varieties may be of interest to supplement the findings on chemical characteristics.

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Supplementary Material Available: Analytical values of some of the *Lathyrus sativus* varieties and cultivars with the content of the toxins–BOAA and "trypsin inhibitory activity" (3 pages). Ordering information is given on any current masthead page.

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Studies on the Proteins of the Mutants of Barley Grain. 3. Fractionation and Characterization of the Glutelin Fraction

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Mutational effects were observed in the glutelin fraction of the barley grain protein. Fractionation of the glutelin of the parent variety, NP-113, its mutants, Notch-1 and Notch-2, and the nutritionally superior Hiproly barley on Sephadex columns indicated that the fraction II (glutelin II) was considerably enriched in the mutants, and this was comparable with glutelin of the Hiproly barley. Data on the amino acid composition also indicated a favorable change for this fraction from the point of nutrition. The in vitro digestibility study of this fraction adduced support for this nutritionally favorable change and suggests that the glutelin II fraction could serve as an index of superior grain type from breeding point of view for nutritional purpose.

Fractionation of the isolated classical protein fractions of barley mutants into subfractions could help in identification of the changes induced by mutagenesis in the strategy of improving the grain protein quality. Earlier work (Singh and Sastry, 1977b) on the alcohol-soluble proteins of these mutants revealed an increase (compared to the parent) in the content of the 35% ethanol-soluble subfraction, which showed relatively superior amino acid composition and in vitro digestibility. These changes were consistent with those in the Hiproly barley of established nutritive quality (Munck et al., 1970; Singh and Sastry, 1977b). The present investigation has been extended to the proteins of the glutelin fractions isolated from the mutants, Notch-1 and Notch-2, their parent, NP-113, and the Hiproly barley, an isolate of Ethiopian origin from the World Barley Collection with superior nutritive quality of its protein.

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MATERIALS AND METHODS

The grain samples, namely, NP-113, Notch-1, Notch-2, and Hiproly, used in this investigation were obtained as described earlier (Singh and Sastry, 1977a). The glutelin fraction from each of the barleys was obtained by extraction with a solvent system comprising acetic acid (0.1 M), urea (3 M), and cetyltrimethylammonium bromide (0.01 M) as described earlier (Singh and Sastry, 1977a). The solvent system was designated as AUC solvent system and the protein AUC–glutelin (Singh and Sastry, 1977a). The determination of this protein content, amino acid composition, and electrophoresis of this protein fraction was also described.

Gel Filtration of Glutelin. Twenty milligrams of the glutelin protein was dissolved in 4 mL of the AUC solvent system. The solution was centrifuged and, after measuring

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Table I. Distribution of Glutelin Protein among the Fractions of the Four Barleys

Variety	Protein recovery (%) Fraction I	Fraction II	Total
NP-113	66.8	30.0	96.8
Notch-1	53.6	44.9	98.5
Notch-2	52.2	45.6	97.8
Hiproly	54.5	44.9	99.4

the total absorption at 280 nm, was applied on the top of a Sephadex G-100 column (2 × 50 cm), previously equilibrated with several changes of the AUC solvent system. Fifty fractions of 3 mL each were collected on an LKB-Radi Rac Siphon Control Automatic Fraction Collector at the rate of 12 mL/h. The optical density of each fraction was monitored at 280 nm. The tubes containing the protein under each peak of the elution profile (Figure 1) were pooled, dialyzed against distilled water, and lyophilized to dryness. The protein eluted under each peak in the order of elution were designated as glutelin I and glutelin II, respectively.

RESULTS

Fractionation of Glutelins. The results of fractionation of the glutelins from the four barleys are presented in Figure 1. Each of these glutelins resolved into two peaks, namely, glutelin I and glutelin II. The elution profiles indicate that glutelin I appeared with the void volume of the column although a slight shift of the peaks to the greater elution volume in the Notch mutants from those of the NP-113 and Hiproly was noted. Likewise, glutelin II of the Notch mutants possessed similar elution characteristics and emerged later than that of NP-113. The Hiproly glutelin II, however, emerged much earlier than those of the Notch mutants, and its peak was closer to and before that of NP-113.

The recoveries of the protein loaded in glutelin I and glutelin II of the different barleys are shown in Table I. The recoveries varied between 96.8 to 99.4%. Glutelin I, thus, represented about 67% in NP-113 while it represented about 52–54% in the mutants and Hiproly. Correspondingly, glutelin II represented only 30% in NP-113 while it represented about 45% in the mutants and Hiproly.

The ratio of glutelin I to glutelin II from NP-113 was 2.2, while it was 1.2 in the mutants and Hiproly. Thus considerable alteration in the proportion of these two fractions of glutelin was evident, and this alteration was in favor of glutelin II.

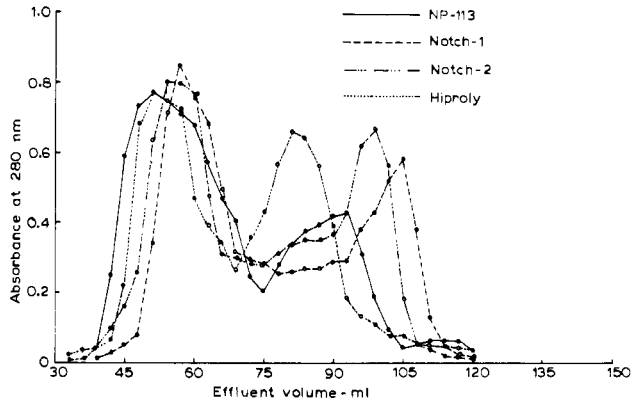

Figure 1. Elution profile of AUC–glutelin of the four barleys from Sephadex G-100 column. The protein eluted in the first and second peaks in the order of emergence from the column are referred to as glutelin I and glutelin II, respectively.

Electrophoresis. Polyacrylamide gel electrophoresis of glutelin I and glutelin II from the four barleys indicated that mostly glutelin I contained components of lower electrophoretic mobility, while glutelin II contained components of high electrophoretic mobility. While a clear resolution of glutelin into glutelin I and glutelin II was not achieved in case of Hiproly, glutelin II of Hiproly showed a predominance of the fast-moving components.

Amino Acid Composition. The amino acid composition of the unfractionated glutelins and glutelin II of the four barleys are presented in Table II. The glutelins of the mutants and Hiproly contained more lysine, histidine, arginine, isoleucine, and tyrosine among the essential amino acids. They contained also more of cystine and glycine. Higher threonine content in Notch-2 and Hiproly and methionine in the mutants were also noted. The nonessential amino acids, glutamic acid and proline, showed a major reduction while serine showed a slight increase in the mutants and Hiproly.

Among the glutelin II of the four barleys, the essential amino acids, lysine, arginine, isoleucine, threonine, and valine, and also the sulfur amino acid, cystine, were invariably in higher proportions in the mutants and Hiproly. Methionine showed only a negligible variation. Histidine showed a reduction in mutants and Hiproly, and tyrosine

Table II. Amino Acid Composition of Glutelins and Glutelins II from the Four Barleys

Amino acid	Amino acid content, g/16 g of N: Glutelin NP-113	Glutelin Notch-1	Glutelin Notch-2	Glutelin Hiproly	Glutelin II NP-113	Glutelin II Notch-1	Glutelin II Notch-2	Glutelin II Hiproly
Lysine	3.92	4.39	4.47	5.68	4.81	5.68	5.69	6.15
Histidine	2.28	2.58	2.51	2.59	2.96	2.64	2.68	2.77
Arginine	5.38	6.54	6.56	6.69	4.64	5.79	6.16	5.55
Aspartic acid	5.63	5.75	5.78	5.40	5.23	5.85	5.31	3.10
Threonine	3.07	3.08	3.19	3.18	3.09	3.26	3.23	3.24
Serine	4.52	4.66	5.45	5.05	5.89	5.31	5.51	5.50
Glutamic acid	21.57	19.91	19.20	18.95	17.25	16.63	16.05	15.54
Proline	10.60	8.71	8.02	7.71	6.66	5.04	5.55	5.88
Glycine	4.98	5.62	5.29	5.12	6.38	6.78	6.59	5.95
Alanine	5.73	5.27	5.54	6.63	7.46	7.17	7.17	7.24
Cystine	2.20	2.72	2.80	2.89	2.45	2.84	2.86	2.98
Valine	6.04	6.03	6.25	6.19	8.09	8.53	8.53	9.01
Methionine	2.07	2.13	2.27	2.05	2.33	2.41	2.36	2.31
Isoleucine	4.11	4.28	4.25	4.39	4.03	4.54	5.87	5.68
Leucine	8.97	8.17	8.29	7.91	9.17	8.68	9.37	9.10
Tyrosine	3.53	3.75	3.97	4.06	3.90	4.54	3.73	3.28
Phenylalanine	5.74	5.06	5.82	5.64	4.58	4.72	5.03	4.64
Chemical score, %	77	77	80	80	77	81	81	81

Table III. In Vitro Digestibility of the Proteins of the Four Barleys

Variety	Seed flour	AUC-glutelin	Glutelin II	Hordein[a]
NP-113	75.2	62.5	68.8	54.3
Notch-1	73.8	62.4	70.9	51.8
Notch-2	81.4	66.9	78.1	63.9
Hiproly	80.5	67.7	77.4	65.6

[a] Cf. Singh and Sastry, 1977b.

showed a reduction in Notch-2 and Hiproly but an increase in Notch-1. Among the nonessential amino acids, serine, glutamic acid, and proline showed a major reduction.

Comparison of the glutelins II with glutelins indicated that the former possessed a nutritionally more favorable composition, particularly in the case of lysine. An increase in the content of essential amino acids like valine, leucine, histidine, methionine, isoleucine, and also the sulfur amino acid, cystine, was noted in glutelin II, although arginine and phenylalanine showed a reverse trend. Tyrosine showed a reverse trend in Notch-2 and Hiproly only. Among the nonessential amino acids, glutamic acid and proline recorded a major reduction and glycine, alanine, and serine an increase.

The chemical score values, also presented in Table II, showed that glutelin II from mutants and Hiproly were superior to those of the parent glutelin. When the glutelins and the glutelin II were compared the chemical score values of those of Notch-2 and Hiproly were higher than that of the NP-113 glutelin.

In Vitro Digestibility. The results of these experiments, presented in Table III, showed that glutelins II had the highest digestibility compared to glutelin, hordein, and the total protein of the flours. It must be pointed out that glutelin II had invariably higher digestibility compared to their respective original glutelins.

DISCUSSION

Like the hordein fraction (Singh and Sastry, 1977b), the glutelin fraction of the mutants also exhibited major changes in their protein make-up. The increase of about 50% in the glutelin II fraction and the resultant alteration in the ratio of glutelin I to glutelin II, due to the increase in glutelin II, is of significance, particularly in the light of the amino acid composition, chemical score values, and the values for in vitro digestibility of the glutelins and glutelin II from the four barleys. The results showed resemblance to those of the Hiproly barley which was of established nutritive quality.

An examination of the amino acid composition pointed out that the essential amino acid composition, particularly, the concentration of the basic amino acid, lysine, among others was more favorable from the nutritional point of view in the glutelin II. The chemical score values and the values for in vitro digestibility of the glutelin II also indicated this nutritionally favorable change. The increase in the content of the essential amino acids were countered by the decrease in the amino acids nonessential and those typical for the prolamine type proteins, namely, glutamic acid and proline. It appears from the composition of the glutelin II that this fraction is responsible for the altered composition of the total glutelin being enriched with proteins containing relatively more favorable amino acid composition. Electrophoretically also this fraction showed components with high mobility which indicated their relative basic nature which might be attributed to an overall increase in level of basic amino acids.

The evidence suggests that in these mutant glutelins a predominance of the synthesis of certain kinds of proteins occurs and, from a breeding point of view, the glutelin II might serve as an index of better grain type for nutritional purpose. An earlier report (Mertz et al., 1974) indicated a variation in the grain lysine contents, and this was related to the changes in free amino nitrogen level. Such variation in lysine content might be due to differences in synthesis of certain kinds of proteins (as observed in the present study) depending upon the level and type of free amino acid pool. In fact, our earlier work (Sastry and Sundaresan, 1973) on one of these high protein barley mutants (Notch-2) and a low protein barley indicated a higher level of free amino nitrogen in the high protein mutants together with a higher level of nucleic acid content and other metabolic changes during grain maturation compared to the low protein barley. The higher level and type of free amino acid pool might preferentially permit synthesis of certain types of protein instead of the other. In this connection the report of Manghers et al. (1973) that the synthesis of glutelin takes place after globulin and before prolamine and that of Bushuk and Wrigley (1971) that glutelin was present at all stages of grain maturation may be relevant. Whichever report might be true, the higher level and the type of free amino acids at the time of glutelin formation might facilitate higher synthesis of some of these glutelins. This suggestion requires establishment by a future study.

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