SUCROSE ESTERS OF FATTY ACIDS

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 65th JECFA (2005) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0 - 30 mg/kg bw for sucrose esters of fatty acids, sucroglycerides and sucrose oligoesters type I and type II was established at the 71st JECFA (2009).

SYNONYMS Sucrose fatty acid esters, INS No. 473

DEFINITION Mono-, di- and tri-esters of sucrose with food fatty acids, prepared from sucrose and methyl and ethyl esters of food fatty acids by esterification in the presence of a catalyst or by extraction from sucroglycerides. Only the following solvents may be used for the production: dimethylformamide, dimethyl sulfoxide, ethyl acetate, isopropanol, propylene glycol, isobutanol and methyl ethyl ketone.

Assay Not less than 80% of sucrose esters

DESCRIPTION Stiff gels, soft solids or white to slightly greyish white powders

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Sparingly soluble in water, soluble in ethanol

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 waterbath 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 are 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 5%
See description under TESTS

Dimethylformamide Not more than 1 mg/kg
See description under TESTS

Dimethyl sulfoxide	Not more than 2 mg/kg See description under TESTS
Ethyl acetate, isopropanol and propylene glycol	Not more than 350 mg/kg, singly or in combination 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 2 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:

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

Dimethylformamide

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

Standard solutions

Prepare a stock solution containing 1.00 mg/ml of dimethylformamide in tetrahydrofuran. Prepare a range of standard solutions containing 0.05, 0.1 and 0.2 µg/ml of dimethylformamide by diluting the stock solution with tetrahydrofuran.

Chromatography conditions

Column: Polyethylene glycol (30 m x 0.32 mm i.d. with a 0.5 µm film)
Carrier gas: Helium
Pressure: 150 kPa (constant pressure)
Detector: Nitrogen/phosphorus detector or thermionic specific detector)
Temperatures:

- injection: 180°
- column: Hold for 2 min at 40°, then 40-160° at 20°/min, hold for 2 min at 160°
- detector: 325°

Injection method: Splitless injection of 1.0 µl with auto-injector, followed by start of purge after 1.0 min.
The retention time of dimethylformamide measured under the above conditions is approx. 6.4 min.

Procedure

Weigh accurately 2 g of sample into a 20-ml volumetric flask, add 10 ml of tetrahydrofuran to dissolve the sample, add tetrahydrofuran to the mark, and mix the solution well. Inject 1.0 µl of the sample solution into the chromatograph.

Standard curve

Prepare daily by injecting 1.0 µl of each of the standard solutions into the chromatograph.

Calculate the concentration of dimethylformamide in mg/kg (C_{DFA}) from:

C_{DFA} (mg/kg) = C x 20 / W

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

NOTE: The nitrogen/phosphorus detector is insensitive to components that do not contain nitrogen or phosphorus. As a consequence, the capillary column can become obstructed with compounds of low volatility, although the baseline of the chromatogram is stable. Accordingly, the column must be reconditioned frequently. Overnight reconditioning (flow carrier gas in the reverse direction at 180°) is required after about every 15 samples.

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. 3.4 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} (mg/kg) = C x 25 / W

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

Propylene glycol

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

Internal standard solution
Prepare a 500 μg/ml solution of ethylene glycol in tetrahydrofuran.

Standard solutions
Prepare a range of standard solutions containing 1, 5, 10, 25 and 50 μg/ml of propylene glycol with 5 μg/ml of ethylene glycol in tetrahydrofuran.

Chromatography conditions
Column: Polydimethylsiloxane (30 m x 0.32 mm i.d. with 0.25 μm film)
Carrier gas: Helium
Flow rate: 1.5 ml/min (Constant flow)
Detector: FID
Temperatures:
- injection: 230°
- column: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
- detector: 270°

The retention times of ethylene glycol and propylene glycol derivatives are approx. 7.6 min and 7.8 min, respectively.

Procedure
Weigh accurately 1 g of the sample into a 10-ml volumetric flask, and add 100 μl of the internal standard solution. Dissolve and make to volume with tetrahydrofuran. Take 0.5 ml of the sample solution in a centrifugation tube, and add 0.25 ml of 1,1,1,3,3,3-hexamethyldisilazane and 0.1 ml of trimethylchlorosilane. After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 μl of this centrifugal supernatant into the chromatograph.

Standard curve
Prepare following the same procedure using 0.5 ml of the standard solutions in place of the sample solution.

Calculate the concentration of propylene glycol in mg/kg (C_{PG}) from:

C_{PG} (mg/kg) = C x 10 / W

where
C = propylene glycol concentration determined (μg/ml); and
W = weight of sample (g).

Methanol, isopropanol, isobutanol, ethyl acetate 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, isopropanol, isobutanol, ethyl acetate 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 (mg/kg)= w_i / W

where

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

W is weight of sample (g).

METHOD OF ASSAY Determine by HPLC using the following conditions:

Procedure

Accurately weigh 250 mg of the sample into a 50-ml volumetric flask. Dilute to volume with tetrahydrofuran and mix. Filter through a 0.45 µm membrane filter. Inject 50 µl of the sample into the pre-stabilized chromatograph.

Chromatography conditions

Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G2000HXL (Tosoh) x 4 column in series or equivalent)
Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 1.0 ml/min
Detector: Refractive index
Temperatures:
- Column: 40°
- Detector: 40°

Record the chromatogram for about 30 min.

Calculate the percentage of sucrose ester content in the sample from:

% sucrose ester = 100 A/T

where

A is the sum of peak areas for the three main components, the mono-, di- and tri-esters, eluting at about 24.9, 23.6 and 22.8 min, respectively; and

T is the sum of all peak areas eluting within 30 min.