Relationship of the Extrusion Temperature and the Solubility and Disulfide Bond Distribution of Wheat Proteins

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Wheat flour was extruded at die temperatures of 60, 90, 120, and 160 °C. The soluble protein content in the extrudates decreased by 66.7% in the extracting buffer (1% sodium dodecyl sulfate in 50 mM sodium phosphate buffer, pH 6.9) as the extrusion die temperature was increased to 160 °C. The most insoluble proteins in the extrudates extruded at die temperatures of up to 120 °C could be resolubilized by using sonication. The changes in the disulfide/protein ratio corresponded with the changes in protein solubility in the extrudates. SDS−PAGEs showed that the content of large molecular weight proteins in the extrudates increased as the extrusion die temperature was increased. However, these proteins completely disappeared after being reduced by β-mercaptoethanol. This study showed the relationship between the extrusion temperature and the solubility and disulfide distribution of wheat proteins.

INTRODUCTION

Wheat is one of most widely cultivated plants. Wheat protein is unique among cereal and other plant proteins in its ability to form a dough with viscoelastic properties ideally suited to making bread, biscuit, pasta, and cereal products (Autran, 1993; Kokini et al., 1994). Protein comprises around 10.3% of wheat flour (Pennington, 1994). Numerous researchers have investigated the physicochemical properties of gliadins and glutenins and their relationships to the bread-making potential of a flour. Studies on the effect of heat on wheat protein interactions have shown that the sulfhydryl−disulfide interchange reaction was responsible for the protein network formation upon thermosetting (Sarwin et al., 1993; Strecker et al., 1995; Weegels et al., 1994a,b). Furthermore, it has been shown that disulfide cross-links played a significant role in the protein network formation in a dough. Disulfide reducing agents (cysteine, glutathione, dithiothreitol, and sulfite), sulfhydryl oxidizing agents (bromate and iodate), and sulfhydryl blocking agents (*N*-ethylmaleimide) can change the physical properties of a dough considerably (Dreese et al., 1988; Gaines, 1990; Sarwin et al., 1993; Tsen, 1969).

Extrusion is one of the most versatile and well-established food processes and is widely used in food and feed industries today to make products such as snacks, cereals, pastas, textured vegetable proteins, pet foods, and animal feeds (Rizvi et al., 1995). Extrusion processing can alter protein structure and solubility by heat, shear force, pressure, and oxygen (Li and Lee, 1996a; Phillips, 1989; Ummadi et al., 1995). Extrusion processing also influences the protein digestibility of products. In vitro, the protein digestibility of wheat can be improved by extrusion at a feed moisture of 15%, a product temperature of 100−150 °C, and a screw speed of 100 rpm (Dahlin and Lorenz, 1993). Some studies have reported that high-temperature drying of pasta (>70 °C) and high-temperature extrusion of wheat flour (>160 °C) resulted in a dramatic decrease in protein

solubility (Aktan and Khan, 1992; De-Stefanis and Sgrulletta, 1990; Dexter et al., 1981; Li and Lee, 1996a). The solubility of semolina proteins decreased by 46.3% after the twin-screw extrusion at a die temperature of 50 °C, a moisture content of 16%, and a screw speed of 900 rpm (Ummadi et al., 1995). The aggregation of proteins during extrusion processing is responsible for the decrease in their solubility, and the formation of new disulfide cross-links during extrusion processing is the major covalent force for the protein aggregations (Li and Lee, 1996a,b; Prudêncio-Ferreira and Arêas, 1993; Strecker et al., 1995). However, the relationship of the extrusion temperature and the disulfide formation and the molecular weight of wheat proteins is not clear. The purpose of this study is to investigate the quantitative relationship of the extrusion temperature and the solubility and disulfide distribution of wheat proteins.

MATERIALS AND METHODS

Materials. Commercial wheat flour (Bouncer flour OS2530), purchased from Bay State Milling Co., Quincy, MA, was used for all experiments. The protein content of the wheat flour was 14% and was determined by using the semi-micro-Kjeldahl method (AACC Method 46-13, 1983). Ellman's reagent and the bicinchoninic acid (BCA) protein assay reagent were purchased from Pierce Chemical Co. (Rockford, IL). The prestained molecular weight standards were purchased from Bio-Rad Laboratories (Richmond, CA). α-Amylase (EC 3.2.1.1, from *Bacillus* species, 2100 units/mg) and other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Springfield, NJ).

Extrusion and Sample Preparation. The extrusion was carried out on a ZSK-30 corotating twin-screw extruder (Werner Pfleiderer Corp., Ramsey, NJ). The unit was equipped with a die having two 3 mm diameter, 5 mm long, openings. The length and diameter of each screw were 900 and 30 mm, respectively. The screw configuration used in the experiments consisted of forwarding elements (L/D = 21.9), two mild mixing elements (L/D = 2.7), six kneading elements (L/D = 3.6), and two reverse elements (L/D = 1.1). The barrel had resistance heaters and five independently controlled heating zones. The barrel also had cooling jackets through which cooling water could be circulated at controlled flow rates (solenoids) to prevent overheating of the barrel. The heaters and five solenoids were controlled using a PID controller. Product

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Table 1. Extrusion Conditions for Experiments

sample ID	feed moisture (% H$_2$O, w/w)	total mass flow rate (g/min)	screw speed (rpm)	die temp (°C)
control[a]				
W-60	30	275	200	60
W-90	30	275	200	90
W-120	30	275	200	120
W-160	30	275	200	160

[a] Unextruded wheat flour.

temperatures were recorded by a thermocouple inserted at the die plate. Wheat flour was fed into the unit with a K-Tron series 7100 volumetric feeding system (K-Tron Crop., Pitman, NJ). A metering pump (U.S. Electric Motors, Millford, CT) was used to add the water into the water inlet.

Wheat flour was extruded at die temperatures of 60, 90, 120, and 160 °C. The feed rate and screw speed were kept constant at 275 g/min and 200 rpm, respectively. Moderate tap water (hardness index = 35) was fed into the extruder to provide a total moisture content of 30% (wet weight basis) (Table 1). The extrudates were collected onto a large scale (about 1 kg for each sample) after the extruder had reached equilibrium conditions, as indicated by the steady die temperature and torque. The extrudates were freeze-dried and were then ground with a laboratory pulverizing mill (Glen Mills Inc., Maywood, NJ) to pass through a 0.2 mm diameter mesh. Liquid nitrogen was poured into the mill to prevent an increase in temperature. Ground samples were sealed and stored at −20 °C in glass bottles for further analyses. After freeze-drying, the moisture content of each ground extrudate was determined according to AOAC Method 934.01 (AOAC, 1990). Approximately 2.0 g of each sample was placed in an isotemp vacuum oven (Model 282A, Fisher Scientific) at 100 ± 2 °C and 300 mmHg for 16 h. The moisture content was calculated as the loss in weight.

Sonication and Protein Extraction. Before sonication, approximately 2.0 g of each ground sample was dispersed in 40 mL of 50 mM sodium phosphate buffer (pH 6.9) containing 4 units/mL α-amylase and shaken for 6 h to digest soluble starches. SDS (0.4 g) was added to the above mixture to a concentration of 1.0% (w/w). The mixture was shaken for an additional 2 h and was then transferred into a 50 mL centrifuge tube for sonication. The sonication was performed according to the method described by Singh et al. (1990) with some modifications. A Model XL2020 sonifier (Heat Systems Inc., Framingdale, NY) was used with a macrotip probe (12.7 mm in diameter) for large-scale sonication (40 mL of the extracting buffer and 2 g of the sample in a 50 mL polycarbonate centrifuge tube). The sonifier generated ultrasonic vibrations with a frequency of 20 kHz. The sonication power was set at output control knob 5, which was appropriate for breaking noncovalent bonding of proteins (Singh et al., 1990). The sonication was performed with a timed/pulsed program, 20 cycles of process for 1 min and a pause for 30 s. A 0 °C water bath was used to prevent heat buildup in the processed sample. After sonication, the mixture was further shaken for 4 h.

The soluble proteins in the wheat flour control and extrudates were extracted with and without sonication. After that, the mixture was centrifuged at 20000g for 40 min at 4 °C, and the supernatant was collected. The protein content in the supernatant was determined by using the BCA protein assay reagent, which was compatible with detergents (up to 1.0% SDS) (Pierce, Catalog & Handbook, 1994−95, p 0-65).

Approximately 2.0 g of each ground sample was dispersed in 40 mL of 50 mM sodium phosphate buffer (pH 6.9) containing 1% SDS and 2% β-mercaptoethanol and shaken for 12 h. The mixture was centrifuged at 20000g for 40 min at 4 °C, and the supernatant was collected. The soluble nitrogen contents of the wheat flour control and extrudates in the 1% SDS plus 2% β-mercaptoethanol aqueous system were determined according to the method of Li and Lee (1996a).

Protein Fractionation. The protein fractionation was conducted on a pressure-modified ultrafilter (Stirred Cells, Model 8050, Amicon Inc., Beverly, MA) equipped with a
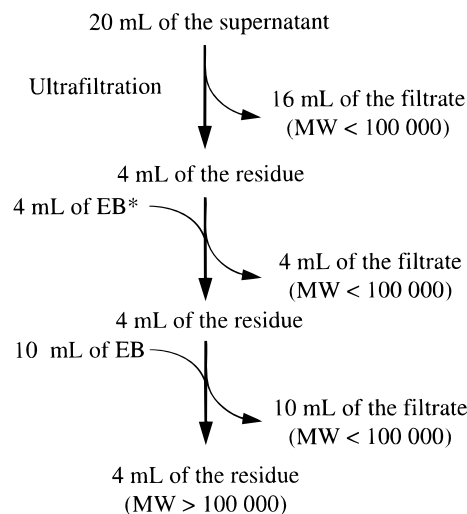

Figure 1. Flow chart for sequentially fractionating soluble proteins in the wheat flour control and extrudates by using a pressure-modified ultrafilter equipped with a macroporous membrane of molecular weight cutoff at 100 000. EB, extracting buffer (1% SDS in 50 mM sodium phosphate buffer, pH 6.9).

macroporous membrane with a molecular weight cutoff at 100 000 (Amicon Inc.). The supernatant obtained with the 1% SDS in 50 mM sodium phosphate buffer with and without sonication was separated into two fractions on the basis of protein molecular weight: >100 000 and <100 000. The fractionation procedures are shown in Figure 1. The protein content in each fraction was determined by using the BCA protein assay reagent.

Sulfhydryl Group and Disulfide Bond Determination. *In the Soluble Fraction.* The total soluble sulfhydryl content (including free sulfhydryl and disulfide) of the wheat flour control and extrudates was determined according to the method of Thannhauser et al. (1987). The soluble free sulfhydryl content was determined according to the Pierce method (Pierce, Immunotechnology Catalog, 1993, p E-56). The detailed procedures were the same as described by Li and Lee (1996a).

In the Solid Phase. The total sulfhydryl content and total free sulfhydryl content of the wheat flour control and extrudates were determined according to the method described by Chan and Wasserman (1993) with some modifications. Approximately 100 mg of a sample with a particle size smaller than 40 mesh was suspended in 4.0 mL of the reaction buffer. The detailed procedures were the same as described by Li and Lee (1996b).

The disulfide content was calculated from the difference between total and free sulfhydryl contents. The insoluble disulfide content was calculated from the difference between total and soluble disulfide contents.

Gradient Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). The SDS−PAGE of proteins was performed according to the method of Bollag and Edelstein (1991) on 3−20% gradient polyacrylamide (w/v) gels. Prestained molecular weight markers for the gradient SDS−PAGE were as follows: myosin (207 000, blue), β-galactosidase (139 000, magenta), bovine serum albumin (84 000, green), carbonic anhydrase (41 700, violet), soybean trypsin inhibitor (32 000, orange), lysozyme (17 900, red), and aprotinin (8 600, blue).

RESULTS AND DISCUSSION

Protein Solubility. The soluble protein contents in the wheat flour control and extrudates are shown in Tables 2 and 3.

a. Without Sonication To Enhance Solubilization of Wheat Proteins. On the basis of the BCA protein assay

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Table 2. Protein Content in the Soluble Fractions of the Wheat Flour Control and Extrudates in the Extracting Buffer of 1% (w/v) SDS in 50 mM Sodium Phosphate Buffer (pH 6.9)

| | protein content[a] (mg/g of control or extrudate on dry basis) in each soluble fraction | | | | | |
| | without sonication | | | with sonication | | |
sample ID	supernatant	>100 000	<100 000	supernatant	>100 000	<100 000
control	132.1 ± 2.1	103.8 ± 0.3	19.1 ± 0.1	129.0 ± 1.0	103.9 ± 0.7	26.0 ± 0.9
W-60	116.3 ± 2.5	99.7 ± 0.4	16.4 ± 0.3	127.3 ± 0.3	105.3 ± 0.5	26.9 ± 0.1
W-90	92.8 ± 0.3	71.2 ± 0.3	21.6 ± 0.3	129.3 ± 1.7	105.8 ± 0.7	30.7 ± 0.8
W-120	69.8 ± 1.9	41.9 ± 0.6	24.0 ± 0.3	125.9 ± 2.1	99.9 ± 0.9	27.3 ± 0.4
W-160	44.3 ± 0.3	25.6 ± 0.2	22.3 ± 0.6	60.3 ± 0.2	43.8 ± 0.2	27.5 ± 0.2

[a] The value is an average of triplicate measurements ± standard deviation.

Table 3. Soluble Nitrogen Content[a] and Protein Content[b] of the Wheat Flour Control and Extrudates in the 1% SDS plus 2% β-Mercaptoethanol Aqueous System

| | 1% SDS + 2% β-mercaptoethanol aqueous system | |
sample ID	soluble N (μmol/g of control or extrudate, dry basis)	soluble protein (mg/g of control or extrudate, dry basis)
control	1566	124.9
W-60	1807	144.2
W-90	1807	144.2
W-120	1719	137.2
W-160	1666	132.9

[a] The value is an average of duplicate measurements. [b] The protein content (mg) was calculated according to the equation protein content (mg) = N content (nmol) × 14 × (5.7/1000).

method, the total soluble protein content in the extrudates in the extracting buffer (1% SDS in 50 mM sodium phosphate buffer, pH 6.9) decreased dramatically as the extrusion die temperature was increased to 160 °C (Table 2). As compared to the soluble protein content in the control, approximately 12.0−66.5% of the proteins in the extrudates became insoluble in the extracting buffer after extrusion at a die temperature of 60−160 °C (Table 2). The decrease in the soluble protein content in the molecular weight >100 000 fraction corresponded with the decrease in the total soluble protein content in the supernatant of the extrudates. However, the soluble protein content in the molecular weight <100 000 fraction was affected little by the extrusion die temperatures, around 20 mg of protein/g of control or extrudate (Table 2). These results demonstrated that, after extrusion, the insoluble aggregated proteins might be formed from the proteins with a molecular weight >100 000, leaving residual proteins with a molecular weight <100 000 that could be extracted.

b. With Sonication To Enhance Solubilization of Wheat Proteins. On the basis of the BCA protein assay method, the total soluble protein content in the extrudates in the extracting buffer changed little, as the extrusion die temperature was increased to 120 °C (Table 2). However, the total soluble protein content in the extrudates decreased dramatically, from 125.9 to 60.3 mg of protein/g of extrudate, as the extrusion die temperature was further increased from 120 to 160 °C (Table 2). The changes of the soluble protein content in the fraction that had a molecular weight of >100 000 corresponded to the changes of the total soluble protein content in the supernatant of the extrudates. The soluble protein content in the fraction that had a molecular weight of <100 000 changed little with the increased extrusion die temperature, around 27 mg of protein/g of control or extrudate (Table 2). These results further confirmed that, after extrusion, the insoluble aggregated proteins might be formed from the proteins with a molecular weight >100 000, leaving residual proteins with a molecular weight <100 000 that could be extracted.

Since the solubility of heated proteins depended on their molecular weights (Pomeranz, 1991), the decrease in wheat protein solubility after extrusion in the extracting buffer indicated the increase in its molecular weight, which might be the result of the aggregation of proteins during extrusion processing. Sonication carried out at a low energy level could disrupt the weak noncovalent bonding forces to reduce the molecular weight of aggregated proteins, which made proteins resolubilize (Jennings, 1978). Most of the insoluble proteins in the extrudates extruded at a die temperature of up to 120 °C could be resolubilized by using sonication, indicating that noncovalent bonding forces contributed to wheat protein aggregations during extrusion processing. Therefore, most of the insoluble proteins in the extrudate extruded at a die temperature of 160 °C could not be resolubilized by using sonication, indicating that the extensive covalent cross-linking of wheat proteins might form. This extensive covalent cross-linking, which could not be disrupted by sonication at a low energy level, resulted in a dramatic increase in protein molecular weight and, subsequently, a decrease in protein solubility. However, almost all of the wheat proteins in the extrudates became soluble in the 1% SDS plus 2% β-mercaptoethanol aqueous system (Table 3). These results further supported our earlier conclusion that the major aggregation forces of wheat proteins during extrusion processing were hydrophobic interactions and disulfide cross-links (Li and Lee, 1996a).

Sulfhydryl Group and Disulfide Bond Distributions. The free sulfhydryl content and disulfide content in both the soluble fraction and the solid phase of the wheat flour control and extrudates are shown in Table 4. The total free sulfhydryl content and disulfide content (including soluble and insoluble, as determined in the solid phase) were affected little by the extrusion die temperatures, around 2.0 and 15.0 μmol/g of control or extrudate, respectively (Table 4). However, the distribution of disulfide bonds in the soluble and insoluble proteins in the extrudates changed greatly with the increased extrusion die temperatures (Figures 2 and 3).

a. Without Sonication To Enhance Solubilization of Wheat Proteins. For the extrudates extruded at die temperatures of 60, 90, 120, and 160 °C, the content of soluble disulfide bonds decreased dramatically from 127.1 to 94.1 and 61.7 nmol/mg of protein and a nondetectable level, respectively (Table 4). In contrast, the content of insoluble disulfide bonds increased from 44.6 to 145.5 nmol/mg of protein (Figure 2). The content of free soluble sulfhydryl groups varied, from 12.0 to 25.4 nmol/mg of protein (Table 4). The changes of the disulfide content corresponded with the changes of the

Table 4. Free Sulfhydryl and Disulfide Contents[a] in the Wheat Flour Control and Extrudates

| | solid phase[b] (μmol/g of control or extrudate, dry basis) | | supernatant[c] (nmol/mg of protein) | | | |
| | | | SH | | SS | |
sample ID	SH	SS	nonsonication	sonication	nonsonication	sonication
control	1.9 ± 0.2	15.5 ± 1.2	13.7 ± 0.5	14.8 ± 0.3	114.5 ± 0.3	126.6 ± 1.3
W-60	2.0 ± 0.2	15.8 ± 0.9	12.0 ± 0.1	15.3 ± 0.5	127.1 ± 0.9	117.8 ± 1.4
W-90	2.3 ± 0.1	15.9 ± 0.5	17.9 ± 0.6	17.9 ± 0.3	94.1 ± 2.6	113.6 ± 0.4
W-120	2.0 ± 0.1	15.5 ± 0.5	14.4 ± 0.8	15.9 ± 0.6	61.7 ± 2.3	110.5 ± 0.8
W-160	1.8 ± 0.1	13.9 ± 0.6	25.4 ± 1.0	30.3 ± 0.6	ND[d]	47.5 ± 1.3

[a] The value is an average of six measurements ± standard deviation. [b] Total free sulfhydryl content and total disulfide content of the wheat flour control and extrudates. [c] Total soluble free sulfhydryl content and disulfide content of the wheat flour control and extrudates in the extracting buffer of 1% SDS in 50 mM sodium phosphate buffer (pH 6.9). [d] Not detectable.
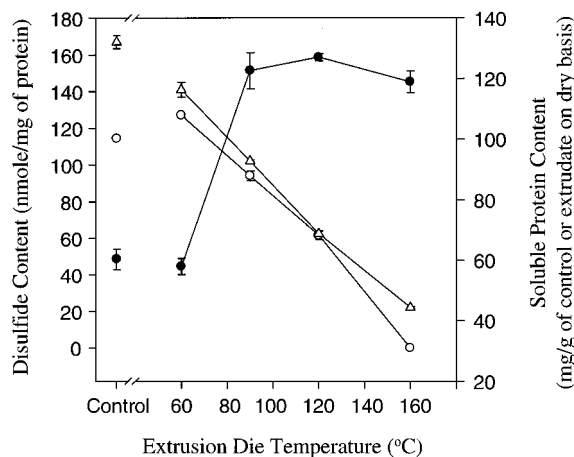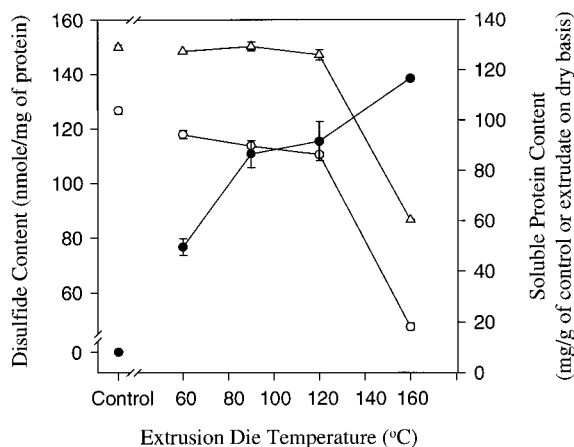

Figure 2. Effect of extrusion die temperature on wheat protein solubility and disulfide bond distribution **without** sonication to enhance solubilization of wheat proteins: (△) soluble protein content; (○) soluble disulfide content; (●) insoluble disulfide content.

Figure 3. Effect of extrusion die temperature on wheat protein solubility and disulfide bond distribution **with** sonication to enhance solubilization of wheat proteins: (△) soluble protein content; (○) soluble disulfide content; (●) insoluble disulfide content.

protein content in the soluble fractions of the extrudates as affected by the extrusion die temperatures (Figure 2).

b. With Sonication To Enhance Solubilization of Wheat Proteins. When sonication was used to solubilize proteins, the content of soluble disulfide bonds decreased slightly from 126.6 nmol/mg of protein in the control to 110.5 nmol/mg of protein in the extrudate, as the extrusion die temperature was increased to 120 °C (Table 4). However, it decreased sharply to 47.5 nmol/mg of protein in the extrudate as the extrusion die temperature was further increased to 160 °C (Table 4).

In contrast, the content of insoluble disulfide bonds increased from a nondetectable level in the control to 138.3 nmol/mg of protein in the extrudate as the extrusion die temperature was increased to 160 °C (Figure 3). The content of soluble free sulfhydryl groups varied from 15.3 to 30.6 nmol/mg of protein in the extrudates (Table 4). These results also showed that the changes in the disulfide content corresponded with the changes in the protein content of the soluble fractions of the extrudates as affected by the extrusion die temperature (Figure 3).

The content of soluble free sulfhydryl groups in the extrudate extruded at a die temperature of 160 °C was almost twice that of the extrudates extruded at die temperatures of up to 120 °C. This indicated that the proteins containing a high level of free sulfhydryl groups were still soluble, probably because they were not involved in aggregations during extrusion processing and had low molecular weights. The parallel changes between the soluble disulfide content and the soluble protein content in the extrudates as affected by the extrusion die temperatures quantitatively supported our previous conclusion (Li and Lee, 1996a,b) that the disulfide cross-link was the primary covalent bonding force in wheat protein aggregations during extrusion processing. During extrusion processing, the wheat proteins denatured and aggregated through the weak noncovalent interaction and interchain disulfide cross-linking. The aggregations of proteins resulted in an increase in their molecular weights and, subsequently, a decrease in their solubility. Under our present experimental conditions, the extensive disulfide cross-linking of wheat proteins occurred at an extrusion die temperature between 120 and 160 °C.

SDS−PAGE. The 3−20% gradient SDS−PAGEs of soluble proteins in the wheat flour control and extrudates were done under reducing and unreducing conditions to check the effect of the extrusion die temperature on the molecular weight distribution and interchain disulfide cross-link of proteins. Since an equal volume of the supernatant was loaded on each well of the gel for each sample, the changes in the relative molecular weight distribution of soluble proteins could be compared from the gels. The results are shown in Figures 4 and 5.

As shown on the unreduced SDS−PAGE (Figure 4A), there are some proteins with a molecular weight of 41 700−207 000 which appeared in the control and the extrudate extruded at a die temperature of 60 °C. These proteins then disappeared in the extrudates extruded at die temperatures of 90, 120, and 160 °C. For the extrudate extruded at a die temperature of 160 °C, the molecular weights of the most soluble proteins were <32 000. As shown in Figure 4B, after reduction by β-mercaptoethanol, the molecular weights of all soluble
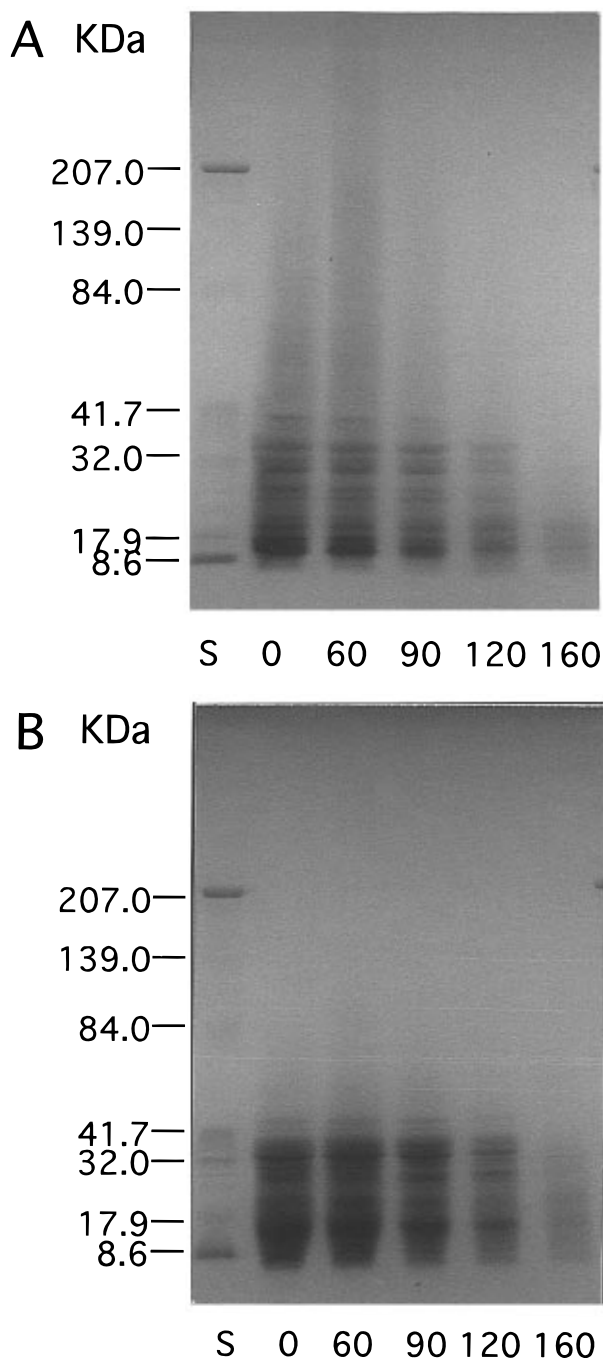
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Figure 4. 3−20% gradient SDS−PAGEs of soluble proteins from wheat flour control and extrudates in the extracting buffer (1% SDS in 50 mM sodium phosphate buffer, pH 6.9) **without** sonication to enhance solubilization of wheat proteins: (A) unreduced proteins; (B) reduced proteins. S, the molecular weight standard; 0, wheat flour control; 60, 90, 120, and 160, wheat flour extrudates extruded at die temperatures of 60, 90, 120, and 160 °C, respectively.

Figure 5. 3−20% gradient SDS−PAGEs of soluble proteins from wheat flour control and extrudates in the extracting buffer (1% SDS in 50 mM sodium phosphate buffer, pH 6.9) **with** sonication to enhance solubilization of wheat proteins: (A) unreduced proteins; (B) reduced proteins. S, the molecular weight standard; 0, wheat flour control; 60, 90, 120, and 160, wheat flour extrudates extruded at die temperatures of 60, 90, 120, and 160 °C, respectively.

proteins were <42 000. These results indicated that most multi-sub-unit proteins linked by disulfide bonds were involved in the aggregation during extrusion processing.

As shown in Figure 5A, most proteins with a molecular weight >42 000 in the extrudates were resolubilized by using sonication. When sonication was used to enhance the solubility of proteins, the content of soluble proteins was affected little in the extrudates by extrusion die temperatures of up to 120 °C (Table 2). However, their molecular weight distributions changed greatly. As indicated by the intensity of the unreduced
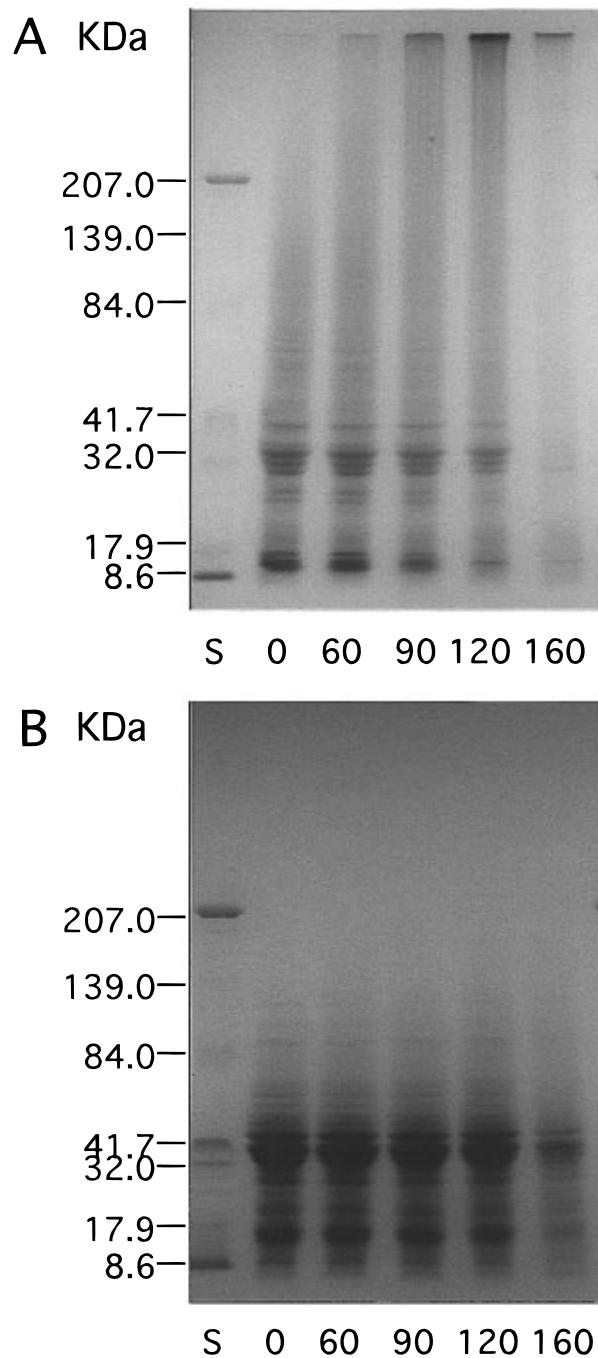
SDS−PAGE (Figure 5A), the content of soluble proteins with a molecular weight >207 000 increased dramatically in the extrudates as the extrusion die temperature was increased up to 120 °C. However, these very large molecular weight proteins completely disappeared after reduction by β-mercaptoethanol (Figure 5B). These results demonstrated that during extrusion processing the aggregation of wheat proteins occurred and the intermolecular disulfide cross-linking was the major covalent bonding force for the aggregation. Furthermore, for the extrudate extruded at a die temperature of 160 °C, most proteins could not be resolubilized by

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using sonication at a low energy level. Almost all of the proteins in the extrudates, however, became soluble in the SDS−mercaptoethanol aqueous system (Table 3). These results indicated that wheat proteins aggregated extensively through intermolecular disulfide bonds, which could not be disrupted by using sonication at a low energy level. This aggregation resulted in proteins with a larger molecular weight and a lower solubility. The range of the extrusion die temperature for the formation of extensive disulfide cross-linking of wheat proteins was between 120 and 160 °C.

In our studies, after extrusion of wheat flour, a decrease in the soluble disulfide content was observed, which corresponded with a decrease in the soluble protein content. Furthermore, the total disulfide content in wheat flour of around 15.0 μmol/g of control or extrudate was affected little by the extrusion die temperatures (Table 4). The SDS−PAGEs of unreduced and reduced soluble proteins showed the aggregation of wheat proteins through disulfide cross-links during extrusion processing. These results indicated that during the extrusion process sulfhydryl−disulfide and disulfide−disulfide interchange reactions might take place, which might contribute to the aggregation of proteins through the interchain disulfide bonds. A small increase in the interchain disulfide formation could result in a large increase in the network formation in wheat gluten and glutenin (Strecker et al., 1995). The disulfide cross-links of wheat proteins directly affected the microstructural and textural properties of wheat flour extrudates (Li and Lee, 1996b). As a disulfide reducing agent, the addition of cysteine weakened the disulfide cross-linking of wheat proteins during extrusion processing, resulting in obvious changes in the microstructures and the textural properties of the wheat flour extrudates (Li and Lee, 1996b). The disulfide cross-linking of gluten was facilitated by the increased temperature and moisture content (Weegels et al., 1994a,b). On the basis of reported results (Koh et al., 1996; Li and Lee, 1996a,b; Strecker et al., 1995; Ummadi et al., 1995; Weegels et al., 1994a,b) and our present results, a mechanism to explain the decrease in wheat protein solubility after extrusion could be proposed. As the extrusion die temperature was increased to 120 °C, wheat proteins denatured continually to expose previously buried hydrophobic residues and other reactive residues. The denatured proteins might aggregate through the hydrophobic interaction and disulfide cross-linking, which might cause an increase in the protein molecular weight and a decrease in the protein solubility. However, the hydrophobic interaction of proteins was very weak and could be disrupted by using sonication at a low energy level to release the hydrophobic sites in proteins. The SDS in the solution could bind the newly released hydrophobic sites to inhibit the further association of denatured proteins, resulting in a reduction of the aggregated protein molecular weight and, subsequently, an increase in the soluble protein content in the extrudates.

As the extrusion die temperature was increased to 160 °C, the wheat proteins aggregated extensively through the disulfide cross-linking, which resulted in a large increase in the protein network formation and, subsequently, a dramatic decrease in the protein solubility. Sonication at a low energy level could not destroy the disulfide cross-links. Therefore, the extensively cross-linked wheat proteins could not be resolubilized by using sonication, but they could be resolubilized by using SDS plus β-mercaptoethanol.

Conclusions. In conclusion, the disulfide cross-linking was the major covalent bonding force for wheat protein aggregations during extrusion processing. The disulfide cross-linking of wheat proteins was facilitated by an increased extrusion die temperature of up to 160 °C. Under present extrusion conditions, the extensive disulfide cross-linking of wheat proteins formed at die temperatures between 120 and 160 °C resulted in a dramatic increase in protein molecular size and, subsequently, a dramatic decrease in protein solubility. It will be interesting to find the precise extrusion die temperature for the formation of the extensive disulfide cross-linking of wheat proteins.

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