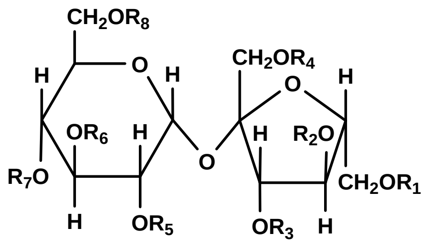
SUCROSE OLIGOESTERS TYPE II

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A group ADI of 0 - 30 mg/kg bw for this substance together with sucrose esters of fatty acids, sucroglycerides, sucrose oligoesters type I and sucrose monoesters of lauric, palmitic or stearic acid was established at the 73rd JECFA (2010).

SYNONYMS Sucrose fatty acid esters; Sucrose oligoesters; INS No. 473a

DEFINITION Sucrose oligoesters type II contains both mono- to tri-esters and tetra- to octa-fatty acid esters of sucrose. Their composition and properties are between Sucrose esters of fatty acids and Sucrose oligoesters type I. They are prepared from sucrose and methyl esters of food fatty acids such as stearic acid, palmitic acid, erucic acid and behenic acid by interesterification in the presence of an alkaline catalyst. Only the following solvents may be used for the production: dimethyl sulfoxide, isobutanol, and methyl ethyl ketone.

Structural formula



R_{1-8}: H or COC_nH_{2n+1}
(mono- to tri-esters: 20-80%
tetra- to octa-esters: 20-80%)

Assay

Total content of mono- to tri-esters: between 20 - 80%
Total content of tetra- to octa-esters: between 20 - 80%
Content of hepta- and octa-esters: not more than 20%
Content of octa-esters: not more than 10%

DESCRIPTION White to red-brown powders, soft solid, stiff gels or colourless to red-brown viscous liquid

FUNCTIONAL USES Emulsifier, stabilizer, tableting aid

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Insoluble in water

Fatty acids Add 1 ml of ethanol to 0.1 g of the sample, dissolve by warming, add 5 ml of dilute sulfuric acid TS, heat in a water bath for 30 min and cool. A yellowish-white solid or oil is formed, which has no odour of isobutyric acid, and which dissolves when 3 ml of diethyl ether is added. Use the aqueous layer separated from the diethyl ether in the Test for sugars.

Sugars To 2 ml of the aqueous layer separated from the diethyl ether in the test for fatty acids, carefully add 1 ml of anthrone TS down the inside of a test tube; the boundary surface of the two layers turns blue or green.

PURITY

Sulfated ash (Vol.4)	Not more than 2% Test 1 g of the sample (Method I)
Acid value (Vol.4)	Not more than 6
Free sucrose	Not more than 1 % See description under TESTS
Dimethyl sulfoxide	Not more than 2 mg/kg See description under TESTS
Isobutanol	Not more than 10 mg/kg See description under TESTS
Methanol	Not more than 10 mg/kg See description under TESTS
Methyl ethyl ketone	Not more than 10 mg/kg See description under TESTS
Lead (Vol.4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Free sucrose

Determine by gas liquid chromatography described in Volume 4 using the following conditions.

Standard solutions

Prepare a stock solution containing 5.0 mg/ml of sucrose in *N,N*-dimethylformamide. Prepare a range of standard solutions containing 0.5, 1.25 and 2.5 mg/ml of sucrose by dilutions of the stock solution with *N,N*-dimethylformamide.

Internal standard solution

Weigh accurately 0.25 g of octacosane into a 50-ml volumetric flask, add 25 ml of tetrahydrofuran to dissolve the octacosane, and add tetrahydrofuran to the mark.

Chromatography conditions

Column: 100%-Dimethylpolysiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injection: 280°
- column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45

min at 300°
- detector: 320°
The retention times of free sucrose and octacosane measured under the above conditions are approx. 18.8 and 19.3 min, respectively.

Procedure
Weigh accurately 20-50 mg of the sample into a centrifugation tube, add 1 ml internal standard solution, 1 ml *N,N*-dimethylformamide, 0.4 ml of *N,O*-bis(trimethylsilyl)acetamide (BSA) and 0.2 ml trimethylchlorosilane (TMCS). After sealing the tube, shake and let stand for 5 min at room temperature. Inject 1 µl into the gas liquid chromatograph.

Standard curve
Prepare silylated standard solutions following the above procedure using 1 ml each of the standard solutions in place of the sample and *N,N*-dimethylformamide . Draw a standard curve by plotting amount of sucrose (mg) in 1 ml of the standard solution (X-axis) vs. ratio of peak area of sucrose/internal standard (Y-axis).

Measure the peak areas for sucrose and internal standard. Calculate the ratio of their peak areas, and obtain the amount of sucrose from the standard curve.

Calculate the percentage of free sucrose from:

$$\text{\% free sucrose} = \frac{\text{amount sucrose determined (mg)}}{\text{weight of sample (mg)}} \times 100$$

Dimethyl sulfoxide

Determine by gas liquid chromatography described in Volume 4 using following conditions.

Standard solutions
Prepare a 0.25 mg/ml stock solution of dimethyl sulfoxide in tetrahydrofuran. Prepare a range of solutions containing 0.1, 0.2, 0.4 and 1.0 µg/ml of dimethyl sulfoxide by dilutions of the stock solution with tetrahydrofuran.

Chromatography conditions
Column: 10% PEG 20M and 3% KOH on Chromosorb W AW DMCS 60/80 mesh (2 m x 3 mm i.d.) or equivalent. Raise the oven temperature to 180° at a rate of 10°/min and let stabilize for 24 to 48 h with 30 to 40 ml/min of nitrogen for conditioning
Carrier gas: Nitrogen
Flow rate: 30 ml/min
Detector: Flame photometric detector (using 394 nm sulfur filter)
Temperatures
- injection: 210°
- column: 160°
The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3 min.

Procedure
Weigh accurately 5 g of the sample into a 25-ml volumetric flask, add 10 ml of tetrahydrofuran to dissolve the sample, add tetrahydrofuran

to the mark, and mix the solution well. Inject 3 µl of the sample solution into the chromatograph.

Standard curve
Prepare daily by injecting 3 µl of each of the standard solutions into the chromatograph.

Calculate the concentration of dimethyl sulfoxide in mg/kg (C_{DMSO}) from:

$$C_{DMSO} \text{ (mg/kg)} = C \times 25 / W$$

where
C is the dimethyl sulfoxide concentration determined (µg/ml); and
W is the weight of sample (g).

Methanol, isobutanol, and methyl ethyl ketone

Determined by gas chromatography with a head space sampler using the following methods.

Standard solutions
Prepare standard solution A containing 4000 mg/l each of methanol, isobutanol, and methyl ethyl ketone by weighing accurately 0.2 g of each solvent into a 50-ml volumetric flask containing approx. 20 ml of water, then adding water to volume. By dilutions of this solution, prepare solutions containing 2000 mg/l (standard solution B) and 1000 mg/l (standard solution C).

Procedure:
Weigh accurately 1 g of the sample into each of four sample vials. To one vial add 5 µl of water, to the second, third and fourth, add, respectively, standard solutions A, B and C, and seal them quickly with a septum. (The concentrations of each solvent after adding 5 µl of standard solutions A, B and C to 1 g of the sample are equal to 20, 10 and 5 mg/kg of sample, respectively). Place the sample vials in a head space sampler and analyse using the following conditions:
Column: 100% Polydimethylsiloxane (30 m x 0.53 mm i.d. with 1.5 µm film)
Carrier gas: Nitrogen
Flow rate: 3.5 ml/min
Detector: FID
Temperatures
- injection: 110°
- column: 40°
- detector: 110°
Head space sampler:
- sample heat insulating temperature: 80°
- sample heat insulating period: 40 min
- syringe temperature: 85°
- sample gas injection: 1.0 ml

Calculation
Plot the relationship between the added amount against the peak area for each solvent using the analytical results. The relationship should be linear. Extrapolate and determine the x-intercept (w_i), and calculate the solvent concentrations (C_i) in the sample from:

$$C_i \text{ (mg/kg)} = w_i / W$$

where

w_i is the x-intercept of relationship line using the standard addition method (µg); and

W is the weight of sample (g).

METHOD OF ASSAY

1. Mono- to tri-esters and tetra- to octa-esters

Determine by HPLC using the following conditions:

Procedure

Accurately weigh 250 mg of the sample into a 100-ml volumetric flask. Dilute to volume with tetrahydrofuran and mix. Filter through a 0.5 µm membrane filter. Inject 80 µl of the sample into the chromatograph.

Chromatography conditions

Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G1000HXL, G2000HXL, G3000HXL, G4000HXL (each 30 cm x 7.8 mm i.d., 5 µm particle for solvent manufactured by Tosoh in series or equivalent)
Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 0.8 ml/min
Detector: RI
Temperatures:
- Column: 40°
- Detector: 40°

Record the chromatogram for about 50 min

Identification of the peaks

More highly esterified components elute earlier and tetra- to octa-esters elute as one peak. Their retention times are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these conditions are described in Table 1. The reference products are available from Mitsubishi Chemical Corporation (Tokyo, Japan) or Dai-ichi Kogyo Seiyaku Co. Ltd (Kyoto, Japan) to confirm the retention time.

Table 1. The retention time (min) of mono-, di-, tri- and tetra- to octa-esters esterified with main fatty acids

Esterified fatty acid	Mono-esters	Di-esters	Tri-esters	Tetra- to octa-esters
Stearic acid	39.0	37.0	35.7	34.9
Erucic acid	38.5	36.3	35.1	34.3
Behenic acid	38.2	36.2	35.0	34.2

Calculate the percentage of mono- to tri esters ($E_{mono\text{-}tri}$) and tetra- to octa-esters ($E_{tet\text{-}oct}$) in the sample from:

$$E_{mono\text{-}tri}\ (\%) = 100\ A_{mono\text{-}tri}/T$$
$$E_{tet\text{-}oct}(\%) = 100\ A_{tet\text{-}oct}/T$$

where

$A_{mono\text{-}tri}$ is the sum of peak areas for mono- to tri-esters;

$A_{tet\text{-}oct}$ is the sum of peak areas for tetra- to octa-esters; and

T is the sum of all peak areas eluting within 43 min.

2. Hepta- and octa-esters

The percentage of the sum of hepta- and octa-esters ($E_{hep+oct}$), and the percentage of octa-esters (E_{oct}) is calculated by two steps. The ratio of hepta- and octa-esrers, and the ratio of octa-esters in sum of tetra- to octa-esters are determined by HPLC. Then $E_{hep+oct}$ and E_{oct} are calculated using $E_{tet-oct}$ obtained above in Method 1.

<u>Procedure</u>
Accurately weigh 1g of the sample into a 50-ml volumetric flask and add a solution for the mobile phase (tetrahydrofuran/methanol=50/50 (vol/vol)) to the mark. Filter through a 0.5 µm membrane filter. Inject 20 µl of the sample into the chromatograph.

<u>Chromatography conditions</u>
Column: reversed phase C18 columns (150mm x 4.6 mm i.d.; ODS-2 manufactured by GL Science or equivalent)
Mobile phase: tetrahydrofuran/methanol=50/50 (vol/vol)
Flow rate: 0.8 ml/min
Detector: RI
Temperatures:
- Column: 40°
- Detector: 40°
Record the chromatogram for about 16 min

<u>Identification of the peaks</u>
The retention times of tetra-, penta-, hexa-, hepta- and octa-esters are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these conditions are described in Table 2. The reference products are available from Mitsubishi Chemical Corporation (Tokyo, Japan) or Dai-ichi Kogyo Seiyaku Co. Ltd (Kyoto, Japan) to confirm the retention time.

Table 2. The retention time (min) of tetra- to octa-esters for some type of SOE Type I

Esterified fatty acid	Tetra-esters	Penta-esters	Hexa-esters	Hepta-esters	Octa-esters
Stearic acid	3.1	3.7	4.8	6.1-7.0*	7.9-10.7*
Erucic Acid	3.3	4.1	5.4	7.5	11.0
Behenic acid	4.5	5.5	7.1	10.2	14.8

*: the retention time range, because highly esterified components have been shown as several unresolved peaks

Calculate the percentage of the sum of hepta- and octa-esters (E_{hep+oc}) and the percentage of octa-esters (E_{oct}) as follows:

$$E_{hep+oct}(\%) = (B_{hep+oct}/T_{tet-oct}) \times E_{tet-oct}$$
$$E_{oct}(\%) = (B_{oct}/T_{tet-oct}) \times E_{tet-oct}$$

where
$B_{hep+oct}$ is the sum of peak areas of hepta-esters and octa-esters;
B_{oct} is the peak areas for octa-esters;
$T_{tet-oct}$ is the sum of peak areas from tetra- to octa-esters; and
$E_{tet-oct}$ (%) is the percentage of tetra- to octa-esters measured by Method 1 described above.