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EFFECTS OF PROTEIN CONTENT, FREEZING, DEHYDRATION AND STABILIZATION ON TEXTURE CHARACTERISTICS OF FREEZE TEXTURIZED SOYBEAN PROTEIN

PANTIPA JANTAWAT[a] AND THANAKORN ROJANAKORN[b]

[a] *Food Technology Dept., Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.*

[b] *Food Technology Dept., Faculty of Technology, Khonkaen University, Khonkaen, Thailand.*

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ABSTRACT

Soy protein slurries at initial solid content of 10, 15 and 20% were freeze texturized by plate freezing or solid carbon dioxide then removal of ice crystals by 95% ethyl alcohol or freeze-drying. The texturized protein was further stabilized in autoclave at 105, 110 and 115°C for 5, 7.5 and 10 minutes. At 10% solid, the alignment of protein fibers was in parallel direction. Fiber wholesomeness and parallelism decreased as the solid content increased. The only difference found in samples frozen by the two methods was that the protein fibers from solid carbon dioxide freezing were more adjacent among themselves. A slightly better wholesomeness of the fibers was resulted when freeze-drying was used to remove ice crystal. Retort heating provided the fibers with higher shear strength. Increasing the heating time and temperature increased compactness of the protein structure.

INTRODUCTION

The fabricating process involves imparting cohesiveness qualities to material lacking cohesiveness such as starch or protein powder. The resulting product acquires a fibrous and chewable structure. To achieve this, several processes have been developed. Freeze texturization is one of the processes used to produce fibrous structure from protein powder. One of the oldest process in the current use is production of Kori-tofu, from soybean (Lillford, 1985)). In the production of Kori-tofu, soybean curds were frozen at -10°C, aged at -2°C for 2-3 weeks and thawed. The resulting product has a porous sponge-like structure. Kori-tofu has more strength and cohesiveness than original tofu and can be dried for extending its shelflife at room temperature. Lilford (1985) also explained that orientated freezing between plates could produce fibrous rather than spongy texture and the extent of fibrosity depended on the freezing rate. According to Lawrence, Consolacion and Jelen (1986), the protein fiber network formed during freezing was an imprint or negative of ice erystal structure and therefore factors which affect the rate of ice crystal growth would also affect the fiber structure. Since heat transfer is possibly be a rate limiting factor during the late stages of freezing (Fennema, 1975), the initial solid content of the protein slurry and freezing rate should affect the protein fiber characteristics. This experiment was therefore designed to examine the textural characteristics of freeze texturized soy protein as affected by the protein slurry solid content, freezing method, dehydration (or ice removal) method and thermal stabilization.

MATERIALS AND METHODS

Effect of Solid Content, Freezing and Dehydration

Soy protein slurry was prepared by precipitating proteins in soymilk with 2M hydrochloric acid at pH 4.7. After centrifugation at 5000 rpm for 7.5 minutes, the protein precipitates were separated and resuspended in distilled water. The pH of the protein slurry was adjusted to 7 with 2 M sodium hydroxide solution. The total solid content of the slurry was adjusted to 10, 15 and 20% w/v with distilled water. Samples at each concentration was divided into 2 portions. One portion was frozen by plate freezing. Four hundred grams of the slurry was poured into aluminium dish (15 cm. diameter, 6.3 cm. height) which was insulated underneath with 5.1 cm thick polystyrene foam. The initial temperature of the slurry was adjusted at 25°C. Temperature during freezing was measured at 1 cm depth from the surface, with thermo-couple. Freezing was carried out at -40°C and continued until the slurry temperature reached -37°C.

Another 400 g portion of the protein slurry at each concentration was frozen by solid carbon dioxide (dry ice) at -80°C, in the same size and shape aluminium dish. Four dishes of the protein slurry were placed in polystyrene box underwhich containing 1 kg of dry ice per one protein dish. The temperature during freezing was recorded in the same manner as the samples frozen by the plate method. Freezing was carried on until the temperature of the protein slurry at 1 cm depth reached -37°C.

Ice crystal removal and initial setting of the protein structure was done by freeze-drying or by substitution of ice with 95% ethyl alcohol. In freeze-drying, the frozen samples were dried in a Virtis sublimator (model 101 SRC) at 30°C, 100 micron (vacuum) pressure. The total sublimating time was 30 hours. Substitution of ice crystals in the slurry with 95% ethyl alcohol was performed by dipping the slurry dishes in 4°C alcohol (1 weight unit of the slurry per 3 weight units of the alcohol) for 3 hours. The dishes were then removed and soaking of the protein structure was continued for another 5 hours. The protein were later dried in a cabinet drier at 60°C for 8 hours to get rid of the adhering alcohol.

Protein structure from all treatments were scanned with scanning electron microscope. Small blocks (1 cm^3) were cut from the finish products. Dehydration of the blocks was carried out in the critical point dryer (CPD) by increasing concentration of ethyl alcohol from 30% by volume to absolute (30, 70, 95% by volume and absolute), for a total time of 2 hours. The dried samples were coated with gold, in the ion sputter apparatus, to 5 m thickness. A JEOL, JSM-35 cm electron microscope operating at 20 kV, 200 x magnifying power was used to examine the sample sections.

Shear strength of the protein samples were determined, using a Mainframe Standard T2001 texturometer. The cutting cell of apparatus was fitted with 7.5x3.8x1.8 cm^3 block of the protein sample. The knife was set at 1 cm distance from the sample. The knife speed was 200 mm per second. Cutting forces were recorded in Newton.Statistical analyses of the shear strength values both the analysis of variance and Duncan's New Multiple Range Test were conducted by the method of Steel and Torrie (1980).

Stabilization of the Protein Structure

Samples of protein structure from a treatment combination, from the previous study were packed in 3 layers of polypropylene bags and all the bags were heat sealed. Packages of sample were heated in autoclave (Tomy, SS-320) at 105, 110 and 115°C for 5, 7.5 and 10 minutes. After cooling to room temperature the protein structure were scanned with electron

microscope and shear strengths were measured and analysed in the same manner as previously described.

RESULT

The structure of native soy protein extracted by sodium hydroxide solution and precipitated with hydrochloric acid was shown in Figure 1. Longitudinal sections of freeze texturized soy proteins as viewed by scanning electron microscopy were shown in figure 2(a-l). The shear strengths of the freeze texturized samples and their statistical analyses were presented in Table 1,2 and 3 respectively.

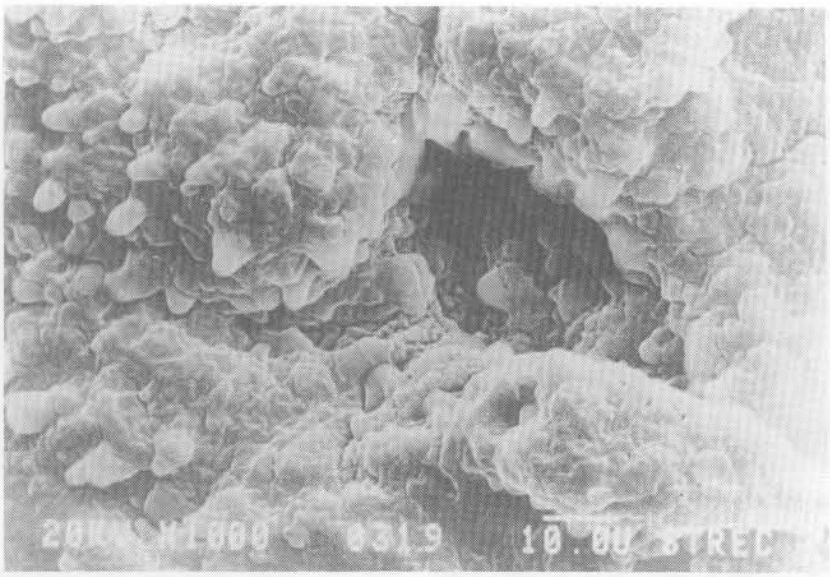
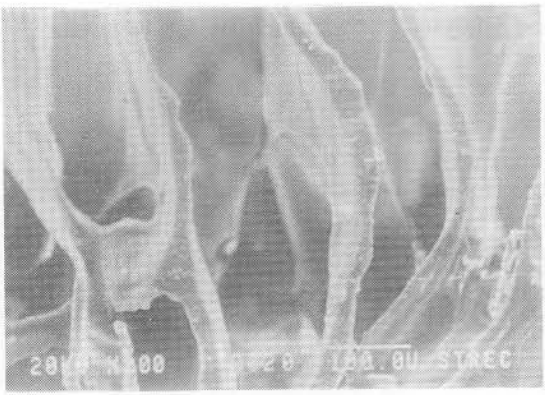
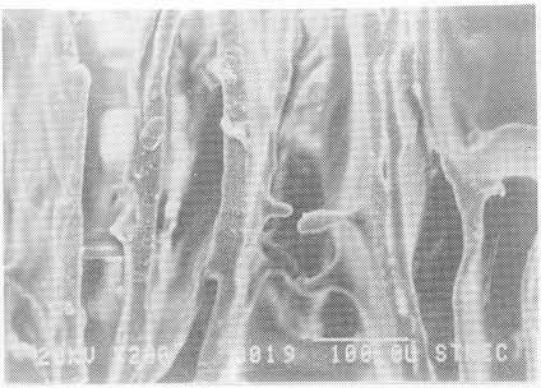


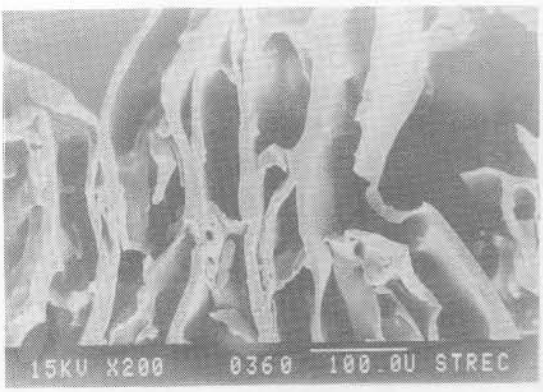
Fig. 1. Micrograph of native soy protein.



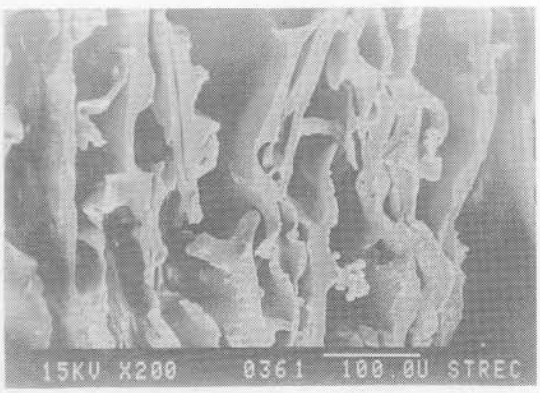
2a. 10% solid-plate-95% EtOH



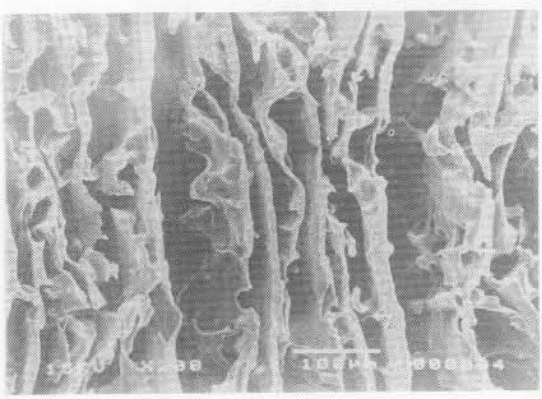
2b. 10% solid-CO_2-95% EtOH



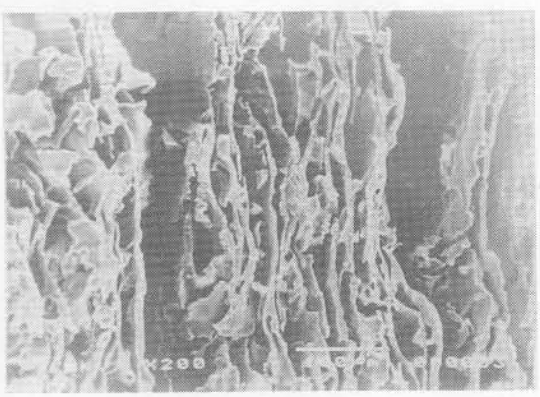
2c. 15% solid-plate-95% EtOH



2d. 15% solid-CO_2-95% EtOH

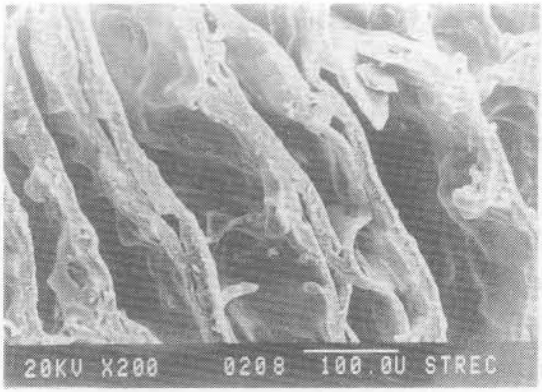


2e. 20% solid-plate-95% EtOH

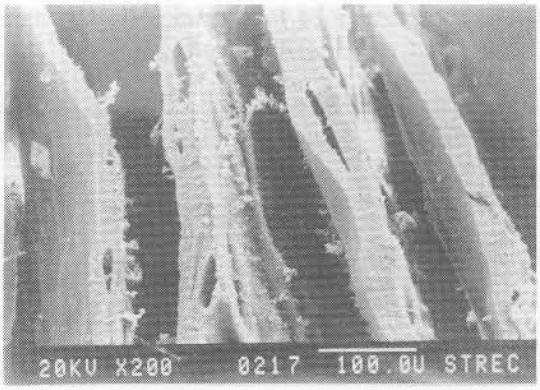


2f. 20% solid-CO_2-95% EtOH

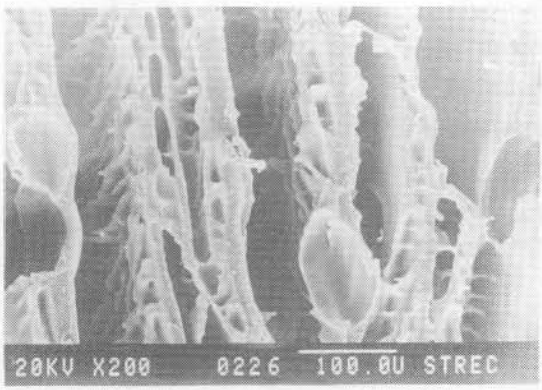
Fig. 2(a-f) Micrographs of freeze texturized soy proteins at various total solid contents, freezing and drying methods.



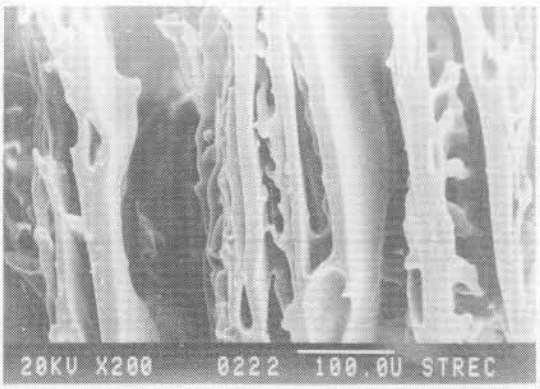
2g. 10% solid-plate-freeze drying



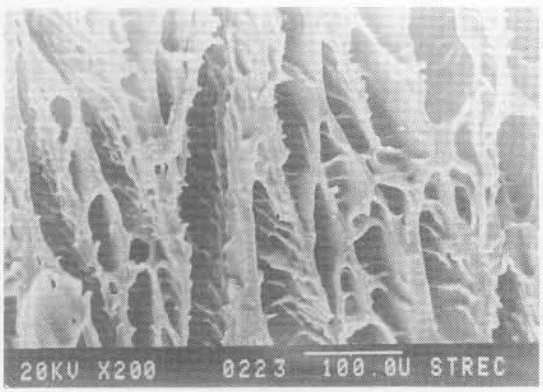
2h. 10% solid-CO_2-freeze drying



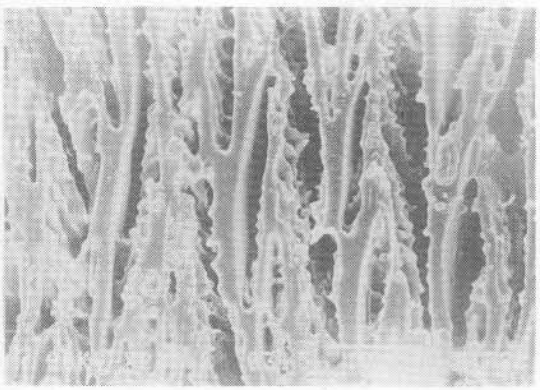
2i. 15% solid-plate-freeze drying



2j. 15% solid-CO_2-freeze drying



2k. 20% solid-plate-freeze drying



2l. 20% solid-CO_2-freeze drying

Fig. 2(i-l) Micrographs of freeze texturized soy proteins at various total solid contents, freezing and drying methods.

Table 1. Effect of total solid content, freezing and dehydration on shear strength of freeze texturized soy protein.

Total Solid (%)	Freezing Method	Dehydration Method	Shear Strength±SD (N)
10	plate	95% EtOH	18.1 ± 1.3
		freeze-drying	18.9 ± 1.7
	solid CO_2	95% EtOH	17.5 ± 1.7
		freeze-drying	18.7 ± 2.1
15	plate	95% EtOH	14.9 ± 0.7
		freeze-drying	15.4 ± 1.4
	solid CO_2	95% EtOH	15.2 ± 1.4
		freeze-drying	15.2 ± 1.2
20	plate	95% EtOH	12.8 ± 1.0
		freeze-drying	12.9 ± 0.9
	solid CO_2	95% EtOH	11.8 ± 1.8
		freeze-drying	12.6 ± 1.1

Table 2. Analysis of variance for shear strength of freeze texturized soy protein.

SOV	df	MS
Total solid (A)	2	66.292*
Freezing method (B)	1	0.603
Dehydration method (C)	1	2.042
AB	2	0.221
AC	1	0.291
BC	2	0.051
ABC	2	0.222
Error	12	3.998

* Significant ($P<0.05$)

Table 3 Effect of total solid on shear strength of freeze texturized soy protein.

Total Solid (%)	Shear Strength ± SD (N)
10	18.2[a]
15	15.2[b]
20	12.5[c]

a,b,c Means with different superscript letters are significantly different ($p<0.05$).

Effect of Solid Content

The native soy protein structure is of globular type. After freeze texturization this structure changed into fibrous network. At 10% solid content the alignment of the protein fibers is in parallel direction. The fiber network diviated from parallelism when the protein content increased to 15 and 20%. Also observed when the protein concentration increased was the decrease of fiber wholesomeness. The shear strengths of the protein pieces decreased as their solid contents increased ($P<0.05$).

Effect of Freezing Method

The electron microscopies of the freeze texturized structures from the two different freezing methods showed a rather similar shape and size of the fibers. The parallelism and wholesomeness of the fibers were more clearly observed in the samples containing 10% solid contents and freezing by both methods. Deviation from parallelism with less wholesomeness were shown in the 15 and 20% solid containing slurries either frozen by plate or by solid carbon dioxide. The only obvious difference found in the samples from the two freezing methods was that protein fibers from solid carbon dioxide freezing were more adjacent among themselves than those of the plate freezing. This result can obviously be observed especially in the 20% solid containing sample. Nonsignificant difference in shear strength was observed among protein pieces from the two freezing methods ($p>0.05$).

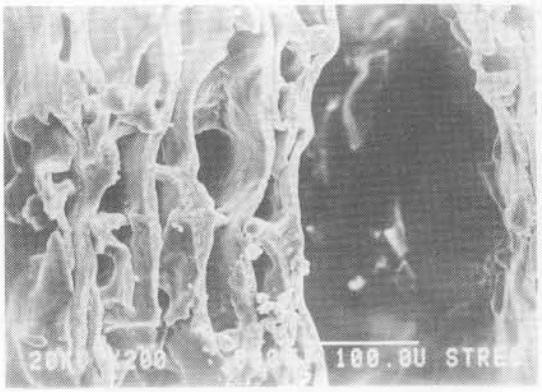
Effect of Dehydration Method

A slightly better wholesomeness of the fibers was resulted when the freeze-drying technique was used. Non significant difference in the strength of samples from the two drying methods was found ($P>0.05$).

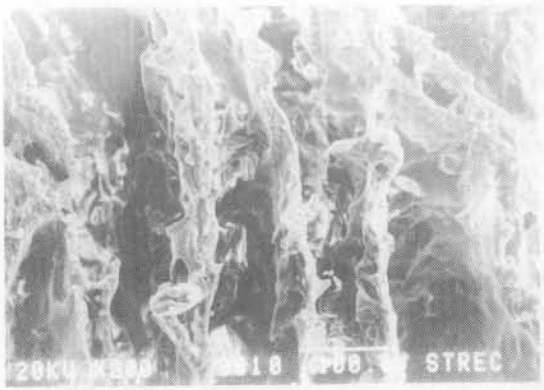
Stabilization of the Protein Structure

The electron microscopies of the longitudinal section of the samples were shown in Figures 3 (a-i). The shear strength and their analyses of variance were in Table 4,5 and 6 respectively.

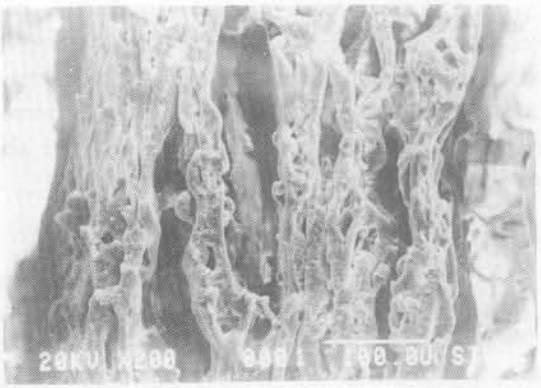
3a. 105°C - 5 min.



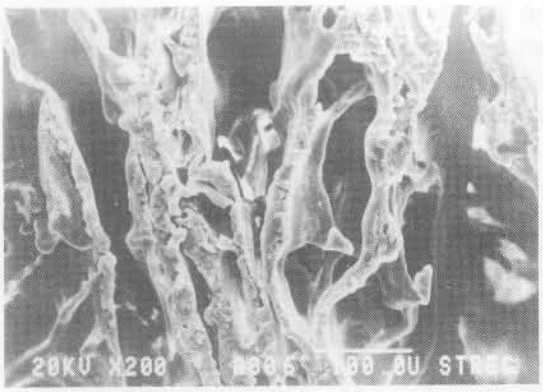
3b. 110°C - 5 min.



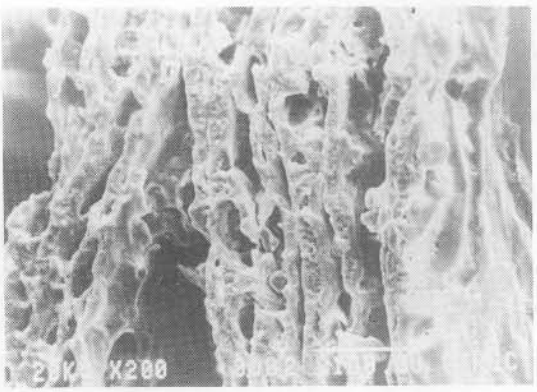
3c. 105°C - 7.5 min



3d. 110°C - 7.5 min.

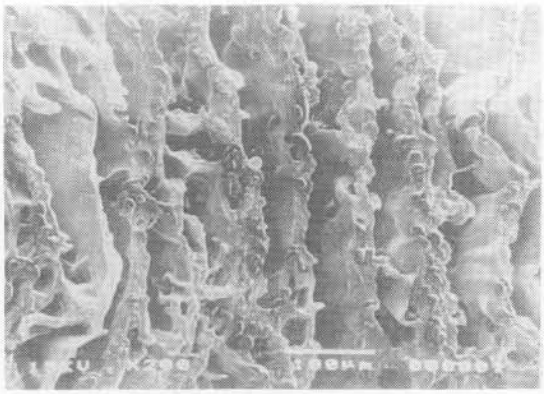


3e. 105°C - 10 min.

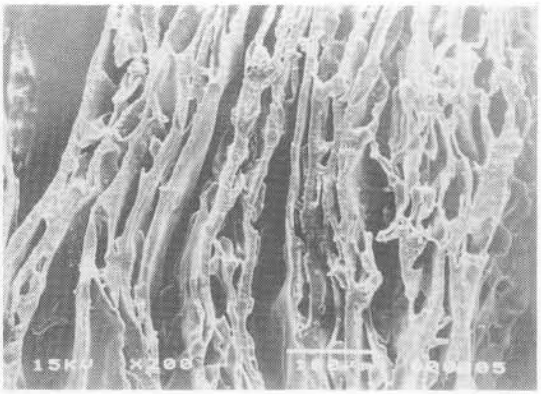


3f. 110°C - 10 min.

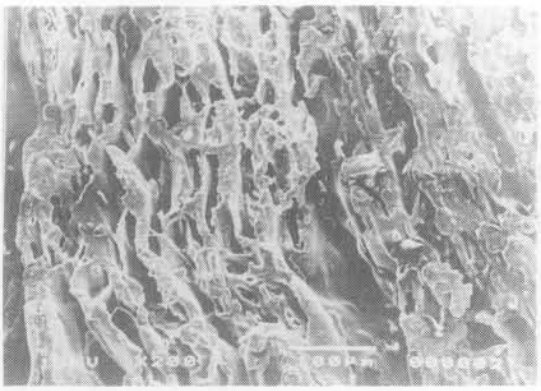
Fig. 3 (a-f) Micrographs of freeze texturized soy protein (10% solid content-plate freezing-95% EtOH) after heat stabilization at various times and temperatures.



3g. 115°C - 5 min.



3h. 115°C - 7.5 min.



3i. 115°C - 10 min.

Fig 3 (g-i) Micrographs of freeze texturized soy protein (10% solid content-plate freezing - 95% EtOH) after heat stabilization at various times and temperatures.

Table 4. Effect of autoclaving temperature and time on shear strength of freeze texturized soy protein.

Temperature (°C)	Time (min)	Shear Strength ± SD (N)
105	5	24.7 ± 1.1
	7.5	27.2 ± 1.6
	10	31.4 ± 1.4
110	5	30.3 ± 1.1
	7.5	32.8 ± 1.6
	10	36.4 ± 1.6
115	5	35.0 ± 1.8
	7.5	38.0 ± 1.8
	10	39.0 ± 1.6

Table 5. Analysis of variance for shear strength of freeze texturized soy protein.

SOV	df	MS
Temperature (A)	2	138.043*
Time (B)	2	47.076*
AB	4	1.662
Error	9	4.688

Significant ($P<0.05$)

Table 6. Effect of heating time and temperature on shear strength of freeze texturized soy protein.

Conditions	Shear Strength ± SD (N)
Temperature (°C)	
105	27.7^{c} ± 3.1
110	33.2^{b} ± 3.1
115	37.3^{a} ± 2.4
Time (min)	
5	30.0^{b} ± 4.4
7.5	32.7^{ab} ± 4.7
10	35.6^{a} ± 3.5

a,b,c means with different superscript letters within each variable are significantly different ($P<0.05$).

Retort heating at 105-115°C for 5-10 minutes resulted in protein fibers of more compact structure. Increase of temperature from 105 to 115°C and time from 5 to 10 minutes provided a denser structure to the protein fibers. The shear strengths of the protein fibers increased as heating time and temperature increased ($P<0.05$).

DISCUSSION

The native soy protein structures are globular in nature. The freeze texturization resulted in changing of this structure into fibrous network. Kelley and Pressey (1966) stated that the main components of soy protein should unfold and partial polymerization needed to be initiated during the protein preparation step so that a successful freeze-induced fiber formation could occur. Alkali extraction of proteins from the soybean tissues resulted in denaturation of the protein globular structure. The polypeptide chains then unfolded into random coils. The sulfhydryl-disulfide interchanges among polypeptide chains resulted in formation of some disulfide bonds. Acid precipitation of the protein later brought polypeptide chains closer to each other and facilitated the formation of chemical bonds such as hydrogen, ionic and hydrophobic, between and within polypeptides.

Effect of Solid Content

Less parallelism and decrease of fiber wholesomeness were observed as the protein solid content of the protein slurry increased from 10 to 15-20%. According to Lugay and Kim (1978), the increasing solid content in the protein slurry interfered with the linearity growth of ice crystals and therefore less water molecules were drawn out of the slurry to bind into ice crystal surface. Proteinaceous materials were less concentrate in the interstitial spaces between the ice crystals and branches of each crystal. Less linear compact of the proteinaceous material in the interstitial spaces was then resulted, upon melting of the ice. The parallelism and strength of protein fibers formed therefore decreased. This result was confirmed by shear strength values of the protein structures (Table 3). The shear strength values decreased from 18.2 N. to 12.5 N. as the solid content of the slurry increased from 10 to 15-20%. This result indicated that strength of freeze texturized protein fibers were adversely affected by increasing quantity of the solid content.

Effect of Freezing Method

Freezing times of soy protein slurries at the solid contents of 10, 15 and 20%, by plate freezing method, were 67, 66 and 60 minutes, while those found when using solid carbon dioxide were 59, 56 and 57minutes, respectively. Slightly shorter freezing times resulted when using solid carbon dioxide at -80°C comparing to the plate freezing at -40°C. In general, slower cooling such as in the plate freezing may result in a high rate of ice crystal growth relative to the rate of nucleation. This will cause a considerable number of larger crystals to form comparing with the faster carbon dioxide freezing. Consequently, in the proteinaceous slurry, relatively larger voids were produced between the protein fibers. Lawrence, Consolacion and Jelen (1986) stated that slow cooling which caused large ice crystals to form should also produce relatively thick fibers in the proteinaceous slurry. However, the thickness of the protein fibers from the two freezing methods used in this experiment was not clearly different. This result could be attributed to the relatively small difference in the freezing rate of the two freezing methods. Besides, the freezing rate provided by the plate freezing although slower than that of the carbon dioxide freezing, could still be considered as relatively fast (Hallowell, 1980). The freezing rate of the plate freezing was 3.9 - 4.2 cm. per hour while that measured for the solid

carbon dioxide was 4.2 - 4.5 cm. per hour. This small margin of difference might also be responsible for the almost resemble pattern of the extent, size and wholesomeness of the fibrosites formed. Result from the shear measurement (Table1-3)also showed a nonsignificant difference between the shear forces of samples from the two freezing methods.

Effect of Dehydration Method

Setting of the fibrous network structure was carried out either by freeze-drying or by soaking the structure in 95% ethyl alcohol. It was evident that a slightly better wholesomeness of the fibers was resulted when the freeze-drying technique was used. In freeze-drying, ice crystals were removed from the proteinaceous structure by sublimation. Since melting of ice was absent there was no contact between water and the proteinaceous structure. Resolubilization of the protein was therefore minimized. Lillford (1985) stated that stabilization mechanism of freeze-drying involved protein crosslinking in the absence of water and denaturation.

On the other hand, the 95% ethyl alcohol is a organic solvent that can depress freezing point of water. Soaking of the frozen texturized proteins in the alcohol resulted in melting of ice crystals. The resulting water then diffused out of the protein matrix while alcohol diffused in. The presence of water in the protein matrix may cause resolubilization of protein fibers before the structure being set by ethyl alcohol. After the considerable quantity of the alcohol diffused in, the mole fraction of water in the matrix was reduced. The ethyl alcohol also decreased the hydrophilic potential of the proteins by causing a conformational change from the hydrate random coil to β-conformation and α-helix which were not dissolved in aqueous solution (Lugay and Kim,1978). This phenomenon resulted in a more stable structure of the protein. After 8 hours of reaction the sample was dries at 60°C for 8 hours. The removal of the water and the drying process may cause shrinkage of the protein structure and thereby provided it with a less straight and wholesome fibrosity.

Result from shear strength measurement showed a nonsignificant difference ($P>0.05$) in the strength of the samples from the two drying methods. The result indicated that melting of ice during the alcoholic treatment has little effect on the strength of the proteinaceous fibers.

Stabilization of the Proteinaceous Structure.

Once the proteinaceous structure of the freeze texturized soy protein was set the product was further stabilized by retorting. The 10% total solid content sample which was plate frozen and set in 95% alcohol was retorted at 105, 110 and 115°C for 5, 7.5 and 10 minutes. Moist heating in the retort at temperature 105-115°C for 5-10 minutes resulted in a more compact structure of the proteinaceous fibers. When the temperature was increased from 105 to 115°C, a denser structure was observed. Steam under pressure might cause the protein fibers to move closer to each other until cross linkings among the fibers could conveniently be occurred. Increase of time from 5 to 10 minutes also help provide the protein structure with a denser fibrosity. Increase of temperature in retort heating was resulted from increase of steam pressure. The higher the pressure of the heating medium the closer was the fiber network. The closer vicinity among fibers resulted in higher potential in the formation of hydrogen, ionic and hydrophobic bonds among the polypeptide reactive groups. Also happenning as a result of the higher heating temperature and time was increasing of the protein coagulation. The protein structure thereby acquired a more compact and strenght. This result was confirmed by the increase in shear strength of the protein pieces at higher heating time and temperature (Table 4-6).

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บทคัดย่อ

ได้นำสารละลายโปรตีนถั่วเหลืองซึ่งมีของแข็งทั้งหมดร้อยละ 10,15 และ 20% (น้ำหนักต่อปริมาณ) มาแปลงเนื้อสัมผัสโดยแช่แข็งด้วยวิธีเพลทสัมผัส (plate freezing) หรือใช้น้ำแข็งแห้ง (solid carbon dioxide) หลังแช่แข็งกำจัดผลึกน้ำแข็งโดยการทำแห้งเยือกแข็ง (freeze drying) หรือใช้สารละลายเอธิลอัลกอฮอล์ 95% (95% ethyl alcohol) จากนั้นเสถียรโครงสร้างด้วยความร้อนชื้นที่ 105, 110 และ 115°C เป็นเวลา 5, 7.5 และ 10 นาที พบว่า โครงสร้างโปรตีนจากสารละลายที่มีของแข็ง 10% มีเส้นใยที่เรียงขนานกันและมีความเป็นอันหนึ่งอันเดียวกัน เมื่อของแข็งในสารละลายเพิ่มขึ้น ลักษณะขนานกันน้อยลง เส้นใยมีลักษณะขาด ไม่ต่อเนื่องเป็นบางแห่ง เส้นใยของโครงสร้างโปรตีนจากการแช่แข็งด้วยน้ำแข็งแห้งจัดเรียงตัวชิดกันมากกว่าตัวอย่างที่แช่แข็งแบบเพลทเล็กน้อย ตัวอย่างที่กำจัดผลึกน้ำแข็งด้วยวิธีทำแห้งแบบเยือกแข็งมีลักษณะเส้นใยเป็นอันหนึ่งอันเดียวกันหรือขาดน้อยกว่าการใช้อัลออฮอล์เล็กน้อย การให้ความร้อนชื้นแก่โครงสร้างโปรตีนทำให้เส้นใยมีความสามารถในการต้านแรงเฉือนเพิ่มมากขึ้น เมื่ออุณหภูมิและเวลาในการให้ความร้อนเพิ่มขึ้นเส้นใยผนึกกันกระชับมากยิ่งขึ้น