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Sucrose Esters of Fatty Acids

SUCROSE ESTERS OF FATTY ACIDS

Prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017), superseding specifications prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010). An ADI of 0 - 30 mg/kg bw for this substance together with sucroglycerides, sucrose oligoesters type I and type II and sucrose monoesters of lauric, palmitic or stearic acid was established at the 73rd JECFA (2010).

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   White to greyish white or pale yellow powder, stiff gel or soft solid

FUNCTIONAL USES   Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4)   Very soluble in ethanol at 50º

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 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

<u>Sulfated ash</u> (Vol.4)	Not more than 2% Test 1 g of the sample (Method I)
<u>Acid value</u> (Vol.4)	Not more than 6
<u>Free sucrose</u>	Not more than 5% See description under TESTS
<u>Dimethylformamide</u>	Not more than 1 mg/kg See description under TESTS
<u>Dimethyl sulfoxide</u>	Not more than 2 mg/kg See description under TESTS
<u>Ethyl acetate,</u> <u>isopropanol</u> <u>and</u> <u>propylene glycol</u>	Not more than 350 mg/kg, singly or in combination See description under TESTS
<u>Isobutanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methyl ethyl ketone</u>	Not more than 10 mg/kg See description under TESTS
<u>Lead</u> (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

<u>Free sucrose</u>	Determine by gas chromatography (Vol. 4) under 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:
  o injection port: 280°
  o column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45 min at 300°
  o 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 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:

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

<u>Dimethylformamide</u>	Determine by gas chromatography (Vol. 4) under 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:
	   o injection port: 180°
	     column: Hold for 2 min at 40°, then 40-160° at 20°/min, hold for 2 min at 160°
	   o 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 (CDFA) from:

$$CDFA\ (mg/kg) = C \times 20/W$$

where

- C is dimethylformamide concentration determined (µg/mL);
- W is weight of sample (g)

Note: The column must be reconditioned frequently. Overnight reconditioning (flow carrier gas in the reverse direction at 180° without the connection of the detector) is required after about every 15 samples.

<u>Dimethyl sulfoxide</u>

Determine by gas chromatography (Vol. 4) under the 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
  o injection port: 210°
  o 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 (CDMSO) from:

$$CDMSO\ (mg/kg) = C \times 25/W$$

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

Propylene glycol (Vol. 4)   Determine by gas chromatography (Vol. 4) under 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:
  o injection port: 230º
  o column: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
  o detector: 270°

The retention times of ethylene glycol and propylene glycol derivatives under the above conditions 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 up to the 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 (HMDS) and 0.1 mL of trimethylchlorosilane (TMCS). After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 µl of the 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 (CPG) from:

$$CPG\ (mg/kg) = C \times 10/W$$

where
- C is polyethylene glycol concentration determined (µg/mL);
- W is weight of sample (g).

<u>Methanol, isopropanol, isobutanol, ethyl acetate and methyl ethyl ketone</u>

Determined by gas chromatography (vol. 4) with a head space sampler under the following conditions.

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 1g 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 port: 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 amounts 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)
- W is weight of sample (g)

METHOD OF ASSAY   Determine by HPLC (Vol. 4) under 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 80 µl of the sample solution into the pre-stabilized 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 in series), Tosoh Co. or equivalent)
- Mobile phase: HPLC-grade degassed tetrahydrofuran Flow rate: 0.8 mL/min
- Detector: Refractive index
- Temperatures:
    o Column: 40°
    o Detector: 40°
- Record the chromatogram for about 50 min.

Typical retention times under the above 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. Typical retention time (min) of mono-, di- and tri-esters esterified with main fatty acids

Esterified fatty acid	Mono-esters	Di-esters	Tri-esters
Lauric acid	40.0	38.2	37.0
Palmitic acid	39.3	37.2	36.0
Stearic acid	39.0	37.0	35.7
Oleic acid	39.1	37.1	35.9

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