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Food Chemistry 106 (2008) 1093–1099
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of lipoxygenase activity in defatted soybean flour on the gelling properties of soybean protein isolate

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Received 24 October 2006; received in revised form 13 July 2007; accepted 16 July 2007

Abstract

Soybean lipoxygenase was inactivated to different degrees by dry heating of defatted soybean flour for 0, 5, 10, 15, 20 and 25 min and soy protein isolates were prepared thereof by isoelectric precipitation of the water extract of the defatted soybean flour. The fluorescence emission intensity at 420 nm of the chloroform–methanol extract of soy protein isolates, which was indicator of the existence of peroxidized lipid, varied in parallel with the lipoxygenase residual activity in defatted soybean flours. The dispersion of soy protein isolate showed an increasing turbidity with the increase of lipoxygenase residual activity in the starting defatted soybean flour, suggesting an elevated tendency to form insoluble aggregates during the preparation of soy protein isolate. Small deformation rheological test revealed that the gelling times were shorter for those soy protein isolates derived from low lipoxygenase activity defatted soybean flours than that of high lipoxygenase activity. Frequency sweep showed that G' of soy protein isolate derived from low lipoxygenase defatted soybean flour was independent of oscillation frequency in contrast to that of derived from non dry-heated defatted soybean flour, the latter showed a marked frequency dependence. Large deformation test revealed that the gel hardness increased about 10 times after dry heating of defatted soybean flour for 20 min. As the increase of the lipoxygenase residual activity, the gel permeability increased markedly, suggesting that soy protein isolate from high lipoxygenase defatted soybean flour produced coarser textured gel, which corresponded well with the results of scanning electron microscopy.

Keywords: Lipoxygenase; Defatted soybean flour; Soy protein isolate; Gelling properties; Aggregates

1. Introduction

Lipoxygenase could catalyze the oxidation of polyunsaturated lipids and ester which contain 1,4-*cis*,*cis*-pentadine structure to pentadienyl, and further result in a pentadienyl radical intermediate, which could interact with other lipids to form many other derivatives such as aldehydes and ketones (De Groot et al., 1975; Feussner & Wasternack, 1998). Off–flavor in soy foods such as green-beany, grassy, is often related to LOX, and could be eliminated by LOX inactivation. In addition, LOX was also found to be linked with the modification of protein structure and as a consequence, of protein functionality. Roubal and Tappel (1966) found the radical derived from lipid peroxidation could attack protein and induce protein cross-linking and aggregating. Obata, Matsuura, and Kitamura (1996) found that –SH degradation and gel-forming ability of soy protein were related to LOX activity. Gowland and Liang (1999) found that the interactions of oxidized soybean oil and soy proteins resulted in decreases in protein solubility while Boatright and Hettiarachchy (1995) reported that solubility of soy protein isolate (SPI) could be increased when antioxidants were added into the alkaline protein

Abbreviations: LOX, lipoxygenase; DSF, defatted soybean flour; SPI, soy protein isolate; SEM, scanning electron microscopy; RFI, relative fluorescence intensity.

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doi:10.1016/j.foodchem.2007.07.050

extraction. Hua, Cui, Wang, Mine, and Poysa (2005) compared the gelling properties of three soy protein isolates and found that SPI prepared from defatted soy flour without heat treatment was inferior to two other SPIs prepared from commercial defatted soy flours. In a further research by Huang, Hua, and Qiu (2006), the formation of soluble aggregates was observed in the model system consisting of lipoxygenase, linoleic acid and soy protein.

Hexane extracted defatted soybean flour (flake) is widely used as the starting material for soy protein production. Since it contains about 3% bound lipids and varying activity of LOX, there is a possibility for LOX initiated lipid oxidation, and then the oxidized products would interact with protein during processing. This study was carried out to find an explicit relation between the gelling properties of the soy protein isolate and the heat inactivation of LOX in defatted soy flour so as to gain a better understanding of, and ultimately a better controlling of the processing conditions which affect the functionalities of soy protein isolates. To the best of our knowledge, there is no such scientific research reported before.

2. Materials and methods

2.1. Materials

Soybean was purchased from a local market. All other reagents and chemicals were of analytical grade.

2.2. Preparation of defatted soybean flour

Soybean was cleaned and then dried in an electric oven at 45 °C for 12 h to a moisture content below 7%. The dried soybean was ground to 60 mesh and defatted with hexane at room temperature and allowed to dry in a fume hood by spreading the defatted flour in thin layers on an enamel plate.

2.3. Inactivation of lipoxygenase in DSF

About 1 g of DSF was firmly stuffed in a small screw-capped Pyrex tube with the dimension of 1 cm × 3 cm. The tubes were placed in a boiling water-bath and taken out at 5, 10, 15, 20 and 25 min and cooled to room temperature by putting the tubes in an ice-bath immediately.

2.4. Measurement of LOX activity in DSF

The LOX activity was measured by the modified method of Axelrod, Cheesbrough, and Laakso (1981). DSF was blended with 50 volumes of deionized water in a blender for 25 min. The mixture was centrifuged at 2000*g* for 15 min to obtain crude enzyme extracts. One milliliter supernatant was diluted with 50 ml distilled water before use, and the LOX activity determined by measuring the formation of conjugated dienes at 234 nm. Substrate was prepared by suspending linoleic acid (Sigma Chemical Co., St. Louis, MO. Assay >99.0% (GC)) in borate buffer (50 mM, pH 9.0) and the suspension was neutralized by dropwise addition of 5 mM NaOH solution and shaken by adding two drops of Tween-20 to it and then diluted to 2.24 mM with borate buffer (50 mM, pH 9.0) before use.

The assay was performed as follows: 0.3 ml diluted supernatant of enzyme extracts was added to 2 ml substrate suspension, the mixture was shaken up rapidly and incubated in water (30 °C) for 3 min. The reaction was terminated by adding 5 ml absolute ethanol to the mixture and then 5 ml distilled water were added to it before the measurement. The reference-blank sample contained all reaction components except that LOX was added after the addition of ethanol to the reaction system. The results were determined from OD_{234} readings recorded using a UV2100 spectrophotometer (UNICO Instruments, Shanghai, China). A unit of enzyme was defined as the amount which produced a change in OD of 0.001 per min at 234 nm in 3.0 ml reaction system. All samples were measured in triplicate and means reported.

2.5. Preparation of soy protein isolates (SPI)

DSF was suspended in deionized water in a liquid solid ratio of 15:1 (v/w) and adjusted to pH 7.0 with 2 mol/L NaOH, the mixture was stirred for 1 h then centrifuged at 3000*g* for 30 min. The supernatant was adjusted to pH 4.5 with 2 mol/L HCl and centrifuged at 3000*g* for 30 min. After washing with water and neutralized to pH 7.0 with 2 mol/L NaOH. All procedures were carried out at room temperature. The samples were freeze-dried and stored in cool place.

2.6. Fluorescence spectroscopy

Organic solvent–soluble fluorescent products from soybean proteins were extracted and analyzed according to the method of Fletcher, Dillard, and Tappel (1973). The freeze-dried samples of SPI (0.3 g) were homogenized with 20 ml of chloroform–methanol (2:1, v/v) at 45 °C for approximately 1 min. An equal volume of water was added, and after thorough vortex mixing, the samples were centrifuged for 10 min at 3000*g* (4 °C). The chloroform-rich layer was pipetted into a small screw-capped Pyrex tube for subsequent fluorescence measurements. Fluorescence intensities were obtained on a Shimadzu RF5301PC fluorescence spectrophotometer (Shimadzu Ltd., Tokyo, Japan) with emission maximum at 420 nm (slit width 5 nm) and excitation at 360 nm (slit width 4 nm).

2.7. Turbidity of soy protein dispersions

Soy protein isolate dispersions were prepared in distilled water to a desired concentration, stirred using a magnetic stirrer at room temperature for 60 min. Absorbance of the dispersions were determined at 600 nm on the UV2100 spectrophotometer (UNICO Instruments, Shanghai, China) and used as an indicator for turbidity.

2.8. Rheological measurements of SPI gels

Gel formation of soy protein dispersions (120 mg/g) was induced by heating from 25 to 95 °C at a heating rate of 10 °C/min, holding the temperature at 95 °C for 30 min, and cooling down to 25 °C at a cooling rate of 10 °C/min. Dynamic rheological measurements were performed in a rheometer (AR1000, TA Instruments, New Castle, DE, USA) using the parallel plate (20 mm radius, 1 mm gap). The storage modulus G' and loss modulus G'' were measured at a constant strain of 0.01, which was within the linear region, and at an angular frequency of 0.63 rad/s. To prevent drying, samples were covered with a cap, sealed with a thin layer of paraffin oil during the procedure. Frequency sweeping measurement was followed temperature sweeping with range changing from 0.1 to 10 Hz at 25 °C.

2.9. Textural measurements of SPI gels

For large deformation measurements, gels were prepared by heating soy protein dispersions (120 mg/g) in cylindrical glass moulds with an inner diameter of 25 mm and a height of 60 mm. The moulds were filled three-quarters full to enable air bubbles to escape, the air bubbles were removed using a vacuum pump and then placed vertically in a water-bath. Sample was heated at 95 °C for 30 min and then cooled to room temperature by immersing in an ice-bath. Before analysis, the gels were removed from the container and transferred to a Texture Analyzer (TA-XT2i, Stable Micro Systems, Godalming, Surrey UK). A cylinder probe with diameter of 10 mm was chosen and the probe speed was set up to 1 mm/s. The tests were performed at 25 °C and by compressing the sample until rapture.

2.10. Permeability measurements

Measurements were performed according to the method developed by van Dijk and Walstra (1986). Gels (75 mg/g) were prepared in open-end glass tubes with an inner diameter of 4 mm and a height of about 30 cm. The tubes were placed in a glass cylinder that was filled with SPI dispersion. The height of the dispersion in the tubes was about 8 cm. The cylinder was closed airtight and placed in a water-bath. Gel formation of SPI dispersions was induced by heating from 25 to 95 °C at a heating rate of 1 °C/min, keeping the temperature at 95 °C for 60 min, and cooling down to 25 °C at a cooling rate of 1 °C/min. Tubes were placed in a rack in a thermostated measuring vessel (25 °C) with reference tubes. The vessel was filled with water, 0.2 or 0.5 M NaCl, respectively, which was used for dissolving the SPI. The level of the solution was higher than the top of each gel, so there was a pressure gradient over the gel. The initial pressure gradient was about 10 kPa/m. The level of the liquid on top of the gel h_t was monitored at regular time intervals by reading the scales on the tubes. Permeability coefficients were calculated with the following equation:

$$B = -\frac{1}{\Delta t}\frac{\eta l}{\rho g}\ln\left[\frac{h_\infty - h_t}{h_\infty - h_0}\right] \quad (1)$$

where h_∞ is the level in the reference tubes, h_0 the level at time 0, ρ the density of the permeating liquid, Δt the time difference, l the distance and η the viscosity of the water or salt solution.

2.11. Microstructure

Gels (as the way of texture measurement, see method 2.9) were cut into 3–5 mm cubes from different areas of each gel and fixed in a 3% glutaraldehyde in 0.05 M sodium phosphate buffer solution (pH 7.0) at 4 °C for 2 h. Three 20 min rinses with cold 0.05 M sodium phosphate solution (pH 7.0) followed primary fixation. A secondary fixation followed for 1.5 h in 1% osmium tetroxide in the 0.05 M sodium phosphate solution (pH 7.0) at 4 °C, succeeded with three 20 min rinses. Samples were dehydrated for 20 min each in 30%, 50%, 70%, and 95% ethanol. Samples were immediately dried by the method of supercritical fluid drying of CO_2. Dried samples were fractured. The specimens were attached to stubs with silver conducting paint and coated with a layer of gold–palladium approximately 30 nm thick. The coated specimens were measured with SEM (Quanta-200, FEI Ltd., Eindhoven, the Netherlands) at an accelerating voltage of 10 kV.

2.12. Statistical analysis

All determinations were carried out for at least two times. Data were analyzed by analysis of standard deviations and variance (ANOVA) using EXCEL.

3. Results and discussion

3.1. Effect of dry heating time on the LOX activity in DSF

Dry heating was used in this study to inactivate the LOX activity so that the maximum natural state of the soy protein was retained. Fig. 1 shows the semi-logarithm graphs of LOX residual activity vs. heating time. With heating time prolonged, the residual LOX activity declined continuously, which was fitted to a simple first order kinetics. Because the heat-resistant ability of LOX increased and the thermal conducting rate decreased in dry heating condition, the apparent inactivation rate calculated according to Fig. 1 was lower than that reported by Ludikhuyze, Indrawati, Van den Broeck, Weemaes, and Hendrickx (1998).

3.2. Fluorescence spectroscopy

Fig. 2 shows the RFI of the chloroform–methanol extract of soy protein isolates prepared from DSFs with different LOX inactivation time. RFI of samples from DSFs heated for 5, 10, 15, 20 and 25 min were reduced by 6.8%, 49.5%, 52.1%, 68.9% and 91.6%, respectively, compared

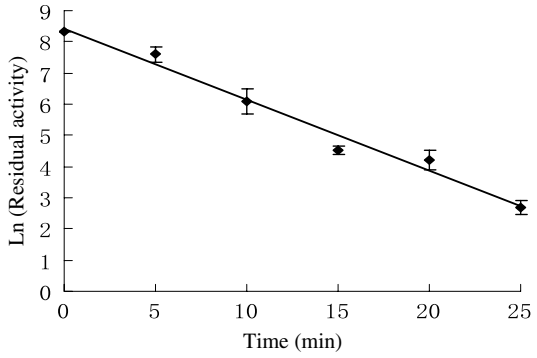

Fig. 1. Heat inactivation of lipoxygenase in defatted soy bean flour. A semi–logarithm graph of ln (residual activity) vs. time.

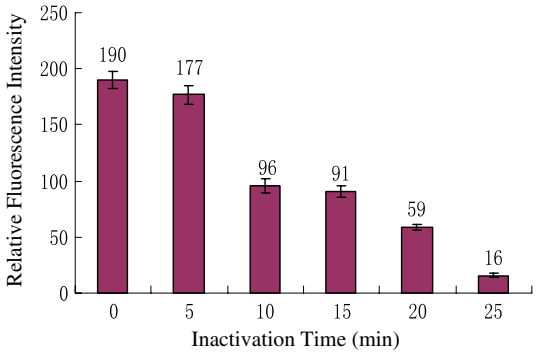

Fig. 2. Relative fluorescence intensity of organic extract from SPI samples. The sample preparation and measurement refer to 2.6.

to the untreated sample. Davies (1987) reported that the oxygen radical could attack protein to form dityrosine, which could be transferred into organic solvent and excited at 320–360 nm to exhibit fluorescence at 410–460 nm. Some other amino acids, such as arginine, lysine, histidine, could also form cross-linking and contribute to the fluorescence (Saeed, Fawthrop, & Howell, 1999). Formation of fluorescent compound during protein amino acids interaction with lipid hydroperoxides was also suggested by Kikugawa, Kato, and Hayasaka (1991). Huang et al. (2006) studied the fluorescence spectroscopy of model reaction systems containing either linoleic acid or LOX or both, in addition to soy protein. They found that linoleic acid and LOX systems as well as soy protein per se exhibited some fluorescence background, but linoleic acid + LOX systems gave much higher fluorescence intensity. Our experiment thus suggested that the lipoxygenase catalyzed lipid oxidation was prevalent in the processing of high LOX activity defatted soybean flour and the DSF heat treatment could effectively reduce the reaction to a much lower level.

3.3. Turbidity and solubility of SPI dispersion

After thorough stirring for sufficient time, those SPIs derived from low LOX activity DSFs formed substantially clear protein solutions, while those prepared from high LOX soy flour gave very opaque suspensions. We found that the turbidity at all SPI concentrations decreased as the LOX inactivation time increased (data not shown). Molina and Wagner (1999) had found that turbidity (OD_{600nm}) could be used to estimate the degree of protein aggregation. More precisely, turbidity was more affected by the formation of those phase separated, or insoluble aggregates. This is confirmed by the results of protein solubility which increased gradually as the inactivation time prolonged (data not shown). The substantially positive correlation between fluorescence intensity and turbidity suggested that the LOX catalyzed lipid oxidation played a major role in the formation of insoluble protein aggregates. In a previous study, Huang et al. (2006) observed the formation of soluble aggregates in the soy protein-LOX-linoleic acid model system by SEC-HPLC and dynamic light scattering. It is not clear right now whether the different aggregation behaviour is caused by the different reaction condition, i.e., model system vs. real system. Another possibility is that the soluble aggregates coagulated further during isoelectric precipitation or/and lyophilyzation steps.

3.4. Rheological properties of SPI gels

Fig. 3 shows the evolution of storage modulus (G') of SPI dispersion as a function of time. A drastic G' value increase after an initial lag time was noticed and it was recognized as the starting point of the gelling process (Kananagh, Clark, & Ross-Morphy, 2000). Since all samples were heated under the same condition, this point could be a measure of the gelling ability. The gel times were about 500 s for SPIs derived from DSFs heated for 15, 20 and 25 min, respectively, but it took about 1000 s to form gel if the DSF had been heat-treated for 10 minutes. For SPI prepared from 5-min heated DSF, gel time was even longer than 2000 s and located at the cooling stage. At the same time, SPI from raw DSF did not form gel at all. It is commonly believed that globular proteins form gels by a multistage process including unfolding, protein association and aggregation, and the cross-linking of aggregates (Clark, Kavanagh, & Ross-Murphy, 2001). For a random

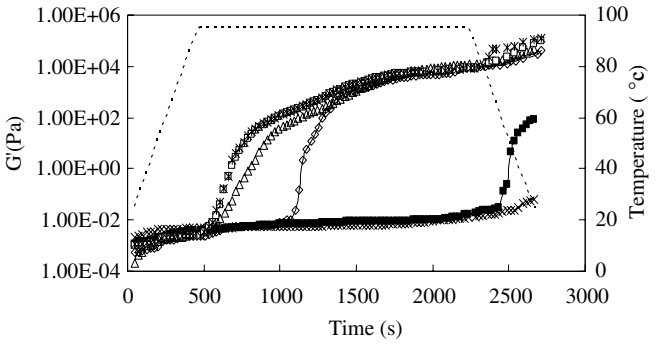

Fig. 3. The increase of storage modulus (G') in gel as a function of time and temperature of SPI gel with different LOX activity. The temperature profile was indicated with a dashed line. Symbols ×, ■, □, △, □, ✻ represent samples with heating time 0, 5, 10, 15, 20 and 25 min, respectively. SPI are dispersed in deionized water (12%, w/w).

cross-linking, classical gel theory predicted that the system exhibited the first sign of gel formation only when the degree of cross-linking approached a critical level, the later being determined by the available sites for cross-linking bond formation. A high cross-linking rate and large f value means short gel time and vice versa. DSF heating time, and hence the LOX catalyzed lipid oxidation affected the gelling time possibly by reducing cross-linking rate or number of bonding sites or both.

In either case, the formation of insoluble aggregates could be a major cause. Benjakul, Visessanguan, Thongkaew, and Tanaka (2005) also found that surimi formed protein aggregation was caused by lipid oxidation during the frozen storage, which could decrease solubility and gelling properties of proteins.

Besides the method mentioned above, there is a sophisticated way to characterize the state of gel which employs both G' and G'' and their frequency dependence (frequency sweep). Winter and Chambon (1986) found that for a typical gel, curves of G' and G'' are parallel to each other with $G' > G''$. In Fig. 4, the G' and G'' curves for SPI prepared from unheated DSF crossed over, while those of 5-min and 10-min heated samples were parallel to each other and essentially independent of the oscillation frequency in the range of 0.1–10 Hz. The result further confirmed that heat treatment of DSF before protein extraction was effective in improving the gelling properties of the resultant SPI.

3.5. Large deformation Textural properties of SPI gel with different LOX activity

The large deformation properties of soy protein gels are important for application in food products, because these are the properties that consumers observe during handling, slicing and eating of the product. Furthermore, mechanical properties provide information about network structure. Table 1 shows the texture properties of SPI gel with different LOX activity. With the extension of the inactivation of LOX in DSF, SPI gel hardness, adhesiveness and cohesiveness increased concomitantly (calculated by Texture Expert Software, Stable Micro Systems, Godalming, Surrey, UK); an almost 10-fold enhancement in gel hardness was observed for 20 min inactivation. Boatright and Hettiarachchy (1995) noticed that adding antioxidants during SPI processing could improve the gel strength of protein, which may be the result of reduced levels of oxidized lipids. The differences in fracture forces among samples as noticed in this experiment also suggested the difference in network structure, as will be shown by electron microscopy measurements that SPI gels from less inactivated DSF consist of thicker and more irregular aggregates than those from low LOX activity DSF.

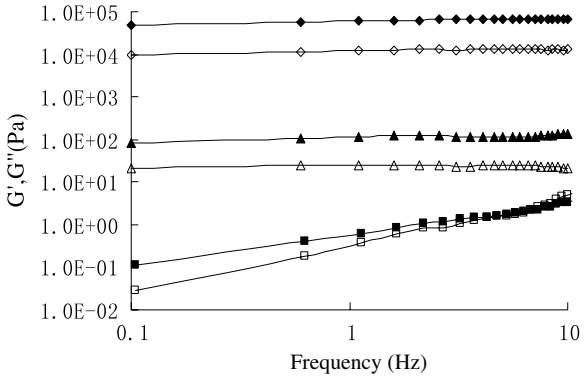

Fig. 4. The G' and G'' as a function of frequency of SPI gel with different LOX activity. The temperature profile is indicated with a dashed line. Symbols ■, ▲, ♦ represent samples with heating time 0, 5 and 10 min, respectively. Solid and hollow symbols represent G' and G''. SPI are dispersed in deionized water (12%, w/w).

3.6. Permeability of SPI gel with different LOX activity

Fig. 5 shows the permeability coefficient B as a function of ionic strength for SPIs derived from DSFs which had been heat-treated for 0, 5, 10, 15, 20 and 25 min, respectively. As the increase in heating time, and consequently the decrease in LOX activity, permeability reduced remarkably. Another feature disclosed is that the different ionic strength dependence for different SPIs. For heating time of 15 min and longer, gel permeability was almost not affected by ionic strength. Otherwise, with high LOX

Table 1
Textural properties analysis (TPA) of SPI gel with different LOX activity

Inactivation time[a] (min)	Hardness[b] (g)	Adhesiveness[c] (g s)	Cohesiveness[d]
0	45.1 ± 0.5 A	−61.3 ± 0.7 A	0.273 ± 0.010 A
5	105 ± 0.6 B	−148 ± 0.8 B	0.340 ± 0.002 B
10	248 ± 3.1 C	−246 ± 1.7 C	0.352 ± 0.004 C
15	323 ± 3.5 D	−327 ± 2.6 D	0.382 ± 0.003 D
20	375 ± 4.4 E	−373 ± 3.2 E	0.386 ± 0.010 E
25	376 ± 4.4 E	−371 ± 3.2 E	0.384 ± 0.010 E

Average of three replications ± standard deviation, the same letter in columns indicates significant difference at $P < 0.05$.

[a] Heating condition: dry heating in a Pyrex screwed tube at boiling water-bath.

[b] Fracturability: the biggest peak force that appeared in the first bite.

[c] Adhesiveness: Negative force area of the first bite.

[d] Cohesiveness: ratio of the positive force areas under the first and second compressions.

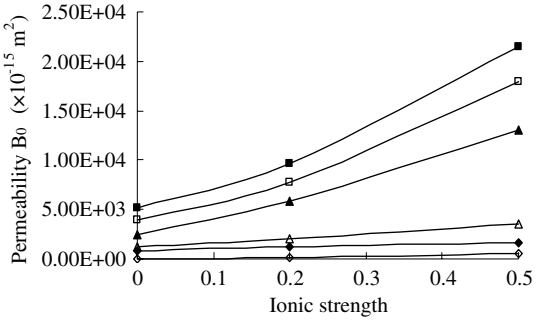

Fig. 5. Permeability of SPI gel (7.5%, w/w) as a function of ionic strength (NaCl). Symbols ■, □, ▲, △, ♦, ◇ represent samples with heating time 0, 5, 10, 15, 20 and 25 min, respectively.

residual activity in DSF, the gel permeability became more and more ionic strength dependent. According to Bremer, van Vliet, and Walstra (1989), permeability coefficient B correlates with the square of the pore radius, which in turn is correlated with the square of the aggregate radius. Thus, small B value represents a fine textured gel and a high water holding capacity, which is desired if the protein is functioning as water absorbing ingredient in applications. In addition, SPI prepared from long-heating time DSF displayed lower ionic strength sensitivity and could be used in high salt foods.

3.7. Microstructure

Effect of DSF heat treatment on the microstructure of SPI gels is shown in Fig. 6. SEM micrograph of native SPI gels showed a coarser and more aggregated gel structure as could be expected from large deformation and permeability tests. Its microstructure was similar to those reported by others for protein gels with a white opaque appearance (Stading, Langton, & Hermansson, 1993; Verheul & Roefs, 1998). As can be seen from SEM images (Fig. 6), LOX inactivation of DSF improved the gel micro-

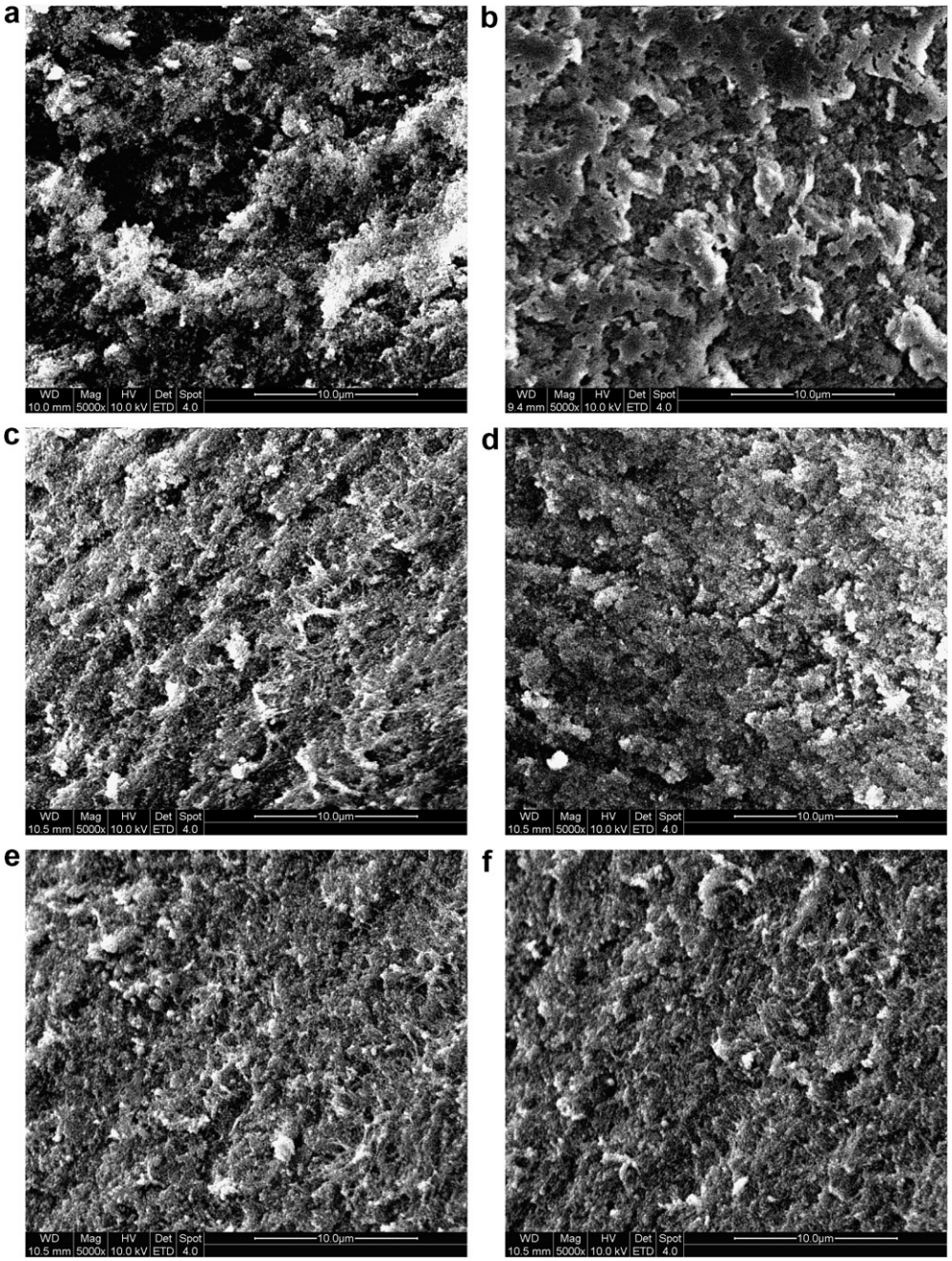

Fig. 6. Scanning electron micrographs of SPI gel (12%, w/w) with different LOX activity. SPI was dispersed at disionized water. Gel was formed at 95 °C for 30 min (before heating, air bubbles was vacuum pumped out from SPI dispersion). (a) Samples without inactivation, (b, c, d, e, f) samples with 5, 10, 15, 20 and 25 min inactivation, respectively. The bars correspond to 10 μm.

structure of SPIs, with the pore and strand size of gels decreased gradually. Further increase of the inactivation time to 15 min and longer resulted in denser networks, where individual strands and pores are hard to distinguish without further magnification.

4. Conclusions

Dry heating of DSF to inactivate the LOX activity proved to be effective in improving the gelling property of soy protein isolate. Based on the facts presented, heat treatment time of 15 min seems to be the minimum required to attain desirable hardness, texture and microstructure. Although lower LOX residual activity and lower relative fluorescence intensity could be achieved by dry heating for 25 min, there were not proportional improvements in protein solubility, turbidity, storage modulus, textural properties, permeability and microstructure. More research is needed to study the chemical and biochemical basis of the process.

Acknowledgements

This research project was financed by funds granted by Natural Science Foundation of China (Project No. 20476040) and Natural Science Foundation of Jiangsu Province, PR China (Project No. BK2005011).

References

Axelrod, B., Cheesbrough, T. M., & Laakso, S. (1981). Lipoxygenase from soybeans. *Methods in Enzymology, 71*, 441–451.

Benjakul, S., Visessanguan, W., Thongkaew, C., & Tanaka, M. (2005). Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. *Food Hydrocolloids, 19*, 197–207.

Boatright, W. L., & Hettiarachchy, N. S. (1995). Soy protein isolate solubility and surface hydrophobicity as affected by antioxidants. *Journal of Food Science, 60*, 798–800.

Bremer, L. G. B., van Vliet, T., & Walstra, P. (1989). Theoretical and experimental study of the fractal nature of the structure of casein gels. *Journal of the Chemical Society, Faraday Transactions 1, 85*, 3359–3372.

Clark, A. H., Kavanagh, G. M., & Ross-Murphy, S. B. (2001). Globular protein gelation-theory and experiment. *Food Hydrocolloids, 15*, 383–400.

Davies, K. J. (1987). Protein damage and degradation by oxygen radicals. *Journal of Biological Chemistry, 262*, 9895–9901.

De Groot, J. J. M. C., Veldink, G. A., Vliegenthart, J. F. G., Boldingh, J., Wever, R., & Van Gelder, B. F. (1975). Demonstration by EPR spectroscopy of the functional role of iron in soybean lipoxygenase-1. *Biochimica et Biophysica Acta - Enzymology, 377*, 71–79.

Feussner, I., & Wasternack, C. (1998). Lipoxygenase catalyzed oxygenation of lipids. *Lipid, 100*, 146–152.

Fletcher, B. L., Dillard, C. J., & Tappel, A. L. (1973). Measurement of fluorescent lipid peroxidation products in biological systems and tissues. *Analytical Biochemistry, 52*, 1–9.

Gowland, R., & Liang, J. H. (1999). Fluorescence due to interactions of oxidizing soybean oil and soy proteins. *Food Chemistry, 66*, 103–108.

Hua, Y. F., Cui, S. W., Wang, Q., Mine, Y., & Poysa, V. (2005). Heat induced gelling properties of soy protein isolates prepared from different defatted soybean flours. *Food Research International, 38*, 377–385.

Huang, Y. R., Hua, Y. F., & Qiu, A. Y. (2006). Soybean protein aggregation induced by lipoxygenase catalyzed linoleic acid oxidation. *Food Research International, 39*, 240–249.

Kananagh, G. M., Clark, A. H., & Ross-Morphy, S. B. (2000). Heat induced gelation of globular protein: part 4 gelation kinetics of low pH beta-lactoglobulin gels. *Langmuir, 16*, 9584–9594.

Kikugawa, K., Kato, T., & Hayasaka, A. (1991). Formation of dityrosine and other fluorescent amino acids by reaction of amino acids with lipid hydroperoxides. *Lipids, 26*, 922–929.

Ludikhuyze, L., Indrawati Van den Broeck, I., Weemaes, C., & Hendrickx, M. (1998). Effect of combined pressure and temperature on soybean lipoxygenase. 1. Influence of extrinsic and intrinsic factors on isobaric–isothermal inactivation kinetics. *Journal of Agricultural and Food Chemistry, 46*, 4074–4080.

Molina, M. I., & Wagner, J. R. (1999). The effects of divalent cations in the presence of phosphate, citrate and chloride on the aggregation of soy protein isolate. *Food Research International, 32*, 135–143.

Obata, A., Matsuura, M., & Kitamura, K. (1996). Degradation of sulfhydryl groups in soymilk by lipoxygense during soybean grinding. *Bioscience, Biotechnology, & Biochemistry, 60*, 1229–1232.

Roubal, W. T., & Tappel, A. L. (1966). Polymerization of proteins induced by free-radical lipid peroxidation. *Archives of Biochemistry and Biophysics, 113*, 150–155.

Saeed, S., Fawthrop, S. A., & Howell, N. K. (1999). Electron spin resonance (ESR) study on free-radical transfer in fish lipid-protein interactions. *Journal of the Science of Food and Agriculture, 79*, 1809–1816.

Stading, M., Langton, M., & Hermansson, A. M. (1993). Microstructure and rheological behaviour of particulate β-lactoglobulin gels. *Food Hydrocolloids, 7*, 195–212.

van Dijk, H. J. M., & Walstra, P. (1986). Syneresis of curd. 2. One dimensional syneresis of rennet curd in constant conditions. *Netherlands Milk and Dairy Journal, 40*, 3–30.

Verheul, M., & Roefs, S. P. F. M. (1998). Structure of whey protein gels, studied by permeability, scanning electron microscopy and rheology. *Food Hydrocolloids, 12*, 17–24.

Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a crosslinking polymer at the gel point. *Journal of Rheology, 30*, 367–382.