A gas chromatographic (GC) method was developed for the determination of sucrose monoesters of fatty acids (mono-SuE) and sucrose acetate isobutyrate (SAIB) in food additive premixes. Mono-SuE and SAIB fractions were prepared by column chromatography with either a C8 or a silica gel solid-phase extraction column. The mono-SuE fraction was acetylated and applied to a wide-bore GC column (0.53 mm × 15 m) by splitless injection for determination. The SAIB fraction was applied to the GC column without derivatization. Gas chromatography/mass spectrometry was used to confirm the identity of GC peaks. The detection limits for mono-SuE and SAIB were 0.005 and 0.01 %, respectively. Mono-SuE (C12, C14, C16, C18, and C18:1) and SAIB were found in commercial food additive premixes and some foods.

Sucrose esters of fatty acids (SuE) are widely used as emulsifiers in food. Their use as food additives is permitted in many countries including the United States, countries of the European Union (EU), and Japan (1, 2). Commercial products are available with a wide range of hydrophilic lipophilic balance (HLB) values, which are controlled by the degree of esterification and the type of fatty acid; thus, emulsifiers are a complex mixture of mono-, di-, and oligoesters with various types of fatty acids. Products with high HLB values are among the most popular hydrophilic emulsifiers for processed food in Japan. We found that the matrixes of SuE premixes for food additive use which we collected contained water, glycerin, and other polar compounds, and that the SuE in these premixes were, therefore, considered to work as hydrophilic emulsifiers. Because monoesters are dominant in hydrophilic SuE, we decided to determine the monoesters (mono-SuE) food additive premixes.

Sucrose acetate isobutyrate (sucrose diacetate hexaisobutyrate, SAIB) is used as a weighting agent in beverages, to prevent flavoring oil from separating from the aqueous phase. The legislation governing its use differs among countries. In EU countries and in the United States, SAIB is regarded as a different substance from SuE. Whereas in Japan, SAIB is regarded as SuE; therefore, package labeling for food additive premixes as “sucrose esters of fatty acid” indicates admixture of either SuE or SAIB. And it is difficult to know which of them were admixed to the food additive premix.

In the present study, we developed a simple method to determine both mono-SuE and SAIB in food additive premixes by gas chromatography (GC). Gas chromatography/mass spectrometry (GC/MS) was used to confirm the identity of GC peaks. Sample solutions for GC and GC/MS were prepared with either a C8 or a silica gel solid-phase extraction (SPE) column, and mono-SuE was determined after acetylation. Levels of SuE and SAIB in food additive premixes and in some foods were determined.

**Experimental**

**Samples**

Eight food additive premixes, one vitamin-enriched rice, and one canned ready-to-drink coffee were collected in the Japanese marketplace. The package labeling for these products indicated admixture of SuE (or SAIB), or indicated admixture to emulsifier for canned ready-to-drink coffee.

**Standards and Reagents**

Standard mono-SuE DK-SS (sucrose monoesters of palmitic [C16] and stearic [C18] acids) was donated by Daichi Kogyo Seiyaku Ltd. (Kyoto, Japan); standard SuE L-1695 (sucrose esters of lauric acid [C12]) and standard O-1570 (sucrose esters of oleic acid [C18:1]) were donated by Mitsubishi Chemical Foods Ind. (Tokyo, Japan); and standard sucrose acetate isobutyrate (SAIB) was donated by Eastman Chemical Japan Ltd. (Tokyo, Japan).

SPE column Bond Elut® (silica gel, 500 mg) was from Varian, Inc. (Palo Alto, CA), LiChrolut RP-select B (C8, ca 40–63 μm, 500 mg) was from Merck KGaA (Darmstadt, Germany), and tetrahydrofuran (THF) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

**Instruments**

A Hewlett-Packard GC-6890 gas chromatograph with a flame ionization detector (Hewlett-Packard, Palo Alto, CA) was used for GC. An Automass 50 mass spectrometer (electron ionization, JEOL Ltd., Tokyo, Japan) and a
Figure 1. Matrixes of the SuE premixes.

Figure 2. Elution profiles of mono-SuE, SAIB, and matrix compounds from a C₈ column.
Figure 3. Gas chromatogram obtained for the methanol–THF (1 + 1) fraction eluted from a C8 column after a solution of a β-carotene premix was applied to the column and water–methanol (1 + 1) was passed through the column. SuE(C16) = sucrose monopalmitate (C16); SuE(C18) = sucrose monostearate (C18).

Figure 4. Elution profiles of mono-SuE, SAIB, and matrix compounds from a silica gel column.
Hewlett-Packard HP 5890 series II gas chromatograph were used for GC/MS.

**Sample Preparation**

**Method 1.**—An SPE RP-select B column was washed with 10 mL methanol and 10 mL water successively before the sample solution was transferred to the column. A 1.0 g portion of sample was dissolved in 5–50 mL of water–methanol (1 + 1). A 1 mL aliquot of the sample solution (<2 mg total SuE and SAIB) was transferred to the column, and the column was washed with 10 mL water–methanol (1 + 1). SAIB and mono-SuE were eluted with 10 mL methanol–THF (1 + 1). The fraction was evaporated to dryness under reduced pressure and acetylated with 0.5 mL pyridine and 0.5 mL acetic anhydride for 30 min at 35°C. After evaporation of the reagents under a nitrogen stream, the residue was dissolved in 2 mL ethyl acetate. A 1 µL aliquot of the ethyl acetate solution was injected into the gas chromatograph.

**Method 2.**—The solid sample was ground to a powder. A 1.0 g portion of sample (powder, liquid, or cream) was extracted twice with 50 mL THF. The THF phases were filtered and concentrated to 20 mL under reduced pressure to prepare a sample solution for column chromatography. A silica gel column was washed with 10 mL diethyl ether–ethyl acetate (3 + 7) before the sample solution was transferred to the column. An aliquot of the sample solution (<2 mg total SuE and SAIB) was evaporated to dryness with a stream of nitrogen. The residue was transferred to the column with a small amount of diethyl ether–ethyl acetate (3 + 7). SAIB was eluted with 10 mL diethyl ether–ethyl acetate (3 + 7). Mono-SuE were eluted with 10 mL ethyl acetate–methanol (1 + 1).

The SAIB fraction was evaporated to dryness under reduced pressure, and the residue was dissolved in 2 mL ethyl acetate. The mono-SuE fraction was also evaporated to dryness and acetylated in the manner described for Method 1. A 1 µL aliquot of the ethyl acetate solution was injected into the gas chromatograph.

Method 1 was applied to samples that were soluble in water–methanol (1 + 1), and contained no diglycerides (DG). Method 2 was applied to samples that were not soluble in water–methanol (1 + 1), or samples that contained DG.

**GC and GC/MS Analyses**

**GC conditions.**—Splitless injection onto a BPX-5 column (0.53 mm × 15 m; liquid-phase thickness, 1 µm; SGE International Pty. Ltd., Ring Wood, Australia) was used. The injector temperature was set at 340°C. The temperature of the flame ionization detector was 360°C. The temperature of the flame ionization detector was 360°C. The column oven temperature program was as follows: 150°C for 2 min; from 150 to 320°C at 10°C /min; from 320 to 350°C at 5/min; hold at 350°C for 20 min. The column head pressure was set at 70 kPa.

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**Figure 5.** Gas chromatograms obtained for a vitamin-enriched rice: (a) THF extract; (b) diethyl ether–ethyl acetate (3 + 7) fraction from a silica gel column; and (c) ethyl acetate–methanol (1 + 1) fraction from a silica gel column. SuE(C_{16}) = sucrose monopalmitate (C_{16}); SuE(C_{18}) = sucrose monostearate (C_{18}).
Figure 6. Mass spectra of sucrose (acetylated), standard SuE (acetylated), and standard SAIB: (a) sucrose; (b) sucrose monolaurate (C_{12}); (c) sucrose monopalmitate (C_{16}); (d) sucrose monostearate (C_{18}); (e) sucrose monooleate (C_{18:1}); and (f) SAIB.
Table 1. Fragment ions (m/z) in the mass spectra of sucrose esters of monofatty acids (FA) and SAIB

<table>
<thead>
<tr>
<th>Compound</th>
<th>FA1</th>
<th>FA2</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SuE</td>
<td>390</td>
<td>530</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Mono-SuE of FA(C12)</td>
<td>524</td>
<td>566</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Mono-SuE of FA(C14)</td>
<td>552</td>
<td>596</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Mono-SuE of FA(C16)</td>
<td>580</td>
<td>614</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Mono-SuE of FA(C18)</td>
<td>608</td>
<td>612</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>SAIB</td>
<td>846</td>
<td>846</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

GC/MS conditions.—GC/MS analyses were performed to confirm the identity of GC peaks. Splitless injection onto a BPX-5 column (0.25 mm × 15 m; liquid-phase thickness, 0.25 μm; SGE International Pty. Ltd.) was used. The injector temperature was set at 340°C. The column oven temperature program was as follows: 150°C for 2 min; from 150 to 350°C at 20°C/min; hold at 350°C for 8 min. The ion source temperature was 210°C, the interface temperature was 300°C, and the scanned masses ranged from 40 to 700 amu. The uncoated capillary tube (0.2 mm × 0.5 m, deactivated) was connected at the end of the separation column in series as a transfer line to the ion source.

Determination of Mono-SuE and SAIB

The level of mono-SuE in the sample was calculated by using the calibration curve obtained with standard sucrose monoester (DK-SS), acetylated under the same conditions as described in the sample preparation section. The level of SAIB in the sample was calculated by using the calibration curve obtained with standard SAIB.

Results and Discussion

Sample Preparation

The characterization of the samples is shown in Figure 1. Sorbitol was determined after acetylation under the same GC conditions as were used for SuE. Other compounds were determined as reported (3–5). Annatto colors contained 85% water and 10% ethanol. The total amounts of polar compounds (water, ethanol, glycerin, propylene glycol, and sorbitol) in β-carotenes, paprika color, flavor premix, and emulsifier premixes were approximately 45–90%. Thus, the SuE in the samples was speculated to be used as a hydrophilic (oil/water type) emulsifier, in which monoesters were dominant. β-Carotenes and the flavor premix contained medium-chain triglycerides (MCT), and emulsifier premixes contained monoglycerides. Because of the diversity of the sample matrices, we used 2 separate methods for sample preparation.

A reversed-phase column was used for the preparation of samples that were soluble water–methanol (1 + 1). A portion of the solution was transferred to the column, and polar substances in the matrices were eluted with water–methanol (1 + 1) before the mono-SuE were eluted. C18, with and without endcaps, and C8 columns were tested as sample preparation columns. Elution profiles of standard mono-SuE from the 3 columns were obtained. DK-SS (mixture of sucrose monooesters of C16 and C18 fatty acids) was diluted with methanol–water (1 + 1) and applied to the columns. Only about 25–50% of the mono-SuE was eluted from any of the columns with 10 mL methanol. Then we used methanol–THF (1 + 1); with 10 mL solution. Almost 100% of the SuE was eluted from the C18 (not endcapped) and C8 columns, but not from the endcapped C18 columns. Because mono-SuE eluted faster from the C8 column than from the C18 column, we decided to use the C8 column for sample preparation.

We examined the elution profiles of SuE, SAIB, and other matrix substances in more detail by changing the wa-
ter-to-methanol ratio (Figure 2). A 10 mL volume of each mixed solvent was used as the eluant. Sorbitol, sucrose, propylene glycol, and glycerin were eluted with water–methanol (8 + 2). SAIB was eluted with water–methanol from (3 + 7) to (1 + 9). MCT were eluted with water–methanol (1 + 9) to methanol–THF (1 + 1). Parts of both SAIB and MCT were eluted in the water–methanol (1 + 9) fraction. As shown in the gas chromatogram for \(\beta\)-carotene in Figure 3, the early GC peaks for SAIB indicated that SAIB partially coeluted with MCT from the GC column. Although SAIB did not coelute by two step elution; elute most part of SAIB with water-methanol (2 + 8), and elute a part of SAIB, indicated as the last peak, together with MCT with methanol–THF (1 + 1) We chose single step elution because the coeluted area was small and considered negligible compared with the total peak area for SAIB. Therefore, we eluted sorbitol, sucrose, propylene glycol, and glycerin with 10 mL water–methanol (1 + 1), and we eluted SAIB and mono-SuE with 10 mL methanol–THF (1 + 1).

DG, which was eluted in the same fraction as mono-SuE from the C18 column, was coeluted with mono-SuE from the GC column. Therefore, samples that contained DG, which was probably a minor component in fat or oil in the sample matrix, and samples which were insoluble in water-methanol (1 + 1) were prepared with the silica gel column, after extraction with THF (4). Elution profiles of target substances and matrix substances from the silica gel column were examined (Figure 4). A 10 mL volume of each mixed solvent was used as the eluant. Most of the MCT was eluted with hexane–diethyl ether (9 + 1). SAIB was eluted with hexane–diethyl ether from (8 + 2) to (6 + 4). Although SAIB was eluted in separate fractions with MCT from the silica gel column (Figure 4), 2 \(\beta\)-carotene samples that contained both SAIB and MCT were prepared with the C8 column, which gave higher recoveries of SAIB, probably because large amounts of polar compounds hindered the extraction of SAIB with THF in Method 2.

DG were eluted with hexane–diethyl ether from (85 + 15) to (8 + 2). Because mono-SuE were eluted with ethyl acetate–methanol (1 + 1), DG were separated from mono-SuE on the silica gel column. Therefore, we transferred the THF extract to the column with a small amount of diethyl ether–ethyl acetate (3 + 7; 4), eluted DG and SAIB with 10 mL of this solvent, and eluted mono-SuE with 10 mL ethyl acetate–methanol (1 + 1).

Gas chromatograms obtained for a vitamin-enriched rice are shown in Figure 5. SuE were coeluted with DG (Figure 5a) without the silica gel column preparation. The DG were eluted from the silica gel column with diethyl ether–ethyl acetate (3 + 7) and separated from the SuE. The gas chromatogram of this fraction (Figure 5b) was obtained; the mono-SuE were then eluted with ethyl acetate–methanol (1 + 1), and the gas chromatogram of this fraction (Figure 5c) was obtained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>SAIB</th>
<th>SuE (C12)</th>
<th>SuE (C16)</th>
<th>SuE (C18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annatto extract</td>
<td>99 ± 4.8</td>
<td>100 ± 2.4</td>
<td>96 ± 4.9</td>
<td>96 ± 4.9</td>
</tr>
<tr>
<td>Vitamin-enriched rice</td>
<td>93 ± 5.9</td>
<td>87 ± 3.4</td>
<td>82 ± 3.6</td>
<td>80 ± 4.3</td>
</tr>
</tbody>
</table>

* SD = standard deviation; n = 4.

Table 3. Concentrations (%) of mono-SuE and SAIB found in commercial food additive premixes and foods

<table>
<thead>
<tr>
<th>Sample</th>
<th>SAIB</th>
<th>Mono-SuE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C12</td>
<td>C14</td>
</tr>
<tr>
<td>Annatto color-1</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>Annatto color-2</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>(\beta)-Carotene-1</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>(\beta)-Carotene-2</td>
<td>7.7</td>
<td>0.56</td>
</tr>
<tr>
<td>Paprika color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavor premix</td>
<td>5.53</td>
<td></td>
</tr>
<tr>
<td>Emulsifier-1</td>
<td>2.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Emulsifier-2</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Vitamin-enriched rice</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Canned ready-to-drink coffee</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>
**GC Conditions**

Splitless injection onto a wide-bore column (0.53 mm id), which we used for the determination of underivatized monoglycerides (4), enabled the determination of acetylated mono-SuE. A BPX-5 column was used because the maximum operating temperature (isocratic) is 350°C, even for a wide-bore column with liquid-phase thickness of 1 μm. Acetylation and trifluoroacetylation were compared for the derivatization. Because the trifluoroacetate split into several peaks, we chose acetylation. Conditions for acetylation were investigated (30, 60, and 120 min at 35 and 60°C, and overnight at room temperature). Because the results were similar, we chose 30 min at 35°C. SAIB was determined without acetylation.

Calibration curves for mono-SuE and SAIB were linear for 5–1000 and 10–1000 μg/mL, respectively. The detection limits for mono-SuE and SAIB in samples were 0.005 and 0.01%, respectively. Triglycerides (TG), which were eluted during sample preparation in the same fraction as mono-SuE or SAIB, were eluted from the GC column by raising the oven temperature to 350°C.

**GC/MS Analysis**

An uncoated capillary tube (0.2 mm× 0.5 m, deactivated) was connected at the output of the separation column in series as a transfer line to the ion source, to prevent mono-SuE from being trapped at the interface. Mono-SuE did not elute from the separation column at the maximum interface temperature of 300°C, which was lower than the elution temperature of mono-SuE.

The mass spectra of standard mono-SuE and SAIB are shown in Figure 6. Fragment ions at m/z 43, 109, 169, 211, and 331 were observed in all mass spectra of mono-SuE. These fragment ions were also observed in the mass spectrum of sucrose octaacetate, in which the fragments of glucopyranose pentaacetate (6) were assigned as shown in Table 1.

The fragment ion at m/z 331 results from [M–CO₂⁻], the loss of an acetyloxy group, where M represents glucopyranose pentaacetate. The fragment ion at m/z 271 arises from the further loss of acetic acid [331–CH₃CO₂⁻]. The further loss of a second acetic acid molecule produces the fragment ion at m/z 211 [331–2CH₃CO₂⁻]. The fragment ion at m/z 169 is produced by the loss of ketene [211–CH₂CO⁻]. And the ion at m/z 109 corresponds to the loss of another acetic acid molecule [169–CH₃CO₂⁻]. Finally, m/z 43 is the acetyl radical [CH₃CO⁻].

Recoveries

A 10 mg portion of DK-SS, L-1695, and SAIB was added to 1 g annatto color (for C₈ column preparation) and to 1 g vitamin-enriched rice (for silica gel column preparation), and the procedure described in the Experimental section was followed. The recoveries from these samples were between 80 and 100% and were considered acceptable (Table 2).
Results for Analyses of Commercial Food Additive Premixes and Foods

The results are shown in Table 3. The emulsifier premixes were not soluble in water–methanol solution. DG in the paprika color were coeluted with mono-SuE. These samples together with the vitamin-enriched rice, a solid sample, were prepared according to Method 2. Other samples were prepared according to Method 1. In 6 samples, both palmitate (C16) and stearate (C18) were found, showing the typical profile of a sucrose ester of a fatty acid derived from hydrogenated oil. A gas chromatogram of paprika color, in which sucrose monolaurate (C12), monomyristate (C14), and monooleate (C18:1) were found, is shown in Figure 7. The myristate (C14) was considered an impurity of the laurate, because of the low concentration. SAIB was found in β-carotene premix-2 and a flavor premix. Palmitate alone was found in β-carotene premix-1 and the canned, ready-to-drink coffee. The palmitate was assumed to have been added to the coffee drink, which is kept hot in vending machines, to prevent spoilage caused by heat-resistant bacteria (7, 8).

Conclusion

A simple GC method for the determination of mono-SuE and SAIB in food additive premixes was developed. A C8 SPE column was used as a preparation column for aqueous samples. A silica gel SPE column effectively removed DG from the SuE. Mono-SuE were acetylated and determined by GC by using a wide-bore column with splitless injection. The identities of GC peaks of mono-SuE and SAIB were confirmed by GC/MS. Mono-SuE of C12, C14, C16, C18, and C18:1, together with SAIB were found in commercial food additive premixes and in some foods.

Acknowledgments

We thank the respective manufacturers of the SuE and SAIB for their generous donation of the products used in this study.

References