CHANGES IN SEMOLINA PROTEINS DURING SPAGHETTI PROCESSING[1]

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ABSTRACT

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Three durum wheats of widely differing spaghetti cooking quality and a hard red spring wheat were processed into spaghetti in a DeMaco laboratory extruder. Samples were taken at various stages throughout the spaghetti-making process and were analyzed for sulfhydryl group and disulfide bond contents. For all four wheats, there was a substantial loss of sulfhydryl groups by the extrusion stage, although, by far, the greatest portion of the loss occurred during the first 6 hr of the 29-hr drying cycle. No relation could be established between cooking quality and the extent of sulfhydryl group loss. Disulfide bond levels did not change significantly during processing for any of the wheats. Changes in protein solubility during spaghetti-making followed a similar pattern for all four wheats. In each case, protein extractability in dilute acetic acid had decreased substantially by the time the dough entered the extruding auger. No further change in solubility occurred during the remainder of processing. Osborne solubility fractionations disclosed an increase in the amount of insoluble residue protein concomitant with a decrease in the amount of salt-soluble protein. Gel filtration elution profiles on Sephadex G-150 in AUC showed no significant changes in the molecular weight (MW) distribution of the proteins during spaghetti processing. This, in conjunction with electrophoretic results, which revealed no qualitative changes in protein electrophoretic patterns during spaghetti processing, suggested that the loss of salt-soluble proteins may be due to binding by the insoluble components of the semolina rather than by polymerization.

It has been well established that protein content (1,2) and gluten quality (3–7) are of prime importance in determining spaghetti cooking quality. However, little information is currently available in the literature concerning the possible effects of the spaghetti-making process on the physicochemical properties of semolina proteins. Accordingly, this investigation was undertaken to determine what changes, if any, occur in semolina proteins during the spaghetti-making process. Three Canadian durum wheats (*Triticum durum* Desf.) representing a wide range of spaghetti cooking quality were chosen for the study. A hard red spring wheat (*Triticum aestivum* L. em Thell) was included for comparative purposes.

MATERIALS AND METHODS

Three durum wheat varieties (Wakooma, Wascana, and Mindum) and a hard red spring wheat variety (Neepawa) were selected for the study (Table I). Spaghetti cooking qualities, as evaluated by the GRL Spaghetti Tenderness Testing Apparatus (8,9), can be classified as follows: Wakooma very good, Wascana good, Mindum fair, and Neepawa poor. The wheats (20-kg samples) were washed, tempered overnight to 16.5% moisture, and milled into

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semolina or farina in a Buhler laboratory mill (10). The mill room was controlled for temperature (22°C) and humidity (60%). Farinograms were performed at 31.5% absorption as described by Irvine *et al.* (11). Ash, Kjeldahl protein contents (N × 5.7), yellow pigment levels and lipoxidase activities were determined by approved AACC methods (12).

Spaghetti-Making

Spaghetti was prepared in the DeMaco (DeFrancisci Machine Corporation) semi-commercial scale laboratory extruder. All four wheat varieties were processed using the following operating conditions:

Extrusion temperature	50°C
Absorption	27%
Extrusion rate	21 rpm
Vacuum	45 cm Hg

Initially, 1000-g batches were premixed at low speed in a Hobart Model C-100 mixer with gradual addition of distilled water. Mixing was continued at

TABLE I
Description and Quality Data for Samples Used in This Study

Property	Variety			
	Wakooma	Wascana	Mindum	Neepawa
Wheat				
Class	Amber Durum	Amber Durum	Amber Durum	Hard Red Spring
Grade	2 CW	3 CW	2 CW	1 CW
Protein[a,b], %	12.8	13.5	13.9	13.2
Ash[a], %	1.56	1.46	1.63	1.26
Semolina yield, %	57.9	55.5	58.8	53.1
Semolina				
Protein[a,b], %	11.8	12.2	12.5	11.2
Ash[a], %	0.52	0.50	0.55	0.39
Yellow pigment[a], ppm	4.96	5.75	4.03	1.75
Lipoxidase[a], $\mu 10_2$/min/g	21	20	18	24
Spaghetti				
Yellow pigment[a], ppm	4.00	4.58	3.34	1.17
Pigment loss, %	19.4	20.3	17.1	33.1
Tenderness index[c], mm/sec × 10^3	37	37	48	47
Compressibility[c], %	71	76	78	100
Recovery[c], %	40	30	22	0
Farinogram[d]				
Mixing time, min	8	5.5	4	6
Maximum consistency, BU	665	690	660	500
Tolerance index, BU	40	90	130	20

[a]Results expressed on a 14% moisture basis.
[b]Protein computed as % N × 5.7.
[c]Evaluated on the GRL Spaghetti Tenderness Testing Apparatus (cooking time, 12 min).
[d]Farinograms performed at 31.5% absorption. BU = Brabender Units.

moderate speed for 8 min after water addition was complete. The premixed dough was then placed in the vacuum mixer of the DeMaco and extruded. The dough passed through two kneading plates as it progressed from the extruding auger to the spaghetti die. An extension tube was added to ensure uniform hydration of the pasta dough prior to extrusion. The spaghetti was dried with a controlled decrease in relative humidity for 29 hr at 39° C.

Sample Preparation

Seven stages during the spaghetti-making process were examined (Table II). Freshly extruded spaghetti was collected as the DeMaco was in operation; the dough from the extruding auger was recovered by quickly dismantling the machine once sufficient spaghetti had been processed. Each sample, including spaghetti at various stages of dryness, was immediately frozen in liquid nitrogen and freeze-dried. The freeze-dried samples, the durum wheat semolina, and hard red spring wheat farina were ground in a coffee grinder to pass through a #100 sieve to ensure uniformity of particle size from sample to sample. The ground samples (about 50 g) were stored in a deep freeze until required for analysis.

Sulfhydryl (SH) and Disulfide (SS) Determinations

Levels of SH and SS were determined by potentiometric titration with $AgNO_3$ as described by Kolthoff *et al.* (13). Total SH was determined by titration in the presence of 8M urea; reactive SH was determined in the absence of urea. Total SS contents were determined by reducing SS groups with sulfite in the presence of urea, titrating for total SH, and then deducting the contribution of total SH in the absence of sulfite.

Results are expressed in μeq per g of protein and represent the average of three separate determinations. The deviation between replicate titrations was less than 10% for each sample.

Protein Extraction

Each sample was extracted in 0.05M acetic acid in a Potter and Elvejhem homogenizer as described by Tanaka and Bushuk (14). It was found that 10 min was sufficient time to achieve maximum extractability. After centrifugation the supernatants were freeze-dried and their protein contents were determined using Nessler's reagent (15). Reproducibility was about 5% for replicate extractions for each sample at each extraction time.

TABLE II
Stages of Spaghetti-Making Examined

Processing Stage	Description
1	Semolina or farina
2	Dough from extruding auger
3	Freshly extruded spaghetti
4	Spaghetti dried 1 hr
5	Spaghetti dried 6 hr
6	Spaghetti dried 12 hr
7	Fully dried spaghetti

Protein Solubility Fractionation

Duplicate samples (10 g) were fractionated by the modified Osborne procedure of Chen and Bushuk (16). This fractionation procedure yields five solubility fractions: albumins (water-soluble), globulins (salt-soluble), gliadins (alcohol-soluble), soluble glutenins (acetic acid-soluble) and insoluble glutenins (insoluble residue). The protein contents (N × 5.7) of the four soluble fractions were determined by the modified Nessler procedure of Williams (15). This method gave inconsistent results for the insoluble residue fraction. Therefore, the protein content of that fraction was determined by the Kjeldahl procedure (12). Reproducibility was better than 10% for each fraction of each sample.

Gel Filtration

Protein extracts were prepared in AUC (0.1M acetic acid, 3M urea, 0.01M cetyl trimethylammonium bromide (17)) as described by Wasik and Bushuk (6). This solvent has been shown to solubilize up to 95% of wheat-flour protein (18). Gel filtration was performed on a 2.5 × 34 cm bed of Sephadex G-150 with upward flow at a rate of 7 ml per hr. Five ml of AUC extract (25 mg protein) was injected for each trial. The effluent was monitored at 280 nm in a LKB 8300 Uvicord II ultraviolet analyzer. MW was determined as described by Whitaker (19) using the following standards: γ-globulin (MW 160,000), bovine serum albumin (dimer MW 136,000, monomer MW 68,000), ovalbumin (MW 43,000), chymotrypsinogen (MW 25,700), cytochrome C (MW 11,700) and N-ethylmaleimide (MW 125).

Polyacrylamide-Gel Electrophoresis

Separation of the albumins, globulins, and gliadins was achieved in an Ortec Model 4200 electrophoresis system using a pH 2.9 cationic gel system (20). Polymerization of the gel was catalyzed by the method of Jordan and Raymond (21). Prior to electrophoresis, proteins were dissolved by shaking overnight at 50° C in upper buffer (10 mg protein/ml) to which 4M dimethyl formamide had been added. The resultant solutions were clarified by centrifugation. On completion of electrophoresis, the gel was stained with Coomassie Brilliant Blue as described by Koenig *et al.* (22).

The soluble glutenins and the insoluble residue proteins were electrophoresed according to the method of Orth and Bushuk (23). Both fractions were dissolved by incubation at 50° C with shaking overnight in electrode buffer to which 1% sodium dodecyl sulfate and 1% β-mercaptoethanol had been added. Owing to the low protein content of the insoluble glutenin, it was necessary to concentrate the solutions obtained from this fraction prior to electrophoresis. This was achieved by means of a Minicon-B15 clinical sample concentrator (Amicon Corporation) containing a membrane rated at 15,000 MW retention. This rating was confirmed by verifying that the electrophoretic profile of the soluble glutenins samples did not change in the region above MW 20,000 after passage through the concentrator. The MWs of the glutenin subunits were estimated according to the procedure of Weber and Osborn (24) using the following standard proteins: phosphorylase a (MW 94,000), bovine serum albumin (dimer MW 136,000, monomer MW 68,000), catalase (MW 60,000), aldolase (MW 40,000), pepsin (MW 35,000), chymotrypsinogen (MW 25,700) and cytochrome C (MW 11,7000). Gels were stained with Coomassie Brilliant Blue according to the method of Koenig *et al.* (22).

RESULTS AND DISCUSSION

Reactive SH Groups

Fabriani *et al.* (25) have observed that durum wheat technological characteristics appear to be related to the ratio of reactive to total SH groups. They have suggested that a high ratio of reactive to total SH groups may be a prerequisite for superior spaghetti cooking quality. Therefore, reactive and total sulfhydryl levels were determined for the three durum wheat semolinas and the Neepawa farina.

The results were inconclusive (Table III). In support of Fabriani's hypothesis, Wakooma, which had the best cooking quality, contained the highest level of reactive SH groups both in terms of amount on a μeq/g protein basis and in terms of the proportion of total SH. Moreover, Neepawa, which had the poorest cooking quality, contained the least reactive SH. However, the difference in the proportion of reactive SH groups among the three durum wheats was marginal. In fact, Mindum, which was inferior in cooking quality, had a greater proportion of reactive SH groups than Wascana.

Total SH Groups

The pattern of SH group loss during spaghetti-making was similar for all four wheats (Fig. 1). The majority of the loss occurred during the drying stage. Significant losses were observed by the extrusion stage, despite the fact that the dough was under a vacuum which limited the oxygen supply. It was not possible to establish a relation between spaghetti cooking quality and SH group loss. Neepawa had the lowest SH group content of the four wheats, confirming that hard red spring wheats contain less SH groups than durum wheats (26).

Total SS Groups

On the basis of results obtained from the Wascana series, it was concluded that no significant change occurred in SS bond content at any stage during the spaghetti-making process. Analysis of the semolina and dried spaghetti from the other wheats verified this result (Table IV). In each case, no change in SS bond content was detectable. This was not unexpected, since it has been shown that SS bond contents of bread doughs are not altered under mixing conditions which result in substantial SH group loss (27).

TABLE III
Comparison of Reactive Sulfhydryl Group Contents for Three Durum Wheat Semolinas and a Hard Red Spring Wheat Farina

Sample	Spaghetti Cooking Quality	Reactive SH	
		μeq/g protein	% total SH
Wakooma semolina	Very good	8.63	74.7
Wascana semolina	Good	6.20	66.2
Mindum semolina	Fair	6.68	69.0
Neepawa farina	Poor	4.76	57.1

Protein Extractability in Dilute Acetic Acid

It has been well documented that the amount of protein extracted from bread doughs by dilute acetic acid increases with mixing (16, 28–31). The rate of

TABLE IV
Effect of Spaghetti-Making on Disulfide Bond Contents

Wheat Variety	Total SS, μeq/g Protein	
	Semolina or farina	Dried spaghetti
Wakooma	93.9	93.5
Wascana	78.2	77.8
Mindum	84.8	84.6
Neepawa	85.9	84.6

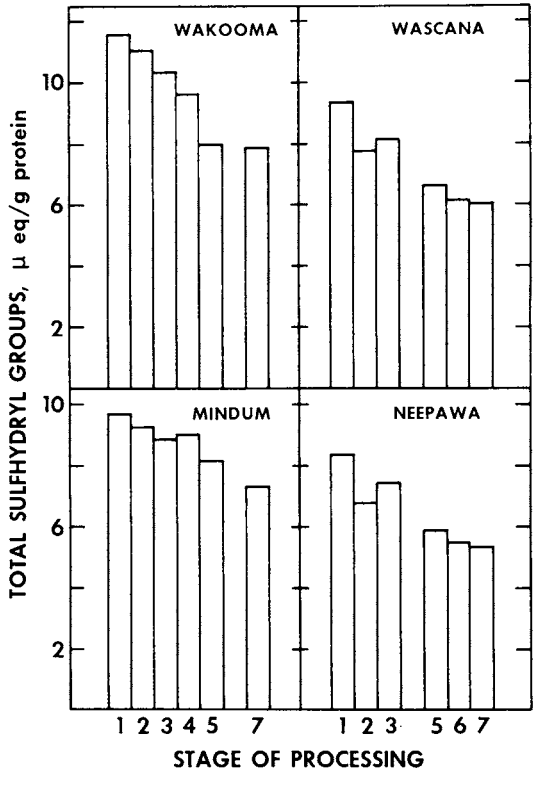

Fig. 1. Change in total sulfhydryl groups during spaghetti-making for three durum wheats (Wakooma, Wascana, and Mindum) and a hard red spring wheat (Neepawa). (Stages of processing described in Table II. Wascana and Neepawa were not sampled at the 1-hr dried stage; Wakooma and Mindum were not sampled at the 12-hr dried stage.)

increase is different for flours of different mixing characteristics. In view of these findings, it seemed desirable to compare the extractability of wheat proteins at various stages during spaghetti-making. The results are presented in Table V. For all four wheats examined, protein extractability had decreased by about 10% by the extruding auger dough stage. No further change in solubility occurred during the remainder of processing. The protein extractabilities of the three durum wheats were essentially the same; the extractability of the Neepawa protein was about 10% less than that of each durum wheat at each stage of spaghetti-making examined. The protein extractability of the Neepawa farina compared closely with previous results for hard red spring flours (16,31).

The observed decrease in protein solubility during spaghetti-making was not anticipated. It took nearly 4 min for the premixed dough to proceed down the auger, pass through two kneading plates, and finally emerge through the spaghetti die. During this time, a great deal of mechanical work was imparted to the pasta dough. Therefore, it is somewhat surprising that there was not an increase in protein solubility due to gluten breakdown during these stages of the spaghetti-making process. Perhaps, due to the low moisture content of pasta doughs, a continuous gluten matrix cannot be formed, rendering it less susceptible to breakdown than in bread doughs.

Protein Solubility Fractionation

In order to determine the nature of the proteins involved in the decrease in acetic acid solubility, Osborne protein solubility fractionations were performed

TABLE V
Effect of Spaghetti-Making on the Extractability of Semolina Protein in 0.05*N* Acetic Acid

		Protein Extractability[a], %	
		Extraction time, min	
Wheat Variety	Processing Stage	5	10
Wakooma	Semolina	73.1	73.8
	Extruding auger dough	65.4	64.1
	Extruded spaghetti	63.8	65.2
	Dried spaghetti	67.3	63.7
Wascana	Semolina	72.5	75.1
	Extruding auger dough	65.3	66.9
	Extruded spaghetti	65.9	65.5
	Dried spaghetti	67.2	66.4
Mindum	Semolina	75.5	76.6
	Extruding auger dough	69.2	69.8
	Extruded spaghetti	68.0	67.8
	Dried spaghetti	69.0	67.7
Neepawa	Farina	65.0	67.1
	Extruding auger dough	60.7	57.8
	Extruded spaghetti	59.9	60.0
	Dried spaghetti	56.9	56.4

[a]Expressed as % total nitrogen (N).

TABLE VI
Effect of Spaghetti-Making on the Osborne Solubility Distribution of Wheat Proteins[a]

	Sample	Albumins, %	Globulins, %	Gliadins, %	Soluble Glutenins, %	Insoluble Residue, %
Wakooma	Semolina	12.1	6.2	41.9	12.0	25.9
	Extruding auger dough	11.3	3.0	39.8	13.8	30.4
	Freshly extruded spaghetti	11.5	2.7	40.5	12.0	30.9
	Spaghetti dried 6 hr	10.8	2.7	40.6	13.3	31.0
	Fully dried spaghetti	11.1	2.4	40.4	12.2	31.2
Wascana	Semolina	9.4	4.9	42.0	12.6	24.1
	Extruding auger dough	8.6	2.2	40.0	12.6	29.6
	Freshly extruded spaghetti	9.6	1.8	41.3	10.8	30.4
	Spaghetti dried 6 hr	9.3	1.7	39.1	12.1	31.8
	Fully dried spaghetti	9.4	1.6	41.4	11.7	32.1
Mindum	Semolina	11.6	5.6	42.6	12.0	24.1
	Extruding auger dough	10.6	2.6	42.8	11.6	27.9
	Freshly extruded spaghetti	10.4	2.8	42.5	12.6	27.7
	Spaghetti dried 6 hr	10.5	2.8	42.3	12.0	28.2
	Fully dried spaghetti	10.8	3.2	41.9	12.2	27.6
Neepawa	Farina	12.6	4.4	35.7	11.6	32.1
	Extruding auger dough	11.0	2.6	34.7	13.5	34.4
	Freshly extruded spaghetti	11.9	2.6	36.6	13.1	33.6
	Spaghetti dried 6 hr	10.9	2.6	34.6	12.5	35.3
	Fully dried spaghetti	12.1	2.2	34.0	12.1	36.4

[a]Expressed as % total nitrogen (N).

on samples from each wheat at five stages of spaghetti-processing. The only significant trends were a decrease in the proportion of globulins concomitant with an increase in insoluble residue protein (Table VI). The decrease in globulins solubility (denatured or bound by insoluble components of flour) was apparent by the extruding auger dough stage for each wheat. Thereafter, the proportion of globulins remained relatively constant. Similarly, the increase in insoluble protein had occurred by the extruding auger dough stage (Table VI). This pattern of change corroborated the acetic acid extraction results (Table V).

The behavior of the samples in the present study differed from a previous report describing changes in the Osborne protein solubility classes of bread doughs during mixing (14). The mixed bread doughs exhibited no change in the amounts of albumins and globulins, an increase in gliadins and soluble glutenins, and a decrease in insoluble residue proteins. This was attributed to depolymerization of glutenin proteins during dough breakdown. Our results (Tables V, VI) give no indication that a similar mechanism is operational during pasta processing.

Gel Filtration

The MW distribution of the proteins from each wheat was determined for semolina, extruding auger dough, freshly extruded spaghetti and fully dried spaghetti samples by gel filtration of AUC extracts on Sephadex G-150. Because of the highly dissociating nature of AUC, it has been assumed that extracted

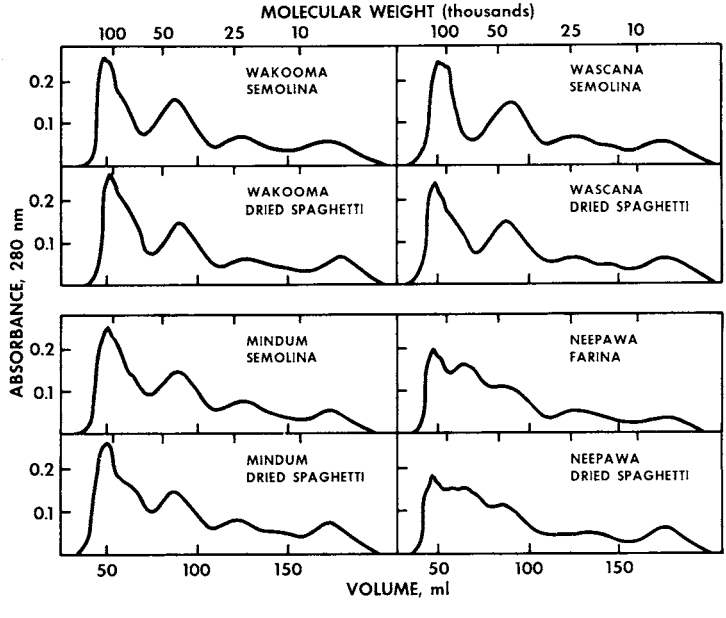

Fig. 2. Gel filtration elution profiles of AUC extracts on Sephadex G-150 for semolina and dried spaghetti from three durum wheats (Wakooma, Wascana, and Mindum) and for farina and dried spaghetti from a hard red spring wheat (Neepawa).

proteins in this solvent exist as a molecular solution and not as aggregates (17). Therefore, if the decrease in protein solubility during spaghetti processing is the result of polymerization, there should be a shift in the elution profile from low MW to high MW. Changes noted were of a very minor nature in each case. As examples, the elution profiles for the semolina and dried spaghetti from each wheat are shown (Fig. 2). The marginal increase in the proportion of high-MW material was not sufficient to establish that protein polymerization had taken place. For each wheat, there was a small progressive increase in low-MW material during spaghetti processing. This was likely due to the action of proteolytic enzymes.

The profiles from the present study are very similar to those reported by other workers for durum (6) and hard red spring wheat (32). Neepawa appeared to have a greater proportion of high-MW material than the durum wheats (Fig. 2). This corroborates the protein solubility data (Tables V, VI), if one assumes that MW increases with decreasing solubility.

Polyacrylamide-Gel Electrophoresis

Polyacrylamide-gel electrophoresis was used to examine each Osborne protein solubility fraction from each wheat at the semolina, extruding auger dough, freshly extruded spaghetti and fully dried spaghetti stages of spaghetti-making. Separation of the albumins, globulins and gliadins was achieved using a pH 2.9 cationic gel system (20). For each wheat, no pronounced changes could be detected for any of the three fractions. To illustrate this, the profiles for Mindum are shown (Fig. 3). A few globulin bands may have changed in relative intensity as spaghetti processing progressed. No new bands appeared. Thus, it is possible that the decrease in globulins during spaghetti processing (Table VI) may be due to binding by the insoluble components of the semolina rather than by polymerization.

The soluble glutenins and insoluble residue proteins were electrophoresed by

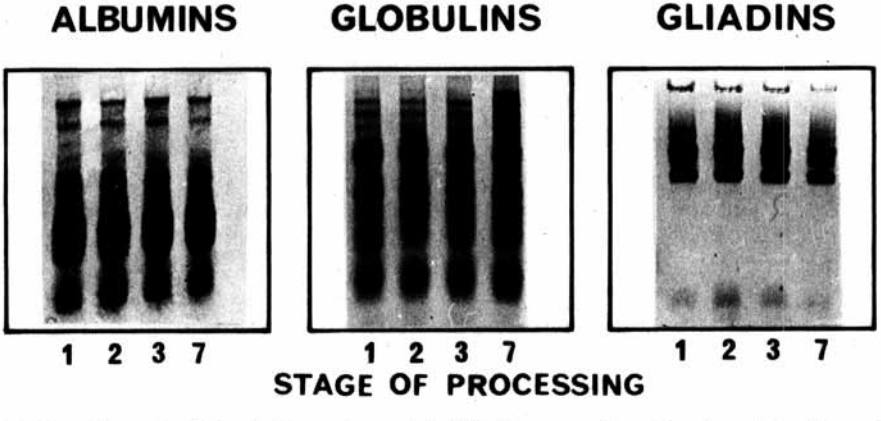

Fig. 3. Polyacrylamide-gel electrophoresis (pH 2.9) patterns for albumins, globulins, and gliadins from Mindum at four stages of spaghetti-making. (Stages of processing described in Table II.)

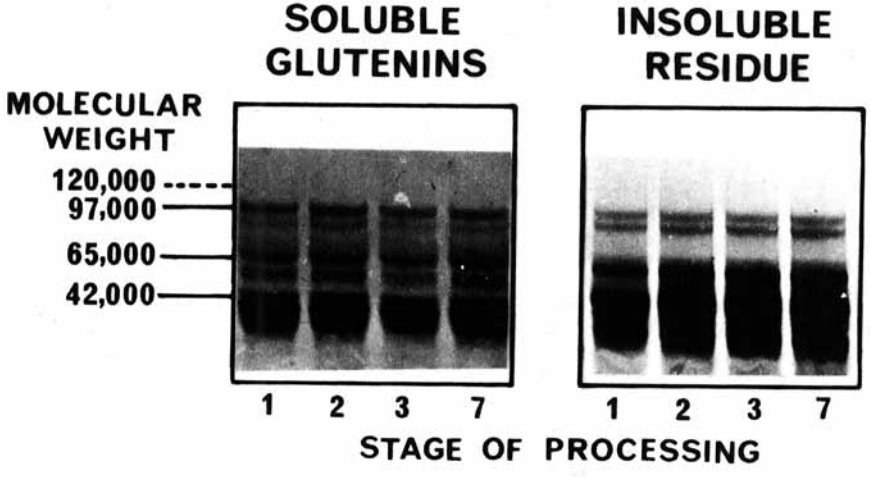

Fig. 4. Sodium dodecyl sulfate polyacrylamide-gel electrophoresis patterns (pH 7.3) for reduced subunits of soluble glutenins and insoluble residue proteins from Mindum at four stages of spaghetti-making. (Stages of processing described in Table II.)

SDS polyacrylamide-gel electrophoresis (23). Due to their very large MW, it was necessary to reduce their SS bands prior to electrophoresis to permit entry into the gel. For all four wheats, no changes were noted in the electrophoretic patterns for either fraction during processing. Results for Mindum are shown as an example (Fig. 4). Subunit MWs were about 10% lower than that noted by other workers (33, 34), which is within the accepted range of reliability established for this method (24).

Durum wheat glutenins are often deficient in the two highest MW bands found in hard red spring wheat glutenins (33–36). In the current study, each durum wheat was found to possess a very faint band (MW 120,000) corresponding to the highest MW glutenin subunit of Neepawa. The second highest MW subunit found in Neepawa (MW 111,000) was not detected in any of the three durum wheats.

GENERAL DISCUSSION

It is difficult to relate the current study with previous reports describing the effect of mixing on bread wheat doughs. First, the nature and amount of mechanical work input are different. Second, there is a great difference in water absorption. The less than 30% water absorption levels of pasta doughs would not be expected to permit the development of gluten to the same extent as that found in higher moisture bread doughs. Therefore, perhaps it is not surprising that no substantial difference could be found in the behavior of semolina proteins during spaghetti processing for wheats of widely differing spaghetti quality. The real determining factor may be the manner in which the proteins withstand the cooking process. Further investigations are required before the factors responsible for spaghetti cooking quality can be properly defined and understood.

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