Effects of Oils on Thermal Gelation of Soybean Protein

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The effects of adding oils to soybean protein were examined with respect to hardness of the formed gel. The gel hardness increased with a decrease in the chain length of the added fatty acid methyl esters or triglycerides. When the gels were observed under a scanning electron microscope, the addition of fatty acid methyl ester with a shorter chain length produced a good, hard gel, while the gel formed with fatty acid methyl ester of a longer chain length was easily broken. There were few differences in the hardness of gels formed after adding various unsaturated fatty acid methyl esters, triolein or edible oils, but these gels were firmer than those without oils. The behavior of the protein solution containing oils on thermo-denaturation was the same as that without oils. The amount of protein adsorbed on oil droplets increased with a decrease in the chain length of the fatty acid methyl ester. The hardness of the gel formed with oils may be strengthened by the protein-oil interaction after thermo-denaturation of protein.

Proteins contained in food materials interact with fats, carbohydrates, etc., and affect the taste, odor and texture of foods. Tofu (soybean curd), one of the soybean protein gels, contains mainly protein and fat,1) and the interactions between both components may create its favorable texture.

Catsimpoolas and Meyer2) investigated the factors affecting the gelation of soybean globulin in the presence of lipids by rheological method. They showed that the apparent viscosities of the heat-treated protein dispersions were increased either by decreasing the fatty acid chain length of the glyceride or decreasing the esterification of the hydroxyl groups of glycerol. Recently, Yamano et al.3) reported that the soybean protein gels prepared by adding palm oil became firmer with the amounts of oils added and tasted better than those without oils.

This study examines the effects of oils on the thermal gelation of soybean protein, especially the differences among chain lengths of fatty acid methyl esters and of triglycerides added.

MATERIALS AND METHODS

Materials. Soybean meal defatted under low-temperature was supplied from Honen Seiyu Co. Soybean protein solution, prepared from an aqueous extract of the defatted meal, was precipitated at pH 4.6, dialyzed against distilled water for 48 hr and lyophilized. Fatty acid methyl esters, tributyrin, and tricaproin were purchased from Tokyo Kasei Co. Tricaprylin and tricaprin were supplied from Nippon Oil and Fats Co. Soybean oil and coconut oil were obtained from Nakarai Chemicals Ltd., Kyoto.

Preparation of thermal gel. Protein solution and oil was mixed for 30 sec with a Vortex mixer and then emulsified for 3 min by sonication. The processes were repeated twice. Each protein and oil in the homogenized dispersion (pH 6.0) was 6.6% concentration by weight. The dispersion was heated at 95°C for 30 min and cooled at 0°C for 10 min. Hardness of the thermal gel formed was measured at room temperature with a Texturemeter (General Food Co. GXT-2) using a visco type plunger and a cup of 24 mm diameter. The clearance between the plunger and the plate was adjusted to 0.3 mm. Hardness was measured from the first chew.

Scanning electron microscopy (SEM). A small piece of protein gel was frozen in liquid nitrogen and lyophilized. The dried protein gel was coated with gold in a sputter-coating device (Eiko IB-3 Ion Coater). A Hitachi S-450 Scanning Electron Microscope was used to examine the gel profile of proteins. Each concentration of protein and oil was 8% (w/w) and pH was 6.0.
Viscosity measurement. The viscosities of protein dispersions were measured with a Haake Rotovisco model RV 11 using a sensor system NV. Protein dispersions were adjusted to pH 7.5 with 1 N NaOH. Protein solution was heated at 95°C for 30 min directly in the viscometer with rotation at shear rate, 175.2 s⁻¹. After a shear stress at 95°C was determined at shear rate, 525.7 s⁻¹, the rotor was immediately cooled, maintained at 20°C for 10 min while again rotating at 175.2 s⁻¹ and a shear stress at 20°C was measured at shear rate, 525.7 s⁻¹.

Differential scanning calorimetry (DSC) measurement. Thermal measurements were made with a Daini Seikosha SSC-560u differential scanning calorimeter using silver hermetic pans. The reference was a sealed sample pan containing a volume (50 µl) of distilled water equal to that of the protein solution.

Determination of protein amount adsorbed on oil droplets. A 4 ml of 3% protein solution and 1 ml of fatty acid methyl ester was mixed for 30 sec with a Vortex mixer, then for 3 min by sonication, and the emulsifying process was repeated twice. The emulsion was immediately heated at 95°C for 30 min in a test tube (15 x 125 mm) with a screw cap. The heat-treated emulsion was centrifuged at 3,000 rpm for 20 min and the floated cream portion was carefully collected. The cream portion was washed twice in distilled water and defatted in chloroform. The amount of protein in a test tube was determined by the Kjeldahl method.\(^4\)

RESULTS AND DISCUSSION

Figure 1A shows the effects of adding fatty acid methyl esters with various chain lengths on thermal gelation of soybean protein. The hardness of the gel became firmer with the decrease of chain length. The protein gels were frozen in liquid nitrogen, lyophilized and observed with a scanning electron microscope (SEM) at 20 kV (Fig. 2). The network structure of the gels formed by adding fatty acid methyl esters were coarse compared with those without oils.

Although the gel treated by liquid nitrogen showed the network structure as shown in the pictures of Fig. 2,\(^5\) the gel fixed with glutaraldehyde before liquid nitrogen-treatment did not show a clear network structure.\(^6,7\) In this experiment, the protein gel was immersed in 2.5% glutaraldehyde, dehydrated in ethanol solutions of increasing strength (50, 70, 80, 90, 95 and 100%), immersed in iso-amyl acetate and dried in a critical point dryer. The SEM images of the gel dried without a process of freeze-drying did not show the clear network structure as that in Fig. 2, and the difference between the presence and the absence of fatty acid methyl esters, or among fatty acid methyl esters with various chain lengths was indistinguishable (not shown here). It seems likely that the fixed gels have fine structures unsolvable by SEM. Consequently, the network structures in Fig. 2 may indicate the crystal size of ice formed during freeze-treatment by liquid nitrogen. The network structure of the gel containing oil was larger than that without oil. The water in gels containing oil might be much more subject to the growth of ice crys-

![Fig. 1. Effect of Adding Various Oils on the Gel Formation of Soybean Protein.](image)

Hardness of gels is an average of three replications. Each concentration of protein and oil was 6.6% (w/w) and pH was 6.0. \(C_n\), carbon number. A: ●, fatty acid methyl ester; ○, triglyceride; control, no oil. B: unsaturated fatty acid methyl ester. (18 : 1), methyl oleate; (18 : 2), methyl linolate; (18 : 3), methyl linolenate. C: TO, triolein; SB, soybean oil; CO, coconut oil.
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Fig. 2. Scanning Electron Micrographs of Soybean Protein Gels Prepared in the Presence of Various Fatty Acid Methyl Esters.

Each concentration of protein and fatty acid methyl ester was 8% and pH was 6.0. Control, no oil; C₆, methyl n-caproate; C₁₀, methyl n-caprate; C₁₄, methyl myristate.

tals compared with that in the gel without oil. It is known that ice crystals grow bigger as the freezing temperature is higher. Since the cooling rate from the outside to the inside of the gel was slower in the presence of oil, the freezing temperature inside the gel might become higher. In reality, it was reported that the protein solution which had added oil underwent the changes of external temperature slowly.³) On the other hand, the structure unit of the gel containing oil might become larger than that without oil since the network formation is inhibited by the presence of oil.

Here, the differences of adding fatty acid methyl esters with various chain length were observed on the SEM images of gels. The gel formed by adding methyl n-caproate showed an unbreakable structure which was able to resist the growth of ice crystals, while with the increase of carbon number, the fragile gel structure was observed. Consequently, it is obvious that fatty acid methyl esters with shorter chain lengths form firmer gels.

Effects of the chain length of triglycerides were investigated. Firmer gels were formed with the decrease of carbon number in triglyceride, but the hardness of gel was lower than those formed by adding fatty acid methyl esters (Fig. 1A). The difference among unsaturated fatty acid methyl esters (methyl oleate, methyl linolate, methyl linolenate) was not observed, and the gels formed by adding triolein or edible oils (soybean oil or coconut oil) showed similar hardness (Fig. 1B, C). Thus, as the chain length or the molecular weight reached a certain size, the hardness of gel approached a constant level. However, all protein solutions with added oil produced a
The melting points of fatty acid methyl ester are below 0°C for the carbon number (Cₙ) 6, 8 and 10, while they are 5°C for C₁₂ and 18.5°C for C₁₄.⁹ It is thought that the hardness of a gel becomes lower since the fatty acid methyl esters having a large carbon number solidify during the cooling process of the gel formation. It was investigated by the rheological method at a temperature above the melting point whether the melting point of fatty acid methyl ester is related to the hardness of gel or not. The apparent viscosities were measured at shear rate, 525.7 s⁻¹. Each concentration of protein and fatty acid methyl ester was 8% (pH 7.5) (Fig. 3). The apparent viscosity at 95°C increased with the addition of fatty acid methyl ester and was significantly higher with the decrease of the chain length. The viscosities increased by the drop in temperature from 95 to 20°C showed a similar tendency to that at 95°C. The protein solution with the addition of fatty acid methyl ester of the longer chain length showed a lower viscosity even at the temperature above the melting point, and this tendency was the same as that of hardness. Consequently, it is obvious that the melting point is not related directly to the difference among the hardness of gels.

It was examined whether the thermodenaturation of protein was affected by the addition of fatty acid methyl ester or not. Figure 4A shows the thermogram in the absence of oil. The main peak at 90.9°C indicates the thermo-denaturation of 11S protein.¹⁰ The temperature of the endothermic peak for 11S protein was 91.6°C when methyl n-caproate (or methyl myristate) was added (Fig. 4B). This denaturation temperature changed little with the addition of oil. As the denaturation enthalpies on the area of endo-thermic peak are similar in spite of the presence or absence of oil, it seems that the effects of oil on gel formation occurs by protein-oil interactions after the proteins have thermo-denatured.

In order to clarify the protein-oil interaction in the protein gel, the amount of protein adsorbed on oil droplets was measured. The protein solution used was 3% concentration which did not form a gel during heat-treatment. The amount of protein adsorbed on fatty acid methyl ester droplets increased with
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Fig. 5. The Amount of Protein Adsorbed on Fatty Acid Methyl Esters (FAME) or Soybean Oil (SB Oil). The oil content was 20% (v/v). The emulsion system was adjusted to pH 6.0. Cn, carbon number.

The decrease of the chain length, and the proteins adsorbed on soybean oil droplets showed the same amounts as that of carbon number 14 (Fig. 5). The increase of hardness by the addition of fatty acid methyl ester may be caused by the protein-oil interaction. It seems that the fatty acid methyl esters with shorter chain length interact strongly with proteins.

When the effects of adding soybean oil to the protein solution was investigated at various pH levels, firmer gels were formed at each pH by adding oil than those to which no oil had been added (Fig. 6). The pH region forming gels shifted slightly to alkaline pH in the presence of 0.3 M sodium chloride, and the hardness of gel became higher with the addition of oil. As salts have functions which shield, more or less, the charges on the surface of protein molecules and which indirectly enhance the protein-protein interaction, the region forming gel may shift to alkaline pH in the presence of salts. But, though oil strengthened the hardness of gel, the shift of pH on gel formation did not occur. It is obvious that oil functions differently than salt, and that it, at least, does not affect the net charge of proteins.

It is still unknown how protein and oil interact, and why protein attaches strongly to the fatty acid methyl ester with shorter chain length, and these remain the problems to be solved.

REFERENCES

4) K. Parnas and R. Wagner, Biochem. Z., 125, 253 (1921).