

Review

The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients

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This review deals with the main proteins of white lupin seed (*Lupinus albus*, L.) and reports on the current knowledge of the structural and functional properties of these proteins with the aim of providing the first comprehensive, accurate and up-to-date survey on this topic. Lupin seed's four main protein families of globulins, termed α -, β -, γ - and δ -conglutins, are reviewed with specific regard to their molecular and biological features. Their nutritional, technological, nutraceutical and allergenic potentials are also considered. The review is intended to provide nutritionists, food technologists and various stakeholders with the molecular background for a better exploitation of this valuable and accessible protein-rich source.

Introduction

The need for plant-derived nutrients is expected to grow due to economic and environmental factors as well as to support the development of new, safe and healthy foods which may respond to the consumers' increasing awareness of the impact of dietary habits on human well-being. Likewise, an increasing demand for plant proteins to be used as food ingredients for the improvement of nutritional, technological and healthy profiles of modern food is emerging, especially in more affluent countries. Although the "historical" role of several legume and cereal grains was as sources of oil for foods, interest in other seed components, including proteins, starches, fibres and minor constituents, is expanding in parallel with industrially available improved separation techniques.

Legume seeds are an abundant source of proteins and, among them, lupin is one of the richest. Indeed, lupin seed deserves greater interest as a result of its chemical composition and augmented availability in many countries in recent years. Lupin is a non-starch leguminous seed with a high protein content, almost as high as that of soybean (about 35% of the dry weight), and a relatively low oil content. Among the lupin seed species, the most cultivated, primarily in Australia, is blue lupin (*Lupinus angustifolius* L.), while the typical European and South American varieties are yellow (*Lupinus luteus* L.) and white (*Lupinus albus* L., Fig. 1) lupins. Blue and yellow lupin seeds are mostly used for feed, while the white lupins are primarily grown for food uses. For this reason, proteins from white lupin seeds will be the focus of this review.

The main proteins of legume seeds are located in the storage vacuoles of the cotyledonary tissues and mostly, but not exclusively, belong to the family of the storage proteins which serve as nitrogen and carbon skeleton sources for the emerging plantlet.

In recent years, seed dietary proteins have started to assume a key position in the interests among nutritionists not solely for their nutrient role, but also for a number of both adverse and beneficial effects that they may exert on the human body, including food intolerance, allergies, inhibition of endogenous hydrolytic enzymes, and hypolipidemic, hypoglycemic, hypotensive, anti-carcinogenic and anti-obesity activities, respectively. In addition, plant proteins can play important techno/functional roles, as witnessed by their increased uses as food ingredients.

On these bases, the aim of this review is to overview the molecular features of the main proteins of lupin in order to

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Fig. 1. *Lupinus albus* in an ancient herbarium.

open the gateway to their optimal exploitation as nutri/techno/functional and nutraceutical ingredients.

The major proteins of white lupin seed

White lupin seeds contain two classes of proteins, which according to Osborne's classification, correspond to the albumin and globulin fractions. Their quantitative ratio is about 1/9 (Blagrove & Gillespie, 1975; Duranti, Restani, Poniatowska, & Cerletti, 1981). Other minor fractions, including prolamins, have been detected (Cerletti, Fumagalli, & Venturin, 1978; Guéguen & Cerletti, 1994). Intervariety changes to this profile are likely, especially at the level of subunit composition (Vaza, Pinheiroa, Martins, & Ricardo, 2004). However, the structural and functional features of the main protein families considered in this review are expected to be conserved among the varieties, as it generally occurs between homologous storage proteins from different legume species.

Albumins comprise a number of protein molecules which represent the functional proteins of a seed. Many of them are metabolic enzymes directly or indirectly related to the storage role of the cotyledonary tissue. Other albumins play important roles in plant defence, such as hydrolase inhibitors and lectins (Casey, 1999; Domoney, 1999) which however are not, or barely, present in lupin seeds.

On the other hand, a storage role has been attributed to the 2S albumin class, meant to provide sulphur at germination (Müntz, 1998).

Globulins are the typical salt-soluble storage proteins of the seeds. This definition may not fit all cases, since both the solubility and the storage role of a protein are not always unequivocally defined, but for the sake of simplicity we will refer to the main lupin seed proteins as globulins in this review.

The first separation of lupin seed globulins dates back to the pioneering work of Blagrove and Gillespie (1975). These Authors used a cellulose acetate electrophoresis technique to separate four main fractions in *L. albus* and *L. angustifolius*. These fractions were named α -, β -, γ - and δ -conglutins on the basis of their electrophoretic mobility. Further separations included isoelectric focusing, which illustrated the acidic nature of α -, β - and δ -conglutins and the basic nature of γ -conglutin, and ion exchange chromatography which showed the extreme heterogeneity of the composition of these proteins (Casero, Duranti, & Cerletti, 1983; Duranti et al., 1981; Restani, Duranti, Cerletti, & Simonetti, 1981) as compared to other legume seed proteins. From those former studies, further research carried out by our own and other laboratories in various countries aimed at improving techniques for the separation of lupin globulins, characterising them at molecular level, elucidating their structure and function, and revealing the unusual properties of some of them.

Until recently, the classification of lupin conglutins into the canonical 11S, 7S and 2S seed globulins was based on relatively empirical criteria, such as the size of their oligomers, their sensitivity to reducing agents and isoelectric points. Nowadays, thanks to the availability of amino acid and nucleotide sequences, an unequivocal definition for each of the four main lupin proteins is possible (Table 1).

The main features of white lupin conglutins, including subunit composition, oligomeric status, post-translational modifications, cellular localisation, physiological role, pattern of deposition and degradation at germination, isolation procedures, nutritional, technological and biological properties as food ingredients are reported in this review. Some of these features are summarised in Table 2.

α -Conglutin

Main molecular features

α -Conglutin is legumin-like lupin globulin and, therefore, belongs to the 11S globulin family. Its presence in white lupin seed amounts to about 35–37% of the total globulins (Duranti et al., 1981). α -Conglutin is an oligomeric protein consisting of hexamers; however, prior to the pro-polypeptide proteolytic conversion to mature subunits, the prevalent form of α -conglutin is a trimer (Duranti, Guerrieri, Cerletti, & Vecchio, 1992). The transition between trimer to hexamer, as the result of a limited proteolytic event, has thoroughly been elucidated by the 3D structures of the homologous soybean proglycinin and glycinin

Table 1. Amino acid sequence entries of *Lupinus albus* conglutins and their best homologous proteins from edible plants

<i>Lupinus albus</i> conglutins		Greatest alignment (BLAST) score with		Protein name
Conglutin	UniProtKB/TrEMBL (acc. n.)	UniProtKB/TrEMBL (acc. n.)	Plant	
α	Q53154	GLYG3 Q9T0P5 Q99304	Soybean Pea Faba bean	Glycinin G3 Legumin A Legumin
β	Q53HY0 and Q6EBC1	B0YJF7 Q4LER6 VCLC	Blue lupin Soybean Pea	Conglutin β β -conglycinin α' subunit Vicilin
γ	Q9FEX1 and Q9FSH9	Q42369 7SB1 Q05929 Q8GT67	Blue lupin Soybean Carrot Tomato	Conglutin γ 7S basic globulin Extra-dermal glycoprotein Xylanase inhibitor
δ	Q333K7	Q99235 Q647G9	Blue lupin Peanut	Conglutin δ Conglutin

acc. n.: accession number.

(Adachi *et al.*, 2003). The 11S oligomers of α -conglutin are in a concentration- and pH-dependent equilibrium with a 7S form corresponding to the trimeric structure (Duranti, Guerrieri, Takahashi, & Cerletti, 1988), as per other legumin-like proteins (Dickinson, Hussein, & Nielsen, 1989).

A number of monomeric units ranging from about 62 to 74 kDa can be seen in a non-reducing SDS-PAGE separation (Fig. 2, lane 1). Mass and charge heterogeneity of the subunits are most probably the result of their multigene family origin as for other globulins (Casey, Domoney, & Ellis, 1985) and post-translational proteolysis (Duranti, Guerrieri *et al.*, 1992). Each monomeric unit of α -conglutin is composed of acidic and basic subunits, also referred to as α and β chains, respectively (Table 2), deriving from the mentioned cleavage of the pro-polypeptide (Fig. 2, lane 2). A single cleavage event likely occurs also in α -conglutin at a highly conserved site among the 11S globulins between

an asparagine and a glycine residue during seed development to give rise to the mature legumin (Müntz, 1998). The two generated subunits are linked by one disulphide bond. The disulphide bond probably already exists in the pro-polypeptide prior to cleavage as per other legumins (Casey *et al.*, 1985; Müntz, 1989). In the α -conglutin of mature *L. albus* seeds, α -polypeptides with M_r 47–52 kDa and 42–44 kDa and β polypeptides of 20–22 kDa are present (Duranti *et al.*, 1988; Johnson, Knight, & Gayler, 1985). Indeed, at least two acidic and five basic chains have been identified by proteomic analysis (Magni *et al.*, 2007).

Contrary to most legumin-like globulins, some of the subunits of α -conglutin are glycosylated (Duranti, Guerrieri *et al.*, 1992; Duranti *et al.*, 1988). The glycosylation consensus sequence resides in the acidic subunit and the region has been directly sequenced (Duranti, Horstmann, Gilroy,

Table 2. Summary of *Lupinus albus* conglutins' main features

Conglutin	Protein family	(%) of total globulins	Native protein			Monomer composition				Protein function in the seed
			M_r , kDa	pI	Quaternary structure	Subunit name	M_r , kDa	pI	Glycosylation	
α	11S (legumin)	35–37	330–430	5.1–5.8	Hexamer	Acidic ^a Basic ^a	42–52 20–22	4.5–4.7 6.7–8.6	Yes/No ^b No	Storage
β	7S (vicilin)	44–45	143–260	5.0–6.0	Trimer	HMW IMW LMW	53–64 25–46 17–20	5.1–5.7 5.3–8.4 4.2–5.0	Yes Yes/No ^b Yes/No ^b	Storage
γ	7S	4–5	200	7.9	Tetramer	Large ^a Small ^a	29 17	8.2–8.9 5.8–6.6	Yes No	Unknown
δ	2S	10–12	13	Acidic	Monomer	Large ^a Small ^a	9 4	4.1–4.3 n.d.	No No	Unknown

S: sedimentation coefficient. HMW, IMW, LMW: high, intermediate and low relative molecular weight, respectively. n.d.: not determined.

^a Covalently linked by disulphide bond(s).^b Not all the polypeptides are glycosylated.

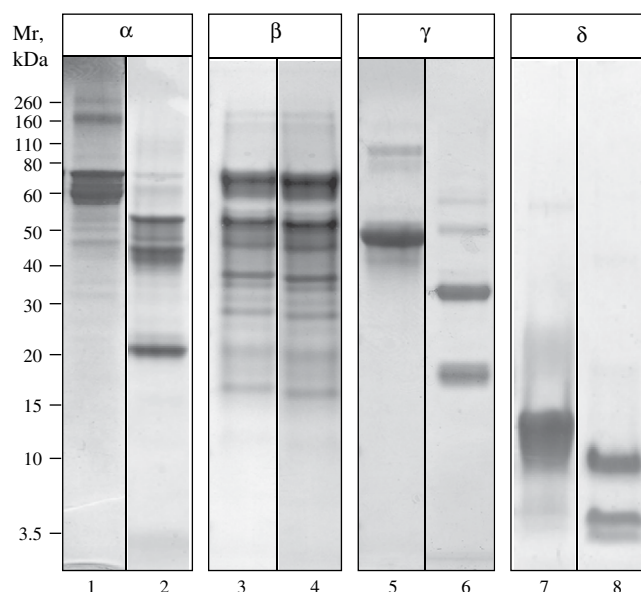


Fig. 2. Electrophoretic pattern of *Lupinus albus* main proteins (α -, β -, γ - and δ -conglutins), as separated by SDS-PAGE under non-reducing (lanes 1, 3, 5 and 7) and reducing (lanes 2, 4, 6 and 8) conditions. See text for purification details.

& Croy, 1995). The α -conglutinin gene sequence, mentioned in Table 1, does not bear any glycosylation consensus sequence, thus confirming the presence of at least two different genes for this protein. However, only one complete, deduced amino acid sequence of α -conglutinin is available in the database (Table 1), so far.

Intracellular localisation and biological function

Lupin α -conglutinin is located in the storage vacuoles of the cotyledonary cells (Duranti, Faoro, & Harris, 1991) and its role as storage protein is confirmed by the extensive proteolytic degradation at germination (Duranti, Cucchetti, & Cerletti, 1984). Interestingly, the basic subunit, which by analogy with the same subunit in the 3D model of soybean glycinin is seemingly located in the protein core (Adachi et al., 2003), is more resistant to proteolysis during germination and in *in vitro* proteolysis assays than the acidic ones (Duranti & Giani, 1989).

The physiology of deposition has not specifically been studied for lupin α -conglutinin, but it is expected to follow the common internal secretory pathway of most storage globulins, including synthesis on membrane-bound polysomes, co-translational removal of the signal peptide, transit into the Golgi stacks, deposition in the developing seed cotyledons storage vacuoles, formerly termed protein bodies, and final maturation through limited proteolysis (Müntz, 1989; Müntz, 1998).

β -Conglutinin

Main molecular features

β -Conglutinin belongs to the 7S globulin family, also named vicilin-like globulins (Blagrove & Gillespie,

1975). Its presence in white lupin seed amounts to about 44–45% (Duranti et al., 1981), thus qualifying β -conglutinin as the most abundant lupin seed protein. β -Conglutinin is a trimeric protein in which the monomers consist of a number of polypeptides ranging from 16 to over 70 kDa. A 64 kDa β -conglutinin precursor has been identified and purified (Duranti, Sessa et al., 1992). This precursor is glycosylated. A large number of proteolytic processing sites give rise to the abundance of 7S mature polypeptides observed (Duranti, Sessa et al., 1992). Recently, 42 spots corresponding to β -conglutinin polypeptides have been identified by proteomic analysis (Magni et al., 2007 and unpublished results). No disulphide bridges are present, as assessed by the identical SDS-PAGE pattern under non-reducing and reducing conditions (Fig. 2, lanes 3 and 4), while a high extent of glycosylation characterises many subunits (Duranti, Goriinstein, & Cerletti, 1990; Duranti, Sessa, & Carpen, 1992).

Two complete, deduced amino acid sequences have been deposited (Table 1). These sequences are very similar, differing by only a few amino acids.

Intracellular localisation and biological function

β -Conglutinin is located in the cotyledonary protein bodies. Apparently the covalent integrity is not a pre-requisite for β -conglutinin to be correctly deposited in the protein bodies, since the mature β -conglutinin from lupin dry seeds appeared already proteolytically cleaved in a number of sites, giving rise to the complex SDS-PAGE pattern seen in Fig. 2 and in 2D-IEF–SDS-PAGE maps (Duranti, Sessa et al., 1992; Magni et al., 2007).

The physiology of deposition has not specifically been investigated for lupin β -conglutinin, but it seemingly follows the pathways common to other storage globulins, as mentioned for α -conglutinin.

The complete degradation at germination strongly supports the storage function of β -conglutinin (Duranti et al., 1984). However, a transient β -conglutinin derived polypeptide chain of 20 kDa has been shown to display lectin-like activity (Rodrigues dos Ramos, Ferreira, Franco, & Teixeira, 1997).

γ -Conglutinin

Main molecular features

γ -Conglutinin is an unusual basic 7S protein, which is equally soluble in water and salt solutions. This protein accounts for about 4–5% of total proteins in mature lupin seeds (Duranti et al., 1981). At neutral pH, the prevalent form of γ -conglutinin is a tetramer (Duranti, Sessa, Scarafoni, Bellini, & Dallochio, 2000), or a hexamer, according to other authors (Blagrove & Gillespie, 1975), probably because of the detected size of the oligomer, which supported both interpretations. At acidic pH, the oligomer dissociates to a monomer of about 50 kDa, with a quick transition around pH 5.0 (Duranti, 1986a, 1986b). Each monomer is in turn composed of two disulphide linked subunits of 17 and 29 kDa (Restani et al., 1981) (Fig. 2, lanes 5 and 6).

Both subunits are heterogenous as a probable result of post-translational proteolysis. However, heterogeneity is much less pronounced than in α - and β -conglutins. Endogenous proteolysis converts the pro-polypeptide into the two mature subunits, as described for *L. angustifolius* γ -conglutin (Ilgoutz, Knittel, Lin, Sterle, & Gayler, 1997). However, the precise site of cleavage has not been identified yet, as trimming of the terminal regions of both subunits is likely to occur (Ilgoutz et al., 1997). Recently, four large and five small chains have been identified by proteomic analysis (Magni et al., 2007 and unpublished results). The 29 kDa subunit is glycosylated with covalently linked mannose and glucosamine units (Eaton-Mordas & Moore, 1978). The corresponding glycosylation consensus sequence is present in both gene sequences mentioned in Table 1 and resides in a cysteine-rich stretch in proximity to the N-terminal region.

Two complete deduced amino acid sequences of γ -conglutin have been deposited (Table 1). However, only the product of one gene (Q9FEX1_LUPAL) has been detected in the mature dry seeds (Scarafoni, et al., 2001).

Intracellular localisation and biological function

Lupin γ -conglutin is located in the protein bodies of developing lupin seeds (Pernollet, 1978; Shewry, Napier, & Tatham, 1995; Weber & Neumann, 1980). However, the protein has also been detected in the extracellular apoplastic regions of germinating lupin cotyledons (Duranti, Faoro, & Harris, 1994). The unusual stability of γ -conglutin during seed germination and in *in vitro* tests with various proteolytic enzymes (Duranti, Gius, Sessa, & Vecchio, 1995) strongly suggests that it is not a storage protein. On the other hand, if the denatured protein is submitted to proteolytic attack, degradation by trypsin and other enzymes is complete. This suggests an “all or none” cleavage mechanism of γ -conglutin (Duranti, unpublished data). Whether this feature reflects some functionality of the protein has yet to be established. The unusual extra-vacuolar location of γ -conglutin adds further evidence that this protein probably plays a non-storage role (Citharel & Delamarre, 1988; Duranti et al., 1991; Duranti et al., 1994). Intense research activity of our group is devoted to elucidating the physiological role of this protein.

γ -Conglutin displays further unique properties of its own: it binds divalent metal ions, especially Zn^{++} and Ni^{++} (Duranti, Scarafoni, Di Cataldo, & Sessa, 2001) which have been shown to promote the refolding of the denatured protein (Duranti, Di Cataldo, Sessa, Scarafoni, & Cecilian, 2002) and, independently, insulin with a K_d of 10^{-5} M over a wide range of pH values (Magni et al., 2004).

δ -Conglutin

Main molecular features

δ -Conglutin belongs to the 2S sulphur-rich albumin family (Blagrove & Gillespie, 1975). Its presence in white

lupin seed was assessed to be around 10–12% (Duranti et al., 1981), but further recent data (Sironi, Sessa, & Duranti, 2005) suggest a lower content of around 3–4%. The previous assessment likely included some contaminant proteins.

δ -Conglutin is a monomeric low M_r protein, consisting of two subunits, one (a) with a molecular mass of about 4 kDa and one (b) with a molecular mass of about 9 kDa (Fig. 2, lanes 7 and 8). The two polypeptide chains are linked by two interchain disulfide bonds $\text{Cys}^{a8}-\text{Cys}^{b24}$ and $\text{Cys}^{a20}-\text{Cys}^{b13}$ or, more likely, $\text{Cys}^{a20}-\text{Cys}^{b12}$. In addition, the large polypeptide contains two intrachain disulfide bridges ($\text{Cys}^{b26}-\text{Cys}^{b68}$ and $\text{Cys}^{b12}-\text{Cys}^{b60}$ or, more likely, $\text{Cys}^{b13}-\text{Cys}^{b60}$) (Salmanowich & Weder, 1997). The presence of an odd cysteine residue may give rise to covalent dimerisation of the monomeric units leading to a stable dimer with an relative mass of about 23–25 kDa, which can be barely seen in the SDS-PAGE patterns under non-reducing conditions (Fig. 2, lane 7).

One complete amino acid sequence of the large subunit and of the small subunit of *L. angustifolius* have been deposited. The sole amino acid sequence of *L. albus* δ -conglutin has been inferred from the gene sequence (Table 1).

Intracellular localisation and biological function

There are neither data on the intracellular localisation of white lupin δ -conglutin nor on its biological function. However, a storage vacuolar localisation of the homologous protein in *L. angustifolius* has previously been described (Gayler, Wachsmann, Kolivas, Nott, & Johnson, 1989). From this finding, a similar location also for the *L. albus* protein can reasonably be inferred.

As far as the physiological role of δ -conglutin is concerned, a storage function has been proposed for this class of proteins. However, structural similarity with the plant cereal inhibitor family, which includes bi-functional trypsin/alpha-amylase inhibitors, may suggest a defence function for this protein in addition to its storage role. Further studies are needed to verify this hypothesis.

Other relevant lupin seed proteins

The proteins described in this review are by far the most abundant in white lupin seeds, making up a total of 85–88% of all seed proteins. Therefore, they are responsible for the main properties of the seeds, flours and other intermediate products deriving thereof. However, other minor proteins can also be relevant to the characteristics and food quality of lupin seeds. By way of example, a Bowman–Birk serine proteinase inhibitor (BBI) has very recently been identified (Scarafoni et al., 2008), suggesting that lupin seeds, contrary to all previous reports, do contain this kind of inhibitor. The overall trypsin inhibitory activity detected in white lupin is approximately 10% of the same activity in soybean seeds and three times as much as that found in garden pea or common bean seeds (Birk, 1993; Hove & King, 1979; Muel, Carrouée, & Grosjean, 1998). The BBI isolated from lupin seeds has the peculiarity of

being a double-headed trypsin inhibitor, instead of the most common trypsin/chymotrypsin type. This deserves further studies to look into the potential anti-carcinogenic or anti-inflammatory effects as other BBIs have shown (Clemente & Domoney, 2008).

Lupin conglutins' isolation procedures

As far as the isolation of the main lupin proteins is concerned, three main protocols have been developed in past years. One is aimed at obtaining each purified protein family on a lab scale; the other one is oriented at an industrial scale preparation of partially purified lupin protein fractions for industrial applications; the third one has been set up in laboratory but it could be scaled up in order to yield three protein fractions at a greater purification level than the industrial scale approach. The protocols are detailed below and are summarised in Table 3.

Lab scale purification

Defatted lupin flour is extracted by stirring in a neutral to slightly alkaline buffer containing NaCl, at a ratio of 1:20 (w/v), for a few hours at 4 °C. The suspension is centrifuged and the supernatant is desalted on a gel filtration column or by ultrafiltration. The desalted extract is fractionated on a weak anion exchanger, such as Whatman DE 52 DEAE-cellulose, equilibrated at pH 7.5. In these conditions, the raw γ -conglutinin fraction is not retained on the column; the β -conglutinin is eluted with an ionic strength of 0.27 M, while α - and δ -conglutinins are eluted at 0.37 M. To separate δ -conglutinin from α -conglutinin, an affinity chromatography step using concanavalin A is used with notable success due to the glycosylation of α -conglutinin. The α - and β -conglutinins can be further purified on a TSK DEAE 5PW column at pH 7.5, to avoid mutual cross-contamination.

Modifications and refinements of this essential purification scheme have been proposed (Melo, Ferreira, & Teixeira, 1994).

The fraction containing γ -conglutinin is adjusted to pH 4.5 with acetic acid and loaded on a Whatman CM-cellulose. γ -Conglutinin is then eluted at an ionic strength of 0.35 M. From this step on, variations in the γ -conglutinin purification procedure can be adopted including selective precipitation with Zn^{2+} (Duranti et al., 2001) and insulin affinity chromatography (Magni et al., 2004) according to the purification level required for this protein.

Pilot plant isolation

The procedure has been developed by the Fraunhofer Institute (Germany) and is described by Wäsche, Müller, and Knauf (2001). To sum up, the protocol involves solubilisation of the proteins at alkaline pH value and subsequent isoelectric precipitation at acidic pH values of the α -, β - and δ -conglutinins which are recovered by large scale centrifugation and are not separated further. This fraction has been termed "Type E" as a result of its excellent emulsifying properties (see below). The remaining supernatant contains primarily γ -conglutinin, which can be recovered by ultrafiltration and stabilized by drying. This γ -conglutinin enriched fraction was termed "Type F" as a result of its excellent foaming properties (see below).

Scalable isolation procedure

This intermediate procedure is also based on the alkaline solubilisation followed by acidic precipitation of the α -, β - and δ -conglutinins leaving the raw γ -conglutinin in the supernatant (Sironi et al., 2005). The raw γ -conglutinin is further purified and recovered by selective precipitation with Zn^{2+} . The precipitated α -, β - and δ -conglutinins are resuspended in sodium acetate, pH 4.0 containing 0.4 M NaCl and 40% ethanol, and the suspension is stirred for 1 h at room temperature. This treatment turns δ -conglutinin into solution that remains in the supernatant upon centrifugation, whereas the α - and β -conglutinins can be pelleted. Recovery

Table 3. Outline of lupin conglutinins' isolation procedures

	Lab scale	Pilot plant isolation process	Scalable isolation process
Raw material	Flour	Flour, flakes	Flour
Protein extraction	Neutral pH, 0.5 M NaCl	Alkaline conditions	Alkaline conditions
Fractionation	Anion exchange chromatography ↓ ConA affinity chromatography ↓ Insulin affinity chromatography	Isoelectric precipitation, pH 4.0-5.0 ↓ ↓ ↓	Isoelectric precipitation, pH 4.0-5.0 ↓ Acidic water/ethanol precipitation ↓ Precipitation with Zn^{2+}
Final protein product, conglutinins	α δ β γ	$\alpha + \beta + \delta$ γ	$\alpha + \beta$ δ γ

—→: insoluble, —•→: soluble. ConA: concanavalinA. Details and references are given in the text.

of δ -conglutin in a solid form is accomplished by cold precipitation, keeping the solution at -20°C overnight.

Nutritional quality of lupin proteins

Lupin seeds have been eaten by humans since ancient times and are commonly consumed in several European and Latin America countries. White lupin represents an important source of proteins for human beings, since its seed is one of the richest in total protein content, with a biological value of 91% of egg proteins (Egaña, Uauy, Cassorla, Barrera, & Yañez, 1992). In the newly developed sweet varieties the presence of anti-nutritional compounds, such as alkaloids and oligosaccharides is relatively low when compared to other lupin species (El-Adawy, Rahma, El-Bedewey, & Gafar, 2001; Hove, 1974). White lupin seeds have a higher essential amino acid index (EAAI) and protein efficiency ratio (PER), based on lysine and tyrosine availability, than blue and yellow lupins (Sujak, Kotlarz, & Strobel, 2006).

Although the results of several studies into the nutritional quality of white lupin flour or protein concentrates deriving thereof have been published (Egaña et al., 1992; Erbas, Certel, & Uslu, 2005; Sujak et al., 2006), no data concerning single protein families are available. For this reason, it is worth to highlight some detail about the presence of the essential amino acid for each protein family (Table 4).

Lupin proteins allergenicity

Allergenicity of lupin proteins is a relevant topic when considering their current and potential food use. Due to the still limited diffusion of whole lupin seeds and their derivatives, no systematic epidemiology studies on the prevalence of lupin allergy have been carried out so far. Therefore, the current evaluation is based on the claims made in some cross reactivity studies which revealed specific acute allergy responses (Radcliffe, Scadding, & Morrow-Brown, 2005), especially in peanut-sensitive patients (Lindvik, Holden, Levik, Cvancarova, & Halvorsen, 2008; Shaw, Roberts, Grimshaw, White, & Hourihane, 2008 and on *in vitro* analyses of the allergenic potential

with available IgEs (Magni et al., 2005). Based on these preliminary studies, the following picture can be depicted. Lupin proteins can elicit food allergy responses in lupin-sensitised patients and in patients sensitised to other legume seeds, especially peanut, as a result of the sequence/structure similarities of the storage proteins. Subjects with primary lupin allergy are, so far, fairly rare, but this may be due to the above-mentioned scarcity of research work so far carried out in the field. Although data are still insufficient to identify the major lupin allergens, β -conglutin, the basic subunits of α -conglutin and γ -conglutin appear to be frequently detected by specific IgEs. In this respect, it is noteworthy that a prevalent lupin allergen, corresponding to *L. angustifolius* β -conglutin, has very recently been deposited in the International Union of Immunological Societies database (available on the Web at <http://www.allergen.org>) under the name Lup an 1. δ -Conglutin, though belonging to the 2S protein family, which is often referred to as a major allergen (Monsalve, Villalba, Rico, Shewry, & Rodríguez, 2004), has not revealed significant immunological reactivity in the tests carried out so far (Magni et al., 2005).

By and large, the overall pattern of lupin allergenicity, which is currently under clinical assessment, is not dissimilar from that which is obtained with soybean, peanut and other edible legume seeds.

The recent inclusion of lupin on the list of ingredients which must appear on the labelling of foodstuffs pursuant to EU law (European Commission Directive 2006/142/EC) is a precautionary measure introduced to protect consumers against acute risks in the consumption of lupin containing foods.

The potential of lupin protein for use as food ingredients

In 1917 a 'lupin' banquet was given in Hamburg at a botanical gathering, at which a German Professor, Dr. Thoms, described the multifarious uses to which lupin might be put. At a table covered with a tablecloth of lupin fibre, lupin soup was served; after the soup came lupin beefsteak, roasted in lupin oil and seasoned with lupin extract, then bread containing 20 per cent of lupin, lupin margarine and cheese made from lupin albumen, and finally lupin liqueur and lupin coffee. Lupin soap was provided for guests to wash their hands, while lupin-fibre paper and envelopes with lupin adhesive were available for writing (Grieve, 1931).

There is no doubt that lupin seed constituents may have several functions, many of which, as we have seen in the quotation taken from Mrs. M. Grieve, can almost certainly be attributed to lupin proteins. As a matter of fact, a number of studies have researched the technological functionality of lupin seed components since 1917. However, these properties appear to be strongly influenced by the actual composition of the analysed fractions, mainly flours or aqueous extracts,

Table 4. Essential amino acid occurrence in white lupin conglutins

Conglutin	Most abundant essential amino acids		Limiting essential amino acids	
	Amino acid	%	Amino acid	%
α	Leu	7.5	Met	0.2
	Ile	5.8	Trp	1.0
β	Leu	7.3	Met + Cys	tr.
	Phe	4.9	Trp	0.1
γ	Leu	9.4	Met	0.8
	Thr	7.4	Trp	1.1
δ	Leu	8.8	Met	0.5
	Cys	6.5	Trp	1.1

tr: traces. Reference: Duranti et al., 1981.

preparation procedures, stabilisation and any other processing treatment, as well as the analytical procedures used. It is, therefore, hard to summarise specific functionalities of lupin proteins. However, the two semi-industrial preparations of lupin proteins already mentioned, i.e. Type E and Type F, are the most characterised protein isolates so far and have been shown to possess specific functionalities (Wäsche *et al.*, 2001). Specifically speaking, Type E, which contains α -, β - and δ -conglutins (Duranti, unpublished results) showed an extremely good emulsifying capacity and emulsion stability, as compared to similar fractions from other legume seeds, while Type F has a great solubility over a wide range of pH values and very good foaming activity and foam stability properties. It has also been observed that high pressure treatment has caused the positive effects of decreasing the size of droplets, flocculation and creaming index (Chapleau & de Lamballerie-Anton, 2003).

The properties of Type E likely derive from multi-subunit composition and extreme fragmentation of β -conglutin polypeptides, which, upon mechanical processing or pressure treatments, may disaggregate and exert their functionalities. As a matter of fact, with more intact proteins such as the soybean β -conglycinin subunits, the activity of hexogenous proteolytic enzymes has been shown to contribute to diminishing viscosity of the solutions and favourably affecting the properties connected with surface activity (Surowka, Zmudzinski, & Surowka, 2004).

The separate lupin protein fractions, by allowing the tailoring of functionalities, are excellent candidates for the development of a variety of food products, including fine bakery foods, sauces, pies, mayonnaise, salad dressings, marshmallows, etc. In particular, lupin protein isolates and flours have been successfully tested as ingredients in the production of muffins in which egg and milk proteins were totally substituted and in diet biscuits with high protein contents (Bez, Schott, & Seger, 2005). More recently, lupin flour and different protein fractions have been included in pasta, dairy, bakery, and meat products (Rojas-Hijazo, Garcés, Caballero, Alloza, & Moneo, 2006). Furthermore, a patent on the preparation of pasta enriched with lupin protein isolates and concentrates has been deposited (Duranti, 2000). Lupin proteins are relevant ingredients of commercially available tofu-like products, gluten-free pasta, bakery products and meat substitutes. The effects of lupin proteins in wine clarification have also been described (Manara & Salvo, 2006). This wide range of food uses is further borne out by the scarce off-flavour generated by lupin flour and, even less, by the isolated lupin protein fractions.

Lupin protein biological activities

A number of studies on the biological effects of lupin protein components are being carried out. As it often happens in this field, the observed nutraceutical effects are attributed to classes of components, such as fibres, starch, oligosaccharides, antioxidants, etc., or even to the

whole seed or flour and only rarely are the molecular determinants of these activities identified (Duranti, 2006; Scarafoni, Magni, & Duranti, 2007). In the area of lupin protein bioactivities, the currently available information is reviewed below.

One example of identification of a specific biological activity is that of γ -conglutin blood glucose lowering effect. As mentioned above, γ -conglutin is capable of interacting with the mammalian protein hormone insulin. Whether strictly related to that finding or not, a significant reduction in glycaemic levels in glucose-overloaded rats was induced by oral treatment with purified γ -conglutin. The γ -conglutin lowering effect at the highest protein dose was comparable to the effect obtained with approximately half a dose of metformin, a well known hypoglycaemic drug (Magni *et al.*, 2004), thus suggesting a kind of pharmacological response brought about by lupin protein. A patent on this has been deposited (Morazzoni & Duranti, 2004). Further studies on the metabolic fate of orally administered γ -conglutin, the meaning of insulin binding capacity and a possible involvement of γ -conglutin in the cell signalling pathway are currently being performed by our group in collaboration with other laboratories.

In addition to these findings, other biological activities have been attributed to lupin proteins, including plasma cholesterol and triglyceride lowering effects (Sirtori *et al.*, 2004), anti-hypertensive properties (Pilvi *et al.*, 2006), angiotensin converting enzyme (ACE) inhibitory activity (Yoshie-Stark, Bez, Wada, & Wäsche, 2004). Research projects are in progress aimed at identifying other bioactive “cryptic peptides” hidden in the polypeptide backbone of lupin storage proteins, such as the aforementioned lectin liberated by β -conglutin during germination (Rodrigues dos Ramos *et al.*, 1997). This research line parallels the biotechnological approaches aimed at incorporating bioactive peptides into the primary structures of plant storage proteins, mostly soybean 7 and 11 S globulins, as it has been shown by S. Utsumi and his group (Maruyama *et al.*, 2003; Prak, Maruyama, Maruyama, & Utsumi, 2006). However, the search of cryptic bioactive peptides does not imply any genetic transformation of the seed.

Conclusions

Understanding food protein structure and other molecular features is a pre-requisite for the targeted, optimal use as functional ingredients. This holds especially true in the case of less common protein food sources, such as lupin seeds. However, contrary to what some people believe, lupin seed is not, technically speaking, a novel food, as it has been used for this purpose both in the Mediterranean and Andean regions for many centuries (Sujak *et al.*, 2006). However, the current use of lupin whole seed as a food is limited to local dietary habits in the Mediterranean area in the form of snack food or accompanying traditional pasta and soup dishes in some regions of Southern Italy, Spain and North African countries. Lupin seed as a food is almost

unknown in Central and Northern European countries, North America or Australia. Nonetheless, lupin's special composition, mostly consisting of protein, fibre and limited amounts of oil, means this seed could play a valuable role as a rich source of a variety of specific ingredients. Various food industries are becoming aware of the potential of lupin seed and a growing number of products including lupin flour and protein or fibre fractions are available.

The mentioned recent inclusion of lupin on the list of potential allergenic foods, already including soybean and peanut (European Commission Directive 2000/13/EC), should be interpreted as a precautionary measure and not as a real limitation, since it simply establishes mandatory labelling of this ingredient in lupin-based foods. With this inclusion, sensitive consumers are becoming alerted to the potential risks, and the many others who are not, can profitably take advantage of the positive features of lupin's components.

Meanwhile, various research activities are currently being carried out into the further characterization of known and less known protein components and the identification of specific bioactivities. The body of information which is accumulating is the gateway to the development of new, safe and healthy foods, allowing better exploitation of this valuable and accessible protein-rich source and responding to consumers' increasing awareness of the impact of dietary habits on human well-being.

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