SUCROGLYCERIDES

Prepared at the 49th JECFA (1997), published in FNP 52 Add 5 (1997) superseding specifications prepared at the 27th JECFA (1983), published in FNP 28 (1983) and FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). 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 INS No. 474

DEFINITION Sucroglycerides are obtained by reacting sucrose with an edible fat or oil with or without the presence of a solvent. They consist of a mixture of mono- and di-esters of sucrose and fatty acids together with mono-, di- and triglycerides from the fat or oil. Only the following solvents may be used in the production: dimethyl formamide, cyclohexane, isobutanol, isopropanol and ethyl acetate.

Assay Not less than 40% and not more than 60% of sucrose esters

DESCRIPTION Odourless, soft, solid masses, white to off-white powders, or stiff gels

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water; soluble in ethanol

Test for 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 is soluble in 3 ml of ether.

Test for sugar To 2 ml of the solution separated from the solid or oil in the Test for fatty acids add 1 ml of anthrone TS carefully down the inside of the test tube. The boundary surface of the two layers turns to blue or green.

PURITY

Sulfated ash (Vol. 4) Not more than 2%
Test 2 g of the sample (Method I)

Acid value (Vol. 4) Not more than 6

Free sucrose Not more than 5%
See description under TESTS

Dimethyl formamide Not more than 1 mg/kg

	See description under TESTS
Cyclohexane and isobutanol	Not more than 10 mg/kg, singly or in combination See description under TESTS
Ethyl acetate and isopropanol	Not more than 350 mg/kg, singly or in combination
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 principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Free sucrose

Determine by *gas liquid chromatography* (see Volume 4) using the following conditions:

Reagents
- Internal Standard: 5 mg/ml cholesterol in chloroform or 10 mg/ml tetracosane in chloroform
- Pyridine (dried over molecular sieve)
- N,O-Bis-(Trimethylsilyl)-acetamide (BSA)
- Trimethylchlorosilane (TMCS)

Procedure
Weigh accurately 20-50 mg of the sample into a silylation vial, add 1 ml internal standard solution, 1 ml pyridine, and 0.5 ml each of BSA and TMCS. Seal vial, and heat at 70° for 30 min. Inject 1 µl into the gas liquid chromatograph.

Conditions
Column:
- length: 0.3 m
- diameter: 4 mm (i.d.)
- material: glass
- packing: Dexil

Carrier gas: Nitrogen
Flow rate: 40 ml/min
Detector: FID

Temperature programme: Hold for 1 min at 160°, then 160-375° at 15°/min.
Measure peak areas for sucrose and internal standard. The response factor (RF) is calculated from a number of gas liquid chromatography runs with standard solutions of sucrose containing internal standard.

Calculation

$$RF = \frac{\text{mg of internal standard} \times \text{area sucrose}}{\text{area internal standard} \times \text{mg sucrose}}$$

and

$$\% \text{ free sucrose} = \frac{\text{mg internal standard} \times \text{area sucrose} \times 100}{\text{RF} \times \text{area internal standard} \times \text{mg sample}}$$

Dimethyl formamide

Determine by hydrolysis to dimethylamine and analysis by *gas liquid chromatography* (see Volume 4) using the following conditions:

Reagents
- Dimethyl formamide
- Dimethylamine hydrochloride
- Methanol
- Ethanol
- Hydrochloric acid
- Sodium hydroxide

Standard solutions
Prepare 4.47 mg/ml (equivalent to 4.0 mg/ml of dimethyl formamide) stock solution of dimethylamine hydrochloride in ethanol, and prepare standard solutions equivalent to 4, 2 and 1 µg/ml of dimethyl formamide, respectively, by dilution of the stock solution with 0.1% sodium hydroxide solution in ethanol.

Sample preparation
The apparatus for the hydrolysis is shown in the Appendix. Weigh accurately about 40 g of the sample into a 1000-ml round-bottomed flask. Add 500 ml of 5% methanolic solution of sodium hydroxide, and attach the flask to the apparatus. Set an Erlenmeyer flask containing 10 ml of 1% methanolic solution of hydrochloric acid to the apparatus. Heat the round-bottomed flask and let the content reflux for 1 hour, then distil to collect about 50 ml of the distillate while cooling water of the reflux condenser is stopped. Evaporate the distillate to almost dryness on a boiling water bath. Dissolve the residue with a small amount of ethanol, add 2.5 ml of 5% ethanolic solution of sodium hydroxide, and dilute to 25 ml with ethanol to prepare a sample solution.

Procedure
Inject 2 µl of the sample solution into the gas liquid chromatograph under the conditions below.

Calibration curve
Prepare a calibration curve by injecting each 2 µl of the standard solutions into the gas chromatograph.

Conditions
Column:
- length: 2 m
- diameter: 2 mm (i.d.)
- material: glass
- packing: 10% amine 220 and 10% KOH on 80/100 weak acid washed

Chromosorb W
- conditioning: Heat to 130° overnight with 5 ml/min of nitrogen flow rate

Carrier gas: Nitrogen
Flow rate: 17 ml/min
Detector: FID

Temperatures
- injection port: 198±5°
- column: 60°

Calculation

$$C_{DFA}\ (mg/kg) = \frac{C\ (\mu g/ml) \times 25\ (ml)}{W\ (g)}$$

where
C_{DFA} is the Concentration of dimethyl formamide;
C is the Concentration of dimethyl formamide detected; and
W is the weight of sample taken.

Cyclohexane and isobutanol

Determine by *gas liquid chromatography* (see Volume 4) using the following conditions:

Reagents
- Dimethylformamide (GLC purity grade)
- Cyclohexane (UV spectrophotometric grade)
- Isobutanol (analytical grade)

Standard solutions
Prepare a 0.1% stock solution of cyclohexane and isobutanol in dimethylformamide by pipetting 130 µl of cyclohexane and 125 µl of isobutanol into dimethylformamide and making up the volume to 10 ml.
Prepare by dilution a range of solutions containing 5, 10 and 20 mg/kg of cyclohexane and isobutanol. Prepare a response curve by injecting 5 µl of these diluted standard solutions into the gas chromatograph under the conditions below.

Sample preparation
Weigh 5 g of sample to the nearest 10 mg into a flask with a ground glass stopper, add 5 g of dimethylformamide and warm to dissolve. Cool and inject 5 µl into the gas chromatograph under the conditions below.

Column
- length: 3 m
- diameter: 4.5 mm
- material: stainless steel
- packing: 20% Carbowax 20 M on Chromosorb G 60/80

Carrier gas: Helium (1.6 bar)
Detector: Flame ionization
Temperatures
- injection port: 130°
- column: 130°
- detector: 200°

Determine the concentration of cyclohexane and isobutanol in the sample solution (50%) by comparison with the standard solutions and multiply the concentration by two to convert the results to correspond to the original sucroglycerides.

Isopropanol and ethyl acetate

Determine by *gas chromatography* (see Volume 4) with a head space sampler using the following conditions:

Reagents

- Isopropanol
- Ethyl acetate

Standard solutions

Take each 1 g of isopropanol and ethyl acetate in a volumetric flask and add water to total volume of 100 ml, and prepare 0.02-0.4 g/100 ml solutions by dilution of this solution.
If necessary, prepare standard solutions containing up to 7 g/100 ml of isopropanol and ethyl acetate.

Procedure

Place 1 g (1.0 ± 0.1 g) of powdered sample in a sample vial. Add 5 µl of water to the sample vial and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the below-mentioned conditions.

Calibration curve

Take 1 g of powdered sucrose esters of fatty acids, solvent free or known residual solvent contents, in a sample vial, add 5 µl of the standard solution and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the following conditions and obtain calibration curves for each solvent.

Column:

- length: 30 m
- diameter: 0.53 mm (i.d.)
- material: Silica capillary
- film: 100% methyl polysiloxane
- conditioning: Heat to 60° for 2-3 h with approximately 10 ml/min of nitrogen

Carrier gas: Nitrogen
Flow rate: 5 ml/min
Detector: Flame ionization
Temperatures:

- injection port: 110°
- column: 40°
- detector: 110°

Head space sampler:

- Sample volume: 1.0 g ± 0.1 g + 5 µl
- Sample heating temp.: 80°
- Sample heating time: 40 min
- Syringe temperature: 85°
- Sample gas injection: 0.4 ml

Calculation

$C_i = A_i \times Cf_i \times 1000$

where

C_i is the Concentration of solvent i (mg/kg);
A_i is the Peak area of solvent i (µv.sec.); and
Cf_i is the Conversion coefficient for solvent i (slope of the calibration curve) (µg/µv.sec).

METHOD OF ASSAY Determine by *high pressure liquid chromatography* (see Volume 4) using the following conditions:

Sample preparation
Add about 250 mg of the sample, accurately weighed to a 50 ml volumetric flask. Dilute to volume with tetrahydrofuran, and mix. Filter through a 0.5-µm membrane filter.

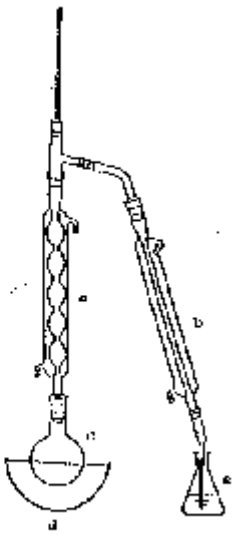
Procedure
Inject 100 µl of the sample into the pre-stabilized high pressure liquid chromatograph.

Conditions
Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G2000 (Supelco) or equivalent)
Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 0.7 ml/min
Detector: Refractive index detector
Temperatures:
Column: 38°
Detector: 38°
Record the chromatogram for about 90 min. Calculate the percentage of sucrose ester content in the sample taken by the formula:

$$100\ A/T$$

where
A is the the sum of peak areas for the three main components, the mono-, di- and triesters, eluting at about 65, 68 and 73 min, respectively; and
T is the sum of all peak areas eluting within 90 min.

Appendix



Apparatus for hydrolysis

a: Reflux condenser
b: Condenser
c: Round bottomed flask
d: Water bath
e: Erlenmeyer flask