J. Agric. Food Chem. 1996, 44, 763–768

Effect of Extrusion Temperature on Solubility and Molecular Weight Distribution of Wheat Flour Proteins

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To study the molecular mechanism of protein interactions during extrusion processing, the changes in molecular weight distribution and quantity of soluble proteins in wheat extrudates as affected by extrusion temperature were investigated. Wheat flour was extruded at die temperatures of 160, 170, and 185 °C. After extrusion, the solubility of wheat proteins decreased dramatically in all tested solvents [water, 0.01 M sodium hydroxide, 0.5 M sodium chloride, 70% ethanol, 0.1 M hydrochloric acid, 0.05 M sodium phosphate buffer (pH 7.0 and 8.0), 6 M urea, 1% SDS, 2% mercaptoethanol] except for an aqueous solvent containing 1% SDS and 2% 2-mercaptoethanol, in which they were almost completely soluble. In the soluble-protein fractions of the extrudates, the content of disulfide bonds decreased dramatically and the content of sulfhydryl groups varied slightly. Both aggregation and fragmentation of wheat proteins occurred during extrusion processing as indicated by SDS–PAGE analysis. The synergistic effect between SDS and 2-mercaptoethanol on solubilizing wheat proteins in extrudates indicated that wheat proteins aggregated primarily through nonspecific hydrophobic interaction and intermolecular disulfide bond formation. This aggregation of proteins might lead to an increase in their molecular weight, which subsequently resulted in a decrease in their solubility. The glutenins and gliadins in wheat flour were mainly responsible for the aggregation of wheat proteins during extrusion processing.

Keywords: *Extrusion temperature; wheat protein; solubility; aggregation; fragmentation*

INTRODUCTION

In recent years, extrusion cooking has been widely employed in the food and feed industries. Extrusion cooking of cereals yields extrudates that can be used as precooked flours to prepare products that require little or no additional cooking, i.e., breakfast products, infant foods, pasta products, and snacks.

In spite of the rapid development of extrusion technology in the past years, information about the molecular mechanism of protein interaction is limited. The structure formation of extrudates is believed to result from a complete restructuring of the polymeric material in an oriented pattern (Kinsella, 1978). The formation of the final molecular network involves the dissociation and unraveling of the macromolecules, which allows them to recombine into an oriented form when the molten mass leaves the extruder die and the superheated water flashes off to make the polymeric material expand (Harper, 1978, 1979, 1981; Kinsella, 1978; Rossen and Miller, 1973). However, the way that protein interacts with protein in the extrusion process is still unclear, and no unified model or mechanism for protein–protein interaction during extrusion processing has been proposed to date. The early studies on the mechanism of protein–protein interaction in the extrusion process tend to consider the protein structure in extrudates that is formed after extrusion similar to a protein gel (Arêas, 1992). However, soy extrudates are not thermally reversible, as are soy gels. The main protein interactions found in these soy gels are disulfide linkages followed by hydrophobic and electrostatic interactions (Fuke et al., 1985; Shimada and Cheftel, 1988). However, the mechanism of protein–protein interaction in soy extrudates is unclear.

Some reports on the protein interactions during soy extrusion claimed that disulfide bonds were of negligible importance in the final structure of extrudates, suggesting that new intermolecular peptide bonds formed during the severe conditions of extrusion (about 180 °C) were responsible for the final structure of these products (Burgess and Stanley, 1976; Simonsky and Stanley, 1982; Stanley, 1986, 1989). These results were based on the detection of an increase in the free sulfhydryl content after soy extrusion and on the decrease in texture formation after free amino and carboxyl groups of proteins were blocked with ninhydrin and citric acid, respectively. In contrast, Baird (1982), studying the effect of heat and shear on viscoelastic properties of soy flour dough, concluded that gelation through cooking and shearing did not involve the formation of a permanent network through covalent chemical bonds. Supporting Baird's finding, Smith et al. (1982) discovered that the addition of as little as 1% anionic polysaccharide to soy grits greatly affected the characteristics of the extruded products, showing the importance of electrostatic interactions for the protein–protein aggregation during extrusion cooking. Also, several investigators have shown that proteins were resolubilized from extruded soy products with a selection of specific chemical actions on protein, indicating that it is mainly disulfide bonds and nonspecific hydrophobic and electrostatic interactions that are responsible for protein–protein interactions during soy extrusion (Baird, 1982; Hager, 1984; Ledward and Mitchell, 1988). So far, it is unclear what the overall mechanism of protein–protein interaction is during the extrusion process.

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0021-8561/96/1444-0763$12.00/0 © 1996 American Chemical Society

Gluten, a class of proteins in wheat flour containing about 2−3% cysteine and cystine (Lâsztity, 1984), is a cohesive and viscoelastic mass, which can be stretched (Belitz et al., 1986). There is an established relationship between the content of disulfide bonds and the strength of gluten. It has been shown that thiol groups and disulfide bonds play an important role in determining gluten and dough properties (Kasarda et al., 1976; Kobrehel et al., 1991). The network of protein−protein interactions through disulfide bonds is strong enough to prevent starch leaching during pasta cooking and to maintain satisfactory surface conditions in cooked pasta (Feillet et al., 1989). It has been demonstrated that both disulfide bond formation and hydrophobic interaction within gluten played an important role in the network formation of dough and baked products (Feillet et al., 1989; Jeanjean et al., 1980; Kobrehel et al., 1991); however, the role of disulfide bonds in the protein−protein interactions in wheat flour extrusion has not been reported. Therefore, it is very important to study the mechanism of wheat protein−protein interactions during the extrusion process.

The objective of the present work was to investigate the effect of high-temperature extrusion on the solubility, disulfide bond content, molecular weight distribution, and fragmentation of wheat proteins and to elucidate the interaction mechanism of wheat proteins in the extrusion process.

MATERIALS AND METHODS

Materials. Commercial wheat flour (Bouncer high-gluten flour OS2530), purchased from Bay State Milling Co., Quincy, MA, was used for all experiments. The protein content of the wheat flour (Bouncer) was 14%, which was determined by using the semimicro-Kjeldahl method (AACC Method 46-13, 1983). α-Amylase (EC 3.2.1.1, from *Bacillus* species, 2100 units/mg), urea, sodium dodecyl sulfate (SDS), 2-mercaptoethanol (2-ME), and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific Co. (Springfield, NJ).

Extrusion. 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 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 processed at die temperatures of 160, 170, and 185 °C. The feed rate and screw speed were kept constant at 225 g/min and 300 rpm, respectively. Moderate tap water (index: 35) was fed into the extruder to provide a total moisture content of 27% (wet weight basis) (Table 1). The extrudates were collected onto a large scale (about 2 kg for each sample) after the extruder had reached equilibrium conditions, as indicated by the steady die temperature and torque. The extrudates were allowed to cool to room temperature and were then ground with a laboratory pulverizing mill (Glen Mills Inc., Maywood, NJ) to pass through a 0.2 mm diameter mesh. Ground samples were sealed and stored at 4 °C in glass bottles for further analysis.

Table 1. Extrusion Conditions for Experiments

sample no.	feed moisture (% H_2O w/w)	total mass flow rate (g/min)	screw speed (rpm)	die temp (°C)	moisture content (%) after extrusion
control[a]					10.87 ± 0.10
1	27	225	300	160	4.65 ± 0.09
2	27	225	300	170	2.66 ± 0.07
3	27	225	300	185	2.67 ± 0.10

[a] Unextruded wheat flour.

Moisture Content. The moisture content of each extrudate was determined immediately after extrusion according to AOAC Method 934.01 (1990). Approximately 2 g of each sample was placed in an Isotemp vacuum oven Model 282A (Fisher Scientific, Springfield, NJ) at 100 ± 2 °C and 300 mmHg for 16 h. The moisture content was calculated as the loss in weight. The moisture content of each sample (Table 1) was an average of triplicate analysis.

Determination of Protein Solubility. Ground samples were dispersed in a solvent at a ratio of 0.5:10 [flour (g): solvent (mL)] and shaken in a water bath at room temperature for 6 h according to the method described by Sefa-Dedeh and Stanley (1979). The mixture was centrifuged at 20000*g* for 40 min at 4 °C, and the supernatant was collected. The nitrogen content in the supernatant was determined according to the semimicro-Kjeldahl method (AACC Method 46-13, 1983) and by using an Orion ammonia electrode (Model 95-12, Orion Research Inc., Boston, MA). All analyses were conducted in duplicate. The protein content in the supernatant extracted by 6 M urea aqueous solution was determined by Bio-Rad protein assay method based on the color change of Coomassie Brilliant Blue G-250 dye in response to various concentrations of protein (Bio-Rad protein assay kit II, Bio-Rad Laboratories, Richmond, CA).

Determination of Sulfhydryl Group and Disulfide Bond Contents in the Soluble Peptides and Proteins. The assay of total sulfhydryl groups and disulfide bonds was carried out according to the method of Thannhauser et al. (1987). A total of 100−200 μL of extract of extrudates was pipetted into 3 mL of the 2-nitro-5-thiosulfobenzoate (NTSB) assay solution. The reaction mixture was incubated in the dark for 25 min at room temperature, and the absorbance was then recorded at 412 nm against a blank of 3 mL of NTSB assay solution and the appropriate amount of solvent. Cystine was used as the standard for the determination of disulfide bonds. The content of free sulfhydryl groups was determined by using Ellman's reagent according to the Pierce Method (Immunotechnology Catalog, Pierce, 1993, p E-56), by mixing 100 μL of extract with 100 μL of assay reagent (4 mg/mL Ellman's reagent in 0.1 N sodium phosphate buffer, pH 8.0) and 5 mL of 0.1 N sodium phosphate buffer (pH 8.0). The reaction mixture was allowed to stand for 15 min, and the absorbance was read at 412 nm. Cysteine was used as the standard for the determination of sulfhydryl groups.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). The SDS−PAGE of protein subunits was performed according to the method of Bollag and Edelstein (1991) on 15% polyacrylamide (w/v) gels. Standard proteins as molecular weight markers for SDS−PAGE were myosin (200 000), β-galactosidase (116 250), phosphorylase B (97 400), serum albumin (66 200), ovalbumin (45 000), carbonic anhydrase (31 000), trypsin inhibitor (21 500), lysozyme (14 400), and aprotinin (6500), all purchased from Bio-Rad Laboratory.

RESULTS AND DISCUSSION

Wheat Protein Solubility. Six solvents [water, 0.01 M sodium hydroxide, 0.5 M sodium chloride, 70% ethanol, 0.1 M hydrochloric acid, 0.05 M sodium phosphate buffer (pH 7.0)] were used to monitor the solubility changes of wheat proteins in extrudates. The soluble

Table 2. Extractable Nitrogen Content (Micromoles per Gram of Dry Base)[a] and Protein Content (Milligrams per Gram of Dry Base)[b] in the Wheat Flour Control and Extrudates in Different Solvent Systems

	solvents											
	water		0.5 M NaCl		0.05 M PBS[c] (pH 7.0)		0.01 M NaOH		0.1 M HCl		70% ethanol	
sample no.	N	protein	N	protein	N	protein	N	protein	N	protein	N	protein
control	139.8	11.2	166.9	13.3	155.9	12.4	1171.1	93.5	245.4	19.6	543.4	43.4
1	55.5	4.4	52.1	4.2	54.1	4.3	324.1	24.5	50.1	4.0	72.3	5.8
2	57.5	4.6	94.5	7.5	72.8	5.8	302.9	24.2	69.8	5.6	76.9	6.1
3	74.7	6.0	95.1	7.6	84.1	6.7	372.4	29.7	104.7	8.4	93.9	7.5

[a] The value is an average of duplicate measurements. [b] The protein content (mg) was calculated according to the equation N content (mg) × 5.7. [c] Sodium phosphate buffer solution.

Table 3. Effect of Specific Chemicals on the Extractable Nitrogen Content (Micromoles per Gram of Dry Base)[a] and Protein Content (Milligrams per Gram of Dry Base)[b] in the Wheat Flour Control and Extrudates

	solvents								
	0.05 M PBS[c] (pH 8.0)		2% 2-ME		1% SDS		1% SDS + 2% 2-ME		6 M urea[d]
sample no.	N	protein	N	protein	N	protein	N	protein	protein
control	335.6	26.7	409.7	32.7	1323.5	105.6	1957.2	156.2	22.53
1	176.8	14.1	243.5	19.4	320.4	25.5	1721.9	137.4	2.35
2	128.8	10.3	273.2	21.8	310.3	24.8	1556.2	124.2	2.59
3	177.1	14.1	262.1	20.9	295.6	23.5	1554.2	124.0	2.57

[a] The value is an average of duplicate measurments. [b] The protein content was calculated according to the equation N content (mg) × 5.7. [c] Sodium phosphate buffer solution. [d] The protein content was determined with Bio-Rad protein assay kit II.

nitrogen content was used as an index of soluble protein content. The contents of soluble nitrogen and wheat proteins in the control and extrudates are shown in Table 2.

After extrusion, the solubility of wheat proteins in the extrudates in the above six solvent systems decreased dramatically. As compared to soluble protein content in the control, approximately 43−86% of the proteins became insoluble in the above six solvent systems after extrusion (Table 2). The proteins soluble in the ethanol aqueous system were relatively more hydrophobic. The decreased degree of solubility of 70% ethanol-soluble proteins in wheat flour during extrusion was the greatest; only 13.3−17.3% of ethanol-soluble proteins were soluble after extrusion. This result demonstrated that the hydrophobic interaction was probably the major force for protein aggregation during extrusion processing. Since the solubility of heated proteins depended on their molecular size, which was affected by heating (Pomeranz, 1991), the decrease of wheat protein solubility after extrusion indicated the increase in protein molecular size, which might result from the aggregation of proteins during extrusion processing. Otherwise, the more hydrophobic the proteins were, the more easily they aggregated by hydrophobic interactions.

On the basis of the above results, we believed that the change in wheat protein solubility during extrusion was a two-step process: (1) unfolding and exposing hydrophobic and reactive sites of molecules and (2) aggregating and increasing the molecular weight, which resulted in the loss of protein solubility.

Jeanjean et al. (1980), after studying the effect of heat treatment on wheat protein solubility, stated that ethanol-soluble proteins were insolubilized through the formation of new bonds between polypeptide chains. The formation of new disulfide bonds was probably involved in this phenomenon because the new bonds could be further disrupted by mercaptoethanol. However, a few polypeptide chains or subunits in the ethanol-soluble fraction, containing few cysteine residues and/or hydrophobic residues, could not participate in the formation of the new insoluble protein complex by hydrophobic interactions and the formation of disulfide bonds and were soluble after heat treatment and extrusion.

As shown in Table 2, in all of the above six solvent systems, the content of soluble proteins in the extrudate at an extrusion temperature of 185 °C was slightly higher than that at extrusion temperatures of 160 and 170 °C. The reason for this might be that the aggregation forces of proteins, the noncovalent bonds (hydrophobic force, electrostatic force, hydrogen bond) and covalent bonds (S−S bond, peptide bond), were partially disrupted during a higher temperature extrusion, reducing the molecular weight of proteins. The fragmentation of protein molecules during extrusion was further demonstrated by SDS−PAGE.

Effect of Urea, SDS, and 2-ME on the Solubility of Proteins in Extrudates. Four reagents with specific chemical actions on protein (6 M urea, 1% SDS, 2% 2-ME, and 1% SDS + 2% 2-ME aqueous solvents) were used to investigate the types of aggregation forces of wheat proteins during extrusion: urea could disrupt hydrogen bonds; SDS could disrupt hydrogen bonds and hydrophobic interactions; and 2-ME could cleave disulfide bonds to sulfhydryl groups. The ground extrudates were dispersed in a 0.05 M sodium phosphate solution (pH 7.0) containing 2 units/mL α-amylase and shaken for 6 h at room temperature to digest soluble starch, and then 1 M sodium hydroxide, urea, SDS, or 2-ME was added to the above systems to a concentration of 0.01 M (pH 8.0), 6 M, 1%, or 2%, respectively, to extract proteins in the control and extrudates. The soluble nitrogen content in the supernatants was determined by using the semimicro-Kjeldahl method. Since urea contains nitrogen, the content of urea-soluble proteins was determined by using the Bio-Rad protein assay kit II. As shown in Table 3, there was 10−14 mg of protein/g of extrudate soluble in a 0.05 M sodium phosphate (pH 8.0) system, about 2 mg of protein/g of extrudate soluble in a 6 M urea system, 19−22 mg of protein/g of extrudate soluble in a 2% 2-ME system, and 23−25 mg of protein/g of extrudate soluble in a 1% SDS system. However, there was approximately 124−137 mg of protein/g of extrudate soluble in a 1% SDS + 2% 2-ME system. Our results have shown that a larger amount of proteins in the wheat flour extrudates became soluble in the SDS−ME system than in the 2-ME system, SDS system, urea system, and sodium

phosphate system (pH 8.0) alone. There was a synergistic effect between SDS and 2-ME on solubilizing proteins in wheat flour extrudates, which demonstrated that the decrease of protein solubility in the extrudates might be caused by the combined effect of the intermolecular disulfide bond formation and hydrophobic interaction of wheat proteins that occurred in extrusion processing. Otherwise, the aggregation of wheat proteins through hydrophobic interactions and disulfide bond formation resulted in an increase in molecular weight and, consequently, a decrease in solubility. Also, our results indicated that the hydrogen bond and electrostatic interaction were not important in the aggregation of wheat proteins during the extrusion process.

Mitchell and Arêas (1992) reviewed the solubility changes of proteins from different sources after extrusion processing in three solvent systems (0.05 or 0.5 M Na_2CO_3, 0.1 or 0.5 M SDS in phosphate buffer, 0.01 or 0.05 M SDS + 0.1 M 2-ME in phosphate buffer). The solubility of casein was affected very little by extrusion processing. The solubility of soy protein isolates was reduced by extrusion processing in all of the above solvents except for a combination of SDS and 2-ME, in which the soy protein isolates were almost completely soluble. Conversely, the solubility of defatted lung proteins was increased by extrusion processing. The solubility of untreated blood plasma was high in the SDS + 2-ME system, but it was reduced to a very low level by extrusion processing. Our results showed that the solubility change of wheat proteins was similar with that of soy protein isolates in the extrusion processing, which was reduced by the high-temperature extrusion processing in all tested solvents except for a combination of 2-ME and SDS, in which it was almost completely soluble.

The mechanism proposed by Burgess and Stanley (1976) of protein−protein interaction during extrusion processing, which stated that disulfide bonds were of negligible importance in forming the final structure of extrudates, has been widely disputed. When extruded soy and whey proteins were solubilized in reagents that exhibited specific chemical actions on protein (disrupting hydrophobic and electrostatic interactions, hydrogen bonds, and disulfide bonds), their solubilized profiles indicated that protein−protein interactions resulted primarily from disulfide bonds formed from cysteine residues and, less importantly, from nonspecific hydrophobic and electrostatic interactions (Martinez-Serna and Villota, 1992; Prudêncio-Ferreira and Arêas, 1993). Camire et al. (1991), investigating the extrusion of cottonseed flour and corn meal, indicated that both noncovalent and covalent forces were responsible for the aggregation of proteins. In agreement with these results, our present results showed that there was a synergistic effect between SDS and 2-ME on solubilizing the wheat proteins of extrudates (Table 3), suggesting that both the disulfide bond formation and the hydrophobic interaction were important for protein−protein interactions during the extrusion process. These interactions resulted in the aggregation of proteins and increased the molecular weight of proteins. The increase in molecular weight of proteins resulted in the decrease in protein solubility.

The disulfide bond content and sulfhydryl group content in the extracts of the control and extrudates are shown in Table 4. For the control and extrudates extruded at die temperatures of 160, 170, and 185 °C, the content of disulfide bonds decreased dramatically from 34.12 to 6.56, 4.53, and 3.62 μmol/mmol of N in the phosphate buffer-soluble fraction and from 12.15 to 2.54, 5.38, and 6.36 μmol/mmol of N in the 1% SDS-soluble fraction. However, the sulfhydryl group content varied only slightly, from 4.26 to 4.74, 4.53, and 3.62 μmol/mmol of N in the phosphate buffer-soluble fraction and from 0.6 μmol/mmol of N to not detectable in the 1% SDS-soluble fraction. Our results, the decrease of total disulfide bond and sulfhydryl group content in the soluble fraction of extrudates, implied the following: (1) the wheat proteins containing a higher level of cysteine/cystine residues were denatured easily by extrusion; (2) the wheat proteins containing a higher level of cysteine/cystine residues aggregated through intermolecular disulfide bonds; and (3) the decrease of total SS and SH content might be the reason for oxidation, degradation, and/or cystine-derived cross-linkage during extrusion processing. Consequently, these interactions caused a modification in the secondary, tertiary, or quaternary structure of the protein molecules, which resulted in the decreased protein solubility.

Table 4. Sulfhydryl Group Content and Disulfide Bond Content (Micromoles per Millimole of N)[a] in Extracts of Wheat Flour Control and Extrudates

sample no.	0.05 M PBS (pH 8.0) solvent		1% SDS aqueous solvent	
	−S−S− bond	−SH group	−S−S− bond	−SH group
control	34.12 ± 1.24	4.26 ± 0.43	12.15 ± 0.61	0.60 ± 0.2
1	6.56 ± 0.45	4.74 ± 0.48	2.54 ± 0.23	nd[b]
2	4.53 ± 0.23	4.53 ± 0.39	5.38 ± 0.34	nd
3	3.62 ± 0.27	3.62 ± 0.32	6.36 ± 0.41	nd

[a] The value is an average of triplicate measurements. [b] Not detectable.

SDS−PAGE. Wheat proteins were denatured after extrusion, resulting in changes of their chemical and physical properties, specifically, the change of solubility. Therefore, the traditional classification of wheat proteins based on their solubility in 70% ethanol was not suitable to be used for extruded wheat proteins. As a consequence, Bushuk and Wrigley's (1971) classification of wheat proteins on the basis of molecular weight was used as the reference, by which proteins larger than 100 000 were considered to be mainly glutenins, those between 100 000 and 25 000 were mainly gliadins and low molecular weight (LMW) glutenins, and proteins smaller than 25 000 were classified as albumins and globulins.

SDS−PAGE of soluble proteins in both the wheat flour control and extrudates was done to check the molecular weight distribution of protein components in different solvent systems: 0.01 N NaOH, 1% SDS, 2% 2-ME, and 1% SDS + 2% 2-ME (Figure 1). Since equal volumes of extracts were loaded for each sample, changes in the relative amounts of protein present in different regions of the gel could be compared. The SDS−PAGE of extracts of extrudates revealed a decrease in the intensity of the glutenin and gliadin regions for 0.01 N NaOH extracts (Figure 1B) and 1% SDS extracts (Figure 1C), and a decrease in the intensity of the gliadin region for 2% 2-ME extracts (Figure 1D). However, this decrease of the intensity in a higher molecular weight region (>25 000) of each column was accompanied with a corresponding increase of the intensity in a low molecular weight region (<25 000), suggesting a pronounced fragmentation of glutenins and/or gliadins into albumins/globulins. This fragmentation of glutenins and/or gliadins might be the reason that the content of soluble proteins in extrudates at an

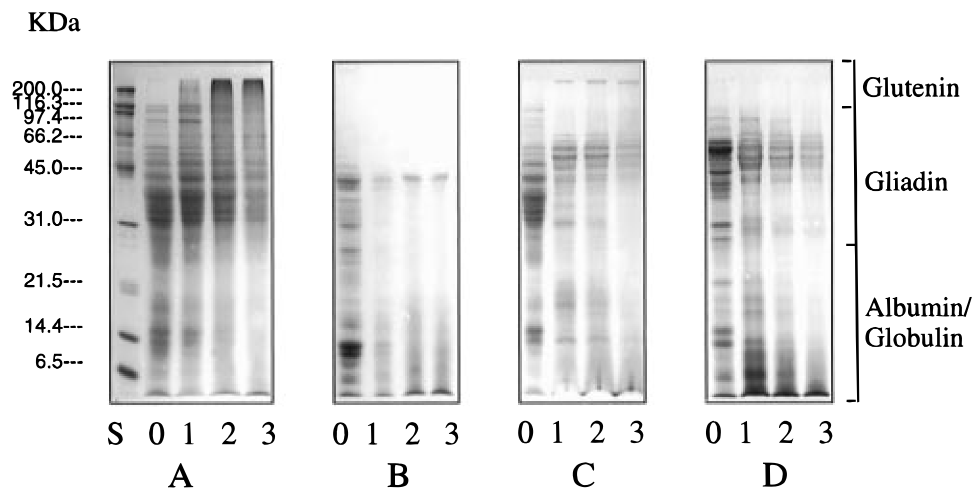

Figure 1. Sodium dodecyl sulfate−polyacrylamide gel (15% acrylamide, 0.4% Bis) electrophoretic patterns of soluble protein fractions from wheat flour control and extrudates in different solvent systems: (A) soluble fractions in 1% SDS and 2% 2-ME aqueous; (B) soluble fractions in 0.01 N NaOH aqueous; (C) soluble fractions in 1% SDS aqueous; (D) soluble fractions in 2% 2-ME aqueous. Lanes: S, molecular weight standard; 0, unextruded wheat flour; 1, wheat flour extruded at 160 °C; 2, wheat flour extruded at 170 °C; 3, wheat flour extruded at 185 °C.

extrusion temperature of 185 °C was a little higher than that at extrusion temperatures of 160 and 170 °C (Table 2). Furthermore, Figure 1 (panels B, C, D; columns 1, 2, 3) showed that the intensity in the glutenin and gliadin regions decreased with the increasing extrusion temperature from 160 to 185 °C. The gliadins with a molecular weight between 45 000 and 66 200 were affected little by the high-temperature extrusion process, probably because they contained few cysteine residues and/or few hydrophobic amino acids. As shown in Figure 1A, the very large molecular weight proteins/protein complexes in extrudates were unable to penetrate the pores of the separating gel, indicating the polymerization of protein−protein and/or protein−nonprotein macromolecules through covalent bonds, such as peptide bonds and isopeptide bonds, during the extrusion process.

Results reported here suggest that both glutenins and gliadins in wheat flour had a functional role in the protein−protein interactions or network formation of extrudates. Glutenins and gliadins seemed to interact through hydrophobic interactions and disulfide bonds and formed the gluten network during extrusion processing.

Conclusions. In conclusion, both the disulfide bond formation and the hydrophobic interaction played a key role in the aggregation of wheat proteins during extrusion processing. The aggregation of proteins caused an increase in their molecular weight, which resulted in a decrease in protein solubility. The fragmentation of proteins also occurred during extrusion processing at die temperatures over 160 °C. Both glutenins and gliadins in wheat flour played an important role in the network formation of extrudates.

ACKNOWLEDGMENT

We thank Dr. M. V. Karwe and group members of the Physical Force Extrusion Project of the Center for Advanced Food Technology, Rutgers University, for extruded samples, Dr. B. Riha for English aid, and Ms. Kirsten-Marie Burr for secretarial aid.

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Received for review August 24, 1995. Revised manuscript received December 7, 1995. Accepted December 27, 1995.[⊗] New Jersey Agricultural Experiment Station Publication D-10114-5-95 supported by State Funds and the Center for Advanced Food Technology (CAFT). CAFT is a New Jersey Commission on Science and Technology Center.

JF950582H

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1996.