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Effects of Phosphorylation and Acetylation on Functional Properties and Structure of Soy Protein

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Abstract

Phosphorylation of soy protein by sodium trimetaphosphate and acetylation of soy protein by acetic anhydride were performed. Then, the functional properties of modified soy proteins were compared with that of unmodified soy protein. Isolated soy protein prepared from defatted soybean flake had protein content of 92.7% as moisture-free basis. The phosphorylated soy protein showed higher solubility, foaming properties, and water holding capacity than unmodified soy protein. Acetylation of soy protein increased emulsification activity and foaming properties greatly, whereas decreased the solubility at pH 8.0. Isoelectric pHs of phosphorylated and acetylated soy protein were shifted to acidic regions(pH 3.0 and pH 4.0) from pH 5.0, which was the isoelectric pH of unmodified soy protein. Soy protein seems to be aggregated during phosphorylation and acetylation procedure, judging form Sepharose CL-4B gel filtration profiles. The modified soy proteins showed increased mobilities to anode direction in disc-gel electrophoresis.
Key words: soy protein, phosphorylation, acetylation, functional properties and structure of protein

Introduction

Soy protein possesses various functional characteristics which can be applied to food processing. These are the intrinsic physicochemical characteristics such as dissolving, foaming, water-binding, and emulsifying property[1]. In recent year, soy protein has been widely used as an essential component in beverages, bakery products, simulated meats, whipped topping etc. for economical and nutritional purpose[2]. However, the possible thermal denaturation of soybean meal during defattening and desolventizing procedure can cause the quality reduction of soy protein. Therefore, it seems to be valuable to develop some procedures improving functional properties of soy protein for the successful and prospective utilization of soy protein as food ingredients.

Chemical modifications using phosphorylation and acetylation have been powerful methods for the quality improvement of soy protein. Sung *et al*[3] have reported that phosphorylation increases solubility, water holding capacity, emulsifying capacity, and foaming property of soy protein. Phosphorus oxychloride treatment also enhanced functional properties of soy protein[4-6]. Narayana *et al*[7] have shown that acetylation enhances water absorption capacity and nitrogen solubility of winged bean flour. Franzen and Kinsella[8] have reported the functionality improvement of soy protein by acetylation. However, reports on the structural changes during chemical modification are scarce.

The main purpose of this research is to trace the structural changes of soy protein during phosphorylation and acetylation.

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Materials and Methods

Preparation of isolated soy protein

Isolated soy protein(ISP) was prepared from defatted soybean meal following the method of Thanh and Shibasaki as described below[9]. Defatted soybean meal was extracted with 10 parts of 0.03M Tris-HCl buffer(pH 8.0) for 1hr and centri-

fuged at 10,000 x g for 20mins. The whole buffer extract was adjusted to pH 4.5 and centrifuged at 10,000 x g for 20 min. The protein precipitate was rinsed by distilled water twice, with recentrifugation. The protein precipitate was suspended in distilled water and adjusted to pH 7.0. The soluble portion was exhaustively dialyzed against distilled water at 4℃ and freeze-dried.

Modification of soy protein by phosphorylation[3]

Sodium trimetaphosphate(STMP) was added to 4% ISP solution as 1% concentration. The reaction mixture was continuously stirred at 35℃ for 3hrs with controlling pH to 11.5. Then, the reaction mixture was precipitated by adjusting pH to 4.5 and centrifuged at 10,000 x g for 20 min. The protein precipitate was washed with distilled water. After neutralization, the phosphorylated soy protein was obtained by dialysis and freeze-drying.

Modification of soy protein by acetylation[10]

Acetic anhydride was added to 2.5% ISP solution by six fold molar excess. During the reaction, the pH was maintained to 7.5. After reaction, 50% ethanol was added to the reaction mixture. The resulting precipitate was centrifuged at 10,000 x g for 20 min. After washing with small portions of 50% ethanol, the acetylated soy protein was obtained by freeze-drying.

Determination of extent of chemical modification

The total amount of serine residue in protein and free pyrophosphate were determined concomitantly against the reaction mixture to determine the extent of phosphorylation. Serine analysis was performed on a LKB 4151 Alpha Plus amino acid analyzer after the sampled soy protein was completely hydrolyzed by 6N HCl at 110℃ for 24hrs. Pyrophosphate was determined as reported previously[11,12]. The ninhydrin assay[8] was used to quantify the extent of acetylation.

Determination of functional properties

Proximate compositions were determined routinely. Solubility[13], emulsification activity[14], foaming properties[15], and water holding capacity[16] were determined for the functionality assessments of unmodified and modified soy protein.

Column chromatographic separation of unmodified and modified soy protein

The glass column previously packed with swollen Sepharose CL-4B resins was eluted with 0.05M phosphate buffer(pH 7.0) overnight. After this step, 6 m*l* of unmodified and modified 5% soy protein were loaded and eluted with the same buffer mentioned above. The fraction time of each tube was 20 min and the fraction volume was 6.5m*l*., The protein content of each tube was automatically recorded by a UV monitor(LKB Co.) at 278*n*m. The molecular weight of each protein fraction was determined by comparing with a calibration curve preapred from eluting marker proteins of Pharmacia Fine Chemicals Co.. The marker proteins used were thyroglobulin (M.W. 669,000), ferritin(M.W. 440,000), catalase(M.W. 230,000), aldolase(M.W. 159,000), and bovine serum albumin(M.W. 67,000).

Disc-gel electrophoresis of unmodified and modified soy protein

Disc-gel electrophoresis of unmodified and modified soy protein was performed according to the method of Davis[17]. Electrophoresis was done at 300V for 1.5 hr and the sample volume for loading was 100μl. After electrophoresis, protein bands were stained with 0.25% Coomassie brilliant blue R-250. Relative mobility was defined as follows.

$$\text{Relative mobility} = \frac{\text{distance traveled by protein, cm}}{\text{distance traveled by tracking dye, cm}}$$

Results and Discussion

Proximate compositions of defatted soybean flake used for ISP preparation are shown in Table 1. Protein, fat, ash, and moisture content were 50.7, 1.6, 6.5, and 6.4%, respectively.

ISP prepared from defatted soybean flake was compared with commercially available ISPs(Ralston Purina Co.) with respect to protein and moisture content(Table 2). As represented in Table 2, the protein content of ISP from our laboratory was 92.7% as moisture-free basis.

The effects of STMP phosphorylation on the functional properties of soy protein are described in Table 3. At pH 8.0, STMP-modified soy protein was more soluble than unmodified soy protein. Foam capacity and foam stability of phosphorylated soy protein were also enhanced than those of unmodified soy protein. Water holding capacity was increased by 3 folds, whereas, emulsification activity was decreased slightly at this experimental condition. It is reported that STMP reacts with hydorxyl group of serine irreversibly under alkaline condition[3]. The net effects of STMP reaction are the introduction of orthophosphate to hydroxyl group of serine and the liberation of pyrophosphate as quantitative basis. In this experiment, 43.5% of hydroxyl group of total serine present in soy protein was phosphorylated by STMP. The enhancement of solubility, foaming properties, and water holding capacity are attributed to the increase in total negative charges resulting from covalent binding of orthophosphate to hydroxyl group of serine[3]. Hirotsuka *et al.* have also reported that functional properties including solubility are increased considerably by the phosphorylation of soy protein[4-6].

Table 1. Proximate compositions of defatted soybean flake (%)

Moisture	6.4
Ash	6.5
Protein	50.7
Fat	1.6

Table 4 shows the changes in functional properties of soy protein by acetylation. Emulsification activity was increased by 2 folds and foaming properties were also increased greatly, whereas solubility at pH 8.0 was decreased slightly. It is reported that acetic anhydride reacts with ε-amino group of lysine during acetylation procedure[10]. The net result is the introduction of acetyl group to ε-amino group of lysine with concomitant decrease in total positive charges. Assays of unmodified and modified soy protein for extents of acetylation showed that nearly all of ε-amino groups present in soy protein were acetylated.

Fig. 1 depicts solubility profiles of unmodified and modified soy protein at varying pH conditions. As depicted in Fig. 1, phosphorylated soy protein was solubilized more easily than unmodified soy

Table 2. Protein contents of the ISPs

Component (%)	Sample					
	ISP prepared	PP90[a]	PP500E[a]	PP590[a]	PP710[a]	PP760[a]
Protein[b]	87.6	86.9[d]	85.3[d]	87.4[d]	86.9[d]	86.9[d]
Protein[c]	92.7	91.5[d]	90.5[d]	92.0[d]	92.0[d]	91.5[d]
Moisture	5.5	5.0[d]	5.5[d]	5.1[d]	5.5[d]	5.0[d]

a: ISP from Ralston Purina Company
b: Protein content determined by N×6.25, wet basis
c: Protein content as moisture-free basis
d: Adoped from Technial Information of Ralston Purina Co.

Table 3. Effects of phosphorylation on the functional properties of soy protein

ISP	Solubility (Abs at pH 8.0)	Emulsification activity (Abs 600 nm)	Foaming properties		Water holding capacity(gH_2O/g product)	Extent of phosphorylation (%)
			Foam capacity(ml)	Foam stability(ml)		
Unmodified ISP	0.384	0.478	25.0	20.0	5.0	
Phosphorylated ISP	0.425	0.412	45.0	27.5	15.3	43.5

Table 4. Effects of acetylation on the functional properties of soy protein

ISP	Solubility (Abs at pH 8.0)	Emulsification activity (Abs 600 nm)	Foaming properties		Water holding capacity(gH_2O/g product)	Extent of acetylation (%)
			Foam capacity(ml)	Foam stability(ml)		
Unmodified ISP	0.325	0.508	17.0	16.9	5.1	
Acetylated ISP	0.302	0.924	39.0	72.0	9.7	100

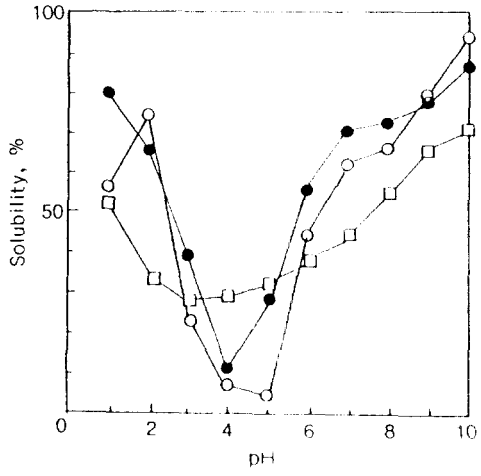

Fig. 1. Solubility profiles of unmodified and modified soy protein. ○——○, unmodified ISP; ●——● phosphorylated ISP; □——□ acetylated ISP; Solubility was determined at 750nm

protein at nearly all pH ranges. The solubility of acetylated soy protein was only higher than that of unmodified soy protein at the pH ranges of 3-6, whereas was lower than that of unmodified soy protein at the other pH ranges. Also, the isoelectric pHs(pIs) of phosphorylated and acetylated soy proteins were shifted to acidic regions(pH 3.0 and pH 4.0) from pH 5.0 which was the pI of unmodified soy protein. In case of acetylated soy prtein, the low solubility below pI seems to be caused by the exhaustive acetylation of ε-amino group of lysine present in soy protein[8,10].

Sepharose CL-4B gel filtration profiles of phosphorylated and acetylated soy protein were compared with that of unmodified soy protein. The molecular weight of each protein fraction was also determined by comparing with the calibration curve(Fig. 2, Fig. 3). As shown in Fig. 3, the unmodified soy protein had 2 major elution peaks. The peak at fraction 45 and 60 corresponded to 11S globulin and 7S globulin, respectively. The molecular weight of 11S globulin fraction was 350,000, whereas that of 7S globulin fraction was 95,000. In case of phosphorylation, a new peak appeared at fraction 23(MW. 2,750,000). The major peak was located at fraction 42. These facts strongly suggest that soy protein is aggregated by phosphorylation procedure. Acetylation also caused the aggregation of soy protein. The molecular weight of major peak which appeared at fraction 21 was 3,500,000. The true mechanism of soy protein aggregation by phosphorylation and

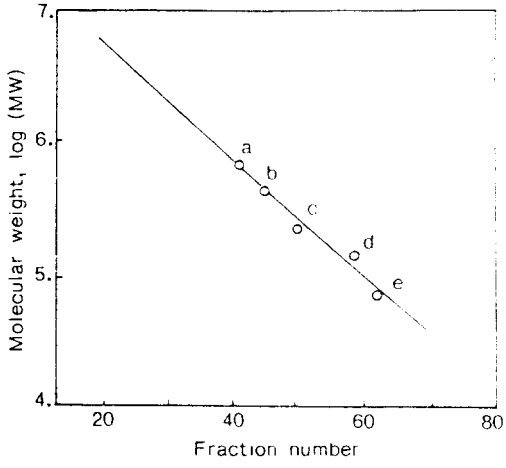

Fig. 2. The calibration curve for gel filtration chromatography. Marker proteins; a, thyroglobulin(M.W. 669,000); b, ferritin(M.W. 440,000); c, catalase(M.W. 230,000); d, aldolase(M.W. 159,000); e, bovine serum albumin(M.W. 67,000).

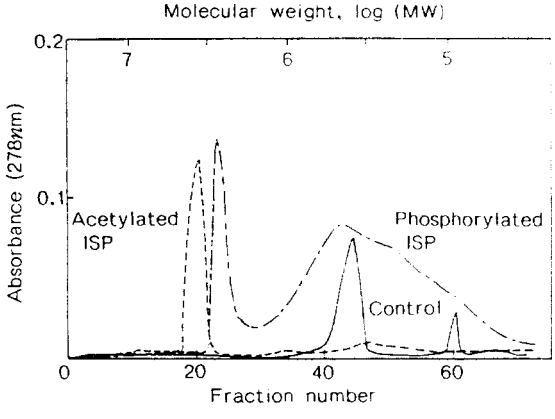

Fig. 3. Sepharose CL-4B gel filtration profiles for unmodifed and modified soy protein.

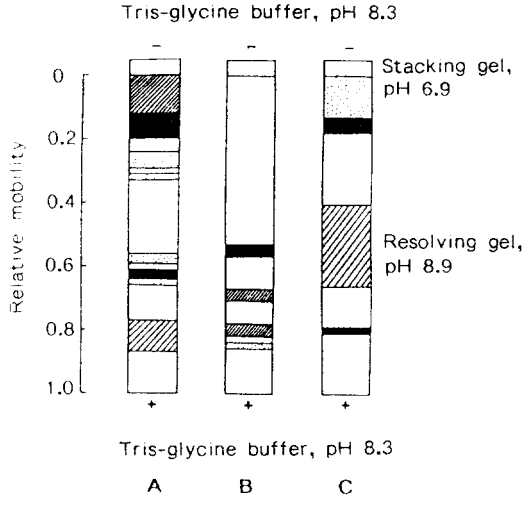

Fig. 4. The disc-gel electrophoresis patterns for unmodified and modified soy protein. A, unmodified ISP; B, phosphorylated ISP; C, acetylated ISP.

acetylation is unclear at this stage. It may be the result of simple association of soy protein fractions or interchain covalent bond formation[18].

Fig. 4 depicts the changes of disc-gel electrophoresis patterns of unmodified and modified soy protein. As shown in Fig. 4, 2 major bands which had relative mobilities of 0.12-0.20 and 0.61-0.64 were found in case of unmodified soy protein. Some other bands differing in band intensities were also observed. All of the protein bands were distributed under relative mobility of 0.32 and over relative mobility of 0.56. The phosphorylation of soy protein by STMP greatly changed electrophoretic pattern, that is, relative mobilities of protein bands were increased conspicuously. The protein bands appearing under relative mobility of 0.32 in case of unmodified soy protein were not found in the electropherogram of phosphorylated soy protein. The great change of electrophoretic pattern seems to be attributed to increased net negative charges produced by covalently bound phosphate anions[3]. Hirotsuka *et al.* have also reported the increases in relative mobilities of protein bands in $POCl_3$-modified soy protein[6]. The acetylation of soy protein also increased relative mobilities of protein bands. One of two major bands advanced considerably compared with unmodified soy protein. As the ε-amino group of lysine consists in the fully protonated form($-NH_3^+$) at pH 8.3 in case of unmodified soy protein due to high pK_a value(10.5), acetylated soy

protein holds relatively higher net negative charges than unmodified soy protein. This fact seems to result in high electrophoretic mobilities of protein bands in acetylated soy protein.

References

1. Hermansson, A.M. : Methods of studying functional characteristics of vegetable proteins. *J. Am. Oil Chemists' Soc.*, **56**, 272(1979)
2. Kinsella, J.E. : Functional properties of soy proteins. *J. Am. Oil Chemists' Soc.*, **56**, 242(1979)
3. Sung, H.C., Chen, H.J., Liu, T.Y. and Su, J.C. : Improvement of the functionalities of soy protein isolate through chemical phosphorylation. *J. Food Sci.*, **48**, 716(1983)
4. Hirotsuka, M., Taniguchi, H., Narita, H. and Kito, M. : Phosphorylation of soybean protein. *Nippon Shokuhin Kogyo Gakkaishi*, **32**(9), 622(1985)
5. Hirotsuka, M., Taniguchi, H., Narita, H. and Kito, M. : Properties of spray dried powder of phosphorylated soy protein isolate. *Nippon Shokuhin Kogyo Gakkaishi*, **32**(9), 626(1985)
6. Hirotsuka, M. and Kito, M. : Phosphorylated soybean protein. *U.S. Patent*, 4, 501, 694(1985)
7. Narayana, K. and Rao, M.S.N. : Effect of acetylation and succinylation on the functional properties of winged bean (Psophocarpus tetragonolobus) flour. *J. Food Sci.*, **49**, 547(1984)
8. Frazen, K.L. and Kinsella, J.E. : Functional properties of succinylated and aceylated soy protein. *J. Agric. Food Chem.*, **24**(4), 788(1976)
9. Thanh, V.H. and ShibasakI, K. : Major proteins of soybean seeds. A straight-forward fractionation and their characterization. *J. Agric. Food Chem.*, **24**(6), 1117(1976)
10. Barman, B.G., Hansen, J.R. and Mossey, A.R. : Modification of the physical properties of soy protein isolate by acetylation. *J. Agric. Food Chem.*, **25**(3), 638(1977)
11. Flaschka, H.A., Barnard, A.J. and Sturrock, D.F. : In *Quantitative Analytical Chemistry*, Barnes and Noble Inc., New York, Vol. 2, p.142(1968)
12. West, T.S. and Sykes, A.S. : In *Analytical Application of Diaminoethantetraacetic Acid*, Brit. Drug House, Ltd., p.74(1960)
13. Sung, H.C., Chen, H.J., Liu, T.Y. and Su, J.C. : Improvement of the functionality of soy protein by introduction of new thiol groups through a papaincatalyzed acylation. *J. Food Sci.*, **48**, 708(1983)
14. Pearce, K.N. and Kinsella, J.E. : Emulsifying properties of proteins; evaluation of a turbidimetric technique. *J. Agric. Food Chem.*, **26**(3), 716(1978)
15. Puski, G. : Modification of functional properties of soy proteins by proteolytic enzyme treatment. *Cereal Chem.*, **52**, 655(1975)
16. Jones, L.J. and Tung, M.A. : Functional properties of modified oilseed-protein concentrates and isolates. *Can. Inst. Food Sci. Technol.*, **16**(1), 57(1983)
17. Davis, B.J. : Disc electrophoresis-II. Method and application to human serum proteins. *N.Y. Acad. Annuals*, **121**, 404(1964)
18. Hermansson, A.M. : Physico-chemical aspects of soy proteins structure formation. *J. Texture Studies*, **9**, 33(1978)

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인산화와 초산화가 대두단백질의 기능특성과 구조에 미치는 영향

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대두단백질을 인산화 및 초산화처리에 의하여 변형시키고, 변형대두단백질의 기능특성을 검토하였다. 인산화 대두단백질은 비변형 대두단백질보다 높은, 용해도, 거품특성, 수분보유력을 나타냈다. 한편, 초산화에 의해서도 유화력과 거품특성이 현저하게 증진되었다. 대두단백질은 인산화와 초산화에 의하여 등전점이 산성쪽으로 변하였으며 단백질의 집괴화현상도 관찰되었다. 또한, 화학변형된 대두단백질을 디스크 전기영동에 의해 분석시 단백질밴드의 상대이동도가 현저하게 증대되었다.