Effect of Extrusion Temperature on the Solubility and Molecular Weight of Lentil Bean Flour Proteins Containing Low Cysteine Residues

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Lentil flour was extruded at die temperatures of 135, 160, and 175 °C. The soluble protein content in the extrudates decreased by 40.1% in the extracting buffer (1% sodium dodecyl sulfate in 50 mM sodium phosphate buffer, pH 6.9) as the extrusion die temperature was increased to 175 °C. The most insoluble proteins in the extrudates extruded at die temperatures of up to 175 °C could be resolubilized by using sonication. The total disulfide content and sulfhydryl content in the extrudates decreased. The SDS−PAGEs showed that the molecular weight distribution of proteins in the lentil flour changed little before and after extrusion as well as during reduction. The results from this study show that the extrusion temperature had less effect on the solubility and molecular weight of the lentil proteins, which contain a lower level of cysteine residues than wheat proteins.

Keywords: *Extrusion temperature; lentil bean flour; protein solubility*

INTRODUCTION

Extrusion is one of the most versatile and well-established food processes and is widely used in the food and feed industries to make products such as snacks, cereals, pastas, textured vegetable proteins, pet foods, and animal feeds (Rizvi et al., 1995). Extrusion processing can alter the protein structure and solubility by heat, shear force, pressure, and oxygen (Li and Lee, 1996a; Phillips, 1989; Ummadi et al., 1995).

It has been reported that the solubility of wheat proteins decreased dramatically as the extrusion die temperature was increased (Li and Lee, 1996a, 1997; Ummadi et al., 1995). The association and cross-linking of proteins during extrusion processing are responsible for the decrease in their solubility (Li and Lee, 1996a, 1997, 1998; Prudêncio-Ferreira and Arêas, 1993; Strecker et al., 1995). The disulfide bond has been identified as the major covalent bond for wheat protein cross-linking during extrusion processing (Koh et al., 1996; Li and Lee, 1996a, 1997, 1998; Strecker et al., 1995). The extensive disulfide-mediated cross-linking of wheat proteins results in an increase in the molecular weight and, subsequently, a decrease in the solubility of wheat proteins (Li and Lee, 1997). A significant correlation has been found between the extrusion temperature and the solubility and disulfide distribution of wheat proteins (Li and Lee, 1997). The disulfide-mediated cross-linking of proteins affected the physical and functional properties and microstructures of wheat flour extrudates (Li and Lee, 1996b).

On the basis of the results of previous studies, it could be hypothesized that the extrusion temperature should have less effect on the solubility and molecular weight changes of proteins that contain a lower level of cysteine residues than those of proteins which contain a higher level of cysteine residues. Wheat proteins are unique because they contain a high concentration of cysteine residues in their amino acid composition, ∼2.3−5.0 mol % of cysteine/100 g of protein (Table 1; Lăsztity, 1984). However, lentil proteins contain relatively low cysteine residues in their amino acid composition, ∼0.8 mol % of cysteine/100 g of protein (Table 1; Adsule et al., 1989). The hydrophobicity of lentil proteins is similar to that of wheat proteins according to the calibration by Eriksson's equation (Eriksson, 1989). To verify this hypothesis, the effect of extrusion temperature on the changes in the solubility and molecular weight of lentil flour proteins was studied.

Table 1. Amino Acid Compositions (Moles per 100 g of Protein) of Wheat Proteins and Lentil Proteins

amino acid	wheat flour[a]	lentil flour[b]
Asp + Asn	1.75	−[c]
Thr	1.9	3.5
Ser	5.55	−
Glu + Gln	38.6	−
Pro	11.6	−
Gly	1.4	−
Ala	1.45	−
Cys	2.3−5.0	0.8
Val (1.87)[d]	3.25	4.2
Met (1.67)	1.4	0.6
Ile (3.15)	4.2	3.6
Leu (2.17)	6.2	6.9
Tyr (2.67)	1.9	3.0
Phe (2.87)	5.2	4.7
His	1.6	2.8
Lys	0.97	7.3
Arg	2.45	8.8
Trp (3.77)	0.35	1.0
% positive charge amino acid	5.02	18.9
% hydrophobic amino acid	22.5	24.0
hydrophobicity (kcal/mol)	56.41	60.43

[a] Lâsztity (1984). [b] Adsule et al. (1996). [c] Not reported. [d] Eriksson (1989).

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MATERIALS AND METHODS

Materials. Commercial lentil bean flour (product 1020), purchased from Brown's Best Foods (Lincoln, NE), was used for all experiments. The protein content of the lentil flour is 25.1%, as quoted by the vendor. Ellman's reagent and the bicinchoninic acid (BCA) protein assay reagent were purchased from Pierce Co. (Rockford, IL). α-Amylase (EC 3.2.1.1, from *Bacillus* species, 2100 units/mg) and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Springfield, NJ).

Extrusion and Sample Preparation. The extrusion was carried out on a ZSK-30 co-rotating twin-screw extruder (Werner Pfleiderer Corp., Ramsey, NJ). The unit was equipped with a die having two 3 mm diameter and 5 mm length openings. The length and diameter of each screw were 900 and 30 mm, respectively. The screw configuration used in the experiment 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 cool water could be circulated at regulated flow rates to prevent overheating of the barrel through the use of solenoid controlled valves. The heaters and five solenoids were controlled using a PID controller. Product temperatures were recorded by a thermocouple inserted at the die plate. Lentil bean flour was fed into the unit with a K-Tron series 7100 volumetric feeding system (K-Tron International, Inc., Pitman, NJ). A metering pump (U.S. Electric Motors, Millford, CT) was used to add the water into the water inlet.

Lentil flour was extruded at die temperatures of 135, 160, and 175 °C. The feed rate and screw speed were kept constant at 225 g/min and 500 rpm, respectively. Tap water (hardness index of 35) was fed into the extruder to provide a total moisture content of 18% (wet weight basis). The extrudates were collected onto a large scale (~1 kg for each sample) after the extruder had reached equilibrium conditions, as indicated by the steady die temperature and torque. The extrudates were ground with a model 700B Waring blender (Waring Products Corp., New Hartford, CT) to a particle size under 40 mesh. Ground samples were sealed and stored at 4 °C in glass bottles for further analysis. After being ground, the moisture content of each extrudate was determined according to AOAC Method 934.01 (AOAC, 1990). Approximately 2.0 g of each sample was placed in an isotemp vacuum oven (model 282A, Fisher Scientific) at 100 ± 2 °C and 300 mmHg for 16 h. The moisture content was calculated using the loss in weight.

The wheat flour control and extrudates used in this study as comparative samples were the same as those reported by Li and Lee (1997).

Sonication and Protein Extraction. The extraction of soluble proteins in the lentil flour control and extrudates in the extracting buffer (1% sodium dodecyl sulfate in 50 mM sodium phosphate buffer, pH 6.9), with and without applied sonication, was performed according to the procedures described by Li and Lee (1997). After extraction, the mixture was centrifuged at 20000*g* for 40 min at 4 °C and the supernatant collected. The protein content in the supernatant was determined by using the BCA protein assay reagent compatible with detergents (up to 1.0% SDS) (Pierce, catalog and handbook, 1994−1995, p 0-65).

Sulfhydryl Group and Disulfide Bond Determination. In the solid phase, the total sulfhydryl content and total free sulfhydryl content of the lentil flour control and extrudates were determined according to the method described by Chan and Wasserman (1993) with some modifications. Approximately 300 mg of a sample with a particle size under 40 mesh was suspended in 4.0 mL of the reaction buffer. The detailed procedures were the same as those described by Li and Lee (1996b). The disulfide content was calculated from the difference between total and free sulfhydryl contents.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). The SDS−PAGE of the proteins was performed according to the method of Bollag and Edelstein (1991) on 15% polyacrylamide (w/v) gels. One hundred microliters of the supernatant obtained with the 1% SDS in 50 mM sodium phosphate buffer was mixed with 20 μL of the SDS−PAGE sample buffer (5×). The mixture was heated in a 100 °C water bath for 10 min and then centrifuged at 13000 rpm for 4 min. After that, the sample solution was loaded into a well of the gel. The sample buffer (5×) contained 0 or 14.4 mM β-mercaptoethanol for unreduced and reduced protein SDS−PAGEs, respectively.

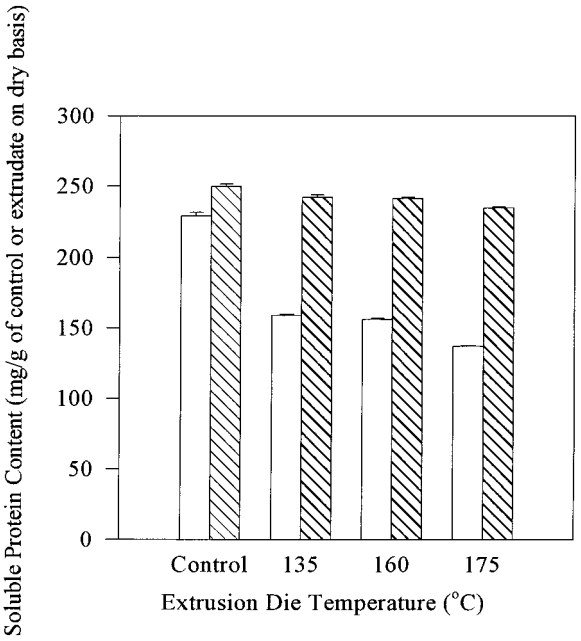

Figure 1. Effect of extrusion temperature (135−175 °C) on the solubility of proteins in the lentil flour with and without sonication applied to enhance solubilization of lentil proteins: (open bars) without sonication; (slashed bards) with sonication.

Oil Absorption Capacity. The oil absorption capacity of the lentil bean flour control and extrudates and of the wheat flour control and extrudates was determined according to the procedures described by Li and Lee (1996b).

RESULTS AND DISCUSSION

Solubility of Proteins in the Extrudates in 1% SDS Aqueous Solvent. The proteins in the lentil flour control and ground extrudates were extracted with the extracting buffer (1% SDS in 50 mM sodium phosphate buffer, pH 6.9) at a ratio of 0.5 g of sample to 10 mL of solvent. As shown in Figure 1, on the basis of the BCA protein assay method, the soluble protein content in the extrudates in the extracting buffer decreased. As compared to the soluble protein content in the control, ~30.6−40.1% of lentil proteins became insoluble after extrusion at a die temperature of 135−175 °C (Figure 1). When sonication was used to enhance the solubilization of proteins, ~95% of the proteins in the lentil flour extrudates were solubilized in the extracting buffer, and the soluble protein content in the extrudates changed little as the extrusion die temperature was increased to 175 °C (Figure 1). In comparison, 52.1 and 66.5% of wheat proteins became insoluble in the extrudate extruded at a die temperature of 160 °C with and without sonication applied to solubilize proteins, respectively (Li and Lee, 1997). This difference might be attributed to the lack of sulfur groups.

The solubility of denatured proteins depended on their molecular weights (Pomeranz, 1991). The decrease in lentil protein solubility after extrusion in the extracting buffer indicated the increase in its molecular weight,

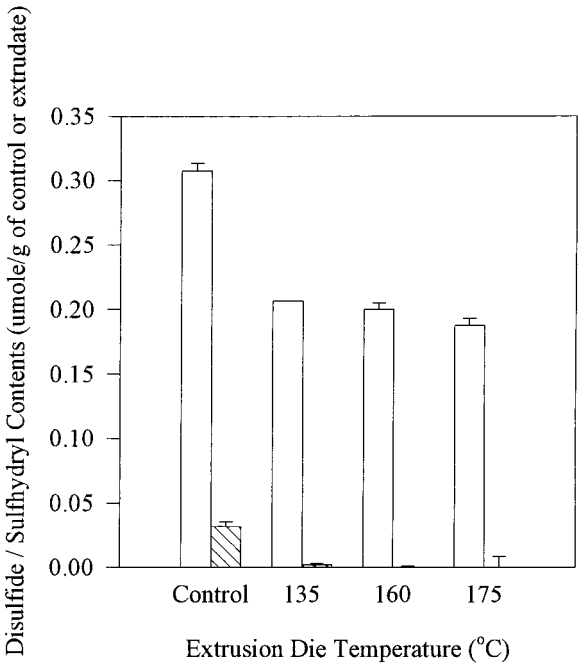

Figure 2. Effect of extrusion temperature (135−175 °C) on the total disulfide and sulfhydryl contents of the lentil flour: (open bars) disulfide; (slashed bars) sulfhydryl.

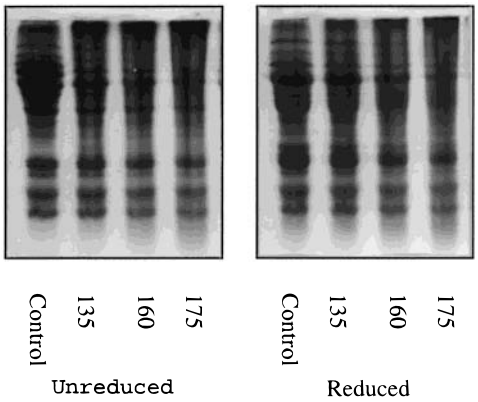

Figure 3. SDS−PAGE (15% acrylamide) of soluble proteins from the lentil flour control and extrudates extruded at die temperatures of 135, 160, and 175 °C in the extracting buffer (1% SDS in 50 mM sodium phosphate buffer, pH 6.9) with sonication applied to enhance solubilization of proteins.

which might be the result of the association of proteins during extrusion processing. Sonication carried out at a low level of power could disrupt the weak noncovalent bonding to reduce the molecular weight of associated proteins, resulting in the resolubilization of proteins (Jennings, 1978). Most of the insoluble proteins in the lentil flour extrudates could be resolubilized by using sonication, indicating that the lentil proteins interacted mainly through noncovalent bonds during the extrusion process.

Total Disulfide and Free Sulfhydryl Contents of the Extrudates. The total disulfide content and free sulfhydryl content (including soluble and insoluble, as determined in the solid phase) in the lentil flour control and extrudates were determined. As shown in Figure 2, the total free sulfhydryl content and disulfide content of lentil flour decreased after extrusion. As the extrusion die temperature was increased to 175 °C, the disulfide content decreased from 0.31 ± 0.006 μmol/g for the control to 0.19 ± 0.006 μmol/g for the extrudate and the free sulfhydryl content decreased from 0.032 ± 0.003 μmol/g for the control to a nondetectable level. The decreases in the disulfide content and free sulfhydryl content might be due to the production of other sulfur-containing compounds during extrusion processing (Riha and Ho, 1996). Li and Lee (1997), however, reported that the total sulfhydryl content (including free sulfhydryl content and disulfide content) of wheat flour changed little as the extrusion temperature was increased to 160 °C.

Molecular Weight Distribution of Unreduced and Reduced Soluble Proteins in the Extrudates. The SDS−PAGEs of the soluble proteins in the lentil flour control and extrudates were done under nonreducing and reducing conditions to check the effect of extrusion temperature on the molecular weight distribution and disulfide-mediated cross-linking of proteins. As shown in Figure 3, the molecular weight of proteins in the lentil flour extrudates increased little as the extrusion temperature was increased and changed little before and after reduction by β-mercaptoethanol. These results suggest that disulfide bonds and other covalent bonds are not important in the interaction of lentil proteins during extrusion, and the noncovalent bonds are responsible for the association and insolubilization of lentil proteins in the extrudates. This is in contrast to our previous results. The molecular weight of wheat proteins in the extrudates (which have more disulfide bonds) increased markedly as the extrusion die temperature was increased to 120 °C, and these new proteins with larger molecular weights disappeared after being reduced by β-mercaptoethanol (Li and Lee, 1997). Therefore, it appears that the disulfide cross-linking contributed to the increase in the molecular weight of wheat proteins after extrusion (Li and Lee, 1997).

Oil Absorption Capacity (OAC) of the Extrudates. The OAC was a measurement of how much oil could be bound to the matrices, which could be used as an index of the hydrophobicity of a food system. As shown in Figure 4, the OAC of the ground lentil flour extrudates increased by 322% as the extrusion temperature was increased to 175 °C. However, the OAC of the ground wheat flour extrudates changed little as the extrusion temperature was increased to 160 °C (Figure 4). These results indicate that the structures of lentil proteins could be modified by extrusion to improve their OAC.

Several papers on the cross-linking of soy proteins during extrusion claimed that disulfide bonds were of negligible importance in the final structure of extrudates, suggesting that new peptide bonds formed under the severe conditions of extrusion (~180 °C) were responsible for the structure of extrudates (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 a decrease in the texture formation after free amino and carboxyl groups of proteins were blocked with ninhydrin and citric acid, respectively. However, this proposed mechanism has been disputed. When extruded soy, whey, and wheat proteins were solubilized in the reagents that exhibited specific chemical actions on proteins (disrupting hydrophobic and electrostatic interactions, hydrogen bonds, and disulfide bonds), their resolubilized profiles indicated that the proteins associated through nonspecific hydrophobic and

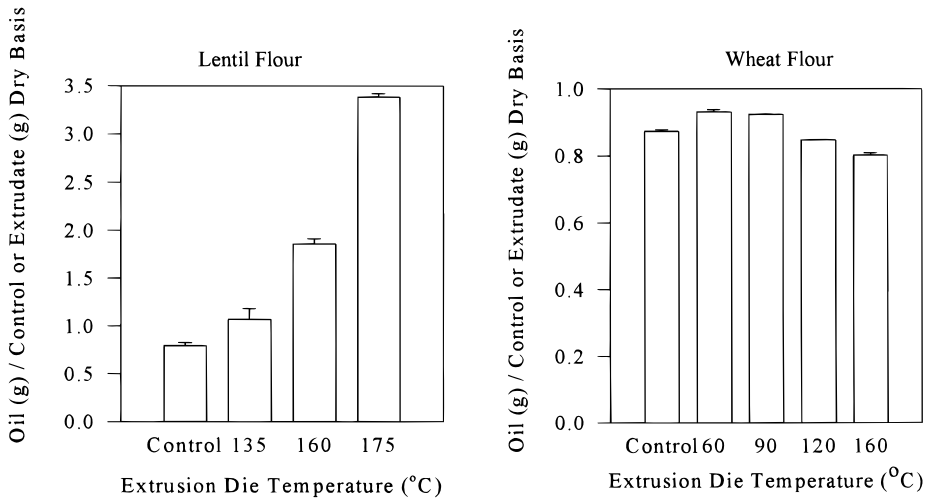

Figure 4. Effect of extrusion temperature on the OAC of the lentil and wheat flours.

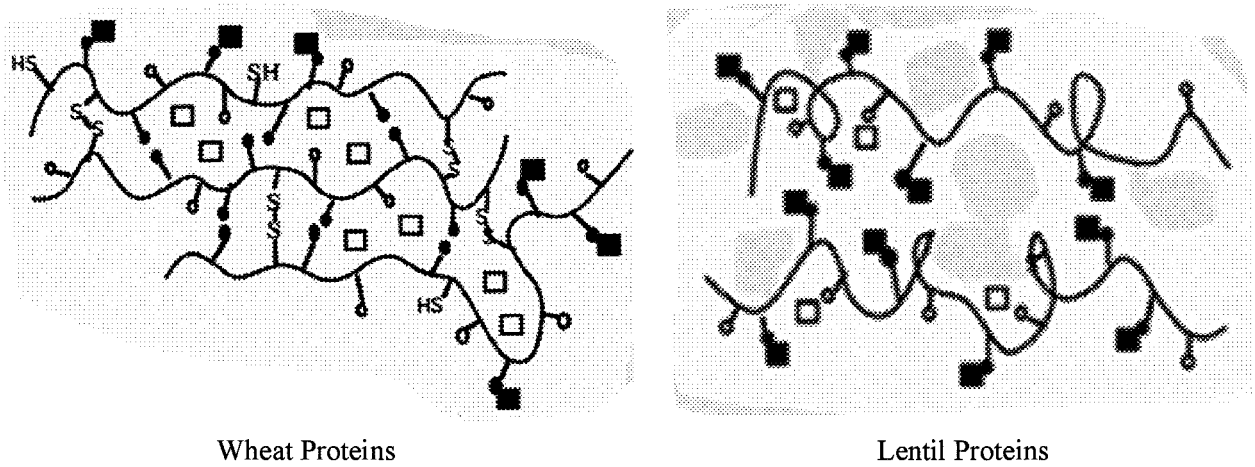

Figure 5. Schematic diagram of the OAC of disulfide cross-linked proteins and noncovalent associated proteins: (●) hydrophobic amino acid side chain; (○) hydrophilic amino acid side chain; (□) water molecule; (■) oil molecule.

electrostatic bonds cross-linked primarily through disulfide bonds formed from cysteine residues (Arĕas, 1992; Li and Lee, 1996a; Prudĕncio-Ferreira and Arĕas, 1993). Li and Lee (1997) reported that extensive disulfide cross-linking of wheat proteins formed at die temperatures between 120 and 160 °C, resulting in a dramatic increase in the protein molecular weight and, subsequently, a dramatic decrease in the protein solubility. Our present results further support that disulfide bonds play an important role in protein cross-linking during the extrusion process. Compared to wheat proteins, lentil proteins contained a lower concentration of cysteine residues; therefore, there were only small changes in the molecular weight and solubility of lentil proteins after extrusion.

The different effect of extrusion on the changes in the solubility, molecular weight distribution, and OAC between lentil proteins and wheat proteins was due to the different contents of cysteine residues in the proteins. Lentil proteins contain a much lower concentration of cysteine residues in their amino acid composition than wheat proteins do. During extrusion processing, lentil proteins associated mainly through noncovalent bonds, whereas wheat proteins associated through noncovalent bonds and were cross-linked through covalent bonds (disulfide bonds) (Figure 5). When sonication was used to break the noncovalent bonds, the lentil proteins in the extrudates mostly disassociated; however, the wheat proteins in the extrudates still cross-linked through disulfide bonds. Therefore, after noncovalent bonds had been broken, there were only small changes in the molecular weight and solubility of lentil proteins after extrusion. There was an increase in the molecular weight and a decrease in the solubility of wheat proteins after extrusion.

The structure of the associated lentil proteins, largely through noncovalent bonds, is less compact than that of wheat proteins associated and cross-linked through noncovalent and covalent bonds (Figure 5). The OAC of the ground extrudates was mostly influenced by protein−oil hydrophobic interactions. Compared to the cross-linked wheat proteins, the associated lentil proteins had more accessible hydrophobic sites and could bind with more oil molecules (Figure 5).

Conclusion. In summary, the extrusion temperature (135−175 °C) had less of an effect on the changes in the molecular weight and solubility of lentil proteins than it did on wheat proteins. Because the sulfhydryl and disulfide contents of the lentil proteins were very low, noncovalent bonds were likely to be responsible for the decrease in lentil protein solubility. Under the extrusion conditions of 135−175 °C die temperatures, unlike that in the wheat flour extrudates, almost all of the insoluble proteins in the lentil flour extrudates became soluble after sonication. These results partially support our hypothesis that the extrusion temperature

has different effects on the changes in the molecular weight and solubility of proteins with various contents of cysteine residues.

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