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Effect of germination on the protein fraction composition of different lupin seeds

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Abstract

Sweet lupin seeds (*Lupinus luteus* cv. 4486 and cv. 4492 and *Lupinus angustifolius* cv. *troll* and cv. *zapaton*) were germinated and investigated according to protein composition, nitrogen and amino acid content of Osborne fractions. In raw lupins, globulins (G) comprised the main fraction of lupins, followed by albumins (A) and glutelins+prolamines (Gt + P). Differences in the protein profile of the Osborne fractions were found among species whilst cultivars did not show electrophoretic differences. Amino acid content of protein fractions was also studied and differences among cultivars were found. In general, Glu, Gly, Arg and Ala (as non-essential amino acids, NEAA) and Lys (as essential amino acid, EAA) were predominant in the A fraction, Glu and Arg (NEAA) and Leu and Thr (EAA) were the main ones in the G fraction; while Asp, Glu, Gly and Arg (NEAA) and Leu and Lys (EAA) were the major components of the Gt + P fraction. Germination increased the protein content of *L. luteus* cv. 4486, *L. angustifolius* cv. *troll* and cv. *zapaton* and caused sharp changes in the protein profile of the Osborne fractions. After germination, the A fraction almost disappeared in the protein profile while G and Gt + P fractions were modified, depending on the lupin species and cultivar.

Keywords: Lupin; Storage proteins; Fractional composition; Amino acids

1. Introduction

Lupin is an economically and agriculturally valuable plant which is able to grow in different soils and climates. Interest in lupin production is increasing, not only because of its strong capacity to fix nitrogen, making accessible macro and micro elements elute to the soil sublayer (Gulewicz, Peretiatkowicz, Bratek-Wiewiórowska, & Wiewiórowski, 1993) but also because of the high protein content of lupin seeds (Duranti & Gius, 1997; Hudson, 1979; Petterson, 1998; Sujak, Kotlarz, & Strobel, 2006). The utilization of lupin seeds can be extended to the production of protein concentrates which, added to other food products, can enrich their nutritional values and improve their technological properties, thus giving higher quality foods (Dijkstra, Linnemann, & van Boekel, 2003; Gladstones, 1998; Torres, Frias, Granito, Guerra, & Vidal-Valverde, 2007b). A recent *in vivo* study has shown that lupin protein is a good quality ingredient, as demonstrated by the biological indices assayed (higher nutritive utilization of protein, improvement in weight gain and the food transformation index), which show that lupin is an excellent protein source for human and animal nutrition (Martínez-Villaluenga, Urbano, Porres, Frías, & Vidal-Valverde, 2007) that could replace soy concentrates in countries where soybean must be imported (Ruiz & Hove, 1976).

Lupin seed protein is considered to be a good source of lysine (Lys) and, generally, poor in the sulfur-containing amino acids (Met and Cys) (Hudson, 1979; Petterson,

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1998). According to Osborne fractionation (Osborne & Campbell, 1898), lupin proteins can be divided into water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamines and acid/alkali-soluble glutelins (Mandal & Mandal, 2000). The main storage proteins of lupin seeds are globulins while prolamines and glutelins are detected in small amounts (Blagrove & Gillespie, 1975; Duranti, Gorinstein, & Cerletti, 1990; Mandal & Mandal, 2000; Peretiatkowicz, Wojtaszek, Stencel, & Gulewicz, 1988b).

The presence of antinutritional compounds, such as alkaloids and α-galactosides, in lupin seeds has played an important role in the utilization of lupin seeds as a protein source for purposes of human and animal nutrition. With the appearance of sweet (low alkaloid content) lupin varieties, the single most important constraint for the consumption of this nutritious food is the high level of α-galactosides (Frias, Diaz-Pollán, Hedley, & Vidal-Valverde, 1995; Martínez-Villaluenga, Frias, & Vidal-Valverde, 2006; Price, Lewis, Wyatt, & Fenwick, 1988) which have been reported to cause several negative nutritional effects, including flatulence, osmotic effects and a reduction in protein utilization and net dietary energy (Martínez-Villaluenga, Frias, & Vidal-Valverde, in press-a).

Germination, among other technological processes, has been widely used for its ability to decrease levels of antinutritional factors present in legume seeds, at the same time improving the concentration and bioavailability of their nutrients (Ghorphade & Kadam, 1989; Urbano et al., 2005a; Vidal-Valverde et al., 2002). The extensive breakdown of seed-storage proteins that takes place during this process improves protein digestibility and the essential amino acid content, thus enhancing the nutritional value of legumes (Duranti, Cucchetti, & Cerletti, 1984; Kuo, Rozan, Lambein, Frias, & Vidal-Valverde, 2004; Rozan, Kuo, & Lambein, 2001). Less information, however, is available about the effect of germination on the profile of albumins, globulins, glutelins and prolamines, or their amino acid composition in legume seeds, which might show that lupin sprouts are an unexploited potential source of dietary protein.

The aim of this work was to study the nitrogen content, protein composition and amino acid content of Osborne fractions in different cultivars of *Lupinus luteus* (cv. 4486 and cv. 4492) and *Lupinus angustifolius* (cv. *troll* and cv. *zapaton*) in order to establish the effect of the genotype. Afterwards, the effect of germination on nitrogen content, protein composition and amino acid content of the Osborne fractions of these lupins was also studied.

2. Materials and methods

2.1. Seeds

Sweet seeds of *L. angustifolius* cv. *troll* were kindly supplied by the Plant Breeding Station in Wiatrowo, near Poznań (Poland). Sweet seeds of *L. angustifolius*cv. *zapaton* and *L. luteus* cv. 4486 and cv. 4492 were provided by the Agrarian Research and Technology Development Service from the Agriculture and Commerce Council of the Junta de Extremadura (Spain).

2.2. Germination procedure

Seeds (10 g) were soaked for 6 h and the imbibed seeds were germinated on a pilot scale germinator (G-120 model, ASL Snijders International S.L., Holland) according to Martinez-Villaluenga, Gulewicz, Frias, Gulewicz, and Vidal-Valverde (in press-b). Germination was performed in darkness, for 5 days at 20 °C. Sprouted seeds were lyophilized and stored under vacuum in plastic bags. The lyophilized raw and sprouted seeds were ground to pass through a sieve of 0.5 mm before analysis.

2.3. Fractionation of raw and sprouted lupin proteins and determination of nitrogen content

Fractionation of protein was carried out according to the method described by Peretiatkowicz et al. (1988b). The general fractionation scheme is shown in Fig. 1. The nitrogen content of the initial material (raw and sprouted seeds) and resulting protein fractions (A, albumins; NPN, non-protein nitrogen; G, globulins; Gt+P glutelins and prolamines; RN, residual nitrogen) was determined by the Kjeldahl method, using the Kjeltec Auto Distillation 2200 apparatus (FOSS TECATOR, Foss, Hillerod, Denmark).

2.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of protein fractions

The precipitated protein fractions obtained according to Fig. 1 were dissolved in TBS buffer (25 mM NaCl and 25 mM TRIS, pH 7.5). Then, fractions were passed through the Amicon filter (10 kDa) after equilibration with TBS buffer. Reduction of protein disulfide bonds was performed by adding 2-mercaptoethanol (5%, v/v) and kept at 100 °C for 3 min. Samples were centrifuged at 4000*g* for 10 min and the aliquots of the supernatants containing 20 μg protein were used to load the gels. SDS-PAGE gel electrophoresis was carried out using the discontinuous system (10% separating/4% stacking gel) described by Laemmli (1970). SDS-PAGE electrophoresis was performed on the Biometra Power Pack P25 apparatus at 7.5 mA (stacking gel) and 14 mA (separating gel) for 3 h. Proteins were fixed in the gel using 10% acetic acid in 50% ethanol and Coomassie Blue stain.

2.5. Determination of amino acid composition of protein fractions

Determination of total amino acids in every Osborne protein fraction was carried out by acid hydrolysis, derivatization and HPLC quantification using the method of Rozan et al. (2001). DL-norleucine (200 μl), of 0.2 mmol/ml