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Effects of α-Chymotrypsin Modification on the Functional Properties of Soy Protein Isolates

Taehyun Ahn* and Sook-Young Lee†**

**National Institute of Crop Science, RDA, Suwon 441-857, Korea*
***Department of Food and Nutrition, Chung-Ang University, Ansung 456-756, Korea*

ABSTRACT: Effects of α-chymotrypsin modification on degree of hydrolysis (DH), solubility, emulsifying capacity and thermal aggregation of laboratory-purified soy protein isolate (SPI) using a lipoxygenase-defected soybean (Jinpum-kong) and commercial soy protein isolate (Supro 500E) were compared. SPIs were hydrolyzed by α-chymotrypsin at pH 7.8 and 37°C for 30 min. DHs of Supro 500E and Jinpum-kong SPI were increased by α-chymotrypsin modification, and DH of Supro 500E was higher than that of Jinpum-kong SPI. DH of α-chymotrypsin treated Jinpum-kong SPI was similar with untreated Supro 500E and DH of treated Supro 500E was the highest. Solubility, emulsifying capacity and thermal aggregation of SPIs were increased by α-chymotrypsin modification, and these changes were highly related to changes in DH. Functional properties of Supro 500E were higher than Jinpum-kong SPI in both of untreated and α-chymotrypsin treated SPIs.

Keywords: soy protein isolate, enzyme modification, solubility, emulsifying capacity, thermal aggregation

Soy protein is the most commercially available vegetable protein due to its high nutritional value and low cost. Soy protein products have been replacing with not only nutrients in food but also industrial materials (non-food products) as renewable natural resources (Kumar *et al.*, 2002). Soy protein related on specific functional properties in many formulated food products, and the major protein fractions such as 2S, 7S, 11S and 15S are classified according to their sedimentation properties (Brooks & Morr, 1985). 7S and 11S among them are 35% and 52% of total protein content, respectively. These are storage proteins and they are found in the protein bodies within the cells of the cotyledons (Kinsella, 1979). 7S and 11S fractions have been contributed to have different effects on soy products and have been the simply reported and referred in soy protein research as β-conglycinin (7S) and glycinin (11S) (Brooks & Morr, 1985).

Many studies have been tried to improve the specific functional properties of soy protein in soy products through physical, chemical, enzymatic and genotype selection method. One of them, enzymatic modification is usually performed through limited proteolysis and is an effective way to enhance the solubility, emulsifying capacity and foaming property of SPI through reactions of several molecular parameters such as mass, conformation, flexibility, net charge, hydrophobicity and interactions with other food components (Richardson, 1977). In general, proteolytic enzymatic modification contributes to decrease molecular weight and viscosity, to increase solubility and emulsifying capacity and to change foaming and gelling properties compare with native soy protein (Chobert *et al.*, 1988; Kim(Lee) *et al.*, 1990; Wu *et al.*, 1998; Ortiz & Wagner, 2002) as well as thermal aggregation of enzymatic modified soy protein provides improved the texture in soy products such as cheese analog using SPI (Kim(Lee) *et al.*, 1992). Under the optimized proteolytic enzymatic modification improves the functional properties of soy protein, while excessive hydrolysis causes loss a part of the functional properties (Wu *et al.*, 1998). In addition, functional properties of soy protein have different behavior and characteristics according to genotype and the content of protein fractions within each genotype (Khatib *et al.*, 2002) as well as these properties are influenced by preparation condition such as protein concentration, chemical and/or thermal treatments, presence of salts, pH and so on (Wagner *et al.*, 2000). And commercial SPI and laboratory-purified SPI show different functional properties due to method of modification such as preparation procedure, thermal and/or chemical treatments and drying methods (Anon *et al.*, 2001). Recently, soybean cultivars that have lipoxygenase-defected genotype have been found and applied on soy products (Kitamura, 1993; Kim *et al.*, 1994; Narvel *et al.*, 2000), however, there are many of research that report about the aroma related off-flavor, certain compounds and/or genetic characteristics in lipoxygenase-defected soybean (Kobayashi, 1995; Nishiba & Suda, 1998; Son *et al.*, 2002; Lee *et al.*, 2005), while few studies are about functional properties in it. Thus, further studies on the functional properties of lipoxygenase-defected soybean are still required.

†Corresponding author: (Phone) +82-31-670-3274 (E-mail) syklee@cau.ac.kr
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The object of this study was to compare the effects of α-chymotrypsin modification on solubility, emulsifying capacity and thermal aggregation in functional properties of SPI between laboratory-purified SPI using a lipoxygenase-defected soybean (Jinpum-kong) and commercial SPI (Supro 500E) which were used to process the cheese analogs.

MATERIALS AND METHODS

Materials

Laboratory-purified SPI was prepared using a lipoxygenase-defected soybean (Jinpum-kong; L-2,3 defected genotype) which were grown and selected at National Institute of Crop Science, Suwon and commercial SPI (Supro 500E) was supplied by Purina Co., Seoul. Protease used was α-chymotrypsin (type II. Bovine pancrease) from Sigma Chemical Co., and all chemicals used were reagent grade.

Preparation of Jinpum-kong SPI

Jinpum-kong SPI was extracted according to modified method of Saio & Watanabe (1973). The defatted Jinpum-kong flour was extracted for 1.5 h at room temperature with water adjusted to pH 8.0 with 6 N NaOH after disperse the defatted Jinpum-kong flour (water : flour ratio, 10:1). The mixture was centrifuged at 10,400 × g for 20 min. The supernatant was adjusted to pH 4.5 with 6 N HCl, then centrifuged at 10,400 × g for 20 min, the precipitate was washed 2 times with water, resolubilized in water by neutralization to pH 7.0 with 6 N NaOH, and then freeze-dried.

Preparation of α-chymotrypsin modified SPI

The SPIs were modified by α-chymotrypsin following the method of Kim(Lee) *et al.* (1990). Each of 20% (w/v) SPI suspensions were adjusted to pH 7.8 using 6 N NaOH, and after adding the enzyme (2% of SPI, w/w), the suspensions were dispersed in distilled water with a water bath using a disperser (GTR-1000, Tokyo Rikalikai Co., Japan) at 37°C for 30 min. After incubation with α-chymotrypsin, the suspensions were heated to inactivate the enzyme at 87°C for 5 min, and then the hydrolysates were neutralized to pH 7.0, freeze-dried, and placed –20°C until analyzed. Untreated SPIs as a control also were prepared in the same method except adding the enzyme.

Degree of hydrolysis (DH)

DH was measured according to modified method of Yamashita *et al.* (1970). Each of 1 g α-chymotrypsin treated SPIs were suspended in 100 mL of distilled water. One mL aliquot of the suspension was assayed for nitrogen by the micro-Kjeldahl method (AOAC, 1975). Ten mL aliquot of the suspension (1% (w/v)) was mixed with 10 mL of 20% trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min. The soluble nitrogen in the supernatant was assayed by the micro-Kjeldahl method. The percent DH was expressed as follows:

$$\text{Degree of hydrolysis}(\%)=\frac{\text{10\% TCA soluble N}}{\text{Total N}}\times 100$$

SDS-PAGE

SDS-PAGE was carried out according to method of Bollag & Edelstein (1991). The samples were loaded in 7.5% acrylamide gel and stained in 0.25% Coomassie brilliant blue R-250 overnight. The standard markers used were phosphorylase b (from rabbit muscle 97,400 daltons), bovine serum albumin (66,200 daltons), ovalbumin (45,000 daltons), carbonicanhydrase (from bovine erythrocytes, 31,000 daltons), trypsin inhibitor (21,500 daltons) and lysozyme (14,400 daltons).

Nitrogen solubility

Nitrogen solubility was estimated by modified method of Kim(Lee) *et al.* (1990). SPIs (0.5 g) were dissolved in 30 mL of 0.1 N NaOH (1.6% (w/v)), and each of 6.0 mL aliquots were adjusted to pH 12 with 1 N NaOH, then were centrifuged at 4000 × g for 20 min. The protein in the supernatant was measured by the biuret method, and assumed to have 100% nitrogen solubility. Hydrolysates (0.5 g) were also dissolved in 30 mL of 0.1 N NaOH (1.6% (w/v)), and each of 6.0 mL aliquots were adjusted to pH 7 with 1 N NaOH, then were centrifuged at 4000 × g for 20 min after stirring at 25°C for 1 h, the supernatant was measured by the biuret method. The percent solubility of enzyme-modified SPIs were obtained as following:

$$\text{Nitrogen solubility}(\%)=\frac{\text{Absorbance at the pH 7.0 solution}}{\text{Absorbance at the pH solution}}\times 100$$

Emulsifying capacity

Emulsifying capacity was estimated by slightly modified method of Kim(Lee) *et al.* (1990). The soybean oil (1 L) was colored with 0.1 g of Oil-Red-O ($C_{26}H_{24}N_4O$) biological stain and the colored oil (20 mL) was added to a 1% suspension of SPIs and enzyme-treated SPIs (25 mL, pH 7.0). And dispersed at low speed using a disperser (GTR-1000, Tokyo Rikalikai Co., Japan) for 1 min, then the speed was increased to high speed. After 30 sec, more oil was added at

a rate about 10 mL/min. The volume (mL) of oil consumed until the inversion of the initially formed emulsion, which was accompanied by sudden changes in oil color and viscosity was recorded and expressed as an emulsion capacity.

Thermal aggregation

Thermal aggregation was determined by method of Krammer & Kwee (1977). After SPIs and enzyme treated SPIs (1 g) were added in 50 mL of distilled water (2% (w/v)), they were agitated using a magnetic stirrer for 15 min. Each of 1.0 mL aliquot were assayed for protein content through measuring the absorbance at 540 nm using the spectrophotometer (Spectronic 1001 plus, Milton Roy, USA) after mixed with 4 mL of biuret solution and placed at room temperature for 30 min (A). And the each of 10 mL suspensions of SPIs and enzyme treated SPIs were placed in cap tubes and heated in a boiling water bath for 20 min, then centrifuged at 2,000 × g for 20 min after cooling to 20°C. The protein content of the supernatant (1 mL) was assayed same as above (B). The percent thermal aggregation was obtained by the difference in the protein content of the supernatant caused by heating:

$$\text{Thermal aggregation (\%)} = \frac{\text{Absorbance before heating (A)} - \text{Absorbance after heating (B)}}{\text{Absorbance before heating (A)}} \times 100$$

Statistical analysis

All experiments were carried out in triplicate and the data were analyzed using SAS program. The data were reported as a mean value ± SEM and also subjected to ANOVA and significant differences were reported at the level of $p < 0.05$.

RESULTS AND DISCUSSION

Changes in DH and SDS-PAGE

DH of Supro 500E was higher than that of Jinpum-kong SPI, especially, untreated Supro 500E (13.5%) was over threefold higher than untreated Jinpum-kong SPI (4.1%) ($p < 0.01$). DH of treated Jinpum-kong SPI (15.4%) was simi-

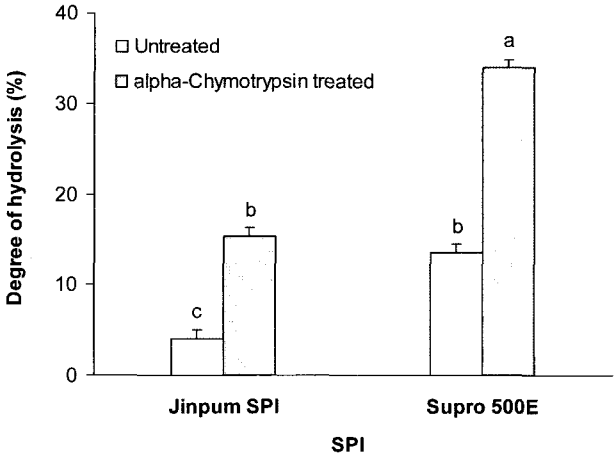

Fig. 1. Changes in DH of SPIs by α-chymotrypsin modification. Values are mean ± SEM and different letter is significantly different at the level of $p < 0.01$.

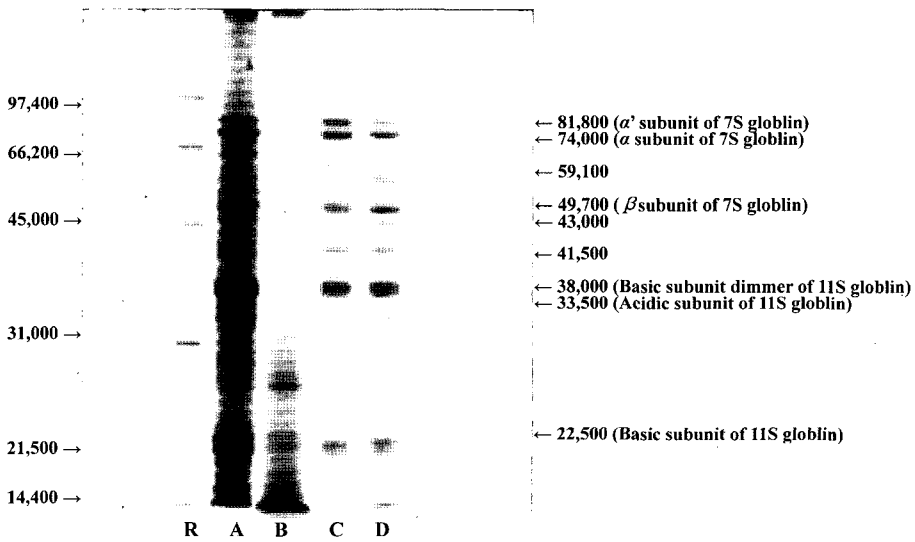

Fig. 2. SDS-gel electrophoretic patterns of reference protein, untreated and α-chymotrypsin treated soy protein isolates. R: Reference protein; phosphorylase b (from rabbit muscle 97,400 daltons), bovine serum albumin (66,200 daltons), ovalbumin (45,000 daltons), carbonic anhydrase (from bovine erythrocytes, 31,000daltons), trypsin inhibitor (21,500 daltons) and lysozame (14,400 daltons). A : Supro 500E, B : α-Chymotrypsin treated Supro 500E, C : Jinpum-kong SPI D : α-Chymotrypsin treated Jinpum-kong SPI

lar with untreated Supro 500E's (13.5%) and DH of treated Supro 500E (34.0%) was the highest. α-Chymotrypsin modification under the optimized condition increased DHs of Jinpum-kong SPI (about fourfold) and Supro 500E (over twofold) ($p < 0.01$) (Fig. 1). High DH of untreated Supro 500E could be supposed that it was already hydrolyzed in manufacturing process of SPI. Kim(Lee) *et al.* (1990) have reported that DHs of untreated commercial SPIs, Ardex F and Supro 710 were 1.35% and 9.44%, after α-chymotrypsin modification at 40°C for 30 min, it were 16.75% and 23.75%, respectively, and the effect of enzyme modification on SPI depended on type of enzymes and SPI used. DH of Supro 710 was similar with the present result of Supro 500E. Qi *et al.* (1997) have also reported that DHs of SPIs were increased by pancreatin modification and varied.

In compared the fractions of 7S and 11S in SDS-PAGE patterns of SPIs, lipoxygenase bands in Jinpum-kong SPI did not show, and after α-chymotrypsin modification, most of 7S fractions were hydrolyzed, while 11S fractions were not hydrolyzed in both of SPIs (Fig. 2). This result indicates that 11S could affect to thermal aggregation related textural properties of cheese analogs. As mentioned above, untreated Supro 500E (Line A in Fig. 2) could be supposed that it was already hydrolyzed in manufacturing process of SPI. In general, the hydration and viscosity properties of SPIs are strongly determined by the amount and properties of the insoluble fraction (Anon *et al.*, 2001), and gelation capacity of SPI is closely related to the relative amounts of the 7S and 11S proteins as well (Arrese *et al.*, 1991).

Changes in solubility

Solubility of Supro 500E was higher than that of Jinpum-kong SPI, and α-chymotrypsin modification increased solubility of Jinpum-kong SPI and Supro 500E ($p < 0.01$) (Fig. 3A). Papain-modified soy protein also showed higher solubility and water hydration capacity than unmodified soy protein (Were *et al.*, 1997) as well as bromelain modification increased solubility of SPI (Ortiz & Wagner, 2002). However, these effects of enzymatic modification are highly affected by pH level (Kim(Lee) *et al.*, 1990; Ortiz & Wagner, 2002). A limited proteolysis increases a negative surface charge of protein and thereby increases solubility and improves emulsifying capacity and stability (Campbell *et al.*, 1996), and solubility of SPI is generally related to DH value of SPI (Kinsella *et al.*, 1979). Supro 500E which has higher DH showed higher solubility. Degree of solubility in processing of soy products is very important because higher solubility is associated with better functional properties. Solubility of SPI is particularly important functional property in soy food system and it is strongly correlated to emulsifying

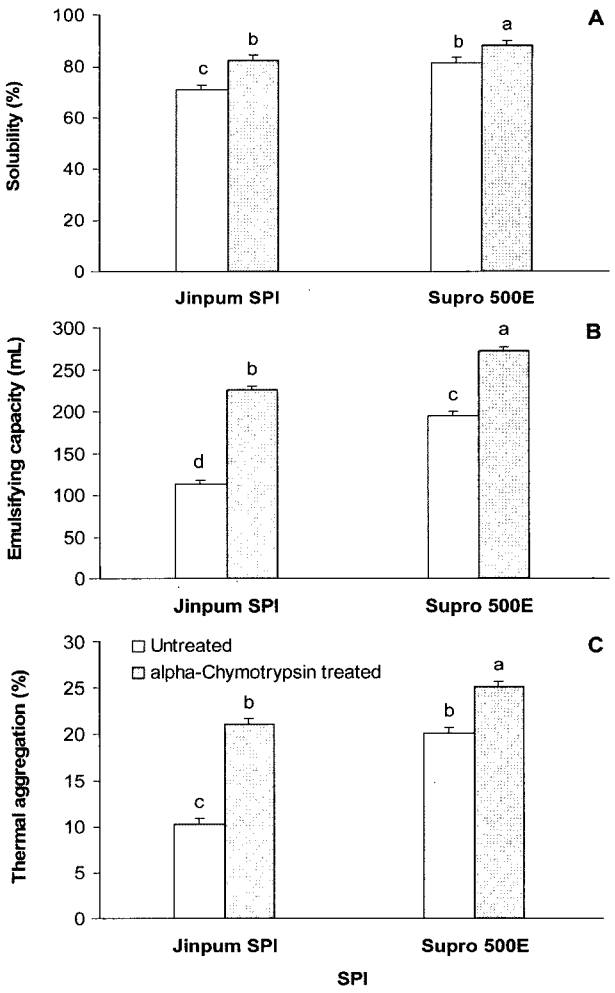

Fig. 3. Changes in solubility (A), emulsifying capacity (B) and thermal aggregation (C) of Jinpum-kong SPI and Supro 500E by α-chymotrypsin modification. Values are mean ± SEM and different letter is significantly different at the level of $p < 0.01$.

capacity (Kinsella *et al.*, 1985).

On the other hand, solubility of SPI depends on cultivar of soybean, solubility of 7S and 11S fractions are significantly different among genotypes and the content of protein fractions within each genotype exhibited different degree of solubility (Khatib *et al.*, 2002) as well as solubility of SPI is influenced by preparation condition such as protein concentration, chemical and thermal treatments, presence of salts, pH and so on (Wagner *et al.*, 2000). In this study also, solubility of laboratory-purified Jinpum-kong SPI from lipoxygenase-defected soybean and commercial Supro 500E were different ($p < 0.01$).

Changes in emulsifying capacity

Emulsifying capacity of Supro 500E was higher than that

of Jinpum-kong SPI, and Jinpum-kong SPI and Supro 500E were increased by α-chymotrypsin modification, particularly, Jinpum-kong SPI was about twice increased ($p < 0.01$) (Fig. 3B). Emulsifying capacity of SPI is mainly related to DH and solubility of SPI (Kinsella *et al.*, 1979; Qi *et al.*, 1997). Papain-modified soy protein showed higher solubility and emulsifying properties than unmodified soy protein (Were *et al.*, 1997). In present study, Supro 500E that has higher solubility than Jinpum-kong SPI showed higher emulsifying capacity than Jinpum-kong SPI. In original, Supro 500E which is used in process of shellfish analogs have a high emulsifying capacity (Mueller, 1992).

Kim(Lee) *et al.* (1990) have reported that emulsifying capacity of SPI was increased by modification of enzymes except rennet treatment, and trypsin or alcalase more effectively increased than α-chymotrypsin and liquozyme. Emulsifying capacity of SPI is mainly determined by surface hydrophobicity and solubility, while the molecular flexibility of the protein is important for emulsion stability (Nakai, 1983). And emulsifying capacity and stability of SPI are differently shown according to major fraction of soy protein, 7S was higher and steadier than 11S fraction in pH 2 up to pH 10 (Aoki *et al.*, 1980).

Changes in thermal aggregation

Thermal aggregation of Supro 500E was higher than that of Jinpum-kong SPI ($p < 0.01$), and α-chymotrypsin modification increased level of thermal aggregation of Jinpum-kong SPI and Supro 500E ($p < 0.01$), particularly, Jinpum-kong SPI was about twice increased (Fig. 3C). The DH, solubility, emulsifying capacity and thermal aggregation of Jinpum-kong SPI were lower than Supro 500E's, whereas it were more effectively enhanced by enzyme modification. Thermal aggregation of SPI needs to interact between certain peptide fractions, and proteolytic enzyme increases thermal aggregation by breaking down the protein, while the insoluble peptide fractions have no gelation ability (Garcia *et al.*, 1990). Thermal aggregation is required in products containing SPI such as sausage and cheese analogs because thermal aggregation is necessary for structure and emulsion stability (German *et al.*, 1982). Thermal aggregation enhanced by enzyme modification contributes to improve the textural properties of cheese analog (Kim(Lee) *et al.*, 1992).

In general, denaturation of SPI is explained by a two-state cooperative transition confined to a narrow temperature range. Oligimeric proteins exhibit additional complexities due to the release of several protomers previously associated. This produces two essentially independent thermal events: first, disruption of the oligomeric structure that itself can result in aggregations, and second, denaturation of the actual monomers initiating further rearrangements and/or aggregations (German *et al.*, 1982). In addition, denaturation is usually accompanied by a decrease of solubility, which results from aggregation of the unfolded molecules, and by changes in certain functional properties such as gelation, foaming and emulsification (Petruccelli & Anon, 1995). Thermal aggregation is increased by SPI concentration and stabilized by SS bonds (Petruccelli & Anon, 1995), moreover, thermal aggregation of SPI according to heating temperature has different behavior between main fraction of proteins. 7S and 11S are totally denatured at 100°C, while 7S is totally denatured but 11S is not denatured at 80°C (Sorgentini *et al.*, 1995).

CONCLUSION

The functional properties of soy protein can be modified by limited enzyme hydrolysis. In accordance with DH of SPI, solubility, emulsifying capacity and thermal aggregation of laboratory-purified SPI from lipoxygenase-defected soybean (Jinpum-kong SPI) and commercial SPI (Supro 500E) were considerably increased. Commercial Supro 500E's were higher than laboratory-purified Jinpum-kong SPI's. α-Chymotrypsin modification enhanced solubility, emulsifying capacity and thermal aggregation of Jinpum-kong SPI, while in Supro 500E, emulsifying capacity and thermal aggregation were enhanced but solubility did not showed remarkable difference. These results indicate that solubility, emulsifying capacity and thermal aggregation in functional properties of SPI are different between SPIs, and effect of α-chymotrypsin modification on SPI is different as well. Nature of soybean is important in terms of quality of soy products and food processing, moreover, enzyme modified SPI could be available to obtain improved textural properties in process of cheese analogs containing SPI.

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REFERENCES

Anon, M. C., D. A. Sorgentini, and J. R. Wagner. 2001. Relationship between different hydration properties of commercial and laboratory soybean isolates. J. Agric. Food Chem. 49 : 4852-4858.

Aoki, H., O. Taneyama, and M. Inami. 1980. Emulsifying properties of soy protein: characteristics of 7S and 11S proteins. J. Food Sci. 45 : 534-538.

Arrese, E. L., D. A. Sorgentini, J. R. Wagner, and M. C. Anon. 1991. Electrophoretic, solubility and functional properties of commercial soy protein isolates. J. Agric. Food Chem. 39 : 1029-1032.

Association of Official Analytical Chemists (AOAC). 1975. Official Methods of Analysis. 12th ed. Association of Official Analytical Chemists. Washington DC. p. 927.

Bollag, D. M. and S. J. Edelstein. 1991. Gel electrophoresis under denaturing conditions: In Protein methods. Wiley-Liss, Inc. pp. 95-127.

Brooks, J. R. and C. V. Morr. 1985. Current aspects of soy protein fractionation and nomenclature. J. Amer. Oil Chem. Soc. 62 : 1347-1354.

Campbell, N. F., F. F. Shih, J. S. Hamada, and W. E. Marshall. 1996. Effect of limited proteolysis on the enzymatic phosphorylation of soy protein. J. Agric. Food Chem. 44 : 759-762.

Chobert, J. M., C. Bertrand-Harb, and M. G. Nicolas. 1988. Solubility and emulsifying properties of caseins whey protein modified enzymatically by trypsin. J. Agric. Food Chem. 36 : 883-892.

Garcia Chacon, E. J., L. D. Satterlee, and M. A. Hanna. 1990. Heat induced gels from partially hydrolyzed soy protein isolate. J. Food Biochem. 14 : 15-29.

German, B., S. Damodaran, and J. E. Kinsella. 1982. Thermal dissociation and association behavior of soy proteins. J. Agric. Food Chem. 30 : 807-811.

Khatib, K. A., T. J. Herald, F. M. Aramouni, F. MacRitchie, and W. T. Schapaugh. 2002. Characterization and functional properties of soy β-conglycinin and glycinin of selected genotypes. J. Food Sci. 67 : 2923-2929.

Kim(Lee), S. Y., P. S. W. Park, and K. C. Rhee. 1990. Functional properties of proteolytic enzyme modified soy protein isolate. J. Agric. Food Chem. 38 : 651-656.

Kim(Lee), S. Y., P. S. W. Park, and K. C. Rhee. 1992. Textural properties of cheese analogs containing proteolytic enzyme-modified soy protein isolates. J. Amer. Oil Chem. Soc. 69 : 755-759.

Kim, Y. H. and S. D. Kim. 1994. Processing characteristics of soybean genotypes lacking lipoxygenase. Korean J. Crop Sci. 39 : 171-174.

Kinsella, J. E. 1979. Functional properties of soy proteins. J. Amer. Oil Chem. Soc. 56 : 242-258.

Kinsella, J. E., S. Damodaran, and B. German. 1985. Physicochemical and functional properties of oilseed proteins with emphasis on soy protein. New Food Proteins 5 : 107-178.

Kitamura, K. 1993. Breeding trials for improving the food processing quality of soybean. Trends Food Sci. Techol. 4 : 64-67.

Kobayashi, A., Y. Tsuda, N. Hirata, K. Kubota, and K. Kitamura. 1995. Aroma constituents of soybean [*Glycine max* (L.) Merri] milk lacking lipoxygenase isozymes. J. Agric. Food Chem. 43 : 2449-2452.

Kramer, A. and W. H. Kwee. 1977. Functional and nutritional properties of tomato protein concentrate. J. Food Sci. 42 : 207-211.

Kumar, R., V. Choudhary, S. Mishra, I. K. Varma, and B. Mattiason. 2002. Adhesives and plastics based on soy protein products. Ind. Crops & Prod. 16 : 155-172.

Lee, H. I., K. C. Kim, and E. H. Park. 2005. Sprout properties and lipoxygenase activity of lipoxygenase-less soybean genotypes. Korean J. Crop Sci. 50(S) : 112-117.

Mueller, I. N. 1992. Isolated soy proteins for surimi based products. Protein Technol Int. A subsidiary of the Ralston Purina Company. Seoul, April, p. 21.

Nakai, S. J. 1983. Structure-function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. J. Agric. Food Chem. 31 : 676-683.

Narvel, J. M., W. R. Fehr, and L. C. Weldon. 2000. Analysis of soybean seed lipoxygenases. Crop Sci. 40 : 838-840.

Nishiba, Y. and I. Suda. 1998. Degradation of vitamin E, vitamin C and Lutein in soybean homogenate: A comparison of normal soybean and lipoxygenase lacking (triple-null) soybean. J. Agric. Food Chem. 46 : 3708-3712.

Ortiz, S. E. M. and J. R. Wagner. 2002. Hydrolysates of native and modified soy protein isolates: structural characteristics, solubility and foaming properties. Food Res. Int. 35 : 511-518.

Petruccelli, S. and M. C. Anon. 1995. Thermal aggregation of soy protein isolates. J. Agric. Food Chem. 43 : 3035-3041.

Qi, M., N. S. Hettiarachchy, and U. Kalapathy. 1997. Solubility and emulsifying properties of protein isolates modified by pancreatin. J. Food Sci. 62 : 1110-1115.

Richardson, T. 1977. Functional changes in proteins following action of enzymes. In: Feeney RE, Whitaker JR, editors. Food proteins: Improvement through chemical and enzymatic modification. American chemical society, Washington DC. p. 185.

Saio, K. and T. Watanabe. 1973. Food use of soybean 7S and 11Sprotein; Extraction and functional properties of their fractions. J. Food Sci. 38 : 1139-1144.

Son, B. Y., Y. H. Lee, S. H. Kim, H. S. Lee, and S. H. Lee. 2002. Effects of planting date and accelerated aging on seed germination-related traits of lipoxygenase-lacking soybean. Korean J. Crop Sci. 47 : 196-200.

Sorgentini, D. A., J. R. Wagner, and M. C. Anon. 1995. Effects of thermal treatment of soy protein isolate on the characteristics and structure-function relationship of soluble and insoluble fractions. J. Agric. Food Chem. 43 : 2471-2479.

Wagner, J. R., D. A. Sorgentini, and M. C. Anon. 2000. Relation between solubility and surface hydrophobicity as an indicator of modifications during preparation processes of commercial and laboratory-prepared soy protein isolates. J. Agric. Food Chem. 48 : 3159-3165.

Were, L., N. S. Hettiarachchy, and U. Kalapathy. 1997. Modified soy proteins with improved foaming and water hydration properties. J. Food Sci. 62 : 821-823, 850.

Wu, W. U., N. S. Hettiarachchy, and M. Qi. 1998. hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. J. Amer. Oil Chem. Soc. 75 : 845-850.

Yamashita, M., S. Arai, J. Matsuyama, M. Gonda, H. Kato, and M. Fujimaki. 1970. Enzymatic modification of proteins in foodstuffs. Part III. Phenomenal survey on α-chymotrypsin plastein synthesis from peptic hydrolysates of soy protein. Agric. Biol. Chem. 34 : 1484-1491.