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Albumin Proteins of Eight Edible Grain Legume Species: Electrophoretic Patterns and Amino Acid Composition

The albumin protein (AP) content of eight edible grain legume species varied from 8.1 to 14.1% of the meal protein. The AP contained from 9.5 to 12.4% nitrogen, 0.09 to 1.72% phosphorus, and 0.2 to 1.8% starch. Although there were differences in the amino acid composition of the species, the AP contained more tryptophan, lysine, threonine, valine, and methionine but less arginine, leucine, and phenylalanine than the globulin proteins isolated from the same legume species. The AP of mung beans, lentils, faba beans, vetch, dry beans, and peas resolved into 20–25 bands and the AP of lathyrus and chickpeas into 19 bands on sodium dodecyl sulfate–polyacrylamide gel electrophoresis with molecular weights estimated mostly between 10 000 and 100 000.

The albumin proteins (AP) are minor and the globulin proteins (GP) major proteins of legume seeds. The GP of a number of legume species have been investigated (Derbyshire et al., 1976; Barker et al., 1976; Stockman et al., 1976; Krishna et al., 1977, 1979; Utsumi and Mori, 1980; Mutschler et al., 1980). In contrast, only a few studies have been conducted on the AP of legumes (Fox et al., 1964; Grant et al., 1976; Sefa-Dedeh and Stanley, 1979; Murray, 1979). Unlike the GP, which are storage proteins, the AP are mostly enzymic or nonstorage proteins. However, recent work (Murray, 1979) showed that AP of pea cotyledons were degraded during germination and thus behaved like the GP or storage proteins.

The AP of peas, faba beans, and chickpeas were richer in sulfur amino acids and lysine than the GP (Jackson et al., 1969; Bajaj et al., 1971) and may be used to improve the methionine–cystine levels of edible grain legumes. However, this view is not shared by Boulter et al. (1973). The functional properties of protein isolates prepared from AP of peas have been reported (Grant et al., 1976).

Fox et al. (1964) reported the electrophoretic patterns of AP isolated from 17 species of legumes (mostly nonedible grain legumes). Nevertheless, most studies on AP have been conducted with peas. Thus, there is a paucity of information on the AP of other legumes, particularly the edible grain legumes which are being used increasingly in human foods. The present study reports on the level, amino acid composition, and electrophoretic patterns of AP isolated from eight species of common edible grain legumes.

MATERIALS AND METHODS

The eight species of edible grain legumes used in the study were the following: chickpeas (*Cicer arietinum* L.), line PI 239859; dry beans (*Phaseolus vulgaris*), cv. Saxa; faba beans (*Vicia faba* L. ssp. minor), cv. Diana; lentils (*Lens culinaris* Medic), type Common Chilean; peas (*Pisum sativum* L.), cv. Trapper; vetch (*Vicia sativa* L.), cv. Alger Somm; mung bean [*Vigna radiata* (L.) Wilezek], line UM MBI; Lathyrus (*Lathyrus sativus* L.), line 2R27. The legumes were grown by Dr. A. E. Slinkard of this department at the University of Saskatchewan experimental plots, Saskatoon. The legume samples were cleaned and ground in a Udy cyclone mill to pass a 1.0-mm screen, and the meal was stored at 5 °C.

The extraction and separation of AP and GP was largely based on the method of Murray (1979). Each meal was vigorously shaken at 5 °C for 10 min with 5.0% potassium sulfate (pH 7.0) in a Udy multiple shaker. The meal to solvent ratio was 1 to 20. The extract was centrifuged at 12000*g* (5 °C) for 20 min; the residue was reextracted once more as described above. The supernatants from the two extractions were combined. An aliquot was taken for total nitrogen determination to calculate salt-soluble nitrogen and the rest of the extract dialyzed at 5 °C for 36 h against several changes of deionized distilled water. The dialyzed extract was centrifuged at 12000*g* for 20 min to obtain water-soluble (AP) and water-insoluble (GP) fractions, which were freeze-dried.

The freeze-dried AP was analyzed in duplicate for total nitrogen, phosphorus, and starch, and the AP and GP were analyzed for amino acids, except methionine, as described previously (Bhatty and Slinkard, 1979). The methionine content of AP and GP was determined by gas–liquid chromatography (Finlayson and MacKenzie, 1976).

Sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis of AP was conducted according to Weber et al. (1968) at an acrylamide concentration of 10%. Molecular weight of the proteins was estimated from a low molecular weight standard (M_r range from 14 300 to 94 000; Pharmacia, Montreal) electrophoresed under identical conditions. Mobilities of the electrophoretic bands were measured relative to that of the tracking dye. The relationship between log molecular weight and relative mobility was linear.

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Table I. Meal, Salt-Soluble, Albumin, Globulin, and Residual Nitrogen Fractions of Eight Grain Legume Species

species	meal N, %	% of meal N: salt-soluble N[a]	albumin N[b]	globulin N[b]	residual N, %	recovery,[c] %	nonprotein N,[d] %
mung beans	4.6	66.5 ± 0.6	8.1	35.4	25.9	92.2	22.8
lathyrus	4.2	68.1 ± 0.9	13.0	33.3	22.8	90.7	21.3
chickpeas	4.3	71.9 ± 2.6	12.2	37.9	22.5	94.0	21.6
lentils	4.6	63.3 ± 1.4	8.1	34.3	28.8	91.9	20.7
faba beans	5.0	62.8 ± 1.8	8.6	42.0	30.0	92.2	11.6
vetch	4.9	67.3 ± 2.2	7.9	37.4	30.6	97.6	21.1
dry beans	4.3	62.8 ± 1.7	13.0	30.5	32.9	95.5	19.1
peas	3.5	62.7 ± 0.8	14.1	30.7	30.0	92.4	17.6

[a] Mean of three separate extractions. [b] Mean of two separate extractions. [c] The sum of salt-soluble and residual nitrogen fractions. [d] Calculated by difference between the sum of albumin and globulin nitrogen fractions and salt-soluble nitrogen.

Table II. Nitrogen, Phosphorus, and Starch Contents of Albumin Proteins Isolated from Eight Legume Species

species	nitrogen, %	phosphorus, %	starch, %
mung beans	9.9	0.09	1.8
lathyrus	12.0	0.09	1.7
chickpeas	10.3	0.60	0.6
lentils	10.2	0.67	0.2
faba beans	11.2	1.72	trace
vetch	12.3	1.27	trace
dry beans	9.5	0.37	0.2
peas	12.4	0.10	trace

RESULTS AND DISCUSSION

Preliminary extraction experiments with four species of the grain legumes showed that the two protein extractions described under Materials and Methods were highly reproducible and solubilized about 98% of the salt-soluble nitrogen. Under these conditions of extraction, the salt-soluble nitrogen content of the eight grain legume species varied from 62.7 to 71.9% with a mean and standard deviation of 65.7 ± 1.5%. A quarter to one-third of the meal nitrogen was not solubilized; the mean nitrogen recovery was 93.3%. The legume species contained 19.5% nonprotein nitrogen (M_r <12 000); the range in this nitrogen fraction was 11.6–22.8% (Table I).

The AP content of the eight legume species varied from 8.1 to 14.1% and the GP from 30.5 to 42.0% of the meal nitrogen. The highest level of AP was found in peas, followed by dry beans, lathyrus, and chickpeas. The other four legume species, mung beans, lentils, faba beans, and vetch, contained lower levels of AP (Table I). The AP content of these legumes, except peas, has not been reported previously. The value of AP found in peas in the present study was identical with that reported for two cultivars by Grant et al. (1976) but was considerably lower than the range of 14–42% reported by Murray (1979). It is unlikely that such a large range exists in the AP of peas or of other legumes; the discrepancies in the published data are more likely due to extraction of AP and its subsequent separation from GP. The AP may not be completely extracted, or there may be coprecipitation of AP with the GP during their separation on dialysis. Alternately, the AP may be contaminated with the GP. Thus, although inter- and intraspecies differences may exist in the AP content of legumes, these differences may be accentuated by the method of AP preparation.

The AP contained 9.5–12.4% nitrogen (59.4–77.5% protein; $N \times 6.25$) and were not pure proteins. The AP contained phosphorus (faba bean and vetch were the highest) and starch (mung bean and lathyrus 3 to 8 times higher than chickpeas, lentils, and dry beans) (Table II). The starch was present probably as a contaminant. Although globulin proteins have been reported to be glycosylated in many legumes (Pusztai and Watt, 1970; Ericson and Chrispeels, 1973; Racusen and Foote, 1977; de a Moreira and Perrone, 1977); nevertheless, carbohydrates have been reported to be present in albumin and globulin proteins of *Phaseolus vulgaris* (Satterlee et al., 1975; Marquez and Lajolo, 1981).

Table III shows the amino acid composition of both AP and GP of the eight legume species. The AP from all the legume species contained more methionine (except dry beans) and tryptophan, the first and sometimes the second limiting amino acids in legumes (Evans and Bandemer, 1967; Bhatty et al., 1976). In addition, the AP contained considerably more lysine and threonine (the first and in some cases the second limiting amino acids in cereals) than the GP. Thus, the AP and to a lesser extent GP of legumes complement cereal proteins for monogastric nutrition. Although there were differences among species, the AP also contained in most cases more valine but less arginine, leucine and phenylalanine, the remaining essential amino acids, than the GP. The methionine content of AP was 43 to 58% greater than the GP in mung bean, lathyrus, chickpeas, faba beans, vetch, and peas. In lentils, the AP contained 150% more methionine than the GP. The amino acid composition of AP of legumes except peas has not been reported, and thus it is difficult to make comparisons. However, the amino acid composition of AP and GP obtained for peas was generally similar, except for aspartic acid and phenylalanine, to that reported for AP and GP by Bajaj et al. (1971).

On $NaDodSO_4$–polyacrylamide gel electrophoresis, the AP resolved into a number of bands (Figure 1); their estimated molecular weight ranges are given in parentheses. The molecular weights estimated beyond the range of the standards (14 000–94 000) can only be approximate as they were obtained by extrapolation. The number of bands and their estimated molecular weights were as follows: mung beans, 25 (12 000–90 000); lathyrus, 19 (14 000–86 000); chickpeas, 19 (8000–78 000); lentils, 25 (10 000–110 000); faba beans, 20 (11 000–96 000); vetch, 21 (11 000–110 000); dry beans, 24 (11 000–105 000); peas, 24 (10 000–95 000). Thus, the AP of six legume species contained between 20 and 25 and of the other two legume species 19 protein bands with molecular weights mostly between 10 000 and 100 000. The band intensities varied among the species; all species contained a heavily stained protein band mostly in the middle of the gels. Similarly, AP from the remaining six species showed heavily stained bands at the origin, except for lathyrus and chickpeas. Again, comparison of electrophoretograms can only be made with those of pea AP which have been published. The number of electrophoretic bands obtained for pea AP (25) in this study was almost identical with the number of bands (24) reported

Table III. Amino Acid Composition (Grams of Amino Acid per 16 Grams of N) of Albumin Proteins (AP) and Globulin Proteins (GP) of Eight Legume Species

	mung bean		lathyrus		chickpeas		lentils		faba beans		vetch		dry beans		peas	
amino acid	AP	GP	AP	GP	AP	GP	AP	GP	AP	GP	AP	GP	AP	GP	AP	GP
tryptophan[a]	1.2	1.0	1.7	1.1	1.6	0.9	1.6	0.9	1.6	1.0	1.9	1.2	2.2	1.3	1.6	0.9
lysine[a]	9.1	7.1	8.6	6.3	9.5	6.0	9.8	6.5	9.4	6.4	9.6	6.2	6.6	7.8	8.8	7.9
histidine	2.3	3.1	2.3	2.9	2.3	3.0	2.4	2.4	3.9	2.7	3.0	2.8	2.1	3.3	2.3	2.6
arginine[a]	4.8	7.8	5.3	10.3	5.6	11.4	5.4	9.6	6.0	10.3	4.7	10.6	3.8	6.4	4.8	10.0
aspartic acid	10.5	14.2	12.1	12.7	12.5	15.2	11.8	12.7	11.5	12.7	11.4	12.7	14.1	14.7	12.0	14.0
threonine[a]	5.1	3.0	5.7	3.7	4.2	3.2	5.9	3.5	5.4	3.6	6.4	3.5	7.1	4.2	5.4	3.6
serine	4.4	6.2	5.3	5.8	4.5	6.6	5.1	6.1	5.3	6.0	5.0	5.8	7.5	4.5	5.1	6.1
glutamic acid	13.6	23.5	14.2	23.9	15.0	25.1	15.9	22.7	16.8	23.7	14.8	23.2	11.2	21.7	14.0	24.9
proline	4.0	4.4	4.6	4.8	3.9	5.0	4.2	4.3	4.9	5.1	4.4	4.9	3.4	4.0	4.5	4.5
glycine	4.7	3.5	5.0	3.9	4.1	4.1	5.4	3.7	5.8	4.2	5.7	4.1	4.5	4.1	5.6	4.1
alanine	6.5	4.1	6.1	3.9	4.1	4.4	7.0	3.6	6.7	4.1	6.2	4.1	5.3	4.0	6.1	4.2
valine[a]	4.9	5.2	5.1	4.3	3.7	4.1	5.4	4.3	4.9	4.6	5.3	4.4	6.1	5.2	4.8	4.6
methionine[a]	1.9	1.2	1.1	0.7	2.1	1.2	1.5	0.6	1.1	0.7	1.2	0.8	1.2	1.2	1.2	0.8
isoleucine[a]	3.7	4.0	4.0	3.9	4.0	4.0	3.9	4.0	3.7	4.1	3.7	4.0	4.0	5.0	3.8	4.2
leucine[a]	5.4	9.1	4.5	8.5	6.8	8.2	5.3	8.8	4.9	9.3	5.3	8.9	6.5	10.5	4.7	9.3
tyrosine	3.1	3.6	4.2	3.3	2.5	2.7	4.2	3.3	3.8	3.7	4.2	3.5	2.8	4.9	4.2	3.6
phenylalanine[a]	3.4	7.3	4.4	4.8	3.7	7.3	2.4	5.6	3.3	4.8	3.4	4.7	5.6	7.3	4.6	5.7
nitrogen recovery, %	76.3	81.3	86.3	83.3	85.4	85.3	84.8	78.8	90.6	92.2	86.9	87.6	81.3	84.4	91.3	85.1

[a] Essential amino acid.

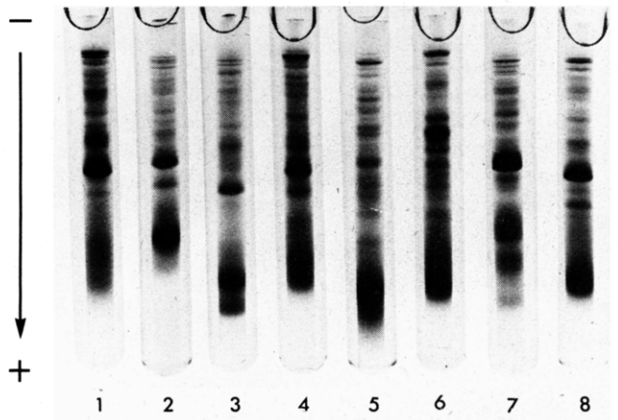

Figure 1. Electrophoretogram of albumin proteins of eight legume species. (1) Mung beans; (2) lathyrus; (3) chickpeas; (4) lentils; (5) faba beans; (6) vetch; (7) dry beans; (8) peas.

for pea albumins by Fox et al. (1964), by using a nondissociating, discontinuous buffer disc electrophoresis system unlike that in the present study. However, Grant et al. (1976) reported only 10 protein bands in pea AP using $NaDodSO_4$–polyacrylamide gel electrophoresis. Thus the AP fraction prepared by Grant et al. (1976) either was more pure or was not fully dissociated in the presence of $NaDodSO_4$.

The traditional role of AP during legume seed germination has been questioned (Murray, 1979). To elucidate and establish the biochemical function of AP during seed germination, it will be necessary to isolate and quantitate these proteins. Such an approach may also result in the isolation of a methionine or other essential amino acid rich AP under independent genetic control which may help to improve the nutritional quality of edible grain legumes. A methionine- and cystine-rich polypeptide has been identified in peanut proteins (Basha and Pancholy, 1981).

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