MODIFICATIONS IN DURUM WHEAT PROTEIN PROPERTIES DURING PASTA DOUGH SHEETING[1]

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ABSTRACT

The effects of pasta dough sheeting on durum wheat protein properties were investigated. Dry gluten content was diminished (from 13 to 0%) by increasing the number of passes through sheeting rolls or by decreasing the roll clearance and the dough moisture content. Simultaneous changes in protein composition occurred—increases in the amount of gliadins and gel proteins, and decreases in the amount of 2-chloroethanol-soluble glutenins. A correlation between the rate of gluten recovery and the gel protein content was observed. Possible reorganizations in the protein network, involving breakage of the glutenin molecules by a stretching effect and disulfide bond interchanges, are discussed.

During pasta processing, the dough obtained by mixing water and semolina is shaped into a suitable form by extrusion through a die; it is forced by a kneading worm into the extrusion head, where the pressure may reach 150 kg/cm^2; consequently, as outlined by Nazarov (1), who observed that disulfide bonds increase during extrusion, the dough is submitted to high mechanical stresses which produce modifications in the protein network. In our experience, the gluten ball cannot be extracted by hand-washing from some commercial pasta products.

This study is the first step of a work aiming to elucidate the changes in properties of durum wheat semolina proteins during pasta extrusion and their consequences on pasta cooking quality. Dough development by extrusion is a very complex phenomenon where vacuum, temperature, pressure, and mixing effects are involved. The modification in protein properties under the effect of mechanical stresses was first examined. Due to the difficulties of controlling extrusion conditions, we simulated the mechanical effect of the extrusion process by sheeting the dough through two rolls.

MATERIALS AND METHODS

A typical durum wheat semolina (protein: 13.6% dry basis), untreated and industrially milled, was used in this investigation.

Dough Sheeting

Semolina and water were blended for 20 min in a small laboratory-made mixer (4-bladed, single-shaft trough mixer; 60 rpm); final moisture content ranged from 30 to 38% (30 to 38 g water/100 g of dough). The mix, which had a granular structure, was kneaded by hand, and then sheeted through a De Lellis sheeter (roll diameter, 10 cm; adjustable clearance, 40 rpm) from 0 to 35 times with various clearances of the rolls ranging from 0.35 to 3.00 mm, without folding the dough between two passes.

After sheeting, the dough was freeze-dried and ground before analysis.

[1]Partly presented at the 59th Annual Meeting, Montreal, Quebec, Oct. 1974.
[2]Also called 2-chloroethanol.

Extraction of Proteins

Albumins and globulins were extracted from 5 g of semolina or ground, freeze-dried dough by stirring with 30 ml of 0.5*M* NaCl (adjusted to pH 6.8 by disodium phosphate) for 1 hr at 4° C. After centrifugation (30 min; 38,000 × *g*), the residue was washed twice with 20 ml 0.5*M* NaCl.

Gliadins were then extracted by a 25-ml volume of 68% ethanol by allowing the mixture to stand overnight; the mixture was stirred for 2 hr before centrifuging, the residue was reextracted by a 25-ml volume of 60% ethanol with stirring for 1 hr, then washed twice with a 10-ml volume of 60% ethanol. Supernatants were pooled.

Following the gliadin solubilization step, two different extraction procedures were used:

1) The residue was thoroughly resuspended with the help of a glass rod in 25 ml of 70% glycolmonochlorydrin[2] (GMC; pH = 1.8) in water and allowed to rest overnight. After a first extraction (2-hr stirring), the insoluble residue was reextracted under similar conditions immediately, then washed twice with 20 ml 70% GMC. Supernatants were pooled and referred to the glutelin fraction (2).

2) The residue was extracted twice with 50 ml of 0.025*M* borate buffer, pH = 10, containing 0.5% sodium dodecylsulfate (SDS), then by 50 ml of SDS-borate solvent to which 0.6% β-mercaptoethanol had been added as described by Wall *et al.* (3) and Landry and Moreaux (4).

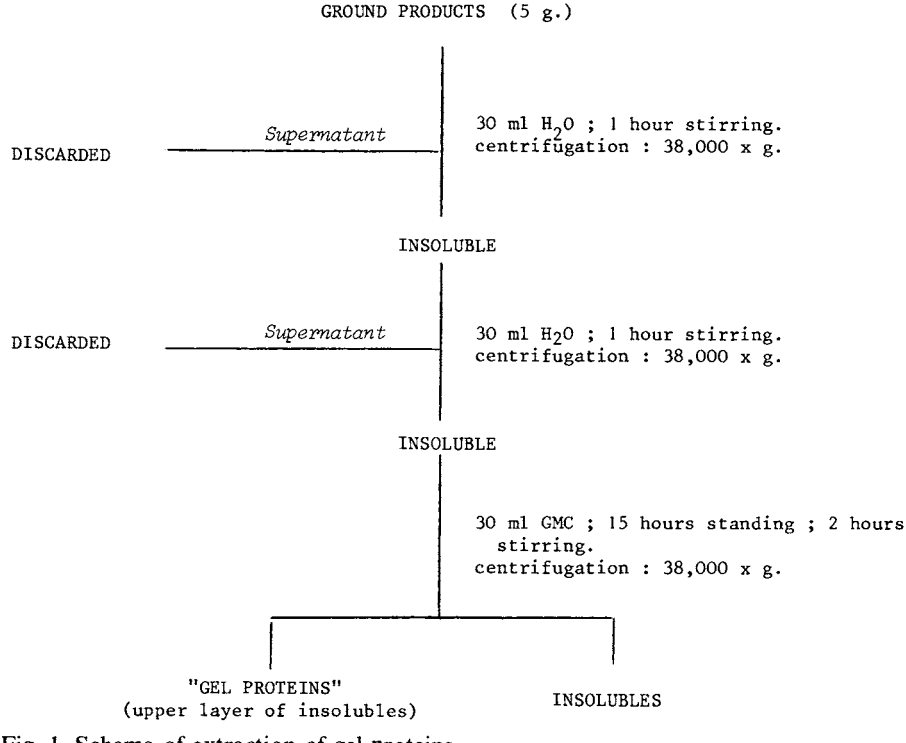

Fig. 1. Schema of extraction of gel proteins.

Gel Protein

The "gel protein" fraction (5) was extracted according to Feillet *et al.* (6) as shown in Fig. 1: semolina or ground freeze-dried dough (5 g) was extracted twice by stirring for 1 hr with 30 ml deionized water and centrifuged 30 min (38,000 × *g*). Supernatants were discarded and insoluble material extracted with 30 ml 70% GMC. After standing overnight, stirring 2 hr, and centrifuging (30 min; 38,000 × *g*), a gel layer formed on the surface of the insoluble residue; its thickness (mm) was measured with a calliper.

The gel layer was then recovered from the upper layer of the centrifuged sediment, weighed, dialyzed, and freeze-dried (Fig. 1).

The highly hydrated "gel-like" layer which remained over the starch after sequential extraction of semolina by 0.5 NaCl, 60% ethanol, and 70% GMC was also referred to as the gel protein fraction.

Analytical Procedures

Nitrogen content of the extracts was determined by a semiautomatic Kjeldahl method (7). Gluten yield was determined by the hand-washing AACC method, except that deionized water instead of tap water was used for mixing and washing. All determinations were duplicated.

RESULTS

Influence of Sheeting on Gluten Recovery

The dough was sheeted from 0 to 35 times with water content (35% on wet basis) and roll clearance (0.35 mm) kept constant. Results are given in Table I.

After a slight increase in the wet gluten yield while the dry gluten content stayed constant (13%), gluten recovery dropped sharply with increasing number of passes and reached zero after 35 passes through the rolls. The water content of wheat gluten did not change significantly.

As illustrated in Fig. 2, a similar modification of the gluten yield (wet or dry) was observed when the setting of the roll clearance was changed from 0.35 to 3.00

TABLE I
Effect of Dough Sheeting (Roll Clearance: 0.35 mm; Dough Water Content: 35% on Wet Basis) on Gluten and Gel Protein Yields

	Gluten			Gel Protein	
No. of Passes through Sheeting Rolls	Wet Gluten Yield % on dry basis	Dry Gluten Yield % on dry basis	Moisture Content % on wet basis	Layer Thickness mm	Gel Protein Yield[a]
0	36.2	12.9	67.1	2	14.8
5	38.0	12.9	66.0	3	15.1
10	32.8	11.1	66.3	5	26.0
15	24.3	8.1	66.5	5.5	30.7
20	16.1	5.3	67.3	7	41.5
25	10.7	3.6	60.9	8.5	46.5
30	5.6	2.0	64.2	11	58.6
35	0	0	...	12	60.3

[a]g wet gel/100 g dry sample.

mm (number of passes:30; dough moisture content: 33% on wet basis) and the dough moisture content from 30 to 40% wb (roll clearance: 0.70 mm; number of passes: 30).

The initial conclusion from this study is that gluten yield, evaluated by the hand-washing technique, can be strongly decreased from 36% (reference sample) to 0%, simply by sheeting a dough under specific conditions.

Changes in the Gel Fraction

Data in Table I indicate that the amount of gel protein, expressed either by the thickness of the layer (mm) or by the wet weight of the gel (g/100 g semolina on dry basis) increased with the number of passes through the rolls; a linear relation occurred between the number of sheetings and the gel thickness ($\rho = 0.99^{**}$). Lowering the roll clearance or the dough moisture content had a similar effect.

Consequently, by plotting the gluten yield of the sheeted dough against the gel protein content, a strong correlation was found between these two variables: after a slight increase, the gluten content decreased, while the thickness of the protein layer increased (Table I).

Possible changes in the gel protein composition brought about by dough sheeting were examined by polyacrylamide-gel electrophoresis, either in aluminum lactate buffer—6*M* urea (pH = 3.2)—or SDS-tris buffer (pH = 8.9) after reduction of protein by mercaptoethanol. No difference was found among the samples.

Solubility of Proteins

The effects of dough sheeting on the amount of soluble proteins in 0.5*M* NaCl (fraction I), 60% ethanol (fraction II), and 70% GMC (fraction III) are shown in Table II.

The solubility of the salt-soluble proteins, not modified by mixing, slowly decreased during sheeting from 20.6% (semolina) to 18.4% (30 passages through the rolls). There was a gradual increase in the amount of fraction II (gliadins). The major changes in the protein solubility were observed in the GMC-soluble fraction, which was markedly reduced (2.5-fold), and in the gel proteins, which

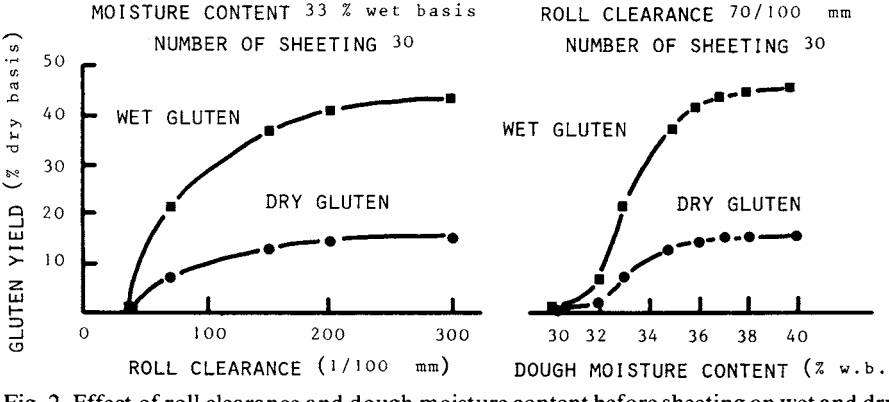

Fig. 2. Effect of roll clearance and dough moisture content before sheeting on wet and dry gluten yield.

TABLE II
Changes in Semolina Protein Solubility during Mixing and Sheeting (Roll Clearance: 0.35 mm; Dough Water Content: 35% on Wet Basis). Results Expressed in Protein % of Semolina Protein

Protein Fraction		Fraction No.	Semolina		Mixed Dough		Sheeted Dough 5 X[a]		15 X		25 X		30 X	
Saline soluble-proteins		I	20.6		20.6		19.9		19.1		18.4		18.4	
Ethanol-soluble proteins		II	41.2		43.4		44.2		46.4		48.5		49.3	
A	GMC-Soluble	III A	27.9		25.0		19.1		14.7		11.0		11.0	
A	Gel proteins	IV A	4.4	38.2	4.4	36.0	9.6	35.9	13.2	34.5	14.7	33.1	14.7	32.3
A	Insoluble[b]	V A	5.9		6.6		7.2		6.6		7.4		6.6	
B	SDS-Soluble	III B	13.2		13.2		14.0		14.0		14.0		14.0	
B	SDS + ME[c]-Soluble	IV B	14.0	38.2	14.7	36.0	14.0	35.9	16.2	34.5	16.2	33.1	16.2	32.3
B	Insoluble[b]	V B	11.0		8.1		7.9		4.3		2.9		2.1	
II + V B			52.2		51.5		52.1		50.7		51.4		51.4	
Gel protein layer, mm			6		6.5		10		14		17		18	

[a]Number of passes through sheeting rolls.
[b]Protein content obtained by difference.
[c]Mercaptoethanol.

increased by more than 300%. For a given sample, the gel thickness is much greater when the flour is previously extracted by saline solution, ethanol, and GMC than when it is extracted under the conditions of Fig. 1. For example, a dough sheeted five times has a gel thickness of 10 mm in the first case, and 3 mm in the second case.

The percentage of fraction VA (residual proteins) was not modified by mixing and sheeting.

When proteins were extracted in the presence of SDS and β-mercaptoethanol after solubilization of fractions I and II, no difference in the amount of fraction IIIB and only slight increases in fraction IVB were observed between samples. However, under these conditions the residual insoluble proteins (fraction VB) diminished greatly. The sums of the two fractions II and VB were constant.

DISCUSSION

Modification in protein solubility may arise from changes in the degree of association between polypeptide chains. Hydrogen bonds, hydrophobic interactions, salt-linkage or disulfide covalent bonds are commonly involved in these phenomena.

It has been stated by several authors (8–10) that common wheat dough-mixing decreased the size of protein complexes. This was attributed to protein disaggregation (9) or depolymerization of the high-molecular-weight glutenin by SH-SS interchange reaction (10,11). More probably, both mechanisms are important.

The results of the present work cannot be easily compared to the data of the other authors for five main reasons: the starting material was durum wheat semolina; the dough moisture content was approximately 32% on wet basis; the mixing step was only intended to blend water and semolina; dough development was achieved by sheeting; and procedure of protein extraction was different from those of other workers.

Our own results of solubility fractionation indicate that the GMC-soluble glutenins (fraction IIIA) became incorporated into the alcohol-soluble and gel-protein fractions as a consequence of sheeting. The increase in gliadin content was consistent with the hypothesis of SS bond breakage in the glutenin molecules. This breakage could arise either from chemical oxidation or from mechanical stresses developed on the oriented protein chains during sheeting. The modification in the protein structure by depolymerization would make the formation of a protein network impossible, and would explain the drop in gluten yield. The observation that gluten was damaged less when the dough water content increased suggests that the mechanical effect of sheeting was the more important phenomenon. Glutenins would be broken by a stretching effect. A similar explanation has been recently proposed by MacRitchie (12) who suggested that glutenin molecules with high-molecular weights would be broken down by mixing.

The formation of gel proteins during sheeting is more difficult to explain, mainly because the properties of this fraction are not yet well understood. According to Mecham and coworkers (5), Cole *et al.* (13,14), and Cole and Ng (15), gel proteins occur in a highly insoluble state and give, after reduction and alkylation, an electrophoresis pattern like that of the similarly treated glutenin;

they contain subunits in the molecular-weight range 55,000–70,000, joined by disulfide bonds. We may postulate that new SS bonds are developed during sheeting, as observed by Nazarov (1), when pasta dough is pressed through a die. Highly reactive new polypeptide chains, resulting from the broken glutenin molecules, would be gathered and oriented by sheeting, then cross-linked by disulfide bonds arising from reactions between the new SS groups; it is also possible that conformational changes in the protein structures would permit hydrophobic groups to interact.

This hypothesis is supported by the results of protein extraction by dissociating (SDS) and reducing (β-mercaptoethanol) agents (Table II).

The most insoluble glutelin fractions (fraction VB) were disrupted into alcohol-soluble proteins (the sum of fractions II and VB is constant). The formation of intermolecular disulfide bonds could account for the slight decrease in soluble protein (fraction I). Furthermore, as expected, if new disulfide bonds were formed during sheeting, a slight increase in the SDS-insoluble material (fraction IVB) was observed after dough sheeting.

CONCLUSION

Sheeting of macaroni dough produces important changes in protein solubility and gluten yield of pasta; we found a marked increase in the gel fraction, while decreases of that component during dough-mixing were observed by several authors (8–11). Consequently, it seems that the mixing and sheeting treatments are quite different. A similar conclusion was reached by Kilborn and Tipples (16), who showed that dough development was achieved by sheeting roll treatment with only 10 to 15% of the net energy required with a dough mixer. With reference to other works (5,6,17) in which the amount of gel protein was correlated with some technological properties of wheat flour, the development of the gel protein fractions by sheeting in our work should be emphasized. Mechanical modifications of the protein properties could be a valuable analytical tool for exploring the relation between protein characteristics and the technological value of wheat. Work is underway to correlate cooking quality of pasta products and the modifications of durum wheat protein caused by sheeting.

Acknowledgments

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