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Properties of lupine proteins relevant to their nutritional performance

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Abstract. Composition, solubility, bound sugar content and quality, subunit composition and structure of the storage proteins of seeds of *Lupinus albus* are discussed. Amino-acid composition is also given for each protein, the various protein classes and the whole flour. These data allow for the characterization of the molecules of the various storage proteins and their contributions to the nutritional properties of the seed. *In vitro* digestibility (by mammalian endopeptidases) is reported and is less than for animal proteins. Possible causes, at the molecular level, for this behaviour and possible means to overcome these effects are examined. The relationships between the above data are considered in view of the nutritional performance of the proteins and of the genetical, agronomic and technological approaches most suited to improve the nutritional quality of lupine seeds as a protein source.

Introduction

Lupines have been used as a human food for centuries in the Andean and in the Mediterranean regions but were one of the last legumes to gain the attention of international agencies and of researchers as a source of proteins for humans. Research on their seed proteins started nearly a century ago but was resumed only in the fifties and developed systematically in recent times. The information obtained allows to relate properties of nutritional relevance, like aminoacid composition and digestibility, to the molecular characteristics of the various types of seed proteins, and to see how these characteristics are influenced by modifications in plant nutrients. In the present paper we shall develop this approach and report recent data on the subject with special reference to work done in our laboratory.

Materials and methods

Enzymic treatments of samples

The enzymes were added in a 1:60 ratio (w:w) to lupine protein. For treatment with trypsin, the protein was dissolved (3 mg/ml) in 0.1 M phosphate buffer pH 7.9, and for pepsin the protein was dissolved in 0.01 N HCl. Blanks

without any enzyme addition were also run. Incubation was at 37°C. At fixed times, 1 ml sample was withdrawn and protein was precipitated by addition of 1 ml 20% trichloroacetic acid. After centrifuging 20 min at 10 000 × g, peptides and aminoacids were assayed in the supernatant by the microbiuret [11] and the ninhydrin reactions [12, 13]. Trypsin (2 × crystallized) and pepsin (crystallized) were from Sigma. All other materials and procedures used were those described in ref. 7 and 14.

Results and discussion

Molecular properties

The seed protein content in lupine is one of the highest among legumes: the actual value depends on the species and varieties and ranges from 30% to 43% of the whole dry seed [3]. These are quite favourable data as compared to other legume seeds: values reported are 35% for the soybean, 21 to 23% for the pea and 26% for the favabean [9]. All of these values are measured by multiplying total nitrogen by 6.25 which may be slightly in excess.

All lupine seed proteins are soluble in water or in salt solution: albumins represent 13% of the total whereas the globulins include the residual 87% [7]. These and the following data refer to *Lupinus albus*. Albumins are separated by cellulose acetate electrophoresis (CAE) into 5 components, whereas sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) resolves at least 18 bands: the proteins may contain more than one subunit each and it is likely that CAE does not yield a complete resolution [2]. Globulins were resolved into 11 species: of these, six are the major components which together represent more than 93% of the total globulins [7]. The other components are present in very small amounts. Properties of the globulin proteins are summarized in Table 1.

Two groups of globulins can be recognized which contain very similar proteins. One is vicilins, 7S proteins [5] which include globulins 4, 5, 6, 7 and together represent 44% of the total globulins. They appear extremely similar in subunit composition but behave as separate molecular species, as shown by differences in molecular weight, behaviour on the ion exchanger, sugar content, etc. [7]. The second group of similar globulins are legumins: i.e., proteins 8 and 9a which include 33% of the total globulins [7]. Two different molecular weights, 11S and 7S, were attributed to legumins [5]. Actually each legumin, as shown in Table 1, contains one light and one heavy component, respectively of molecular weight 187 and 372 kd for globulin 8 and 250 and 437 kd for globulin 9a. For each legumin, the two components display an identical aminoacid composition but differ in some of the subunits contained. Probably the heavy component is the original structure: the high molecular weight subunits are split post-translationally

Table 1. Molecular weight and distribution of protein and neutral sugar in the globulins of *Lupinus albus*[a]

Globulin	Component on cellulose acetate electrophoresis	M.W. (kd)	Protein % of total globulin	Neutral sugar mg/100 mg peptide
1[b]	γ	199	6.0	4.3
2	–	n.d.	0.5	36.9
3	–	123	0.7	15.5
4	β	270	10.0	2.4
5	β	275	2.1	3.7
6	β	335	30.2	2.1
7	β	143	1.9	3.4
8	α	187–372	21.2	1.5
9a	α	250–437	12.0	0.8
9b	δ	44	12.5	0.7
10	–	226	2.6	21.5
11	–	n.d.	0.4	21.5

[a]Molecular weights, given in kilodaltons, were determined on proteins from Lupinus albus var. Multolupa; other data are from ref. 7; n.d. = not determined.
[b]globulin 1 = conglutin γ

and this changes the association of the subunits yielding the molecule of smaller molecular weight. On the other hand, the two legumins differ in subunit composition, behaviour on the ion exchanger and to a small extent in aminoacid contained. Other major globulins of interest for our purpose are globulins 1 and 9b, which will be discussed later.

CAE does not resolve among themselves the vicilins and the legumins which appear as single bands, band β and band α, whereas protein 1 and 9b correspond respectively to bands γ and δ [7]: therefore previous authors who used this technique recognized in lupine only a limited number of the component proteins.

All globulins are glycosylated. Some of the minor components are indeed proteoglycans with a very high sugar content (globulins 2, 3, 11). The other proteins range from 1% to 4% carbohydrate. Globulin 1 has the highest sugar content, vicilins are intermediate, and legumins and globulin 9b have the lowest values, as shown in Table 1. The carbohydrate is covalently bound and is associated only with some of the different subunits which are present in the molecule. As far as the vicilins are concerned, the sugar appears bound to subunits which represent only a minor part of the total and the major components do not contain significant amounts of bound sugar; this is an aspect of the heterogeneity of these proteins [14]. In legumins, the carbohydrate is bound to the high molecular weight subunits.

Monosaccharides found in the different proteins are shown in Table 2. The tvpe of sugar is similar in groups of related proteins: vicilins are rich in mannose, legumins contain also galactose, glucose and glucosamine. However each protein in the group has a typical distribution. This specific sugar composition may be one of the causes for the individual identity of different vicilins and legumins. Proteins 1 and 9b contain also pentoses [7].

Table 2. Monosaccharide composition of carbohydrate in globulins, %[a]

Sugar		Vicilins			Legumins		
	1	4	5	6	8	9a	9b
Xylose	3.9	–	–	–	tr	tr	3.3
Arabinose	20.8	–	–	–	tr	tr	7.5
Galactose	61.2	–	tr	tr	2.9	20.1	31.2
Mannose	10.7	96.3	72.0	94.2	79.9	68.9	41.5
Glucose	2.8		22.9	0	6.3	1.3	13.1
N-Acetil glucosamine	0.6	3.7	5.1	5.8	10.9	9.7	3.4

[a]Data from ref. 7.

All globulins are oligomeric proteins and contain several subunits. In vicilins each subunit is formed by a single polipeptide and no interchain disulfide bridge was found, which agrees with the practical absence of cysteine in these proteins. On the other hand in legumins and in globulins 1 and 9b some subunits contain dipeptide linked protomers [14].

Separation of subunits according to molecular weight (as done by SDS-PAGE and by gel filtration in 8 M urea) or to charge (as done by isoelectric focussing (IEF)) indicates that all these proteins are microheterogeneous. This heterogeneity is far more relevant in vicilins than in legumins. For the different vicilins, the subunit compositions is too complicated to establish a molecular structure. Proposed subunit structures for the legumins and globulins 1 and 9b is given in Table 3.

Aminoacid composition

As shown in Table 4, proteins in the groups of vicilins and of legumins are relatively similar as far as their aminoacid compositions are concerned. Differences with globulins not belonging to these groups are, on the other hand, relevant. The two major vicilins, proteins 4 and 6, differ significantly only in isoleucine and tyrosine content. Proteins 5 and 7, which as subunit composition and molecular weight [7, 14] appear strictly related to 4 and 6 respectively, are also alike to those proteins as far as aminoacids are concerned. The two legumins differ for their arginine, threonine and methionine contents.

The overall aminoacid composition is therefore characteristic and constant for each protein though the molecules differ in relation to the subunits contained. This suggests that the subunits were generated by different fragmentation of a common larger parent polypeptide. The number of aminoacid residues in this possible parent polypeptide of globulin 8 and 9a and in each subunit of globulin 1 was calculated and is given in Table 5.

Nutritionally speaking, vicilins are extremely poor because of a lack of sulfur containing aminoacids and tryptophan, and low valine, lysine and threonine levels. This produces a low MEAA index. Legumins are better,

Table 3. Subunit structure of lupine globulins

	M.W (kd) of native protein	M.W. (kd) of the protomers (S-S bound)	% of total	Number of subunits
		(45.0 + 19.0)	14	
Globulin 8	187 ⇌ 372	9.0* + (38.5 + 19.0)	68	
		21.0 + (26.0 + 19.0)	18	
		(45.0 + 19.0)	5	
		6.0* + (36.5 + 19.0)	46	
Globulin 9a	250 ⇌ 437	6.0* + 21.0 + (15.5 + 19.0)	25	
		16.5 + (25.5 + 19.0	24	
Globulin 1	199	(28.8 + 16.1)		4
Globulin 9d	44	13.0 + (11.0 + 11.0)		1

*Not seen in SDS-PAGE

because of only a minor deficit in lysine, sulfur containing aminoacids and valine: indeed their MEAA index is above 80%. Peculiar is globulin 9b for its absence of tryptophan, low lysine, threonine, methionine, valine and aromatic aminoacids but very high cysteine and glutamate. This unbalanced composition yields a low MEAA value. Glutamic acid is found also in vicilins and legumins as is frequently the case with plant proteins. Globulin 1, which is also an important seed constituent, has a very balanced aminoacid composition which results in a MEAA value of 99%. The index for potential bitter taste is always below 1300, which is the critical level for beginning of bitter taste.

The total globulin extract is low in methionine and tryptophan but still has a MEAA index of 74 (Table 6). The aminoacid pattern of lupine flour is better than for total globulins, which causes a higher MEAA value. This is due to the presence of albumins, which display a more balanced aminoacid composition. It is interesting that the aminoacid composition calculated for the flour from the composition of albumins and of total globulins and their respective percent as seed protein (for total globulins from the aminoacid content in each separate fraction and the percent protein in the fraction) after adjusting to 100% total recovery, is very similar to the determined values. This confirms the values determined for the components and the recoveries obtained.

If a comparison of essential aminoacids is made with other lupine species (data are shown in Table 7), all appear to behave similarly. The best pattern is shown by *L. luteus* which also has the highest protein content. Favourable results are given also by *L. mutabilis*. Comparison with soy proteins is also favourable, both for essential aminoacids and protein yield; the soybean however appears richer in tryptophan and lysine.

In summary, the various protein groups of lupine seeds have quite different chemical nutritional value and for the whole seed this value depends on the ratio between the various proteins. This ratio can be modified by

Table 4. Aminoacid composition of seed globulins in *Lupinus albus*, moles %[a]

	Vicilins						Legumins				Essential a.a. pattern, FAO (1973)
	1	3	4	5	6	7	8	9a	9b	11	
His	4.46	2.87	1.06	1.57	1.19	1.13	1.59	1.78	1.63	2.11	–
Lys	5.43	5.29	3.82	3.52	3.28	2.89	3.61	3.30	0.96	6.83	5.4
Arg	2.90	9.34	10.01	9.64	10.79	10.50	9.03	7.67	8.80	7.24	–
Asp	11.10	9.11	13.75	12.43	12.04	13.24	10.08	10.45	10.14	6.67	–
Thr	7.80	5.15	3.48	3.25	3.01	3.12	4.28	5.37	1.57	3.85	4.0
Ser	10.47	8.90	7.33	8.55	7.18	6.40	6.19	5.24	6.64	8.42	–
Glu	8.59	18.85	21.62	23.35	22.88	25.43	22.31	21.44	38.38	10.47	–
Pro	6.50	5.78	4.78	4.48	4.84	4.40	5.04	5.05	2.39	5.66	–
Gly	6.75	8.47	6.01	7.41	7.09	5.82	6.90	7.58	3.66	11.78	–
Ala	5.91	4.75	4.41	4.17	4.08	4.23	3.91	4.27	1.28	5.51	–
Cys	2.71	0.90	tr.	0.03	0.00	0.00	1.38	1.26	7.44	0.00	3.5
Val	6.73	4.43	3.59	2.77	3.44	3.66	4.31	4.43	2.03	3.22	
Met	0.63	0.25	tr.	tr.	0.00	0.00	0.16	0.48	0.46	3.40	5.0
Ile	4.68	3.40	5.08	3.51	4.26	4.66	5.87	5.51	3.49	5.49	4.0
Leu	8.92	5.96	7.60	6.73	7.28	7.14	7.62	8.79	9.19	11.36	7.0
Tyr	2.09	2.63	3.38	3.95	5.04	4.78	3.91	3.55	1.01	3.43	5.0
Phe	3.65	2.83	4.05	3.63	3.60	2.60	3.18	3.06	0.92	4.46	
Trp	0.68	1.07	0.03	0.00	0.00	0.00	0.62	0.78	0.02	0.00	1.0
Amide N, % of total aminoacids[b]	16.70	–	23.95	–	22.63	–	–	17.83	27.75	–	
MEAA	99.5	80.8	33.2	25.9	26.4	82.8	84.0	35.1	91.5	100	
Index for potential bitter taste	1202	1088	1132	1090	1159	1135	1197	1183	856	1322	–

[a] Calculated from ref. 7
[b] Unpublished results

Table 5. Aminoacid residues per subunit

	1	8	9a
His	16	10	11
Lys	20	23	21
Arg	10	57	48
Asp	40	63	65
Thr	28	27	34
Ser	38	39	33
Glu	31	141	134
Pro	23	32	31
Gly	24	43	47
Ala	21	25	27
Cys	10	9	8
Val	24	27	28
Met	2	1	3
Ile	17	37	34
Leu	32	48	55
Tyr	8	25	22
Phe	13	20	19
Trp	2	4	5

Table 6. Determined and calculated aminoacid composition of seed fractions in *Lupinus albus*, %[a]

	Defatted flour		Total globulin extract		
	determined	calculated	determined	calculated	Albumins
His	2.13	1.90	1.76	1.85	2.06
Lys	4.36	3.26	3.90	3.46	5.33
Arg	11.06	11.07	11.81	11.42	12.40
Asp	10.76	10.47	10.55	10.81	11.79
Thr	3.86	3.23	3.08	3.26	4.89
Ser	5.22	5.05	5.35	5.24	5.23
Glu	25.07	25.68	27.40	24.84	26.24
Pro	4.06	4.04	4.38	3.92	4.01
Gly	3.96	3.46	3.68	3.55	1.97
Ala	3.04	2.69	2.69	2.49	3.57
Val	3.35	3.35	3.16	3.22	3.57
Cys	1.48	1.46	1.48	1.40	1.68
Met	0.51	0.33	0.27	0.23	1.01
Ile	4.24	4.55	4.50	4.52	3.75
Leu	7.31	7.69	7.36	7.54	7.51
Tyr	4.57	4.57	5.58	4.80	1.71
Phe	3.86	3.77	3.58	3.75	3.11
Trp	0.44	0.42	0.49	0.43	0.35
MEAA	78	–	74	–	81
Potential bitter taste index	1102	–	1147	–	1041

[a]Data from ref. 6.

Table 7. Essential aminoacid content of different species of lupine seeds and soybean, %

	Albus	Angustifolius[a]	Luteus[a]	Mutabilis[a]	Soybean[a]
Lys	4.4	5.2	6.1	6.0	6.4
Thr	3.9	3.5	4.0	4.0	3.9
Cys + Met	2.0	2.3	3.1	2.7	2.6
Val	3.4	4.0	4.3	4.0	4.8
Ile	4.2	3.8	4.6	4.7	4.5
Leu	7.3	6.6	9.0	7.4	7.7
Tyr + Phe	8.3	7.1	6.9	7.9	8.1
Trp	0.4	0.9	0.9	0.8	1.2
MEAA	78	87	95	92	96

[a]Data from ref. 10.

selection and, as we will briefly mention in what follows, by the field treatment of the plant: the data reported become therefore one of the leading criteria for the improvement of this crop. Selection must aim to modify favourably the ratio of legumins to vicilins. These globulins represent by far the major proteins in the seed and their relative amounts can be affected with a certain ease. The harvesting time is also important since legumins seem to be deposited after vicilins as shown by Danielsson [4] on *Pisum sativum.* Also field treatment may be effective in improving the ratio between the two groups, and globulins 1. Indeed it has been observed on *Lupinus angustifolius* that when soil nutrients are poor in sulfur, legumins and conglutin γ (i.e. globulin 1) decrease in the seed and nearly disappear, and are substituted by vicilins, thus changing the nutritional value of the seed proteins for man [8].

In vitro digestibility

An alternative approach to evaluating the nutritional performance of lupine proteins was to study their breakdown by mammalian digestive proteolytic enzymes. As shown in Table 8 pepsin is less effective on them than on casein and the decrease differs for the various proteins: vicilins and globulin 1 are less digested than legumins and globulin 9b. Lupine proteins, but not casein, gradually form some TCA soluble peptide independently of enzymic action, particularly if they are incubated at the low pH used for pepsin. This may denote partial dissociation of the oligomeric structure of the proteins during incubation. The interruption of covalent continuity within each constituent part of the oligomer due to the proteolytic nicking that we mentioned earlier, is likely to contribute to the lability of the molecules. Moreover, some proteolytic activity in the preparations is also likely.

Trypsin is affected to a larger extent than pepsin and the pattern differs for the various proteins (Table 9). Decreased proteolysis is evident from the very start of incubation for globulin 1 and the vicilins, but legumins and globulin 9b appear less digested than casein only after 3 hours incubation.

Table 8. Proteolysis by pepsin
TCA soluble peptides produced, determined by microbiuret, are given, net of blank, as % of initial protein

Digestion time, hrs at 37 °C	Casein	Total globulin extract	Globulin				
			1	4	6	8	9a + 9b
1	35	34	32	34	34	36	30
3	44	38	33	36	36	44	37
6	47	40	39	37	37	44	37
8	48	41	40	37	38	44	46
24	50	41	40	41	38	44	48
Neutral sugar, mg/100 mg peptide	0.3	2.3	4.3	2.4	2.1	1.5	0.8

Table 9. Proteolysis by tripsin of lupine seed globulins
TCA soluble peptides and aminoacids produced by trypsin are given, net of blanks, as % of the initial protein. Assays are respectively by the microbiuret (μbi.) and ninhydrin (nin.) methods.

Hours at 37°C	1		4		6	8	9a + 9b		Casein	
	μbi.	nin.	μbi.	nin.	μbi.	μbi.	μbi.	nin.	μbi.	nin.
0.5	4.8		4.6		9.8	19.7	15.6		11.5	
1	6.8	6.0	5.6	5.7	10.7	22.5	18.0	5.5	21.8	2.7
3	8.8	7.1	11.9	6.7	12.7	24.9	20.0	8.5	36.4	3.0
6	8.6	8.0	5.6	9.9	16.1	26.0	22.0	8.5	40.6	3.5
24	4.1	9.4	5.6	16.0	8.0	16.6	15.8	48.9	48.9	3.5
Neutral sugar mg/100 mg peptide	4.3		2.4		2.1	1.5	0.8		0.3	

Longer times make the difference more relevant, and soluble peptides formed become smaller in size and react with ninhydrin, whereas biuret reactivity is lost. This is not the case for casein. The mentioned effects are most evident for vicilins and for globulin 1 but less evident for legumins.

The different aminoacid composition of the proteins, and the presence of large amounts of aminoacids which do not favour attack by endopeptidases, like glutamate, is probably one of the causes of the modified activity of the enzymes. Moreover decreased proteolysis parallels the content in bound sugar of the globulins, particularly as far as trypsin is concerned. Also, the native conformation of the proteins is in part responsible, since the inhibition largely disappears after heat denaturation.

In conclusion, the results reported show that the poorer nutritional performance of lupine proteins depends, not only on the aminoacid composition, but also on reduced digestibility by proteolytic enzymes. By in-

dicating possible causes at the molecular level of this behaviour and elucidating the molecular characteristics of the various storage proteins of the seed, these studies clarify the chemical basis for the nutritional performance and allow a documented approach to improve performance. This can be done by genetical means; i.e., selection of varieties with more favourable protein composition, and with agronomic criteria. In this case, since the ratio of vicilins to legumins is highly sensitive to the sulfur content in the soil, by using a proper fertilizer, we may improve significantly the nutrional protein spectrum of the plant. As far as bound carbohydrate is concerned, Basha and Beevers [1] have shown that the carbohydrate component in glycoproteins from pea changes during seed ripening and thus the period of harvesting may be important to have a more favourable composition of the proteins. A further approach are technological treatments which properly denature the proteins to make them more suitable for attack by digestive mammalian proteases.

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