000). While the potential of the bean protein is high, it is associated with antinutritional factors and other substances that are harmful to health (Pröll, Petzke, Ezeagu, & Metges, 1998), such as inhibitors of proteases, lectins, anti-vitamins, saponins, tannins, flatulence factors, allergens, phytic acid and toxins (Vasconcelos, Trentim, Guimarães, & Carlini, 1994).

Among the antinutritional factors, polyphenols are the main contributors to the low digestibility of the bean. Polyphenols are part of the composition of many plants and are considered antinutritional factors of great importance. They are highly chemically active and may react reversibly or irreversibly with proteins, impairing the digestibility and bioavailability of essential amino acids. The most important phenolic substances found in plants are phenolic acids, flavonoids and tannins. In legumes, tannins are prevalent and have the ability to bind to proteins through hydrogen bonds, thereby preventing their digestibility (Reddy & Butler, 1989).

Besides proteins, tannins form complexes with starch and digestive enzymes, reducing the nutritional value. Tannins are attributed with other harmful effects in the diet, such as undesirable food and decreased palatability due to astringency (Chung, Wong, Wei, Huang, & Lin, 1998).

Many studies have shown that the bean seed coat has a greater phenolics content than has the cotyledon (Aparicio-Fernandez, Yousef, Loarca-Piña, De Mejia, & Lila, 2005). According to Ranilla, Genovese, and Lajolo (2007), in general, the condensed tannins, anthocyanins and flavonols are mostly found in seed coats while the phenolic acids are concentrated mainly in the cotyledons. The seed coat colour pattern and the type of cultivar of *P. vulgaris* L. represent an important influence on the variability of phenolic profiles and levels. In most cases, the coloured beans have higher concentrations of phenolics (Sutivisedsak et al., 2010). This study evaluated the interaction between phaseolin and polyphenols of extracted fractions of bean seeds with different colours.

2. Materials and methods

2.1. Samples

The varieties of common bean (*P. vulgaris* L.) seeds that were used in this study were BRS Supremo (black colour), Carioca Pontal (brown colour) and WAF 75 (white colour). All seeds were donated by EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária). The

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samples were milled in a knife mill and passed through a 30 mesh sieve with the purpose of removing the larger particles. This flour was stored in polyethylene bags, sealed, kept under refrigeration (4 °C), and used within two months.

2.2. Extraction of phaseolin

Phaseolin was extracted according to the methods of Ahn, Sen, and Whitaker (1991). The samples were prepared with 6 g of raw bean flour after adding 100 ml of cold distilled water. Then, 23.78 g of ammonium sulphate were added in order to precipitate the proteins. The bean samples were agitated for an hour and a half in an orbital shaker and then filtered. We then added 2.378 g of ammonium sulphate to the solution and allowed it to agitate for a further hour. The samples were then centrifuged at 30,000*g* for 30 min at 4 °C. The precipitate that formed in the solution was discarded and we used only the supernatant. To this solution, 8.71 g of ammonium sulphate were added and the solution was stirred for a further hour. Once again, the samples were centrifuged under the same conditions described above but, in this step, the precipitate of the solution was used Added to the precipitate was a minimum volume of phosphate buffer, pH 7. Then, the samples were placed in dialysis membranes where they remained for three days in cold water- which was changed several times to remove the salts present in the medium. After this step, the samples were freeze-dried and stored refrigerated at 6 °C.

2.3. Extraction of polyphenols

The extraction was performed according to Cardador-Martinez, Loarca-Piña, and Oomah (2002). In order to perform this extraction, 10 g of lyophilised flour were weighed out and combined with 100 ml of methanol. The mixture was stirred for 24 h at 25 °C. After that, the samples were centrifuged for 10 min at 5449*g*. The supernatant was placed in a balloon and the methanol was evaporated in a rotary evaporator at 35 °C with a vacuum of 26 lb in^{-2}. The extracts were frozen at −20 °C and lyophilised.

2.4. Fractionation of polyphenolic crude extract

The material obtained in the extraction was separated into 6 fractions by open column chromatography with a vacuum, using a methodology proposed by Aparicio-Fernandez, Manzo-Bonilla, and Loarca-Piña (2005).

The solution was placed in an open column with silica gel. The following reagents were added and the fractions were collected after each passage: (1) 100% petroleum ether, (2) petroleum ether:ethyl acetate (1:1, v/v), (3) 100% ethyl acetate. The solvents used for fractions 4–19 were ethyl acetate with a gradient of increasing concentration (2%, 5% 8% 12% 15% 20% 25% 30% 35% 40% 45%, 50%, 60%, 70%, 80%, 90%) and methanol/water (1:1, v/v). The fraction (20) contained methanol and water (1:1, v/v), (21) 100% methanol, and (22) only water.

The extracts were grouped in the following order: fraction A (1–3), fraction B (4–8), fraction C (9–12), fraction D (13–17), fraction E (18–20) and fraction F (21, 22) according to results presented by Aparicio-Fernandez, Manzo-Bonilla, et al. (2005) and Aparicio-Fernandez, Yousef, et al. (2005). In order to evaporate, the mixtures were placed in a balloon on a rotary evaporator.

2.5. Digestibility of phaseolin

The digestibility of the protein was determined by the method of Akeson and Stahmann (1964), which is assessed *in vitro* by determining the rate of enzymatic hydrolysis through the associations of pepsin and pancreatin in order to simulate the conditions existing in the gastrointestinal tract. Initially, 0.05 g of phaseolin were weighed and added to 3.3 ml of an acidic solution of pepsin. The samples were maintained for 3 h at 37 °C in a shaking water bath. Then, the samples were neutralised with 3.3 ml of 0.1 N NaOH and added to 3.3 ml of pancreatin. The samples were kept for 24 h at 37 °C in a shaking water bath. In the next stage, 2 ml of the mixture were withdrawn and transferred to a centrifuge tube. Added to this mixture were 3.3 ml of picric acid (1%). The material was centrifuged for 30 min at 13950*g*. The Bradford method for protein determination was then used by pipetting 20 μl of the sample into a quartz cuvette and adding 1 ml of Bradford reagent solution. After 2 min, a reading was obtained on the spectrophotometer at 595 nm.

The analysis of digestibility was originally done only with phaseolin, and was later repeated with the addition of polyphenolic extracts, being first added to 2.5 mg of polyphenolic crude extract and in the following analysis, being added to 2.5 mg of the polyphenol fractions of phaseolin.

2.6. Electrophoresis by SDS–PAGE

The electrophoresis were performed in polyacrylamide gel at a concentration of 10%. Added to the gel were 20 mg of phaseolin. For the preparation of the polyphenol–phaseolin mixture, 2 mg of polyphenols (dissolved in 10% ethanol) and 20 mg of phaseolin were added. The gels were stained in a solution of Coomassie Brilliant Blue R250 for 2 h and then bleached in a solution of methanol and acetic acid.

2.7. Statistical analysis

The trials were randomised. For the results, we used the SAS software (1996) for analysis of variance by *F* test and comparison of means by the Tukey test ($p \leqslant 0.05$).

3. Results and discussion

3.1. Protein digestibility

Protein digestibility is a nutritional parameter that evaluates the use of a protein source. This is influenced by several factors, for example, phenolic compounds, inhibitors of protein, and heat treatment (Antunes, Bilhalva, Elias, & Soares, 1995).

Table 1 shows the digestibility of phaseolin before and after the addition of polyphenolic crude extract for the three bean cultivars under study. The results of the first analysis proved to be superior to those reported by Genovese and Lajolo (1998), who obtained results from 9.8% to 22.5% for the digestibility of phaseolin obtained from raw bean. According to Genovese and Lajolo (1998), in the raw bean, phaseolin is highly resistant to hydrolysis *in vitro*. This

Table 1
Phaseolin digestibility (%) (means ± standard deviation) of different cultivars of common bean (*Phaseolus vulgaris* L.), before and after the addition of the crude extract of polyphenols.

Cultivars	Digestibility (%) of phaseolin	Digestibility (%) of phaseolin–polyphenol
BRS Supremo	76.2 ± 1.5[1]a[2]A[3]	15.1 ± 3.5^{bB}
BRS Pontal	75.8 ± 2.1^{aA}	18.8 ± 2.7^{abB}
WAF 75	76.8 ± 1.3^{aA}	23.1 ± 2.2^{aB}

[1] Means ± standard deviation.
[2] Different small letters vertically for the same analysis, indicate significant differences ($p \leqslant 0.05$) with the cultivar.
[3] Different capital letters indicate horizontal significant difference ($p \leqslant 0.05$) with respect to the treatment used.

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