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Effect of Polysaccharides on Rheological Properties of Soy Protein Isolate Emulsion Gels*

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The effects of x-carrageenan (KC), sodium alginate (SA), xanthan gum (XG) and XG/locust bean gum (XG+LG) mixture on rheological properties and microstructure of soy protein isolate (SPI) emulsion gels were investigated. Rheological properties were determined with texture profile analysis, breaking test and creep-recovery curve analysis. KC increased the hardness and instantaneous elastic modulus, and showed the most effectiveness on the gels. While, XG showed the lowest effect on hardness. XG+LG addition gave the maximum in breaking force and deformation at heating from 20 to 60 min. Scanning electron microscope was used to evaluate the changes in the microstructure of SPI-emulsion gels with polysaccharides. Preparations were carried out by critical point drying and cryofracturing methods. The fat droplets in the gels were not thoroughly fixed by glutaraldehyde and it was shown that they were dislodged from the fractured surfaces, leaving vacuoles, These observations also showed a wide variation among emulsion gels with various polysaccharides on a microstructural basis, which may be correlated to rheological properties.

INTRODUCTION

Polysaccharides are used in the food industry as texture modifying agents in many types of products. They have a wide range of functional properties and are used for several different purposes, including as stabilizers, thickeners and structure forming (gelling) agents. For example, oil-in-water emulsion systems, polysaccharieds are widely used as stabilizers and emulsifier to prevent creaming and coalescence. Such as, xanthan gum or sodium alginate is used in the preparation of mayonnaise and salad cream. Lippi and Taranto (1980) reported that emulsifying activity and stability of soy protein isolate (SPI) could be improved by addition of sodium alginate. The effect of xanthan gum upon the rheological properties and stability of oil-in-water emulsion was evaluated by Hennock et ***al.*** (1980). In the heat-induced gelation of emulsion meat products, such as frankfurters and sausage, xanthan gum was more effective than carrageenan, locust bean gum and low methoxy pectin in preventing water loss from a low-fat meat emulsion (Wallingford and Labuza, 1983), and both xanthan gum and carrageenan were shown to stabilize the texture of frankfurters held in a vinegar pickle (Fox ***et al.,*** 1983). These reports suggested that select gums were compatible with meat batters. Foegeding and Ramsey (1986) studied the effect of seven gums on

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stability and textural properties of low-fat meat batters. Changes of rheological and water-holding ability of gelled meat batters containing gums after various heating temperature were also investigated by them (1987), and the results indicated that gums were specific in affecting texture and water-holding ability.

The scanning electron microscope (SEM) is a relatively new research tool which allows scientists to view specimens at very high resolution with three dimensional detail. Theno and Schmidt (1978) were the first among scientists who made an effort to establish whether or not a meat emulsion is a true one in SEM micrographs of meat emulsion. These researchers compared microstructures of three commercial frankfurters and concluded that they are true meat emulsions. The microstructure of liver sausage was evaluated by Ray et al. (1981). Basgall et *al. (1983)* also reported an alternative to critical point drying method for preparing meat emulsion for SEM. However, researches with SEM to correlate the rheological properties and microstructure had been very limited thus far with respect to emulsion products. The purpose of this study was to evaluate the effects of several polysaccharides and heating time on rheological properties and microstructure of the SPI-emulsion gels.

MATERIALS AND METHODS

Materials

Soy protein isolate (SPI) was purchased from Fuji Oil Co., Ltd. and salad oil from Nissin Saled Oilmills Ltd. Sodium alginate (SA), x-carrageenan (KC), xanthan gum (XG), and locust bean gum (LG) were guaranteed grade reagents obtained from Wako Pure Chemical Industries Ltd.

Preparation of emulsion gels

Forty grams of SPI was mixed with 200 ml of 0.25% (w/v) polysaccharides solution, and then 60 ml of salad oil were added slowly to the protein/polysaccharide mixtures under continuous mixing in a mixer (MK-6000, Matsushita Electric Co., Ltd.). The emulsions were then transferred to the casing (Teepak. Wienie-Pak Co., diameter =21.4 mm) and stood at 4°C for 1 day. The emulsions were heated at 80°C for various time. After heating, the samples were removed from water bath and allowed to stand in tap water for cooling to approximately 15°C. The gels were left overnight at 4°C. Prior to measuring the rheological properties, the gels were equilibrated at 20°C for **1 hr.**

Determination of rheological properties

(1) Texture profile analysis

Textural characteristics of the emulsion gels were measured with a rheometer (Rheoner RE-3305, Yamaden Co., Ltd.) by means of General Food objective texture profile analysis. The speed of the moving stage was set at 1 mm/sec in both upward and downward directions. The compression ratios of the cylindrical specimen (20 mm in height, 23 mm in diameter) were 15% deformation. A full scale load range of 199.9 g was used and two consecutive bites were taken. The textural characteristics such as hardness, cohesiveness and adhesiveness were evaluated according to Bourne's definition (1978).

Each estimation was performed at least five times in duplicated samples and data obtained were averaged.

(2) *Determination of* ***breaking parameters***

The breaking parameters of emulsion gels were measured with a rheometer at a constant table speed of 1 mm/sec using a spherical plunger with diameter of 5 mm for the sample of 20 mm height. From a force-deformation curve, two parameters were obtained, breaking force was defined as the force at the breaking point (g) and breaking deformation at the breaking point (mm), according to Toda et ***al.*** (1971).

(3) Creep-recovery curve ***analysis***

The creep-recovery curve analysis was performed with a rheometer as recommended by Mohseinin and Mittal (1977). Moving stage speed was 1 mm/sec on a constant force. Such a compression involved linear 15% of the 3 mm height of the cylindrical gels, and tests were carried out at 20°C. The viscoelastic parameters such as instantaneous elastic modulus, retarded elastic modulus, viscosity and time, and permanent viscosity were obtained.

Preparations of gel for scanning electron microscopy (SEM)

Samples were prepared by cutting to a piece with 2 x 2 mm surface from the center of each SPI-emulsion gels with a razor blade, followed by two different methods for SEM : (1) critical point drying and (2) cryofracturing.

(1) ***Critical Point drying method*** : The procedure of Jones and Mandigo (1982) was used with slight modifications. Emulsion gel specimens from each treatment were fixed in 2% glutaraldehyde using a 0.1 M phosphate buffer (pH 7.2) for 12 hr at 4°C, followed by three washings with 0.1 M phosphate buffer (pH 7.2). All specimens were then post-fixed for 6 hr in 1.0% osmium tetroxide solution (phosphate buffered), and followed by three more washing with 0.1 M phosphate buffer. Specimens were then dehydrated in graded series of ethanol of 60, 70, 80, 90%, and three changes of 100% for 10 min each and treated with isoamyl acetate for 1 hr. Samples were applied to critical point drying with liquid CO, as the transitional medium (HCP-1, Hitachi Co.). Each piece was then broken such that the broken side would used for SEM observation.

(2) *Cryofracturing* ***method*** : The procedure of Edward ***et al.*** (1983) was also used with slight modifications. Emulsion gel specimens were immersed in liquid nitrogen, and cryofractured by sharply striking with a precooled metal blade. Samples were immediately removed from the liquid nitrogen and fixed in 2% glutaraldehyde fixative buffered with 0.1 M phosphate buffer (pH 7.2) for 12 hr at 4°C. In order to preserve the protein matrix, all the samples were fixed and were post-fixed with 1% osmium tetroxide solution for 1 hr to stabilize the lipid. After fixation, the samples were washed for three times with 0.1 M phosphate buffer. Samples were dehydrated in a graded ethanol series of 25, 50, 75, 90, 100, 100, and 100% (ethanol : water, v/v) for 10 min at 25°C, followed by a graded Freon 113 series of 25, 50, 75, 90, 100, 100, and 100% (Freon 113 : ethanol, v/v) for 10 min each at 25°C. The samples were dried in a vacuum desiccator attached to a running water aspirator for 12 hr.

The dried specimens were mounted on 15 mm aluminum stubs with double-sided adhesive tape and silver paint for increased conductivity. Then, specimens were coated with gold, by applying with a diode sputtering unit (HUS-4GB, Hitachi Co.). The specimens were viewed under a scanning electron microscope (MSM-6, Akashi

Co.) operated at an accelerating voltage of 20 KV, at 5000 x magnification. An average of five places from each treatment was observed in order to obtain representative micrographs.

RESULTS AND DISCUSSION

1. Textural characteristics of SPI-emulsion gels with polysaccharides

The effects of 0.25% (w/v) KC, SA, XG and XG+LG (1: 1) mixtures on texture

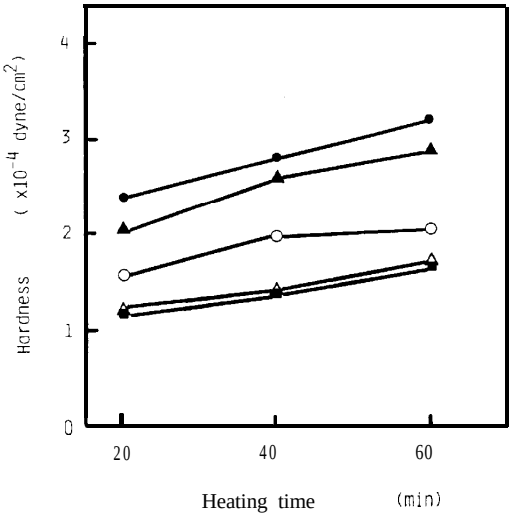

Fig. 1. Effect of heating time and different polysaccharides on hardness of SPI-emulsion gels. (■: control, ● : KC, ○: SA, △: XG, ▲: XG+LG)

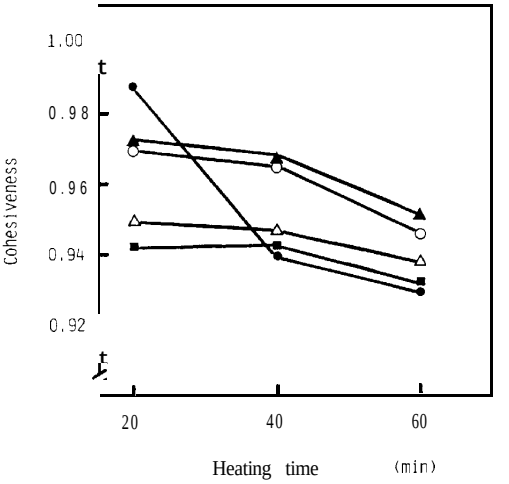

Fig. 2. Effect of heating time and different polysaccharides on cohesiveness of SPI-emulsion gels. (See Fig. 1 for symbols)

of SPI-emulsion gels were studied. As shown in Fig. 1, the hardness increased by all treatments at heating time from 20 to 60 min. Analysis of data obtained indicated that a significant differences among them were observed. All samples with polysaccharides showed hardness greater than that of control (polysaccharides-free), and no differences were shown between the XG and control-samples. KC-samples became the most hardest by heating from 20 to 60 min and showed 2 times higher than control, at heating 60 min. It indicated that the KC had the most effect on hardness of emulsion gels.

In the cohesiveness determination of the gels (Fig. 2), the XG+ LG-sample gave values higher than the other samples, except at heating 20 min. And, it was showed that almost the values decreased slightly accompanying heating time. But, the values of KC-sample decreased sharply at heating 40 min, and from this point the minimum was obtained.

As shown in Fig. 3, the adhesiveness varied a little with elongation of heating time. The control-sample exhibited the remarkably decrease by about 75% for heating from 20 to 60 min. The addition of KC resulted in increase with elongation of heating time. However, the other samples showed a decrease tendency during heating from 40 to 60 min.

2. Breaking parameters of SPI-emulsion gels with polysaccharides

Breaking test involves measuring either the depth of penetration of a body sinking into the gels, and the force required to push a penetrating body into a gels at a constant speed. The results of breaking test are shown in Figs. 4 and 5. The breaking force of the gels with polysaccharides as a function of heating time was shown in Fig. 4. XG+ LG-sample showed the highest resistance to penetration and control-sample gave the lowest value at heating from 20 to 60 min. These observations indicated that the gels with polysaccharides showed a significantly higher breaking force than that of without

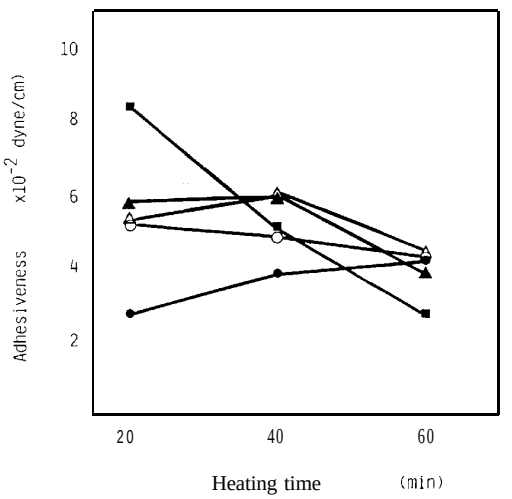

Fig. 3. Effect of heating time and different polysaccharides on adhesiveness of SPI-emulsion gels. (See Fig. 1 for symbols)

polysaccharides after various heating time. Also, these results showed that the values increased slightly with elongation of heating time in all samples. The order of the value was XG + LG > KC > XG > SA > control.

The breaking deformation was shown in Fig. 5. Similarly, the XG+LG-sample showed the highest value, and the control-sample exhibited the second one higher than the other samples. In XG, KC and SA-samples, the values decreased with elongation of heating time, but SA-sample showed slightly increased at heating for 60 min. The order of the value was XG + LG > control > XG > KC > SA.

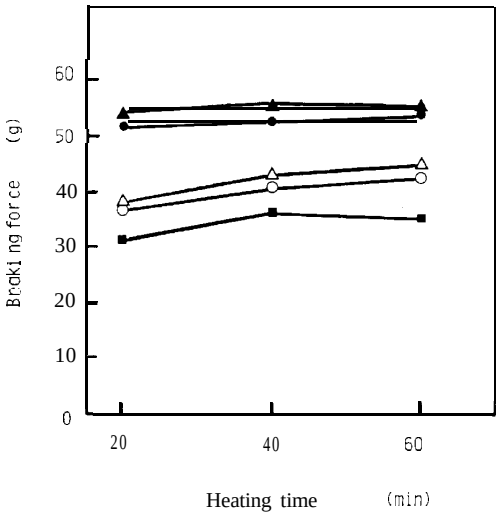

Fig. 4. Effect of heating time and different polysaccharides on breaking force of SPI -emulsion gels. (See Fig. 1 for symbols)

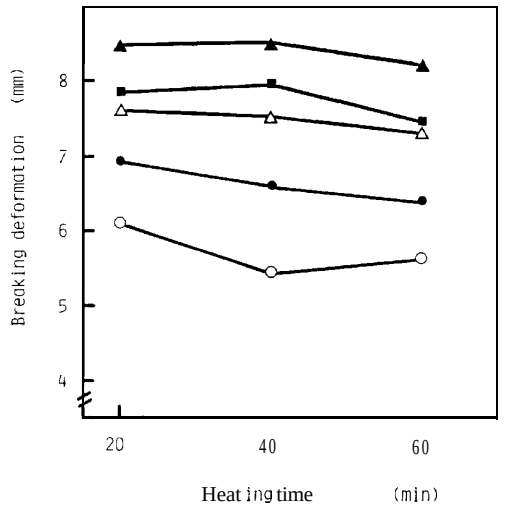

Fig. 5. Effect of heating time and different polysaccharides on breaking deformation of SPI-emulsion gels. (See Fig. 1 for symbols)

3. Creep compliance curves for SPI-emulsion gels with different polysaccharides and heating time

Fig. 6 showed the creep compliance curves of the SPI-emulsion gels with control, KC, SA, XG and XG+LG after heating for 20, 40 and 60 min, respectively. The results showed an evident similarity among all samples. The curves essentially consist of two parts. When a fixed stress was applied, the elastic compliance rose instantaneously to a value J_0, equal to reciprocal of the instantaneous elastic modulus. After that, there was a time dependence build up which consists of the equilibrium compliance and the viscous compliance. It wsa found that the creep compliance curves could be fitted by a model consisting of a Maxwell element in series with one or more Voigt elements as described by the following equation :

$$J(t) = J_0 + J_i(1 - e^{-t/\tau_{k1}}) + t / \eta_N$$

where J_0 is the instantaneous elastic compliance, J_1 is the retarded elastic modulus, τ_{k1} is the retardation time associated with the Voigt elements, and η_N is the Newtonian viscosity of Maxwell dashpot. The results illustrated that the compliance values decreased with elongation of heating from 20 to 60 min for all samples, and the facts indicated that gels' elastic properties were enhanced and became more viscous flow characteristics by heating and resulted in the changes of gels network structures.

4. **Viscoelastic parameters of SPI-emulsion gels with polysaccharides**

All the SPI-emulsion gels exhibited viscoelasticity with or without polysaccharides under the test conditions applied. And, all the samples in this experiment were considered six-elements. The results from creep-recovery curves was shown in Tables **1** ~ 3, indicating the effect of polysaccharides on SPI-emulsion gels which heated at 80°C for 20, 40 and 60 min, respectively. The KC-sample showed the maximum instantaneous elastic modulus (E_0) and control-sample showed the minimum. The order of the value was KC > XG + LG> SA > XG> control, unlike hardness and breaking force. Also, the results indicated that E_0 values increased with elongation of heating time, since all the samples showed the instantaneous elastic modulus with 10^5 dyne/cm² order and Newtonian viscosity with 10^8 dyne/cm² order, it was illustrated that those gels have as cross-linked rubber-like materials.

The effect of polysaccharides on viscoelastic deformation of gels was also shown in Tables 1 ~ 3. The deformation proportion was combined with instantaneous, retarded and permanent deformations. It was shown that no regular variation of retarded and permanent deformation by heating was observed for all the samples. However, the instantaneous deformation proportion decreased with heating time from 20 to 60 min for any samples. This means that with elongation of heating time, the elastic properties of gels could be enhanced and became more resistant against the force applied to prevent the deformation of gels.

5. Scanning electron microscopic structure

Microstructures of emulsion gels with polysaccharide were observed by SEM. Figs. 7-1, 2a, 2b, Figs. 7-3a, 3b and Figs. 7-4a, 4b showed the micrographs of control, XG + LG and SA-samples, respectively. Fig. 7-l showed the fractured surface of control-sample which had been fixed solely with glutaraldehyde. A noticeable lipid

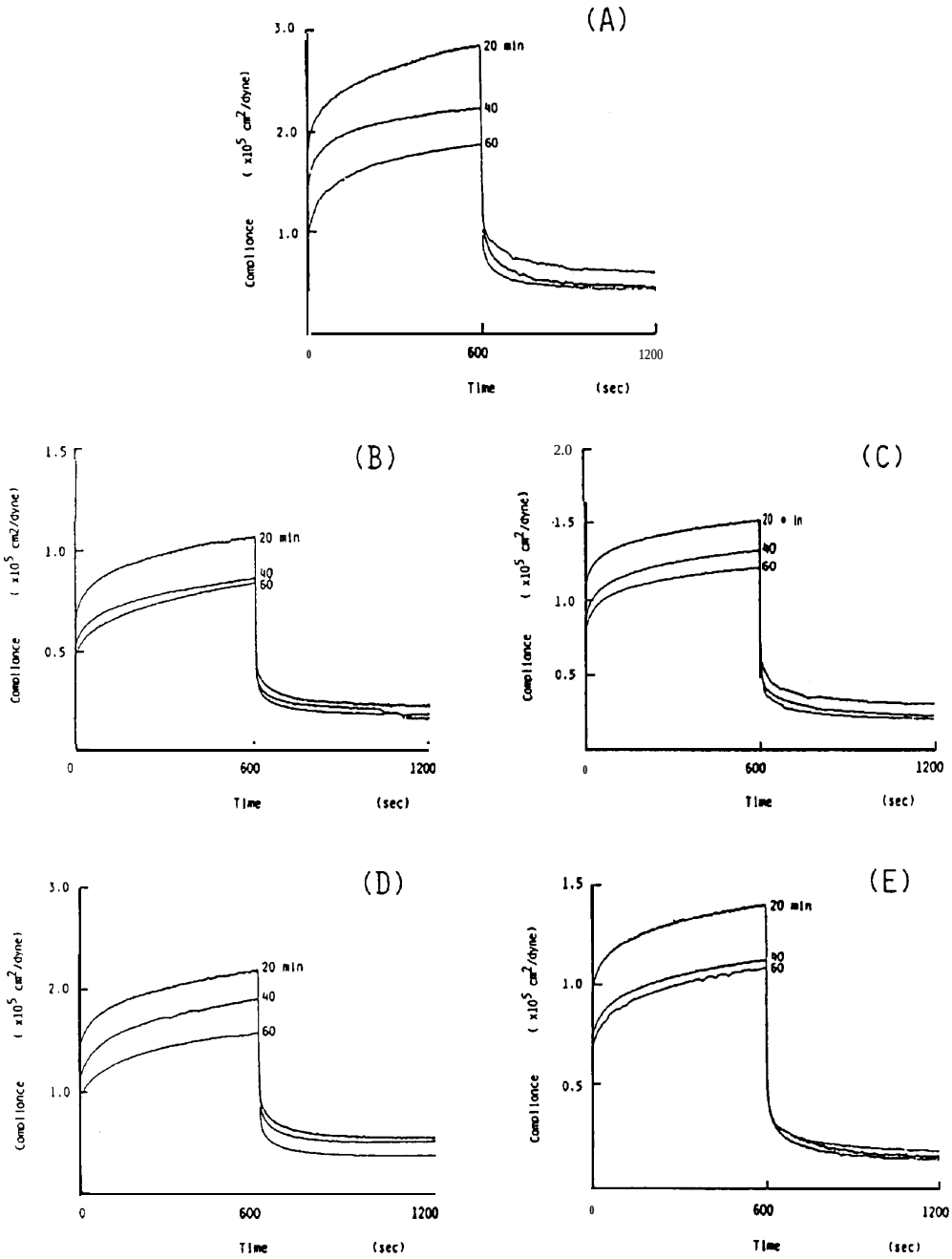

Fig. 6. Creep compliance curves of the SPI-emulsion gels with different polysaccharides as function of heating time. (A : control, B : KC, C : SA, D : XG, E : XG+ LG)

Table 1. Effect of polysaccharides on viscoelastic parameters and deformation of SPI-emulsion gels heating at 80°C for 20 min.

Polysaccharides	CTL*	KC	XG	SA	XG+LG
E_0 (dyne · cm^{-2} × 10^{-5})	0.69	1.67	0.79	1.00	1.12
E_1 (dyne · cm^{-2} × 10^{-5})	2.53	4.98	2.83	4.89	4.79
η_1 (poise ×10^{-7})	2.28	5.61	2.92	4.59	4.44
τ_{k1} (sec)	90.0	112.7	103.2	93.9	92.7
E_2 (dyne · cm^{-2} × 10^{-5})	4.00	7.27	3.61	5.64	6.91
η_2 (poise ×10^{-6})	5.36	7.06	3.86	5.42	7.02
τ_{k2} (sec)	13.4	9.7	10.7	9.6	10.2
η_N (poise ×10^{-8})	2.14	4.36	2.07	4.19	3.82
Deformation (%)					
Instantaneous	59.9	55.8	57.6	64.9	63.6
Retarded	29.1	31.4	29.3	25.8	25.4
Permanent	11.0	12.8	13.1	9.3	11.0

* CTL : Control.

Table 2. Effect of polysaccharides on viscoelastic parameters and deformation of SPI-emulsion gels heating at 80°C for 40 min.

Polysaccharides	CTL*	KC	XG	SA	XG+LG
E_0 (dyne · cm^{-2} × 10^{-5})	0.79	2.11	1.05	1.32	1.53
E_1 (dyne · cm^{-2} × 10^{-5})	2.24	6.54	2.51	4.19	4.92
η_1 (poise × 10^{-7})	2.15	6.26	2.10	4.06	5.10
τ_{k1} (sec)	95.9	95.7	83.8	97.0	103.7
E_2 (dyne · cm^{-2} × 10^{-5})	2.62	10.5	4.12	5.03	7.11
η_2 (poise ×10^{-6})	2.68	9.74	3.69	4.70	6.84
τ_{k2} (sec)	10.2	9.3	8.9	9.4	9.6
η_N (poise ×10^{-8})	3.69	4.12	1.83	4.39	4.49
Deformation (%)					
Instantaneous	56.4	54.3	49.5	56.8	57.7
Retarded	36.5	29.2	33.4	33.0	30.6
Permanent	7.1	16.5	17.1	10.2	11.7

* CTL : Control.

Table 3. Effect of polysaccharides on viscoelastic parameters and deformation of SPI-emulsion gels heating at 80°C for 60 min.

Polysaccharides	CTL*	KC	XG	SA	XG+LG
E_0 (dyne · cm^{-2} × 10^{-5})	1.22	2.23	1.30	1.44	1.66
E_1 (dyne · cm^{-2} × 10^{-5})	2.08	6.02	2.46	4.29	4.89
η_1 (poise × 10^{-7})	2.10	7.51	3.07	4.35	6.98
τ_{k1} (sec)	104.1	124.8	125.0	101.3	106.5
E_2 (dyne · cm^{-2} × 10^{-5})	5.03	12.3	4.30	5.39	6.98
η_2 (poise ×10^{-6})	6.08	13.8	3.39	5.77	7.22
τ_{k2} (sec)	12.1	11.3	7.9	10.7	10.3
η_N (poise × 10^{-8})	1.79	3.72	3.14	5.58	4.07
Deformation (%)					
Instantaneous	44.3	52.7	48.4	57.0	55.1
Retarded	37.8	28.4	39.7	34.3	31.6
Permanent	17.9	18.9	11.9	8.7	13.3

* CTL : Control.

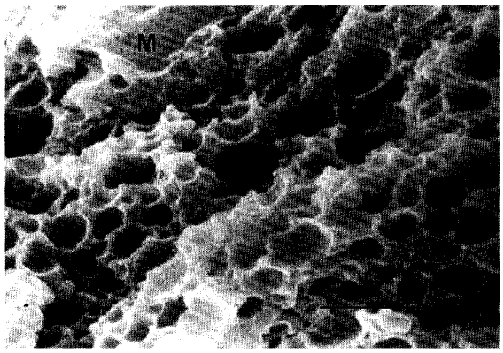

Fig. 7-1. Microstructure of SPI-emulsion gel (control). Sample was fixed with glutaraldehyde only, as followed by critical point drying.
M = protein matrix, E = empty vacuoles, Magnification = 5,000 ×, Bar = 5 μm.

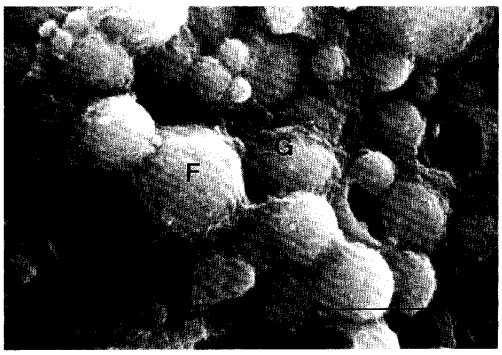

Fig. **7-2a.** Microstructure of SPI-emulsion gel (control). Sample was fixed with glutaraldehyde and post-fixed with osmiun tetroxide, as followed by critical point drying.
M = protein matrix, F= fat droplet, G = protein encapsulated fat droplet, Magnification = 5,000 ×, Bar= 5 μm.

material could not be observed, although the protein matrix was well preserved. The surface is pock-marked with empty voids, and a fine structure of protein matrix of the gels could be observed, but lipid could not.

Figs. 7-2a, 3a and 4a depict the fractured surfaces of control gel and those with XG+LG and SA, respectively. They had been fixed in glutaraldehyde and post-fixed in osmium tetroxide to preserve the lipid structure followed by critical point drying. Both the protein and lipid component are well preserved. For control-sample, as shown in Fig. 7-2a, it is evident that a large number of round fat droplets was presented and size from 3.75 μm to 0.6 μm in diameter. The fat droplets are evenly distributed throughout the gel matrix, (F) denotes fat droplets. It was also presumed that oil droplets had been contained in most of empty voids, as shown in Fig. 7-1, but dissolved away, inducing variation of size during fixation in glutaraldehyde. As shown in Fig. 7-3a for XG+ LG-sample, smaller globules of fat droplet were embedded in

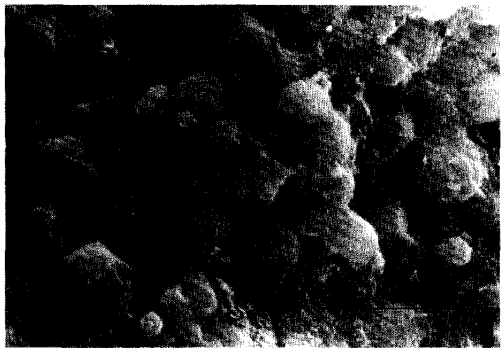

Fig. 7-3a. Microstructure of SPI-emulsion gel with XG+LG mixture. Sample was fixed with glutaraldehyde and post-fixed with osmiun tetroxide, as followed by critical point drying.
M = protein matrix, F= fat droplet, G = protein encapsulated fat droplet, Magnification= 5,000 ×, Bar= 5 μm.

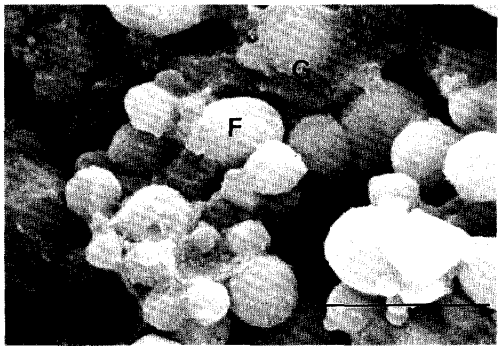

Fig. 7-4a. Microstructure of SPI-emulsion gel with SA. Sample was fixed with glutaraldehyde and post-fixed with osmiun tetroxide, as followed by critical point drying.
M=protein matrix, F=fat droplet, G=protein encapsulated fat droplet, Magnification= 5,000 ×, Bar= 5 μm.

protein matrix, and the shape of droplets was not shown clearly, compared to control. The micrograph also reveals that a thick, dense protein-polysaccharides film surrounding by the fat droplet would be fixed with OsO_4. SA-sample (Fig. 7-4a) indicated different structure compared to control and XG+ LG-sample. The micrograph revealed that the gel had a porous surface and fat droplets of irregular sizes which were filled in the textured structure. The fat droplets are bound into the protein matrix structure and appeared shiny.

The cryofractured surface of gels was then observed for control, XG+LG and SA -samples, respectively. As shown in Fig. 7-2b, the network structures of gel could be seen clearly and the fine structure of protein matrix is well preserved. It also appeared that well dispersed fat droplets were laden by protein matrix and small uniform round

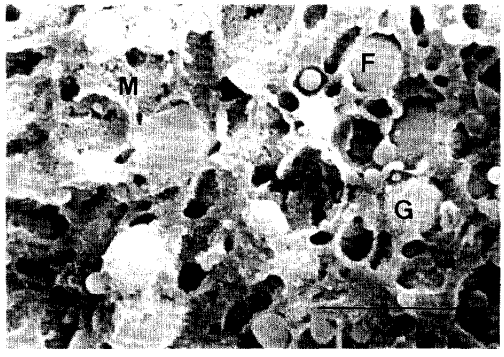

Fig. **7-2b.** Microstructure of SPI-emulsion gel (control). Sample was cryofractured then fixed with glutaraldehyde and post-fixed with osmiun tetroxide, as followed by vacuum drying.
M= protein matrix, F= fat droplet, G= protein encapsulated fat droplet, Magnification = 5,000 ×, Bar = 5 μm.

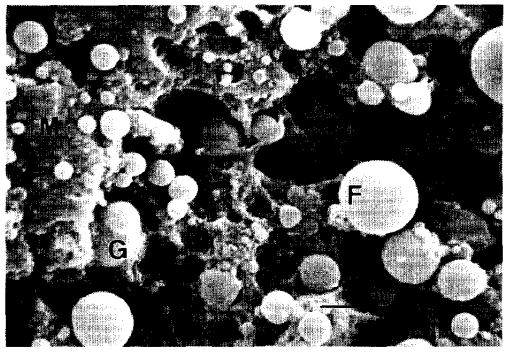

Fig. 7-3b. Microstructure of SPI-emulsion gel with XG+LG mixture. Sample was cryofractured then fixed with glutaraldehyde and post-fixed with osmiun tetroxide, as followed by vacuum drying.
M =protein matrix, F= fat droplet, G = protein encapsulated fat droplet, Magnification= 5,000 ×, Bar= 5 μm.

fat droplet (F) were stood out from the exposed fracture face. XG+LG-sample was shown in Fig. 7-3b, the gel also showed that the fine protein matrix and fat droplets embedded partly in matrix. And, many number of larger and smaller droplets appeared to have been draw out from the exposed fracture face. SA-sample was shown in Fig. 7-4b, which was significant different from control and XG+ LG-sample, and the fat droplets were not as fine as that observed in the XG+LG-sample, but the droplets appear to be bound throughout the matrix.

From the observations in this experiment, it was found that the SPI-emulsion gels with polysaccharides would be firmer than the control-sample and suggested that either the protein, fat or both are interacting with the polysaccharides. Protein-polysaccharide interactions was observed between anionic polysaccharides and casein,

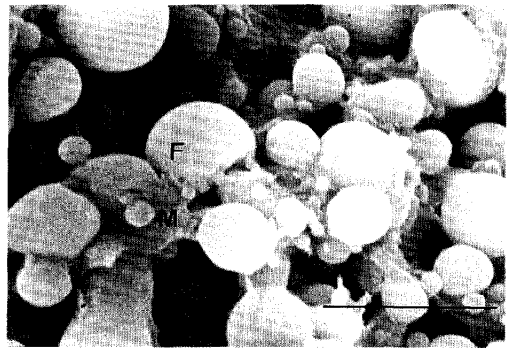

Fig. 7-4b Microstructure of SPI-emulsion gel with SA. Sample was cryofractured then fixed with glutaraldehyde and post-fixed with osmiun tetroxide, as followed by vacuum drying.
M = protein matrix, F= fat droplet, Magnification= 5,000 ×, Bar = 5 μm.

but not between casein and neutral polysaccharides (Elfak et al. 1979). Among polysaccharide-gels applied in this investigation, the KC added gel was the most resistance to compression force in contrast to control-sample, so that the KC seemed to be most effective to obtain the maximum hardness and instantaneous elastic modulus. This suggested that the sulfate groups in KC would interact strongly with protein, and resulted in increase of the rheological properties.

Xanthan gum has the unusual property of forming dimensionally stable gels in combination with locust bean gum (Rocks, 1971). It was observed that, for emulsion gels with XG+LG, the maximum cohesiveness and breaking force could be obtained, and hardness and instantaneous elastic modulus were the second. This may be attributed to the specific interaction of xanthan gum and locust bean gum. But, Foegeding and Ramsey (1986) reported that xanthan gum could interfered with the gelation process and significantly decreased all textural properties of meat batters. Similar findings were reported by Whiting (1984) who found that addition of 0.1 or 0.3% of xanthan gum to the frankfurter batters decreased cooking loses and gel strength. In our findings, the XG-sample showed the minimum for most of rheological parameters but a little harder than control.

In observation of emulsion gel structures by SEM, the use of osmium tetroxide post-fixation provides a quick method to distinguish protein from lipid in emulsion gel systems and may be useful for the examination of structural changes in the lipid and protein components of many food products. On the other hand, the use of critical point drying and cryofracturing methods dipict different micrographs, and these may be caused by the different method of procedure of SEM observation.

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