Cultivar Differences in Proteins of Oriental Mustard (*Brassica juncea* [L.] Coss.)[1,2]

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ABSTRACT

The variability in the amino acid composition of the lipid-free meals from the seeds of five cultivars of *Brassica juncea*, Burgonde, Ekla, Lethbridge 22A, Primus and Stoke has been studied. Arginine, tyrosine and histidine were the more variable amino acids. The meals were extracted with 0.5 M NaCl and 64-68% of the meal nitrogen was released. The extracts were fractionated by molecular exclusion chromatography to produce (a) purified preparations of the major storage protein, a globulin having a sedimentation coefficient of 12S; and (b) a fraction containing low molecular weight proteins. No difference in the relative proportions of these two components was observed. The amino acid composition of the low molecular weight protein fraction varied more than that of the 12S protein.

Brassica juncea (L.) Coss, Oriental or Brown mustard, is grown primarily for its use as a condiment but has considerable potential as a source of oil (1). The main barrier to it is the presence of the glucosinolate sinigrin. Although a process has been developed for producing a bland product from the meal (2) and the amino acid composition compares favorably with that of soybean (3), little use has been made of mustard seed meal as a source of protein.

Some of the proteins from seeds of the species *B. campestris* and *B. napus* have been isolated and characterized (4-6). The saline soluble proteins were fractionated by molecular exclusion chromatography into two fractions. One fraction consisted almost entirely of the major storage protein, a large molecular weight globulin having a sedimentation coefficient of ca. 12S, and the other fraction consisted of low molecular weight basic proteins. Varietal differences in the composition of the 12S protein have been examined in three *B. campestris* and three *B. napus* cultivars or selections (7). Furthermore the seed protein amino acid compositions resulting from crosses between two *B. campestris* cultivars suggested that the amino acid composition of seed storage proteins may be changed by intercultivar crossing (8). There is therefore some potential for improving the amino acid composition of the meals derived from seeds of *Brassica* species, not only by intercultivar crossing but also by interspecific crossing. This "requires considerably more investigation of the stability of the phenotypes produced" (8), but also requires more information on the variation in the composition of the proteins from seeds of *Brassica* species other than *B. campestris* and *B. napus*.

The characterization of the 12S protein and the major component of the low molecular weight protein fraction from *B. juncea* seeds has been reported (9), but only one cultivar was examined. The present report describes the variations in the amino acid composition of the meal, the 12S protein and the total low molecular weight protein fraction from the seeds of five cultivars of *B. juncea*.

[1]One of six papers presented in the symposium "Rapeseed Marketing and Breeding," AOCS Meeting, Ottawa, September 1972.

[2]NRCC No. 13392.

TABLE I

Nitrogen, Lipid and Protein Content of Seeds, Meals and 0.5 M NaCl Extracts from Five *B. juncea* Cultivars

	Seed		Meal	Extract	
Cultivar	N[a]	Lipid[a]	N[a]	N[b]	Protein[c]
Burgonde	4.3	33.8	6.5	68.3	26.6
Ekla	4.6	31.8	6.8	68.3	29.3
L22A	4.9	34.9	7.5	63.9	29.5
Primus	4.3	37.1	6.8	65.6	25.3
Stoke	4.2	40.9	7.1	64.9	23.3

[a]Wt %.
[b]% of meal N.
[c]Wt % of meal.

EXPERIMENTAL PROCEDURES

Materials

The cultivars studied were Burgonde, Ekla, Lethbridge 22A (L22A), Primus and Stoke. The L22A and Stoke cultivars were yellow-seeded, the remainder brown-seeded. The seed came from a replicated field trial at Saskatoon except for the L22A cultivar, which came from southern Alberta.

Extraction and Purification of Proteins

The lipid-free meals and protein extracts were prepared as described previously (9), with the exception that 0.5 M NaCl solution (20 ml/g meal) was used to extract the protein. The purification of the 12S protein by chromatography on Sephadex G100 and Sephadex G200 (superfine

TABLE II

Fatty Acid Composition of Oils from Seeds of Five *B. juncea* Cultivars (%)[a]

	Carbon number and degree of saturation					
Cultivar	16:0	18:1	18:2	18:3	20:1	22:1
Burgonde	3.5	22.4	24.4	13.7	12.1	19.8
Ekla	3.3	19.5	22.1	14.7	12.3	23.7
L22A	3.2	20.1	23.7	14.1	11.7	23.0
Primus	3.2	21.7	23.0	13.3	12.3	22.9
Stoke	3.2	23.8	23.0	12.5	11.6	21.7
Coefficient of variation[b]	4.0	8.0	3.7	6.0	3.0	6.9

[a]Fatty acids, each constituting less than 2% of the total, have been omitted.
[b]Expressed as a percentage of the data above.

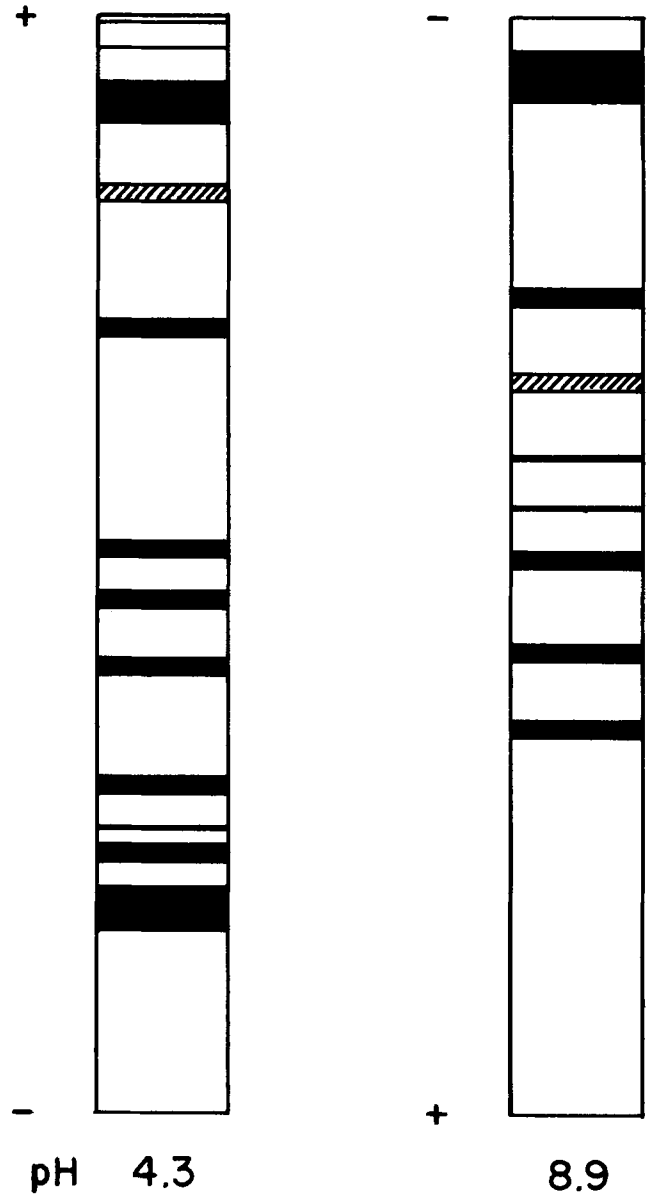

FIG. 1. Protein patterns obtained by polyacrylamide gel electrophoresis of 0.5 M NaCl extracts of seeds of *B. juncea* cv. Burgonde.

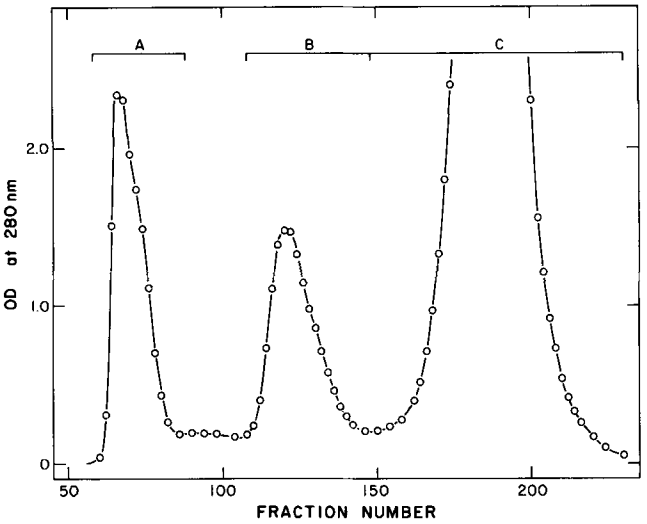

FIG. 2. Elution profile obtained by G100 chromatography of 0.5 M NaCl extract of oil-free meal from seeds of *B. juncea* cv. Burgonde.

grade) was also previously described (9). The fraction containing the low molecular weight proteins (fraction B from G100 column) was dialyzed against distilled water in a DOW Hollow Fiber Dialyzer-Ultrafilter and freeze-dried. Fraction C, which contained less than 5% of the meal nitrogen, was not examined.

Analytical Methods

Nitrogen was determined by the micro-Kjeldahl method and protein by the method of Itzhaki and Gill (10). Total seed lipid was determined by NMR spectroscopy (11). Fatty acid analyses were determined by gas liquid chromatography (GLC) using an ethylene glycol succinate column. Polyacrylamide gel electrophoresis of the extracts was performed at pH 4.3 as previously described (9) and at pH 8.9 by the method of Davis (12).

Amino acid analysis was performed by the method of Moss et al. (13). Hydrolysis was performed under the following conditions: 105 C, 22 hr, 5.7 NHCl, sealed tube. No correction for hydrolytic losses was applied.

RESULTS AND DISCUSSION

The properties of the seeds, meals and extracts are

TABLE III

Amino Acid Analyses of Meals from Five *B. juncea* Cultivars[a] (mg/g N)

Need heading	Burgonde	Ekla	L22A	Primus	Stoke	Coefficient of variation
Arg	255	415	386	284	250	24
Tyr	95	147	135	125	97	19
His	184	183	147	210	144	16
Glu	1200	1116	975	972	978	9.9
Ser	186	232	220	210	188	9.6
Met	30	37	38	35	38	9.4
NH_3[b]	138	156	158	170	171	8.4
Pro	411	373	365	359	332	7.7
Lys	333	295	308	288	274	7.4
Leu	410	363	367	357	340	7.0
Asp	418	374	369	364	349	6.9
Ile	226	210	224	195	200	6.5
Val	269	245	272	241	277	6.1
Gly	245	269	238	265	241	5.5
Phe	252	243	229	228	219	5.5
Thr	205	202	213	193	185	5.4
Ala	222	250	219	213	230	5.2
N[c]	81	86	81	81	76	

[a]Each value is mean of at least three analyses on each of two separate hydrolysates.
[b]Determined from analyses performed by ion exchange chromatography.
[c]% of meal N recovered.

TABLE IV

Amino Acid Composition of 12S Protein from Seeds of Five *B. juncea* Cultivars (mol%)[a]

Need heading	Burgonde	Ekla	L22A	Primus	Stoke	Coefficient of variation[b]
Met	0.16	0.22	0.19	0.16	0.16	15.0
His	1.68	1.80	1.56	1.70	1.42	9.0
Tyr	1.41	1.49	1.41	1.23	1.49	7.5
Arg	4.37	3.92	4.11	3.74	4.13	5.9
Val	5.07	5.28	5.62	5.55	5.16	4.5
Ser	4.23	4.24	4.06	4.49	4.16	3.8
Thr	3.20	3.34	3.53	3.36	3.37	3.5
Ile	3.86	4.03	4.01	4.21	4.00	3.0
Asp	7.98	8.18	8.35	8.51	8.53	2.8
Phe	3.25	3.29	3.47	3.33	3.42	2.7
Lys	2.37	2.44	2.31	2.30	2.36	2.4
Leu	7.06	7.10	6.91	6.72	6.95	2.1
Gly	7.66	7.28	7.44	7.55	7.64	2.1
Ala	5.28	5.33	5.13	5.25	5.32	1.5
Glu	14.6	14.4	14.4	14.6	14.3	1.0
Pro	4.90	4.83	4.86	4.90	4.94	1.0
NH_3	22.9	22.5	22.6	22.4	22.6	1.0
N in sample, %	16	16.5	16.5	16.7	16.6	
N recovered, %	90	87	91	88	88	

[a]Data have been normalized by being expressed as mol%.

[b]Coefficient of variation is expressed as a percentage of the data presented in the other columns.

shown in Table I. The seed nitrogen content varied from 4.2 to 4.9% and the lipid content from 31.8 to 40.9%. The oil content of the L22A cultivar is lower than the expected value of ca. 40%. No explanation can be offered. The two yellow-seeded cultivars, L22A and Stoke, had the largest meal nitrogen content, although because of the variation in oil content this was reflected in the total seed nitrogen content only in L22A. Despite the differences in the meal nitrogen, the amounts of nitrogen extracted varied only from 44 to 48 mg per gram of meal. Thus, although the L22A seeds contained 10 mg N per gram of meal more than seeds of the Burgonde cultivar, a large proportion of this nitrogen was not in a saline soluble form. This also applied to the seeds of the Stoke cultivar, from which less nitrogen was recovered than from the Ekla seeds. The amount of protein extracted varied from 23 to 30% of the seed weight and the ratio of saline extractable protein to seed nitrogen varied from 5.5 (Stoke) to 6.4 (Ekla). The application of a constant factor to convert seed nitrogen to saline soluble protein could thus lead to considerable error.

The fatty acid composition of the oils (Table II) was within the range of other published values for *B. juncea* seeds (14). Oleic acid was the most variable of the fatty acids present in substantial amounts.

Examination of the extracts by polyacrylamide gel electrophoresis did not detect any differences in the numbers and electrophoretic properties of the major proteins present. A typical example is shown in Figure 1. This observation does not eliminate the possibility of variation in the amounts of these proteins present in very minor amounts.

TABLE V

Difference Indices[a] Calculated from Composition of 12S Proteins Isolated from Seeds of Five *B. juncea* Cultivars

Cultivar	Stoke	Primus	L22A	Ekla
Burgonde	1.5	2.2	2.0	2.1
Ekla	1.2	1.8	1.7	
L22A	1.2	1.6		
Primus	1.8			

[a]A difference index is defined as 50 times sum of absolute values of differences in fractional content of each amino acid. Values for ammonia were excluded from calculation.

The elution profiles obtained by Sephadex G100 chromatography were all similar, and no significant differences in the relative proportions of the three fractions obtained were observed. The relative proportions of the fractions were similar to those previously published (9). A typical example is shown in Figure 2.

The amino acid compositions of the meals are shown in Table III, in order of decreasing variability. The most variable of these amino acids essential to humans was methionine, but it was present in the smallest proportion and was also the one with the largest error in measurement (±5% vs. ±3% for all the other amino acids). The other essential amino acids varied within a narrow range of ±7% for lysine to ±5% for threonine. Histidine and arginine, which are essential for young chicks, were considerably more variable. The amount of nitrogen recovered ranged from 76 to 86% of the meal nitrogen. When the recovered amino acids are expressed as a weight per cent of the meal the results are as follows: Burgonde 31.6, Ekla 34.7, L22A 36.5, Primus 32.3 and Stoke 32.0. Based on the amino acid recovery from purified protein samples (Tables IV and VI) it was assumed that the recovery of amino acids from the meals was of the order of 90%. After applying this correction, the factors to convert meal nitrogen to meal protein are then Burgonde 5.4, Ekla 5.7, L22A 5.4, Primus 5.3 and Stoke 5.0. The values for Burgonde, L22A and Primus cultivars are in good agreement with the value of 5.4 determined by Tkachuk for *B. hirta* (15). The values for the Ekla and Stoke cultivars indicate that there may be substantial cultivar variation in the ratio of meal nitrogen to meal protein.

The amino acid compositions of the 12S proteins are shown in Table IV in order of decreasing variability. Except for methionine, the variations in the amount of each amino acid is considerably less than that in the meal, and only those amino acids having a coefficient of variation greater than 6% can be considered to show variation exceeding analytical error. Methionine, valine, threonine and isoleucine are the only amino acids essential to humans showing significant variations but, as observed for the meals, histidine and arginine show considerable variation.

The relationship between the compositions of the various 12S proteins can be examined by multivariate statistical analysis, such as the method of Metzger et al. (16). This relationship is expressed by a difference index,

TABLE VI

Amino Acid Composition of B Fraction
Isolated from Seeds of Five *B. juncea* Cultivars (mol %)

Need heading	Burgonde	Ekla	L22A	Primus	Stoke	Coefficient of variation
Met	0.33	0.28	0.58	0.23	0.40	37.0
Tyr	0.91	0.71	0.84	0.64	0.74	13.9
Ser	3.63	4.07	4.67	4.70	4.24	10.5
Asp	2.38	2.16	2.70	2.48	2.09	10.4
Leu	6.12	6.36	5.83	5.03	5.37	9.5
Pro	9.10	8.56	8.74	7.95	7.11	9.4
Thr	2.70	2.93	3.12	2.91	2.46	8.9
His	2.52	2.83	2.99	3.07	3.18	8.8
Lys	4.81	4.74	5.36	4.22	4.92	8.5
Phe	2.76	2.44	2.41	2.20	2.36	8.4
Gly	5.38	6.43	6.42	5.61	5.83	8.0
NH_3	21.7	22.0	20.7	24.7	24.6	7.9
Arg	4.78	5.01	4.68	5.38	4.73	5.9
Val	3.90	3.47	3.58	3.65	3.89	5.2
Glu	21.4	20.3	19.32	19.3	20.4	4.4
Ile	2.96	2.91	3.21	2.97	2.91	4.2
Ala	4.60	4.81	4.87	4.95	4.75	2.8

which is defined as 50 times the sum of the absolute values of the differences in the fractional content of each amino acid in a protein pair. The smaller the difference index, the more closely related are the two proteins. The compositions of the 12S proteins isolated from seeds of several *Brassica* species and from seeds of several cultivars of *B. napus* and *B. campestris* have been examined by this method (9). Difference indices obtained by interspecific comparisons ranged from 0.8 to 4.7. The data presented in Table III present an opportunity to examine the relationship between the compositions of the 12S proteins isolated from seeds of *B. juncea* cultivars. The difference indices are shown in Table V and vary from 1.2 to 2.2. These difference indices are clearly smaller than those obtained when comparing 12S proteins isolated from seeds of different species. Thus the taxonomic and genetic relationships among the *Brassica* species and cultivars are reflected in the relationship between the compositions of the respective storage proteins.

The amino acid composition of the low molecular weight protein fraction is shown in Table VI. This composition was considerably more variable than that of the 12S protein. No significance can be attached to the slightly greater variation for histidine in the 12S protein than in the B fraction. With methionine, serine, aspartic acid, leucine, proline, threonine, lysine, phenylalanine and glycine, the composition of the B fraction varies more than that of the whole meal. Therefore a disproportionate amount of the variation of these amino acids in the meal can be attributed to the B fraction. For the amino acids tyrosine, histidine, arginine, valine, glutamic acid, isoleucine and alanine, the variation in the meal is greater than that in either the 12S protein or the B fraction. Therefore it may be concluded that there is an even greater variation in the proportions of these amino acids in those proteins not examined.

The observation that the amino acid composition of the low molecular weight protein fraction is more variable than that of the 12S protein must be considered in any attempt to improve the amino acid composition of *Brassica* seed meals by plant breeding.

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REFERENCES

1. Downey, R.K., JAOCS 48:718 (1971).
2. Urs, M.K., and H.A.B. Parpia, Presented at the FAO/WHO/UNICEF Protein Advisory Group Meeting, Geneva, 1969, Document 2.35/1.
3. Sims, R.P.A., JAOCS 48:733 (1971).
4. Bhatty, R.S., S.L. MacKenzie and A.J. Finlayson, Can. J. Biochem. 46:1191 (1968).
5. Goding, L.A., R.S. Bhatty and A.J. Finlayson, Ibid. 48:1096 (1970).
6. Lonnerdal, B., and J.C. Janson, Biochim. Biophys. Acta 278:175 (1972).
7. Finlayson, A.J., R.S. Bhatty and C.M. Christ, Can. J. Bot. 47:679 (1969).
8. Goding, L.A., R.K. Downey and A.J. Finlayson, Can. J. Plant Sci. 52:63 (1972).
9. MacKenzie, S.L., and J.A. Blakely, Can. J. Bot. 50:1825 (1972).
10. Itzhaki, R.F., and D.M. Gill, Anal. Biochem. 9:401 (1964).
11. Conway, T.F., and F.R. Earle, JAOCS 40:265 (1963).
12. Davis, B.J., Ann. N.Y. Acad. Sci. 121:428 (1964).
13. Moss, C.W., M.A. Lambert and F.J. Diaz, J. Chromatogr. 60:134 (1971).
14. Appelqvist, L.-Å., JAOCS 48:851 (1971).
15. Tkachuk, R., Cereal Chem. 46:419 (1969).
16. Metzger, H., M.B. Shapiro, J.E. Mosimann and J.E. Vinton, Nature (London) 219:1166 (1968).

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