Phytochemistry, Vol. 25, No. 3, pp. 641–644, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00

PROTEIN SUBUNITS AND AMINO ACID COMPOSITION OF WILD LENTIL

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(*Revised received* 15 *July* 1985)

Key Word Index—*Lens*; leguminosae wild lentil species; protein subunits; albumin proteins; globulin proteins; protein and free amino acid composition.

Abstract—Three wild species of lentil, *Lens orientalis*, *L. ervoides* and *L. nigricans* were investigated for protein subunits of the albumin protein fraction (APF), globulin protein fraction (GPF) and for protein and free amino acid composition. The APF and GPF formed 12.7–16.8% and 34.7–49.0%, respectively, of the meal nitrogen. SDS-PAGE showed APF to contain 15 to 20 major and a similar number of minor protein subunits ranging in M_r at least from 14 400 to 94 000. The GPF was also heterogenous and contained some subunits having M_r similar to APF subunits but none < 15 000. The three wild lentil species were distinguishable by their protein subunit composition. The protein amino acid composition of the wild species was identical and similar to that of the cultivated lentil. The wild species, like the cultivated species (*L. culinaris*), contained major amounts of free arginine, glutamic and aspartic acids, serine and a number of unidentified amino acids. *L. orientalis*, *L. nigricans* and the cultivated lentil contained two acidic and two basic unidentified amino acids. However, *L. ervoides* was distinctly different in that it contained only the two acidic plus one neutral unidentified amino acid, but none of the two basic unidentified amino acids.

INTRODUCTION

The cultivated lentil (*Lens culinaris* Medik) is an important source of dietary protein in the developing countries. Improvements in protein content and amino acid composition of cultivated lentil will enhance its nutritional quality. Such an improvement is largely dependent on the availability of genetic variability within the cultivated lentil species and secondly within the exotic or wild species of lentil which are crossable with the cultivated lentil.

Four wild species of lentil have been reported [1]. The cultivated lentil has been investigated for biochemical and nutritional properties [2–8]. No such studies have been reported for the wild species which have been investigated mostly from the standpoint of cytology and cytogenetics [9]. The objectives of the present study were to determine if there were differences in protein subunits of the two major protein fractions of lentil (albumins and globulins) and amino acid composition among the wild species, and in free amino acids between the cultivated lentil and the three wild lentil species.

RESULTS AND DISCUSSION

The three wild lentil species contained 24.2–26.2% protein (N × 6.25), similar to the mean protein content of a large number of cultivated lentil samples grown under widely varying environmental conditions [8]. The solvent system used for the extraction of albumin and globulin protein fractions solubilized 67.1–78.4% of the meal nitrogen. Further extractions solubilized only little additional meal nitrogen. The soluble nitrogen fraction contained 18.9–22.9% albumin protein nitrogen and 51.7–62.5% globulin protein nitrogen. The remaining nitrogen (16.6–29.4%) diffused out on dialysis and was analogous to non-protein nitrogen with M_r < 3500. The values for the albumin (12.7–16.8%) and globulin (34.7–49.0%) protein nitrogen fractions were lowered when these were expressed as percent of the meal nitrogen, as 21.6–32.9% of the meal nitrogen was not solubilized. The yields of the albumin and globulin protein fractions from the wild lentil species were greater than from the cultivated lentil [14]. However, *L. nigricans* was lower in both the albumin and globulin protein nitrogen fractions, expressed either as percent of soluble or meal nitrogen, than the other two species. Conversely, *L. nigricans* contained more non-protein nitrogen than *L. orientalis* and *L. ervoides*. The implications of this difference were not clear, but may relate to a reduced ability of this species to convert nitrate nitrogen to protein nitrogen through the nitrate reduction mechanism. However, lentil containing more albumin protein nitrogen may be nutritionally superior, as this fraction contains more essential amino acids than the globulin protein fraction [14].

The three lentil meals (Fig. 1: tracks 1–3) contained 15–20 major and an equal number of minor protein subunits ranging in M_r at least from 14 400 to 94 000. The albumin protein fraction (APF) of the three species (tracks 4, 5, 6) were equally heterogeneous. The globulin protein fraction (GPF; tracks 7, 8, 9) contained some subunits which had M_r similar to those present in the APF, but none with M_r < 14 400. These smaller M_r subunits were present only in the APF, though some were present in the meal protein as well. The SDS-PAGE patterns showed heterogeneity of the wild lentil proteins like those of the cultivated lentil reported previously [14]. Proteins of many legume species have been investigated and reported to contain subunits ranging in M_r from 20 000 to 63 000 [15, 16]. The legume protein, legumin (11 S) alone is hexameric containing six subunit pairs (M_r

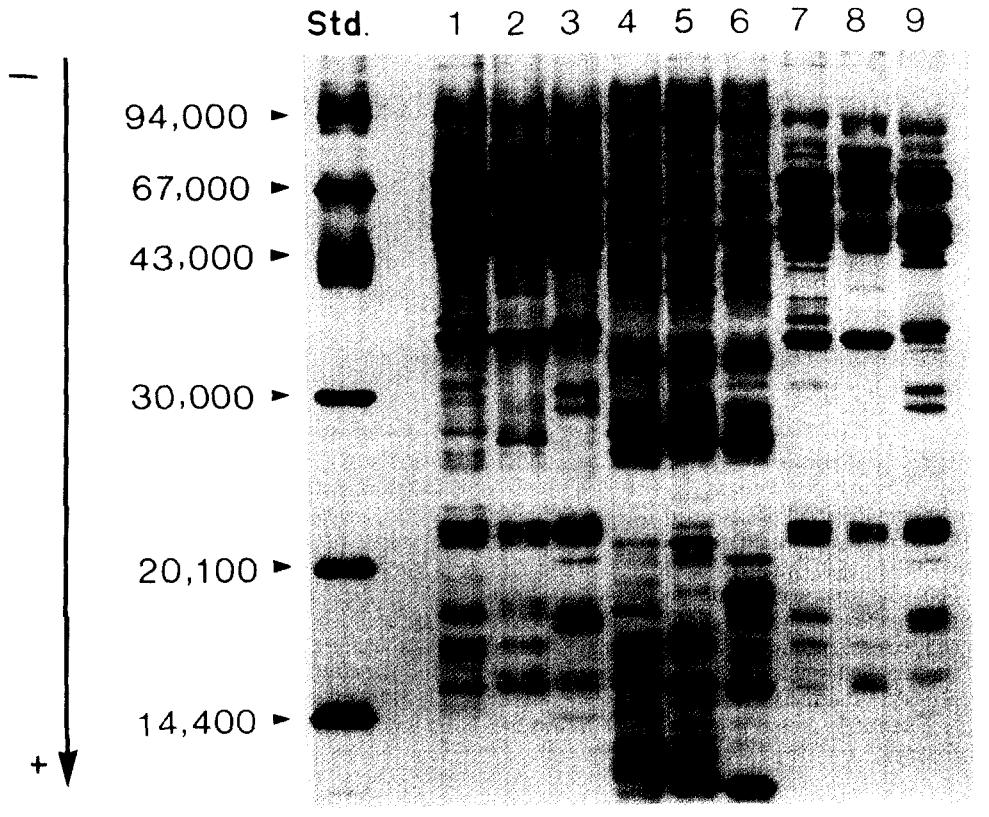

Fig. 1. SDS-PAGE of meal, albumin and globulin protein fractions of three wild species of lentil. Std. Pharmacia low M_r standard; tracks 1, 2, 3 meals; 4, 5, 6 albumin protein fractions; 7, 8, 9 globulin protein fractions of *L. orientalis*, *L. ervoides* and *L. nigricans*, respectively.

20 000 to 40 000) linked by disulphide bonds [17]. Another storage protein of legumes, vicilin (7 S) is also heterogeneous and may contain many subunits [18]. However, the major proteins of cultivated or wild lentil have not been investigated in detail though they have been reported to contain 2, 7 and 11 S proteins [19].

A close examination of the electrophoregram of APF (tracks 4, 5, 6) and of GPF (tracks 7, 8, 9) suggested interspecific differences in the protein subunits. The APF of *L. orientalis* (track 4) contained a major subunit at an *M* of 0.22 (M_r *ca* 55 000) which was not present in the APF of the other two species. Another subunit (*M* 0.26; M_r *ca* 43 000) was present only in *L. ervoides* (track 5). There were other differences as well among the three species in smaller M_r (*ca* 14 400 to 20 100) subunits, some of which gave different staining intensities.

Similarly, there were major and minor differences in the subunit composition of the GPF (tracks 7, 8, 9). *Lens ervoides* (track 8) contained a major protein subunit (*M* 0.13; M_r *ca* 70 000) which was absent from *L. nigricans* (track 9) and present only as a minor band in *L. orientalis* (track 7). Both *L. orientalis* and *L. ervoides* contained heavily stained bands at an *M* value of 0.20. A band of similar *M* appeared to be absent in *L. nigricans*. Further differences among the GPF of the three species were noticed at *M* values of 0.48 (M_r *ca* 30 000), 0.68 (M_r *ca* 20 000), 0.79, 0.85 and 0.89 (M_r *ca* 14 400 to 20 000).

The amino acid composition of the three species was almost identical (Table 1) and generally similar to that of the cultivated lentil [4]. Each wild species contained about a quarter of the total amino acids as aspartic and glutamic acids, followed by leucine, glycine, alanine, serine and lysine in that order. The wild species were deficient in methionine, cystine and tryptophan like the cultivated lentil. However, the free amino acid composition of the three species was quite variable. Arginine, aspartic acid, serine and glutamic acid were the major free, non-protein amino acids. *Lens nigricans* contained three times more arginine, but only about half the aspartic acid of the other two species. This species also contained the lowest levels of serine. Glutamic acid was the major free amino acid in *L. ervoides*.

The free amino acid composition of the wild lentils was compared with that of three cultivars of the cultivated lentil (Table 1). Arginine, aspartic acid, threonine, serine and glutamic acid were again the major amino acids. The free amino acids of lentil, like those of other legumes, may vary considerably with growing conditions. However, the presence of free arginine, aspartic and glutamic acids and threonine and serine in major concentrations, both in wild and cultivated lentil species, suggested that these amino acids acted as nitrogen reservoirs during protein synthesis. The amides glutamine and asparagine and free arginine are usually the major transport compounds present in plants [20]; threonine is the precursor for synthesis of isoleucine [21].

In addition to free protein amino acids, the wild and cultivated lentil species contained a number of unidenti-

Table 1. Protein and free amino acid composition (mol %) of wild and cultivated species of lentil

Amino acid	Protein amino acids Wild			Free amino acids Wild			Cultivated		
	L. orientalis	*L. ervoides*	*L. nigricans*	*L. orientalis*	*L. ervoides*	*L. nigricans*	*L. culinaris* Medik Laird*	Chilean*	Eston*
Tryptophan	0.57	0.66	0.60	—	—	—	—	—	—
Lysine	6.76	6.74	6.65	—	—	—	0.83	0.64	0.00
Histidine	2.45	2.34	2.31	1.76	—	0.95	2.15	2.10	2.68
Arginine	6.19	6.29	6.61	17.57	16.15	46.28	12.78	16.55	14.55
Aspartic acid	12.94	13.16	13.82	22.41	25.13	10.70	24.20	21.00	22.84
Threonine	4.23	4.37	4.18	3.27	6.50	1.13	7.92	5.71	5.23
Serine	6.87	6.72	6.97	27.53	18.93	15.39	17.48	13.27	20.41
Glutamic acid	12.61	12.64	12.71	20.42	31.75	18.91	23.80	27.92	23.94
Proline	4.75	4.73	4.44	—	—	—	—	—	—
Glycine	7.68	7.63	7.39	1.57	1.56	1.84	2.59	2.60	2.36
Alanine	6.86	6.88	6.97	4.65	1.80	4.67	5.29	5.92	5.36
Cystine	1.08	1.11	0.99	—	—	—	—	—	—
Valine	5.98	5.94	6.03	3.38	1.61	1.07	1.39	2.00	1.13
Methionine	0.79	0.89	0.83	—	—	—	—	—	—
Isoleucine	4.69	4.63	4.79	—	—	—	—	—	—
Leucine	8.11	8.00	8.31	—	—	—	—	—	—
Tyrosine	2.43	2.40	2.41	—	—	—	0.56	0.68	0.50
Phenylalanine	4.98	4.85	4.31	1.01	1.04	0.65	0.99	1.60	0.98

NH_3 excluded for the calculation of molar ratios. The standard error of the mean varied from 0.01 to 0.03 mol % for protein amino acids and from 0.05 to 2.33 mol % for free amino acids.

*Licensed cultivars of lentil in Canada.

fied amino acids. The concentrations of the unknown amino acids were greater in the cultivated lentil (three cultivars investigated) than in the wild lentil. This difference may partly be due to different growing conditions of the wild and cultivated species. The wild species were grown in hydroponics for rapid seed increase. The three cultivars of the cultivated species were grown under field conditions. Nevertheless, the unidentified amino acids were consistently present in the wild and cultivated lentil species. Two of the unidentified amino acids were acidic and the other two basic. The first unidentified amino acid eluted immediately from the column and the second between aspartic acid and threonine. Of the two basic, unidentified amino acids, one eluted between histidine and lysine and the second shortly before arginine. Sulser and Stute [22] and Sulser and Sager [23] have reported the presence of three free basic amino acids in lentil in addition to free lysine (very low), histidine and arginine. The major free basic amino acid was γ-hydroxyarginine, the minor amino acids were γ-hydroxyornithine and homoarginine. γ-Hydroxyarginine (peak before arginine) was most likely present in the cultivated lentil and in two of the wild species (*L. orientalis* and *L. nigricans*). *Lens ervoides* did not contain this amino acid nor the one which eluted between histidine and arginine. However, it contained a neutral amino acid eluted between alanine and valine which was absent in both the other wild or the cultivated lentil. Thus, *L. ervoides* was unique in containing one neutral and two unidentified acidic amino acids, and none of the two basic amino acids present in the other species. This may be a distinguishing feature of this lentil species. Experiments are in progress to identify the unknown amino acids detected in the lentil species. Their physiological and nutritional significance, if any, is not known.

EXPERIMENTAL

Three species of wild lentil, *Lens orientalis* (Boiss.), *L. ervoides* (Brign.) and *L. nigricans* (M. Bieb.) were supplied by Dr. A. E. Slinkard of this Department. *Lens orientalis* and *L. ervoides* were originally obtained from G. Ladizinsky, Hebrew University, Rehovot, Israel, and *L. nigricans* from F. Muehlbauer, University of Washington, Pullman, USA. The former two species had originated in Israel, the latter in Rodez, France. Each species was multiplied at Saskatoon by growing in hydroponics using a modified Hoagland nutrient solution [10].

The lentil seed was ground through a Udy cyclotec mill to pass 1 mm screen and the meals stored at 5°. Total nitrogen content of the meals was determined by the official method [11]. Free amino acids were extracted from 1 g meal by shaking for 1 hr in a wrist-arm shaker with 40 ml of 70% EtOH. The extract was centrifuged at 10 000 *g* for 20 min and the supernatant fraction dried by rotary evaporation. The residue was dissolved in the Beckman dilution buffer (pH 2.2). For protein amino acid analysis, unextracted meals were hydrolysed and prepared for amino acid analysis including cystine, methionine and tryptophan as described previously [2]. All amino acids were determined with a Beckman 121 C amino acid analyser. (Data in Table 1 are means of duplicate determinations.)

Albumin and globulin protein fractions were extracted at 5° from each meal by shaking vigorously for 15 min in a Udy shaker with 20 vols of 50 mM Tris–HCl buffer (pH 8.1) containing 0.5 M NaCl, 5 mM monothioglycerol, 2 mM EDTA-Na_2 salt and 5 mM of *p*-chloromercuriphenylsulphonic acid. The extract was centrifuged at low speed (5000 *g*; 10 min), the residue re-extracted

twice again as described above. The three supernatant fractions were combined, an aliquot was taken for the determination of total nitrogen [11] to calculate soluble nitrogen and the rest dialysed in a spectropore dialysis tubing (M_r cut off 3500) against H_2O for 48 hr with three changes of H_2O daily. The non-diffusible fraction was centrifuged at 15 000 g for 20 min to separate the albumin (water-soluble) and the globulin (water-insoluble) protein fractions, which were freeze dried.

SDS-polyacrylamide slab gel electrophoresis of meal, albumin and globulin proteins was performed using a discontinuous buffer system [12] with the following exceptions: separation gel, 15% (acrylamide: bisacrylamide ratio 37.5 : 1); stacking gel, 4.5%; 1 mM EDTA-Na_2 was added to all gels and running buffers. The sample preparation, gel staining and destaining (5 hr by shaking) were according to ref. [13]. Electrophoresis was run for about 22 hr at a voltage of 10 mM. The M_r of the protein subunits was estimated from Pharmacia standard (M_r range 14 400–94 000) electrophoresed under identical conditions. The apparent relative mobility (M) was expressed as the ratio of the distance (mm) travelled by the protein to that of total gel length (100 mm).

Acknowledgements—H. Braitenbach conducted the amino acid analysis of the samples, and G. Pocha provided technical assistance.

REFERENCES

1. Goshen, D., Ladizinsky, G. and Muehlbauer, F. J. (1982) *Euphytica* **31**, 49.
2. Bhatty, R. S., Slinkard, A. E. and Sosulski, F. W. (1976) *Can. J. Plant Sci.* **56**, 787.
3. Terrago, J. F. and Gregorio, N. (1976) *Plant Physiol.* **58**, 618.
4. Bhatty, R. S. and Slinkard, A. E. (1979) *Can. Inst. Food Sci. Technol. J.* **12**, 88.
5. Sosulski, F. W., Elkowics, L. and Reichert, R. D. (1982) *J. Food Sci.* **47**, 498.
6. Bhatty, R. S., Nielsen, M. A. and Slinkard, A. E. (1983) *Can. Inst. Food Sci. Technol. J.* **16**, 104.
7. Bhatty, R. S. and Christison, G. I. (1984) *Qual. Plant Foods Hum. Nutr.* **34**, 41.
8. Bhatty, R. S. (1984) *J. Agric. Food Chem.* **32**, 1161.
9. Slinkard, A. E. (1985) *Lentil Experimental News Service (LENS)* (in press).
10. Shia, G. Y.-H. (1976) Ph.D. thesis, University of Saskatchewan, Saskatoon, Sask., Canada.
11. Association of Official Analytical Chemists (1980) *Methods of Analysis*, 3rd edn. The Association, Washington, DC.
12. Laemmli, U. K. (1970) *Nature* **227**, 60.
13. Brinegar, A. C. and Peterson, D. M. (1982) *Arch. Biochem. Biophys.* **219**, 71.
14. Bhatty, R. S. (1982) *J. Agric. Food Chem.* **30**, 620.
15. Derbyshire, E., Wright, D. J. and Boulter, D. (1976) *Phytochemistry* **15**, 3.
16. Sathe, S. K., Deshpande, S. S. and Salunkhe, D. K. (1984) *CRC Crit. Rev. Food Sci. Nutr.* **20**, 1.
17. Croy, R. R. D., Derbyshire, E., Krishna, T. G. and Boulter, D. (1979) *New Phytol.* **83**, 29.
18. Croy, R. R. D., Gatehouse, J. A., Evans, E. D. and Boulter, D. (1980) *Planta* **148**, 57.
19. Chakraborty, P., Sosulski, F. and Bose, A. (1979) *J. Sci. Food Agric.* **30**, 766.
20. Lea, P. J. and Miflin, B. J. (1980) in *The Biochemistry of Plants—A Comprehensive Treatise* (Miflin, B. J., ed.) Vol. 5, p. 570. Academic Press, New York.
21. Bryan, J. K. (1980) in *The Biochemistry of Plants—A Comprehensive Treatise* (Miflin, B. J., ed.) Vol. 5, p. 407. Academic Press, New York.
22. Sulser, H. and Stute, R. (1974) *Lebensm-Wiss. Technol.* 7, 322.
23. Sulser, H. and Sager, F. (1976) *Specialia* **15**, 423.