Changes in Solubility and Distribution of Semolina Proteins Due to Extrusion Processing

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ABSTRACT

The effects of extrusion processing on wheat protein solubility and molecular weight distribution were investigated. Enriched semolina was extruded using a twin-screw extruder at two different temperatures (50 and 96°C). A modified Osborne fractionation showed a marked decrease in albumin, globulin, gliadin, and glutenin fractions with a concomitant increase in the insoluble residue fraction following extrusion. The effect on protein solubility was greater with the higher extrusion temperature. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of reduced and unreduced protein fractions suggest that conformational changes occurring during extrusion may be responsible for the increase in the insoluble residue fraction.

Extrusion processing of wheat is widely used in the manufacture of breakfast cereals, infant foods, crispbread, snacks and sweets, and pasta products. Extrusion processing has the potential to alter protein structure, solubility, and digestibility by heat, pressure, and shear (Phillips 1989). It has been previously reported that extrusion processing decreased the solubility of semolina proteins (Dexter and Matsuo 1977). Various studies have also reported that high-temperature drying of pasta resulted in decreased protein solubility (Dexter et al 1981, De-Stefanis and Sgrulletta 1990, Aktan and Khan 1992). Although it is well established that extrusion processing decreases the solubility of semolina proteins, limited information is available on the molecular weight distribution and the possible conformational changes that result in decreased protein solubility following extrusion.

The purpose of this study was to determine the effects of extrusion processing at two different temperatures on the solubility and distribution of proteins in semolina. The role of disulfide linkages in the effects of extrusion on protein solubility and distribution also was investigated.

MATERIALS AND METHODS

Extrusion

Semolina (30 mesh), milled from durum wheat with ≈65% extraction, and enriched with niacin, iron, thiamin, and riboflavin was obtained from the North Dakota Mill & Elevator, Grand Forks, ND. Semolina was extruded to make a product in the shape of small o's. A corotating twin-screw extruder (Creusot-Loire, model 45) was used at two different temperature ranges (47–50°C and 92–96°C). Water was injected into the feed at the rate of 0.12 L/min. The screw was operated at 900 rpm. The feeder was set to deliver 2.06 kg of semolina per minute. The final product was dried in a vat dryer-blower to a moisture content of 8–9%. The extruded semolina cereals were milled in a coffee mill to a coarse powder (able to pass completely through a 30 mesh sieve) before analyses.

Protein Fractionation and Determination

Albumins, globulins, gliadins, glutenins, and the insoluble residue were fractionated from raw semolina and extruded semolina cereals according to the Osborne procedure as modified by Chen and Bushuk (1970). The protein fractions were freeze-dried and stored in a desiccator at room temperature before all analyses. The protein content of raw and extruded semolina and each of the protein fractions was determined by the micro-Kjeldahl method (AOAC 1980) using a protein conversion factor of N × 5.83.

Electrophoresis

The protein fractions from raw semolina and extruded semolina were subjected to electrophoresis with or without a reducing agent (5% 2-mercaptoethanol) on 17.5% (w/v) gels using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Ng and Bushuk (1987). The weights of protein fractions were adjusted so that the same amount of protein was loaded into each well. Gels were stained for protein using Coomassie Brilliant Blue-R. Protein extract from the flour of cultivar Neepawa was used as a reference for molecular weights.

RESULTS AND DISCUSSION

Protein Distribution

Results of the protein fractionation of raw semolina and semolina cereal extruded at 50 and 96°C are presented in Table I. The recovery of samples is reported on the basis of weight and protein content. Recovery by weight was 93–95%, and protein recovery range was 93–98%. The variability in recovery may be attributed to the loss of low molecular weight proteins during dialysis and loss of sample during analysis. In raw semolina, protein was present in the highest amount in the alcohol-extractable protein fraction (gliadin) (41.8% of total protein), followed by the insoluble fraction (27.7%), glutenin (14.2%), albumin (11.7%), and globulin (3.6%). These results are in general agreement with the findings of Chen and Bushuk (1970). The distribution of proteins differed in the extruded semolina cereals compared to raw semolina (Table I). Extrusion processing at both experimental temperatures caused a marked decrease in the percentage of total protein present as albumin, globulin, gliadin, and glutenin fractions with a corresponding increase in the insoluble residue fraction. Extrusion at a higher temperature (96°C) caused a greater increase in insoluble residue than did the lower temperature (50°C). Previously, Dexter and Matsuo (1977) reported that extrusion of semolina at 50°C increased the amount of insoluble residue protein concomitant with a decrease in the amount of salt-soluble protein. An increase in the insolubility of protein components following high-temperature drying of pasta was also reported (Dexter et al 1981, De-Stefanis and Sgrulletta 1990, Aktan and Khan 1992). The marked decrease in the solubility of

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semolina proteins seen after extrusion in the present study (Table I) may have implications for the food industry. For example, some baby foods prepared by extrusion cooking may have low solubility upon rehydration if prepared under high temperature extrusion. Extrusion may also influence protein digestibility of products. Dahlin and Lorenz (1993) reported that in vitro protein digestibility of extruded wheat was improved by extrusion. Products extruded at 100–150°C showed the highest protein digestibility. Another example is the role of proteins in determining the cooking quality of the product. The cooking quality of an extruded product is reported to be affected by the relative amount of insoluble protein (Sgrulletta and De-Stefanis 1989, De-Stefanis and Sgrulletta 1990) and low molecular weight glutenin proteins (Feillet et al 1989) and the glutenin-to-gliadin ratio (Wasik and Bushuk 1975).

Electrophoresis

The molecular weight (MW) distribution profiles obtained from SDS-PAGE under reduced or unreduced conditions for albumins of raw and extruded semolina are presented in Figure 1 (lanes 2–4, 11–13). After extrusion of raw semolina, the reduced SDS-PAGE pattern of albumins showed a disappearance of the high molecular weight (HMW) (estimated at 61,500) polypeptides and an appearance of a low molecular weight (LMW) polypeptides MW polypeptide (estimated at 49,800). Although the patterns were similar for both extruded semolina cereals, extrusion at 96°C resulted in bands of greater intensity than those from extrusion at 50°C. These results may be due to the depolymerization of some proteins extruded at 50°C, with subsequent LMW polypeptides eluting off the gel during electrophoresis. Alternatively, at 96°C, aggregation of proteins may occur resulting in relatively greater intensity of bands, even though equivalent amounts of total protein were loaded per gel lane for both temperatures. There were differences between the SDS-PAGE patterns of reduced and unreduced raw (lane 2 vs. 11) or extruded (lane 3 vs. 12, 4 vs. 13) semolina, indicating that the extrusion temperatures used in the present study did not destroy all disulfide bonds linked to the proteins.

Under reduced conditions (Fig. 1, lane 5), the globulin fraction of raw semolina contained five major regions of polypeptides with estimated MW of 34,600, 37,000, 45,200, 49,800, and 61,500. Several fainter bands in the range of MW 34,600–67,500 were also observed. Extrusion temperatures of 50 and 96°C affected protein patterns similarly, decreasing intensity of some bands in the HMW region (49,800–63,300) and increasing intensity of some bands in the LMW region (<45,200) (lanes 5 vs. 6 and 7). There were differences noted between the SDS-PAGE patterns of reduced and unreduced globulin fractions from raw (lane 5 vs. 14) or extruded (lane 6 vs. 15, 7 vs. 16) semolina, suggesting that disulfide bonds still played a role in extruded products in the present study.

The SDS-PAGE patterns of the reduced gliadin fraction of semolina, shown in Figure 1 (lanes 8–10), were similar before and after extrusion at 50 or 96°C. However, a band representing a polypeptide of estimated MW 67,500 appeared fainter after extrusion at 96°C when compared to those of raw semolina or extrusion at 50°C. These results confirm the findings of Aktan and Khan (1992), who observed few changes in the SDS-PAGE patterns of gliadin fractions of dried pasta compared to those of semolina. In the present study, the patterns of the unreduced fractions from raw and extruded semolina (lanes 17–19) were similar to the reduced gliadin fraction pattern. In addition, a few HMW glutenin subunits appeared in the raw semolina (lane 8) but not in either of the extruded semolina cereals. These subunits could have aggregated after extrusion at the temperatures used. As for globulins, there were apparent differences between the SDS-PAGE patterns of reduced and unreduced gliadin fractions from raw (lane 8 vs. 17) or extruded (lane 9 vs. 18, 10 vs. 19) semolina.

The reduced glutenin fraction of raw semolina appeared to have six major regions of polypeptides: MW 37,000, 45,000–50,000, 65,000, 92,400, 100,000 and 120,000 (Fig. 2, lane 2). After extrusion, a disappearance of bands in the HMW region (>65,000), an increase in intensity of the band at 65,000, and an appearance of some bands in the LMW region (<45,200) were observed (lane 2 vs. 3 and 4). Aktan and Khan (1992) also observed similar SDS-PAGE patterns in dried pasta when compared to semolina; the intensity of HMW (66,000–110,000) glutenin bands decreased as the intensity of LMW (<31,000) bands increased with an increase in drying temperature. In the present study, extrusion at 50 and 96°C showed similar patterns (lane 3 vs. 4). However, the 65,000 band was more intense after extrusion at the higher temperature (96°C) than at the lower temperature (50°C). There were differences in the SDS-PAGE patterns of reduced versus unreduced extruded semolina cereals in the 35,000–45,000 region, but not above 45,000 (lane 3 vs. 9, 4 vs. 10). In raw semolina, the unreduced pattern (lane 8) did not show the HMW polypeptides (above 65,000) and some LMW polypeptides (45,000–50,000) that were seen in the reduced fraction (lane 2).

The reduced insoluble residue of raw semolina produced an SDS-PAGE pattern with band ranges of MW 45,000–50,000 and 92,000–128,000 (Fig. 2, lane 5). After extrusion, the intensity of all bands was greater, and bands appeared in the range of MW 61,000–67,500 (lane 5 vs. 6 and 7). Extrusion at 96°C (lane 7) produced slightly more intense bands when compared to those at the extrusion temperature of 50°C (lane 6), although both showed similar SDS-PAGE patterns. In the unreduced SDS-PAGE pattern of the insoluble residue of raw semolina (lane 11), no bands were visible. The unreduced insoluble fraction of extruded semolina cereals showed only two sets of very faint bands at MW 34,600 and 65,000 (lanes 12 and 13).

Results indicate that polypeptides of albumins, globulins, and glutenins of semolina were most likely aggregated after extrusion at 50 or 96°C (Figs. 1 and 2, unreduced). In contrast, the MW distribution of gliadin proteins seemed to be unaffected by extru-

TABLE I
Protein Fractionation of Raw and Extruded Semolina[a]

		Extrusion Temperatures	
	Raw Semolina	50°C	96°C
Protein (%)	14.2	14.4	14.9
Albumin fraction			
Weight (g/10 g of prod)	0.5	1.07	0.92
Protein content (%)[b]	31.3	14.8	2.9
Total protein (%)[c]	11.7	10.8	2.0
Globulin fraction			
Weight (g/10 g of prod)	0.09	0.03	0.24
Protein content (%)	54.6	49.9	9.6
Total protein (%)	3.6	1.4	0.7
Gliadin fraction			
Weight (g/10 g of prod)	0.95	0.47	0.54
Protein content (%)	62.1	22.5	9.4
Total protein (%)	41.8	7.3	3.4
Glutenin fraction			
Weight (g/10 g of prod)	0.33	0.19	0.18
Protein content (%)	60.1	20.7	32.4
Total protein (%)	14.2	2.8	4.0
Insoluble fraction			
Weight (g/10 g of prod)	7.38	7.59	7.73
Protein content (%)	5.3	14.1	16.1
Total protein (%)	27.7	74.0	83.2
Recovery			
Weight (%)	92.8	95.1	94.7
Protein (%)[d]	98.6	96.2	93.3

[a] Values represent means of two replicates.
[b] Protein content (N × 5.83).
[c] Protein (%) × fraction wt [(g) × 10 / total protein].
[d] Sum of % total protein in each fraction.

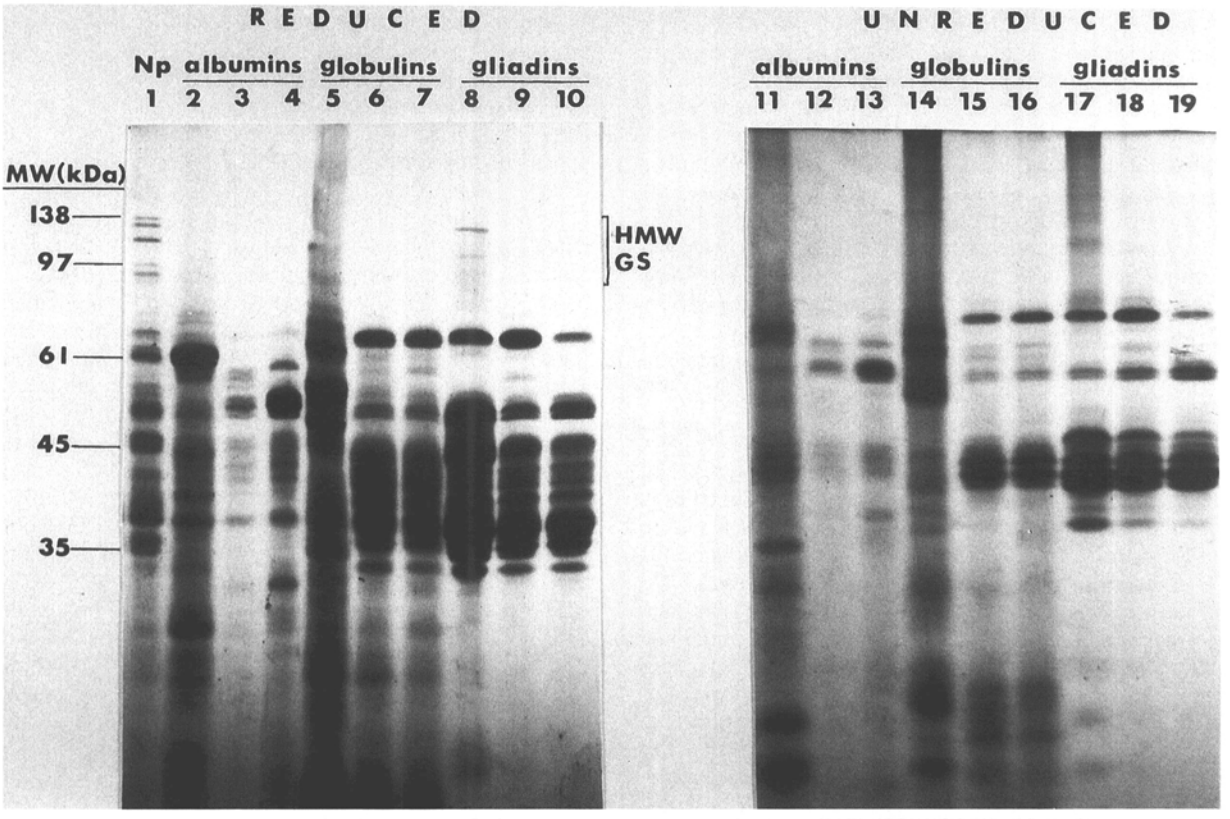

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoretic patterns of reduced (with 2-mercaptoethanol) and unreduced albumin, globulin, and gliadin fractions from raw and extruded semolina. Np = protein extract from flour of cultivar Neepawa; lanes 2,5,8,11,14,17 = fractions from raw semolina; lanes 3,6,9,12,15,18 = fractions from semolina extruded at 50°C; lanes 4,7,10,13,16,19 = fractions from semolina extruded at 96°C; HMW-GS = high molecular weight glutenin subunits.

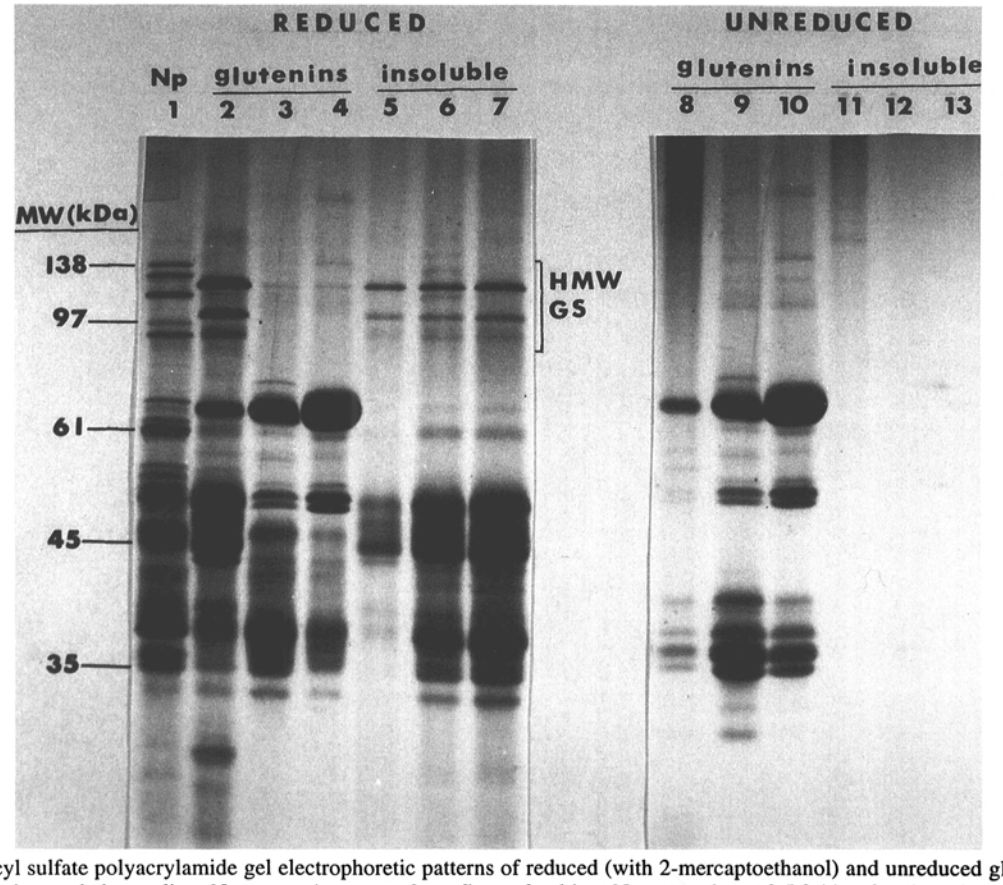

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoretic patterns of reduced (with 2-mercaptoethanol) and unreduced glutenin and insoluble fractions from raw and extruded semolina; Np = protein extract from flour of cultivar Neepawa; lanes 2,5,8,11 = fractions from raw semolina; lanes 3,6,9,12 = fractions from semolina extruded at 50°C; lanes 4,7,10,13 = fractions from semolina extruded at 96°C; HMW-GS = high molecular weight glutenin subunits.

sion at either experimental temperature (same pattern for raw and both extruded cereals) (Fig. 1, lanes 17–19). The differences in electrophoretic patterns of the albumin and globulin fractions for reduced versus unreduced fractions demonstrate that disulfide linkages likely played a major role in the structural changes that occurred during extrusion.

In the glutenin fraction, under reduced conditions, the HMW polypeptides seen in the raw semolina are not visible in the extruded cereals. In contrast, under reduced conditions, the insoluble fractions from extruded cereals show a greater intensity of bands in the same region when compared to those of the insoluble fraction from raw semolina. Thus, these differences suggest that extrusion may have caused the HMW extractable glutenin polypeptides to aggregate and contribute to the nonextractable glutenin (insoluble) fraction of semolina.

The insoluble fraction from raw and extruded semolina produced different electrophoretic patterns under unreduced and reduced conditions. In both raw and extruded semolina, there was an apparent lack of bands under unreduced conditions and an appearance of bands under reduced conditions, indicating the presence of disulfide bonds between polypeptides in the insoluble fraction. The results of this study also suggest that extrusion may have promoted the formation of additional disulfide bonds which, when broken, release the polypeptides; hence there is an increase in band intensity for the insoluble fraction of extruded semolina when compared to that of raw semolina. In contrast, Dexter and Matsuo (1977) reported that extrusion of semolina at 50°C did not produce any significant changes in disulfide bond levels or molecular weight distribution of proteins. Also, no qualitative changes in protein electrophoretic patterns were observed. It was concluded that the loss in protein solubility following extrusion may have been due to binding by the insoluble components and not due to polymerization.

The role of disulfide bonds in thermal extrusion effects on protein solubility has been studied previously. However, most of these studies were conducted using soy proteins. Hager (1984) showed that disulfide bonds contribute to the new, extended protein networks produced by extrusion of soy concentrate. Rhee et al (1981) reported that extrusion at 138°C reduced protein solubility; an increase in protein solubility was achieved by the use of 2-mercaptoethanol and SDS, suggesting that disulfide linkages may be responsible for the decrease in protein solubility after extrusion.

CONCLUSIONS

Extrusion processing at both 50 and 96°C caused a marked increase in the percentage of total protein present in the insoluble glutenin fraction. Results of this study also indicate the presence of disulfide linkages between polypeptides in the insoluble fraction that may have been promoted by extrusion. Such information may prove to be relevant in the improvement of cooking quality of extruded foods or in the use of extrusion processing to manufacture weaning foods and infant formulas.

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