Hanguk Nonghwahak Hoechi
(J. Korean Agric. Chem. Soc.)
34(4), 353~359(1991)

Microstructural analyses of soyprotein fibers

J. C. Kim, S. J. Cho*, P. H. Byun**, S. K. Yoon**,
K. C. Rhee*** and S. M. Byun

Department of Life Science, Korea Advanced Institute of Science and Technology(KAIST), Tajeon 305-701, Korea
*Department of Home Economics Education, Seo Won University, Cheong Ju 360-140, Korea
**Department of Food Science and Nutrition, Dong Duk University, Seoul 100-273, Korea
***Food Protein Research Institute, Texas A & M University, U.S.A.

Abstract : As a tool for the texture analyses of the soyprotein fibers, the scanning electron microscopical microstructure were studied. With the results of TPA(Texture Profile Analysis), microstructural analyses of the soyprotein fibers showed that the disulfide and hydrogen bonds are one of the most important factors determining the shape and maintenance of fiber struture. The microstructures of the hydrated soyprotein dispersion and dope, as starting materials of the soyprotein fiber were presented(Received November 26, 1991, accepted December 21, 1991).

Disulfide bonds are the most stable covalent cross-link in the structure of protein. Although the direct evidences lack, the intermolecular disulfide bonds of protein have been implicated as being responsible for the texturized soyprotein products.[1-6] Moreover, by extruding soyprotein dope through fine spinnerets, the polypeptide chains are brought close together favoring the hydrogen and ionic bonds.[7]

Reports on the texture profile and microstructure analyses of a number of foods have appreared in the literature. Stanley et al[8] have studied the textural properties and ultrastructures of rehydrated spun soyfibers and Lee and Rha[9] have studies soyprotein aggregates. However, effects of chemical modification on the microsturcture and texture of soyprotein fibers have not been reported.

In this paper we, therefore, have studies those of soyprotein fiber and result indicated that an examination of product microsturcture has been a satifactory method for evaluation and prediction of textural properties. As the starting materials of the soyprotein fiber, the microstructures of the hydrated soyprotein dispersion and dope are also presented.

Materials and Methods

Materials

The soyprotein isolate (SPI), commercial name as Promine-D from Central Soya Co.(U.S.A.) was used to prepare the hydrated soyprotein dispersion (HSD), dope (D), and texturized soyprotein fiber (TP). All the other chemicals used were obtained from commerical sources and were of analytical grades.

Preparation of hydrated soyprotein dispersion

Promine-D was mixed with distilled water by Waring blender at room temperature and hydrated at 4℃ for 24 hr in refrigerator and equilibrated at 20℃ of a water bath fo 2 hr. For the study of the

Key words : Microstructual analyses, soyprotein fiber
Corresponding author : S. M. Byun

effects of protein concentration and chemical modification on the properties of HSD, HSDs were prepared as described in Table 1.

Preparation of dope

The sodium hydroxide solution was added to HSD on a water bath at 20℃ and mixed thoroughly with Waring blender. HSD of slightly yellow color changed gradually to dough of drarkly brown color and the final NaOH concentration was 0.6% (w/v), considered as the standard of the dope series. To examine the effects of protein concetration and denaturation by the NaOH solution, the dopes were also prepared as in Table 1.

Preparation of texturized soyprotein fiber (TP)

Using a protein spinning apparatus designed in our laboratory,[7] the dope was deaerated at 40℃ for 1 hr and chilled to room temperature and spinned through the spinnerete and newly formed fibers were fixed at a coagulating bath whose composition was 20% (w/v) NaCl and 1 N acetic acid. Then the excess of coagulating solutions were washed out and soyprotein fibers were stored in freezer at 40℃. The freezed soyprotein fibers were thawed in a refrigerator at 4℃ for 14 hr before use.

Scanning electron microscopic analysis (SEM)

Scanning electron microscopic obervation was carried outi in Texas A & M Univ.(U.S.A.) with JEOL-JSM-V3. Pretreatments of samples for SEM were such that the samples were freezed with lequid nitrogen, lyophilized and coated with gold palladium.[10] Conditions for observation were the voltage of 25 KV and the ratio of magnification 30~3,000 times, respectively.

Texture analyses

Texture analyses of TP were carried out at the Cereal Engineering Lab. of Korea Institue of Food Science and Technology, with G. F.-texturometer. For the pretreatment of the samples of modified soyprotein fibers, lyophilization and crushing with a electric mill to size of 20~40 mesh were carried out.[11] Samples were rehydrated with the distilled water (water : lyphilized sample=3 : 1 on dry wei-

Table 1. Notation of sample

	Notation	Description	Measure	Remark
HSD	HSD-15	15% Protein concentration	M	Standard
	HSD-15-A*	HSD-15 in 0.1 N HCl	M	
	HSD-15-G	1% Glutaraldehyde treated HSD-15	M	
	HSD-15-Ca	HSD-15 in 20 mM $CaCl_2$	M	
	HSD-15-H	HSD-15 heated at 85℃ 30 min	M	
D	D-12	12% Protein concentration	M	
	D-15	15% Protein concentration	M	Standard
	D-18	18% Protein concentration	M	
	D-15-3	HSD-15 in 0.3% NaOH	M	
TP	TP-15-9	TP-15 in 0.9% NaOH	M, T	
	TP-15	15% Protein concentration	M, T	Standard
	TP-15-Na-10	TP-15 in 10% NaCl	M, T	Standard
	TP-15-C	TP-15 in 0.5 M phosphate buffer	M, T	Control
	TP-15-M	TP-15 0.01 M Mercaptoethanol	M, T	
	TP-15-U	TP-15 in 0.6 M Urea	M, T	
	TP-15-S	TP-15 in 1% Sodium sulfite	M, T	
	TP-15-O	TP-15 in 2% H_2O_2	M, T	
	TP-15-H	TP-15 Heated at 85℃ 30 min	M, T	
	TP-15-Az	TP-15 in 0.1% Sodium azide	M, T	

* Chemical treatment, M: Scanning electron microscope, T: G.F.-texturometer.

ght basis). Textural parameters such as hardness, cohesiveness, elasticity, gumminess and chewiness were calculated by the method of Lee et al.[12]

Determination of protein

The contents of the soluble soyprotein, when the texturized soyprotein fibers were chemically modified, were determined by the procedure of Lowhan and Cater.[13]

Results and Discussion

Effect of chemical modification on hydrated soyprotein dispersion

Fig. 1 shows the scanning electron microphotographs (SEP) of the chemically modified HSD. HSD-15 shows fibrous, uniform, and cross-linked structure to some extent, meanwhile glutaraldehyde treated HSD-15-G shows the sheeted structure, resulted in cross-linking of the protein aggregates. The mechanism of covalent bonding by glutaraldehyde is considered as "shift base" formation. The reaction can be carried out in a neutral or acidic solution at room temperature.[14] The calcium chloride treated HSD, HSD-15-Ca, however, shows that the structure is the shape of aggregates of globular protein to some extent and is not fibrous structure. Heat treated HSD (HSD-15-H) indicates more fine three dimensional network than that of HSD-15. This result is well coincident the results of Lee and Rha.[9] Finally, SEP of HSD-15-A shows the structure of aggregates of globular protein. This phenomena are considered that folded soyprotein aggregates by shear stress be changed to native and unfolded states which are caused by decreasing the pH to isolelectic point of soyprotein (pH 4.6). Rha[15] has reported that a globular protein is deformed under shear. When stress is removed, normal or

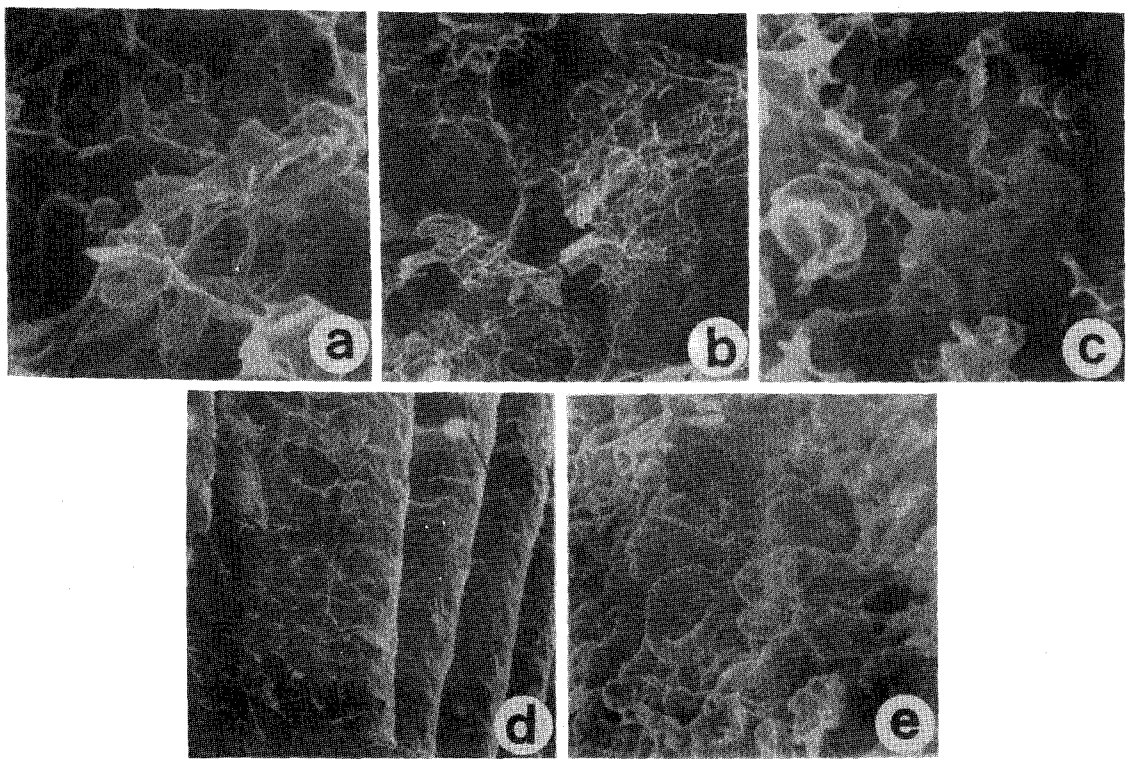

Fig. 1. Scanning electron microscopic pictures of hydrated soyprotein dispersions treated variously.
(a) : HSD-15 (300x), (b) : HSD-15-G (300x), (c) : HSD-15-Ca (300x), (d) : HSD-15-H (120x), (e) : HSD-15-A (300x)

recoversable stress returns, at least partly, to ist original state. With the studies of dynamic rheological propreins of HSD, HSD-15-G shows nearly elastic behaviors because of extremely low value of loss tangent, tan ϕ, which indicates highly developed three dimensional network structure (data not shown).[16] This statement is well agreed with the result of SEM analysis, namely as shown in Fig. 1(c) and Fig. 1(d).

Effects of protein and alkaline conentrations on dope

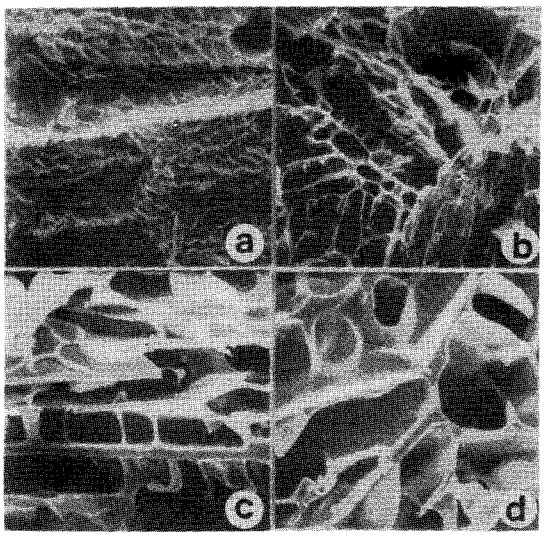

Fig. 2. Scanning electron microscopic pictures of alkali treated soyprotein solution (dope).
(a) : D-15, (b) : D-12, (c) : D-15-3, (d) : D-18, Magnification ratio : 300x

Fig. 2 illustrates electron microscopic pictures of various dopes which were prepared according to the concentrations of protein as well as treatment with the NaOH solution. The structure of D-15 standard dope may have the fine three dimensional network and fiber-like structure(Fig. 2(a)).

From the rsults of dynamic properties for the dope,[16] it appeared that the three dimensional network were developed, in sequence of D-12, D-15-3, D-15 and D-18 (data not shown). But the differences of the dope microstructure were not significant.

Texture profile anlaysis (TPA)

As shown in Table 2, TP-15-9 shows remarkable increase in the hardness and decrease in the cohesiveness as compared with TP-15. Also TP-15-Na-10 coagulated in 10% NaCl-1 N acetic acid bath shows remarkable decrease in the hardness and increase in the adhesiveness and springness. This indicates that TP-15-Na-10 is more flexible than TP-15.

In general, it is considered that the cohesiveness is related to intramolecular as well as intermolecular reaction.[9] In the point of view, a decrease of the cohesiveness of TP-15-9 seems to be caused by the decrease of intermolecular reations, resulted from the dissociation of protein molecules, in the excess of NaOH concentration. Otherwise increase of the cohesiveness for TP-15-Na-10 may be caused by the insufficient stabilization of the steostructure

Table 2. Texture profile analysis of soyprotein fiber by G.F-texturometer

Notation of sample	Textural parameter					
	Hardness (kg/wt/volt)	Cohesiveness	Elasticity (mm)	Adhesiveness (cm^2)	, Gumniness (×100)	Chewiness (×10)
TP-15	1.52 ± 0.06	0.86± 0.01	17.3± 1.0	−*	1.31 ± 0.07	22.7 ± 2.5
TP-15-9	1.67 ± 0.02	0.81± 0.01	17.3± 0.5	−	1.35 ± 0.03	23.4 ± 1.2
TP-15-Na-10	1.26 ± 0.04	0.90± 0.04	19.0± 0.1	−	1.13 ± 0.08	21.5 ± 1.6
TP-15-C	0.189± 0.002	0.520± 0.004	10.4± 0.5	0.500± 0.051	0.095± 0.002	0.99± 0.03
TP-15-S	0.188± 0.002	0.527± 0.017	11.0± 1.0	0.527± 0.052	0.099± 0.002	1.09± 0.12
TP-15-Az	0.187± 0.003	0.518± 0.006	9.8± 0.2	0.440± 0.005	0.097± 0.001	0.95± 0.03
TP-15-H	0.265± 0.001	0.517± 0.005	12.0± 0.1	0.200± 0.005	0.137± 0.002	1.64± 0.03

Expermental conditions : Chart speed, 750 mm/min; Clearance, 4.5 mm; Voltage, 0.5 V or 2.0 V; Plunger, 5 cm lutice; Plat from, flat; Sample wt., 10g; Sample height, 18 mm; *No appearance.

of soyprotein fiber in the acid-salt coagulating bath.

For chemically modified TP samples, the TPA of TP-15-O, TP-15-U, and TP-15-M were not tested because of insufficient texture. For the TP-15-S and TP-15-Az, there are not remarkable differences in the textural parameters, but only in the case of TP-15-Az, the springeness increased. It is considered that sodium azide acts as a coagulant on the soyprotein fibers. For TP-15-H, heated at 85℃ for 30 min, should remarkable increase in the harndness and in the spningeness to some extent. This results are similar to that from the heat treatment of meats, reported by Lee et al.[12] The structure of TP-15-H seems to be developed by the heat-treatment, caused by the denaturation.

Solubility of soyprotein fiber

Table 3 indicates the solubilities of SPI, alkaline treated SPI, and soyprotein fibers in 0.5 M phosphate buffer (pH 7.6) and the chemical reagents. Kelly and Pressey[1] and Byun et al[7] considered that the exchange of intra- or intermolecular disulfide bonds were carried out in the soyprotein dispersions with unfolding of protein, when NaOH added. Because of the facts that the solubility of SPI treated with NaOH, are remarkably smaller than those

Table 3. Solubility of various preparations of soyprotein

Sample	Treatment	Amount soluble (%)
Acid precipitated protein	P.B.[b]	71.5
	P.B.+M.E.[c]	88.7
	P.B.+6 M urea	96.1
SPI treated at pH 12 for 30 min and reprecipitated[a]	P.B.	34.7
	P.B.+M.E.	43.6
	P.B.+6 M urea	77.3
	P.B.+M.E.+6 M urea	84.4
Soyprotein fiber TP-15-M	P.B.	0.6
	P.B.+M.E.	4.2
TP-15-U	P.B.+6 M urea	13.6
TP-15-O	P.B.+H_2O_2 (2% v/v)	6.5
TP-15-S	P.B.+Na_2SO_3 (1% w/v)	0.2
TP-15-Az	P.B.+Sodium azide (0.1% w/v)	0
TP-15-H	P.B.+Heated 30 min at 85℃	0

[a] : Prepared by the method of Byun et al.[7]
[b] : 0.5 M Phosphate buffer, pH 7.6
[c] : 0.01 M Mercaptoethanol

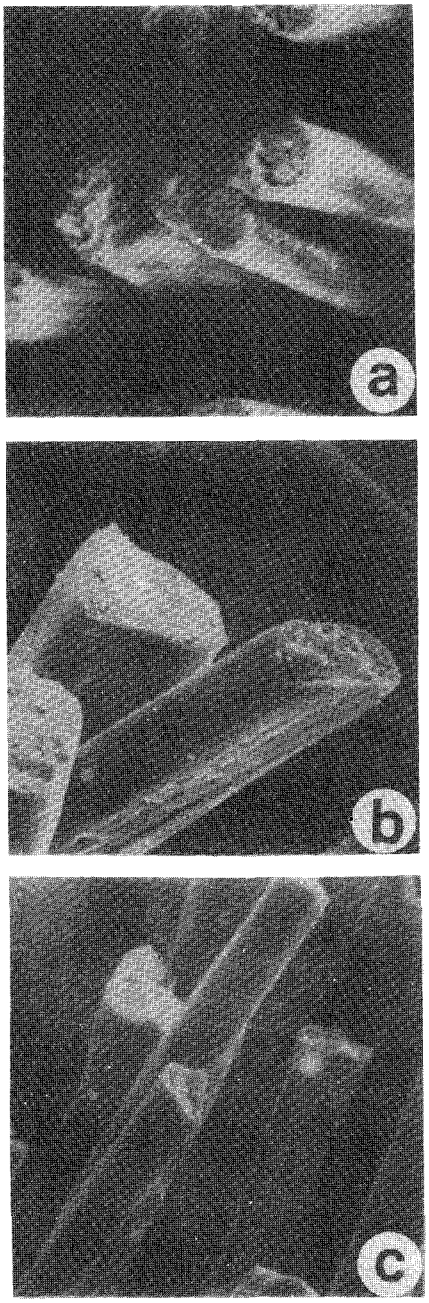

Fig. 3. The shapes of soyprotein fibers produced with SPI.
(a) : TP-15, (b) : TP-15-9, (c) : TP-15-Na-10, Magnification ratio : 300x

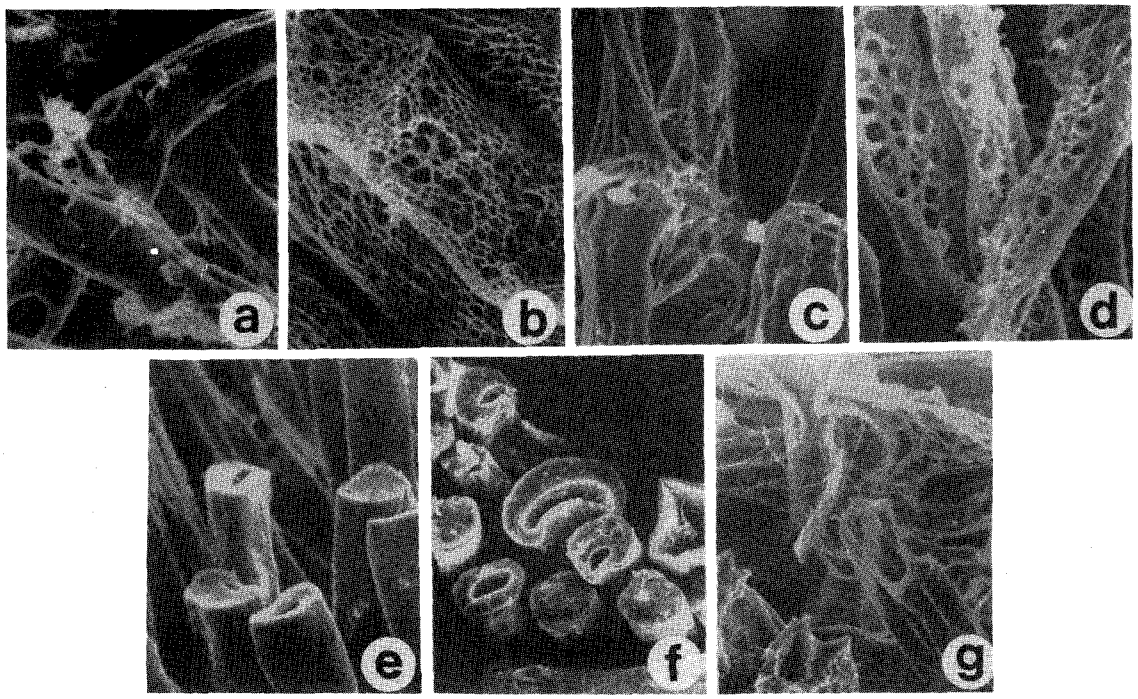

Fig. 4. Effects of chemical modification on the shape of soyprotein fibers.
(a) : TP-15-C, (b) : TP-15-M, (c) : TP-15-O, (d) : TP-15-U, (e) : TP-15-Az, (f) : TP-15-S, (g) : TP-15-H, Magnification ratio : 300x

of SPI itself and the mecaptoethanol treated SPI, it is considered that the disulfide bond interchanges are one of the most important factors, with the hydrogen bond formation, determining the properties of SPI and dope. The solubilities of the chemically modified soyprotein fiber were given as follows; The solubilities of TP-15-U, TP-15-O, and TP-15-M were 13.6, 6.5. and 4.2% (w/w). responsible force for hydrogen bond, non specific cleavage, and disulfide bond, respectively. The hydrogen bonds of the secondary intermolecular association as well as intramolecular association[1,7,17] and the disulfide bonds concerning the primary structure force have been implicated as being responsible for the texturized soyprotein.

Microstructure of soyprotein fiber

Microscopic photographs (SEP) of the texturized soyprotein fiber, nonchemically modified are shown in Fig. 3. There are not remarkable changes in SEP of the soyprotein fiber. But SEP of TP-15-9 shows rather the coarse surface structure. This result confirms that TP-15-9 shows remarkable increase in the hardness.

During the solubility test sodium ion seems to be a potential coagulant (refer to Table 3). To eliminate the effect of sodium ion on the microstructures, the distilled water was used instead of phophate buffer. Fig. 4 shows the scanning electron microscopic picture of various types of soyprotein fibers which were treated with various kinds of chemical modification reagents; TP-15-C, control shape; TP-15-M, sponge-like shape; TP-O, melted shape of fiber; TP-15-U, network-like shape; TP-15-Az and TP-15-S, conventional soyprotein fiber like shapes. The effects of chemicals on the structural changes appreared to be breakage of disulfide and hydrogen bonds mainly, and also be caused by sodium ion. Sodium ion changed the patterns of coagulation of soyprotein. These results with those of solubility test of soyprotein as shown in Table 3.

References

1. Kelly, J. J. and Pressey, R. : Cereal Chem., 43 : 195 (1966)
2. Chiang, J. P. C. and Sternborg, M. : Cereal Chem., 51 : 465(1974)
3. Jenkins, S. L. : U. S. Patent, 3, 496, 858(1970)
4. Wolf, W. J. : J. Agr. Food Chem., 18 : 969(1970)
5. Pomeranz, Y., Finney, K. F. and Hosensy, R. C. : Science, 167 : 964(1970)
6. Bloksma, A. H. : Cereal Chem., 52 : 170(1975)
7. Byun, S. M., Kwon, J. H., Kim, C. H. and Lee, Y. H. : Kor. J. Food Sci. Technol., 10 : 143(1978)
8. Stanley, D. W., Cumming, D. B. and deMan, J. M. : Can. Inst. Food Sci. Technol. J., 10 : 143(1978)
9. Lee, C. H. and Rha, C. Y. : J. Food Sci., 43 : 79 (1978)
10. Toranto, M. V., Ceglar, G. F. and Rhee, K. C. : J. Food Sci., 43 : 767(1978)
11. Breene, W. M. and Barker, T. G. : J. Texture Studies, 6 : 459(1975)
12. Lee, Y. H., Lee, K. and Lee, S. R. : Korean J. Food Sci. Technol., 6 : 42(1974)
13. Lowhon, J. J. and Carter, C. M. : J. Foo Sci., 37 : 778(1972)
14. Linn, M. : in "*Enzymology*" edited by Weetall, H. H. pp. 1-48, Marcel Dekker, New York(1975)
15. Rha, C. K. : Food Technol., 33 : 71(1979)
16. Kim, J. C., Cho, S. J., Byun, P. H. and Byun, S. M. : Kor. J. Food Sci. Technol., Accepted for publication(1991)
17. Brown, A. G. and Menkart, J. : in "*Ultrastructure of Protein Fibers*", pp. 5-19, edited by Borasky, R., Academic, New York(1963)

대두 단백섬유의 미세구조 연구

김지천 · 조숙자* · 변평화** · 윤석권** · 이기춘*** · 변시명(한국과학기술원 생명과학과, *서원대학교 가정교육학과, **동덕여자대학교 식품영양과, ***텍사스 A & M 대학교 식품단백연구소)

초록 : 대두단백 섬유의 texture 분석을 위한 도구로서, 그들의 미세구조를 관찰하였다. 미세구조와 texture profile analysis 결과, 대두단백 섬유의 구조를 유지 및 결정하는 인자중 disulfide 결합과 수소결합이 가장 중요한 인자중의 하나임을 확인하였다. 이와함께 대두단백 섬유의 원료물질인 대두단백질 수화물과 dope의 미세구조도 관찰하였다.