

## Isolation of Wheat High Molecular Weight Glutenin Subunits from Durum Wheat

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Durum wheat is an important crop used for the production of pasta, couscous, and some types of bread. The fact that durum wheat can be used for such purposes is due to the unusual biochemical characteristics of gluten proteins, which form a network in doughs by giving them viscoelastic properties. Payne et al (1987) showed an association between specific high molecular weight glutenin subunits (HMW-GS) and the quality of the flours in bread wheats. A similar relationship between HMW-GS and flour quality has been demonstrated in durum wheats (Boggini and Pogna 1989, Liu and Rathjen 1996). There are currently large markets for durum wheat, both for domestic consumption and for export to developing countries (Liu et al 1996). Screening for good quality HMW-GS is thus important for wheat quality assessment and has been performed by SDS-PAGE (Payne et al 1987, Kasarda et al 1998), reverse-phase HPLC (Marchylo et al 1989), and capillary electrophoresis (Sutton and Bietz 1997).

Some methods of extraction and purification of glutenins from bread wheats have been published whose aim was to separate HMW-GS from low molecular weight glutenin subunits (LMW-GS) (Marchylo et al 1989, Mélas et al 1994, Larré et al 1997, Verbruggen et al 1998). We have applied the most recent method (Verbruggen et al 1988) to purify the HMW-GS from flours of durum wheats. However, our results revealed a high contamination of the HMW-GS fraction by other proteins in relation to the cultivar used. In this article, we therefore provide a modified procedure for extracting HMW-GS from durum wheats.

### MATERIALS AND METHODS

The samples used in this study were the Italian durum wheat cultivars Cirillo, Colosseo, Creso, Grazia, and Solex. Seeds were ground in a break roller-mill (Labormill 4RB, Italy) and the flour was stored at 4°C in the dark. Two different isolation procedures for HMW-GS were used.

Procedure 1 was based on a modification to the method of Marchylo et al (1987). All extractions were done at 60°C in screw-capped Teflon-coated centrifuge tubes (28.5 × 104 mm) by continuously agitating the samples with a Teflon-coated stirring paddle at 200 rpm. The flour (1.5 g) was routinely extracted 2× with 0.5M NaCl (7.5 mL) for 30 min. The suspension was then centrifuged for 10 min at 10 000 × g and the precipitate was extracted 2× with 50% (v/v) propan-1-ol (7.5 mL). The suspension was centrifuged as above. The precipitate was extracted with 50% (v/v) propan-1-ol containing 1% (w/v) dithiothreitol (DTT) for 30 min and the suspension was centrifuged for 30 min at 10,000 × g. The supernatant was adjusted to 60% (v/v) propan-1-ol, mixed, and then rested for 1 hr at 4°C. The precipitate was collected by

centrifugation (10,000 × g for 30 min), resuspended with 1 mL of 60% (v/v) propanol-1-ol, placed in a fresh micro test tube, vigorously mixed, and recentrifuged as above. The pellet was freeze-dried. In some experiments, Procedure 1 was applied to chloroform-defatted flours according to Verbruggen et al (1998).

Procedure 2 was based on the method of Verbruggen et al (1998). SDS-PAGE was done using 11% resolving gel and 4% stacking gel. Freeze-dried HMW-GS (1 mg) were resuspended in a 0.5 mL of 62 mM Tris-HCl, pH 6.8, buffer containing 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 0.1% bromophenol blue, boiled for 3 min, and loaded (2 µg) on SDS slab gels. The BDH calibration kit of molecular weight standards was applied to the gels. The gel separation was performed in mini-gel apparatus (C.B.S. Scientific Co., Solana Beach, CA). Runtime was 30 min at 150V and then 50 min at 200V. The gels were stained overnight with Coomassie brilliant blue R250, destained with 7.5% acetic acid, and then scanned and processed by a digital image analysis program (SigmaGel, Jandel Corporation, San Rafael, CA).

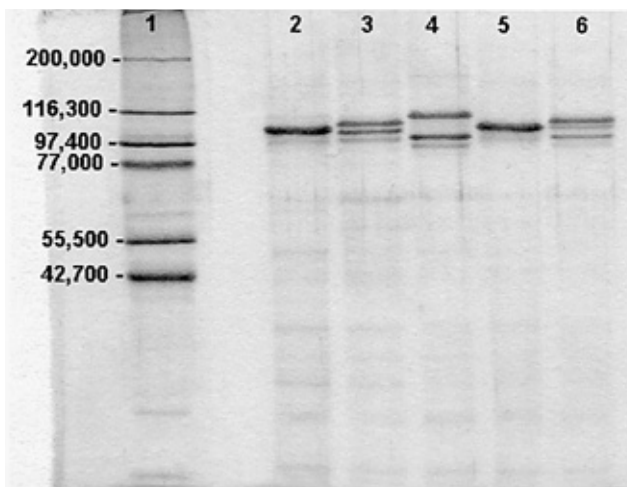
### RESULTS AND DISCUSSION

Although durum wheat has been and continues to be used in the formulation of several types of bread in southern Italy, durum wheat cultivars with satisfactory characteristics as well as an acceptable pasta-making quality are still not available. To establish the breadmaking quality, several Italian durum wheat cultivars have been studied, and the related influence of HMW-GS has been determined (Boggini and Pogna 1989). The cultivars examined in this study were chosen because they are widely used in Italy and also because of their different HMW-GS compositions and consequent capacity to affect breadmaking quality. In particular, according to Payne et al (1987), Boggini and Pogna (1989), and N. E. Pogna (*personal communication*), they can be assigned as follows: Cirillo 20, Colosseo 13+16, Creso 6+8, Grazia 20, and Solex 15\*+16\*. Cultivars that have subunit 7+8 are known to produce higher bread volumes than cultivars with either subunit 20 or subunit 6+8 (Boggini and Pogna 1989).

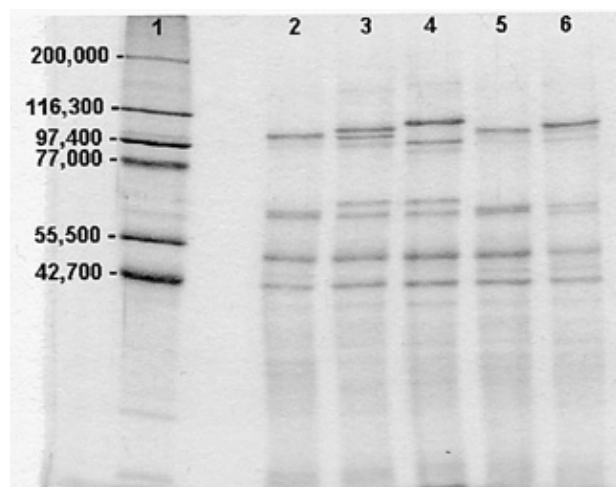
Figures 1 and 2 illustrate the protein patterns we obtained for the HMW-GS extracted from the five cultivars by Procedures 1 and 2, respectively. The main differences between the two procedures were that the flours were not defatted and preextracted by salt and that an additional extraction with propan-1-ol was performed in Procedure 2. Furthermore, the alcoholic extraction was performed at 60°C in Procedure 1 and at 30°C in Procedure 2. The defatting treatment proved to be unable to modify the electrophoretic pattern of the extracted proteins (data not shown). The preextraction with salt of the flours was performed to remove salt-soluble proteins and to modify gluten protein properties that were altered significantly upon the addition of salt (Fu et al 1996). A visible manifestation of the changed gluten protein conformation following salt treatment was the considerable swelling of the residue with 50% propan-1-ol. Extraction with 50% propan-1-ol was performed to remove gliadin, while extraction of the residue with

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**Fig. 1.** SDS-PAGE (11% T; 1% C) of high molecular weight glutenin subunits extracted from five Italian cultivars of durum wheat with Procedure 1. Lanes 1–6, respectively: molecular weight standards, Cirillo, Colosseo, Creso, Grazia, Solex. Gel dimensions: 1 × 110 × 80.



**Fig. 2.** SDS-PAGE (11% T; 1% C) of high molecular weight subunits extracted from five Italian cultivars of durum wheat with Procedure 2. Lanes 1–6, respectively: molecular weight standards, Cirillo, Colosseo, Creso, Grazia, Solex. Gel dimensions: 1 × 110 × 80.

50% propan-1-ol containing 1% DTT solubilized primarily HMW-GS and LMW-GS (Marchylo et al 1989). These proteins were selectively precipitated by increasing the alcoholic solution. Densitometric analysis of the area of HMW-GS and total area of glutenin subunits, obtained with Procedure 1, showed a high purity of the HMW-GS fraction depending on the cultivar used. The contamination was higher ( $\approx 10\%$ ) for Cirillo and Grazia and lower ( $\approx 5\%$ ) for Colosseo, Creso, and Solex. The same analysis performed on the HMW-GS extracted with Procedure 2 revealed high contamination at 70–90% depending on the cultivar used. The major contaminating proteins, in accordance with their electrophoretic mobility, were gliadins and LMW-GS.

These results clearly demonstrate that the method of Verbruggen et al (1998), which was devised for the isolation of HMW-GS from common wheat, is not applicable to durum wheat. The difference in performance of the two procedures might be due to the inclusion of the salt preextraction in Procedure 1 or to the highest temperatures used during salt and alcoholic extractions. We investigated both possibilities by performing Procedure 1 without salt preextraction and Procedure 2 with salt preextraction at 30°C. The contamination of the HMW fraction increased in Procedure 1 and was high in Procedure 2 (data not shown). This indicates that the temperature had a great influence during the preextraction with salt and in the following extraction steps. Three combinations of temperatures were used: 1) salt preextraction at 30°C followed by propan-1-ol extraction at 60°C; 2) salt preextraction at 60°C followed by propan-1-ol extraction at 30°C; 3) salt preextraction at 60°C followed by propan-1-ol extraction at 60°C. The electrophoretic analyses (data not shown) indicated that the beneficial effect of the salt preextraction on contamination by non-HMW proteins was obtained only if this step and the alcoholic extractions were performed at 60°C. A combined effect of salt preextraction and temperature during salt and alcohol extractions appears to modify the extractability of the gluten. Although the method of preparation of purified subunits of glutenin presented in this article was on an analytical level, we also proved it to be effective on a preparative level as well. In conclusion, we think that the good recovery of purified HMW-GS makes this method suitable for isolation from durum wheat flour. It may be especially useful when HMW-GS are required without alkylation for use in physicochemical and technological experiments.

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