Solubility Testing of Sucrose Esters of Fatty Acids in International Food Additive Specifications

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We investigated the solubility of 10 samples of sucrose esters of fatty acids (SEFA) products that are commercially available worldwide as food additives (emulsifiers). Although one sample dissolved transparently in both water and ethanol, other samples produced white turbidity and/or precipitates and did not meet the solubility criterion established by the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Expert Committee on Food Additives (JECFA). When the sample solutions were heated, the solubility in both water and ethanol increased. All of the samples dissolved transparently in ethanol, and dispersed and became white without producing precipitates in water. The present study suggests that the current solubility criterion of the JECFA SEFA specifications needs to be revised.

Key words: sucrose fatty acid ester; solubility; specification; emulsifier; food additive

Sucrose esters of fatty acids, in which hydroxyl groups are esterified with fatty acids, are used worldwide as emulsifiers and stabilizers in food, cosmetic, and pharmaceutical products. For food additives, fatty acids derived from edible fats and oils (lauric acid, palmitic acid, stearic acid, oleic acid, etc.) are used. Stearic acid-type products are preferentially used because of their flavor and functional characteristics. As a sucrose molecule has 8 hydroxyl groups, it can produce esters ranging from mono- to octaesters. Commercial products produced using the methods described in the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Expert Committee on Food Additives (JECFA)—“Sucrose esters of fatty acids” specifications—are thought to be mixtures of these sucrose esters. It is known that products in which the monoester content is higher or the chain length of the fatty acids esterified to sucrose is shorter exhibit more hydrophilic properties. A representative structure of sucrose monostearate is shown in Fig. 1.

Sucrose esters of fatty acids are approved for use as a food additive in a number of countries, and the specifications for its use are established in those countries to ensure the quality and stability of the products. In particular, the specifications of the JECFA are considered international food additive specifications. According to the specifications of the JECFA, European Union (EU), and United States, “sucrose esters of fatty acids” must contain a total content of mono-, di-, and triesters of not less than 80%. A substance that has a lower mono- to triester content (i.e., that has a much higher esterified composition [tetra- or higher esters]) is designated a “sucrose oligoesters.” In Asian countries, including Japan, China, South Korea, and Taiwan, there is no restriction on ester composition, and all products are approved under the single designation “sucrose esters of fatty acids” (Table 1).

In this study, we use the term SEFA to indicate that the product is hydrophilic and composed of sucrose esters of fatty acids for which the total mono- to triester content is not less than 80%, as defined by the JECFA, EU, and United States. SEFA has been approved in a number of countries and are used in oil-in-water emulsion-type foods (e.g., whipped cream), wheat products (e.g., bread, biscuits, and cakes), and confectionaries (e.g., chewing gums and candies).

The JECFA first evaluated the safety of SEFA in 1969. Subsequently, safety evaluations were carried out based on newly available animal and human studies in the 44th (1995) and 49th (1997) meetings. Solubility testing is required for the identification of SEFA according to the JECFA specifications, and this requirement has not been changed for nearly 40 years. Japan, China, South Korea, and Taiwan do not have a solubility criterion in their specifications; however, solubility testing in the JECFA specifications is now commonly required when SEFA products are used in foods sold worldwide.

In this study, we investigated the solubility of samples of commercially available SEFA products based on the JECFA methods. Most SEFA product samples did not dissolve in either water or ethanol and thus did not meet the JECFA SEFA solubility criterion. In particular, we found that SEFA product

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samples that were test materials in the safety studies evaluated by the JECFA did not meet the solubility criterion of the JECFA SEFA specifications. We discuss some of the factors that may have influenced these results.

MATERIALS AND METHODS

Samples and Reagents  
Ten commercially available SEFA products were provided by Japanese and European companies. Six samples of RYOTO SUGAR ESTERS (L-1695, L-595, P-1670, S-1670, S-1170, and S-570) were obtained from Mitsu­bishi-Kagaku Foods Corporation (Tokyo, Japan). They were manufactured by Mitsubishi Chemical Corporation (Tokyo, Japan). Two samples of DK ESTER (F-110 and F-50) were obtained from DKS Co., Ltd. (Kyoto, Japan). Two samples of DUB SE (11S and 5S) were obtained from Stearinerie Dubois SA (Boulogne-Billancourt, France). S-1170 and S-570 were test materials in the safety studies evaluated by the JECFA. Except for the solubility criterion, all samples met the JECFA SEFA specifications.

The water used was deionized (≤1 µS/cm) and obtained from a water purification system (Classpure C-10P, Mitsubishi Rayon Aqua Solutions Co., Ltd., Tokyo, Japan). Ethanol (99.5% (w/w)) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Properties of Individual Samples

1) Analysis of Monoester Content

The monoester contents of the 10 samples were analyzed by gel permeation chromatography (GPC) according to the JECFA test methods using an HLC-8320GPC (Tosoh Corporation) equipped with a refractive index detector, TSKgel G10000HXL, TSKgel G2000HXL, TSKgel G3000HXL and TSKgel G4000HXL columns (all 300×7.8 mm i.d., 5 µm) (Tosoh Corporation) were tandemly connected and used for the separations. The SEFA samples were dissolved in tetrahydrofuran at a concentration of 5 mg/mL. The injection volume was 80 µL, the flow rate was 0.8 mL/min, and the mobile phase was HPLC-grade degassed tetrahydrofuran. The columns and refractive index detector temperatures were set at 40°C. The percentage of monoester content in each sample was calculated by the following formula:

\[
\%\text{ sucrose monoester} = 100 \times \frac{A}{T}
\]

where \(A\) is the peak area of the monoester and \(T\) is the sum of all peak areas eluting within 43 min of initiating elution.

2) Analysis of Fatty Acid Composition

The fatty acid composition of the 10 samples was analyzed by gas chromatography with flame ionization detection (GC-FID) according to the Standard Methods for the Analysis of Fats, Oils, and Related Materials, as edited by the Japan Oil Chemists’ Society.

Fatty acid composition was analyzed on an Agilent 7890B GC (Agilent Technologies Japan, Ltd., Tokyo, Japan) equipped with an Agilent 7693 autosampler (Agilent Technologies Japan, Ltd.) and a flame ionization detector (FID). An Agilent J&W DB-WAX column (30 m×0.53 mm i.d., 1 µm) (Agilent Technologies Japan, Ltd.) was used for the separations. SEFA samples were silylated using a Fatty Acid Methylation Kit (100 tests) (Nacalai Tesque, Inc., Kyoto, Japan). One microliter of sample was injected into a splitless liner using an inlet temperature of 240°C and a 4 mL/min nitrogen gas flow rate. The FID temperature was 240°C. The percentage of fatty acid composition in each sample was calculated by the following formula:

\[
\%\text{ fatty acid} = 100 \times \frac{A}{T}
\]

where \(A\) is the peak area of the fatty acid, and \(T\) is the sum of all peak areas eluting within 60 min of initiating elution.

Solubility Testing

Solubility was investigated according to the JECFA test methods. The solubility criterion of the JECFA describes SEFA as “sparingly soluble in water, soluble in ethanol.” Following the descriptive terms for solubility according to JECFA and shown in Table 2, solubility testing in water was conducted at the lowest-limit concentration of “sparingly soluble” (1% (w/w)) and that in ethanol at the lowest-limit concentration of “soluble” (3.3% (w/w)).

1) Solubility Testing in Water

A sample of SEFA product (1 g) was placed in a 200-mL conical flask, to which 100 g of water (at approximately 20°C) was added. After sealing with Parafilm, the flask was shaken lightly to disperse the sample. Subsequently, the flask was placed in a 20°C water bath (constant temperature immersion-type controller Thermomate BF-500, Yamato Scientific Co., Ltd., Tokyo, Japan) and shaken for 5 min by hand. Solubility was assessed visually.

2) Solubility Testing in Ethanol

A sample of SEFA product (1.6 g) was placed in a 100-mL conical flask, to which 48 g of ethanol (at approximately 20°C) was added. After sealing with Parafilm, the flask was shaken lightly to disperse the sample. Subsequently, the flask was placed in a 20°C water bath and shaken for 5 min by hand. Solubility was assessed visually.
Effect of Heating on Solubility Solubility was also tested after samples were heated to increase the solubility.

1) Solubility Testing in Water
   A sample of SEFA product (1 g) was placed in a 200-mL conical flask, to which 100 g of water (at approximately 20°C) was added. After sealing with Parafilm, the flask was shaken lightly to disperse the sample. Subsequently, the flask was placed in a 60°C water bath and shaken for 5 min by hand. Solubility was assessed visually.

2) Solubility Testing in Ethanol
   A sample of SEFA product (1.6 g) was placed in a 100-mL conical flask, to which 48 g of ethanol (at approximately 20°C) was added. After sealing with Parafilm, the flask was shaken lightly to disperse the sample. Subsequently, the flask was placed in a 50°C water bath and shaken for 5 min by hand. Solubility was assessed visually.

Measurement of Transmittance A sample of SEFA product (1 g) was placed in a 200-mL conical flask, to which 100 g of water (at approximately 20°C) was added. After sealing with Parafilm, the flask was shaken lightly to disperse the sample. Subsequently, the flask was placed in a 60°C water bath and shaken for 5 min by hand. The inside small bubbles resulting from shaking was removed by sonication for 10 min in an ultrasonic cleaner (2510J-MT, Yamato Scientific Co., Ltd.). Immediately after this step, the sample was placed in a 1-cm quartz cell, and the light transmittance was measured at a scan width of 0.2 nm over the range of 350 to 780 nm using a spectrophotometer (UV-1800, Shimadzu Corp., Kyoto, Japan).

RESULTS

Properties of Individual Samples To investigate the properties of each SEFA product sample, the monoester content was analyzed by gel permeation chromatography, and the fatty acid composition was analyzed by gas chromatography with flame ionization detection. The results are shown in Table 3. The lauric acid-type samples (L-1695 and L-595) consisted of nearly 100% lauric acid. As for the palmitic acid- and stearic acid-type samples, they consisted of a mixture of palmitic and stearic acid. The monoester content of these samples ranged from 27 to 80%. The monoester content of the L-1695, P-1670, and S-1670 samples was approximately 80%.

Solubility Testing The solubility of each SEFA sample was evaluated according to the JECFA test methods. The
results are shown in Table 4. The results of solubility tests of L-1695, P-1670, S-1170, and S-570 are also shown in photographs (Fig. 2). Nine samples produced white turbidity and precipitates in water, and 6 samples produced precipitates in ethanol. Only the L-1695 sample dissolved transparently in both water (1% (w/w)) and ethanol (3.3% (w/w)) and met the solubility criterion of the JECFA SEFA specifications (sparingly soluble in water, soluble in ethanol). P-1670 dissolved transparently in ethanol but produced white turbidity in water; thus, it did not meet the solubility criterion of the JECFA SEFA specifications. Only L-1695 met the solubility criterion of JECFA SEFA specifications.

**Effect of Heating on Solubility** We also investigated the effect of temperature on solubility, as the solubility of emulsifiers is generally known to increase following heating.10) The melting points of the SEFA product samples ranged from 35 to 50°C (data not shown). Thus, we investigated their solubilities at 50°C in ethanol in this study. As for the solubilities in water, there are some emulsifiers of which solubility criterion in water is specified under heating conditions such as warm water in the JECFA specifications although warm water is not defined in the JECFA test methods and procedures.11) In this study, we investigated their solubilities at 60°C in water because the temperature of warm water is defined as from 60 to 70°C in the Japanese Pharmacopoeia. The results are shown in Table 5. The results of the solubility tests are also shown in photographs (Figs. 3, 4). L-1695 dissolved transparently in both water and ethanol, as expected. In ethanol, other samples dissolved transparently. As shown in Fig. 3, in water, P-1670 and S-1670 dispersed to assume a slightly white appearance, whereas other samples, such as L-595, S-1170, S-570, F-110, F-50, 11S, and 5S also assumed a white appearance without producing precipitates. Thus, the solubility of the SEFA samples increased following heating; however, none of the samples except L-1695 dissolved transparently in water.

**Measurement of Light Transmittance** After the water solubility of heated samples was tested, the light transmittance of the sample solutions was measured. The results are shown in Fig. 5. Samples that dissolved transparently or dispersed to assume a slightly white appearance, such as L-1695 and S-1670, showed more than 90% light transmittance in the wavelength range of 350 to 700 nm. Moreover, the light transmittance of L-1695 in the identical wavelength range was higher than that of S-1670. On the other hand, S-570 and S-1170 dispersed to assume a white appearance and showed less than 35% and less than 90% light transmittance, respectively, in this wavelength range. Thus, samples with a less turbid appearance showed higher light transmittance in the wavelength range of 350 to 700 nm.

**DISCUSSION**

In this study, we investigated the solubility of 10 SEFA samples using the JECFA test methods and found that 9 of these samples produced white turbidity or precipitates and did
not dissolve transparently in water. The present study showed that most of the SEFA products commercially available worldwide do not meet the solubility criterion of the JECFA SEFA specifications. Although we do not know the details of the process that the JECFA used to establish the solubility criterion as “sparingly soluble in water, soluble in ethanol,” we considered that very pure sucrose monoesters of fatty acids, such as those used as reagents, had been used to establish the solubility criterion. Sample L-1695, which was the only sample that dissolved transparently in both water and ethanol and met the solubility criterion of the JECFA SEFA specifications, has a high content of lauric acid and high content of monoesters. However, S-1670 and P-1670, which also have a high monoester content, produced white turbidity or precipitates in water. These results suggest that not only the content of monoesters but also the type of fatty acids affects the solubility in water.

Although L-1695 meets the solubility criterion of the JECFA SEFA specifications, this product is rarely used as a food additive because of its strong bitter taste. It is often used as a raw material in preparing antifogging agents for food containers or detergents for washing foods. Generally, stearic acid-type SEFA products are used as food additives; however, they did not dissolve transparently and did not meet the solubility criterion of the JECFA SEFA specifications. Furthermore, the stearic acid-type samples S-570 and S-1170, which also did not meet the solubility criterion of the JECFA SEFA specifications, were used as test materials in the safety studies of SEFA evaluated by the JECFA. Accordingly, the current
JECFA solubility specification for SEFA needs to be revised. As the JECFA specifications are international standards, it is extremely important that worldwide commercially available food additives meet the specifications from the perspectives of quality and safety assurance. In addition, according to the Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS agreement), if an international trade dispute would occur, judgment will most likely be made based on the international standard. Therefore, the fact that worldwide commercially available SEFA products do not meet the current solubility criterion of the JECFA SEFA specifications may cause international trade conflicts.

In our present study, we found that the solubility of SEFA product samples in water or ethanol increased when the samples were heated. In water at 60°C, all of the samples dispersed and became white without producing precipitates, and in ethanol at 50°C, all of the samples dissolved transparently. We conclude that when the sample solutions were heated close to the melting points of the SEFA products, the solubility increased due to an increase in the molecular movement of the hydrophobic groups (i.e., the fatty acid moieties). In addition, the results of measurement of light transmittance showed that the appearance of the water-solubility at 60°C for SEFA product samples is involved in the light transmittance in the wavelength range of 350 to 700 nm.

CONCLUSION

Only 1 of 10 SEFA product samples tested in the present study met the solubility criterion of the JECFA SEFA specifications. The solubility improved upon heating; that is, the heated samples dissolved transparently in ethanol and dispersed and became white without producing precipitates in water. This study suggests that the current solubility criterion of the JECFA SEFA specifications needs to be appropriately revised, as most of the SEFA products commercially available worldwide do not meet the current solubility criterion of the JECFA SEFA specifications.

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Conflict of Interest Chiye Tatebe, Kyoko Sato and Hirosi Akiyama have no conflict of interest. Yukino Nagai, Satoshi Kawano and Masaaki Tomida are employees of the Mitsubishi Chemical Corporation, Chiyoda-ku, Tokyo, Japan. Kenichiro Motoda is an employee of the Mitsubishi-Kagaku Foods Corporation, Yokohama, Japan.

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