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# Mechanism of the Chemical Composition Changes of Yuba Prepared by a Laboratory Processing Method

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Yuba is a filmlike soybean food made from heated soymilk that contains oil bodies (average diameter, 270 nm), particulate protein (>40 nm; average diameter, 70 nm), soluble protein (<40 nm), and carbohydrate (molecular size). Three varieties of soybean were used to make yuba. The carbohydrate in the remaining soymilk increased sharply while lipid increased a little. The particle size distributions of oil body showed the trend that smaller oil bodies were concentrated in the remaining soymilk, and the percentage of soluble protein in whole protein increased in the remaining soymilk. These results could be explained well with diffusion theory. The temperature gradient and concentration gradient originating from the heat treatment were considered to cause the net particle diffusion from the surface to the bottom soymilk. Lipids, which mainly exist as oil bodies, are easily incorporated into yuba films because a few of these less dense droplets diffuse downward, causing the lipid concentration in the soymilk to change a little. Carbohydrate at the surface quickly diffuses downward, causing the carbohydrate concentration increase in the soymilk beneath the developing yuba. Protein (particulate and soluble) in the soymilk was intermediate between lipid and carbohydrate.

**KEYWORDS: Yuba; soymilk; diffusion theory; oil body; protein; carbohydrate**

## INTRODUCTION

The traditional soybean food yuba has long been considered a luxury in China and Japan. Yuba is composed of protein (57.6%), lipid (24.1%), and carbohydrate (11.9%), the combination of which contributes to its flavor and physical properties (*1*). Because of its meatlike texture and good nutritional value, its popularity is on the rise.

When soymilk is heated, water on the soymilk surface evaporates and a film forms on the soymilk surface. Once this film is collected, a new film begins to form. Because this process can be repeated, several films can be collected from a given container of soymilk. These films are called yuba, and these successive yuba sheets vary in composition. The later a yuba forms, more carbohydrate is incorporated (*2*). This yuba is generally called "sugar yuba" by producers in China. Wu and Bates (*3*) prepared yuba by using 5 L of soymilk, and yubas were collected after heating soymilk for 1, 2, 3, 4 (water was added), 5 (lipid was added), and 6 h. After each yuba was collected, some soymilk was collected. The lipid, protein, and carbohydrate contents in yubas and soymilks were determined. It was found that lipid and protein were incorporated into yuba readily, while carbohydrate instead readily concentrated in the remaining soymilk. It is known that protein forms the yuba network and carbohydrate is just a solute in soymilk. However, the effect of lipid (oil body) on yuba formation is still not clear. Although a carbohydrate solution cannot form a carbohydrate film by heating, it can be incorporated into yuba. Thus, yuba composition clearly correlates with soymilk composition regardless of the effects of lipid, protein, and carbohydrate on yuba formation (*4*).

Okamoto and Watanabe (*5*) proposed a hypothesis for the mechanism of yuba formation in which the hydrophobic amino acid residues of the soy proteins become exposed to the surface upon heating (*6*) at the same time that water evaporates on the soymilk surface. The yuba then develops as a result of several intermolecular forces (*7*, *8*). This explains yuba formation but cannot explain why yubas collected successively from soymilk have different compositions. Therefore, yuba formation cannot be due solely to changes in protein structure but must also be influenced by other factors. If we can clarify why the yuba composition changes, this would be very beneficial to yuba industrialization in which the uniform properties are very important. It could also be used to control yuba composition.

It has been reported (*9*−*12*) that lipids in soymilk exist mainly as oil bodies (average diameter, 270 nm), proteins exist as particulate ( > 40 nm; average diameter, 70 nm) and soluble proteins ( < 40 nm), and carbohydrates exist in molecular form. Oil bodies have been reported (*9*, *13*−*15*) to have a matrix of triacylglyceride (TAG) at their core and to be covered by a monolayer of phospholipid and a layer of alkaline protein known as oleosin. Because yuba forms from oil bodies, particulate proteins, soluble proteins, and carbohydrates, the chemical properties and particle sizes of these components might also be important in yuba formation. The

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aim of this study was to investigate why yubas taken successively from soymilk have different compositions.

## MATERIALS AND METHODS

**Materials.** Soybeans [*Glycine max* (L.) Merr.] used in this study were of Suzuyutaka (2007), Fukuyutaka (2007) and Yumeminori (2007). They were stored at 4 °C before use. The protein compositions of the three soybeans were different. The $\beta$-conglycinin (7S) to glycinin (11S) ratio decreased in the order of Suzuyutaka, Fukuyutaka, and Yumeminori. It was considered that −S−S− is very important to yuba formation. 7S cannot easily form −S−S−, but 11S can easily form −S−S−. Thus, the protein compostion could have some effect on yuba formation, such as the yuba film formation rate.

**Soymilk Preparation.** Soybeans (20 g) were soaked with deionized (DI) water (Organo Co., Tokyo, Japan) for 18 h at 4 °C. The soaked water was not discarded, and DI water was added to a total mass of 300 g. The water to soybean ratio of 14:1 was optimal to elute soybean composition (*16*). Soaked beans were ground twice for 2 min each time with an Oster blender (Oster, Milwaukee, WI). The homogenate was filtered through a cotton sheet, and the filtrate was heated at > 95 °C for 5 min and cooled with tap water. This was designated as soymilk.

**Yuba Preparation.** Soymilk (250.00 g) was put into a stainless steel container (15 cm × 13.5 cm × 4.5 cm) and was allowed to settle in a water bath (Isotemp Fisher general purpose water bath, Fisher Scientific, Boston, MA) at 85 °C. The water level outside of the container exceeded the soymilk level inside the container. The first yuba film was collected after 15 min. Successive yuba films were taken at 10 min intervals.

Up to 12 sheets of yuba were collected from Suzuyutaka soymilk. After the first and the sixth yubas were taken, 12 mL of soymilk was immediately collected with a Finnpipette from the top of the soymilk surface without stirring and cooled with tap water. The final soymilk was also collected. These soymilk aliquots were named Ss1, Ss6, and Ss12. Up to nine sheets of yuba were collected from the Fukuyutaka soymilk. After the first and the fifth yubas were taken, 12 mL of soymilk was collected with the same method as Suzuyutaka. The final soymilk was also collected. These soymilk aliquots were named Sf1, Sf5, and Sf9. Up to eight sheets of yuba were collected from Yumeminori soymilk. After the first and the fifth yubas were taken, 12 mL of soymilk was collected with the same method as Suzuyutaka. The final soymilk was also collected. These soymilk aliquots were named Sy1, Sy5, and Sy8. These showed that the soybean variety had an effect on the yuba film formation rate. Yuba could quickly form from Yumeminori (11S-rich) soymilk (250 g, 85 min), while yuba could not quickly form from Suzuyutaka (7S-rich) soymilk (250 g, 125 min). To collect soymilk uniformly, we collected soymilks in the way as stated above (Ss1, Ss6, and Ss12; Sf1, Sf5, and Sf9; and Sy1, Sy5, and Sy8).

**Lipid, Protein, and Carbohydrate Determination in Soymilk.** Fourier transform infrared spectroscopy (FT-IR) (SPECTRUM2000, PerkinElmer, Ltd., Beaconsfield, England) using attenuated total reflectance (ATR) sampling was used to determine the lipid, protein, and carbohydrate concentrations in soymilk simultaneously (*17*). The determination of protein concentration was performed by using amide II absorbance at wavenumber 1545 $cm^{-1}$ for protein, ester absorbance at 1745 $cm^{-1}$ for lipid, and C−C·C−O absorbance at 1000 $cm^{-1}$ for carbohydrate. Soymilk (0.5 mL) was placed on the ZnSe crystal cell. The IR region measured was from 4000 to 800 $cm^{-1}$ with 4 $cm^{-1}$ resolution; a total of five scans were made. The IR spectrum of DI water was used as the background. One sample was measured three times.

**Concentration Ratios of Lipid, Protein, and Carbohydrate in Soymilk.** To represent the extent by which the soymilk concentrations became progressively more concentrated during yuba formation as compared with the S1 (Ss1, Sf1, and Sy1), concentration ratios were calculated using the following equations:

$$\text{concentration ratio of lipid in } S_x = \text{lipid concentration in } S_x/\text{lipid concentration in S1} \quad (1)$$

$$\text{concentration ratio of protein in } S_x = \text{protein concentration in } S_x/\text{protein concentration in S1} \quad (2)$$

$$\text{concentration ratio of carbohydrate in } S_x = \text{carbohydrate concentration in } S_x/\text{carbohydrate concentration in S1} \quad (3)$$

The unit of concentration is g/100 g soymilk; $x$ = 5, 6, 8, 9, or 12.

**Separation of Soluble and Particulate Protein (Fukuyutaka).** The Sf1 viscosity was measured with Viscomate (VM-1G, Yamaichi Electronics Co., Ltd., Tokyo, Japan). The viscosities of Sf5 and Sf9 were prepared to the Sf1 viscosity with DI water. These two soymilks were named Sf5v and Sf9v. They (9.00 g each) were ultracentrifuged (156000*g*, 30 min) (*18*) and separated into floating (oil body), supernatant (soluble protein), and precipitate (mainly particulate protein) fractions. About 3 mL of supernatant was collected with a syringe from the three samples. The protein concentrations of these three supernatants, Sf5v, and Sf9v were measured by FT-IR. The floating fraction was collected with a pipet.

**Oil Body Preparation (Fukuyutaka).** The floating fractions collected above were solved in 30 mM Tris-HCl buffer (pH 8.6) to a final weight of 20.00 g, and sucrose (5.00 g) was added to make the concentrations of 20%. They were mixed till sucrose totally solved and were centrifuged (59800*g*, 30 min). The floating fraction (oil body) was collected and named as oil body 1, oil body 5, and oil body 9. They were used as the samples of particle size distribution.

**Particle Size Distribution of Oil Body.** The particle size distribution of oil body was measured using a laser light scattering instrument (Coulter LS230, Beckman Coulter, Fullerton, CA). A prefiltered (0.2 $\mu$m membrane filter, Advantec Toyo Co. Ltd., Tokyo, Japan) 30 mM Tris-HCl buffer (pH 8.6) was transferred into the instrument and was circulated at 50 L/min. Glass was selected for the optical model of light scattering. The refractive index (1.332) was used. After the background light scattering was determined, the oil body sample was dropped into the instrument. The particle size distribution was measured three times and displayed after about 5 min. The particle volume ratio (%) of the sample was shown against the particle diameters.

**Yuba Preparation from Fukuyutaka.** Soymilk (250.00 g) was put into a stainless steel container (15 cm × 13.5 cm × 4.5 cm) and was allowed to settle in a water bath (Isotemp Fisher general purpose water bath, Fisher Scientific) at 85 °C. The first film of yuba was collected after 15 min. The temperature of the soymilk surface center was measured, and it was 65 °C (the lowest in soymilk). The point where two container walls and the bottom joined together had the highest temperature (about 80 °C). Successive yuba films were taken at 10 min intervals. Up to 10 sheets of yuba were collected and named Y1−Y10. Every yuba started to form in the middle of the soymilk surface and developed gradually. The yuba surface area increased gradually from Y2 to Y10. After Y10 was collected, the remaining soymilk depth became very small, and the Y11 stuck on the container wall. So, it was not used as a yuba sample. After each yuba film was collected, the container was taken from the water bath carefully, and the water on the outer surface of this container was removed

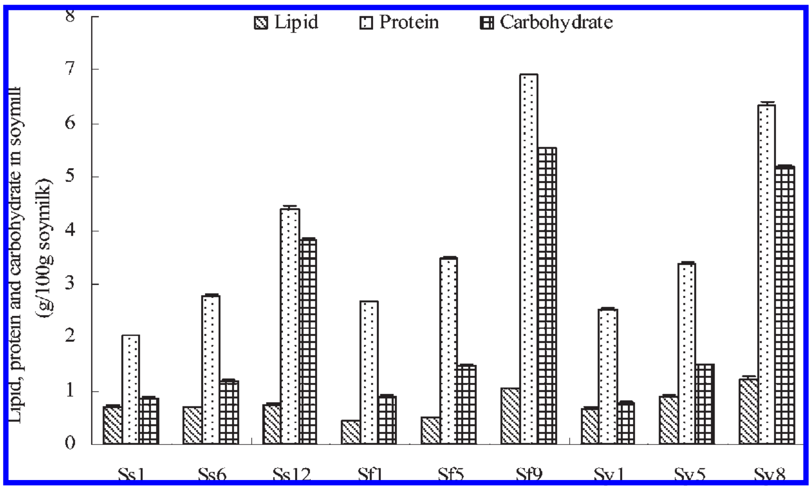


**Figure 1.** Lipid, protein, and carbohydrate concentrations (means ± standard deviations, $n = 3$) in Ss1, Ss6, Ss12, Sf1, Sf5, Sf9, Sy1, Sy5, and Sy8. Differences (except Suzuyutaka lipid) are significant ($P < 0.05$).

with a dry towel. Its weight was measured and put back into water bath as soon as possible. Then, the wet yuba was measured immediately. This experiment was repeated more than three times.

Wet yuba was dried at 105 °C for 3.5 h and cooled in a desiccator for 30 min. The dry yuba was very brittle, and its weight was measured. The TAG content of yuba was measured by the Soxhlet method, and hexane was used as the solvent. The sample for this determination was prepared by grinding the yuba in a miller for 5 min. The carbohydrate and protein contents were measured by subtracting TAG from the weight of dry yuba.

The contents of lipid, protein, and carbohydrate were also determined by FT-IR, which was used for soymilk above. The sample was prepared like this: Yuba was soaked in DI water for at least 12 h at 4 °C and then homogenized for 10 min at 2000 rpm with a homogenizer (Potter-Elvehjem type). The homogenate and the DI water used to rinse the homogenizer were pooled, and DI water was added to a final mass of 20 g. The homogenate was heated at > 95 °C for 5 min and cooled with tap water. This was used as a sample for FT-IR, and the contents of lipid, protein, and carbohydrate of each yuba were calculated. This result showed the same trend as the above method.

## RESULTS AND DISCUSSION

**Effect of Soybean Variety on Soymilk Composition Change in Yuba Making.** Suzuyutaka, Fukuyutaka, and Yumeminori were used to make yuba. The concentrations of lipid, protein, and carbohydrate of Ss1, Ss6, Ss12, Sf1, Sf5, Sf9, Sy1, Sy5, and Sy8 were determined (**Figure 1**). The lipid concentration increased little in Suzuyutaka soymilk, while it increased a little in Fukuyutaka and Yumeminori soymilk. Protein and carbohydrate concentrations increased sharply as the soymilk volume decreased. These observations were similar in all of the varieties tested.

The ratios of lipid, protein, and carbohydrate concentrations in Ss6, Ss12, Sf5, Sf9, Sy5, and Sy8 to their respective concentrations in soymilk S1 (Ss1, Sf1, and Sy1) were determined (**Figure 2**). This parameter may be very meaningful in elucidating the yuba formation process. If yuba did not form on the surface of the soymilk and only the water was lost by evaporation during heating, the concentration ratios of lipid, protein, and carbohydrate would remain the same over time. Therefore, the efficiencies of lipid, protein, and carbohydrate incorporation into the yuba can be estimated from the ratios of the soymilk concentrations before and after yuba collections. The concentration ratio for lipid changed the least in every soybean variety tested, while the ratio of protein remaining in the soymilk with each yuba collected increased more than lipid and that of carbohydrate increased the most. This suggests that lipid distributed between the yuba and the soymilk uniformly, whereas carbohydrate did not, with more remaining in the soymilk than became incorporated into the yuba. Thus, the soybean variety did not have an obvious effect on the ways in which lipid, protein, and carbohydrate were incorporated into yuba, although it had some effect on the yuba formation, such as the yuba film formation rate.

As stated above, soymilk mainly contains oil bodies, particulate proteins, soluble proteins, and carbohydrates. It was considered that the sizes of these compositions might have some effect on the ways in which lipid, protein, and carbohydrate were incorporated into yuba. The oil body, which was the largest, was readily incorporated into yuba, and carbohydrate, which was the smallest, was readily concentrated in the remaining soymilk. In one word, the smaller the particle size is, the more easily the particle is concentrated.

**Particle Size Distribution of Oil Body (Fukuyutaka).** It was known that the oil body surface was oleosin, a kind of alkaline protein, so oil bodies should have the same surface properties. However, oil bodies in soymilk are of several sizes; there are large ones and also small ones. Thus, oil bodies with different sizes might be concentrated in different ways. **Figure 3** shows the particle size distributions of oil body 1, oil body 5, and oil body 9. The size range of oil body was from 0.122 to 0.657 $\mu$m, and the average diameter was about 0.270 $\mu$m. It was shown that the small oil body percentage of oil body 9 was larger than those of oil bodies 5 and 1, while the large oil body percentage of oil body 9 was less than those of oil bodies 5 and 1. This was in agreement with the hypothesis above. Suzuyutaka and Yumeminori also showed the same trends.

**Separation of Particulate and Soluble Protein (Fukuyutaka).** Protein exists as soluble (< 40 nm) and particulate proteins (> 40 nm; average diameter, 70 nm) in soymilk. Particulate protein is larger than soluble protein. **Figure 4** shows that the soluble protein percentages in Sf1, Sf5v, and Sf9v are about 53.5, 56.5, and 60.0%, respectively. Thus, this was also in

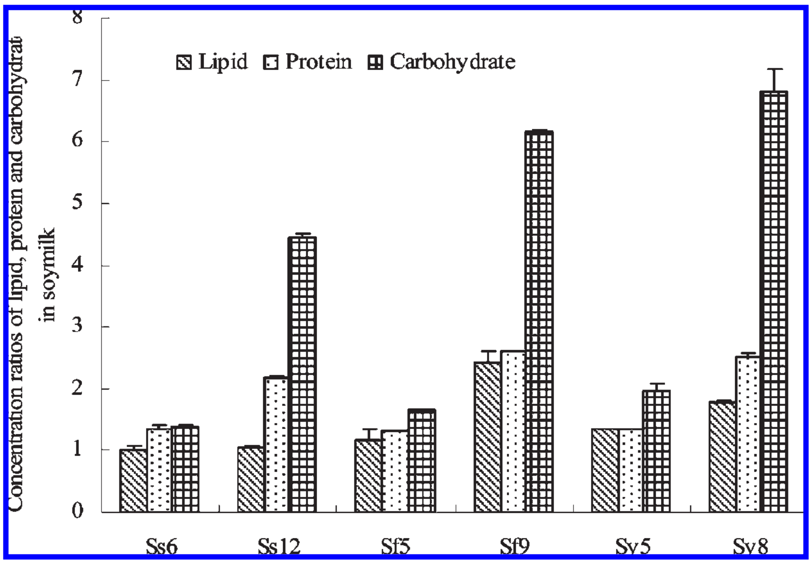


**Figure 2.** Concentration ratios (means ± standard deviations, $n = 3$) of lipid, protein, and carbohydrate in Ss6, Ss12, Sf5, Sf9, Sy5, and Sy8. Differences (except Suzuyutaka lipid) are significant ($P < 0.05$).

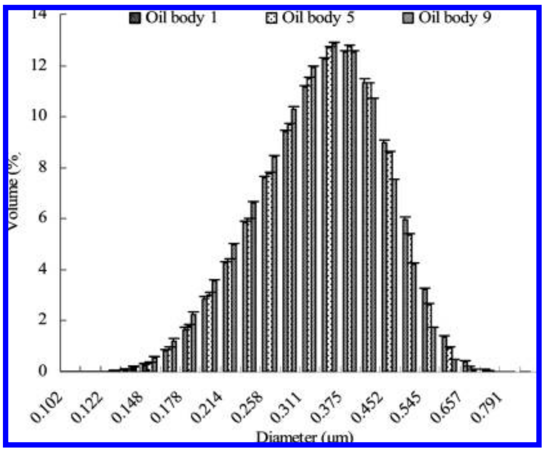


**Figure 3.** Particle size distributions (means ± standard deviations, $n = 3$) of oil body 1, oil body 5, and oil body 9 (Fukuyutaka). Differences between oil body 1 and oil body 9 are significant ($P < 0.05$).

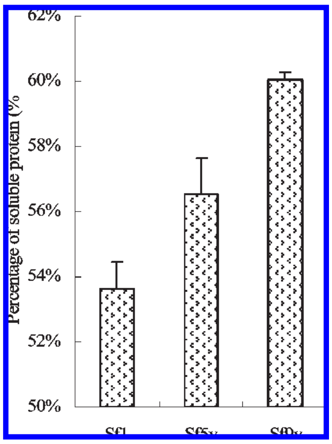


**Figure 4.** Percentages (means ± standard deviations, $n = 3$) of soluble protein in whole protein of Sf1, Sf5v, and Sf9v (Fukuyutaka). Differences are significant ($P < 0.05$).

agreement with the hypothesis above. Suzuyutaka and Yumeminori also showed the same trend as Fukuyutaka.

**Model for Yuba Formation.** From these results, we propose a model for yuba formation. Actually, the chemical change of protein is very important to yuba, but we want to focus on the sizes of soymilk compositions in this article.

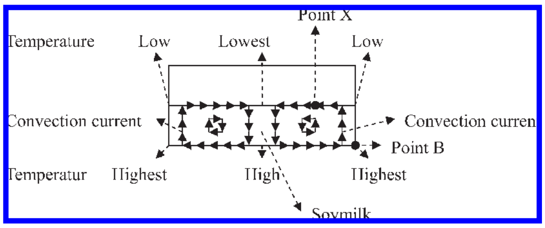


**Figure 5.** Schematic of the convection current caused by the temperature gradient in heated soymilk. The middle of the soymilk surface has the lowest temperature, and the temperature at point B has the highest temperature.

In unheated soymilk, oil bodies, particulate proteins, soluble proteins, carbohydrates, the other molecules, and irons move randomly, and these particles disperse uniformly. By heat treatment, a temperature gradient forms and the convective heat transfer happens in soymilk. The bottom soymilk whose temperature is higher than the top soymilk becomes less dense and rises. The top soymilk, which is cooler, moves to replace it. This cooler top soymilk is then heated, and the process continues, forming convection currents (**Figure 5**). The driving force is considered as buoyancy, a result of differences in soymilk density. Because the soymilk surface temperature is lower than the bottom temperature, this would cause the net particle diffusion from the surface to the bottom. At the same time, water molecules on the soymilk surface evaporate, and this causes the concentration of the surface soymilk. As a result, the concentration gradient forms, and particles in the surface soymilk have net diffusion downward. In whole, the temperature and concentration gradients cause net particle diffusion from the surface to the bottom and make soymilk establish an equilibrium, and there is one more forced stirring effect caused by the yuba collection process.

As stated above, particles in soymilk have different sizes. Lipids exist as oil bodies (average diameter about 270 nm), proteins exist as particulate proteins (>40 nm; average diameter about 70 nm) and soluble proteins (<40 nm), and carbohydrates exist in molecular form. According to the diffusion theory, their diffusion rates depend on $D$, which

**Table 1.** Weight Change of Remaining Soymilk, Yuba in the Yuba Making Process

| | first | second | third | fourth | fifth | sixth | seventh | eighth | ninth | tenth |
|---|---|---|---|---|---|---|---|---|---|---|
| yuba taken time (min) | 15.00 | 25.00 | 35.00 | 45.00 | 55.00 | 65.00 | 75.00 | 85.00 | 95.00 | 105.00 |
| remaining soymilk (g) | 222.26 | 203.13 | 183.09 | 163.71 | 143.77 | 123.69 | 103.33 | 83.90 | 62.10 | 40.82 |
| soymilk depth (mm) | 10.98 | 10.03 | 9.04 | 8.08 | 7.10 | 6.11 | 5.10 | 4.14 | 3.07 | 2.02 |
| wet yuba (g) | 1.70 | 1.37 | 1.53 | 1.68 | 1.78 | 1.90 | 1.98 | 2.18 | 2.31 | 2.44 |
| evaporated water (g) | 26.04 | 17.76 | 18.51 | 17.70 | 18.16 | 18.18 | 18.38 | 17.25 | 19.49 | 18.84 |
| dry yuba (g) | 0.46 | 0.41 | 0.45 | 0.50 | 0.56 | 0.61 | 0.69 | 0.78 | 0.82 | 0.90 |

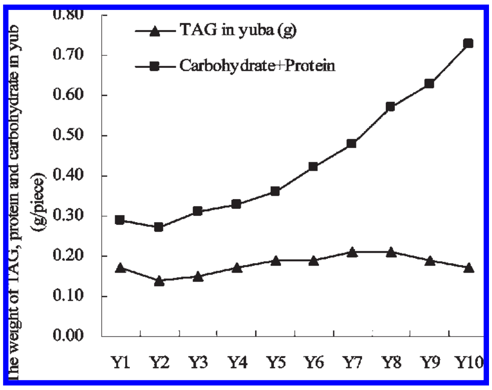


**Figure 6.** Weight changes (g/each yuba) of TAG, carbohydrate, and protein from Y1 to Y10.

could be determined by eq **4**.

$$D = RT/(6\pi\eta\, rN) \quad (4)$$

where $D$ is the diffusion coefficient (m$^2$/s), $R$ is a gas constant (J K$^{-1}$ mol$^{-1}$), $T$ is the absolute temperature (K), $\eta$ is viscosity (Pa s), $r$ is the radius of the molecule (m), and $N$ is Avogadro's number (mol$^{-1}$). The larger the $D$ is, the larger the rate is. $R$, $T$, $\pi$, and $N$ are constant. $\eta$ is different among different points in being heated soymilk. However, particles at the same point are in the same viscosity condition. So, their diffusion coefficients can be determined merely by their radius. The larger the radius is, the smaller the $D$ is. So, the $D$ increases in the order of oil body, particulate protein, soluble protein, and carbohydrate. In addition, oil body tends to float at the top layer because of its lower density than water.

Thus, we want to explain the yuba formation below: There is a point X (**Figure 5**) on the soymilk surface, and there are oil bodies, particulate proteins, soluble proteins, and carbohydrate molecules at this point. By the heating treatment, yuba starts to form on the surface. At the same time, oil bodies, particulate proteins, soluble proteins, and carbohydrates diffuse downward. The oil body is the slowest, and carbohydrate is the fastest. So, most oil bodies are incorporated into yuba film, while most carbohydrates diffuse downward and are concentrated in the remaining soymilk (**Figures 1** and **2**). Protein is the intermediate between lipid and carbohydrate. This also could be used to well explain why the particle size distribution of the oil body changes in the remaining soymilk (**Figure 3**).

This research is a laboratory process. In industrial situations, water and freshly prepared soymilk are injected into the processed soymilk system during yuba making to compensate for the water evaporated, and new solids are injected into the systems. **Figure 1** shows that the lipid percentage in remaining soymilk decreases in yuba making. As a result, the lipid percentage in yuba would decrease. Thus, lipid should also be added to make yuba almost have the same compositions with each other when water and freshly prepared soymilk are injected into the processed soymilk system. In addition, new yubas could be produced by replacing the soybean lipid with many kinds of other lipids, such as sesame lipid. In this way, the defatted soybean flour, which is mostly used as a feed, might be used to produce new yubas by using various lipids. This is considered very meaningful to the soybean industry.

**Yuba Preparation from Fukuyutaka. Table 1** shows the changes of remaining soymilk and wet yuba weight in the yuba making process. The wet yuba weight increased from Y2 to Y10. About 18 g of water could be evaporated in 10 min. The dry yuba weight also gradually increased from Y2 to Y10. This showed that more and more lipids, proteins, and carbohydrates were incorporated into the yuba. The remaining soymilk concentration (**Figure 1**) increased in the yuba-making process, and viscosity would also increase with the soymilk concentration. The viscosity increase caused the diffusion coefficients of the particles to decrease. As a result, the diffusion quantity of particles from soymilk surface would decrease and the dry yuba weight would increase.

**Figure 6** shows that carbohydrates and proteins increase obviously, while lipids increase from Y2 to Y8 and decrease from Y8 to Y10. Carbohydrates and proteins could be concentrated more quickly than oil bodies (**Figures 1** and **2**). So, more and more carbohydrates and proteins could be incorporated into yuba just as stated above. It was considered that the lipid content in each yuba was determined by the balance between the yuba surface area and the oil body diffusion coefficient. In the beginning, the yuba surface area increased gradually but could not increase continually because of the constant soymilk surface. **Figure 3** shows that the smaller oil bodies gradually increase in the yuba-making process, which could cause the diffusion coefficient to increase for oil body. The yuba surface area increase caused more oil bodies to be incorporated into yuba, while the large diffusion coefficient caused more oil bodies to be concentrated in the remaining soymilk, which caused the lipid changes in **Figures 6** and **2** (especially Sf9).

Lipids, proteins, and carbohydrates exist in different sizes as oil bodies, particulate proteins, soluble proteins, and carbohydrate molecules in soymilk. According to diffusion theory, the diffusion coefficient is mainly determined by the particle size, in which larger particles diffuse more slowly. As a result of the temperature gradient and concentration gradient, particles have a net diffusion from the surface to the bottom. Oil bodies are considerably larger particles and hardly diffuse at all into the soymilk beneath, which accounts for the minimal change in lipid distribution observed (**Figure 1**) during yuba formation. Greater attention has been paid in the past to the role of the protein components of the yuba film network (*21*, *22*) without consideration of the diffusion of the oil bodies, particulate proteins, soluble proteins, and carbohydrates. The soluble protein becomes concentrated, and the yuba composition changes with collection of successive yubas in a manner that is consistent with diffusion theory. Carbohydrates are the smallest components and diffuse the most readily. Therefore, it had the largest concentration ratio (**Figure 2**). As a result, lipids, proteins, and carbohydrates were concentrated at different rates with the formation of successive yubas. Thus, successive yuba sheets have different compositions depending on how long it took for a given yuba to form.

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