Effect of Cysteine on the Functional Properties and Microstructures of Wheat Flour Extrudates

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The effect of the concentration of added cysteine (0, 0.25, 0.5, 0.75, 1.0, and 1.5% (w/w)) on the functional properties and microstructures of wheat flour extrudates was studied in order to elucidate the relationship of the disulfide cross-linking, the extrudate microstructure, and the functional property of proteins through the high-temperature short-time extrusion. The added cysteine markedly affected the functional properties and microstructures of the extrudates during twin-screw extrusion. After extrusion, the disulfide content in the extrudate (including in both protein and cystine) increased by nearly two times. The expansion ratio of the extrudates decreased by 25%. The bulk density of the extrudates first dropped by 50% and then remained constant. The expansion volume of the extrudates first increased by 25% and then returned back. The water-holding capacity of ground extrudates decreased by 16%. The oil-absorption capacity of ground extrudates first increased by 135% and then remained constant. The "whiteness" value of the extrudates increased by 3.9 color units. The gumminess and cohesiveness of the extrudates increased. For the microstructures of the extrudates, the cell size decreased and the cell wall thinned; also, the cell surface morphology changed. The information generated in this study could be applied to predict and control the functional properties and textural characteristics of extruded products.

Keywords: *Cysteine; extrusion; disulfide cross-linking; microstructure; functional properties*

INTRODUCTION

High-temperature short-time extrusion has been widely used to texturize starch and protein-based materials to produce a variety of convenience foods (Harper, 1981, 1986). The cereal industry produces such products as ready-to-eat (RTE) breakfast cereals and expanded snack foods made from wheat, corn, rice, and oat, as their microstructures and textural characteristics are critical to product quality (Clark and Lee-Tuffnell, 1986).

In spite of the rapid development of extrusion technology in 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). During extrusion, the flour completely disaggregates through mechanical mixing to form a homogeneous suspension. Consequently, the proteins are denatured, dissociated, and unraveled, allowing alignment of the denatured protein molecules in the direction of the flow. The proteins then cross-link at the die end of the extruder to impart a network to the extrudates (Martinez-Serna and Villota, 1992). However, the way that protein cross-links with protein in the extrusion process is still unclear, and no unified model or mechanism for protein−protein interactions during extrusion processing has been proposed to date.

Several reports on protein interactions in soy extrusion claimed that disulfide bonds were of negligible importance in the final structure of extrudates, suggesting that new peptide bonds formed in the severe conditions of extrusion (about 180 °C) were responsible for the 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 of the free sulfhydryl content after soy extrusion and on the decrease in texture formation after blocking free amino and carboxyl groups of proteins with ninhydrin and citric acid, respectively. However, this proposed mechanism has been widely disputed. When extruded soy and whey proteins were solubilized in reagents which exhibited specific chemical actions on proteins (disrupting hydrophobic and electrostatic interactions, hydrogen bonds, and disulfide bonds), their resolubilized profiles indicated that protein interactions resulted primarily from the disulfide bonds formed from cysteine residues and, less importantly, nonspecific hydrophobic and electrostatic interactions (Hager, 1984; Jeunink and Cheftel, 1979; Arêas, 1992; Martinez-Serna and Villota, 1992; Prudêncio-Ferreira and Arêas, 1993). Supporting this finding, Strecker et al. (1995) have shown that with a small increase in disulfide bond formation a large increase in network formation resulted in wheat proteins, which provided insight into the extent that disulfide bonds contributed to the polymerization of wheat proteins. So far, it is still unclear what the overall mechanism of protein interaction is during the extrusion process, which has prevented the application of the extrusion process in improving functional and textural properties of underutilized protein sources.

Wheat flour has a unique composition compared to other feedstocks. Though protein comprises 10−14% (weight base) of the total flour, it is responsible for the viscosity and elasticity of dough to a great extent (Greenwood and Ewart, 1975; Funt Bar-David and Lerchenthal, 1975). Gluten, a class of proteins in wheat flour, contains about 2−3% cysteine and cystine (Lâsztity, 1984). Gluten consists of two major groups: the gliadins and the glutenins. The gliadins consist of a mixture of many proteins of rather low and medium

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Table 1. Extrusion Conditions for Experiments

sample ID	added cysteine (%)	die temp (°C)	feed moisture (% wet basis)	feed rate (g/min)	screw speed (rpm)	moisture after extrusion (% dry basis)
EWSH-0 [a]	0.0	185	16	225	500	3.87 [b] ± 0.10 [c]
EWSH-0.25	0.25	185	16	225	500	3.80 ± 0.11
EWSH-0.5	0.5	185	16	225	500	3.35 ± 0.09
EWSH-0.75	0.75	185	16	225	500	3.31 ± 0.02
EWSH-1.0	1.0	185	16	225	500	2.84 ± 0.07
EWSH-1.5	1.5	185	16	225	500	2.55 ± 0.10

[a] EWSH: Extrudates of wheat flour with added cysteine. [b] The mean of triplicates. [c] The standard deviation (SD).

molecular weight that are monomers with either no disulfide bonds or intrachain disulfide bonds. The glutenins consist of a mixture of many proteins that vary in molecular weight from 100 000 to millions, and most of the molecules are made up of a number of polypeptide chains connected by disulfide bonds (Tatham et al., 1990). The hydrated gluten is a cohesive, viscoelastic mass, which can be stretched (Belitz et al., 1986). There is an established relationship between the disulfide bond content and the strength of gluten. It has been shown that sulfhydryl 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 interaction 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 the disulfide bond formation and hydrophobic interactions of gluten played a functional 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 interaction in wheat flour extrusion is unclear. Therefore, it is very important to understand the function of the disulfide bond in the network formation of wheat flour extrudates, because it relates the microstructures, and the physical and functional properties of extrudates.

In this experiment, cysteine, a SH-containing amino acid, has been added to wheat flour. The sulfhydryl group in cysteine or protein is an active site; it can react with another to form disulfide bonds and also exchange with disulfide bonds. Theoretically, the protein−protein interaction through the formation of disulfide bonds can be reduced by the added cysteine, since cysteine can react with protein through the formation of disulfide bonds. The objective of this work is to investigate the effect of the concentration of added cysteine on the microstructures and functional properties of wheat flour extrudates and to elucidate the role of disulfide bonds to protein interactions during high-temperature short-time extrusion processing.

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 was 14%; it was determined by using the semi-micro-Kjeldahl method (AACC Method No. 46-13, 1983). Ellman's reagent was purchased from the Pierce Co. (Rockford, IL). L-Cysteine and other reagents were obtained from the Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Springfield, NJ).

Extrusion. L-Cysteine was mixed thoroughly with wheat flour to the concentrations of 0, 0.25, 0.50, 0.75, 1.0, and 1.5% (w/w). 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 length 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 a controlled flow rate (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 Corp., Pitman, NJ). A metering pump (U. S. Electric Motors, Millford, CT) was used to add the water. The extrudate was pelletized by a two-blade cutter at the face of the die. The following conditions were used: 16% moisture content (wet basis), 185 °C die temperature, 500 rpm screw speed, and 225 g/min mass flow rate (Table 1).

Sample Preparation. The extrudate for each concentration of added cysteine was collected onto a large scale (about 2 kg) 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 broken into about 10 cm long strands and transferred to Ziplock bags. Some of the extrudates were ground with a 700B Waring blender (Waring Products Corp., New Hartford, CT) in order to pass through a 40 mesh sieve. Ground samples were sealed and stored at 4 °C in glass bottles for further analysis.

Moisture Content. The moisture content of each extrudate was determined immediately after extrusion according to the AOAC method (934.01, 1990). Approximately 2 g of each sample was placed in a 282A Isotemp Vacuum Oven (Fisher) 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.

Sulfhydryl and Disulfide Analysis. The contents of the free and total sulfhydryl groups in the solid phase of the extrudates (including in both protein and cysteine/cystine) were determined according to the method of Chan and Wasserman (1993) with some modifications. Approximately 100 mg of the ground extrudate with a particle size smaller than 40 mesh was suspended in 5.0 mL of reaction buffer. The reaction mixture was incubated under N_2 in the dark for 1.0 h at room temperature and then centrifuged at 13 000 rpm for 20 min in a microcentrifuge (Eppendorf centrifuge 5415C, Brinkmann Instruments, Inc.). The supernatant was collected and diluted at a ratio of 1:10. The absorption of the diluted supernatant was determined at 412 nm against a blank in a U-3110 spectrophotometer (Hitachi Instruments Inc., CT). The content of the sulfhydryl groups was calculated by using the absorption and the extinction coefficient of 1.36×10^4 M^{-1} cm^{-1}. The disulfide bond content of the extrudate was calculated as the difference in the sulfhydryl group content before and after the reduction of disulfide bonds with sulfite.

Functional Properties. *Expansion Ratio.* The expansion ratio (ER) was calculated as the cross-sectional diameter of an extrudate divided by the diameter of the die opening (Dahl and Villota, 1991). Fifty measurements were performed on random segments of each sample.

Bulk Density. The bulk density (BD) was determined by placing the ground extrudate in a 10 mL graduated cylinder,

tapping 10 times with a glass rod at the 2, 4, 6, 8, and 10 marks, and then recording the resulting volume and weight of the extrudate. The bulk density was expressed in g/cm^3 (g/mL) (Dahl and Villota, 1991). Triplicate measurements were performed on each sample.

Expansion Volume. The expansion volume (EV) was calculated as the volume per unit weight for each sample. The weighed extrudate strands (about 2.5 g) were put into a 100 mL cylinder, and then glass balls (diameter = 0.5 mm) were used to fill the residual space. The extrudate volume (mL) = 100 (mL) - the volume (mL) of the glass balls. The apparent expansion volume of the extrudate was calculated as follows: EV = the volume (mL) of strands divided by the weight of the apparent strands (gram on a dry basis). Four measurements were performed on each sample.

Color. The color of ground extrudates was determined on a Minolta CR-210 Chroma Meter (Minolta Corp. Ramsey, NY). *L*, *a*, and *b* values were recorded with a white plate as standard. A "whiteness" index was calculated on the basis of the following equation (Fujii et al., 1973): "whiteness" = 100 - $[(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$, where (*) denote the corresponding experimental value for each sample. Six measurements were performed on each sample.

Water-Holding Capacity. The water-holding capacity (WHC) was determined according to the method described by Artz et al. (1990) with some modifications. Approximately 2 g of the ground extrudate was mixed with 40 mL of water. The mixture was allowed to stand for 40 min at 25 °C and then centrifuged at 18000*g* for 30 min at 25 °C. The liquid retained by the solids was measured. The water-holding capacity was expressed as grams of water retained per gram of the extrudate on a dry basis. Triplicate measurements were performed on each sample.

Oil Absorption Capacity. Oil absorption capacity (OAC) was measured according to a modified method of Tjahjadi et al. (1988). Approximately 0.5 g of the ground extrudate and 10.0 mL of soybean oil were added to a 15 mL conical graduated centrifuge tube. The contents in the tube were mixed for 3 min with a vortex mixer to disperse the sample into the oil. After a holding period of 30 min, the tube was centrifuged for 25 min at 3000*g*. The separated oil was then removed with a pipet, and the tube was inverted for 25 min to drain the oil prior to reweighing. Oil absorption capacity was expressed as grams of soybean oil bound per gram of the extrudate on a dry basis. Triplicate measurements were performed on each sample.

Texture Measurement. Textural attributes of an extrudate were characterized by using the textural profile analysis (TPA) (Bourne and Comstock, 1981), which was carried out on a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a TA-25 probe (2 in. diameter cylinder). A 1.5 cm long strand of an extrudate was compressed longitudinally to 20% of its initial height (the cross-section diameter) at a speed of 2.0 mm/s. The waiting time between the two bites was 3 s. The data were automatically analyzed by a computer program (XTRA dimension software, Stable Micro Systems, Haslemere, England). Forty measurements were performed on each sample.

Scanning Electron Microscopy. A scanning electron microscopy (SEM) (Amray 1830I SEM, Amray Inc., Bedford, MA) was used to exam the microstructures of extrudates in a cross-section. An extrudate strand was cut in the cross-section, mounted on the SEM studs using the silver colloidal paste, and coated with the gold platinum prior to examination. The cross-section and the cell wall closeup of an extrudate were viewed and photographed on an ETEC autoscan scanning electron microscope (Amray Inc., Bedford, MA) operating at an accelerating voltage of 10 kV (Faubion and Hoseney, 1982). Duplicate examinations were performed on each sample. The cell size and cell wall thickness of an extrudate were measured directly from the SEM pictures with a ruler. The ranges of cell size and cell wall thickness of each sample were based on fifty and ten measurements, respectively.

Statistical Analysis. Dunnett's method for multiple comparisons against a single control group was used in this experiment to estimate the difference among the tested groups (Glantz, 1992). The statistically significant difference was defined as $P < 0.05$.

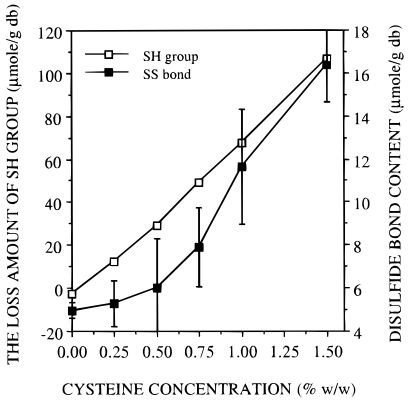

Figure 1. Effect of the concentration of added cysteine on the formation of disulfide bonds (−■−) and the loss of cysteine (−□−) in wheat flour extrudates: plot symbol, the mean; error bar, the standard deviation.

The replicate analyses were made on the extrudates from each of the duplicate extrusion runs. The results of the analyses on the extrudates from each of the duplicate extrusion runs were similar. The data reported in this paper were the analytical results of the extrudates from one extrusion run.

RESULTS AND DISCUSSION

Changes of Sulfhydryl Groups and Disulfide Bonds in the Extrudates. The contents of SS and SH in the ground extrudates were determined. As the concentration of added cysteine in wheat flour was increased to 1.5%, after extrusion, the content of the disulfide bonds increased by nearly two times and the loss of free sulfhydryl groups increased linearly (r^2 = 0.998) (Figure 1). About 2.67 ± 0.25 μmol of SH/g of the control extrudate (without added cysteine) was generated (Figure 1), which might result from the reduction of disulfide bonds during the extrusion process.

Cysteine is a heat unstable, SH-containing amino acid; it can degrade into acetaldehyde, ammonia, and hydrogen sulfide under high temperature and aqueous conditions (Shu et al., 1985). Consequently, a considerably large number of sulfur-containing compounds can be produced (Zhang et al., 1988). In this study, the loss of SH groups (cysteine) may be due to the formation of SS bonds, the degradation of cysteine, and the production of other sulfur-containing compounds during the extrusion processing. The increase in disulfide bonds may be due to the oxidation of SH groups between cysteine and cysteine, protein and cysteine, and protein and protein.

Physical and Functional Properties of the Extrudates. *Expansion Ratio.* The Expansion Ratio (ER) of the extrudates was significantly ($P < 0.05$) affected by an addition of cysteine. As shown in Figure 2, the ER of the extrudates decreased by about 25% as the concentration of added cysteine was increased to 1.5%. When an extrudate was cut at the die into a 1 cm long strand during extrusion, with an increase in the concentration of added cysteine, the shape of the cut extrudate changed from the small sphere to the irregular jagged particle (Figure 3). Unlike the control extrudate, the extrudate with the added cysteine failed to resist the expansion forces, expanding at their cut surface when it exited the die during the extrusion processing. The increased expansion through the cut

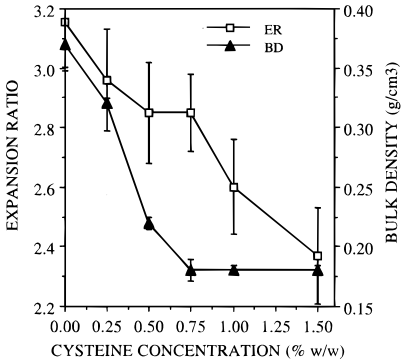

Figure 2. Effect of the concentration of added cysteine on the expansion ratio (−□−) and the bulk density (−▲−) of wheat flour extrudates: plot symbol, the mean; error bar, the standard deviation.

Figure 3. Effect of the concentration of added cysteine on the appearance of wheat flour extrudates cut at the die of the extruder: EWSH-0, 0% added cysteine; EWSH-0.5, 0.5% added cysteine; EWSH-1.0, 1.0% added cysteine; EWSH-1.5, 1.5% added cysteine.

surface directly indicated a weaker matrix in the extrudate, which was caused by the added cysteine.

Bulk Density. The bulk density (BD) of the ground extrudates, which described the degree of compactness of the matrix, decreased by nearly 50% as the concentration of added cysteine was increased to 0.75% ($P < 0.05$) and then changed little with the further increased concentration of added cysteine (Figure 2). As shown in Figure 2, the BD break point of the extrudates occurred at a concentration of 0.75% added cysteine, at which the BD of the extrudates changed little with the further increased concentration of added cysteine. The decrease in BD of corn extrudates has been concluded to be due to inherently weaker matrix because of a reduction in the average of the molecular weight of starch and protein (Barrett and Peleg, 1992). In our study, the decrease in BD of wheat flour extrudates indicated that cysteine might result in a reduction in the molecular weight of wheat proteins during the extrusion processing. Probably, as a reducing reagent, cysteine reduced the molecular weight of wheat proteins during the extrusion processing by two ways: (a) inhibiting the disulfide bond polymerization of proteins; (b) cleaving the native intermolecular disulfide bond of proteins. The BD break point of the extrudates might reflect a condition in which the cysteine−protein interaction was saturated due to the limited cysteine/cystine residues in wheat proteins.

Expansion Volume. The expansion volume (EV) of the extrudates, which was expressed as the volume per unit weight, increased by 25.6% as the concentration of added cysteine was increased to 0.75% and then returned to the original values as the concentration of added cysteine was further increased to 1.5% (Figure 4). The EV break point of the extrudates occurred at the same concentration of 0.75% added cysteine as that of the bulk density. The changes in EV might be the result of the weakened internal networks of the extrudates: the thinned cell wall and the less compact matrix caused by the added cysteine. First, as the concentration of added cysteine was increased to 0.75%, the weakened internal networks of the extrudates could resist less to the forces exerted by gas expanding at the die up to a point at which the extrudates could possess maximum air bubbles with the thinnest cell wall, which resulted in the initial increase in EV of the extrudates. Second, as the concentration of added cysteine was increased from 0.75 to 1.5%, the further weakened networks of the extrudates could not maintain the intact cell structure, the cell wall started to collapse, and the cell size became smaller, which resulted in the decrease in EV of the extrudates.

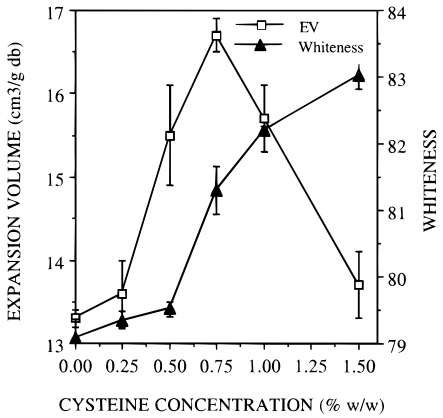

Figure 4. Effect of the concentration of added cysteine on the expansion volume (−□−) and the whiteness (−▲−) of wheat flour extrudates: plot symbol, the mean; error bar, the standard deviation. The *L*, *a*, and *b* values were determined with a light reflectance instrument (Minolta CR-210 chroma meter) and the whiteness value was calculated as "whiteness" $= 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$.

The ER, BD, and EV all described the degree of expansion of the extrudate. The ER considered expansion only in the direction of perpendicular to the extrudate flow. The BD considered expansion in all directions of an extrudate matrix (Falcone and Phillips, 1988). The EV considered expansion in all directions of an extrudate, including the air bubble size and number, and the cell wall thickness and compactness. Therefore, one might expect that these three properties of extrudates would be interrelated. In this paper, the relationship of ER, BD, and EV of the extrudates will be discussed later in more detail from the microstructure viewpoint.

Color. The color of the extrudates with added cysteine became visibly whiter than that of the control extrudate. The *L*, *a*, and *b* values of the extrudate were significantly ($P < 0.05$) affected by the added cysteine. The whiteness value of the extrudate increased by 3.9 color units as the concentration of added cysteine was increased to 1.5% (Figure 4). This indicated that cysteine could reduce the brown color formation in wheat flour extrudates.

Because of the high heat and shear force, extrusion cooking altered the nature of many food constituents, such as starch and protein, by changing their physical and chemical properties (Harper, 1979). The resulting changes in conformation, together with the partial

degradation of starch and protein, could result in an increase in the availability of reactive groups which could go onto take part in chemical reactions, such as the Maillard reaction (protein−sugar) and protein−protein interactions. Andersson et al. (1981) found a 70−80% decrease in total sugar content (sucrose, glucose, and fructose) after the extrusion cooking of a mixture of wheat starch, wheat gluten, and wheat bran. Since higher recoveries of total sugar were obtained when gluten was excluded from the above feed mixture, they concluded that the losses of glucose and fructose were at least partly due to these sugars taking part in the Maillard reaction. For the extrusion of ready-to-eat cereals and expanded snack foods, the material might reach temperatures of 120−180 °C during processing and the moisture content was typically in the range of 12−18% (Sgaramella and Ames, 1993). The Maillard reaction was active under extrusion conditions with high temperature and 12−18% moisture content (Sgaramella and Ames, 1993).

It has been reported that certain SH-containing compounds (L-cysteine, *N*-acetyl-L-cysteine, and reduced glutathion) could inhibit the Maillard browning in heated amino acid−glucose systems, fruit juices (apple, grape, orange, grapefruit, pineapple), and protein-containing foods (barley flour, soy flour, casein) (Friedman and Molnar-Perl, 1990; Molnar-Perl and Friedman, 1990). Our results showed that the SH-containing amino acid (cysteine) could reduce the brown color development in extrusion processing. Because of its strong nucleophilic reactivity and the ability to dissipate free radicals (Cilliers and Singleton, 1990; Dudley and Hotchkiss, 1989; Golan-Glodhirsh and Whitaker, 1984), cysteine might inhibit the brown color development during the extrusion processing by (a) interacting with intermediates formed during the Maillard reaction and/or (b) dissipating free radicals formed during heating and shearing.

Sulfites have been used to inhibit enzymatic and nonenzymatic browning reactions (Wedzicha et al., 1991). Sulfite substitutes were being needed since sulfites were reported to induce asthmatic crises in 4−8% of exposed asthmatics, and their use was being discontinued (Friedman, 1994). Our study has shown that cysteine, a SH-containing amino acid, was effective in preventing browning of extruded wheat flour, which indicated its potential for preventing long-term food browning under processing conditions.

Water-Holding Capacity. The water-holding capacity (WHC) was a measurement of how much water could be bound to the matrices in a particular food system. As shown in Figure 5, the WHC of the ground extrudate decreased by 16% as the concentration of added cysteine was increased to 1.5%. The greatest drop of WHC occurred at the concentration of 0.25% added cysteine. The residual moisture content of the extrudate decreased by approximately 34% as the concentration of added cysteine was increased to 1.5% (Table 1).

Furthermore, it has been observed that the strands of the control extrudate held their original shape during hydration, even after being centrifuged at 18000*g* for 30 min. The strands of the extrudate with added cysteine disintegrated rapidly in water, giving a cream-like consistency.

The WHC of extrudates was mostly influenced by protein−water interactions, water−water interactions, and physical capillary actions due to "conformational voids" (Dahl and Villota, 1991). In our study, the decreases in WHC and residual moisture content of the extrudates could certainly indicate that the network formation of wheat proteins was weakened by the added cysteine. As a result of this weakened protein network, the extrudates could not hold their shapes in water and could not permit the high retention of water by physical capillary actions. However, the control extrudate could retain water by physical capillary actions because of its intact and strong protein network.

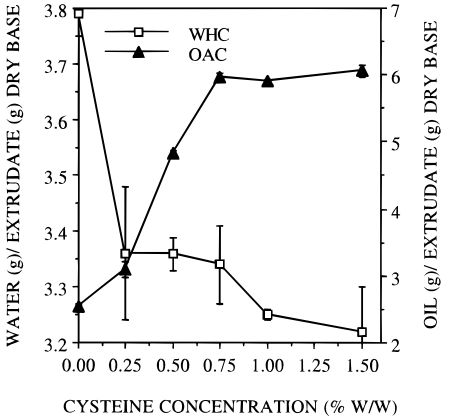

Figure 5. Effect of the concentration of added cysteine on the water-holding capacity (−□−) and the oil absorption capacity (−▲−) of ground wheat flour extrudates: plot symbol, the mean; error bar, the standard deviation.

Oil Absorption Capacity. The oil absorption capacity (OAC) was a measurement of how much oil could be bound to the matrices in a particular food system, which could be used as an index of the hydrophobicity of a food system. As shown in Figure 5, the OAC of the ground extrudate increased by 135% as the concentration of added cysteine was increased to 0.75% and then changed little as the concentration of added cysteine was further increased to 1.5%. The OAC break point of the extrudate occurred at the concentration of 0.75% added cysteine.

The OAC of ground extrudates was mostly influenced by protein−oil hydrophobic interactions. In this study, the weakened protein network caused by the added cysteine could expose more hydrophobic sites to bind with oil, resulting in the increase in OAC of the extrudates.

Texture Characteristics of Extrudates. Figure 6 showed a typical force−time curve obtained from texture profile analysis (TPA) during compression of the extrudates. The control extrudate was rigid, as indicated by the steep initial slope, sharp multi-mini peak, and definite fracturability peak in the curve (Figure 6, A). In contrast, the extrudates with a concentration of over 0.75% added cysteine were more elastic, as indicated by the low initial slope and no fracturability peak in the curves (Figure 6).

As shown in Table 2, the added cysteine markedly affected the textural properties of wheat flour extrudates. The concentrations of 0.5−1.5% added cysteine significantly ($P < 0.05$) affected the gumminess and cohesiveness of the extrudates. As the concentration of added cysteine was increased to 1.5%, the gumminess and cohesiveness of the extrudate increased by nearly 50 and 24%, respectively, and the fracturability of the extrudate decreased. In general, the extrudate with the concentration of 1.5% added cysteine gave the highest values for all textural parameters except springiness. The extrudate with the concentration of 0.25% added cysteine gave the lowest values for all textural param-

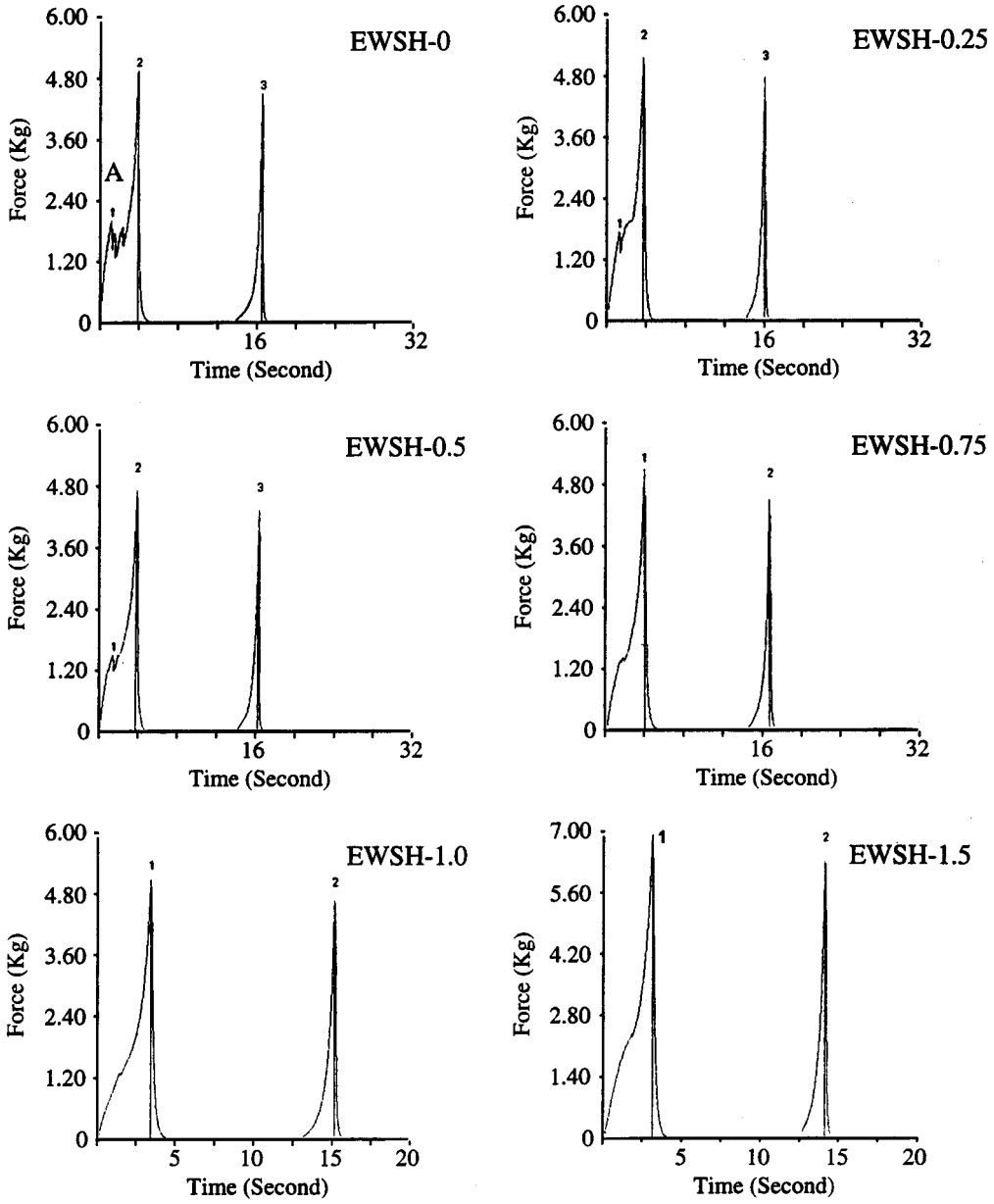

Figure 6. Effect of the concentration of added cysteine on the TPA curve of wheat flour extrudates: EWSH-0, 0% added cysteine; EWSH-0.25, 0.25% added cysteine; EWSH-0.5, 0.5% added cysteine; EWSH-0.75, 0.75% added cysteine; EWSH-1.0, 1.0% added cysteine; EWSH-1.5, 1.5% added cysteine.

Table 2. Effect of Additions of Cysteine on the Textural Characteristics of Wheat Flour Extrudates

	% added cysteine					
textural characteristic	0.0	0.25	0.5	0.75	1.0	1.5
springiness	0.598 [a] ± 0.039 [b]	0.507 ± 0.044	0.560 ± 0.03	0.584 ± 0.027	0.580 ± 0.030	0.577 ± 0.038
gumminess (kg/mm^2)	1.554 ± 0.136	1.422 ± 0.171	1.601 ± 0.202	1.689 ± 0.146	1.899 ± 0.210	2.338 ± 0.328
fracturability (kg/mm^2)	2.189 ± 0.275	1.763 ± 0.216	1.570 ± 0.212	1.553 ± 0.174 [c]	1.537 ± 0.253 [d]	1.893 ± 0.434 [e]
cohesiveness	0.284 ± 0.010	0.278 ± 0.017	0.314 ± 0.012	0.329 ± 0.011	0.348 ± 0.013	0.351 ± 0.011
hardness (kg)	5.473 ± 0.407	5.105 ± 0.454	5.092 ± 0.569	5.144 ± 0.473	5.460 ± 0.625	6.682 ± 1.010
chewiness (kg/mm)	0.930 ± 0.111	0.724 ± 0.120	0.894 ± 0.111	0.984 ± 0.072	1.098 ± 0.103	1.341 ± 0.145
modulus (10 dyn/cm^2)	1.304 ± 0.129	1.153 ± 0.138	1.018 ±0.098	1.105 ± 0.114	1.199 ± 0.186	1.848 ± 0.324

[a] The mean of forty measurements. [b] The standard deviation (SD). [c] Nine of forty measurements taken showed no fracturability peak. [d] Sixteen of forty measurements taken showed no fracturability peak. [e] Twenty-four of forty measurements taken showed no fracturability peak.

eters. Both the highest and the lowest values of textural parameters of the extrudates will be explained later from the microstructure analysis.

Microstructures of Extrudates. The scanning electron microscopy (SEM) was used to examine the effect of cysteine concentration on the extrudate microstructures (cell size, cell wall thickness, and morphology). The SEM photographs of all extrudates showed porous structures, in which the air pockets were surrounded by laminar sheets of solid material, presumably gelatinized protein and starch (Figures 7 and 8). As the concentration of added cysteine was increased, the cell of the extrudate became consistently smaller in size and greater in number, and the cell wall of the extrudate became thinner and more broken (Figures 7 and 8). As shown in Table 3, the cell size of the extrudate changed

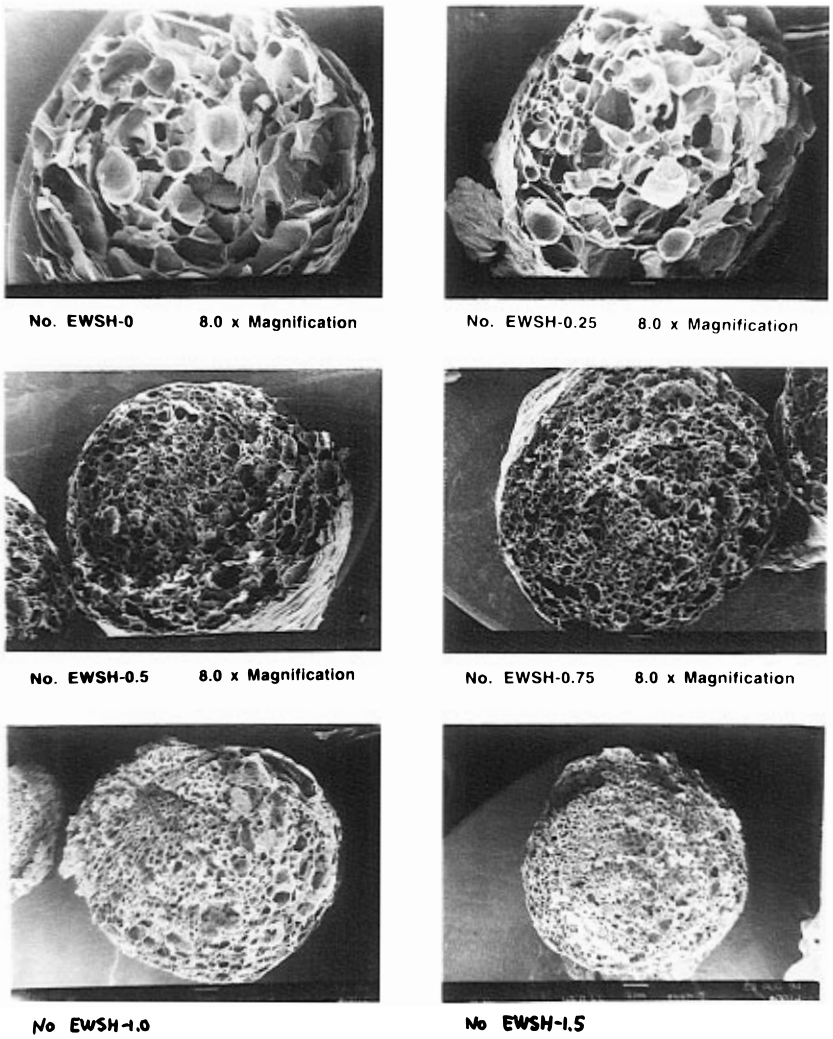

Figure 7. Effect of the concentration of added cysteine on the cell size distribution in the cross-section of wheat flour extrudates. All pictures are SEM photographs of the extrudates (the magnification is ×8.0): EWSH-0, 0% added cysteine; EWSH-0.25, 0.25% added cysteine; EWSH-0.5, 0.5% added cysteine; EWSH-0.75, 0.75% added cysteine; EWSH-1.0 (bottom left), 1.0% added cysteine; EWSH-1.5 (bottom right), 1.5% added cysteine.

little, from 0.75−1.75 to 0.5−1.65 mm in diameter, as the concentration of added cysteine was increased from zero to 0.25%. The diameter then dropped quickly to less than 0.5 mm as the concentration of added cysteine was further increased to 1.5%. The cell wall thickness of the extrudate decreased dramatically, about 90%, as the concentration of added cysteine was increased to 0.75%. Above 0.75% added cysteine, the cell wall thickness changed little.

The cell surface structure of the extrudate (Figure 8) showed a stretched morphology at zero concentration of added cysteine, a smooth and continuous morphology at low concentrations of added cysteine (0.25−0.5%), and a broken morphology at higher concentrations of added cysteine (>0.5%). All changes in microstructures of the extrudates might be a response to the changes in chemical compositions and protein interactions caused by a varied concentration of added cysteine. Strecker et al. (1995) stated that disulfide bonds were important in polymerization of wheat proteins during heating and shearing. The cysteine might affect the microstructures of wheat flour extrudates by reducing the molecular weight of wheat proteins as discussed before. Therefore, as a result, the network formation of wheat proteins in the extrudate matrix was weakened, which resulted in the reduction of cell wall thickness and strength, and the matrix compactness.

In our study, the changes in physical, functional, and textural properties of extrudates corresponded with the changes in microstructures of extrudates, which were caused by the added cysteine. The decreased cell size and the thinned cell wall could result in the decreases of ER and BD, respectively. The decreased cell size could result in a decrease of EV. Conversely, the thinned cell wall could result in an increase of EV. Overall, as the concentration of added cysteine was increased to 0.75%, the cell wall thinned predominantly, resulting in the initial increase in expansion volume. In addition, as the concentration of added cysteine was increased above 0.75%, the cell wall remained constant, but the cell size decreased predominantly, resulting in the decrease in expansion volume. Compared to that of the control extrudate, the SEM photographs of the extrudate with the concentration of 0.25% added cysteine revealed the similar cell size and thinned cell wall (Figures 7 and 8). The thinned cell wall had lower resistance to physical compression during texture measuring, resulting in the lowest values in textural parameters of the extrudate. The SEM photographs of the extrudate with the concentration of 1.5% added cysteine revealed a coarse mass of the smallest cells surrounded by the thinnest cell walls (Figures 7 and 8). Although the individual cell wall was the thinnest, there were many of them to support the structure. As a result, this

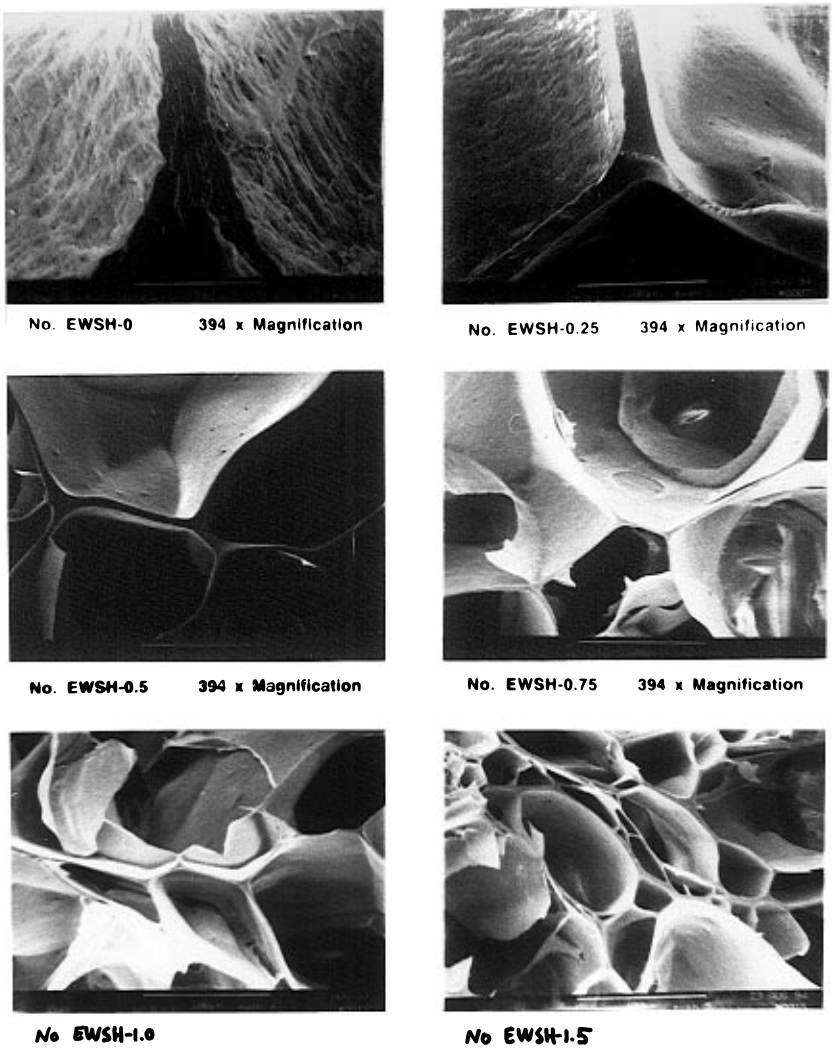

Figure 8. Effect of the concentration of added cysteine on the cell wall thickness in the cross-section of wheat flour extrudates. All pictures are SEM photographs of the extrudates (the magnification is ×394): EWSH-0, 0% added cysteine; EWSH-0.25, 0.25% added cysteine; EWSH-0.5, 0.5% added cysteine; EWSH-0.75, 0.75% added cysteine; EWSH-1.0 (bottom left), 1.0% added cysteine; EWSH-1.5 (bottom right), 1.5% added cysteine.

Table 3. Effect of Additions of Cysteine on the Cell Size and Cell Wall Thickness of Wheat Flour Extrudates

added cysteine (% (w/w))	cell wall thickness[a] (μm)	cell size[b] (mm)
0.00	23−36	0.75−1.75
0.25	15−20	0.5−1.65
0.50	5−10	0.1−0.75
0.75	2−4	0.06−0.88
1.00	<3	0.06−0.63
1.50	<3	0.025−0.5

[a] Based on 10 measurements directly from the SEM pictures with a ruler. [b] Based on 50 measurements directly from the SEM pictures with a ruler.

structure had higher resistance to physical compression during texture measuring, resulting in the highest values in textural parameters of the extrudate. The effect of the concentration of added cysteine on the microstructures of wheat flour extrudates demonstrated that the disulfide bond played an important role in protein−protein interactions during the extrusion processing.

CONCLUSION

In conclusion, the addition of cysteine had a very marked effect on the physical and functional properties and microstructures of wheat flour extrudates, which demonstrated that the disulfide bonds played an important role in protein interactions during the extrusion processing. On the basis of the properties of gluten and cysteine, the ways in which cysteine affects the properties of wheat flour extrudates during extrusion might be (1) breaking the interchain disulfide bond of gluten and greatly lowering the molecular weight and (2) inhibiting the disulfide bond polymerization of proteins and greatly reducing the network formation.

During the extrusion processing, the protein interactions resulted primarily from the disulfide bonds formed from cysteine residues and, less importantly, nonspecific hydrophobic interactions (Martinez-Serna and Villota, 1992). With a small increase in disulfide bond formation, a large increase in network formation resulted in wheat proteins (Strecker et al., 1995). On the basis of our results and the reported results for protein interactions during the extrusion processing (Martinez-Serna and Villota, 1992; Strecker et al., 1995), a hypothesis of the molecular mechanism of wheat protein interactions to impart the three-dimensional structures to the extrudates was proposed. The reaction sequence is depicted in Figure 9. During extrusion processing, wheat flour formed a homogeneous suspension through mechanical mixing. Consequently, the proteins were

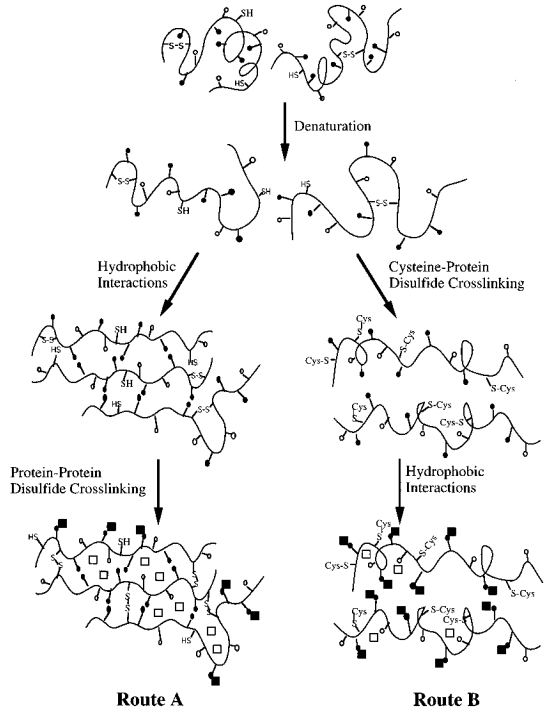

Figure 9. Schematic diagram of a protein molecule denaturing, aligning in the direction of flow and cross-linking through hydrophobic interactions and disulfide bond formations with another protein or cysteine molecule during extrusion processing: −●, the hydrophobic amino acid side chain; −○, the hydrophilic amino acid side chain; □, the water molecule; ■, the oil molecule; route A, without addition of cysteine in wheat flour; route B, with addition of cysteine in wheat flour.

denatured, dissociated, and unraveled allowing alignment of the denatured protein molecules in the direction of the flow. The denatured wheat proteins aggregated, first through the hydrophobic interaction, followed by the disulfide cross-linking (Figure 9, route A). The disulfide cross-linking was probably not the first step in the aggregation of wheat proteins, since the chance that sulfhydryl groups reacted with each other without any further catalysis was unlikely on the basis of their concentration. It was likely, however, that the hydrophobic interaction played a key role in the disulfide cross-linking in wheat proteins during the extrusion processing. First, the denatured proteins associated together by the hydrophobic interaction of hydrophobic residues. Then, the sulfhydryl groups in the associated proteins reacted with each other and/or exchanged with disulfide bonds to form interchain disulfide bonds (Figure 9, route A). The resulting network of wheat proteins that involved covalent bonding made the extrudate possess a strong and compact matrix, which could resist stretching of the cell wall caused by gas expanding at the die and retain water by physical capillary actions (Figure 9, route A).

However, in the presence of a large excess of added cysteine compared to the protein disulfide and sulfhydryl groups, the sulfhydryl group in cysteine could break the native interchain disulfide bonds of gluten to produce protein−SH or protein−SS−cysteine and greatly reduce the molecular weight of gluten (Figure 9, route B). Also, the disulfide cross-linking between protein and protein was inhibited by the interaction of cysteine and protein during the extrusion processing. As a result, the reduction in the extent of cross-linking between protein molecules made the extrudate possess a weakened network, which could not resist the stretching of the cell wall caused by gas expanding at the die. The cell size and the cell wall thickness decreased to a level which could be supported by the strength of the weakened network. The weakened cross-linking between proteins made the denatured proteins expose more hydrophobic sites to bind with oil. Thus, the changes in microstructures resulted in the changes in functional and textural properties of the extrudates. All break points of BD, EV, and OAC of the extrudates were at the concentration of 0.75% added cysteine, which might reflect a condition that the disulfide cross-linking between cysteine and protein was saturated due to the limited amount of cysteine/cystine in wheat proteins.

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