N sodium hydroxide. Each mL of 1 N sodium hydroxide is equivalent to 46.03 mg of CH_2O_2.

Acceptance criteria: NLT 85.0% of CH_2O_2

IMPURITIES

Inorganic Impurities

- **SULFATE**

Sample: 2.1 mL (2.5 g)

Control: 100 µg of sulfate

Analysis: Add about 10 mg of sodium carbonate to the *Sample* and to the *Control* contained in separate beakers. Evaporate to dryness on a steam bath.

Acceptance criteria: Any turbidity produced by the *Sample* residue does not exceed that shown in the *Control.* (NMT 0.004%)

Organic Impurities

- **ACETIC ACID**

Sample: 1 mL

Analysis: Dilute the *Sample* to 100 mL with water. Transfer 50 mL of this solution into a 250-mL boiling flask and add 5 g of yellow mercuric oxide. While continuously stirring, boil the mixture under a reflux condenser for 2 h, cool, filter, and wash the residue with about 25 mL of water. Add phenolphthalein TS to the combined filtrate and washings, and titrate with 0.02 N sodium hydroxide.

Acceptance criteria: NMT 2.0 mL of 0.02 N sodium hydroxide is required to produce a pink color. (NMT 0.4%)

SPECIFIC TESTS

- **DILUTION TEST**

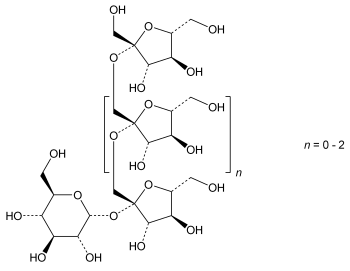
Analysis: Dilute 1 volume of sample with 3 volumes of water.

Acceptance criteria: No turbidity develops within 1 h.

Fructooligosaccharides, Short Chain

First Published: FCC 6

scFOS



DESCRIPTION

Fructooligosaccharides, Short Chain (scFOS), are indigestible carbohydrates synthesized from sucrose and fructose through an enzymatic process or from Inulin by partial enzymatic hydrolysis. These carbohydrates are a mixture of polysaccharides consisting of a sucrose molecule (glucose-fructose disaccharide, GF_1) linked to one (GF_2), two (GF_3), or three (GF_4) additional fructose units added by β2-1 glycosidic linkages to the fructose unit of sucrose for the synthesized scFOS. The scFOS from Inulin consists of oligosaccharides with the same structure but a slightly larger range of polymerization (from GF_2 to GF_9 and from F_3 to F_9). They are very soluble in hot and cold water, and almost insoluble in most organic solvents.

Function: Bulking agent; source of dietary fiber; sweetener; prebiotic

Packaging and Storage: Store tightly closed containers in a cool, dry place.

IDENTIFICATION

- **PROCEDURE**

Acetate buffer (pH 4.5 ± 0.05): Transfer 22 mL of 0.2 M sodium acetate and 28 mL of 0.2 M acetic acid into a 100-mL volumetric flask, and dilute to volume with water.

Standard solution: 3.0 mg/mL of GF_2, 4.5 mg/mL of GF_3, 0.50 mg/mL of GF_4 scFOS Reference Standards (Waco Pure Chemical Industries, Ltd., Osaka, Japan, or equivalent) and 0.50 mg/mL each of fructose, glucose, and sucrose in water.

Sample stock solution: 10 mg/mL using a sample previously dried to constant weight

Digested sample solution: Transfer 10 mL of *Acetate buffer* and 10 mL of the *Sample stock solution* into a 25-mL volumetric flask. Add 150 units of Fructozyme SP230 enzyme (Novozymes, Denmark), or equivalent. Digest for 30 min at 60°, cool, and dilute to volume with water.

Mobile phase: Acetonitrile–water (65–70% to 35–30%)

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index [NOTE—Use a detector with a sensitivity of 8×10^{-5}.]

Column: 25-cm × 4-mm (id) 5-µm LiChrospher 100 NH2 column (Merck Corp.), or equivalent

Column temperature: 35°

Flow rate: 1 mL/min

Run time: 12 min

Injection volume: 20 µL

Sample loop: 20 µL

Analysis: Separately inject the *Digested sample solution* and the *Standard solution* into the chromatograph and record the chromatograms. Determine the percentage of fructose and the percentage of glucose in the *Digested sample solution* using the following formula:

$$\text{Result} = 100(C_{ST} \times A_{SA})/(A_{ST} \times W)$$

C_{ST} = concentration of fructose or glucose in the *Standard solution* (mg/100 mL)

A_{SA} = area of the corresponding sugar peak in the chromatogram of the *Digested sample solution*

A_{ST} = area of the corresponding sugar peak in the chromatogram of the *Standard solution*

W = weight of sample (g) contained in each 100 mL of the *Sample stock solution*

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Correct the percent fructose and percent glucose results for the mono- and disaccharide content (obtained in the *Assay* below), and for moisture.

Acceptance criteria: The sample releases greater than 67% fructose and less than 33% glucose upon enzymatic digestion.

ASSAY

- **PROCEDURE**

Mobile phase: Acetonitrile–water (65–70% to 35–30%)

Standard solution: 3.0 mg/mL of GF_2, 4.5 mg/mL of GF_3, 0.50 mg/mL of GF_4 scFOS Reference Standards (Waco Pure Chemical Industries, Ltd., Osaka, Japan, or equivalent) and 0.50 mg/mL each of fructose, glucose, and sucrose in water

Sample stock solution: 10 mg/mL using a sample previously dried to constant weight

Chromatographic system, Appendix IIA

Mode: High-performance liquid chromatography

Detector: Refractive index [NOTE—Use a detector with a sensitivity of 8×10^{-5}.]

Column: 25-cm × 4-mm (id) 5-µm LiChrospher 100 NH2 column (Merck Corp.), or equivalent

Column temperature: 35°

Flow rate: 1 mL/min

Run time: 12 min

Injection volume: 20 µL

Sample loop: 20 µL

Analysis: Separately inject the *Sample solution* and the *Standard solution* into the chromatograph, and record the area responses for each scFOS. Calculate the percentage of each scFOS, from trimers to nonamers, in the sample taken using the formula:

$$\text{Result} = 100(C_{ST} \times A_{SA})/(A_{ST} \times W)$$

C_{ST} = concentration of the scFOS of interest in the *Standard solution* (mg/100 mL)

A_{SA} = area of the corresponding sugar peak in the chromatogram of the *Sample solution*

A_{ST} = area of the corresponding sugar peak in the chromatogram of the *Standard solution* (for oligomers without a specific standard, use the average area response of the peaks of the standards)

W = weight of sample (g) contained in each 100 mL of the *Sample solution*

Calculate the total percentage of scFOS in the sample by adding the individual percentages of each scFOS, from trimers to nonamers.

Acceptance criteria: NLT 85.0% scFOS (≥ 30.0% trimer, ≥ 45.0% tetramer, and ≥ 5.0% pentamer and larger), with the remainder being glucose, fructose, and sucrose, on the dried basis

IMPURITIES

Inorganic Impurities

- **LEAD,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIIB

Acceptance criteria: NMT 1 mg/kg

SPECIFIC TESTS

- **RESIDUE ON IGNITION (SULFATED ASH),** Appendix IIC

Analysis: Ignite sample at 525° for 2 h.

Acceptance criteria: NMT 0.1%

- **TOTAL SOLIDS,** *Water Determination, Karl Fischer Titrimetric Method,* Appendix IIB

Analysis: Calculate the percent *Total Solids* by the formula:

$$\text{Result} = (W_U - W_W) \times 100/W_U$$

W_U = weight of the sample taken (mg)

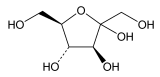
W_W = weight of the water determined (mg)

Acceptance criteria: NLT 95.0%

Fructose

First Published: Prior to FCC 6

D-Fructose
Levulose
Fruit Sugar



$C_6H_{12}O_6$ Formula wt 180.16

CAS: [57-48-7]

DESCRIPTION

Fructose occurs as white, hygroscopic, purified crystals or as a purified crystalline powder. It is a natural constituent of fruit, and is obtained from glucose in corn syrup by the use of glucose isomerase. Its density is about 1.6. It is soluble in methanol and in ethanol, freely soluble in water, and insoluble in ether.

Function: Nutritive sweetener

Packaging and Storage: Store in tight containers protected from humidity.

IDENTIFICATION

- **A. PROCEDURE**

Sample solution: 100 mg/mL

Analysis: Add a few drops of a *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.

Acceptance criteria: A copious red precipitate of cuprous oxide is formed.

- **B. INFRARED ABSORPTION,** *Spectrophotometric Identification Tests,* Appendix IIIC

Reference standard: USP Fructose RS

Sample and standard preparation: *K*

Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.

ASSAY

- **ANGULAR ROTATION,** *Optical (Specific) Rotation,* Appendix IIB

Sample: 10 g, previously dried

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