

# Changes in Disulfide and Sulfhydryl Contents and Electrophoretic Patterns of Extruded Wheat Flour Proteins

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## ABSTRACT

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Twin-screw extrusion of wheat flour and the effects on the flour proteins were studied using flour samples containing 9, 20, and 30% protein. Vital gluten containing 70% protein was used to achieve the flour protein levels. The three flour samples were extruded with a twin-screw extruder at a combination of processing parameters (exit die temperatures of 120, 140, and 160°C, and screw speeds of 240, 320, and 400 rpm). Increasing extruder exit die temperatures resulted in increased sulfhydryl content of the 9 and 20% protein content flour samples, but appeared to have little or no effect on the 30% protein content flour sample. Similarly, disulfide content decreased, albeit disproportionately, following the same trend. Both sulfhydryl and disulfide contents of extruded samples were lower than

those of the nonextruded samples and could imply denaturation of protein, aggregation through intermolecular disulfide bonds, or oxidation during extrusion processing. Total cysteine content of extruded samples decreased by ≈16% relative to nonextruded samples, but otherwise remained almost unchanged among all extruded samples. The loss of total cysteine in extruded samples could represent the generation of hydrogen sulfide, volatile organic compounds, or flavor compounds during extrusion. SDS-PAGE analysis of total proteins showed a shift from the higher to lower molecular weight regions for certain protein bands. Both depolymerization and protein aggregation occurred at higher shear forces during extrusion.

Wheat flour is an important raw material in the manufacture of breakfast cereals, infant foods, snack foods, and pasta products through extrusion processing. The extrusion process has the potential to change protein structure, solubility, and digestibility through a combination of shearing, heat, and pressure (Phillips 1989, Wen et al 1990). Covalent bond formation between polypeptide chains, or between polypeptide chains and other constituents, is considered to be the chemical basis of extrusion texturization (Jeunink and Cheftel 1979). Previous studies have concluded that during the extrusion process, proteins are denatured and the forces that stabilize the tertiary and quaternary structures are weakened through a combination of increased heat and shear within the extruder, resulting in change in protein conformation (Harper 1986, Ledward and Mitchell 1988, Camire et al 1990, Camire 1991, Dahl and Villota 1991).

The cross-linking of proteins during extrusion is responsible for the textural characteristics of extruded products, and disulfide bonds are one obvious source of cross-links (Koh et al 1996). Disulfide cross-links have been reported in spun soy fibers and in heated soy protein systems, and the texture of extruded soy granules has been improved through the addition of elemental sulfur or potassium or sodium sulfites which are believed to increase disulfide cross-links (Jenkins 1970).

Sulfhydryl groups and disulfide bonds also contribute to the stability of the native conformations of proteins and are thus important in maintaining structure and functional properties of native proteins (Thannhauser et al 1987, Synowiecki and Shahidi 1991), as well as the texture of cereal-based products (Chan and Wasserman 1993). Several studies have attempted to relate the disulfide and sulfhydryl contents of extrudates to some properties of the extrudates. Cumming et al (1973) have shown that a sulfhydryl-disulfide interchange reaction is involved in the insolubilization of soy protein during extrusion texturization. Hager (1984) and Neumann et al (1984) have also shown that intermolecular disulfide bonds in soy proteins contribute to the new and extended protein networks produced by extrusion of soy concentrate. Ummadi et al (1995) reported

differences between SDS-PAGE patterns of reduced and nonreduced fractions of albumins, globulins, and glutenins from raw and extruded semolina, and attributed these differences to the role played by disulfide linkages during extrusion.

Disulfide-sulfhydryl interchange reactions during extrusion processing have also been related to the thermal polymerization of gluten proteins during extrusion. Strecker et al (1995) evaluated the polymerization reactions of wheat gluten proteins during extrusion through analysis of solubility and disulfide bond content, and concluded that the reaction mechanism in wheat gluten during extrusion was dominated by polymerization reactions contributed mainly by disulfide bond formation. In an experiment to study effects on sulfhydryl groups of extruded wheat flour proteins, Koh et al (1996) added cysteine to hard red winter wheat flour. After extrusion, there was a marked increase in protein sulfhydryl content and a decrease in protein disulfide content which was possibly due to disulfide-sulfhydryl interchange.

For the extrusion processing of wheat flour-based products to be more controllable and predictable, there is the need to gain further information on the changes in wheat proteins as a result of extrusion processing. In the present study, the effects of twin-screw extrusion processing on the sulfhydryl and disulfide contents, and the molecular weight patterns of the total wheat flour proteins were examined for wheat flours of three different protein contents.

## MATERIALS AND METHODS

### Materials

Soft white wheat grain (cv. Harus) harvested in Michigan in 1994 was milled to obtain straight-grade flour with a 67% extraction rate and 9% protein content on a Buhler MLU-202 automatic mill (Buhler AG, Uzwil, Switzerland). The wheat grain was tempered overnight to 14% moisture before milling. Vital gluten (70% protein) was obtained from Midwest Grain Products (Atkinson, KS).

Calculated amounts of vital gluten were added to the wheat flour to obtain flour-gluten samples containing 20 or 30% protein. Moisture and Kjeldahl nitrogen contents of wheat flour, vital gluten and flour-gluten samples were determined according to AACC Approved Methods 44-19 and 46-11A, respectively (AACC 1995) (Table I). Ellman's reagent, [5,5'-dithiobis(2-nitrobenzoic acid)] (DTNB), ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific Co. (Springfield, NJ) and were of analytical grade.

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## Extrusion Conditions

Each sample was extruded in duplicate on an corotating and intermeshing twin screw extruder (MP19TC-25, APV Baker, Grand Rapids, MI) with barrel diameter of 19 mm and length-to-diameter (L/D) ratio of 25:1. Samples were fed into the extruder with a twin-screw volumetric feeder (K2M, K-Tron Corp., Pitman, NJ). Sample feed rate and feed moisture content were maintained constant at 2 kg/hr and 19%, respectively. Extrusion process variables were screw speed (240, 320, and 400 rpm); extruder exit die temperature (120, 140, and 160°C); and feed sample protein content (9, 20, and 30%). Temperatures at the four extruder barrel zones preceding the exit die were maintained constant at 40, 60, 80, and 100°C, respectively. The extrusion screw configuration used in all experiments consisted of single and twin lead screws and paddles as shown in Table II. This screw configuration was shown in preliminary experiments to produce adequately expanded cereal products.

After extrusion, samples were dried in a convection fan oven at 40°C to ≈8% moisture, cooled to room temperature, and ground in a cyclone sample mill (Udy Corp., Fort Collins, CO) through a 0.5-mm screen. Samples were then stored in airtight plastic freezer bags at 4°C till further analysis.

## Disulfide-Sulfhydryl Analysis

Disulfide (SS) and sulfhydryl (SH) contents of nonextruded and extruded samples were determined according to a modified solid-

phase colorimetric assay method of Chan and Wasserman (1993). For free sulfhydryl content determination, samples (≈30 mg) were suspended in 1.0 mL of reaction buffer consisting of 8M urea, 3 mM EDTA, 1% SDS, and 0.2M Tris-HCl (pH 8.0) (Buffer A). Samples were vortexed for 30 sec and placed on a constant agitation shaker at room temperature. After 1 hr, 0.1 mL of 10 mM DTNB in 0.2M Tris-HCl (pH 8.0) (Buffer B) was added to each sample, and shaking continued for another 1 hr. Samples were then centrifuged at 13,600 × g for 10 min at room temperature, and the absorbance of the supernatant was read at 412 nm against a blank consisting of 1.0 mL of buffer A and 0.1 mL of buffer B.

For determination of total sulfhydryl (SH + reduced SS) content, a reaction buffer was used that consisted of 8M urea, 3 mM EDTA, 1% SDS, and 0.2M Tris-HCl (pH 9.5), with 0.1M Na<sub>2</sub>SO<sub>3</sub> and 0.5 mM 2-nitro-5-thiosulfobenzoate (NTSB<sup>2-</sup>) synthesized from DTNB according to the procedure of Thannhauser et al (1987).

**TABLE I**  
Moisture and Protein Contents of Wheat Flour, Vital Gluten, and Flour-Gluten Samples

Sample	Moisture (%)	Protein Content (%) <sup>a</sup>
Wheat flour	10.3	9.1
Vital gluten	9.2	69.8
Flour-gluten, 20% protein	10.1	20.3
Flour-gluten, 30% protein	9.9	29.7

<sup>a</sup> Dry basis.

**TABLE II**  
Screw Configuration<sup>a</sup> Used in Extrusion Experiment

Feed Zone	Exit Die Zone								
	8D	1.75D	8D	0.75D	0.75D	2D	1D	0.75D	2D
Length (mm)	(152)	(33.25)	(152)	(14.25)	(14.25)	(38)	(19)	(14.35)	(38)
Screw elements	TL	FP	TL	FP	RP	SL	FP	RP	SL
Degrees	...	30	...	60	-30	...	60	-30	...

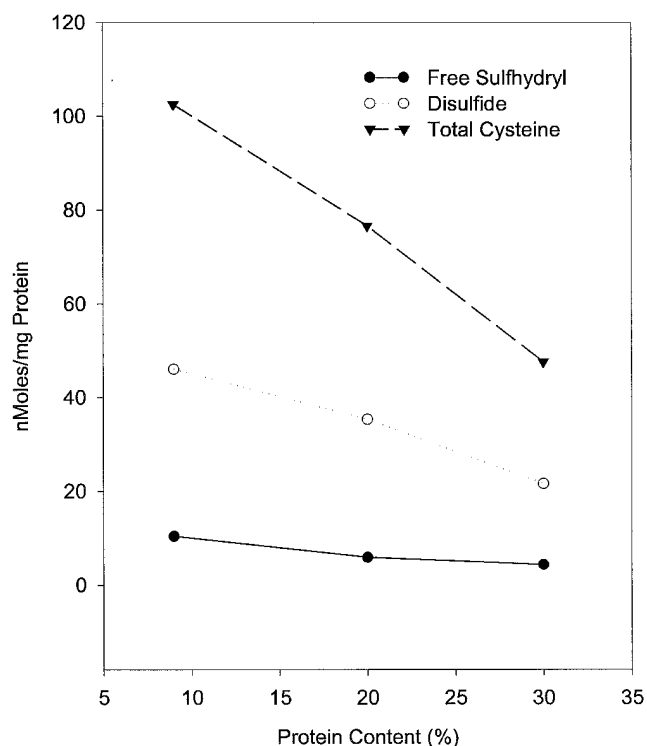
<sup>a</sup> TL = twin lead; FP = forward paddle; RP = reverse paddle; SL = single lead; D = extruder barrel diameter.

**TABLE III**  
Effects of Extruder Exit Die Temperature, Screw Speed and Sample Protein Content on Free Sulfhydryl, Disulfide, and Total Cysteine Contents of Extruded Wheat Flour

Exit Die Temp. (°C)	Screw Speed (rpm)	9% Protein			20% Protein			30% Protein		
		Free Sulfhydryl	Disulfide	Total Cysteine	Free Sulfhydryl	Disulfide	Total Cysteine	Free Sulfhydryl	Disulfide	Total Cysteine
NE <sup>a</sup>		10.43±0.31a <sup>b</sup>	46.04±0.11	102.51±0.19	5.90±0.18	35.35±0.40	76.62±0.14a	4.37±0.12	21.62±0.07c	47.60±0.17ab
120	240	6.95±0.07	38.36±0.11	83.66±0.15b	3.58±0.11	36.35±0.24	76.27±0.37a	2.79±0.01c	22.70±0.05ab	48.18±0.12a
140	240	9.78±0.12	36.84±0.11	83.44±0.10b	5.00±0.14ab	32.96±0.09b	70.91±0.33b	2.94±0.06b	21.97±0.15c	46.86±0.35cd
160	240	10.98±0.04	35.43±0.05b	81.82±0.14	9.42±0.04	31.91±0.02	73.24±0.01	2.93±0.03bc	22.37±0.08b	47.66±0.20a
120	320	7.55±0.02	41.02±0.04	89.57±0.50	4.03±0.11c	33.41±0.09a	70.84±0.08bc	2.79±0.01c	22.77±0.25a	48.33±0.25a
140	320	8.78±0.25b	37.49±0.12a	83.75±0.49b	4.85±0.07b	32.65±0.30b	70.14±0.68cd	3.18±0.01a	22.85±0.04a	47.87±0.08a
160	320	10.60±0.21a	35.13±0.22b	80.85±0.23	10.65±0.21	29.49±0.27	69.62±0.33d	3.13±0.04ab	21.74±0.26c	46.59±0.35d
120	400	7.98±0.25	40.23±0.02	88.42±0.29a	4.08±0.18c	33.41±0.21a	70.89±0.24b	3.38±0.12a	20.66±0.35	44.68±0.17
140	400	9.08±0.03b	37.50±0.25a	84.07±0.47b	5.20±0.14a	32.89±0.23b	70.98±0.60b	3.30±0.03a	21.75±0.21c	46.79±0.41d
160	400	13.29±0.27	37.73±0.25a	88.73±0.23a	9.93±0.11	28.41±0.08	66.74±0.07	3.36±0.08a	21.93±0.28c	47.21±.48bc
LSD		0.33	0.39	0.76						

<sup>a</sup> NE = Nonextruded

<sup>b</sup> Data shown are mean ± standard deviation of three replicates, reported in nmol/mg of protein. Values followed by the same letter in the same column are not significantly different (*P* < 0.05).



**Fig. 1.** Variation in free sulfhydryl, disulfide, and total cysteine contents in nonextruded wheat flour samples of varying protein content.

Reaction buffer (1 mL) was added to 30 mg of each sample and vortexed for  $\approx 30$  sec at room temperature. Samples were then shaken in the dark for 1 hr and subsequently centrifuged at  $13,600 \times g$  for 10 min at room temperature. A 0.1-mL aliquot of the supernatant was diluted with 0.9 mL of reaction buffer, and the absorbance was read at 412 nm.

Free SH and total SH contents were calculated from the absorption readings using a molar absorption coefficient of  $13,600 \text{ M}^{-1} \text{ cm}^{-1}$  (Chan and Wasserman 1993) as:  $A = \epsilon bc$ , where  $A$  is the absorbance readings,  $\epsilon$  is the molar extinction coefficient,  $b$  is the cell thickness, and  $c$  is the concentration. Disulfide content was calculated as half the difference between total SH and free SH contents using the formula  $SS = (TS - SH)/2$ , where  $SS$  is disulfide content,  $TS$  is total sulfhydryl content (free SH + reduced SS), and  $SH$  is free sulfhydryl content.

### SDS-PAGE

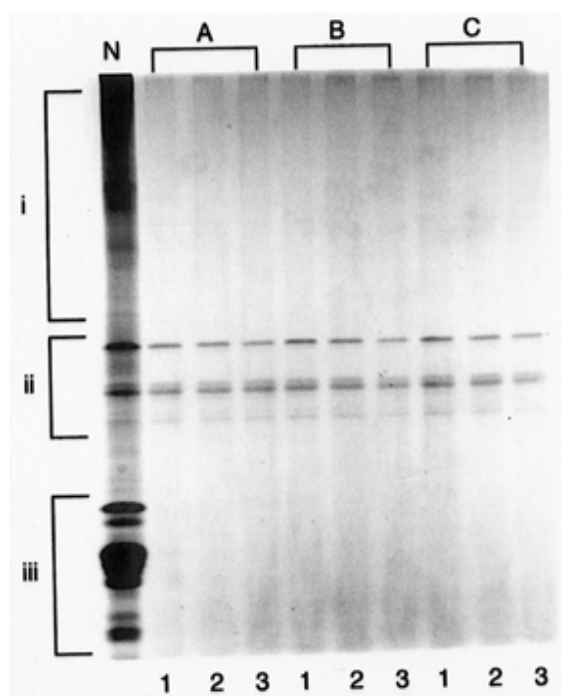
Total reduced proteins were extracted from ground extrudates and nonextruded flour according to Pogna et al (1990). Each sample (50–60 mg) was stirred in 1 mL of extraction buffer consisting of 0.67 mL of distilled water, 0.05 mL of 2-mercaptoethanol (2-ME), and 0.28 mL of stock solution containing 0.2M Tris-HCl (pH 6.8), 7% (w/v) SDS, 30% (v/v) glycerol, and 0.04% (w/v) Pyronin Y.

For nonreduced total proteins, extraction buffer did not contain 2-ME. The extraction mixture was vortexed for 30 sec and placed on a constant agitation shaker at room temperature for 2 hr. Samples were then heated at  $80^\circ\text{C}$  for 30 min and allowed to cool to room temperature. Aliquots (25  $\mu\text{L}$ ) of extracted samples were loaded onto 15% (T = 15.1%, C = 0.58%) SDS separating gels with 4.5% (T = 4.5%, C = 1.3%) stacking gels. Gel dimensions were  $16 \times 18$  cm with a thickness of 0.75 cm. The gels were run at room temperature for 18 hr at a constant current of 12.5 mA per gel.

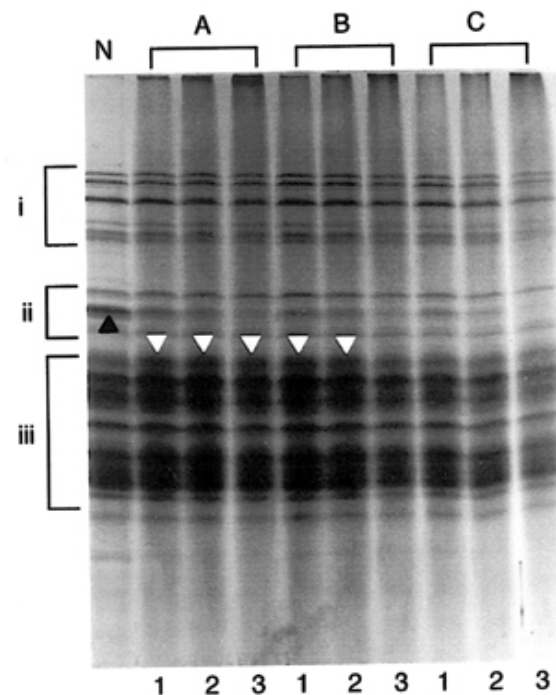
After the run, gels were stained according to the procedure of Redaelli et al (1995). Each gel was placed overnight in a staining solution consisting of 15 mL of Coomassie Brilliant Blue R-250 (4 g dissolved in 1L of 95% ethanol), 25 mL of 60% trichloroacetic acid (TCA), and 210 mL of distilled water. Gels were then washed several times with deionized water and photographed.

### Statistical Analysis

Samples were independently analyzed at least in duplicate. Analysis of variance (ANOVA) was performed using the general linear models (GLM) procedure (SAS Institute, Cary, NC). Treatment mean comparisons were evaluated using Student's  $t$  test.



**Fig. 2.** SDS-PAGE patterns of nonreduced total protein of extruded wheat flour containing 9% protein. N = nonextruded flour sample. Screw speeds (rpm): A = 240; B = 320; C = 400. Exit die temperatures: 1 =  $120^\circ\text{C}$ ; 2 =  $140^\circ\text{C}$ ; 3 =  $160^\circ\text{C}$ . High molecular weight protein region (i), midregion (ii), and low molecular weight region (iii).



**Fig. 3.** SDS-PAGE patterns of reduced total protein of extruded wheat flour containing 9% protein. N = nonextruded flour sample. Screw speeds (rpm): A = 240; B = 320; C = 400. Exit die temperature: 1 =  $120^\circ\text{C}$ ; 2 =  $140^\circ\text{C}$ ; 3 =  $160^\circ\text{C}$ . High molecular weight protein region (i), midregion (ii), and low molecular weight region (iii).

**TABLE IV**  
Statistical  $F$ -Values ( $P \leq 0.01$ ) for Analyses of Free Sulfhydryl, Disulfide, and Total Cysteine Contents in Wheat Flour Extrudates

Source	df	$F$ -Values		
		Free Sulfhydryl	Disulfide	Total Cysteine
Screw speed	2	63.34	29.79	6.63
Temperature (T)	2	2183.09	955.43	154.40
Screw speed (S) $\times$ T	4	20.27	43.20	39.96
Protein (P)	2	6936.16	31980.65	47513.58
S $\times$ P	4	23.58	183.36	202.98
T $\times$ P	4	612.06	265.85	55.16
S $\times$ P $\times$ T	8	25.64	47.92	76.23
$R^2$		0.998	0.999	0.999

## RESULTS AND DISCUSSION

### Disulfide and Sulfhydryl Analysis

Figure 1 depicts the relationships between the protein content and sulfhydryl, disulfide, and total cysteine contents of nonextruded flour samples. Increasing the total protein content of flour resulted in decreases in free sulfhydryl, disulfide, and total cysteine contents of nonextruded samples. The albumin and globulin fractions of wheat flour contain higher amounts of total cysteine (6.2 and 5.4 g/16 g of nitrogen, respectively) than the gluten proteins of gliadin and glutenin (2.7 and 2.2 g/16 g of nitrogen, respectively) (Bushuk and Wrigley 1974). Thus, it is reasonable to expect that increasing the flour protein content from 9 to 30% using vital gluten is essentially equivalent to dilution of the total SH content per unit weight of sample. The effect of this is declines in free SH content, disulfide content and total cysteine content as protein content is increased in the nonextruded flour.

Table III lists the data for sulfhydryl, disulfide and total cysteine contents of nonextruded and extruded samples from flour and flour-gluten samples with 9, 20, and 30% protein content. Analysis of variance (Table IV) indicates that both main and interaction effects of variables on free sulfhydryl, disulfide, and total cysteine contents were significant ( $P \leq 0.01$ ). At a constant screw speed of 240 rpm, increasing the die temperature from 120 to 160°C increased free SH content from 6.95 to 10.98 nM/mg of protein (Table III), an increase of  $\approx 58\%$ . Similarly, increases of 40 and 67% in free SH were noted over the same temperature ranges at constant screw speeds of 320 and 400 rpm, respectively, for the extrudates containing 9% protein. There were much higher increases in free SH for the 20% protein content extrudates, and relatively much smaller increases for 30% protein content sample, as exit die temperature was increased. It is evident from Table IV that temperature and protein content were the major factors affecting the free SH and SS contents during the extrusion process.

When nonextruded samples are compared with extrudates, at all screw speed levels, free SH contents in nonextruded samples were higher than in samples extruded at 120 and 140°C for the 9 and 20% protein content samples, and at all three temperature levels for the 30% protein samples (Table III). The decrease in SH content after extrusion could imply that wheat proteins were denatured during extrusion, aggregated through intermolecular disulfide bonds, or oxidized (Li and Lee 1996). Within all extruded samples, increasing exit die temperature also increased free SH at all screw speed levels while, correspondingly, SS contents decreased. This was particularly evident for the 9 and 20% protein content samples.

The decrease in free SH content of extrudates relative to non-extruded samples, and the opposite increases with increasing exit die temperatures among all extruded samples concomitant with decreases in SS, are in general agreement with the findings reported by Chan and Wasserman (1993). These authors showed a decrease in the SH group content of extruded corn meal proteins relative to that of nonextrudates. However, among extrudates, samples extruded at a higher temperature showed an increase in free SH content relative to those extruded at a lower temperature, and this was accompanied by slight decreases in SS group content. Burgess and Stanley (1976) had previously reported slight decreases in SS and an increase in SH contents on extrusion of soy meal. However, the data from the present study contrast with the findings of Synowiecki and Shahidi (1991), who reported a decrease in the content of sulfhydryl groups and an increase in the content of disulfide bonds on heating seal meat proteins. Together, these results would suggest that the process of sulfhydryl-disulfide changes occurring in seal meat proteins, as described by Synowiecki and Shahidi (1991), may be essentially one of disulfide cross-linking of protein molecules due to oxidation of sulfhydryl to disulfide groups, while the extrusion of wheat proteins, as reported in the present study, appeared to result in the reduction of disulfide groups to sulfhydryls. Secondly, the involvement of shear force in extrusion, as in the present

study, as opposed to ordinary heating alone, may be a significant factor in the differences between the results. In general, the apparent increases in free SH group content accompanied by small decreases in SS group content in the present study, with increasing temperatures, may be indicative of molecular fragmentation of S-S bonds due to the combination of heating and shearing forces during the extrusion processing of wheat proteins.

Data in Table III also show that the total cysteine contents of all extrudates were lower than that of the nonextruded flour in the 9% protein sample, and varied between 81.82 and 89.57 nM/mg of protein compared with 102.51 nM/mg of protein for the nonextruded sample. On average, extrusion reduced total cysteine content by  $\approx 16\%$  the original content in the 9% flour protein sample. Total cysteine content of the 30% flour protein sample was virtually unaffected by extrusion. This sample contains much higher vital gluten as compared with the 9 and 20% protein content samples, and the protein content is composed mainly of the gluten proteins (gliadins and glutenins). This finding is in general agreement with Schofield et al (1983), who reported that the level of total cysteine groups in gluten essentially remained constant irrespective of temperature and was unaffected by heating to 100°C.

The decrease of 16% in total cysteine content in extruded 9% protein content samples relative to the nonextruded samples and the slight variations in total cysteine contents for the 20 and 30% protein samples suggest that some cysteine was lost during extrusion. This could represent cysteine loss at the extruder die as hydrogen sulfide or as volatile organic compounds such as those found as flavor compounds in extrudates (Koh et al 1996). Extruded cereals are generally believed to have less flavor than those prepared by traditional baking, and this is attributed to loss of produced or added aroma volatiles when the melt leaves the extruder die and becomes an expanded solid material (Bredie et al 1997). Chan and Wasserman (1993) and Weegels et al (1994) have suggested that conformational changes in the protein structure during extrusion might inhibit access of colorimetric reagent in the presence of SDS during analysis of SS and SH contents. It has also been suggested that the cysteine in the extrudates is more prone to oxidation after extrusion (Chan and Wasserman 1993), leading to the formation of more noncovalent disulfide bonds. The present data did not support this theory because there were no noticeable increases in disulfide group content after extrusion.

### SDS-PAGE

The electrophoretic patterns of nonreduced total proteins of samples containing 9% protein are shown in Fig. 2. In all extruded samples, bands in the high molecular weight protein region (i) and in the low molecular weight region (iii) were not visible. There was no major difference in the intensity of visible bands in the mid-region (ii) among all extruded samples. However, the intensities of these bands were visibly less than those of corresponding bands from the nonextruded sample. Similar observations were made in the electrophoretic patterns of the nonextruded samples containing 20 and 30% protein (data not shown) even though these two contained more protein bands than the 9% protein content sample in view of their mixture with vital gluten. It is evident in this study that, without reduction of disulfide bonds, extractability of low molecular weight proteins in SDS was markedly decreased after extrusion, which is consistent with previous studies (Koh et al 1996). Dexter and Matsuo (1977) reported a decrease in protein extractabilities of four different flours after extrusion.

Previous studies have demonstrated reductions in solubility as a function of temperature for soy protein (Hager 1984) and corn meal protein (Racicot et al 1981). The disappearance of the bands at the lower molecular weight region after extrusion may suggest that extrusion might have depolymerized the lower molecular weight proteins into even much smaller units which then eluted off the gel into the running buffer. Or, the proteins might have polymerized into

larger aggregates, making those proteins too big to enter the running gel. However, gels run under nonreduced conditions in which running was terminated when the tracking dye just reached the bottom of the running gel showed similar patterns. This suggests that polymerization was the more likely process to have occurred during extrusion. Ummadi et al (1995) observed the aggregation of polypeptides of albumins, globulins, and glutenins of semolina under nonreduced conditions after extrusion.

Figure 3 shows the electrophoretic patterns of reduced 9% protein content samples. After reduction of disulfide bonds with 2-ME, strong and distinct bands appeared at the high molecular weight protein region (i) in both nonextruded and extruded samples. There were no visible differences among intensities of bands in this region. The electrophoretic patterns of both 20 and 30% protein samples were similar (data not shown). However, comparing the bands of nonextruded samples with those of the extruded samples, it was observed that a single band (black arrow) in the middle region (ii) of the nonextruded sample lightened or disappeared after extrusion. Simultaneously, there was an appearance of heavy bands (white arrows) in the lower region (iii) of extruded samples. There was a very light band at the same location in the nonextruded sample. Therefore, it appears that proteins in the middle region depolymerized into lower molecular weight units during the extrusion process, which then appeared in the extruded products in the lower region. However, examination of Fig. 3 shows that samples extruded at the highest screw speeds (C) did not show the disappearance-appearance pattern described, which might indicate that a combination of high shearing forces and temperatures might have provided alternate formation of new disulfide bonds through cross-linking reactions or by additional cystine formation from cysteine. Thus, it appears depolymerization of proteins was followed by protein aggregation, especially at higher shear forces.

The marked differences between SDS-PAGE patterns of reduced and nonreduced samples, and the decrease in protein disulfide content after extrusion are, in part, inconsistent with thiol-disulfide interchange reactions. According to Koh et al (1996), the characteristics of such interchange reactions include net increase in protein SH, net reduction in cross-linking, and no increase or decrease in disulfide bonds. Thus, reduction with 2-ME produces no change on SDS-PAGE patterns under such conditions. The fact that net protein disulfide content decreased and the SH content increased with extrusion in the present study indicates that extrusion might have resulted in shear-mediated scission of disulfide bonds. This observation, together with changes in SDS-PAGE patterns, suggest that thiol-disulfide interchange had only a minor effect under the extrusion conditions used in this study. Possible explanations for the absence of thiol-disulfide interchange reactions may be the low water content and very short reaction times of extrusion. Studies in doughs have shown that the thiol-disulfide interchange reactions require longer periods of mixing time and also require sufficient water (Stewart and Mauritzen 1966, Jones and Carnegie 1971).

## CONCLUSIONS

Extrusion processing of wheat flour resulted in a decrease in protein disulfide content and an increase in the free sulfhydryl content, while total cysteine content remained almost unchanged. SDS-PAGE patterns of the total proteins showed marked differences between reduced and nonreduced samples, indicating that there was very little or no thiol-disulfide interchange reaction during extrusion processing under the conditions of this study, and that the intramolecular scission of disulfide bonds seemed to be the predominant occurrence during the extrusion process. Intramolecular fragmentation of proteins, as evidenced by SDS-PAGE patterns, led to the formation of low molecular weight protein aggregates. This information could be helpful in determining the relationship between disulfide and free sulfhydryl contents of an extruded product and the textural characteristics of the extrudates.

## ACKNOWLEDGMENTS

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