NOTE

PROTEINS

Factors Affecting the Extractability of the Glutenin Macropolymer

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The poor extractability of wheat proteins is a major concern of many cereal chemists and a serious drawback for wheat protein research. Complete extraction is possible using reducing agents in combination with denaturing agents. This method, however, is detrimental for the polymeric nature of the protein. Some research (Singh et al 1990, Gao and Bushuk 1992) has shown that nearly complete extraction of protein is possible without using reducing agents. We regard their statements, that no reduction takes place in the polymer using their procedures, with some caution and concern. In our hands, we have observed that virtually no depolymerization or increased extractability of the isolated glutenin macropolymer (the sodium dodecyl sulfate [SDS] unextractable glutenin polymers) occurred over a period of six months. At this point, we still leave open to discussion whether or not the glutenin macropolymer is a covalently cross-linked polymer or a noncovalently linked and highly entangled polymer. In this view, depolymerization can be either splitting of covalent bonds or disentanglement, or both. (See also discussion by Tsen 1967).

To investigate the effect of several treatments on the polymeric state of the glutenin, we isolated the glutenin macropolymer from an overstrong cultivar (Fresco) and a medium quality cultivar (Camp Remy) according to Graveland et al (1980) by making a suspension of the flour in 1.5% (w/v) SDS in a 1:20 (w/v) ratio. This suspension was centrifuged for 30 min at 23,500 × *g* in a Sorvall RC-5B Refrigarated Superspeed centrifuge. The glutenin macropolymer was gently scraped off the starch pellet, mixed gently with 1.5% (w/v) SDS, and centrifuged as before. The glutenin macropolymer was scraped from the small remaining starch pellet, resulting in washed glutenin macropolymer. This procedure was followed to remove most of the SDS-extractable material from the polymer, because this could affect the stability of the polymer. In one series, the first supernatant was added back to the washed glutenin macropolymer. In the other, 1.5% SDS solution was added instead. The glutenin macropolymer suspensions were treated according to Gao and Bushuk (1992) in urea-SDS at 55°C for 24 hr according to Singh et al (1990) by tip-probe sonication for 30 sec and by several other treatments (Table I).

The results show that the treatments increase the extractability to various extents. Mild sonication for 30 min or 24 hr standing at ambient temperature do not have an effect on the extractability. Stirring for 24 hr or shaking for 60 min increase the extractability by 25% at best. Boiling and 30 min of Polytron treatment increase the extractability of glutenin macropolymer from Fresco by 30% at maximum. In contrast, the glutenin macropolymer from Camp Remy becomes 63–80% extractable in 1.5% SDS after these treatments. Mechanical treatments with a high energy output or treatment with 6*M* urea and 6% SDS increase the extractability of the glutenin macropolymer of both cultivars by 45–79%. It is likely that the mixture of 6*M* urea and 6% SDS increases the extractability by an improved unfolding and complexation of SDS and breaking of hydrogen bonds (Gao and Bushuk 1992), and not by depolymerization. However, in contrast to the findings of Gao and Bushuk (1992), no complete extractability was achieved. In contrast to the urea-SDS extraction, the treatments using high mechanical shear are likely to increase the extractability of the polymer by splitting covalent bonds. Although Singh et al (1990) claim that peptide bonds are broken only after prolonged sonication, their results (Singh et al 1990, Fig. 3 therein) show that the SDS-polyacrylamide gel electrophoresis (PAGE) bands of the high-molecular mass glutenin subunits decrease in intensity after 30 sec of sonication. The disappearance of the high-molecular mass glutenin subunits is according to the postulation of MacRitchie (1975) that under shear the largest polymers are depolymerized first. From polymer chemistry, we know that covalent bonds of synthetic polymers are readily mechanochemically degraded by shearing forces, e.g. by ball milling, slicing, sawing, high-speed stirring, or ultrasonic energy (Sohma 1989). Because disulphide bonds are weaker than peptide bonds (MacRitchie 1975), it is likely that tip-probe sonication for as short as 15 sec results in cleavage of disulphide bonds as well.

On the other hand, Table I shows that in the SDS-extractable part of flour, components such as glutathione or cysteine (Sarwin et al 1992) are not able to depolymerize the glutenin macropolymer in 1.5% SDS. In contrast, the results indicate that the SDS-extractable part is counteracting the increase in extractability. One could speculate that there are also "active components" present in flour, such as proposed by Graveland et al (1980, 1984), which are actively cross-linking the proteins or protecting it against depolymerization.

On the basis of these observations, we conclude that the covalent bonds in the glutenin macropolymer are important for its poly-

TABLE I
Effect of Various Treatments on the Extractability of the Glutenin Macropolymer

	Glutenin Macropolymer Remaining After Treatment (%)[a]			
	1.5% SDS		First Supernatant	
Treatment	Fresco	Camp Remy	Fresco	Camp Remy
No treatment (direct)	100	100	100	100
Standing, 24 h	99	100	110	105
Sonication bath, 30 min	100	97	110	102
Magnetic stirring, 24 hr	81	75	95	78
Shaking, 60 min	75	nd[b]	97	nd
Urea-SDS, 55°C, stirring, 24 hr	nd	21	47	33
Boiling, 10 min	75	20	103	nd
Polytron, 30 sec	78	37	70	33
Sonication tip, 30 sec	36	31	55	nd

[a] Glutenin macropolymer (1.5 g, containing 3.01, 2.89, 3.53, and 3.30% protein, respectively; N × 5.7) treated in sodium dodecyl sulfate (SDS) solution or supernatant (7 ml). Remaining gel recovered by centrifugation for 10 min at 15,000 × *g*. The standard error of the determination was in general less than 5%.
[b] Not determined.

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meric nature. They are probably more important than noncovalent entanglements. This is in contrast to the view of Eckert et al (1993), who speculates that the polymeric nature of the glutenin macropolymer is merely by entanglements. Nevertheless, even if covalent bonds were not important, the polymer is entangled in such a way that disentanglement of the SDS-unextractable polymer is difficult to achieve by relatively mild methods. Finally, we want to stress that the glutenin macropolymer is indeed an extremely large and, therefore, unique polymer that depolymerizes by cleavage of covalent bonds when treated with sufficient mechanical shear.

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