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The Mechanisms for Yuba Formation and Its Stable Lipid

YEMING CHEN* AND TOMOTADA ONO

The United Graduate School of Agricultural Sciences, Iwate University, Ueda 3, Morioka, Iwate 020-8550, Japan

Yuba is a protein−lipid film formed on soymilk surface by heating. It is characteristic of textured structure and long shelf life (3−6 months) in the dry state at room temperature. It was known that soymilk contained oil bodies, protein particles and soluble protein as main components. In this study their roles on Yuba formation were examined. Film was formed by heating an oil body suspension, but it was dispersed again by mixing; Tosan 205 soluble protein (mainly α′ and α subunits of β-conglycinin) formed film after a long time heating; Yumeminori soluble protein (mainly acidic peptides of glycinin) did not form a film even after all water was evaporated; Tosan 205 and Yumeminori non-lipid soymilks (containing protein particles and soluble protein) formed films by heating. Thus, it was concluded that protein particles were the most important for Yuba formation. In addition, Yuba was treated by liquid nitrogen, vacuum freeze-drying and observed by field emission scanning electron microscope (FESEM). The FESEM pictures showed that oil bodies, protein particles and soluble protein themselves were the "blocks" for Yuba network formation; the Yuba network was formed from protein, but oil bodies were incorporated in the network.

KEYWORDS: Soybean; Yuba; oil bodies; protein particles; soluble protein; particle size; amphipathic structure

INTRODUCTION

Film is formed on soymilk surface by heating. After the film is lifted, continued heating produces successive films, each of which is in turn removed. These films are dried and known as Yuba. Yuba contains 57.6% protein and 24.1% lipid (*1*). But it would have different protein and lipid concentrations if prepared from different soybeans (*2*). In addition, several pieces of Yuba lifted from one pan of soymilk could have different protein and lipid concentrations. The mechanism has been clarified with diffusion theory by Chen et al. (*3*).

Yuba is used not only for food but also for cosmetics (facial mask) and the coatings of other food (especially Japanese food). But the mechanism of Yuba formation is still not clear. Wu and Bates (*2*) gave a hypothesis that Yuba formation was based on protein denaturation, endothermic polymerization of protein and lipid−protein interaction. Okamoto and Watanabe (*4*) gave a hypothesis that soymilk surface protein molecules exposed hydrophobic amino acid residues to the air upon heating at the same time that water was evaporated; then protein molecules arranged on the soymilk surface to form film. They all did not well know about the existing states of soymilk lipid and protein in the 1970s and gave the mechanism of Yuba formation from a molecular viewpoint. Ono et al. (*5*, *6*) reported that soymilk was composed of oil bodies, protein particles (>40 nm), soluble protein (<40 nm) and carbohydrate. According to Huang's famous oil body model (*7*, *8*), oil body had a triacylglycerol (TAG) matrix core, covered by a layer of phospholipids and embedded by oil body intrinsic oleosins. It was reported (*3*, *9*) that oil bodies and protein particles had size ranges of 121−657 nm and 40−258 nm, respectively. In this study, we aimed to explain the mechanism of Yuba formation from the viewpoint of colloidal science; the roles of soymilk oil bodies, protein particles and soluble protein on Yuba formation were examined, respectively; then Yuba structure was observed by field emission scanning electron microscope (FESEM) and the mechanism of Yuba formation was explained.

*To whom correspondence should be addressed. Tel and fax: +81-19-621-6168. E-mail: chenyeming19821213@gmail.com.

MATERIALS AND METHODS

Materials. Soybeans Tosan 205 (2007), Suzuyutaka (2007) and Yumeminori (2007) were used. Tosan 205 was a glycinin deficient soybean while Yumeminori was an α′, α deficient soybean. Suzuyutaka was one typical soybean variety in Iwate, Japan. All three soybeans were stored at 4 °C until use.

Soymilk Preparation. Soybean (20 g) was soaked in deionized (DI) water at 4 °C for 18 h. It was ground for 2 min at 13,900 rpm with an Oster blender (Oster, Milwaukee, WI) and filtrated through a two-layer Kim-Wipe sheet (Nippon Paper Crecia Co. Ltd., Tokyo, Japan). The filtrate was deemed as raw soymilk. Raw soymilk was heated at >95 °C for 5 min and deemed as soymilk.

Soymilk Ultrafiltrate Preparation. The soymilk ultrafiltrate was prepared by using a stirred ultrafiltration cell (Micon-8200, Millipore co., Bedford, MA) with a membrane (limited molecular weight 10 kDa). This ultrafiltrate was used to disperse the soymilk oil bodies.

Preparation of Soymilk Oil Body Suspension. Sucrose (30 g) was added into soymilk (120 g), mixed and centrifuged (50000*g*, 45 min). The floating fraction (oil bodies) was collected and washed twice (50000*g*, 45 min; DI water, 20% sucrose (w/w)). Then the floating fraction was collected and dispersed into 60 mL of soymilk ultrafiltrate. This oil body suspension was used as sample.

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Defatted Soybean Meal Preparation (Tosan 205 and Yumeminori). About 600 g of soybean was put into a paper bag and was dried in a decompression oven (DPF-31, Yamato Scientific Co., Tykyo, Japan) at 50 °C for 24 h. The dried soybean was ground by New Power Mill (PM-2005, Osaka Chemical Co. Ltd., Osaka, Japan) at 20,000 rpm for 20 s. The powder was passed through a 500 μm sieve. Then the passed powder was put into a thimble filter (Advantec 84, 60 × 200 mm, ADVANTEC Co. Ltd., Tokyo, Japan). The filter was put into a Soxhlet extractor, and hexane was used as solvent. The extraction was done overnight. Then the filter was taken out of the Soxhlet extractor and put into a decompression chamber to deodorize hexane for about 24 h. The defatted soybean meal was used to prepare non-lipid soymilk.

Preparation of Non-Lipid Soymilk. DI water (180 g) was added to defatted soybean meal (20 g) and ground for 2 min. It was filtrated, and the filtrate was heated at >95 °C for 5 min. This was used as non-lipid soymilk.

Preparation of Soluble Protein. Soymilk (8.80 mL) was put into ten 10 mL centrifuge tubes, respectively. They were treated by ultracentrifugation (156000*g*, 30 min) and separated into three fractions: floating, supernatant (soymilk soluble protein) and precipitate. About 3 mL of supernatant was carefully collected from each centrifuge tube with a syringe. So about 30 mL of supernatant (soluble protein) was obtained.

SDS−PAGE. SDS−PAGE was used to examine the protein components of soluble protein. SDS−PAGE was conducted with the method by Laemmli (*10*) with the concentrations of the stacking and running gels being 5% and 12.5%, respectively. The buffer in the reservoir contained 0.025 M Tris, 0.192 M glycine and 0.1% SDS, while the buffers in the stacking and running gels were 0.125 M Tris-HCl (pH 6.8) and 0.38 M Tris-HCl (pH 8.8), respectively. 0.01% samples contained 0.25 M Tris-HCl (pH 6.8), 1% SDS, 2% 2-mercaptoethanol, glycerol and bromophenol blue. This stood for overnight. Each sample was put into a sample well in the stacking gel and electrophoresed.

Coomassie brilliant blue G-250 was used for staining protein in gel by the method of Blakesley and Boezi (*11*). Gel was destained using tap water and dried on a filter paper (No. 2, Advantec Toyo Co., Tokyo Japan) in decompression condition at 75 °C.

Yuba Preparation. The samples (oil body suspension, non-lipid soymilk, soluble protein and Suzuyutaka soymilk) above were put into stainless containers (10 × 6 x 5 cm), respectively. Then they were put into an 85 °C water bath (Isotemp Fisher general purpose water bath, Fisher Scientific, Boston, MA). The surfaces of oil body suspension, non-lipid soymilk and soluble protein were observed.

The first Yuba was lifted from Suzuyutaka soymilk after 15 min heat treatment; the second was after 25 min; the third was after 35 min, and it was washed with DI water slightly to remove the residual soymilk. Then it was immersed into liquid nitrogen and treated by vacuum freeze-drying for two days.

Oil Body and Yuba Observations by Field Emission Scanning Electron Microscope (FESEM). Soymilk oil body suspension obtained above was diluted 200 times with DI water. Ten microliters of diluted oil body suspension was spread on the surface of a specimen stub, dried in a decompression chamber (HUS-5, high vacuum evaporator, Hitachi Co., Tokyo, Japan). Then it was coated with 5 nm of osmium by an osmium plasma coater (OPC 40, Filgen, Inc., Nagoya, Japan). This was used as FESEM sample.

Dried Yuba was cut into fine pieces about 2−3 mm. One specimen stub was spread by dotite paint (XC-12 carbon-20G, JEOL DATUM Ltd., Tokyo, Japan). Yuba pieces were located on the surface in different ways before the paint was dried. These pieces were coated with 5 nm of osmium by the osmium plasma coater above. This was used as FESEM sample. A field emission scanning electron microscope (JSM-7001F, JEOL DATUM Ltd., Tokyo, Japan) was used for the observations.

RESULTS AND DISCUSSION

The effect of Oil Body on Yuba Formation. In the 1970s, researchers (*12*, *13*) did not well know about the soymilk lipid. So they examined the effect of soybean oil on Yuba formation. But soybean oil existed as oil bodies in soymilk. Thus, the effect of oil bodies on Yuba formation was examined. Oil body suspension was put into a stainless container and put into an 85 °C water bath. Film was formed on the suspension surface. But it was dispersed again by mixing with a magnetic stirrer.

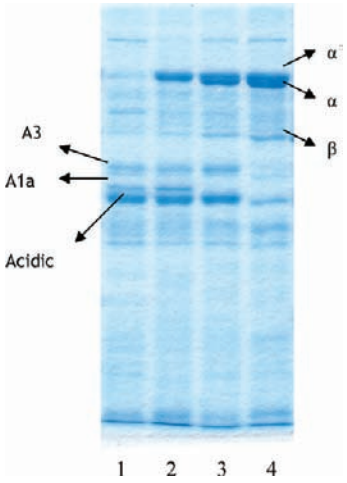

Figure 1. The protein compositions of soluble protein (lanes 1, 2, 3, and 4) obtained from Yumeminori, Fukuyutaka, Suzuyutaka, and Tosan 205 soymilks by ultracentrifugation (156000*g*, 30 min), respectively.

On one hand, water was evaporated, which caused concentration of surface oil bodies. So they had a trend to diffuse downward. But they were so large (average diameter about 380 nm) that they diffused slowly (*3*). As a result, surface oil bodies were gradually concentrated and interacted with each other to form an oil body film. On the other hand, it was known that oil body had a hydrophilic and negatively charged surface at neutral pH (*14*). So the interactions among oil bodies should be weak and could be destroyed by mixing. Thus, it was considered that large size was meaningful for film formation; oil body film was weak because of oil bodies' hydrophilic and negatively charged surfaces at neutral pH.

The Effect of Protein on Yuba Formation. Film was formed from oil body suspension by heating, but it was dispersed by mixing. It revealed oil bodies could not form a strong network for Yuba. In this section, non-lipid soymilks (Tosan 205 and Yumeminori) were prepared, added into two stainless containers and put into an 85 °C water bath, respectively. Films were formed on surfaces of Tosan 205 and Yumeminori non-lipid soymilks. These two films could not be dispersed by mixing. This revealed that protein formed a strong network for Yuba, which was in agreement with the results reported (*13*).

The Effect of Soluble Protein on Yuba Formation. In the section above, it was shown that non-lipid soymilk (containing protein particles and soluble protein) could form a strong network for Yuba. Because soluble protein could be easily obtained by ultracentrifugation (156000*g*, 30 min), the effect of soluble protein on Yuba formation was examined. The effect of protein particle on Yuba formation could be inferred from the results of this section and the section above.

The supernatants (Tosan 205 and Yumeminori soymilks) were obtained by ultracentrifugation (156000*g*, 30 min). They were deemed as Tosan 205 and Yumeminori soluble protein, respectively. Tosan 205 soluble protein mainly contained α, α′ subunits of β-conglycinin (**Figure 1**, lane 4) while Yumeminori soluble protein mainly contained acidic peptides of glycinin (**Figure 1**, lane 1). Tosan 205 and Yumeminori soluble protein were put into two stainless containers, respectively. Then they were put into the 85 °C water bath. It was found that film could not be formed from Yumeminori soluble protein even after all water was evaporated. Tosan 205 soluble protein formed film after a long heating time.

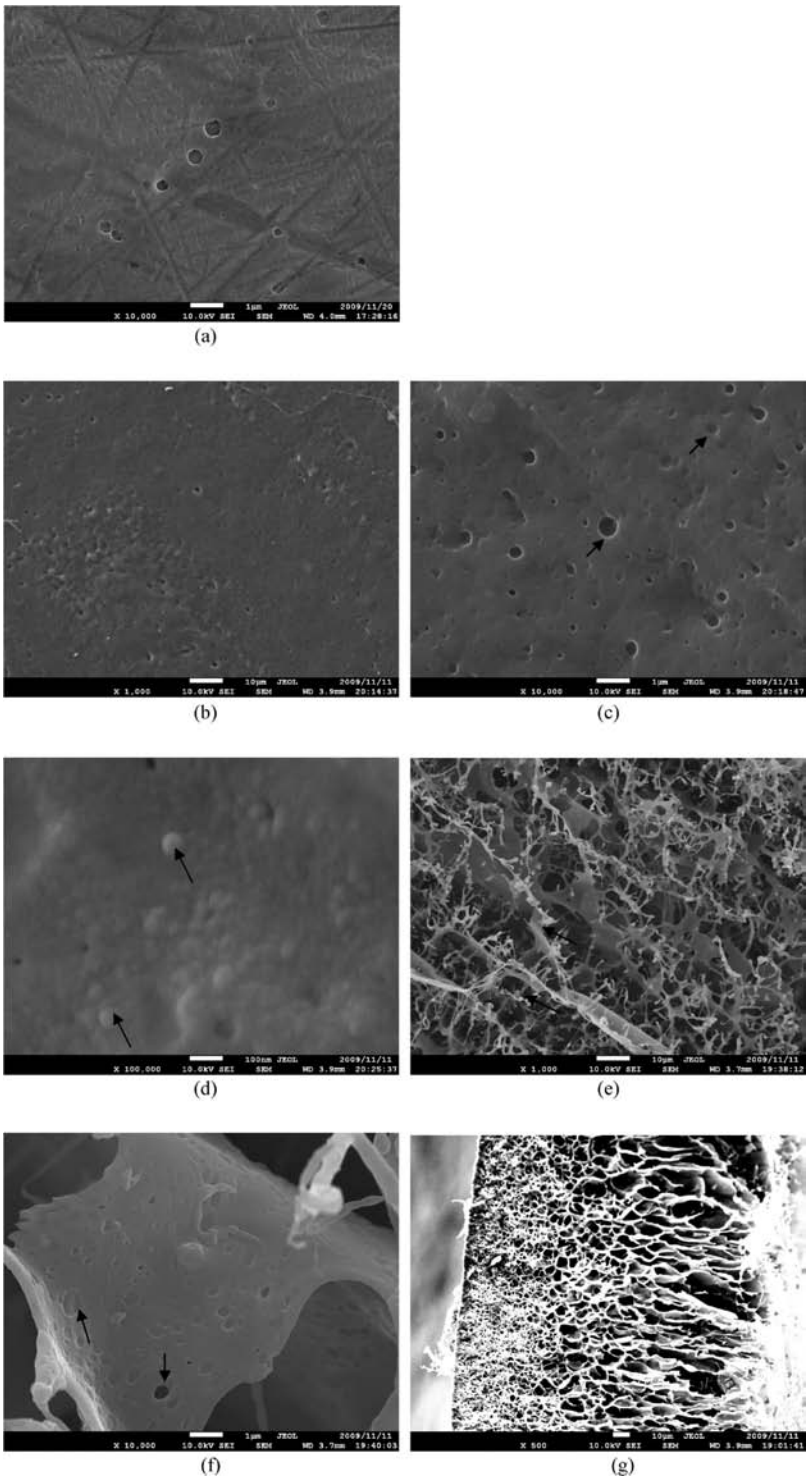

Figure 2. The FESEM pictures of soymilk oil bodies and Yuba: (**a**) soymilk oil bodies; (**b**) Yuba surface exposed to the air (×1000); (**c**) Yuba surface exposed to the air (×10000); (**d**) Yuba surface exposed to the air (×100000); (**e**) Yuba reverse surface immersed in soymilk (×1000); (**f**) one protein wall of Yuba reverse surface immersed in soymilk (×10000); (**g**) Yuba section (left, surface; right, reverse surface; ×500).

Acidic peptides were considered as hydrophilic protein (*17*−*19*). But it was known that α, α′ subunits of β-conglycinin had two regions, one extension region (N-terminus; hydrophilic) and one core region, while β subunit of β-conglycinin just had a similar core region. β was considered as hydrophobic protein (*15*−*17*). Thus, α and α′ should have an amphipathic structure, which was different from acidic peptides.

It was known that an amphipathic molecule existed by itself when its concentration was below the critical micelle concentration (CMC). But it would form various large micellar structures when its concentration was above the CMC. This should be the reason why Tosan 205 soluble protein (α′ and α) formed a film after a long heating time (concentration). Thus, it was considered that amphipathic structure was also meaningful for film formation. In order to clarify it, 2% soybean phospholipid (typical amphipathic molecule; much smaller than α′ and α) solution was prepared and put into a stainless container. The container was put into an 85 °C water bath. Film was formed on the surface although it was

dispersed again by mixing. Thus, it was concluded that amphipathic structure was also meaningful for film formation in addition to particle size.

Films could be formed from Yumeminori and Tosan 205 nonlipid soymilks, which contained protein particles and soluble protein. But film was not formed from Yumeminori soluble protein (mainly acidic peptides), and film was formed from Tosan 205 soluble protein (mainly α, α′) slowly. Thus, it was concluded that the protein particle was the most important in Yuba network formation.

It was known that surface hydrophobicity of soybean protein was increased by heating. But protein particles could exist stably in soymilk. Thus, it was considered that protein particle should be amphipathic. In addition, protein particle had a large size of about 100 nm (*9*). The large size and amphipathic structure were reasons for the most important role of protein particle in Yuba network formation. It was suggested that α/α′ would take part in Yuba network formation after they were concentrated to some extent by heating. Oil bodies, acidic peptides and carbohydrate were incorporated into the network.

Generally, disulfide was considered important for Yuba. Okamoto and Watanabe reported (*20*) that disulfide was meaningful for the Yuba strength but was not indispensable for Yuba formation. Thus, the disulfide was not considered in this study.

Oil Bodies and Yuba Observations by FESEM. Figure 2a is a picture of oil bodies. They had sizes of < 500 nm and appeared black in the FESEM picture (background was the specimen stub surface). It was shown that the Yuba surface, which was exposed to air, was dense (**Figure 2b**), and there were many black and gray circles (< 500 nm; **Figure 2c**). It was suggested that they were oil bodies or holes which were caused by water evaporation. **Figure 2d** showed that the Yuba surface was not smooth but formed by arranged white globules (< 100 nm). They were considered as protein particles and soluble protein (α/α′). The size of protein particles was < 100 nm owing to their shrinkage caused by vacuum freeze-drying. Because the size of protein particles (average size, about 100 nm) reported by Ren et al. (*9*) was in an aqueous medium. Thus, **Figure 2c** and **Figure 2d** revealed that oil bodies, protein particles and soluble protein were the "blocks" for Yuba formation but not their molecules.

Figure 2e and **Figure 2f** showed Yuba reverse surface, which was immersed in soymilk. Its structure was loose and composed of large holes and protein walls. The protein walls were similar to Yuba surface (**Figure 2c**). There were many gray circles (< 500 nm) on the protein wall surface (**Figure 2f**), and they were considered as oil bodies. **Figure 2g** showed Yuba section structure. It showed that the Yuba surface network was dense while the reverse surface network was loose.

Yuba Formation Mechanism. In all, Yuba was formed as below: surface evaporation and convection current were caused by heating. Surface evaporation caused the concentration of surface soymilk, and convection current caused surface soymilk particles (oil bodies, protein particles and soluble protein) to gather in the middle of the soymilk surface. Thus, Yuba film began to form in the middle and gradually grew large and thick.

Then we wanted to explain the arrangement of soymilk particles in Yuba formation. It was stated above that large particle size and amphipathic structure were meaningful for film formation. In addition, Okamoto and Watanabe (*21*) reported that Yuba just could form on the evaporation surface. Therefore, it was considered that hydrophobic parts of protein particles (or concentrated α and α′) were exposed to the gas phase of evaporation surface, and hydrophilic parts were immersed in the liquid phase. Then they interacted with each other by molecular interactions to form the Yuba network. Oil bodies were so large that they diffused slowly. As a result, they were incorporated into the protein network. The oil bodies would be wrapped more closely by protein network if Yuba was dried later. This should be the reason for the long shelf life.

At last, we wanted to explain why Yuba has the structure in **Figure 2g**. It was considered that the Yuba network (Yuba surface and protein walls) was evaporation surfaces in Yuba formation (*21*). Soymilk surface was the initial evaporation surface (**Figure 2b**). After surface film was formed, evaporation was still quick and many separate small evaporation surfaces were formed owing to the steric hindrance of surface film. So many small protein walls were formed on these small separate evaporation surfaces, and the structure here was dense (holes were small). With the growth of Yuba film thickness, water evaporation gradually became difficult. As a result, large bubbles of water vapor were formed and the Yuba network was formed on the evaporation surfaces of these large bubbles. This was the reason why Yuba had the structure in **Figure 2g**.

It was considered that this study would give some hints to the textured food production from vegetal protein. In addition, the flavor and functional components could be emulsified and dispersed into soymilk or non-lipid soymilk to produce various Yuba-like foods. This was meaningful for the utilization of defatted soybean meal.

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