Amino Acid Composition of Seed Proteins of *Lupinus albus*

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The amino acid pattern of lupine flour, of separated albumins and globulins, and of isolated and purified globulin fractions was established. The essential amino acid scores and modified essential amino acid index were calculated. Albumins have a balanced distribution of amino acids but represent only 12.8% of total seed protein and therefore their nutritional contribution is limited. Globulins are 87.2% of total proteins and were divided into five major fractions. Three of these (43.4% of total globulin) lack sulfurated amino acids and are very poor in tryptophan. However one component representing 34.6% of total seed proteins and 45.0% of total globulins has a fairly good content of essential amino acids, being relatively poor only in tryptophan. This species is a promising candidate for improving protein quality in lupine.

The great success of soya as a protein source stimulated active interest in other legumes. A promising seed is lupine: it grows in conditions unfavorable to soya and its different varieties adapt to various soils and climates. Selection and modern agricultural practice increased its yields and its protein and oil content and made it worth considering economically.

In a previous report we showed that the protein content of *Lupinus albus* favorably compares with that of soya and of other legumes. Its globulins represent 87.2% of the proteins of the kernel. They can be separated in fractions which behave as single molecular species on gel filtration, ion exchange, and electrophoresis, but each contains several subunits, some of which appear in different fractions (Cerletti et al., 1978).

We have subsequently investigated the nutritional potential of these proteins, and in this article we report their amino acid composition.

EXPERIMENTAL SECTION

White lupines with an alkaloid content of 2.26% in the flour from dehulled seed (Pompei and Lucisano, 1976) were used. The pentane-treated flours were extracted twice with water at pH 5.0 to separate albumin. The residue was extracted with 1.0 M NaCl at pH 7.0; the globulins solubilized were gel filtrated on a column of Sephadex G 25 equilibrated with 50 mM phosphate buffer, pH 7.5, removing excess salts and colored low-molecular-weight compounds. They were then fractionated on a column of Whatman DE 52 cellulose equilibrated with 50 mM phosphate buffer, pH 7.5: fractions 1, 4, 5, 6, and 7_1 and 7_2 were eluted in order with the buffer alone or containing increasing concentrations of NaCl (0.1, 0.15, 0.25 M). All operations were performed at 20 °C. Details on these procedures are given in a previous paper (Cerletti et al., 1978). Fractions 7_1 and 7_2 which are partly resolved on the ion exchanger, and also by gel filtration on Ultrogel AcA 34, and which differ by only one subunit, were analyzed together as fraction 7.

The eluates were desalted by dialysis against water at pH 7.0 and freeze-dried. Aliquots of 3–5 mg of protein were hydrolyzed 24 h at 110 °C in 3 mL of 6 N hydrochloric acid in glass vials sealed in vacuo. The hydrolyzed samples were filtered with 3–4 volumes distilled water in an Uniphore N 348-1301 filtration kit, dried at 40 °C under reduced pressure, and resuspended in a proper volume of 0.2 N citrate buffer, pH 2.2.

Amino acid analysis was performed according to Spackman et al. (1958) with an automatic Beckman Multichrom B amino acid analyzer. Cysteine and cystine were determined as cysteic acid and methionine as the sulfone after oxidizing the protein according to Hirs (1967) previous to hydrolysis. Tryptophan was assayed according to Spies and Chambers (1949).

The amino acid scores for essential amino acids were calculated referring to the provisional amino acid scoring pattern (F.A.O., 1973). The modified essential amino acid index (MEAA) was calculated according to Mitchell (1954), omitting histidine.

RESULTS AND DISCUSSION

Albumins represent only 12.8% of total seed proteins and contain a large number of different molecular species; therefore their contribution to the nutritional properties is limited and they do not appear a promising candidate for improving protein quality or yield in lupine.

The globulin fractions considered in the present paper make up 94.5% of total globulins, the rest belonging to four other smaller peaks which were not analyzed. Table I gives the amino acid composition of lupine flour, of total albumins, of the isolated globulin fractions, and of the total globulin extract. Values for lupine flour and for total globulins were determined and also calculated, for the flours from the composition of albumins and of total globulins and their respective percent as seed protein, for total globulins from the amino acid content in each separate fraction and the percent globulin recovered in the fraction, adjusting this item to 100% total recovery.

Albumins and globulin fractions 1 and 7 have a distinctive amino acid composition, whereas the pattern in fractions 4, 5, and 6 is similar. These fractions indeed show striking similarities in subunit composition and in ionization behavior as evidenced by ion-exchange chromatography, electrophoresis (Cerletti et al., 1978), and electrofocusing (Cerletti and Restani, 1979).

The large content in glutamate and low lysine in fractions from 4 to 7 gives reason of their acidic character (isoelectric points of the subunits between 5.7 and 6.3) as compared to fraction 1 (isoelectric point of both subunits 7.9).

Values determined on and calculated for the flour agree within the limits of the experimental error for 14 amino acids and for the others they diverge only very slightly; values for total globulins agree for 11 amino acids and diverge significantly only for two (methionine and tryptophan). These findings indicate a fair fitting of the found amino acid content and protein distribution in each fraction.

The nutritional parameters deductible from the amino acid content are given in Table II for the total globulin extract and for fractions 1, 7, and 6, the latter representing

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Table I. Amino Acid Composition of Lupine Seed Proteins (%)[a]

	total globulin extract		globulin fractions[b]						lupine flour	
			1	4	5	6	7			
	determ.	calcd	(5.92)	(9.96)	(2.1)	(31.39)	(45.07)	albumins	determ.	calcd
Lys	3.90	3.84	6.34	4.10	3.78	3.56	3.67	5.40	4.36	4.09
His	1.76	1.93	5.53	1.21	1.79	1.37	2.05	2.09	2.13	1.80
Arg	11.81	11.12	4.04	12.80	12.35	14.00	9.62	12.57	11.06	11.91
Asp	10.55	11.17	11.81	13.43	12.17	11.96	10.01	11.95	10.76	10.73
Thr	3.08	4.12	7.43+	3.04	2.85	2.68	5.01	4.96+	3.86	3.32
Ser	5.35	5.10	8.79	5.65	6.61	5.62	4.09	5.30	5.22	5.34
Glu	27.40	23.30	10.10	23.34	25.27	25.14	23.67	26.61	25.07	27.30
Pro	4.38	4.63	5.98	4.04	4.64	4.14	4.91	4.07	4.06	4.34
Gly	3.68	4.01	4.05	3.31	4.09	3.36	4.61	2.00	3.96	3.46
Ala	2.69	3.10	4.21	2.88	2.73+	2.71	3.30	3.62	3.04	2.81
Cys	1.48	1.86	2.62	Tr	0.03	0.00	3.57	1.70	2.13+	1.51
Val	3.16	4.04	6.30	3.09	2.39	3.00	4.10	3.62	3.35+	3.22
Met	0.27	0.19	0.75	Tr	Tr	0.00	0.30	0.52	0.51	0.30
Ile	4.50	4.65	4.91	4.89	3.93	4.17	4.92	3.80	4.26	4.41
Leu	7.36	7.88	9.35	7.31	6.49	7.12	8.11	7.62	7.31	7.39
Tyr	5.58	4.92	3.02	4.49	5.27	6.80	3.95+	1.73+	4.57+	5.09
Phe	3.85	4.63	4.82	4.91	4.41	4.43	4.07	3.15	3.86	3.76
Trp	0.49	0.33	1.11	0.04	0.00	0.00	0.53	0.19	0.44	0.45

[a] Data are the average of three determinations on separate hydrolyses. Variability coefficients were less than 10% except where marked +: here they ranged between 10 and 15%. [b] Values in parentheses give the percent of each fraction in the total extract.

Table II. Essential Amino Acid Scores in Globulin Fractions Isolated from White Lupine Seeds

	total globulin extract	globulin fractions		
		1	6	7
Lys	70.91	115.27	64.73	66.73
Thr	77.00	185.75	67.00	125.25
Cys + Met	50.00	96.29	0	110.57
Val	63.20	126.00	60.00	82.00
Ile	112.50	122.75	104.25	123.00
Leu	105.14	133.57	101.71	115.86
Tyr + Phe	157.17	130.67	187.17	133.67
Trp	49.00	111.00	0	53.00
MEAA	73.43	99.53	26.72	85.66

also fractions 4 and 5 which have a similar composition and are quantitatively less important.

Essential amino acid scores and the MEAA value of the total extract are rather poor: tryptophan is limiting and second limiting are the sulfur-containing amino acids. Fraction 6 has even worse scores and lacks both tryptophan and sulfur-containing amino acids, but fraction 7 has a reasonably good composition as compared to the F.A.O. pattern, its major deficiency being tryptophan. Fraction 1 has a very good pattern, sulfur-containing amino acids being limiting, followed by tryptophan: unfortunately this fraction represents less than 10% of total globulin. It is a stimulating finding, however, that fraction 7, which includes 34.6% of total seed protein, has a better pattern than the total globulins. This offers some hope that amino acid imbalance may partly be corrected by breeding and selection.

Amino acid analyses for whole lupine seeds and for kernels are reported in the literature (Hudson et al., 1976; Hill, 1977; Boulter and Derbyshire, 1971): for *Lupinus albus* they agree fairly well with our data on lupine flour.

Assays are also reported based on fractionation procedures that, as already shown (Cerletti et al., 1978), resolve incompletely the globulins: in *Lupinus angustifolius* Blagrove and Gillespie (1975) described three globulin fractions: conglutin α, with similar electrophoretic mobility to our fraction 7, contained limited amounts of tryptophan and of sulfurated amino acids, conglutin β that moves as our fraction 6, was lacking in methionine and tryptophan and conglutin γ had a fair content of both these compounds and moved as our fraction 1. Gerritsen (1956) found that methionine and tryptophan were absent from the globulins with sedimentation constant 7.8 in *Lupinus angustifolius* and 7.4 in *Lupinus luteus* while a heavier fraction with sedimentation coefficient 11.6 in either variety contained both amino acids, which however were in order the first and the second limiting one; in our results fractions 4, 5, and 6 that lack tryptophan and methionine have a smaller molecular weight than fraction 7 that contains both these components (Cerletti et al., 1978). These observations suggest that similarities may exist in seed protein composition between different varieties of lupine.

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