

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). An ADI of 0-30 mg/kg bw was established at the 49th JECFA (1997)

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

Not more than 350 mg/kg, singly or in combination

isopropanol

Lead (Vol. 4)

Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

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

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

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:

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

where

C_{DFA} = Concentration of dimethyl formamide

C = Concentration of dimethyl formamide detected

W = 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 μ l 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 μ l 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 \pm 0.1 g + 5 μ l
- 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 = Concentration of solvent i (mg/kg)

A_i = Peak area of solvent i ($\mu\text{v}.\text{sec}.$)
 C_{f_i} = Conversion coefficient for solvent i (slope of the calibration curve)
($\mu\text{g}/\mu\text{v}.\text{sec}.$)

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 = the sum of peak areas for the three main components, the mono-, di- and triesters, eluting at about 65, 68 and 73 min, respectively

T = 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

