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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.
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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

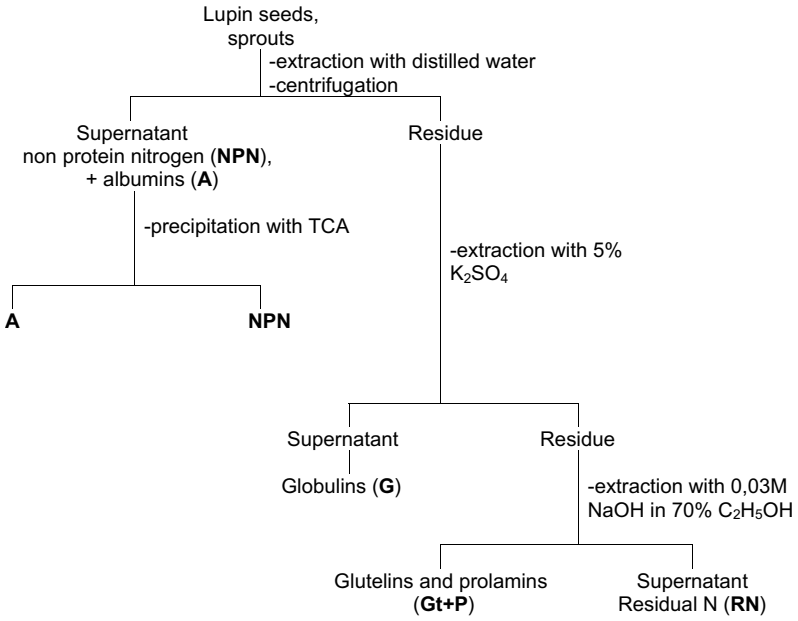

Fig. 1. Scheme of lupin seed and sprout proteins fractionation.

(Sigma), were added to 50 mg of sample as internal standard. Protein hydrolysis was done, following a classical method with 6 M HCl for 21 h at 110 °C in a vacuum-closed vial. Hydrolysates were dried under vacuum and rinsed twice with water. For amino acid derivatization, PITC (phenylisothiocyanate 99%, Aldrich) was used.

The chromatographic system consisted of an Alliance Separation Module 2695 (Waters, Milford, USA), a Photodiode Array detector 996 set at 254 nm (Waters, Milford, USA) and a personal computer running the Empower 2 for Microsoft Windows chromatographic software (Waters). The sample (20 μl) was injected onto a C_{18} reversed phase Alltima 250 × 4.6 mm i.d., 5 μm size column (Alltech) equipped with a guard column (Alltech). The linear gradient system with buffer A (0.1 M ammonium acetate, pH 6.5) and buffer B (0.1 M ammonium acetate/ acetonitrile/methanol: 44/46/10, v/v/v) at pH 6.5, allowed separation of the amino acids using a constant temperature of 43 °C.

2.6. Statistical analysis

Multi-way analysis of variance (ANOVA) was conducted using the MATLAB programme, version 6.5 (MathWorks Inc.) available at Poznań Supercomputing and Networking Center of the Institute of Bioorganic Chemistry, PAS, Poznań, Poland.

3. Results and discussion

3.1. Protein content of lupin seeds and sprouts

Table 1 presents the protein content in raw and sprouted lupins. *L. luteus* cv. 4486 and cv. 4492 presented similar protein contents (45.9% and 47.5%, respectively) but these were higher than those of *L. angustifolius* cv. *troll* and cv. *zapaton* (35.8% and 31.7%, respectively). These results confirm that lupins are protein-rich seeds and agree with data found in the literature for different lupin seeds (Kadam, Chougule, & Salunkhe, 1989; Martínez-Villaluenga et al., 2006; Petterson, 1998; Torres, Frias, & Vidal-Valverde, 2005).

Table 1
Protein contents of raw and sprouted lupin seeds[a]

Seeds	Seeds	Sprouts
Lupinus luteus cv. *4486*	45.7 ± 0.38^{a}	49.7 ± 0.46^{b}
Lupinus luteus cv. *4492*	47.5 ± 0.10^{a}	47.5 ± 0.10^{a}
Lupinus angustifolius cv. *troll*	35.8 ± 0.24^{a}	39.3 ± 0.64^{b}
Lupinus angustifolius cv. *zapaton*	31.7 ± 0.38^{a}	34.3 ± 0.19^{b}

[a] Mean values are expressed as % of dry matter of two determinations ±standard deviation. The same superscript in the same row means no significant difference ($P \leqslant 0.05$).

Germination brought about a slight, but significant ($P \leqslant 0.05$), rise in protein content, except for *L. luteus* cv. 4492 (Table 1). Different authors have shown that the germination process increases the protein content of lupin, peas, pigeon peas, green gram, bengal gram and horse gram, although other authors have observed no changes or lower protein content in sprouts, and results seem to depend, not only on the seed cultivar, but also on the germination conditions (Dagnia, Petterson, Bell, & Flanagan, 1992; Martinez-Villaluenga et al., in press-b; Torres, Frias, Granito, & Vidal-Valverde, 2007a; Urbano et al., 2005b).

3.2. Protein fractions of lupins

3.2.1. Protein fractions of raw lupin seeds

Tables 2 and 3 show the nitrogen contents of the different Osborne fractions of the four lupin cultivars studied. The predominant fractions in both *L. luteus* cultivars were globulins (G), which amounted to 42% of total N while the residual nitrogen (RN) and glutelin+prolamine (Gt + P) fractions contributed to about 20% each, and the albumin (A) and non-protein nitrogen (NPN) fractions represented only about 10% each (Table 2). In *L. angustifolius* cultivars, the G fraction also contributed the most to the total nitrogen (42% and 51% for cv. *troll* and *zapaton*, respectively), followed by the A fraction (18 %) and NPN and Gt + P fractions (10% each). However, the RN fractions corresponded to 17% and 12% of the total nitrogen for cv. *troll* and *zapaton*, respectively (Table 3). Most of the authors coincide in high values for the globulin fraction in legume

Table 2
Nitrogen contents of Osborne fractions of raw and sprouted lupins[A]

Fractions	*Lupinus luteus* cv. 4486		*Lupinus luteus* cv. 4492	
	Seeds	Sprouts	Seeds	Sprouts
Albumins	6.88 ± 0.26^{a}	6.75 ± 0.11^{a}	8.55 ± 0.21^{a}	9.41 ± 0.33^{b}
Globulins	30.1 ± 2.05^{a}	32.7 ± 0.73^{a}	31.8 ± 2.28^{b}	26.5 ± 2.30^{a}
Glutelins and prolamines	15.6 ± 0.97^{b}	8.02 ± 1.31^{a}	12.8 ± 1.18^{b}	8.10 ± 0.25^{a}
Residual nitrogen	14.6 ± 1.45^{b}	8.23 ± 0.10^{a}	15.4 ± 1.12^{b}	7.46 ± 0.19^{a}
Non-protein nitrogen	5.47 ± 0.19^{a}	23.7 ± 0.18^{b}	8.12 ± 0.33^{a}	24.5 ± 0.22^{b}
Total nitrogen of fractions	72.6 ± 2.22^{a}	79.4 ± 1.82^{b}	76.6 ± 2.49^{a}	75.9 ± 2.18^{a}
Total nitrogen of legumes	73.4 ± 0.15^{a}	79.5 ± 0.73^{b}	76.0 ± 0.15^{a}	76.0 ± 0.15^{a}
Recovery total nitrogen (%)	98.9 ± 3.35^{a}	99.9 ± 1.77^{a}	104 ± 3.33^{a}	99.9 ± 3.17^{a}

[A] Mean values are expressed as mg N/g d m of two determinations ±standard deviation. The same superscript in the same row for each lupin cultivar means no significant difference ($P \leqslant 0.05$).

Table 3
Nitrogen contents of Osborne fractions of raw and sprouted lupins[A]

Fractions	*Lupinus angustifolius* cv. *troll*		*Lupinus angustifolius* cv. *zapaton*	
	Seeds	Sprouts	Seeds	Sprouts
Albumins	11.3 ± 0.56^{a}	17.1 ± 0.84^{b}	8.90 ± 0.78^{a}	7.58 ± 0.29^{a}
Globulins	23.6 ± 0.92^{b}	18.1 ± 0.76^{a}	26.5 ± 0.86^{b}	21.9 ± 0.22^{a}
Glutelins and prolamines	6.15 ± 0.78^{a}	6.75 ± 0.55^{a}	5.21 ± 0.37^{a}	6.01 ± 0.55^{a}
Residual nitrogen	9.80 ± 0.19^{b}	4.02 ± 0.07^{a}	6.24 ± 1.58^{b}	5.27 ± 0.12^{a}
Non-protein nitrogen	5.96 ± 0.00^{a}	15.8 ± 0.44^{b}	4.88 ± 0.40^{a}	14.8 ± 0.29^{b}
Total nitrogen of fractions	56.8 ± 0.49^{a}	61.7 ± 0.72^{b}	51.7 ± 0.28^{a}	55.5 ± 0.30^{b}
Total nitrogen of legumes	57.3 ± 0.37^{a}	62.9 ± 1.02^{b}	50.7 ± 0.60^{a}	54.8 ± 0.29^{b}
Recovery total nitrogen (%)	99.1 ± 1.08^{a}	98.1 ± 1.20^{a}	102 ± 1.86^{a}	101 ± 0.01^{a}

[A] Mean values are expressed as mg N/g d.m. of two determinations ±standard deviation. The same superscript in the same row for each lupin cultivar means no significant difference ($P \leqslant 0.05$).

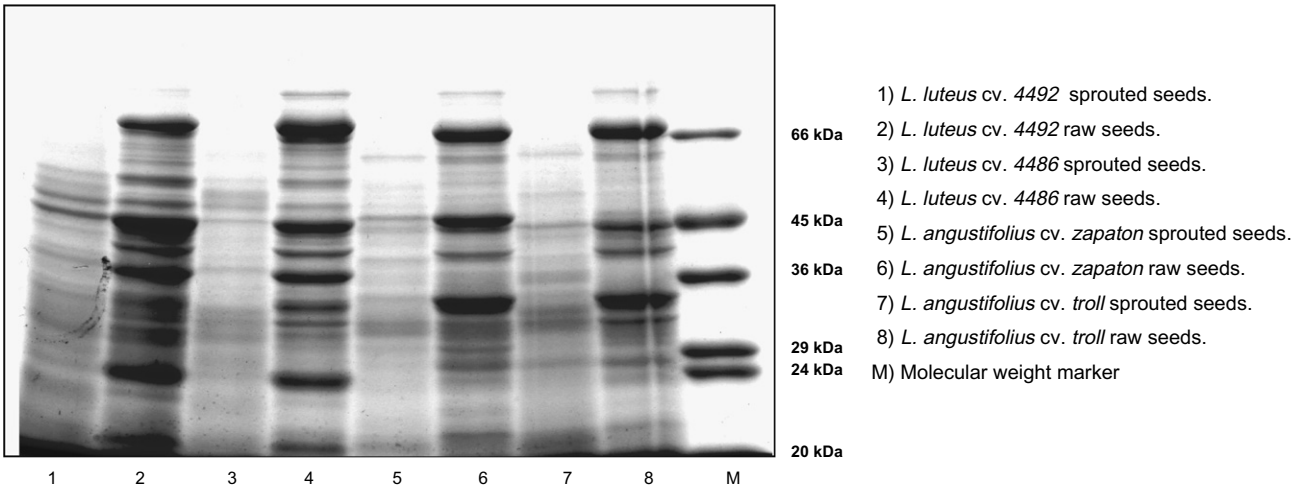

Fig. 2. SDS-PAGE electrophoregrams of albumin fractions of raw and sprouted *L. luteus* and *L. angustifolius* seeds.

seeds, although this seems to depend on the kind of material (species and cultivar) (Martinez-Villaluenga et al., in press-b; Peretiatkowicz, Wojtaszek, Michalski, & Gulewicz, 1988a; Peretiatkowicz et al., 1988b; Schroeder, 1982). The content of the other protein fractions was more diverse and Peretiatkowicz et al. (1988a) showed a complete dependence of the protein fractional composition on genotypes of different lupins. Moreover, Sell, Steinhart, and Paschke (2005) reported that the maturation stage of the seed depends on environmental factors and leads to variation in the protein fraction composition. Other factors, e.g., lipid content, particle size, flour solvent ratio and extraction temperature, seem to be critical variables in determining the Osborne fractions (Barba de la Rosa, Gueguen, Paredes-López, & Viroben, 1992; Chagas & Santoro, 1997).

Figs. 2–4 (lines 2, 4, 6 and 8) show the protein profile of A, G and Gt + P fractions of raw lupin seeds using the SDS-PAGE. As shown in Fig. 2 (lines 2 and 4) the main proteins of the A fraction of *L. luteus* are proteins, with molecular weights of about 69, 45, 40.5, 36, 33.5, 30, 24 and 21.5 kDa whilst, in cultivars of *L. angustifolius*, they are proteins of 69, 45, 40.5, 33.5, 32, 30, 26.5 and 22 kDa in both cultivars (Fig. 2, lines 6 and 8). The main seed proteins in the G fraction in *L. luteus* (Fig. 3, lines 2 and 4) corresponded to molecular masses of 64, 53, 50, 47 and

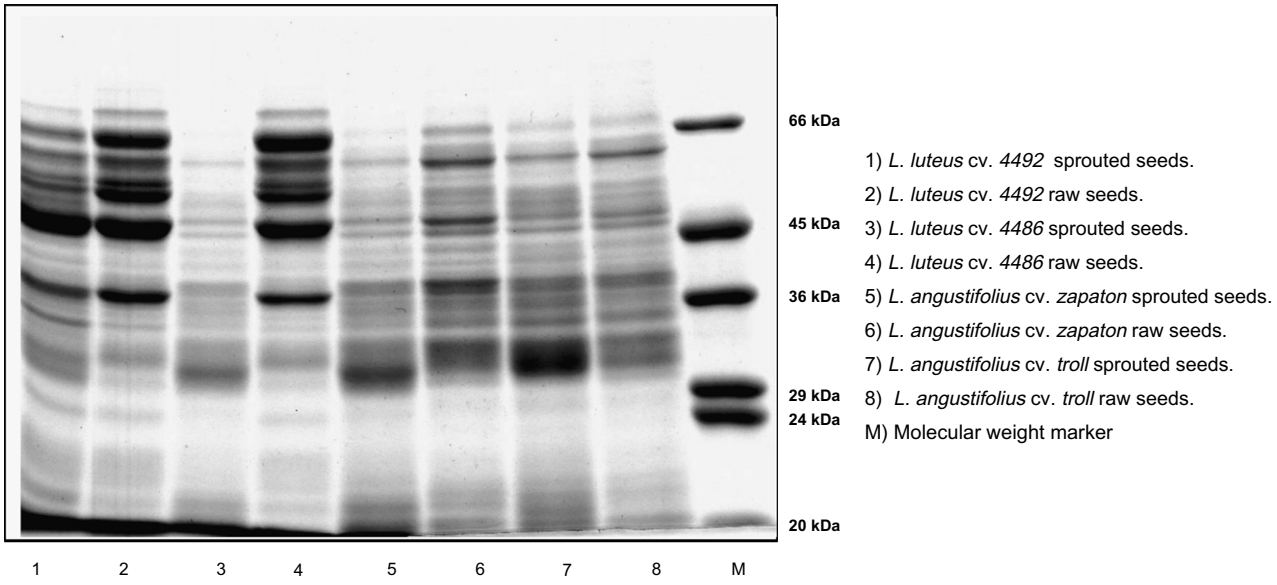

Fig. 3. SDS-PAGE electrophoregrams of globulins fractions of raw and sprouted *L. luteus* and *L. angustifolius*.

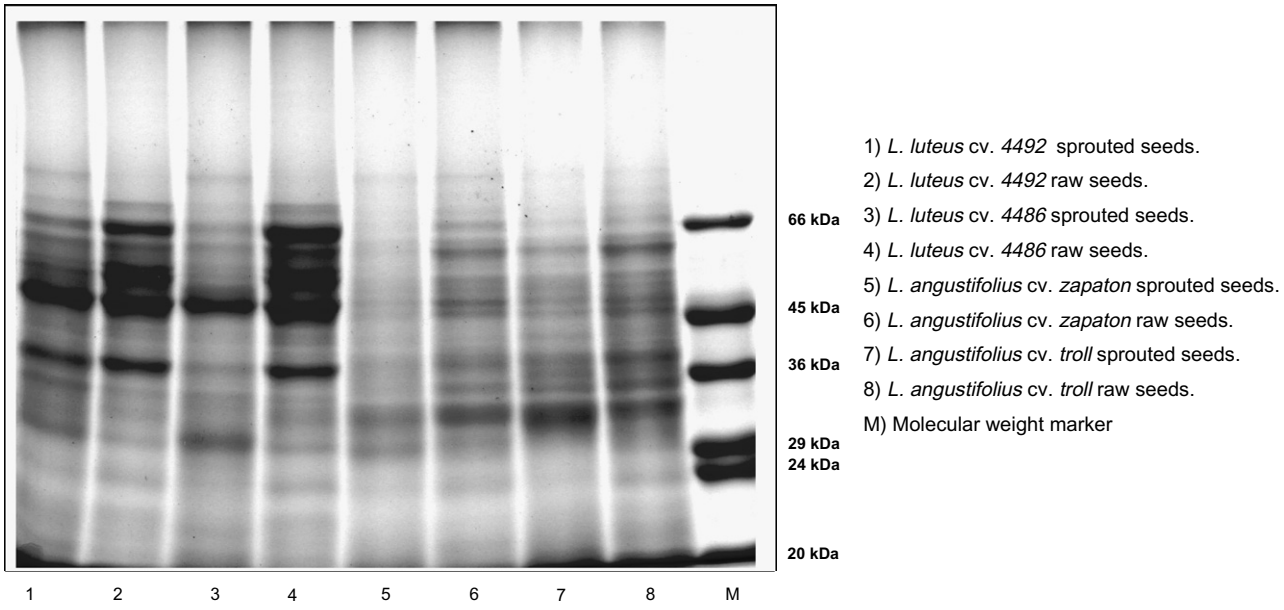

Fig. 4. SDS-PAGE electrophoregrams of glutelins and prolamins fractions of raw and sprouted *L. luteus* and *L. angustifolius* seeds.

36 kDa whilst, in *L. angustifolius,* they were 64, 47 and 38.5 kDa although with less intensity. Proteins of 50–45 kDa were predominant in the G fraction of lupin cultivars studied. Gt + P fractions, however, were composed of proteins with molecular weights ranging between 66 and 45 kDa for *L. luteus* (Fig. 4, lines 2 and 4) and between 62 and 31.5 kDa for *L. angustifolius* (Fig. 4, lines 6 and 8). As shown, differences in the protein profiles of the Osborne fractions were found among species, whilst cultivars did not show apparent differences. Similar results were found in pea cultivars by Martinez-Villaluenga et al. (in press-b).

The globulin fraction, which is predominant in lupin seeds, contains two major proteins: conglutin α and conglutin β (11 S and 7S, respectively), and lupin-specific proteins called conglutin γ and conglutin δ (10S and 2S, respectively) (Blagrove & Gillespie, 1975; Petterson, 1998; Restani, Duranti, Cerletti, & Simonetti, 1981). Lupin conglutins differ in size, amino acid composition and presence of disulphide bonds in their structure (Cerletti, Duranti, Guerrieri, & Giani, 1988; Lambert & Yarwood, 1992; Restani et al., 1981; Sepulveda, Davila, & Rodriguez, 1999). Therefore, lupin globulins with a specific composition, for instance, enriched in α, β and γ conglutins, are highly soluble protein isolates with outstanding emulsification, salt tolerance and foaming properties (Wäsche, Müller, & Knauf, 2001; Wright & Burnstead, 1984), functional characteristics that could be of interest for the food processing industry. For the other lupin fractions (A and Gt + P), there is a lack of detailed information in lupin seeds, information that is available for other legumes (Martinez-Villaluenga et al., in press-b).

3.2.2. Protein fractions of lupin sprouts

Tables 2 and 3 show the *N* content of each Osborne fraction for sprouted lupins. Figs. 2–4 (lines 1, 3, 5 and 7) show the protein composition of the A, G and Gt + P fractions in sprouted lupins using SDS-PAGE and Fig. 5 shows

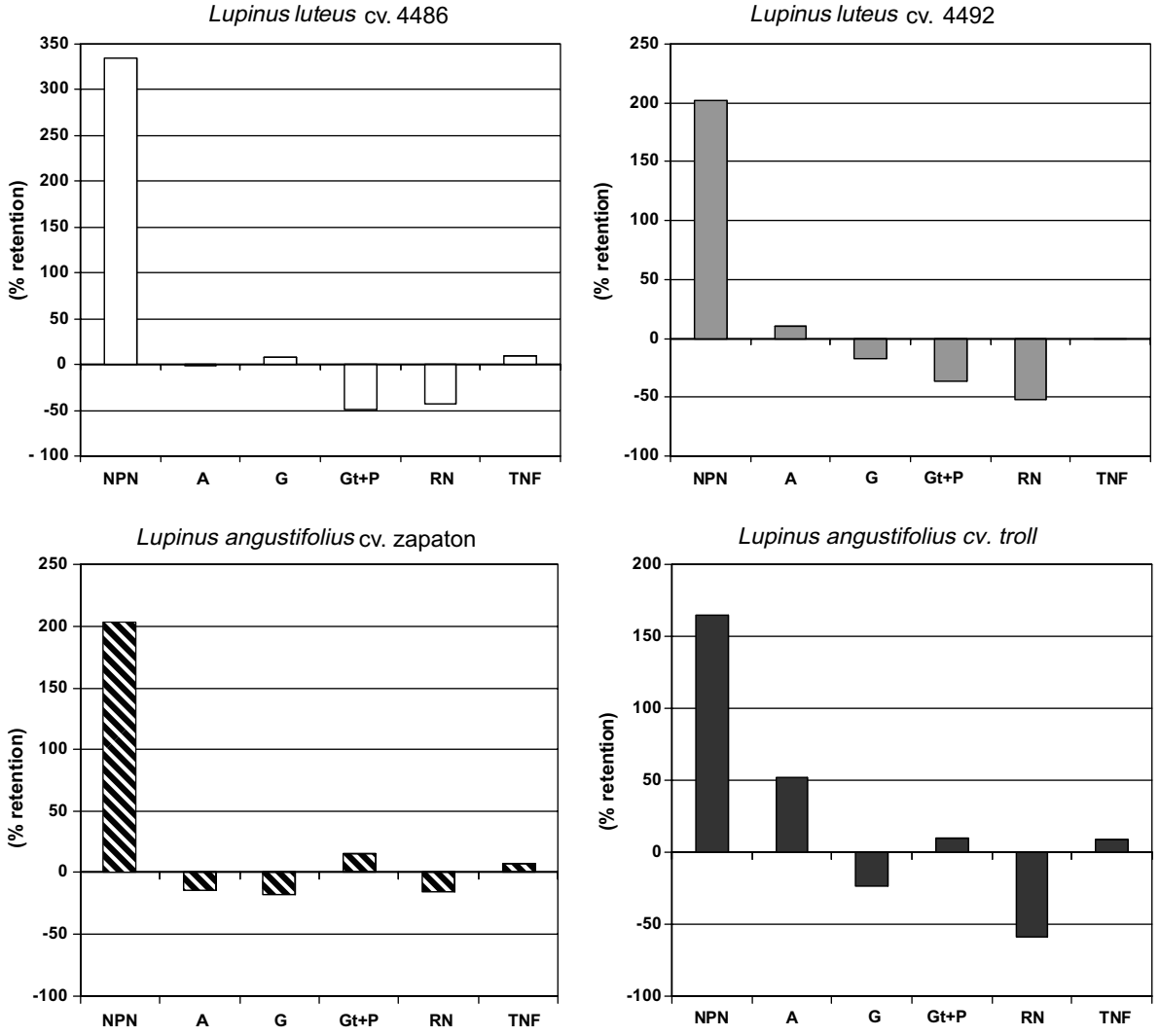

Fig. 5. Effect of germination on the nitrogen content of Osborne fractions of lupins. **NPN** – non-protein nitrogen, **A** – albumins, **G** – globulins, **Gt + p**-glutelins + prolamins, **RN** – residual nitrogen.

the relative nitrogen content of different lupin Osborne fractions vs. ungerminated lupins.

The germination process brought about large changes in the N content of Osborne fractions in the lupins studied (Tables 2 and 3, Fig. 5). In cultivars of *L. luteus*, the total *N* content of the A fraction only increased significantly ($P \leqslant 0.05$) in sprouted cv. 4492, although the total *N* content for the G fraction increased in sprouted cv. 4486 and decreased in sprouted cv. 4492 whilst, for RN and Gt + P fractions, sharp decreases were found and the N content of the NPN fraction increased almost 4-fold for sprouted cv. 4492 and 6-fold for sprouted cv. 4486. In *L. angustifolius*, germination caused an increase in the *N* content of the A fraction of cv. *troll* (52%), and a significant ($P \leqslant 0.05$) decrease (15%) in cv. *zapaton*. The *N* content of the G and NR fractions dropped after germination of both cultivars while, for Gt + P fractions, it did not significantly change ($P \leqslant 0.05$), and the *N* content of NPN fractions increased sharply (Table 3, Fig. 5). The high recovery found for total nitrogen content (98.1–104%) (Tables 2 and 3) was remarkable. Martinez-Villaluenga et al. (in press-b) reported a decrease in albumin fraction and no changes in the globulin fraction as a consequence of pea germination and similar results were obtained by Portari, Tavano, Silva, and Neves (2005) for albumins and globulins of chickpea sprouts.

Changes observed in protein fractions during the germination of lupins (Tables 2, 3, Fig. 5) can be attributed to extensive breakdown of seed-storage proteins (Ahmed, Abdel-Rahim, Abdel-Fatah, Erdmann, & Lippmann, 1995; Duranti et al., 1984; Ferreira, Melo, & Teixeira, 1995). The decreases found in RN fractions could be due to the fact that, during germination, degradation of the protein cell wall causes peptides and amino acids to decrease. As is known, the protein cell wall is not soluble in solvents used in Osborne fractionation and, together with nucleic acid compounds (purines, pyrimidines) and polyamines, is part of the RN fraction. The degradation of cell wall proteins, as well as the Gt+P fraction, during germination, may result in the rise in NPN found in *Lupinus* cultivars that, constituted mainly by free amino acids and peptides, may be of considerable importance from a nutritional point of view. According to Duranti et al. (1984), germination causes N shifts from the storage globulins to other *N*-compounds and the rate of breakdown differs for each globulin.

Germination also caused important changes in the protein profile of the Osborne fractions in all the lupins studied, as was confirmed by SDS-PAGE (Figs. 2–4). High molecular weight bands corresponding to the A fraction almost disappeared after germination of lupin seeds (Fig. 2, lines 1, 3, 5 and 7). However, behaviour of the globulin fraction profile depends not only on the lupin species but also on the cultivar (Fig. 3, lines 1, 3, 5 and 7). The intensity of some of the bands was found to decrease during germination but other new ones appeared. For *L. luteus* cv. 4492, bands with molecular weights above 38.5 kDa remained, although with a lower intensity after germination. However, for *L. luteus* cv. 4486 and *L. angustifolius* cv. *zapaton* and cv. *troll*, the intensity of these bands decreased sharply. Moreover, for all the studied lupin seeds, germination caused a rise

Table 4
Contents of amino acids in albumin fractions of raw and sprouted lupins[A]

Amino acids	*L. luteus* cv. 4486		*L. luteus* cv. 4492		*L. angustifolius* cv. *troll*		*L. angustifolius* cv. *zapaton*	
	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts
Non essential amino acids								
Asp	1.75^{a}_{1}	8.50^{b}	7.75^{a}_{2}	10.40^{b}	8.45^{b}_{3}	6.66^{a}	8.36^{a}_{3}	11.4^{b}
Glu	6.65^{a}_{1}	15.0^{b}	16.9^{a}_{3}	20.2^{b}	15.5^{a}_{2}	17.3^{b}	16.9^{a}_{3}	16.6^{a}
Ser	5.46^{a}_{3}	5.28^{a}	5.02^{a}_{2}	5.12^{a}	4.68^{a}_{1}	5.23^{b}	$4.76^{a}_{1,2}$	4.85^{a}
Gly	12.6^{b}_{3}	9.38^{a}	10.4^{b}_{2}	8.29^{a}	9.03^{b}_{1}	8.25^{a}	9.46^{b}_{1}	8.38^{a}
Arg	8.26^{b}_{2}	7.90^{a}	7.20^{b}_{1}	8.10^{a}	8.45^{b}_{2}	5.45^{a}	8.51^{a}_{2}	8.80^{b}
Ala	7.11^{b}_{4}	5.31^{a}	6.48^{b}_{3}	4.92^{a}	5.89^{a}_{2}	8.75^{b}	5.39^{a}_{1}	6.22^{b}
Pro	$1.20^{a}_{1,2}$	1.68^{b}	1.23^{a}_{2}	1.90^{b}	1.10^{a}_{1}	2.90^{b}	3.46^{a}_{3}	3.65^{b}
Gln	0.77^{b}_{2}	0.53^{a}	0.58^{b}_{1}	0.48^{a}	ND	ND	ND	ND
Essential amino acids								
His	1.22^{a}_{2}	1.24^{a}	1.28^{b}_{2}	1.20^{a}	0.97^{a}_{1}	0.94^{a}	1.00^{a}_{1}	0.99^{a}
Val	5.89^{b}_{3}	4.82^{a}	5.11^{b}_{2}	3.93^{a}	4.13^{a}_{1}	4.07^{a}	4.04^{b}_{1}	3.94^{a}
Met	0.58^{b}_{3}	0.33^{a}	0.24^{a}_{1}	0.51^{b}	0.51^{b}_{2}	0.30^{a}	1.03^{b}_{4}	0.77^{a}
Cys	0.14^{a}_{1}	0.25^{b}	0.17^{a}_{1}	0.35^{b}	0.25^{b}_{2}	0.22^{a}	0.28^{a}_{2}	0.33^{b}
Ile	4.07^{a}_{3}	4.15^{a}	3.61^{b}_{1}	3.40^{a}	3.56^{a}_{1}	4.61^{b}	3.86^{a}_{2}	4.36^{b}
Leu	6.54^{a}_{2}	7.53^{b}	6.50^{a}_{2}	7.22^{b}	4.96^{a}_{1}	6.58^{b}	5.20^{a}_{1}	6.03^{b}
Phe	4.73^{a}_{2}	5.74^{b}	4.52^{a}_{2}	4.72^{b}	3.73^{a}_{1}	4.49^{b}	3.86^{a}_{1}	4.78^{b}
Tyr	5.26^{a}_{3}	5.08^{a}	4.22^{a}_{2}	4.16^{a}	$4.12^{a}_{1,2}$	4.18^{a}	3.93^{a}_{1}	4.26^{b}
Lys	12.2^{a}_{2}	12.2^{a}	7.46^{a}_{1}	10.43^{b}	19.4^{b}_{4}	8.52^{a}	16.3^{a}_{3}	16.1^{a}
Thr	6.78^{b}_{2}	5.11^{a}	6.14^{b}_{2}	4.91^{a}	3.72^{a}_{1}	7.74^{b}	3.75^{b}_{1}	3.30^{a}

[A] Mean values of g/100 g of protein fraction. The same superscript in the same row for each lupin cultivar means no significant difference ($P \leqslant 0.05$). ND = not detected.

Table 5
Contents of amino acids in globulin fractions of seeds and sprouted lupins[A]

Amino acids	*L. luteus* cv.4486		*L. luteus* cv. 4492		*L. angustifolius* cv. *troll*		*L. angustifolius* cv. *zapaton*	
	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts
Non essential amino acids								
Asp	5.07^{a}_{4}	6.22^{b}	4.52^{a}_{3}	4.49^{a}	2.53^{a}_{1}	6.05^{b}	4.67^{b}_{2}	2.72^{a}
Glu	19.3^{b}_{4}	18.8^{a}	17.2^{b}_{3}	15.4^{a}	8.59^{a}_{1}	12.7^{b}	9.56^{a}_{2}	13.3^{b}
Ser	3.72^{a}_{4}	3.68^{a}	3.10^{a}_{3}	3.04^{a}	2.23^{a}_{1}	3.08^{b}	2.16^{a}_{2}	2.91^{b}
Gly	4.47^{a}_{4}	4.86^{b}	3.99^{a}_{2}	4.48^{b}	2.66^{a}_{1}	4.09^{b}	2.79^{a}_{3}	4.21^{b}
Arg	9.46^{b}_{4}	8.08^{a}	7.12^{a}_{2}	7.20^{a}	5.84^{a}_{1}	7.58^{b}	5.32^{a}_{3}	7.59^{b}
Ala	2.34^{b}_{2}	2.00^{a}	4.86^{b}_{3}	2.16^{a}	1.84^{a}_{1}	2.06^{b}	1.59^{a}_{1}	1.61^{a}
Pro	0.05^{a}_{1}	2.42^{b}	1.13^{a}_{3}	2.21^{b}	0.10^{a}_{1}	1.76^{b}	1.24^{a}_{2}	1.69^{b}
Gln	0.22^{a}_{3}	0.21^{a}	0.19^{a}_{2}	0.18^{a}	ND_1	ND	ND_1	ND
Essential amino acids								
His	0.90^{b}_{4}	0.73^{a}	0.69^{a}_{3}	0.79^{b}	0.46^{a}_{1}	0.68^{b}	0.49^{a}_{2}	0.52^{b}
Val	2.28^{b}_{4}	1.91^{a}	1.86^{a}_{3}	1.86^{a}	1.30^{a}_{1}	1.69^{b}	1.66^{b}_{2}	1.21^{a}
Met	0.03^{a}_{1}	0.07^{b}	0.22^{a}_{2}	0.24^{b}	0.05^{a}_{1}	0.09^{b}	0.07^{a}_{1}	0.07^{b}
Cys	0.20^{a}_{1}	0.53^{b}	0.14^{a}_{1}	0.27^{b}	0.19^{b}_{1}	0.11^{a}	0.21^{b}_{1}	0.12^{a}
Ile	2.04^{a}_{3}	2.19^{b}	1.68^{a}_{2}	2.09^{b}	1.47^{a}_{1}	2.41^{b}	2.35^{b}_{4}	1.75^{a}
Leu	5.00^{a}_{4}	5.15^{b}	4.20^{a}_{3}	4.84^{b}	2.37^{a}_{1}	3.52^{b}	3.48^{b}_{2}	2.68^{a}
Phe	2.93^{a}_{4}	2.91^{a}	2.17^{a}_{3}	3.08^{b}	1.49^{a}_{1}	2.44^{b}	2.07^{b}_{2}	1.78^{a}
Tyr	2.06^{a}_{4}	2.29^{b}	1.43^{a}_{2}	1.91^{b}	1.36^{a}_{1}	2.09^{b}	1.84^{b}_{3}	1.49^{a}
Lys	2.90^{b}_{4}	0.15^{a}	0.17^{b}_{1}	0.11^{a}	1.64^{a}_{2}	3.59^{b}	2.71^{a}_{3}	3.16^{b}
Thr	3.79^{b}_{4}	2.41^{a}	3.25^{b}_{3}	2.36^{a}	1.86^{b}_{2}	1.55^{a}	1.34^{b}_{1}	0.94^{a}

[A] Mean values of g/100 g of protein fraction. The same superscript in the same row for each lupin cultivar means no significant difference ($P \leqslant 0.05$). ND = not detected.

in band intensities for proteins of lower molecular weights, between 37 and 20 kDa (Fig. 3, lines 1, 3, 5 and 7). For the Gt + P fraction, an evident decrease in the protein bands was observed for cultivars of *L. angustifolius*. However, *L. luteus* cultivars were characterized by the disappearance of bands above 45 kDa, whilst those of 45 and 38 kDa remained almost unchanged and a band of lower molecular weight appeared (Fig. 4, lines 1, 3, 5 and 7). These results agree with literature studies which report that the germination effect on the protein fraction depends on the species and type of legume studied.

A reduction has also been reported for the number of protein bands in the albumin fraction during germination

Table 6
Contents of amino acids in glutelin + prolamine fractions of seeds and sprouted lupins[A]

Amino acids	*L. luteus* cv. 4486		*L. luteus* cv. 4492		*L. angustifolius* cv. *troll*		*L. angustifolius* cv. *zapaton*	
	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts
Non essential amino acids								
Asp	9.83^{b}_{4}	2.83^{a}	6.67^{a}_{1}	7.19^{b}	9.20^{b}_{3}	6.95^{a}	7.33^{b}_{2}	7.13^{a}
Glu	22.5^{b}_{4}	9.27^{a}	20.2^{b}_{2}	14.3^{a}	21.7^{b}_{3}	13.3^{a}	13.0^{b}_{1}	11.5^{a}
Ser	5.19^{a}_{2}	6.38^{b}	5.72^{a}_{3}	5.80^{a}	5.68^{b}_{3}	4.88^{a}	4.53^{a}_{1}	4.61^{b}
Gly	7.77^{a}_{2}	10.9^{b}	9.13^{a}_{3}	9.23^{a}	8.04^{b}_{2}	7.71^{a}	7.35^{a}_{1}	7.34^{a}
Arg	9.74^{a}_{2}	10.9^{b}	11.1^{b}_{3}	9.16^{a}	13.8^{b}_{4}	8.68^{a}	8.27^{a}_{1}	8.73^{b}
Ala	5.17^{a}_{4}	6.42^{b}	4.73^{a}_{3}	6.78^{b}	3.92^{a}_{2}	4.82^{b}	3.69^{a}_{1}	4.77^{b}
Pro	2.12^{a}_{3}	5.95^{b}	1.71^{a}_{2}	4.75^{b}	1.07^{a}_{1}	3.09^{b}	2.43^{a}_{4}	3.05^{b}
Gln	0.48^{a}_{2}	0.68^{b}	0.53^{b}_{3}	0.38^{a}	ND_1	ND^{a}	ND_1	ND
Essential amino acids								
His	1.24^{a}_{3}	1.34^{b}	1.49^{b}_{4}	1.69^{a}	1.17^{b}_{2}	1.12^{a}	0.92^{a}_{1}	1.10^{b}
Val	3.13^{a}_{1}	5.03^{b}	3.93^{a}_{3}	4.61^{b}	3.47^{a}_{2}	3.70^{b}	3.32^{a}_{2}	3.74^{b}
Met	0.77^{a}_{4}	1.05^{b}	0.09^{a}_{1}	1.24^{b}	0.63^{b}_{2}	0.58^{a}	0.67^{a}_{3}	1.17^{b}
Cys	0.16^{a}_{2}	0.18^{a}	0.15^{a}_{2}	0.14^{a}	0.26^{b}_{3}	0.06^{a}	0.07^{a}_{1}	0.09^{b}
Ile	3.07^{a}_{1}	4.98^{b}	3.62^{a}_{2}	4.59^{b}	4.19^{a}_{3}	4.31^{b}	3.56^{a}_{2}	4.10^{b}
Leu	6.37^{a}_{2}	9.87^{b}	7.79^{a}_{3}	8.33^{b}	6.52^{b}_{2}	6.32^{a}	5.22^{a}_{1}	6.04^{b}
Phe	4.26^{a}_{2}	6.37^{b}	5.11^{a}_{3}	5.64^{b}	4.51^{a}_{2}	4.64^{b}	3.78^{a}_{1}	4.52^{b}
Tyr	3.74^{a}_{2}	5.62^{b}	4.18^{a}_{3}	4.59^{b}	4.07^{a}_{3}	3.79^{a}	3.16^{a}_{1}	3.73^{b}
Lys	10.4^{b}_{4}	6.77^{a}	3.90^{a}_{1}	5.98^{b}	7.63^{a}_{3}	8.21^{b}	7.01^{b}_{2}	10.5^{b}
Thr	4.46^{a}_{3}	5.47^{b}	5.39^{b}_{4}	5.12^{a}	3.48^{b}_{2}	2.96^{a}	2.82^{b}_{1}	2.67^{a}

[A] Mean values of g/100 g of protein fraction. The same superscript in the same row for each lupin cultivar means no significant difference ($P \leqslant 0.05$). ND = not detected.

in different legumes seeds that results from proteolytic action taking place during this process. Martinez-Villaluenga et al. (in press-b) found a decrease in albumin bands of above 45 kDa and an increase in the band of 31 kDa after the germination of different cultivars of *Pisum sativum*. This fraction is mainly composed of non-desirable proteins, such as lypoxygenase, trypsin inhibitors, lectins, antigenic and allergenic compounds, that interfere with the nutritional and safety quality of legumes (Sell et al., 2005; Tzitzikas, Vincken, Groot, Gruppen, & Visser, 2006) and, therefore, germination is suggested as a process for obtaining hypoallergenic food and it improves nutritional quality by degrading these proteins (Tzitzikas et al., 2006; Yamada et al., 2005). From a technological point of view, the isolated albumin fraction from sprouted lupins is interesting since it confers good emulsion properties and high solubility (Sathe & Salunkhe, 1981).

Our results for the G fraction of lupin during germination coincide with those of Martinez-Villaluenga et al. (in press-b) who showed a decrease in the protein bands of the globulin fraction in different cultivars of *P. sativum*. These findings suggest that convicilin (60 kDa), vicilin (50–45 kDa) and legumin (43–35 kDa) undergo a differential proteolytic process during germination, as previously reported for γ-, β- and α-conglutins for *Lupinus albus* (Chandna & Matta, 1994). Similarly, Cosme, Cunha-Queda, and Bruno de Sousa (1993) reported the disappearance of conglutins during the germination of *L. albus* and *L. luteus*. All these results suggest that knowledge of the composition of specific protein fractions may be of special interest for the food industry (for functional ingredients). Therefore, changes in the globulin content and composition during germination suggest that lupin sprouts may also have a favourable protein composition for food applications and also increase the nutritive value of the processed food.

3.2.3. Amino acid composition of protein fractions of raw seeds

The amino acid composition in the albumin fraction (A) of raw lupin seeds is shown in Table 4. The predominant non essential amino (NEAA) acids of the A fraction of all raw lupin cultivars were Glu, Gly, Arg and Ala. Lys was the major essential amino acid (EAA), followed by Leu, Thr, Val, Tyr and Phe, whilst Met and Cys were in low amounts. These results agree with those reported by Peretiatkowicz et al. (1988a) in the albumin fraction of three *Lupin* species. Amino acid content in the albumin fraction has been reported in different cultivars of *P. sativum* (Martinez-Villaluenga et al., in press-b; Schroeder, 1982), *Vigna mungo* (Mahajan, Malhotra, & Singh, 1988), *Phaseolus lunatus* (Gallegos-Tintore, Pacheco-Aguirre, Betancur-Ancona, & Chel-Guerrero, 2004) and *Lathyrus maritimus* (Chavan, McKenzie, & Shahidi, 2001), where the major amino acids were Asp, Glu, Gly and Lys. Differences among lupin species and varieties were also found and, while in A fraction of *L. luteus* significant differences ($P \leqslant 0.05$) in most of the NEAA were found between cultivars, *L. angustifolius* only presented significant ($P \leqslant 0.05$) differences in the Glu, Ala and Pro contents between cultivars. Among EAA, the albumin fraction of all *Lupinus* seeds studied had similar His, Cys, Leu, Phe

Table 7
Contents of amino acids in non-protein fractions of seeds and sprouted lupins[A]

Amino acids	*L. luteus* cv. 4486		*L. luteus* cv. 4492		*L. angustifolius* cv. *troll*		*L. angustifolius* cv. *zapaton*	
	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts
Non essential amino acids								
Asp	0.18^{a}_{2}	4.98^{b}	0.09^{a}_{1}	1.52^{b}	0.09^{a}_{1}	1.91^{b}	0.22^{a}_{3}	4.98^{b}
Glu	0.65^{a}_{2}	2.13^{b}	0.53^{a}_{1}	1.56^{b}	0.74^{a}_{3}	1.86^{b}	0.97^{a}_{4}	2.13^{b}
Ser	0.12^{a}_{1}	0.91^{b}	0.16^{a}_{2}	0.83^{b}	0.18^{a}_{3}	0.49^{b}	0.24^{a}_{4}	0.91^{b}
Gly	0.28^{a}_{1}	0.82^{b}	0.34^{a}_{3}	0.83^{b}	0.31^{a}_{2}	0.63^{b}	0.53^{a}_{4}	0.82^{b}
Arg	0.90^{a}_{2}	3.07^{b}	0.16^{a}_{4}	3.16^{b}	1.06^{a}_{3}	1.48^{b}	0.84^{a}_{1}	3.07^{b}
Ala	0.13^{a}_{1}	0.82^{b}	0.16^{a}_{1}	1.04^{b}	0.56^{a}_{2}	0.77^{b}	0.64^{a}_{3}	0.82^{b}
Pro	0.01^{a}_{1}	0.02^{b}	0.01^{a}_{1}	0.09^{b}	0.01^{a}_{1}	0.01^{a}	0.07^{b}_{2}	0.02^{a}
Gln	ND^{a}_{1}	0.04^{b}	0.01^{a}_{2}	0.04^{b}	0.01^{b}_{2}	ND^{a}	ND^{a}_{1}	0.04^{b}
Essential amino acids								
His	0.05^{a}_{1}	0.31^{b}	0.07^{a}_{2}	0.29^{b}	0.05^{a}_{1}	0.14^{b}	0.05^{a}_{1}	0.13^{b}
Val	0.07^{a}_{1}	0.40^{b}	0.09^{a}_{2}	0.37^{b}	0.09^{a}_{2}	0.28^{b}	0.17^{a}_{3}	0.29^{b}
Met	0.01^{a}_{1}	0.01^{a}	0.04^{a}_{2}	0.03^{a}	0.01^{a}_{1}	0.09^{b}	0.01^{a}_{1}	0.04^{b}
Cys	0.01^{a}_{1}	0.01^{a}	0.01^{a}_{1}	0.01^{a}	0.03^{a}_{2}	0.02^{a}	0.01^{a}_{1}	0.26^{b}
Ile	0.06^{a}_{1}	0.17^{b}	0.08^{a}_{1}	0.26^{b}	0.07^{a}_{1}	0.19^{b}	0.19^{a}_{2}	0.27^{b}
Leu	0.08^{a}_{1}	0.51^{b}	0.15^{a}_{2}	0.59^{b}	0.08^{a}_{1}	0.25^{b}	0.22^{b}_{3}	0.16^{a}
Phe	0.03^{a}_{1}	0.18^{b}	$0.07^{a}_{1.2}$	0.25^{b}	0.04^{a}_{1}	0.12^{b}	0.11^{a}_{2}	0.44^{b}
Tyr	0.15^{a}_{2}	0.12^{a}	0.06^{a}_{1}	0.12^{b}	0.19^{a}_{3}	0.24^{b}	0.31^{b}_{4}	0.14^{a}
Lys	0.04^{a}_{3}	0.04^{a}	0.02^{a}_{2}	0.06^{b}	0.01^{a}_{1}	0.46^{b}	0.22^{b}_{4}	0.01^{a}
Thr	0.11^{a}_{1}	1.08^{b}	0.41^{a}_{4}	1.12^{b}	0.21^{a}_{2}	0.66^{b}	0.32^{a}_{3}	0.46^{b}

[A] Mean values of g/100 g of protein fraction. The same superscript in the same row for each lupin cultivar means no significant difference ($P \leqslant 0.05$). ND = not detected.

and Thr contents and even *L. angustifolius* cultivars also contained significantly similar amounts of Val. Differences in the amino acid content of the albumin fraction among varieties and cultivars have also been studied by different authors in other legumes (Martinez-Villaluenga et al., in press-b; Schroeder, 1982).

The amino acid content in the globulin (G) fraction of *Lupinus* is shown in Table 5. Lupin globulins presented Glu and Arg as the major NEAA, and Leu and Thr as the main EAA. This amino acid profile differs from those found in the globulin fraction of three *Lupin* species (Peretiatkowicz et al., 1988a) where Asp, Ile, Lys, Phe and Tyr were also present at a high level, and from those of *P. sativum* cultivar globulins (Martinez-Villaluenga et al., in press-b) where Asp, Glu, Gly and Arg, Leu, Phe, Lys and Thr were present in the highest amounts. However, in the globulin fraction of other legumes, Asp, Glu, Arg, Leu and Lys were the major amino acids (Chavan et al., 2001; Gallegos-Tintore et al., 2004; Schroeder, 1982). When lupin cultivars were compared in relation to the amino acid content of the G fraction, large differences were observed; most of the NEAA and EAA differed significantly ($P \leqslant 0.05$), with the exception of Ala, Gln (NEAA), Met and Cys (EAA) between cultivars of *L. angustifolius*, and of Cys of cultivars of *L. luteus*. Peretiatkowicz et al. (1988a) have also found differences in the amino acid contents of the globulin fraction of different species of *Lupinus*. Differences were also shown in the amino acid content of the globulin fraction of different cultivars of *Phaseolus vulgaris* and *P. sativum* (Martinez-Villaluenga et al., in press-b; Schroeder, 1982).

Table 6 shows the amino acid content in the glutelin+prolamine fractions (Gt + P) of raw lupins. Asp, Glu, Gly and Arg were the major NEAA, and Leu and

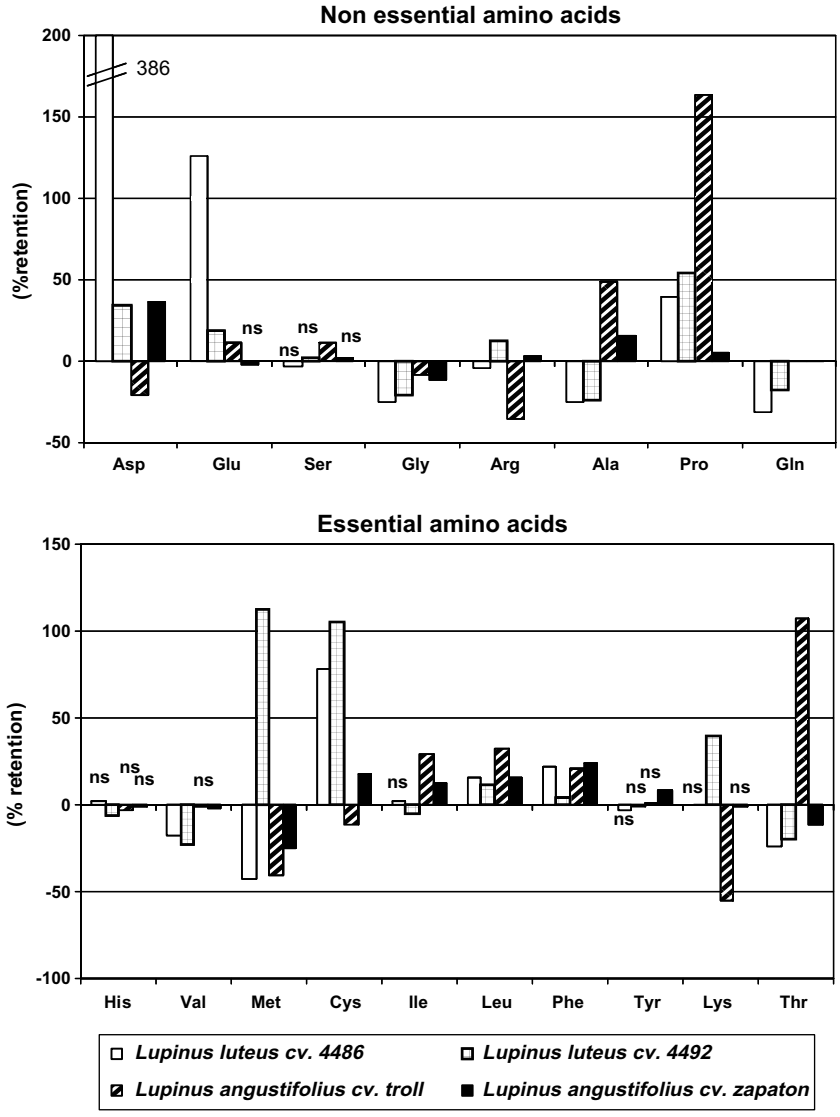

Fig. 6. Effect of germination on the amino acid content of albumin fraction of lupins. ns = no significant differences ($P \leqslant 0.05$) between raw and sprouted seeds.

Lys the main EAA. In the glutelin + prolamine fraction of *L. angustifolius* cv. *mirela*, Peretiatkowicz et al. (1988b) found Ile, Leu, Lys, Phe, Tyr and Val as major EAA and Asp, Glu and Arg as main NEAA. When the amino acid contents of the Gt + P fraction were compared among cultivars, only the content of Cys was not significantly ($P \leqslant 0.05$) different between *L. luteus* cv. 4486 and cv. 4492. Differences among species and cultivars in the glutelin + prolamin fraction have also been reported by Peretiatkowicz et al. (1988a).

In the non-protein nitrogen (NPN) fraction of raw lupins, the content of NEAA and EAA was very low (Table 7), results that differ from those reported by Peretiatkowicz et al. (1988b) in the fraction of *L. angustifolius* cv. *mirela*, but are in accordance with those reported for this fraction in different cultivars of *P. sativum* (Martinez-Villaluenga et al., in press-b). Among the cultivars, the contents of NEAA of the Gt+P fraction differed significantly ($P \leqslant 0.05$) and the contents of EAA depended, not only on the species, but also on the cultivar.

3.2.4. Amino acid composition of protein fractions of sprouted lupins

The germination process had different effects on the amino acid contents of protein fractions of lupins (Tables 4–7, Figs. 6–9). In the albumin (A) fraction of *L. luteus* cv. 4486 and cv. 4492, germination brought about significant increases ($P \leqslant 0.05$) in NEAA, such as Asp, Glu and Pro, and decreases in Gly, Arg, Ala and Gln contents (Table 4, Fig. 6). Among the EAA, sprouting caused an increase in Cys, Leu and Phe in sprouts of *L. luteus* while Val was also increased in cv. 4486, and Met and Lys in sprouted cv. 4492, compared with their contents in the raw cultivars. In *L. angustifolius* cultivars, the A fraction showed increases in Ala and Pro (as NEAA), and in Ile, Leu and Phe (as EAA) as a consequence of germination (Table 4, Fig. 6).

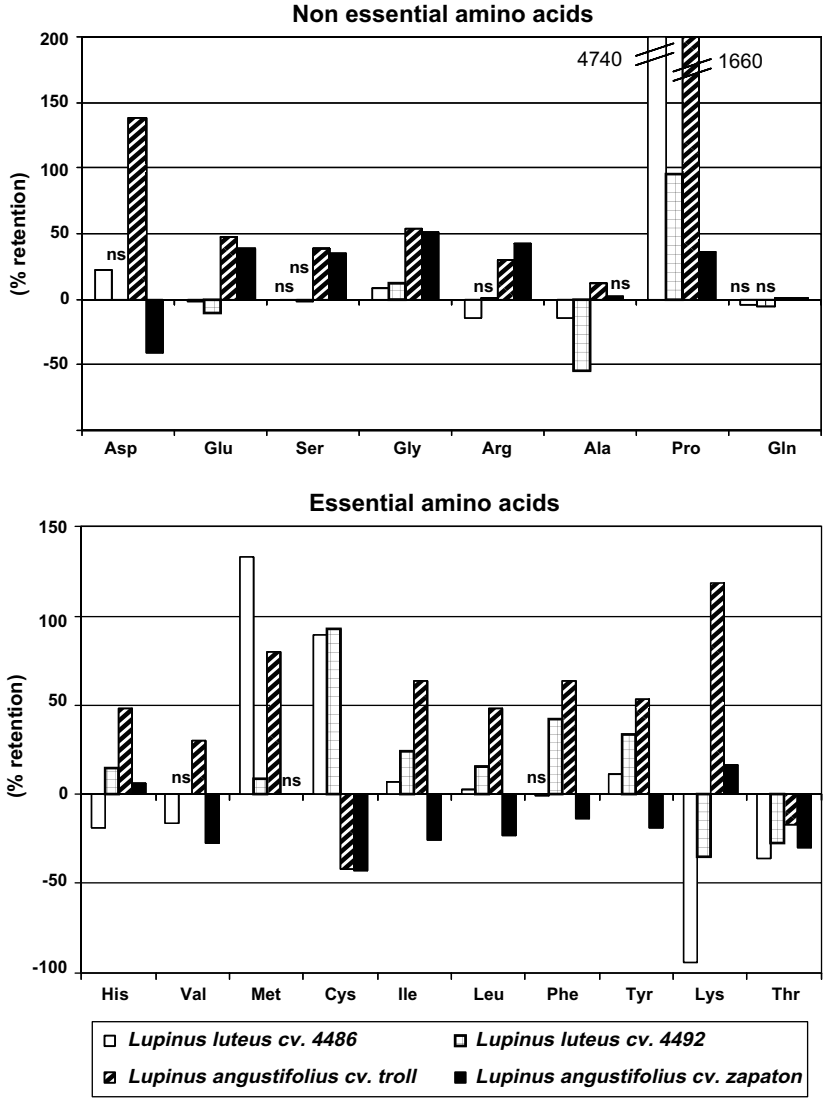

Fig. 7. Effect of germination on the amino acid content of globulin fraction of lupins. ns = no significant differences ($P \leqslant 0.05$) between raw and sprouted seeds.

The effects of germination on the amino acid content of the globulin (G) fraction of lupins are shown in Table 5 and Fig. 7. Sprouts of *L. luteus* cv. 4486 showed significantly ($P \leqslant 0.05$) higher contents of Asp, Gly and Pro among the NEAA and of Met, Cys, Ile, Leu, Phe and Tyr among the EAA than did the ungerminated seed, although the contents of Glu, Arg, Ala, His, Val, Lys and Thr were lower than in the raw seeds. Similar results were observed for *L. angustifolius* cv. *zapaton*. In this case, although most of the NEAA increased after germination, among the EAA only Lys underwent a significant ($P \leqslant 0.05$) increase. Moreover, the G fraction of *L. luteus* cv. 4492 and *L. angustifolius* cv. *troll* showed a nutritional improvement after germination since most of the EAA increased (Table 5, Fig. 7).

In the glutelin + prolamine (Gt + P) fractions of *L. luteus* cv. 4486, *L. luteus* cv. 4492 and *L. angustifolius* cv. *zapaton* (Table 6, Fig. 8), the germination process brought about a general improvement compared with the raw seeds. In this sense, the contents of most of the EAA increased, with the exception of Lys in *L. luteus* cv. 4486, His and Thr in *L. luteus* cv. 4492 and Thr in *L. angustifolius* cv. *zapaton*. Concerning the effect of germination on the NEAA of the Gt + P fraction, *L. luteus* cv. 4486 showed increases in Ser, Gly, Arg, Ala, Pro and Gln, *L. luteus* cv. 4492 increased contents of Asp, Ala and Pro and *L. angustifolius* cv. *zapaton* increased contents of Ser, Arg, Ala and Pro. The germination of *L. angustifolius* cv. *troll* only caused a rise in His, Met, Cys, Leu and Thr as EAA.

The effect of lupin germination on the amino acid content of the non-protein nitrogen fraction (NPN) is recorded in Table 7 and shown in Fig. 9. All the lupin cultivars studied had increased contents of most of the free protein amino acids after germination, possibly due to their release by proteolysis from storage proteins during germination, as previously reported by Martinez-Villaluenga et al. (in press-b).

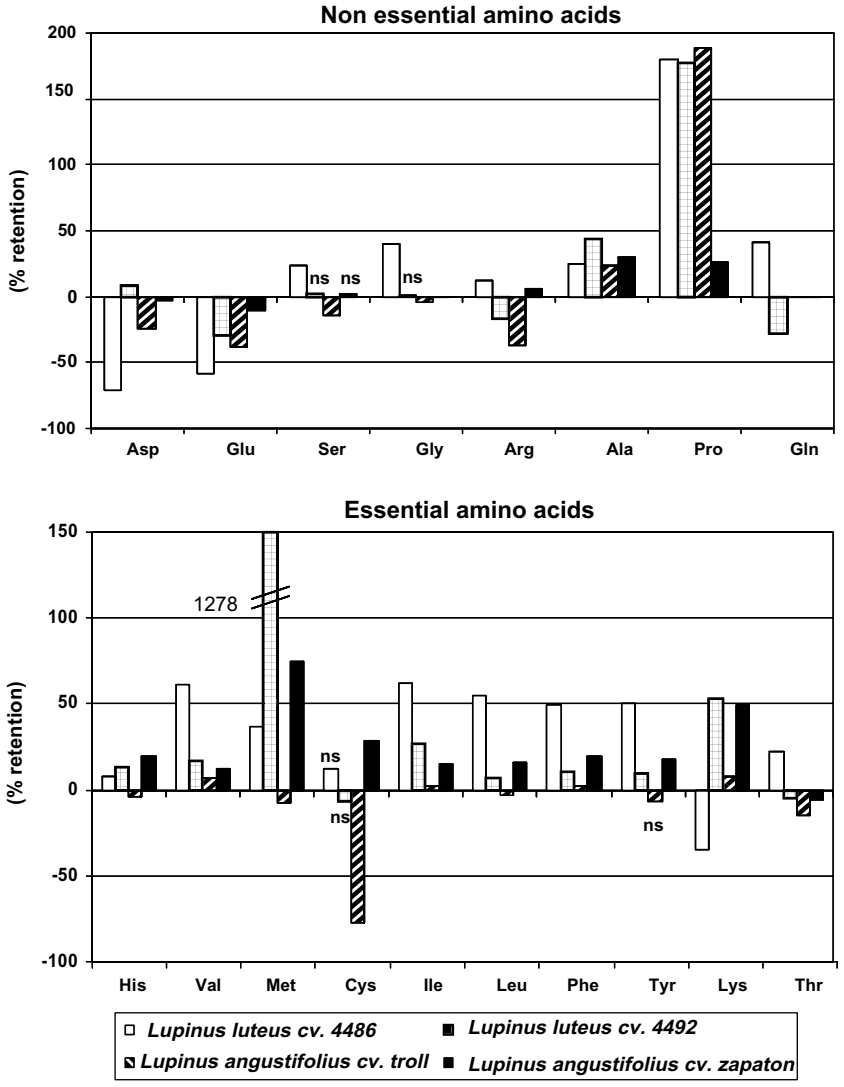

Fig. 8. Effect of germination on the amino acid content of glutelin + prolamine fraction of lupins. ns = no significant differences ($P \leqslant 0.05$) between raw and sprouted seeds.

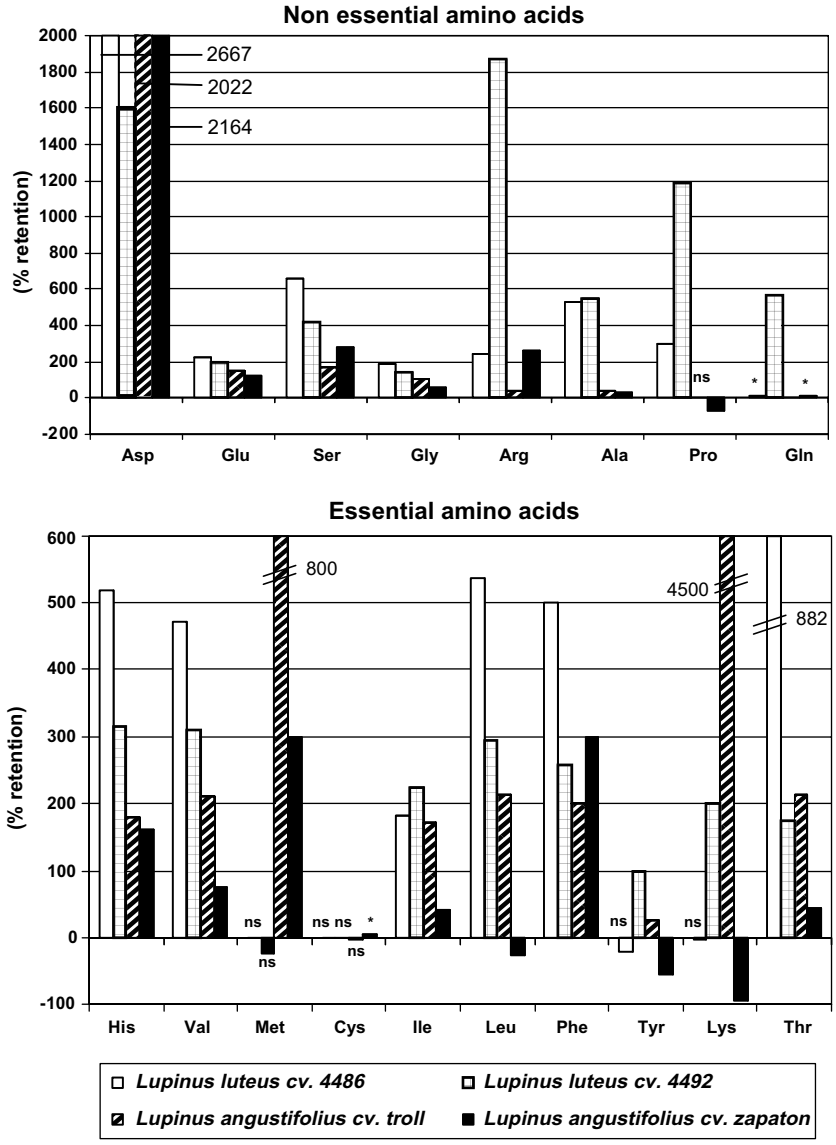

Fig. 9. Effect of germination on the amino acid content of non-protein *N* fraction of lupins. ns = no significant differences ($P \leqslant 0.05$) between raw and sprouted seeds (*) 0.04 g/100 g non-protein *N* fraction.

Germination is a biotechnological process in which metabolic enzymes, such as proteinases, are activated and can bring about release of some amino acids and peptides and the synthesis and utilization of others to form new proteins. As a consequence, the nutritional quality of proteins is enhanced, which is why germination has been suggested as a technological procedure for improving the nutritional quality of legumes.

4. Conclusions

Results obtained in this paper suggest the feasibility of obtaining the protein fraction from raw and germinated lupin cultivars by the Osborne procedure. The individual fractions from germinated seeds have protein bands and amino acid profiles different from the raw material. The protein fractions are an important unexploited source of protein with functional properties and, together with their nutritional qualities, could be a valuable product for exploitation by the food industry.

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