# The Role of Low Molecular Weight Glutenin Proteins in the Determination of Cooking Quality of Pasta Products: An Overview[1]

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**ABSTRACT**

Low molecular weight glutenin (LMWG) proteins are those proteins that correspond to large aggregates in wheat and upon reduction yield subunits with apparent molecular weights of 12,000–60,000. Estimation of their content, by combining sequential extraction, chromatography, and electrophoresis, showed that they make up a major fraction of durum wheat gluten and that their content in durum wheat of γ-45 type is higher (28%) than in the γ-42 type (15%). The discovery of a recombination within the *Gli-B1* locus between γ-gliadins and LMWG indicated that γ-gliadins 42 and 45 are only genetic markders of pasta firmness and elasticity. LMWG strongly aggregate through heat treatments and contribute to pasta firmness and elasticity. Sulfur-rich glutenin proteins were also found associated to surface condition of cooked pasta, and a new model was proposed to explain their contribution to the aggregation of LMWG through hydrophobic and disulfide bonds.

The cooking quality of durum wheat pasta products and the baking quality of common wheat flour depend mainly on gluten proteins (Feillet 1980, 1984).

Among gluten proteins, low molecular weight glutenins (LMWG) are subunits of large aggregates that, upon reduction with mercaptoethanol, yield polypeptides with apparent molecular weights of 12,000–60,000 (Khan and Bushuk 1979, Payne and Corfield 1979, Bietz and Wall 1980). They differ from high molecular weight glutenin (HMWG) subunits in size and because most LMWG are encoded by genes on the short arms of homoeologous group 1 chromosomes, whereas the genes encoding HMWG are located on the long arms of group 1 chromosomes (Payne et al 1984b).

LMWG subunits are not easily identified by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) because they overlap ω- and γ-gliadin bands. Nevertheless, because they are aggregative proteins (whereas gliadins are monomeric and nonaggregative), it has been possible to specifically identify LMWG subunits and to determine their contribution to the total protein pool of durum wheats by combining differences in solubility, ion-exchange chromatographic profiles and SDS-PAGE patterns (Autran et al 1987).

## CLASSICAL VIEWS ON THE GENETIC BASIS AND BIOCHEMICAL CONTROL OF PASTA COOKING QUALITY

Pasta cooking quality is related to three main groups of parameters: matter losses and water absorption (or swelling) during cooking, viscoelastic behavior and firmness after cooking, and pasta disintegration or surface condition of cooked pasta (Feillet 1986).

It is now well documented that firmness and surface condition are independent parameters (Autran et al 1986) and that viscoelasticity of cooked pasta correlates to protein content and to the γ-gliadin electrophoretic type. Glutens from durum varieties with the γ-45 component have high firmness and viscoelasticity. These varieties yield pastas that, after cooking, are rated good or very good in firmness. An opposite situation prevails with pasta processed from varieties containing the γ-42 component (Damidaux et al 1980).

γ-Gliadins are under the genetic control of the *Gli-B1* locus, a family of closely linked genes located on the short arm of the 1B chromosome; this locus also contains the genes that code for ω-gliadins and LMWG (Payne et al 1984b, Shewry et al 1986, Payne 1987). Unlike the *Glu-B1* locus coding for HMWG, for which 13 different alleles were identified (Branlard et Autran, *unpublished*) and for which associations between allelic types and pasta quality are still controversial (Autran and Feillet 1987, du Cros 1987), the *Gli-B1* locus has two major known allelic types in the world collection: the allele "42", that codes for γ-gliadin 42, ω-gliadins 33-35-38, and the LMWG quadruplet referred to as LMW-1; and allele "45", that codes for γ-gliadin 45, ω-gliadin 35, and the LMWG triplet referred to as LMW-2 (Autran and Berrier 1984, Payne et al 1984a).

This raises the question as to whether good pasta quality, which was formerly associated with γ-gliadin 45, is actually caused by the presence of this protein or whether it is due to the closely linked genes coding for ω-gliadin 35 or LMW-2 (Payne et al 1984a).

Despite biochemical and physicochemical studies of gliadins 42 and 45 that have led to contradictory hypotheses (Godon and Popineau 1981; Cottenet et al 1983, 1984), it was not clear which proteins directly influence quality since genetic recombination at *Gli-B1* was not observed until recently. The discovery of a variety, Berillo, that contained γ-gliadin 42, ω-gliadin 35, and LMW-2 (the LMWG type linked to gliadin 45) and had high gluten elastic recovery (Table I), supports the conclusion that gliadin 42 is only a genetic marker, and suggests that LMWG aggregative proteins are the direct causal agents of gluten viscoelasticity and firmness (Pogna et al 1988).

Should LMWG directly impart gluten characteristics, a further question will arise: Are the differences in gluten viscoelasticity due to differences in the amounts of LMWG, in their physicochemical and functional properties, or in both parameters?

## LMWG CONTENT

Autran et al (1987) determined the amount of LMWG in two varieties, Calvinor (type 42) and Agathe (type 45), by combining solvent extraction, ion-exchange chromatography, and densitometry of SDS-PAGE patterns. A higher content of glutenins and a lower content of gliadins in good-quality durum wheats compared with poor-quality durums were confirmed, but

**TABLE I**
**Low Molecular Weight (LMW) Glutenin Subunits Allelic Types and Gluten Elastic Recovery**

| Varieties | γ-Gliadins | ω-Gliadins | LMW Glutenin | Gluten Elastic Recovery |
|---|---|---|---|---|
| Type 42 | 42 | 33-35-38 | LMW-1 | low |
| Type 45 | 45 | 35 | LMW-2 | high |
| Berillo[a] | 42 | 35 | LMW-2 | high |

[a] Pogna et al 1988.

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only minor quantitative differences between γ-42 and γ-45 gliadins were found.

The contributions of the main types of glutenin subunits to the total protein content were 10.2 and 11.5% (HMWG) and 15.1 and 27.7% (LMWG) for varieties belonging to γ-type 42 and γ-type 45, respectively (Table II). Furthermore, when expressed as percent of total glutenin, the data demonstrated that the strong viscoelasticity of Agathe gluten was related to a high percentage of LMWG in glutenin. Conversely, HMWG occured in higher concentrations in glutenins of type-42 durum wheats (Table II).

A second approach consisted of extracting proteins by an SDS-phosphate buffer without reducing agents to solubilize mainly LMWG aggregates, along with gliadins and salt-soluble proteins, and to fractionate the supernatant by size-exclusion chromatography as described by Huebner and Bietz (1985).

Four peaks were obtained having molecular weights from 800,000 to 13,000 as determined by comparing elution characteristics to those of standard proteins (Fig. 1). SDS-PAGE showed that peaks 1 and 2 were mainly composed of aggregated LMWG. Most of the extracted HMWG were also present in peak 1 (Fig. 2).

The comparison of 20 durum wheat samples (four varieties, each grown at five locations) showed a highly significant correlation between contents of the first (P1) or second (P2) peaks, gluten firmness, and gluten elastic recovery (Table III). Furthermore, analysis of variance showed that the variability in P1, P2, and P3 contents was almost exclusively genetically determined, whereas growing location had no effect (Table III).

This relationship is shown in Figure 3, where the amount of P1 (in percent of total proteins soluble in SDS-phosphate buffer) is plotted against gluten elastic recovery.

On the basis of P1 content, durum wheats can be divided into two groups; most of those with a high P1 content are of gliadin type-45, whereas those with a low P1 content belong to gliadin type-42. Studies are in progress to further identify all proteins in P1 and attempt to explain their contribution to the viscoelastic behavior of gluten.

## FUNCTIONAL PROPERTIES OF LMWG

Temperature is an important parameter in pasta technology. Drying operations are frequently performed above 70° C, and during cooking pasta is left in boiling water for about 10 min (depending on shape). We therefore focused our investigations on the behavior of pasta proteins under well-defined hydrothermic conditions (Autran and Berrier 1984, Kobrehel et al 1985, Feillet 1987).

We first found that submitting pasta (30% moisture content) to a heat treatment for 120 min led to a steep decrease of solubility in SDS. Initial solubility was restored by further extraction with mercaptoethanol. This can be explained by formation of disulfide bonds between pasta proteins during heat treatment.

In another experiment, pasta having 24, 18, and 12% moisture contents was left for 2 hr at 90° C. The stronger the hydrothermic treatment (i.e., the pasta humidity), the larger the loss of solubility

**TABLE II**
**Protein Composition (%) of Durum Wheat**

| Component[a] | Cultivar Calvinor (γ-42 type) | Cultivar Agathe (γ-45 type) |
|---|---|---|
| Total wheat proteins | | |
| Salt-soluble proteins | 23.5 | 19.2 |
| Gliadins | 33.4[b] | 22.22[c] |
| HMWG subunits | 10.2 | 11.5 |
| LMWG subunits | 15.1 | 27.7 |
| Minor subunits | 8.2 | 10.3 |
| Insoluble | 9.6 | 9.1 |
| Total glutenin | | |
| HMWG subunits | 30.5 | 23.2 |
| LMWG subunits | 45.1 | 56.0 |
| Minor subunits | 24.4 | 20.8 |

[a] HMWG = High molecular weight glutenin; LMWG = low molecular weight glutenin.
[b] Including γ-42, 3.9%.
[c] Including γ-45, 2.6%.


**Fig. 1.** Size-exclusion high-performance liquid chromatography elution curve of sodium dodecyl sulfate-phosphate buffer extract of durum wheat, cultivar Kidur.

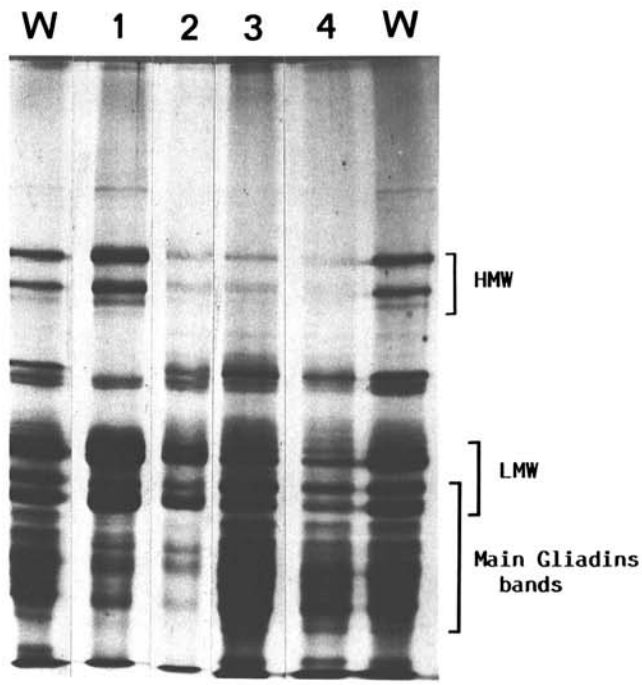


**Fig. 2.** Sodium dodecyl sulfate-polyacrylamide gel electrophoretic characterization of the four peaks obtained by size-exclusion high-performance liquid chromatography. W = whole extract; digits are peak numbers.

**TABLE III**
**Statistical Analysis of Size-Exclusion High-Performance Liquid Chromatography (HPLC) Data from Four Durum Wheat Cultivars Grown in Five Locations**

| HPLC Peaks | Correlation (r) with Gluten Property | | % of Variability Assignable to | | F Test | |
|---|---|---|---|---|---|---|
| | Firmness | Elastic Recovery | Variety | Growing Location | Variety | Growing Location |
| P1 | 0.81 | 0.88 | 91.0 | 1.4 | **[a] | NS[b] |
| P2 | 0.75 | 0.82 | 91.6 | 4.1 | ** | NS |
| P3 | −0.85 | −0.86 | 93.8 | 1.2 | ** | NS |

[a] ** = $P < 0.01$.
[b] Not significant.

in sodium myristate, which disrupts hydrophobic bonds. In addition, most residual proteins were soluble in mercaptoethanol.

PAGE in acidic buffer fractionations of unreduced protein extracts more accurately revealed which proteins aggregate during heat treatment. ω-Gliadins, which have a very low sulfur content, are very heat resistant (Wrigley et al 1980). Streaks and slot proteins, however, rapidly disappear upon heat treatment (Fig. 4).

To identify slot material, we sliced out the first millimeter of gel after the slot and dissolved the proteins in Tris-SDS-mercaptoethanol buffer. Most slot material consisted of proteins with molecular weights from 35,000 to 50,000, i.e., in the range of major LMWG subunits (Fig. 5). The results were confirmed by size-exclusion high-performance liquid chromatography of SDS-phosphate (unreduced) extracts (Fig. 6). All protein peaks decreased when the intensity of heat treatment was increased. The phenomenon especially affected peaks 1 and 2, which rapidly disappeared from the elution curves (Table IV). The heat sensitivity of LMWG aggregates was thus confirmed.

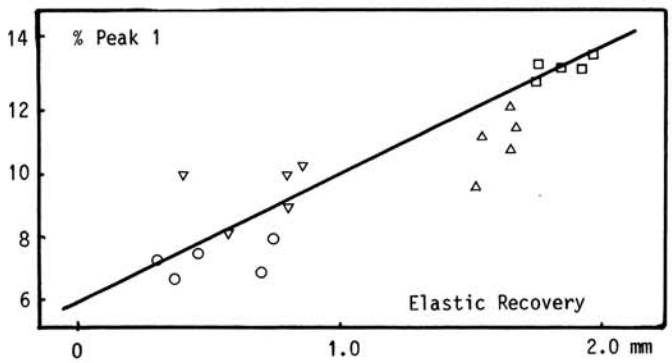


**Fig. 3.** Relation between percentage of sodium dodecyl sulfate-phosphate-soluble proteins of size-exclusion high-performance liquid chromatography peak 1 and gluten elastic recovery for 20 durum wheat samples.

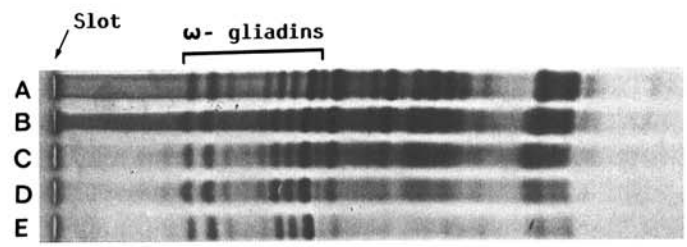


**Fig. 4.** Effect of heat treatments on low molecular weight glutenin aggregation: polyacrylamide gel electrophoresis in acidic buffer (pH 3.2) of chloro-2-ethanol-soluble proteins. Semolina (**A**); pasta dried at 55° C (**B**); pasta left for 2 hr at 90° C at 13% (**C**), 18% (**D**), and 24% (**E**) moisture content, respectively.

## ROLE OF DURUM WHEAT SULFUR-RICH GLUTENINS

Although different biochemical analyses (protein content, γ-gliadin type, SDS-sedimentation test) can predict firmness or viscoelasticity of gluten or cooked pasta (Damidaux et al 1980, Feillet 1984, Autran et al 1986), no equivalent method could, until now, specifically predict surface condition, the second parameter of pasta cooking quality. The finding of Kobrehel et al (1987, 1988) of a durum wheat sulfur-rich glutenin (DSG) permits further understanding of the biochemistry of the surface condition of cooked pasta.

DSGs were solubilized in a purified form by low concentrations of sodium myristate (2.5 mg per gram of flour) from flour previously treated with 0.5*M* NaCl and 60% ethanol. These proteins may also be solubilized by 0.01*N* acetic acid, but the extracts then also contain other proteins (mainly LMWG). DSG

**TABLE IV**
**Changes in the Aggregation Profile of Sodium Dodecyl Sulfate-Phosphate Soluble Proteins as Estimated by Size-Exclusion High-Performance Liquid Chromatography upon Heat Treatments (in % of total area under the elution curve)**

| | Peak | | | | |
|---|---|---|---|---|---|
| Sample | P1 (MW> 800)[a] | P2 (MW 250) | P3 (MW 43) | P4 (MW 13) | Others (MW< 13) |
| Semolina | 24 | 18 | 38 | 11 | 10 |
| Control pasta | 3 | 11 | 70 | 14 | 3 |
| Pasta left for 2 hr at 90° C | | | | | |
| 13% mc | 1 | 0 | 69 | 22 | 8 |
| 18% mc | 2 | 0 | 38 | 29 | 11 |
| 24% mc | 0 | 0 | 37 | 31 | 31 |

[a] Estimated molecular weight × $10^3$.

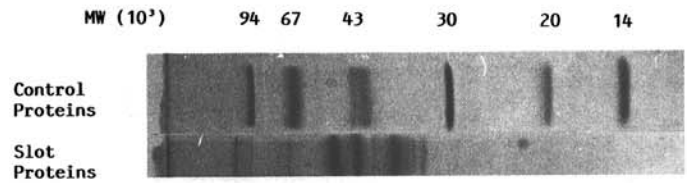


**Fig. 5.** Effect of heat treatments on low molecular weight glutenin aggregation: sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of reduced "slot proteins" (remaining in the sample well during PAGE in acidic buffer, pH 3.2).

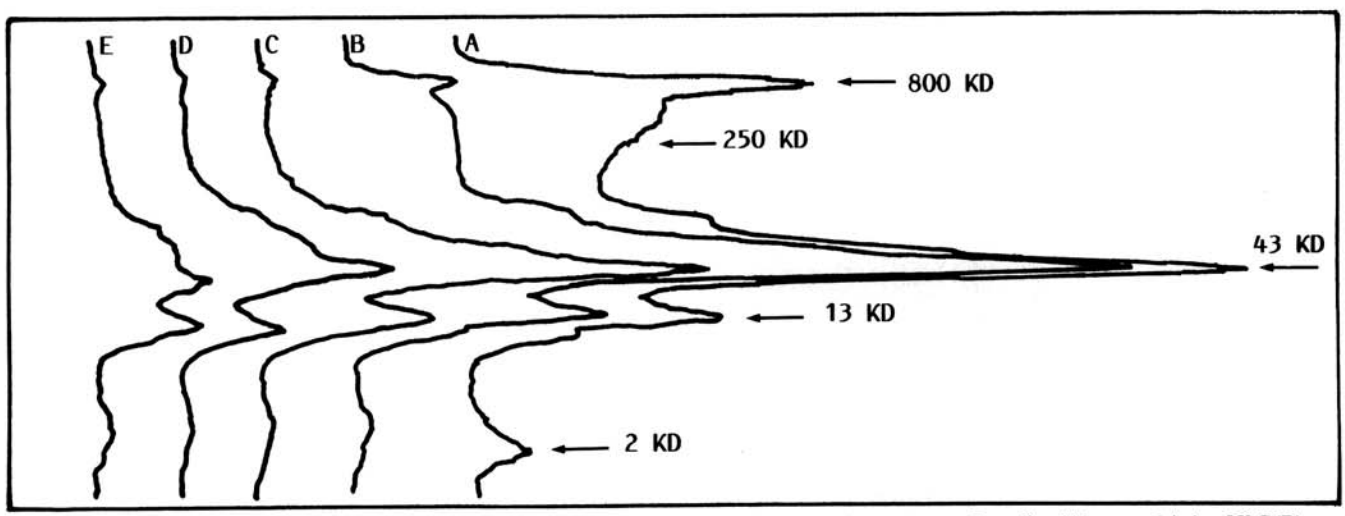


**Fig. 6.** Size-exclusion high-performance liquid chromatography of sodium dodecyl sulfate-phosphate extracts. Semolina (**A**); pasta dried at 55° C (**B**); pasta left for 2 hr at 90° C at 13% (**C**), 18% (**D**), and 24% (**E**) moisture content, respectively.

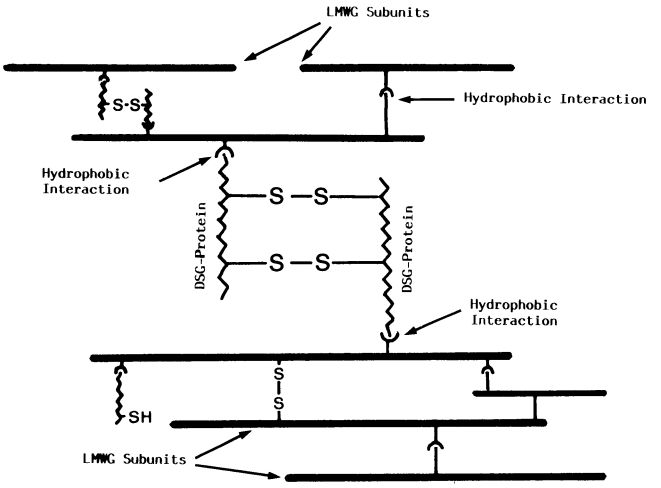


**Fig. 7.** Proposed role of DSG proteins in aggregation of low molecular weight glutenin (after Alary 1988).

proteins, as their solubility properties showed, are not disulfide cross-linked, either to HMWG or to LMWG subunits. However, they are part of durum wheat gluten proteins, and their amino acid composition is similar to the amino acid composition of other glutenins (Kobrehel et al 1988). The content of SH+SS groups in DSG ranged from 140 to 180 mol per gram of protein and was higher in durum wheat varieties with good cooking quality than in those of poor quality.

Alary and Kobrehel (1987) also found a highly positive correlation between content of SH+SS groups in glutenin and the surface condition of cooked pasta; higher SH+SS contents occurred with higher surface condition scores. Alary (1988) proposed that DSG played a functional role in preventing disaggregation of the cooked pasta surface. DSG proteins were not extractable from heat-treated pasta with sodium myristate, but they were extractable with mercaptoethanol. These results are in agreement with those reported by Jeanjean et al (1980) who identified, among others, a gluten protein referred to as "fraction D" that was extracted with mercaptoethanol from heat-treated gluten but was not extractable without a reducing agent. According to the electrophoretic mobility, the proteins of fraction D are likely to correspond to DSG proteins.

## CONCLUSIONS

Our results lead to six major conclusions: 1) γ-Gliadins-42 and γ-45 are only genetic markers of pasta firmness and elasticity. 2) The LMWG content of durum wheat is genetically controlled; two families of durum wheats that differ significantly in LMWG content have been identified. 3) LMWG content is responsible for the viscoelastic properties of heat-shaped gluten in durum wheat. 4) LMWGs strongly aggregate upon heat treatment, thereby contributing to pasta firmness and viscoelasticity. 5) Durum wheat gluten contains cysteine-rich proteins (DSG). 6) SH+SS content of DSG proteins and surface condition of cooked pasta are significantly correlated.

On the basis of physicochemical studies (changes in protein solubility upon pasta drying, SDS-PAGE, and size-exclusion high-performance liquid chromatography) we postulate that DSG proteins contribute to aggregation of LMWG (and possibly of HMWG) through hydrophobic and disulfide bonds. These bonds are sufficiently strong to prevent starch leaching during pasta cooking and to maintain a satisfactory surface condition of cooked pasta. We propose the model in Figure 7 to explain the contribution of the DSG-LMWG complex in promoting retention of good surface condition of pasta during cooking.

This model of the protein network in cooked pasta is based upon the hypothesis that 1) a tight protein network will prevent starch leaching during pasta cooking; 2) DSG and LMWG link through hydrophobic bonds that are disrupted easily by sodium myristate buffer but not by acetic acid (this hypothesis is sustained by our results of solubility and electrophoresis); and 3) DSGs are joined to each other through S–S bonds and, thereby, indirectly link LMWG molecules.

Our present investigations are aimed at solving several important problems: developing an accurate and rapid method to evaluate the LMWG content in durum wheats; verifying differences in LMWG content of type 42 and type 45 durum wheats for many samples; explaining how the amount of LMWG is genetically controlled; understanding the mechanism of LMWG aggregation upon heat treatment and quantifying the chemical bonds (S–S or hydrophobic?) involved; and isolating and sequencing genes encoding DSG and LMWG proteins.

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