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In vitro and *in vivo* protein hydrolysis of beans (*Phaseolus vulgaris*) genetically modified to express different phaseolin types

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Abstract

Experiments were conducted to study whether phaseolin type could influence proteolysis susceptibility and nutritional value of total bean protein. The DOR-390 bean cultivar was genetically modified to express different phaseolin types (S, T or I). Beans were soaked and autoclaved. A sequential hydrolysis was carried out *in vitro* with pepsin and pancreatin. Differences in the degree of protein hydrolysis among bean lines started at 30 min and remained until 240 min, with the S bean proteins presenting lower values ($P < 0.05$). Subsequently, rats were fed with diets containing beans expressing different phaseolin types as the only source of protein for N digestibility and nutritional value determination. No differences ($P > 0.05$) in ileal protein digestibility and rat growth were observed. In conclusion, the differences in *in vitro* hydrolysis between bean lines expressing different phaseolin types had no consequences on growth and N retention in rats.

Keywords: *Phaseolus vulgaris*; Phaseolin; Nutritional value; *In vitro* hydrolysis

1. Introduction

The common bean (*Phaseolus vulgaris*) is a staple food for many people of Latin America and Central Africa. It is rich in protein with high lysine content as well as in minerals, starch and dietary fibre (Leterme & Muñoz, 2002).

Phaseolin, its major storage protein represents 40–50% of the total seed protein (Chagas & Santoro, 1997). Its nutritive value is limited by low sulphur-containing amino acid and a high resistance of raw phaseolin to proteolysis. Thermal treatment drastically improves phaseolin digestion (Montoya et al., 2006).

More than 40 phaseolin types have been classified by electrophoresis according to their subunit composition. The S (Sanilac) and T (Tendergreen) phaseolin are the two major phaseolins found in cultivated beans. They typically have three subunits with a MW ranging from 45.6 to 54.4 kDa (Chagas & Santoro, 1997; Salmanowicz, 2001). They differ in subunit composition: the S type has 2α, 2β and 2γ subunits which appear as three main bands in SDS-PAGE (Brown, Ma, Bliss, & Hall, 1981). However, the S phaseolin may appear as two main bands when less resolution equipment or conditions are used (Gepts, 1988; Koening, Singh, & Gepts, 1990; Montoya et al., 2006). The T phaseolin has only 1α, 1β and 1γ subunits, appearing as three main bands in SDS-PAGE (Brown et al., 1981). Finally, the I (Inca) type is unique among the phaseolin family in that it lacks the largest polypeptide

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(52 kDa; Koening et al., 1990). Thus it appears with two bands only in SDS-PAGE.

Plant breeders would like to improve the nutritional quality of beans. They screen bean accessions for possible differences between phaseolin types in methionine content or digestibility. However, phaseolin type will be integrated in breeding programmes only if evidence is made that genetic modification will result in an improvement of the nutritional value of whole cooked beans. The susceptibility to hydrolysis *in vitro* varies considerably among phaseolin types (Montoya, unpublished data). These differences could be due to differences in protein subunit composition (Fukuda et al., 2005) or in amino acid composition.

Differences between phaseolin types have been reported for *in vivo* digestibility (S and T phaseolins in whole bean; Begbie & Ross, 1993) and *in vitro* hydrolysis (S, T and I purified phaseolins; Montoya, Lallès, Beebe, Souffrant, & Leterme, in press). However, it is difficult to compare the value of different phaseolin types in whole beans, since the latter vary in size, colour and composition. In order to overcome that problem, the DOR-390 bean variety with small black seeds and naturally expressing the S phaseolin type, has been genetically modified to express either the T or the I phaseolin types.

The present work aimed to study the *in vitro* and *in vivo* proteolysis of cooked beans differing in their phaseolin types only and the possible consequences on the nutritional value of beans in rats.

2. Materials and methods

2.1. Production of bean lines expressing different types of phaseolins

The initial bean was the DOR-390 cultivar, a bean with small black seeds, which normally expresses the S phaseolin type. Through backcrossing, the T and I phaseolin types were introduced in the DOR-390 bean line. The source of the T phaseolin was G19892 of the CIAT gene bank, a wild bean accession from Argentina. The source of I phaseolin was G23585, a wild bean from Ecuador. The F1 plants of the backcross-2 generation were analyzed by electrophoresis to identify the heterozygotes carrying the T or I phaseolin. The beans were self-pollinated and the individual F2 plants were analyzed by electrophoresis. Lines with T and I phaseolin in the homozygous state were selected. Then, the lines with the three different phaseolin types were grown in order to produce enough material for the study.

2.2. Preparation of the beans and the purified phaseolins

The beans were soaked in distilled water (1:3, w:v) overnight at room temperature. The following day, the water was changed and the beans were washed, autoclaved in distilled water (1:3, w:v) at 121 °C (15 psi) for 20 min, frozen and freeze-dried. The beans were then ground (0.5 mm-mesh) and kept at −20 °C until use.

The phaseolins were isolated as described by Montoya et al. (2006). Briefly, flour prepared with dehulled beans (1 g/20 ml) was extracted with 0.5 M NaCl in 0.025 M HCl at pH 2.0. Then, it was centrifuged at 20,000*g* for 20 min. The supernatant fraction was mixed with five volumes of distilled water (1:5) at 4 °C and centrifuged for 20 min at 4 °C and 20,000*g*. The final precipitate was suspended in 0.5 M NaCl and dialyzed against distilled water (4 °C) for 24 h, and then frozen and freeze-dried.

2.3. In vitro hydrolysis experiment

2.3.1. Enzymatic hydrolysis protocol

Porcine pepsin (Merck No. 107197) and pancreatin (a mixture of pancreatic enzymes, Sigma No. P1750), were used to study the kinetics of *in vitro* protein hydrolysis of the beans. Casein was used as a reference protein. Samples were mixed with HCl 0.1 N (pH 2.0; 9.4 mg protein/ml HCl) and pre-incubated for 30 min at 39 °C in a water-bath under continuous stirring. Then, pepsin was added to the medium with an enzyme to protein ratio of 1:67 (w:w). Aliquots were taken after 0, 30 and 120 min of pepsin hydrolysis. Phosphate buffer saline (0.2 M, pH 8.0) was then mixed (1:1) with the remaining incubation medium and pancreatin was added with an enzyme to protein ratio of 1:30 (w:w). Aliquots were taken 20, 120 and 240 min after pancreatin addition (i.e. at times 140, 240 and 360 min after pepsin addition).

Aliquots were immediately added with trichloroacetic acid [TCA, 7.5% (w:v) final concentration] for precipitating protein. After centrifugation at 21,000 *g* for 10 min, the supernatants were collected for TCA-soluble N determination by the Kjeldahl method.

2.4. Sample treatment and SDS-PAGE electrophoresis

Other aliquots collected as above were immediately mixed with SDS 20% with a final concentration of 7% (v:v). They were incubated at 100 °C for 3 min and centrifuged at 21,000 *g* for 5 min. The supernatants were frozen at −20 °C.

Electrophoresis was conducted in 62.5 mM Tris–HCl buffer with 3.4 mM SDS for 3 h under a current of 70 V, as previously described (Salgado et al., 2003), except that we used concentration and separation gels with 4.8% and 15% polyacrilamide, respectively. Molecular weight (MW) standards (14.2–66.0 kDa; MW-SDS-70L, Sigma) were added to specific wells in each gel. The MW of each protein band detected visually after enzymatic hydrolysis was determined using a linear regression obtained from MW standards migration (Salgado et al., 2002).

2.5. In vivo experiments

2.5.1. Diet preparation

The diets of Experiment 1 (digestibility trial) were formulated to contain 100 g protein/kg dry matter (DM). In