Characterization of Triticale Protein

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Cereal Chem. 62(3):174–178

ABSTRACT

Proteins from triticale endosperm and germ tissues were individually fractionated on the basis of solubility in several different solvents. Protein content and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) studies were done for each fraction. Gels were stained with Coomassie Brilliant Blue and Periodic Acid-Schiff (PAS) reagents. Molecular weights of the protein subunits were estimated in each fraction. Coomassie Brilliant Blue metachromasia was observed only in the storage proteins, which suggests that this behavior could be due to high proline content in these proteins.

Plant and cereal proteins have low levels of some essential amino acids, particularly lysine and tryptophan, limiting their use in human nutrition (Righetti and Bianchi Bosisio 1981). Triticale, a hybrid cereal species, is produced by combining the genomes of wheat (*Triticum*) and rye (*Secale*). Triticale offers higher yields, good nutritive quality, and advantages in other important characteristics, and is a good alternative to wheat. With respect to lysine content, triticale is significantly superior to commercial wheats (Bushuk and Larter 1980).

Chen and Bushuk (1970a–c) studied the endosperm proteins in one line (6A 190) of triticale and its parental species. They reported that, for some characteristics, triticale proteins were intermediate between the wheat and rye parents. By comparison of the disc electrophoretic patterns of various soluble protein fractions of the triticale and those of the wheat and rye parents, these authors found that all the protein components of the interspecific hybrid were present in the parents.

The purpose of this study was to characterize the endosperm and germ proteins of a triticale line (47746).

MATERIALS AND METHODS

Triticale used in this study (line 47746) was grown at the experimental station of the Facultad de Agronomía, Universidad Nacional de La Plata, Argentina.

The germ and the endosperm (with aleurone and testa) were manually separated from the grains. Because the amount of material was small, the separated germs and endosperms were ground with a mortar and pestle.

Protein Extraction

Proteins were extracted from the two fractions as shown in Figure 1 based on the Landry and Moureaux (1970) method (Sequence D) as modified by Kapoor and Heiner (1982). In each extraction step, the solvent volume to sample weight ratio was approximately 1 for endosperm and 26 for germ. The supernatants were separated from the corresponding precipitates by centrifugation (20 min, 2,000 × *g*). All treatments were carried out at 4° C.

Protein Content

Residue and whole seed protein contents were determined using Kjeldahl Büchi equipment. Conversion factors were N × 5.7 for endosperm and seed, and N × 6.25 for the germ.

The protein content of soluble fractions, except those with 2-mercaptoethanol (2-ME), was determined directly by the method of Lowry et al (1951), using soluble bovine albumin as a standard. Protein content of fractions containing 2-ME was also determined by this method, but after elimination of 2-ME by evaporation in a boiling water bath (de Wreede and Stegemann 1980). The initial volume was restored with distilled water. Protein standards were prepared under similar conditions.

Electrophoresis

Slab sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the Laemmli discontinuous buffer system (Laemmli 1970, Laemmli and Favre 1973) at a gel concentration of 12.6%, using a Pharmacia gel electrophoresis apparatus GE-2/4. Gel slabs were fixed in a solution of isopropanol, acetic acid, and water (25:10:65) and stained with 0.02% Coomassie Brilliant Blue R-250 in 7% acetic acid. Periodic Acid-Schiff reagent (PAS) was used to stain glycoproteins (Pharmacia 1980).

Rod gel electrophoresis was performed according to the method described by Gabriel (1971) with 0.1% (w/v) SDS at a gel concentration of 10%. The gels were fixed and stained with 0.1% Coomassie Brilliant Blue R-250 in methanol 25%, acetic acid 10%, and water (v/v). The Coomassie Brilliant Blue stained gels were scanned in a Shimadzu dual wavelength TLC Scanner CS-910 (sample wavelength 570 nm and reference wavelength 395 nm) attached to a C-R 1A Chromatopac Shimadzu integrator.

Molecular weights of the bands were estimated by use of the Pharmacia protein molecular weight calibration kit. The standard proteins used and their molecular weights were as follows: phosphorylase-b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), α-lactalbumin (14,400).

RESULTS AND DISCUSSION

Table I shows the protein content of endosperm and germ fractions extracted with different solvents. These results are the average of four different extractions; the protein content of each extraction was determined in duplicate.

As fractions C and D were extracted from separate samples of the same material (see Fig. 1, first precipitate), and because some proteins are soluble in hydrophobic bond-disrupting agents and also in alcohol (Wall 1979), it is possible that some of the same proteins would be included in fraction C as well as in fraction D.

Results in Table I show that albumins (fraction A) and globulins (fraction B) constituted 33% of the extracted endosperm proteins and 59% of the extracted germ proteins.

Fractions extracted with borate buffer (fraction D_1) and 2-ME-SDS-borate buffer (fraction D_2) constituted 31% of germ proteins extracted (Table I), and probably consist mainly of proteins related to membranes and cell walls (Doll 1977).

The alcohol-soluble fraction of endosperm contains the most important storage proteins and is localized in special protein stores called protein bodies (Doll 1977). This fraction constituted 29% of the extracted endosperm proteins whereas it constituted only 10% of the proteins of the germ extracted under similar conditions (Table I).

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According to Wall (1979), after extraction of flour with nonreducing extractants, appreciable protein remains associated with the insoluble residues. Subsequent extraction with reducing agents such as 2-ME in SDS solution solubilizes almost all of the remaining protein. Thus, this protein should be extracted in the fraction D_2. Differences in the extraction conditions and especially in particle size probably contributed to the low level of extracted protein (48% vs., for example, 81% reported by Chen and Bushuk 1970a and 88–94% by Kapoor and Heiner 1982). However, the percentage of the extracted protein of each fraction (Table I) is in accordance with those reported in the literature (Chen and Bushuk 1970a, Simmonds 1978). No published data were found for triticale germ protein.

Electrophoresis

Two electrophoretic methods were applied, as described in Materials and Methods. The Laemmli system provided a higher resolution, and protein fractions obtained were run in gel slabs following this method. Figure 2 shows the electrophoretic patterns and Figures 3 and 4 depict the 570 nm spectrophotometric scannings corresponding to some fractions.

Water-soluble and salt-soluble proteins (albumins and globulins). The electrophoretic patterns corresponding to endosperm (Fig. 3a) and germ (Fig. 3c) albumins (fraction A) differ in the number of bands as well as in their corresponding mobilities and consequently in their estimated molecular weight (mol wt). The endosperm pattern of Figure 3a shows major and minor bands.

The electrophoretic pattern of the albumin fraction (fraction A) of the germ was more complex than that of the corresponding endosperm fraction. This may be explained by the presence of water-soluble enzymes in the germ that are necessary for plant development. By contrast, endosperm globulins (fraction B) present a higher diversity of protein bands than germ globulins, as seen in the electrophoretic patterns (Figs. 2 and 3).

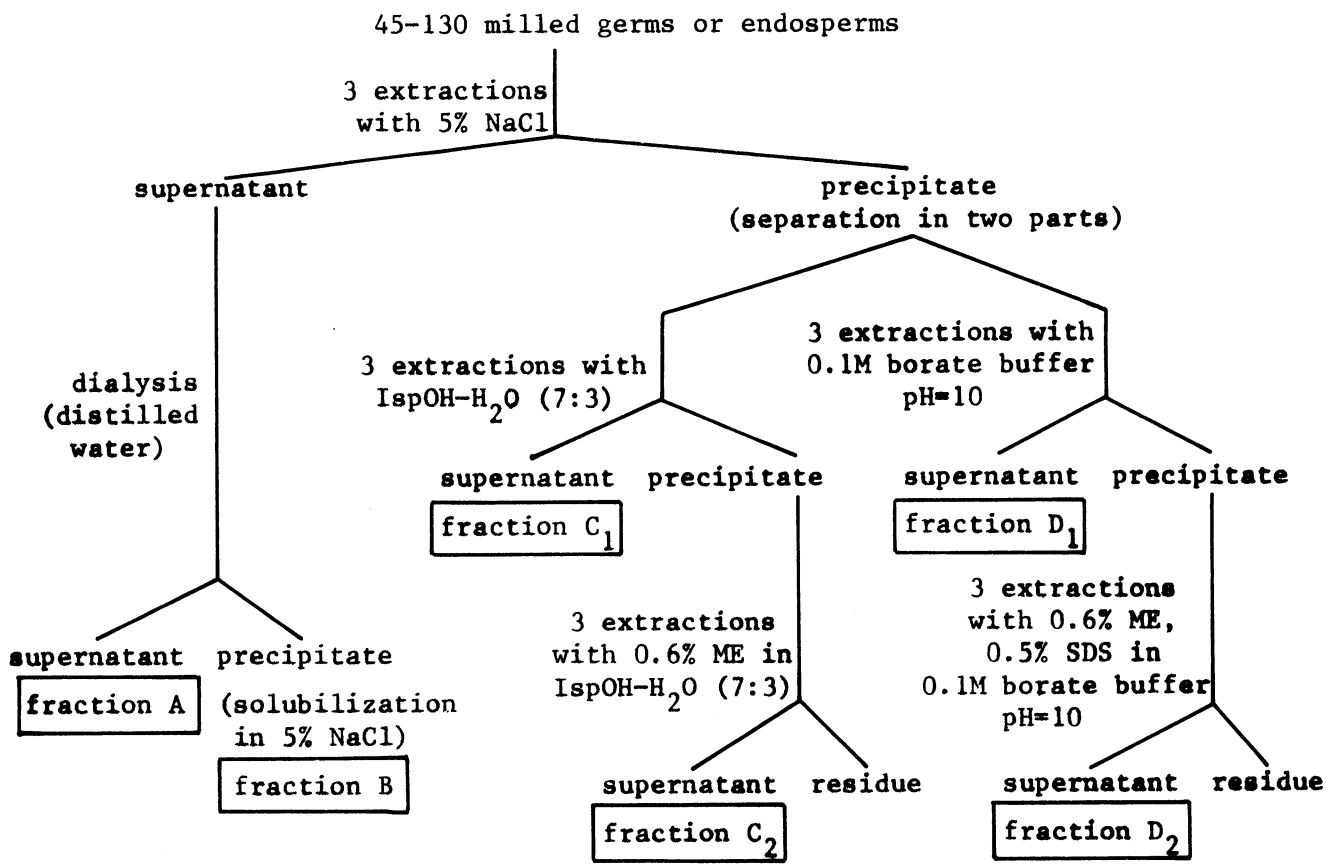

Fig. 1. Scheme of protein extraction method: fraction A, albumins plus non-protein nitrogen; fraction B, globulins; IspOH, isopropanol.

TABLE I
Triticale[a] Protein Content

	Water-Soluble (Albumins) Fraction A	Salt-Soluble (Globulins) Fraction B	Isopropanol-Soluble Fraction C_1	ME-Isopropanol-Soluble Fraction C_2	Borate Buffer-Soluble Fraction D_1	ME-SDS-Borate Buffer-Soluble Fraction D_2	Total Extracted Protein	Residue
Germ								
Protein per seed (μg)	59 ± 11	26 ± 13	11 ± 2	4 ± 2	29 ± 7	14 ± 5	143	80[b]
Percentage of total extracted protein	41	18	8	2	21	10	...	...
Endosperm								
Protein per seed (μg)	389 ± 36	79 ± 8	306 ± 51	105 ± 11	232 ± 27	319 ± 79	1,430	1,496[c]
Percentage of total extracted protein	27	6	22	7	16	22	...	...

[a] Average seed weight: 41 mg. Total seed protein content (μg), calculated as N × 6.25: 3,347.
[b] Calculated as N × 6.25.
[c] Calculated as N × 5.7.

Alcohol-soluble proteins (fractions C_1 and C_2). No bands were obtained in SDS-PAGE of the isopropanol- and 2-ME-isopropanol-soluble germ fractions.

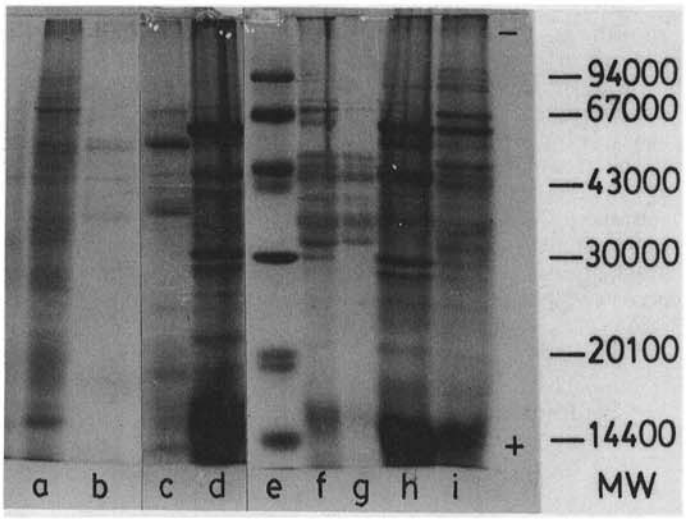

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of germ and endosperm triticale proteins extracted with different solvents: **a** = fraction A (germ); **b** = fraction B (germ); **c** = fraction B (endosperm); **d** = fraction A (endosperm); **e** = marker proteins; **f** = fraction C_1 (endosperm); **g** = fraction C_2 (endosperm); **h** = fraction D_1 (endosperm); **i** = fraction D_2 (endosperm); **MW** = molecular weight.

The fraction C_1 pattern of Figure 4a shows that most protein subunits of this endosperm fraction appear at a range of about mol wt 22,000–86,000, forming two distinctive groups: one with an mol wt range of 60,000–86,000, and the other with a range of 22,000–60,000 mol wt. These molecular weight ranges are similar to those reported for wheat gliadins (Godon and Guilbot 1979), the first group corresponding to ω-gliadins.

Fraction C_2 (Fig. 4c), extracted by 2-ME-isopropanol, showed an electrophoretic pattern similar to that of fraction C_1, although the fraction C_2 pattern lacks some bands corresponding to high and low MW subunits (Figs. 2 and 4).

Borate buffer- and 2-ME-SDS-borate buffer-soluble proteins (fractions D_1 and D_2). Rod SDS-PAGE of germ proteins extracted with borate buffer gave electrophoretic patterns with a major band of about 43,000 mol wt and some other minor bands of higher and lower molecular weight (results not shown).

The electrophoretic pattern corresponding to fraction D_1 endosperm proteins (Fig. 4b) shows a high number of bands, most of them with mol wt lower than 65,000. These protein subunits would be associated by noncovalent bonds (Godon and Guilbot 1979).

SDS-PAGE of reduced glutenin components of wheat showed that glutenin is a complex mixture of about 15 different protein subunits with mol wt ranging from 12,000 to 133,000 (Wall 1979). The molecular weight ranges of the triticale fraction D protein subunits (Figs. 4b and d) are very similar to those found for wheat glutenins (Wall 1979).

The electrophoretic patterns in Figure 4 corresponding to the storage proteins of the triticale endosperm show a large number of bands. Storage proteins of most cereals are quite heterogeneous as a result of the polyploid nature of the tissue or the absence of strong

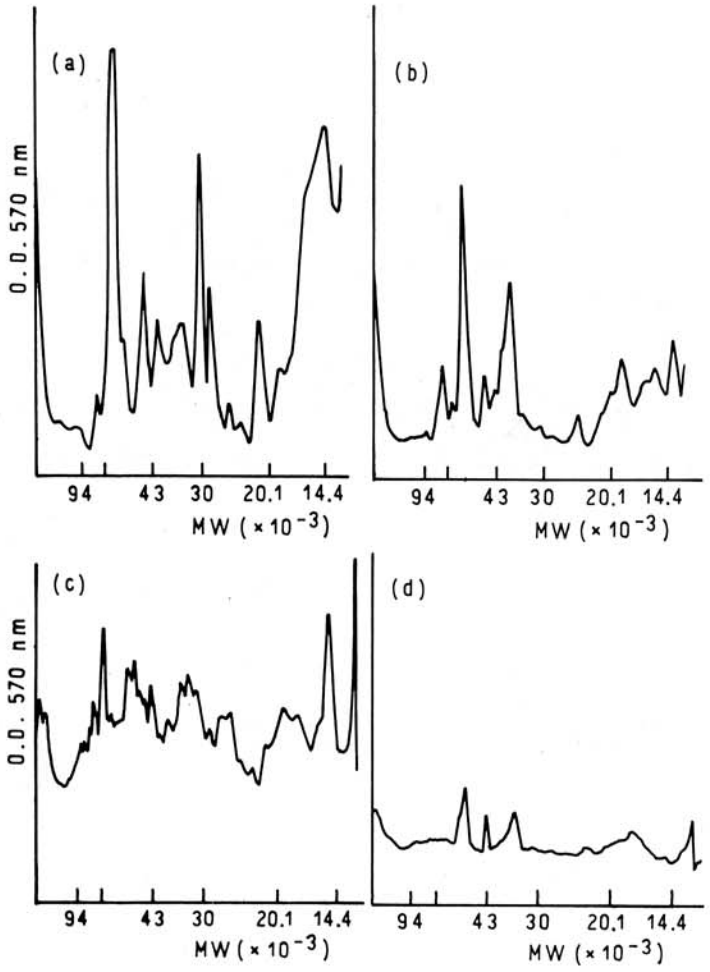

Fig. 3. Densitometric tracings of the electrophoretic patterns of germ and endosperm triticale proteins: **(a)** = fraction A (endosperm); **(b)** = fraction B (endosperm); **(c)** = fraction A (germ); **(d)** = fraction B (germ); **MW** = molecular weight; **O.D.** = optical density.

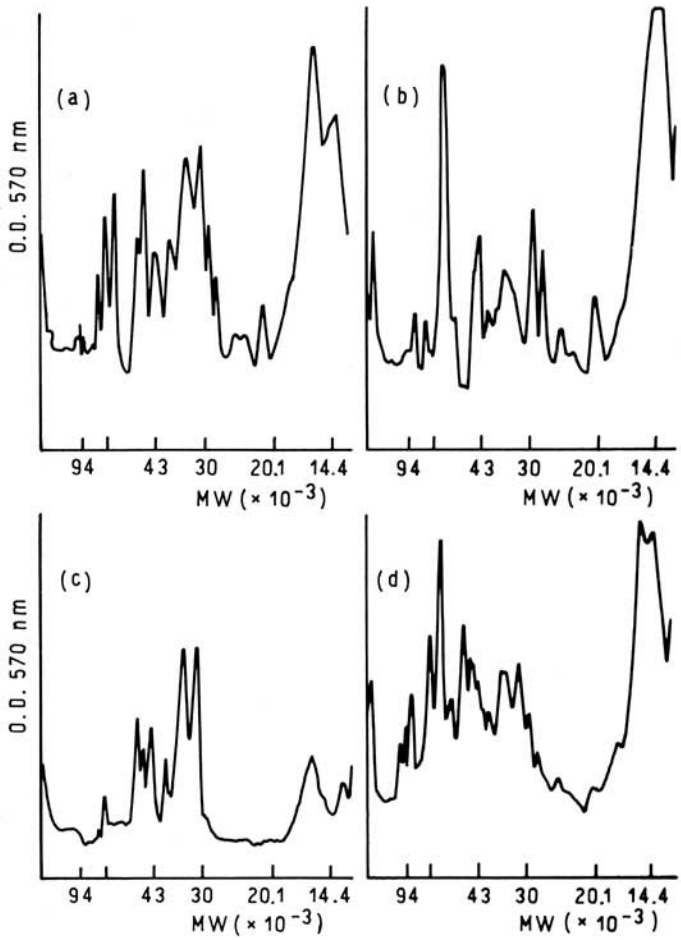

Fig. 4. Densitometric tracings of the electrophoretic patterns of endosperm triticale proteins: **(a)** = fraction C_1; **(b)** = fraction D_1; **(c)** = fraction C_2; **(d)** = fraction D_2; **MW** = molecular weight; **O.D.** = optical density.

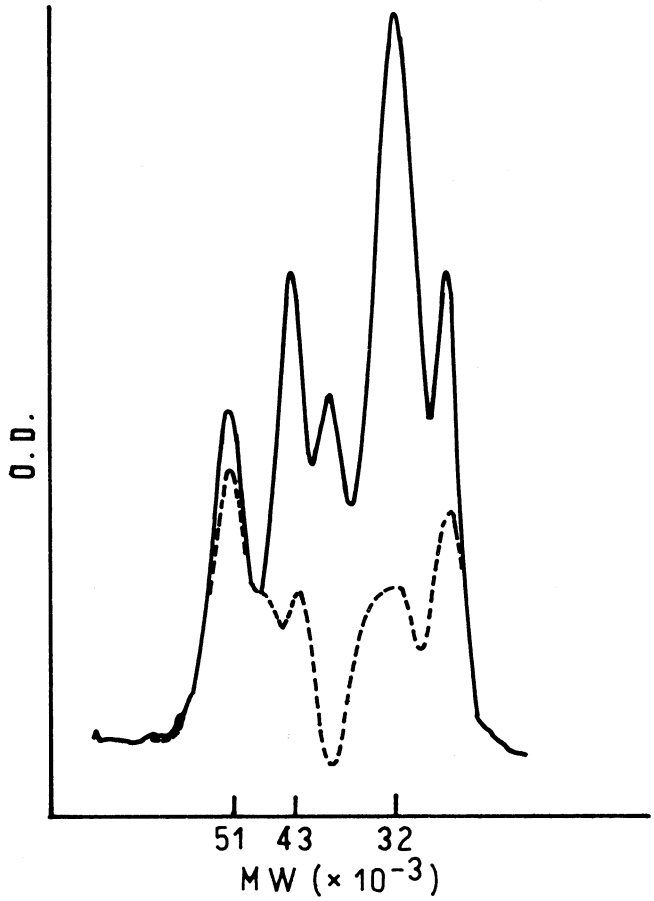

Fig. 5. Densitometric tracings of a portion of the electrophoretic pattern corresponding to endosperm triticale protein (fraction C^2). The gels were scanned at both 520 (---) and 610 nm (——) and the scan tracings superimposed. MW = molecular weight; OD = outer diameter.

evolutionary pressure to conserve a particular amino acid composition.

In endosperm fractions A and C most of the protein bands of SDS-PAGE were stained when treated with PAS reagent, which suggests the presence of glycoproteins. In the other fractions only some of the bands stained intensely with PAS treatment. It is possible that some SDS could still remain in the gel after washing, and the presence of adhering substances such as starch would give a positive reaction with PAS. However, the electrophoretic patterns of PAS-stained gels were different in terms of relative intensity from those of the equivalent gels stained with Coomassie Brilliant Blue.

Relatively large amounts of carbohydrates have been found in purified gluten fractions. McMaster and Bushuk (1983) reported the proportions and compositions of carbohydrates associated with the alcohol-soluble and the alcohol-insoluble fractions of a gluten preparation. Glycoproteins have various functions in the plant: as enzymes (such as peroxidase), as transporters, for storage (seed proteins), and others (Lamport 1980).

Metachromatic staining. A metachromatic behavior was observed during the destaining process of rod gels corresponding to fractions C_1, C_2, D_1, and D_2. This phenomenon, a progressive color change from blue to red, was particularly evident for proteins with a mobility corresponding to the mol wt range 40,000–50,000, whereas the rest of the proteins remained unchanged. This metachromasia has been observed in collagen and procollagen, and also in calf thymus histone 1 (Duhamel et al 1980).

The red and blue bands can be distinguished spectrophotometrically by comparing absorption scans of the same gel at two different wavelengths, 520 and 610 nm, according to the method used by Duhamel et al (1980). The blue bands exhibit greater absorbance at 610 than at 520 nm, whereas the red ones exhibit greater absorbance at 520 than at 610 nm. The superimposed absorption scans at these two wavelengths of a portion of the fraction C_2 electrophoretic pattern are shown in Figure 5. Duhamel et al (1980) proposed a model in which they attributed this behavior to the closely spaced proline residues in both collagen and histone 1. Abnormally low electrophoretic mobilities in SDS gels of these proteins were attributed to the same cause (Duhamel et al 1980).

Both triticale and wheat flours have high proline contents (Chen and Bushuk 1970a). Wheat gliadins have about 15–30% proline with respect to total amino acid residues (Kasarda et al 1978). Proline would conceivably play a role in determining the secondary and tertiary structures of the gliadins (Kasarda et al 1978). Higher molecular weight values in gliadins obtained by SDS-PAGE were attributed to the high content of proline in the gliadins that impairs their ability to form rigid helixes in SDS solution (Wall 1979). This behavior was also observed in glutenins (Bietz and Huebner 1980).

If this relationship could be verified, the metachromatic effect might be used to detect storage proteins that exhibit abnormally low electrophoretic mobilities in SDS.

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[Received April 23, 1984. Revision received December 13, 1984. Accepted December 17, 1984.]