Emulsifying Properties of Soybean β -Conglycinin and Glycinin: Evaluation by Turbidimetry

Fumio YAMAUCHI, Yasushi OGAWA, Yoshiro KAMATA
and Kazuo SHIBASAKI

*Department of Food Chemistry, Faculty of Agriculture,
Tohoku University, Sendai 980, Japan*

Received January 22, 1981

The emulsifying properties of soybean β -conglycinin (7S protein) and glycinin (11S protein) were investigated by turbidimetric measurements after their emulsification with oil by an ultrasonic instrument. The emulsifying properties were also evaluated by emulsion stability and microscopic observations. The turbidity of the 7S protein-stabilized emulsion showed a larger value than that of the 11S protein-stabilized emulsion with less protein quantity. The pH-turbidity curve of the 7S protein-stabilized emulsion decreased sharply at pH 5.0 and was closely related to its emulsion stability. However, the pH-turbidity curve of the 11S protein-stabilized emulsion had a broad concave curve near pH 6.0. The low turbidities at the isoelectric point were ascribed to large oil particles in the case of 11S protein and to the flocculation of small oil particles in the case of 7S protein. At ionic strengths between 0.1 and 0.4, the turbidity of the 11S protein-stabilized emulsion decreased considerably, while that of the 7S protein-stabilized emulsion was unchanged. The turbidity changed according to the type of dilution buffer, even after emulsification.

Studies on the functional properties of soybean proteins have been investigated by many workers, and the emulsifying properties are one of the most important for many food applications.^{1,2)} These emulsifying properties are commonly evaluated by the methods of emulsifying capacity, emulsion stability and emulsion activity.^{1,2)} Yamauchi *et al.*³⁾ applied these three methods to the native and acetylated proteins of an acid precipitated protein (APP), 7S protein and 11S protein of the soybean. The native 7S protein always showed higher emulsifying properties than the 11S protein in various protein concentrations. Aoki *et al.*⁴⁾ have also obtained the results that 7S protein has higher emulsifying properties than 11S protein using these methods for various pH's of the protein solutions.

However, the mechanism of the emulsion formation of oil by a protein is difficult to understand by these methods. Pearce and Kinsella⁵⁾ have investigated the emulsifying properties by a turbidimetric technique. This method can evaluate the phenomenon of

emulsion formation related to the interfacial area of emulsion per unit weight of the protein. By modifying this method, we have proposed a method⁶⁾ which can measure the emulsifying properties of a small quantity of protein after emulsification with an ultrasonic instrument and followed by turbidimetry. The results with this method showed a parallel relationship between the turbidity and emulsion stability of APP.

Using turbidimetry, we describe here the effects of protein concentration, pH and ionic strength (μ) on the emulsifying properties of 7S and 11S protein-stabilized emulsions. In some experiments, an APP was used for the reference and the criteria of emulsifying stability and microscopic investigation were also used to confirm these results.

MATERIALS

11S protein (glycinin) was prepared by a rough fractionation at pH 6.4 and low μ ,⁷⁾ and purified by ammonium sulphate fractionation at 51~66% saturation.⁸⁾ 7S protein (β -conglycinin) was also prepared by rough frac-

tiation⁷⁾ and then chromatographed on a DEAE Sephadex A-50 column.⁹⁾ APP was prepared by extraction from defatted meal with a 0.03 M Tris-hydrochloric acid buffer (pH 7.8), followed by precipitation at pH 4.5 and by washing with the buffer. All the proteins were dialyzed and lyophilized. A commercial oil (mainly rape seed oil and others) was obtained from Ajinomoto Co. Inc.

METHODS

Measurement of emulsion turbidity. The standard method for measurement⁶⁾ is as follows. A mixture of a 1% protein solution (1.4 ml in a potassium phosphate buffer at pH 8.0, $\mu=0.1$) and oil (0.6 ml) was emulsified with a sonicator (Ohtake Seisakusho, Model 5202, 20 kHz) at 60 W for 30 min while cooling in an ice-water bath. To the emulsified solution, 8 ml of the phosphate buffer was added (the first dilution). A portion (0.02 ml) of the first diluted emulsion was further diluted with 10 ml of the same buffer (the second dilution). The turbidity of the emulsion was measured in a 1-cm pathlength cuvette at a wavelength of 500 nm with a spectrophotometer (Hitachi, Model 124). Unless otherwise stated, the above standard method was carried out in this study.

Measurement of emulsion stability.⁶⁾ The emulsion stability was measured using the method essentially described by Acton and Saffle.¹⁰⁾ Protein solutions and oil were emulsified according to the same method described above. The first diluted emulsions were permitted to stand for 24 hr at room temperature. A portion (2 ml) of the emulsion was removed from the bottom and the moisture content analyzed by lyophilization.

$$\text{Emulsion stability} = \frac{100 - M_{\text{test}}}{100 - M_{\text{original}}} \times 100\%$$

where M_{test} = percentage of moisture after 24 hr, and M_{original} = percentage of moisture in the original emulsion.

Microscopic observation. Micrographs of the emulsion were taken using an optical microscope (Nikon, model NE, 200 $\times$).

RESULTS

Effects of protein concentration and oil volume fraction on turbidity

In the standard procedures, the protein concentration was taken at 0.5, 1.0 and 2.0% and the oil volume fraction on 0.1, 0.3, 0.5 and 0.7. The total volume of the protein solution and the oil was always 2 ml. The standard procedure with 1.4 ml of the protein solution and 0.6 ml of oil corresponds to a 0.3 oil volume fraction. The results for 7S protein, 11S protein and APP are shown in Fig. 1.

The turbidity of those emulsions initially increased with protein concentration and, thereafter, decreased. The APP and 11S protein-stabilized emulsions showed the largest turbidity at an oil volume fraction of 0.3 with various protein concentrations. APP showed a lower turbidity than the others, and the 7S and 11S protein-stabilized emulsions gave nearly the same turbidity. At 1.0 and 2.0% protein concentrations, the APP-stabilized emulsion showed the same turbidity as the 11S protein-stabilized emulsion. The 7S protein-stabilized

FIG. 1. Effect of Oil Volume Fraction on Turbidity.

Proteins were dissolved in a potassium phosphate buffer (pH 8.0, $\mu=0.1$) and emulsified with various ratios of oil volume by an ultrasonic instrument. Protein concentration, 0.5% (a), 1.0% (b) and 2.0% (c). Emulsions stabilized by 7S protein (Δ), 11S protein ($\bullet$) and APP ($\circ$). Oil volume fraction = oil volume/volume of protein solution plus oil volume.

FIG. 2. Effect of Protein Concentration on Turbidity. Proteins were dissolved in a potassium buffer (pH 8.0, $\mu=0.1$) and emulsified at an oil volume ratio of 0.3 by an ultrasonic instrument. Emulsions stabilized by 7S protein (○), 11S protein (●) and APP (△).

FIG. 3. Effect of pH on Turbidity and Emulsion Stability of 7S Protein-stabilized Emulsion.

Protein (1%) was dissolved in water and adjusted to pH 1~10 with 0.5N hydrochloric acid or 0.5N sodium hydroxide. After emulsification by an ultrasonic instrument, the turbidity at 500 nm and emulsion stability were measured.

FIG. 4. Effect of pH on Turbidity and Emulsion Stability of 11S Protein-stabilized Emulsion.

Experimental methods were the same as those of Fig. 3.

emulsion gave a characteristic turbidity at an oil volume fraction of 0.5, where the turbidity increased with protein concentration, and showed the largest value among all the oil volume fractions and all the proteins.

The protein concentration in the standard procedure was varied from 0.5 to 5.0% and the turbidity measured as shown in Fig. 2. The turbidity of the 7S protein-stabilized emulsion was slightly higher than that of the 11S protein-stabilized emulsion at concentrations under 2%. However, all three proteins gave almost similar turbidity values in all concentrations with an oil volume fraction of 0.3.

Effects of pH on turbidity and emulsion stability

Proteins (1%, 7S and 11S proteins) were dissolved (partially suspended) in water and adjusted to pH's between 1 and 10 with 0.5N hydrochloric acid or 0.5N sodium hydroxide solution. The turbidity and emulsion stability of these emulsion are shown in Figs. 3 and 4. The turbidity and emulsion stability for 7S protein were closely related to each other and sharply decreased at the isoelectric point of the protein. The turbidity and stability for 11S protein were also related to each other, that is, they decreased towards pH 6.0 and then increased, giving a concave curve. In the region of lower and higher pH's, how-

ever, the relationship between turbidity and emulsion stability was different from that for 7S protein. In order to observe the state of the emulsion particles at the isoelectric point and at pH 8.0, the 7S and 11S proteins were dissolved and emulsified in a 0.2M sodium acetate buffer (pH 5.0) containing 0.1M sodium chloride and a potassium phosphate buffer (pH 8.0, $\mu=0.1$). The turbidity of the emulsions stabilized by 7S protein and 11S protein was measured at different pH's after the first and second dilutions with two buffers; either the same buffer which dissolved the

TABLE I. TURBIDITY OF EMULSIONS STABILIZED BY 7S PROTEIN AND 11S PROTEIN AT DIFFERENT pH'S

Protein	Emulsifying buffer (EB)	Dilution buffer	Turbidity (500 nm)	Micrograph
1% 11S	Potassium phosphate pH 8.0, $\mu=0.1$	The same as EB	0.42	Fig. 6a
		0.1% SDS in EB	0.80	Fig. 6b
	0.2 N sodium acetate pH 5.0 containing 0.1 M NaCl	The same as EB	0.00	Fig. 6c
		0.1% SDS in EB	0.00	Fig. 6d
3% 11S	Water pH 8.0 adjusted by NaOH	The same as EB	0.83	—
		0.2 N sodium acetate pH 5.0	0.41	—
1% 7S	Potassium phosphate pH 8.0, $\mu=0.1$	The same as EB	0.68	Fig. 5a
		0.1% SDS in EB	0.88	Fig. 5b
	0.2 N sodium acetate pH 5.0 containing 0.1 M NaCl	The same as EB	0.03	Fig. 5c
		0.1% SDS in EB	0.46	Fig. 5d
3% 7S	Water pH 8.0 adjusted by NaOH	The same as EB	0.86	—
		0.2 N sodium acetate pH 5.0	0.30	—

FIG. 5. Micrographs of 7S Protein-stabilized Emulsion at Different pH's.

Dilution buffers: a, pH 8.0; b, pH 8.0 with 0.1% SDS; c, pH 5.0; d, pH 5.0 with 0.1% SDS. Emulsifying buffers are shown in Table I.

protein or 0.1% SDS as shown in Table I. The micrographs of these emulsions are shown in Figs. 5 and 6.

FIG. 6. Micrographs of 11S Protein-stabilized Emulsion at Different pH's.

Dilution buffers: a, pH 8.0; b, pH 8.0 with 0.1% SDS; c, pH 5.0; d, pH 5.0 with 0.1% SDS. Emulsifying buffers are shown in Table I.

At pH 8.0, the turbidity of the 7S protein-stabilized emulsion was greater than that of the 11S protein-stabilized emulsion. The larger

turbidity may be ascribed to smaller and/or less flocculated oil particles which are stabilized by proteins. The results of the micrographs are consistent with the fact that the 7S protein-stabilized emulsion showed less flocculation than the 11S protein-stabilized emulsion (Figs. 5a and 6a). When the SDS solution was added, the emulsion turbidity was increased, especially for the 11S protein, and its flocculation was decreased (Figs. 5b and 6b).

At pH 5.0, the emulsions of both proteins showed low turbidities. These corresponded to large particles for the 11S protein-stabilized emulsion (Fig. 5c) and to small particles, but high aggregates, for the 7S protein-stabilized emulsion (Fig. 6c). When the SDS solution was added, the 11S protein-stabilized emulsion did not increase in turbidity. However, the 7S protein-stabilized emulsion increased in turbidity to a small extent. The 11S protein-stabilized emulsion showed apparently similar micrographs to those with no SDS in the buffer (Figs. 5d and 6d).

After the proteins (3%) were emulsified at pH 8.0 (in water adjusted by 0.1N sodium hydroxide), the dilution effect was determined by dilution with 0.2M sodium acetate buffer (pH 5.0) as shown in Table I. Both the 7S and 11S protein-stabilized emulsions decreased their turbidities at pH 5.0 to less than the half the values at pH 8.0 with a 3% protein concentration. This means that the proteins on the surface of an oil particle retained the ability to change their properties even after emulsification.

Effects of ionic strength on turbidity and emulsion stability

Proteins (2.1%) of 7S and 11S were dissolved in a phosphate buffer (pH 8.0) in various μ 's from 0.05 to 0.5 adjusted by sodium chloride. The turbidity was measured after dilution with the buffer of the same μ at emulsification. These turbidities together with the emulsion stabilities are shown in Fig. 7.

The turbidity of the 7S protein-stabilized

FIG. 7. Effects of Ionic Strength on Turbidity and Emulsion Stability of 7S Protein- and 11S Protein-stabilized Emulsions.

Proteins (2.1%) were dissolved in phosphate buffers (pH 8.0, $\mu=0.05$) with various μ adjusted by sodium chloride. After emulsification, the emulsions were diluted with the same buffer as those used in emulsification. Emulsions stabilized by 7S protein (a) and 11S protein (b).

emulsion was not changed with μ . However, the emulsion stability of the 7S protein-stabilized emulsion gradually increased with μ . Although the turbidity of the 11S protein-stabilized emulsion was almost the same as that of the 7S protein-stabilized emulsion at $\mu=0.05$, it decreased considerably at $\mu=0.1$ and further decreased up to $\mu=0.4$. The emulsion stability of the 11S protein-stabilized emulsion also decreased in this region, giving nearly a zero value from $\mu=0.1$ to 0.3. Thereafter, both the turbidity and the emulsion stability of the 11S protein-stabilized emulsion increased with μ .

Proteins (2%) of 7S and 11S were dissolved in a potassium phosphate buffer (pH 8.0, $\mu=0.1$) and emulsified. When the emulsions were diluted, μ was changed from 0.1 to 2.0 by the addition of sodium chloride to the phosphate buffer. In order to check the emulsion state with no flocculation, 0.1% SDS-water solution was used for dilution as a reference at $\mu=0$. Their turbidities are shown in Fig. 8.

The turbidity of the 7S protein-stabilized

FIG. 8. Effect of Ionic Strength in Dilution on Turbidity.

Proteins (2%) were dissolved in a potassium phosphate buffer (pH 8.0, $\mu=0.1$). After emulsification, the emulsions were diluted with a phosphate buffer adjusted to various μ 's with sodium chloride. At $\mu=0$, 0.1% SDS was used for dilution. Emulsions stabilized by 7S protein (Δ), 11S protein ($\circ$) and APP ($\bullet$).

emulsion at $\mu=0.01$ was nearly the same as that at $\mu=0.1$, increased slightly at $\mu=0.3$ and thereafter, decreased until $\mu=2.0$. The turbidity of the 11S protein-stabilized emulsion decreased considerably at $\mu=0.3$, then, increased and reached the same value of the 7S protein-stabilized emulsion at $\mu=2.0$.

DISCUSSION

In Fig. 1, the 7S and 11S protein-stabilized emulsions showed almost the same turbidities at an oil volume fraction of 0.3. However, the 7S protein-stabilized emulsion (1% and 2%) showed the largest turbidity at an oil volume fraction of 0.5. Larger oil volume fractions correspond with less protein. This means that the 7S protein has more emulsifying ability than the 11S protein. This is consistent with the results that the emulsifying capacity, the emulsion stability and the emulsifying activity of the 7S protein-stabilized emulsion were all higher at any protein concentration³⁾ and the former two properties were also higher at any pH's⁴⁾ compared with those of the 11S protein-stabilized emulsion.

The turbidity and emulsion stability of the 7S protein-stabilized emulsion (Fig. 3) showed a sharp decrease at pH 5.0, whereas these

properties for the 11S protein-stabilized emulsion gave a broad decrease at pH 6.0. This pH point of minimum turbidity resembles the maximum point of the precipitation curve for 7S protein (pH 5.0) and 11S protein (pH 5.7).⁷⁾ In these relationships, the 11S protein-stabilized emulsion always showed the maximum or minimum point with higher pH and broader curves than the 7S protein-stabilized emulsion. Aoki *et al.*⁴⁾ have reported that the emulsifying properties and the solubility of APP, 7S and 11S proteins were not always correlated with each other. However, the pH point was plotted for every 2.5 (pH) unit interval and consequently, closer pH points are needed in order to find out the real minimum point of the emulsion properties and their solubility.

The micrographs of the emulsions showed the particle size and the flocculated state of emulsified oil particles. These figures related well with their turbidities. Larger particles and/or flocculation of the oil particles gave lower turbidities.⁵⁾ At the isoelectric point, the low turbidity of the 11S protein-stabilized emulsion was dependent upon the large oil particles, while that of the 7S protein-stabilized emulsion was dependent upon the flocculated small particles.

Both the turbidity and the emulsion stability of the 11S protein-stabilized emulsion showed a characteristic decrease at low μ 's between 0.1 and 0.3. Generally, an increase of μ causes flocculation of the oil particles by the reduction of an electric bi-layer on the surface of oil particle stabilized by a protein.¹¹⁾ However, the increase in turbidity of the 11S protein-stabilized emulsion over $\mu=0.4$ cannot be explained by this reason. The turbidity of the 7S protein-stabilized emulsion did not decrease with an increase of μ . This may be explained by another reason for the inhibition of flocculation, for example, the effect of hydration of the surface of the particles.¹²⁾

When dilutions are carried out under conditions of pH or μ different from these employed in emulsification, the turbidity is mainly dependent upon the conditions at di-

lution, such as pH (from 8.0 to 5.0, Table I) and μ (Fig. 8). This means that a protein is changed by the conditions in an emulsion and the altered protein actually works on the surface of an oil particle even after emulsification.

REFERENCES

- 1) F. Yamauchi, *Nippon Shokuhin Kogyo Gakkaishi*, **26**, 266 (1979).
 - 2) J. E. Kinsella, *J. Am. Oil Chemists' Soc.*, **56**, 242 (1979).
 - 3) F. Yamauchi, N. Hishinuma, H. Ono and K. Shibasaki, *Nippon Shokuhin Kogyo Gakkaishi*, **25**, 446 (1978).
 - 4) H. Aoki, O. Taneyama and M. Inami, *J. Food Sci.*, **45**, 534 (1980).
 - 5) K. N. Pearce and J. E. Kinsella, *J. Agric. Food Chem.*, **26**, 716 (1978).
 - 6) Y. Ogawa, F. Yamauchi and K. Shibasaki, *Nippon Shokuhin Kogyo Gakkaishi*, **27**, 631 (1980).
 - 7) V. H. Thanh and K. Shibasaki, *J. Agric. Food Chem.*, **24**, 1117 (1976).
 - 8) W. J. Wolf, G. E. Babcock and A. K. Smith, *Arch. Biochem. Biophys.*, **99**, 265 (1962).
 - 9) V. H. Thanh, K. Okubo and K. Shibasaki, *Plant Physiol.*, **56**, 19 (1975).
 - 10) J. C. Acton and R. L. Saffle, *J. Food Sci.*, **35**, 852 (1970).
 - 11) V. H. Lange and C. P. Kurzendorfer, *Fette Seifen Anstrichmittel*, **76**, 121 (1974).
 - 12) M. Karel, *J. Food Sci.*, **38**, 756 (1973).
-