SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS OF REDUCED GLUTENIN OF DURUM WHEATS OF DIFFERENT SPAGHETTI-MAKING QUALITY[1]

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ABSTRACT

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of reduced durum wheat glutenins that had been purified by fractional salt precipitation of the AUC (acetic acid, urea, and cetyltrimethylammonium bromide)-soluble flour proteins revealed distinct varietal differences in the molecular-weight (mol wt) distribution and relative concentration of the six largest subunits. These differences appeared to be related to the spaghetti-making quality of the variety from which the glutenin had been isolated. The glutenin of those varieties with excellent spaghetti-making quality had more of subunit 6 (mol wt 53,000) than subunit 5 (mol wt 60,000). Those varieties with low or mediocre spaghetti-making quality appear to have less subunit 6 relative to subunit 5.

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The usefulness of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for examining glutenin subunits obtained after reduction of disulfide bonds has been demonstrated by recent publications (1–4). Using this technique, Bietz and Wall (1) showed that the reduced glutenins of a number of classes of hexaploid bread wheat had very similar patterns. However, the one durum wheat examined in their study was deficient in two high-molecular-weight (mol wt) subunits. These workers (1,2) and others (3) have suggested that the absence of these subunits in durum wheats could be related to their lack of breadmaking quality. The possible relationship of the durum wheat glutenin subunit structure to spaghetti-making quality has not been investigated.

Recent work (5–8) on durum wheat proteins and studies on their relation to spaghetti-making quality have shown that the gluten proteins appear to be very important in controlling the quality of pasta products. Moreover, the same studies have suggested that the glutenin fraction may be responsible for controlling such quality parameters as farinograph dough development time, tolerance index, and spaghetti firmness. This article reports results of an SDS-PAGE study of reduced glutenins of 15 durum wheat varieties of different quality and is an extension of the companion article (8) on gel filtration studies of the proteins of the same varieties.

MATERIALS AND METHODS

Wheat Samples and Quality Data

The wheat varieties used and their pedigrees are given in Table I of the previous publication (8). Quality data for the varieties is given in Table II of the same publication (8).

Milling

The wheats were milled into flour on a Brabender Quadrumat Junior mill following overnight tempering to 16.5% moisture.

Extraction and Purification of Glutenin

Flour proteins were extracted with AUC (0.1*M* acetic acid, 3*M* urea, and 0.01*M* cetyltrimethylammonium bromide) following the method of Bushuk and Wrigley (9). Extracted glutenin was purified by fractional salt precipitation of the AUC-soluble proteins (4).

SDS-PAGE

The method of Weber and Osborn (10) as applied to glutenin by Bietz and Wall (1) and to slab gels by Orth and Bushuk (3) was used to determine the number and molecular weight of the subunits of each glutenin preparation after reduction with β-mercaptoethanol. After electrophoresis, protein bands were stained with Coomassie brilliant blue and photographed on high-contrast copy film (64 A.S.A.). The gels were also scanned on a Joyce-Loebl Chromoscan Scanning densitometer (Model MK.11).

RESULTS AND DISCUSSION

In order to highlight the subunit bands that are important to the discussion, both the densitometer profiles and schematic representations of the bands will be

presented. The subunits will be identified by their molecular weights and numbered in order of decreasing molecular weight. Those bands (subunits) not relevant to this discussion will not be identified. Use of the same number in discussing particular subunits does not imply that the subunit is identical in a different preparation. The numerical system used simply gives the order of the bands from top to bottom, and does not imply that bands of equal molecular weight are identical or consist of a single component.

The SDS-PAGE patterns of the reduced glutenins prepared from the 15 varieties are shown in Fig. 1 in order of decreasing overall spaghetti-making quality. Detailed examinations of the gel patterns revealed that although the number of subunits was relatively constant (about 14 for all 15 varieties), there were distinct varietal differences among the glutenin subunit patterns. The differences were particularly evident in the intensity and the molecular-weight distribution of the first six subunits. It was possible to classify the 15 varieties into three groups on the basis of gel patterns and densitometer profiles of the six largest glutenin subunits. This classification, in general, parallels the classification of the varieties by subjective and objective technological tests into three groups having excellent, intermediate, and mediocre spaghetti-making quality (8). The first six subunits were the only bands that showed significant variations in molecular weight and/or intensity; accordingly, these were used in the investigation of the possible relationship between the SDS-PAGE properties and spaghetti quality.

Those varieties which had the best overall spaghetti-making quality, L 592, Candealfen, Pobulacion Tangarog, and Tunisian, had glutenin subunits with the following traits in common:

1. The first two glutenin subunits (bands), 1 (mol wt 117,500) and 2 (mol wt 97,000), of all four varieties in this group had the same molecular weight.

2. The intensity of subunit 6 (mol wt 53,000) was greater than subunit 5 (mol wt 60,000). The densitometer profile of reduced L 592 glutenin (Fig. 2) is typical of the varieties of this category.

No information as to pedigree was available on the four varieties of this high-quality group. It is not known if any of them are related genetically.

DT 316, DT 406, Pelissier, DT 332, DT 412, and Hercules had intermediate spaghetti-making quality based on technological tests (8). The SDS-PAGE patterns of glutenin subunits of these varieties had the following characteristics in common:

1. The first four glutenin subunits 1 (mol wt 126,000), 2 (mol wt 115,000), 3 (mol wt 105,000), and 4 (mol wt 93,000) had the same molecular weights for all six varieties. The difference in molecular-weight distribution of the high-molecular-weight subunits was the most striking difference between this and the high-quality group (compare results for L 592 and DT 316, Fig. 2).

2. The molecular weights of subunits 5 (mol wt 60,000) and 6 (mol wt 53,000) of this quality group were identical to the molecular weights of analogous subunits in the high-quality group. Also, the intensities of these subunits in this quality group were approximately the same as subunits 5 and 6 of the high-quality group. However, in the case of DT 412 (not shown), the next lowest quality variety in the intermediate quality group, subunit 5, was becoming more prominent. This increase of subunit 5 appears to be a trend in the transition from high to low spaghetti-making quality varieties.

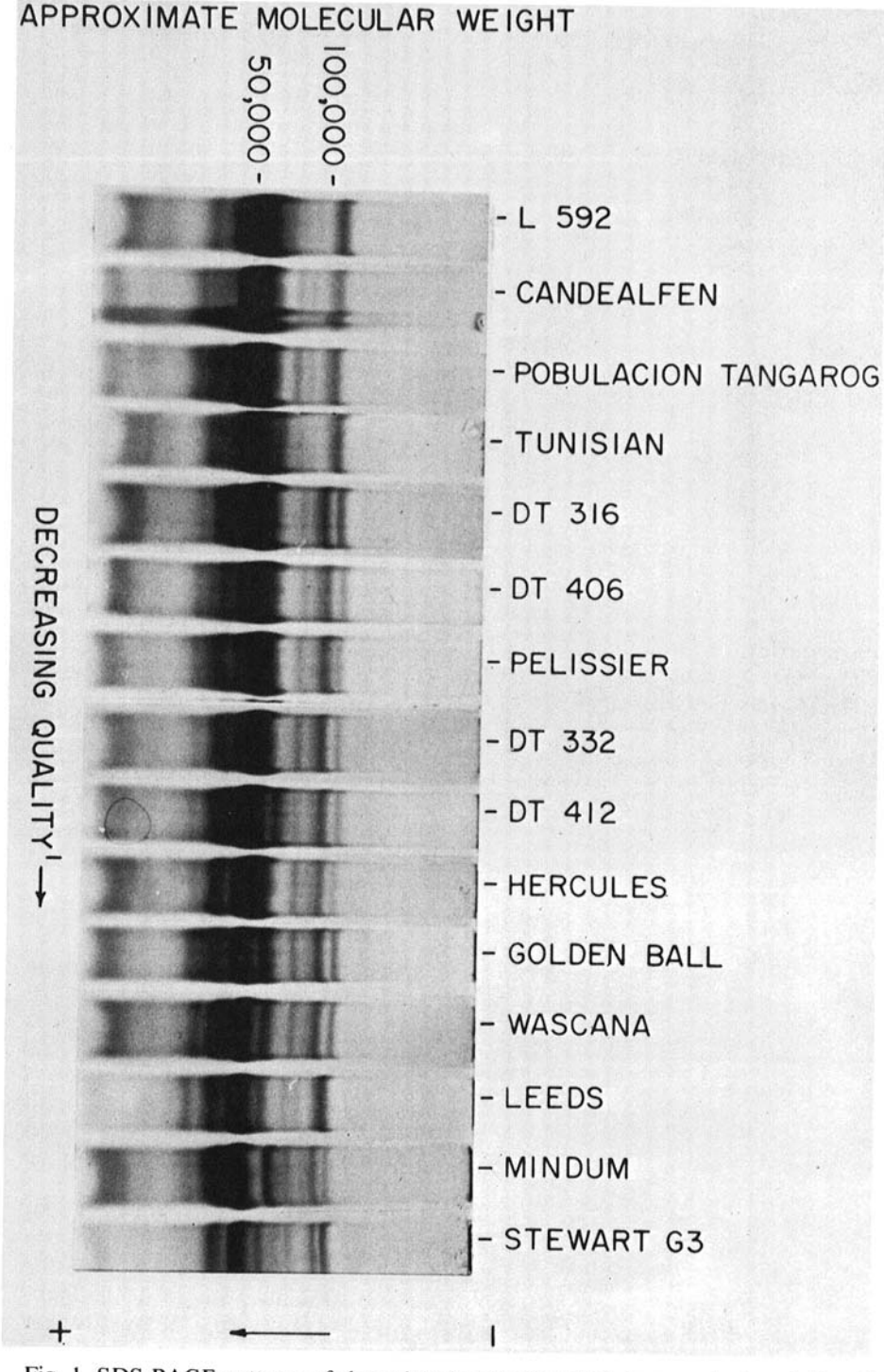

Fig. 1. SDS-PAGE patterns of the reduced glutenins of 15 durum wheat varieties of different spaghetti-making quality.[1] As determined by standard quality test methods.

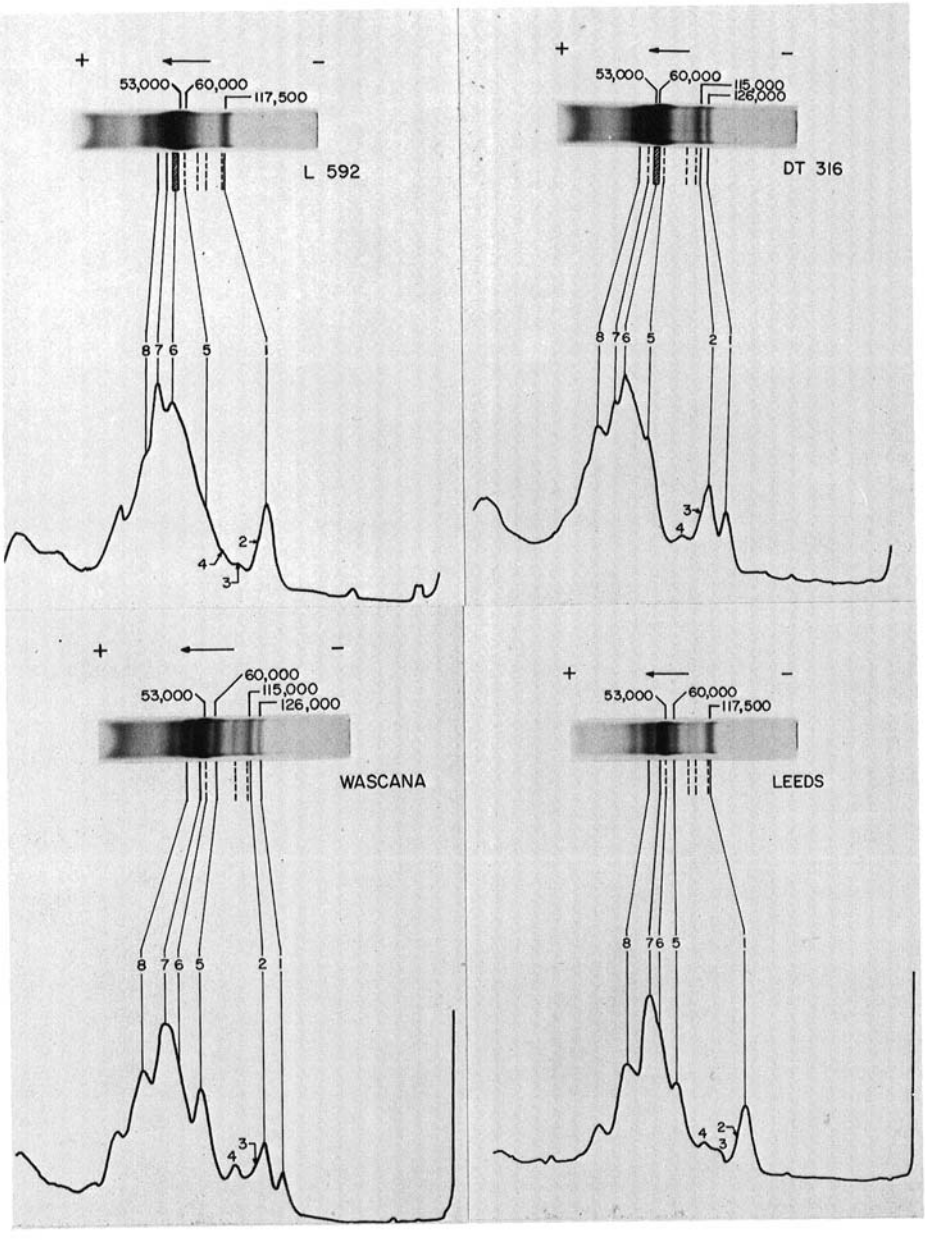

Fig. 2. SDS-PAGE patterns of reduced glutenin and densitometric records of a variety with excellent spaghetti-making quality (L 592), a variety with intermediate spaghetti-making quality (DT 316), and two varieties with mediocre spaghetti-making quality (Wascana and Leeds).

All varieties in this group, except Pelissier, are genetically related. Pelissier's parentage is not known except that it was originally imported from Algeria.

Varieties that were rated as having mediocre spaghetti-making quality on the basis of technological tests (Golden Ball, Wascana, Leeds, Mindum, and Stewart 63) had glutenin subunit patterns with similar features. The SDS-PAGE patterns (Fig. 1) of these varieties, except Leeds, which will be discussed separately, have two characteristics in common:

1. The first six glutenin subunits had the same molecular weights as the first six subunits of varieties in the intermediate quality group.
2. The intensity of subunit 6 (mol wt 53,000) was only slightly greater than or equal to the intensity of subunit 5 (mol wt 60,000). For most of the varieties in this group, subunit 5 appeared as a distinct peak, whereas in the patterns of the varieties of the other two quality groups, peak (band) 5 was not resolved from peak (band) 6. It cannot be stated with certainty if the resolution of subunits 5 and 6 in this group is due to differences in either or both subunits.

Of this group of varieties, Wascana, Mindum, and Stewart 63 are genetically related, not only to each other but also to some of the varieties in the intermediate quality group. The pedigree of the variety Golden Ball is not known. DT 316 (intermediate quality) and Wascana (mediocre) (Fig. 2) are selections from the same cross. However, their spaghetti-making quality and SDS-PAGE patterns were quite different. Wascana glutenin appears to have more of subunit 5 than DT 316 glutenin.

The SDS-PAGE pattern of Leeds glutenin (Fig. 2) is qualitatively similar to the pattern characteristic of the high-quality group of durum wheats (compare results for Leeds and L 592, Fig. 2). However, the concentration of subunit 6 in Leeds glutenin is much lower than that of the equivalent subunit of the high-quality varieties (Fig. 2, compare Leeds with L 592 and DT 316). This might account for its inferior quality relative to the high-quality group. These data suggest that the low-molecular-weight subunits may have a greater effect than the high-molecular-weight subunits on the functional properties of glutenin in the spaghetti-making process.

The precise relation between spaghetti-making quality and the first 6 subunits remains to be established. It is possible that the first six subunits of durum wheat glutenin could function in a manner similar to that postulated by Huebner *et al.* (11) for bread wheat glutenin. These workers suggested that the high-molecular-weight subunits of glutenin could be vital to the formation of the insoluble residue protein (insoluble glutenin) of bread wheats. Residue protein is important for determining the breadmaking quality of bread wheats (12). An alternate hypothesis might be that the first six glutenin subunits are involved in the formation of a specific conformation which has an effect on spaghetti-making quality. These speculations require further investigation.

Literature Cited

1. BIETZ, J. A., and WALL, J. S. Wheat glutenin subunits: Molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cereal Chem. 49: 416 (1972).
2. BIETZ, J. A., HUEBNER, F. R., and WALL, J. S. Glutenin—the strength protein of wheat flour. Baker's Dig. 47: 26 (1973).
3. ORTH, R. A., and BUSHUK, W. Studies of glutenin. II. Relation of variety, location of growth,

and baking quality to the molecular weight distribution of subunits. Cereal Chem. 50: 191 (1973).
4. WASIK, R. J., and BUSHUK, W. Studies of glutenin. V. Note on additional preparative methods. Cereal Chem. 51: 112 (1974).
5. MATSUO, R. R., and IRVINE, G. N. Effect of gluten on the cooking quality of spaghetti. Cereal Chem. 47: 173 (1970).
6. MATSUO, R. R., BRADLEY, J. W., and IRVINE, G. N. Effect of protein content on the cooking quality of spaghetti. Cereal Chem. 49: 707 (1972).
7. WALSH, D. E., and GILLES, K. A. The influence of protein composition on spaghetti quality. Cereal Chem. 48: 544 (1971).
8. WASIK, R. J., and BUSHUK, W. Relation between molecular-weight distribution of endosperm proteins and spaghetti-making quality of durum wheats. Cereal Chem. 52: 322 (1975).
9. BUSHUK, W., and WRIGLEY, C. W. Glutenin in developing wheat grain. Cereal Chem. 48: 448 (1971).
10. WEBER, K., and OSBORN, M. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406 (1969).
11. HUEBNER, F. R., DONALDSON, G. L., and WALL, J. S. Wheat glutenin subunits. II. Compositional differences. Cereal Chem. 51: 240 (1974).
12. ORTH, R. A., and BUSHUK, W. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49: 268 (1972).

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