Low Molecular Weight Durum Wheat Glutenin Fractions Rich in Sulfhydryl Plus Disulfide Groups

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Cereal Chem. 65(1):65–69

ABSTRACT

Durum wheat glutenin fractions with sulfhydryl (–SH) plus disulfide (S–S) groups content of 185 and 140 μmol/g of protein for cultivars Mondur and Kidur, respectively, were extracted from gluten with Na-tetradecanoate (2.5 mg/g of gluten) after extraction of gliadins. Amino acid analyses confirmed the high content of cysteine in these as compared to other glutenin fractions; some intervarietal differences were also noted. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), without reduction, showed these proteins to consist of two major low molecular weight proteins, DSG-1 and DSG-2 (durum-wheat sulfur-rich glutenin fractions) of 14.1 and 17.7 kDa, respectively. After reduction, SDS-PAGE showed DSG-1 to be composed of two subunits of lower mobility than DSG-1, having apparent molecular masses of 14.6 and 14.7 kDa. The SDS-PAGE mobility of DSG-2 increased after reduction to approximately 15.8 kDa. At pH 3.1 the electrophoretic mobility of DSG-2 was greater than that of DSG-1; both ran faster than gliadins under similar conditions.

Few studies have concerned the sulfhydryl (–SH) and disulfide (S–S) content of durum wheat proteins. Tsen and Anderson (1963) showed noticeable differences between thiol contents of soft, hard, and durum wheat proteins. According to Fabriani et al (1970) the cooking quality of pasta was better when the ratio of reactive S–S to total S–S content was higher in a cultivar. Fabriani et al (1975) found that the thiol group content of water-soluble durum wheat proteins decrease as a consequence of milling and pasta manufacturing. More recently, Alary and Kobrehel (1987) reported that the surface state of cooked pasta, which is an important aspect of cooking quality of pasta products, is positively correlated with the amount of –SH plus S–S groups in glutenins of various cultivars. In the present work, we investigated whether some glutenin proteins contain higher amounts of –SH plus S–S groups than other fractions of glutenin, and consequently are more important in determining pasta cooking quality.

MATERIALS AND METHODS

Semolina Samples

Durum wheat semolina samples of two cultivars, Mondur and Kidur, were obtained in 70% yield in a pilot mill.

Extraction of Proteins

Proteins from semolina were extracted sequentially by using a modified Osborne method as described by Alary and Kobrehel (1987). Albumins plus globulins, gliadins, and glutenins were obtained with 0.5*M* NaCl, 68% (v/v) ethanol, and Na-tetradecanoate (80 mg for 1 g of semolina), respectively.

Extraction of Gluten

Gluten G-1 was extracted from semolina by the hand-washing procedure (Mauze et al 1972) except that instead of tap or salted water, distilled water was used for washing. Gluten G-2 was extracted by washing with distilled water as for gluten G-1, but starting from a residue obtained after extraction of albumins and globulins as in the sequential-extraction method.

Determination of Protein Content

A semiautomatic Kjeldahl method was used as described by Feillet (1967). Protein content was calculated as N × 5.7.

Fractionation of Glutenin

Freeze-dried gluten samples were reduced into powder in a Miag experimental mill by adding dry ice to the samples during grinding. Gliadins were extracted with 68% (v/v) ethanol (10 ml/g of gluten). Various amounts of Na-tetradecanoate and 10 ml of distilled water were then added to the residues. Other experimental conditions were as for sequential extraction of proteins (Alary and Kobrehel 1987).

Electrophoresis of Proteins

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done as described by Payne and Corfield (1979). Proteins were analyzed either after reduction or without reduction. For two-dimensional electrophoreses, the first run was done with nonreduced proteins. The strip of gel containing the proteins was put in a buffer (20-ml 1*M* Tris, 2 g of SDS, 10 ml of glycerol, and 2.5 ml of 2-mercaptoethanol brought to 100 ml with distilled water) at 37° C for 15 min. The gel strip with reduced proteins was then applied to the top of the vertical gel and fixed in place with 1% agarose; the run in the second dimension was as for the first dimension. Electrophoresis at pH 3.1 was performed as described by Bushuk and Zillman (1978) except that the run lasted 3 hr.

Determination of -SH Plus S-S Content

The amounts of –SH plus S–S groups in protein samples were determined together by an amperometric technique (Alary and Kobrehel 1987).

Amino Acid Analysis

The experimental conditions were as described by Bidlingmeyer et al (1984).

RESULTS AND DISCUSSION

-SH Plus S-S Content of Protein Fractions

Results of the sequential extraction of proteins from semolina and the –SH plus S–S contents of the protein fractions are shown in Table I. For both cultivars, Mondur and Kidur, the highest

TABLE I
Sulfhydryl Plus Disulfide Contents of Protein Fractions (Albumins + Globulins, Gliadins and Glutenins) Obtained by Sequential Solubilization from Semolina with 0.5*M* NaCl, 68% Ethanol, and Na-Tetradecanoate[a]

Cultivar	Protein Fractions	Protein Content (% of total protein)	-SH Plus S-S Content (μmol/g of protein)
Mondur	Albumin + globulin	18	144
	Gliadin	37	107
	Glutenin	32	92
Kidur	Albumin + globulin	22	145
	Gliadin	34	102
	Glutenin	29	80

[a] Eighty milligrams of Na-tetradecanoate and 10 ml of distilled water per gram of semolina.

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TABLE II

Sulfhydryl Plus Disulfide Contents of Gliadins and Glutenins Extracted from Gluten G-1[a] and G-2[b] of Cultivar Mondur

Gluten Fractions	Gluten G-1 Protein Content (% of gluten proteins)	Gluten G-1 -SH Plus S-S Content (μmol/g of protein)	Gluten G-2 Protein Content (% of gluten proteins)	Gluten G-2 -SH Plus S-S Content (μmol/g of protein)
Gliadin	53	101	57	107
Glutenin	33	113	31	107

[a]Gluten extracted from semolina by the hand washing procedure.
[b]Gluten obtained from a residue after extraction of albumins plus globulins.

TABLE III

Sulfhydryl Plus Disulfide Contents of Glutenin Fractions Solubilized at Various Na-Tetradecanoate Concentrations

Cultivars	Quantity of Na-Tetradecanoate (mg/100 mg of gluten)	Glutenin Solubilized (% of the total protein)	-SH Plus S-S Content (μmol/g of glutenin)
Mondur	2.5	4.2	185
Kidur		5.1	140
Mondur	5	8.6	171
Kidur		10.9	128
Mondur	10	32.1	122
Kidur		29.8	91
Mondur	66	33.3	112
Kidur		31.0	86

amounts of −SH plus S−S groups were found in the albumins plus globulins and the least in glutenin. However, significant varietal differences between the −SH plus S−S contents were found only for glutenin. These results are comparable to those of Alary and Kobrehel (1987), and to those of Beckwith and Wall (1966) and Stachelberger (1978) for gluten proteins from bread wheat.

The amount of −SH plus S−S groups was higher in glutenin extracted from gluten G-1 washed from semolina than in the glutenin sequentially extracted from semolina (Table II). Because gluten G-1 was obtained by the usual hand-washing procedure, it was supposed that some albumins or globulins (or both) rich in −SH plus S−S groups (Table I and Alary and Kobrehel 1987) might have been incorporated into the gluten network and subsequently extracted with glutenins. To avoid such a possible contamination, gluten G-2 was prepared from the residue remaining after extraction of albumins and globulins. The amount of −SH plus S−S groups in this glutenin was slightly lower than in glutenin G-1 (Table II), but still considerably higher than in glutenin obtained by sequential extraction from semolina (Table II). Consequently, the higher −SH plus S−S content in the glutenin extracted from gluten G-1 cannot be fully explained by a contamination of the gluten with albumins and globulins.

No experimental evidence was found to explain the higher −SH plus S−S contents of glutenins extracted from glutens G-1 or G-2 as compared with glutenin extracted sequentially from semolina. It is possible that some glutenin proteins, very low in or lacking −SH and S−S groups, could be washed out, proportionately increasing the −SH plus S−S content in the remaining glutenin. Considering that the differences between −SH plus S−S contents of various glutenins could not be caused by contamination with albumins or globulins (Table II), our further studies were done on glutenin obtained from hand-washed gluten G-1.

It should be mentioned that Bietz et al (1975) found differences in subunit composition of glutenin from a gluten ball as compared with that extracted from bread wheat flour. Moreover, Bietz and Wall (1975) showed that 30–51% of sequentially extracted glutenin is noncovalently bound and consists of gliadins and albumins plus globulins. Also shown were differences in subunit compositions of glutenins extracted under different conditions.

Results in Tables I and II also show that the −SH plus S−S contents of gliadin extracted from gluten G-1 (101 μmol/g) were a bit lower than for the gliadins extracted sequentially from semolina or gluten G-2 (each 107 μmol/g).

TABLE IV

Amino Acid Compositions[a] of Durum Wheat Sulfur-Rich Glutenin (DSG) Proteins and Their Corresponding Residues from Cultivars Mondur and Kidur

Amino Acid	DSG Proteins Mondur	DSG Proteins Kidur	Residues Mondur	Residues Kidur
Alanine	7.0	5.8	5.9	6.3
Arginine	3.2	2.6	2.2	2.2
Aspartic acid[b]	4.3	3.7	3.1	3.2
Cysteine	5.4	4.3	2.4	2.1
Glutamic acid[c]	20.2	26.7	27.2	27.5
Glycine	10.6	8.7	9.8	11.9
Histidine	1.3	1.2	1.3	1.3
Isoleucine	3.4	3.4	3.7	3.6
Leucine	6.9	6.8	6.9	6.1
Lysine	1.4	1.4	1.5	1.6
Methionine	1.5	1.1	1.4	1.2
Phenylalanine	2.3	3.0	2.9	2.9
Proline	12.8	14.0	12.9	12.1
Serine	6.7	7.0	8.1	7.8
Threonine	4.5	3.8	3.3	3.8
Tyrosine	2.8	2.0	1.8	1.6
Valine	6.5	5.3	5.5	5.0

[a]Mole percent of the total protein. Tryptophan was not determined.
[b]Includes asparagine.
[c]Includes glutamine.

Fractionation of Glutenin with Na-Tetradecanoate

After extraction of gliadins from gluten G-1, glutenins were solubilized in the presence of Na-tetradecanoate in distilled water. For both cultivars Mondur and Kidur, as concentration of Na-tetradecanoate increased, the percentage of glutenin solubilized increased in a parallel manner (Table III). These results can be compared to those already reported on solubility of wheat proteins (Kobrehel and Matignon 1980, Kobrehel 1980) or more specifically of gluten proteins with sodium salts of fatty acids (Kobrehel and Bushuk 1977, Wasik et al 1979).

Some varietal differences were also noticeable; glutenin solubility of Mondur at low soap concentrations was slightly lower than for Kidur; this tendency was reversed at higher concentrations of Na-tetradecanoate.

For both cultivars, as percentage of solubilized glutenin increased at higher soap concentrations, the −SH plus S−S content of glutenin fractions decreased. The highest amounts of −SH plus S−S groups, 185 and 140 μmol/g of glutenin, for Mondur and Kidur, respectively, were in glutenins solubilized with the lowest concentration of Na-tetradecanoate, 2.5 mg/g of gluten. These −SH plus S−S rich glutenin fractions could not be solubilized in distilled water without Na-tetradecanoate, and they were not extractable from gluten without a previous extraction of gliadins. The extractability of these fractions will be discussed more thoroughly in relation with their electrophoretic composition.

The −SH plus S−S contents of glutenin fractions from Mondur were considerably higher than for those of Kidur (Table III). The decreased −SH plus S−S content of fractions solubilized with increasing amounts of Na-tetradecanoate was also more pronounced for Mondur than for Kidur.

These results indicate that solubilization of glutenin proteins by Na-tetradecanoate is not random, because fractions rich in −SH plus S−S groups were solubilized preferentially at low soap

concentrations, and fractions soluble only at higher soap concentrations contained a much smaller number of −SH plus S−S groups. Subsequent amino acid analyses and electrophoretic studies confirmed these differences.

Amino Acids Analysis

Amino acid compositions of glutenin fractions solubilized with 2.5 mg of Na-tetradecanoate per 100 mg of gluten that contained the highest amount of −SH plus S−S groups, fraction DSG (durum wheat sulfur-rich glutenin proteins), and of glutenin not soluble under these conditions (residue) were analyzed (Table IV). On the whole, both soluble and residue fractions had amino acid compositions characteristic of glutenin (Wu and Dimler 1963, Ewart 1967, Huebner et al 1974, Danno et al 1978). Cysteine contents of the soluble fraction of both cultivars were much higher than of the corresponding residues. Glutenin fraction A, isolated from bread wheat by Huebner et al (1974), also had a relatively high cysteine content and was composed of low molecular mass proteins, some less than 30 kDa, whereas fraction B, composed of high molecular weight glutenins, contained less cysteine.

Some differences were also found between the cultivars. Cysteine content was higher in the soluble fraction of Mondur, confirming amperometric results; conversely, glutamic acid content was considerably higher in Kidur. Amino acid compositions of residual glutenins were similar for the two cultivars.

Compositions of cysteine-rich soluble fractions differed considerably from those of other sulfur-rich proteins, such as purothionin (Nimmo et al 1974) or ligolin (Frazier et al 1981). Despite some similarities, differences between compositions of glutenin fractions isolated by others (Bietz and Wall 1973, Danno et al 1976, Arakawa et al 1977, Huebner et al 1977) or of S proteins (Zawistowska et al 1986) and those of our cysteine-rich glutenin fractions were apparent.

Electrophoretic Analyses

As shown in Figure 1, the SDS electrophoregram of the glutenin fraction containing the highest amount of −SH plus S−S groups (lane c) has two major low molecular weight protein bands. With the increased amount of soap for glutenin solubilization, more and more proteins of higher molecular weights were solubilized. As in the case of the total protein extraction from flours with soaps (Kobrehel and Matignon 1980, Kobrehel 1980), high molecular weight glutenin subunits required the highest amounts of soaps to be solubilized.

Comparison of electrophoretic patterns of the −SH plus S−S rich glutenin fractions with electrophoregrams of their residues showed (Fig. 2) that the two low molecular weight glutenin proteins were practically quantitatively solubilized at the lowest soap concentrations for both cultivars; only traces of these proteins were observable in the residues. The molecular masses of these durum wheat sulfur-rich glutenin proteins (DSG-1 and DSG-2) were, as determined by SDS-PAGE without reduction, 14.1 and 17.7 kDa, respectively. By using a two-dimensional SDS-PAGE (Fig. 3) (nonreduced proteins in the first dimension, reduced proteins in the second), it was shown that DSG-1 actually has two subunits; in reduced form, both protein chains were slightly less

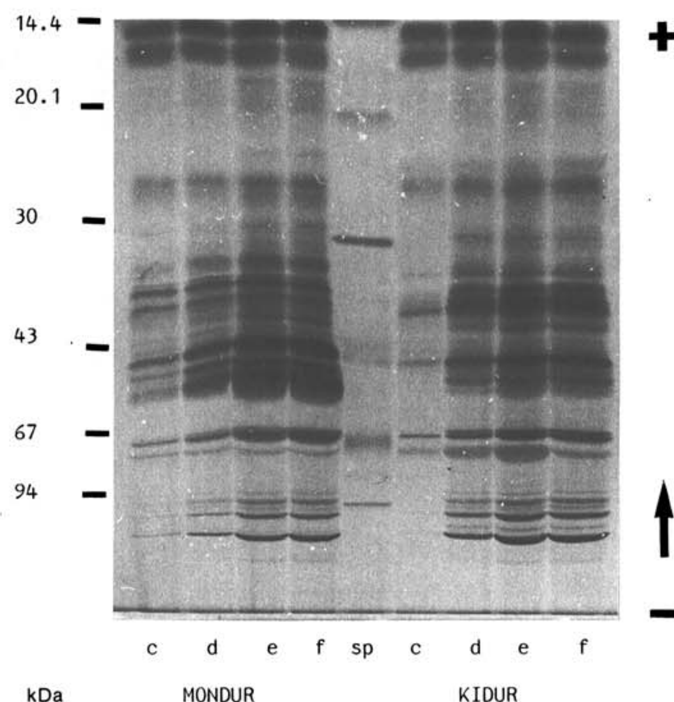

Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of glutenin fractions of cultivars Mondur and Kidur, solubilized with 10 (**c**), 20 (**d**), 33 (**e**), and 66 (**f**) mg of Na-tetradecanoate per gram of gluten. **SP** = standard proteins.

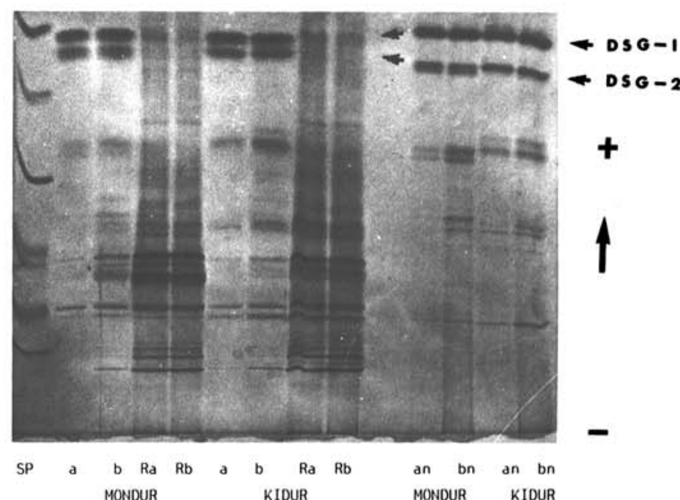

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of glutenin fractions solubilized with 2.5 (**a**, reduced; **an**, nonreduced; **Ra**, residue reduced) and 5 mg (**b**, reduced; **bn**, nonreduced; **Rb**, residue reduced) of Na-tetradecanoate per gram of gluten. **SP** = standard proteins.

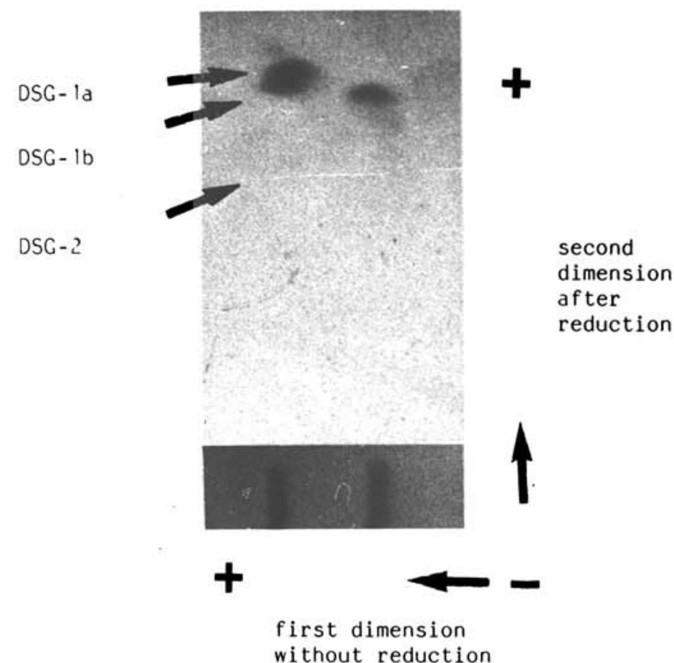

Fig. 3. Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the durum wheat sulfur-rich glutenin fractions. In the first dimension proteins were nonreduced; in the second they were reduced.

mobile than the nonreduced proteins. Apparent molecular masses in reduced form were approximately 14.6 and 14.7 kDa versus 14.1 kDa in nonreduced form. Such a change in mobility can result from unfolding of globular polypeptides upon reduction. Similarly, higher mobilities for nonreduced than reduced proteins have been noted for legume proteins (Sauvaire et al 1984) and animal proteins (Allore and Barber 1984).

As opposed to DSG-1, DSG-2 was less mobile in its nonreduced than in its reduced state, and yielded only one band upon two-dimensional SDS-PAGE (Fig. 3). Its approximate molecular mass in reduced state was 15.8 kDa versus 17.7 kDa before reduction. Various explanations are possible for increased mobility of DSG-2 after reduction. Disulfide bond disruption may permit new secondary interactions, different than the native ones, conveying to the reduced protein a more globular shape than it had when not reduced.

Electrophoreses of sequentially extracted proteins from gluten and from semolina were performed to check whether DSG proteins are present in the different protein fractions. Only traces of proteins were extracted from gluten with distilled water (Fig. 4). Besides gliadins, the ethanol extract contained a very low amount of DSG proteins, and the extract obtained afterward with Na-tetradecanoate (2.5 mg/g of gluten) was principally composed of DSG proteins. Other results (not shown) demonstrated that low amounts of Na-tetradecanoate did not efficiently extract DSG proteins when gliadins were not previously removed from gluten.

In the water-soluble fraction of semolina, a protein band with similar mobility to DSG-1 and a fainter band with mobility close to that of DSG-2 were present (Fig. 4). Results were similar when instead of distilled water 0.5*M* NaCl was used for the first extraction. With ethanol, as in the case of sequential extraction of gluten proteins, only traces of DSG proteins were extracted along with the gliadins. The second extractions with ethanol contained no additional DSG proteins (results not shown). Thus, because DSG proteins can be present in low amounts in albumin-globulin or in gliadin extracts, they are not covalently linked to other glutenins through S−S bonds; however, their amino acid composition is of the glutenin type, and their extractability in the presence of low amounts of Na-tetradecanoate seems to be quite characteristic for them.

Electrophoresis at pH 3.1 of nonreduced DSG proteins solubilized with 2.5 mg of Na-tetradecanoate per gram of gluten (Fig. 5, lane 2) showed only two major components: the faster moving protein was DSG-2 and the slower was DSG-1, as identified by SDS-PAGE. Both components run faster than gliadins under similar conditions (Bushuk and Zillman 1978; and the slower components in Fig. 5, lane 1).

CONCLUSIONS

Our results show durum wheat glutenin to contain proteins varying greatly in amounts of −SH plus S−S groups. In the DSG fractions, representing 13 and 16% (Table III) of the glutenin for cultivars Mondur and Kidur, respectively, the amount of −SH plus S−S groups was higher than in gliadins of these cultivars and, for Mondur, even higher than that of its albumin plus globulin fraction (Tables I and III).

When higher amounts of Na-tetradecanoate were used to solubilize glutenin, lower −SH plus S−S contents were found in the solubilized proteins (Table III). More high molecular weight glutenin subunits were also solubilized at higher soap concentrations (Fig. 3), suggesting a general inverse relationship between subunit molecular weight and −SH plus S−S content.

Considering the results of Alary and Kobrehel (1987) showing that the −SH plus S−S contents of glutenins are highly significantly correlated to the surface state of cooked pasta, the sulfur-rich glutenin fraction composed of two major low molecular weight proteins (DSG-1 and DSG-2) is probably involved in the cooking quality of durum wheat semolina; there may be a functional relationship between the −SH plus S−S content of these proteins and the surface state of cooked pasta. Further investigations are necessary to determine the respective role of DSG-1 and DSG-2 and to further isolate and characterize these proteins. DSG-1 and DSG-2 show significant differences upon electrophoresis, and DSG-1 seems to be composed of two subunits. Further studies could reveal additional heterogeneity and possible differences in their −SH plus S−S content.

Molecular weights of DSG-1 and DSG-2 are similar to those of wheat endosperm CM proteins (Garcia-Olmedo and Carbonero 1970, Redman and Ewart 1973, Rodriguez-Loperena et al 1975, Salcedo et al 1978), S proteins (Zawistowska and Bushuk 1986, Zawistowska et al 1985), and to some extent to ligolin (Frazier et al

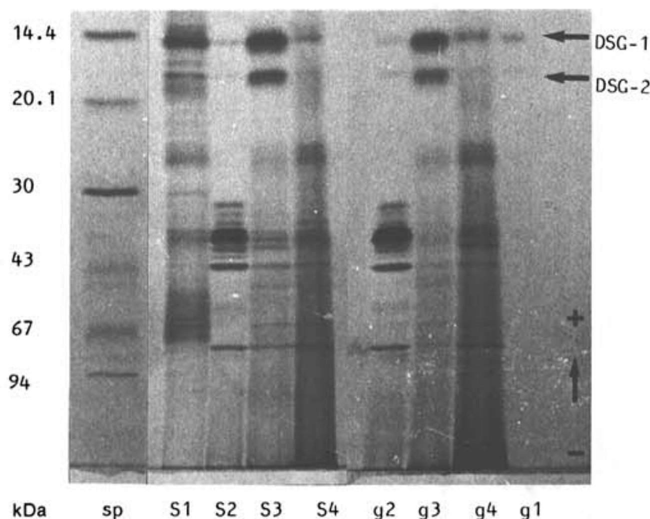

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sequentially extracted nonreduced protein fractions from semolina (**s**) and from gluten (**g**) cultivar Kidur. Extractions were performed with distilled water (**s1, g1**), 68% ethanol (**s2, g2**), 2.5 mg Na-tetradecanoate (**s3, g3**), and 80 mg Na-tetradecanoate (**s4, g4**). **SP** = standard proteins.

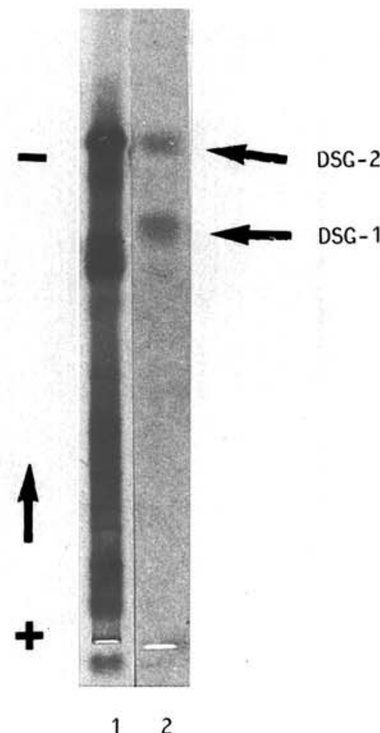

Fig. 5. Acidic polyacrylamide gel electrophoresis of the durum wheat sulfur-rich glutenin fractions solubilized with 66 mg (**1**) and 2.5 mg (**2**) Na-tetradecanoate.

1981); however, amino acid compositions of DSG-1 and DSG-2 differ considerably. Moreover, CM-proteins (Rodriguez-Loperena et al 1975, Salcedo et al 1978) and S proteins (Zawistowska and Bushuk 1986) are soluble in 70% ethanol, whereas in our experiments the DSG fractions were not extractable with ethanol from either gluten or semolina. Thus, DSG-1 and DSG-2 appear to represent a unique protein type.

Our results also showed that Na-tetradecanoate, at low concentration, preferentially interacts with the DSG fraction of glutenin proteins. This suggests that DSG proteins, like ligolin, CM proteins, and S proteins, may interact strongly with lipids. Further separation and characterization of DSG-1 and DSG-2, which is in progress, should reveal further information on their lipid binding, functional and chemical properties.

ACKNOWLEDGMENT

The authors gratefully acknowledge J. Landry (Laboratoire de Chimie Biologique, INRA, 78850 Thiverval-Grignon, France) for performing the amino acid analyses.

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[Received February 5, 1987. Revision received August 21, 1987. Accepted August 31, 1987.]