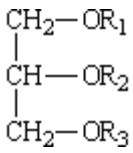
CITRIC and FATTY ACID ESTERS of GLYCEROL

Prepared at the 35th JECFA (1989), published in FNP 49 (1990) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). An ADI 'not limited' was established at the 17th JECFA (1973)

SYNONYMS Citric acid esters of mono- and di-glycerides, citroglycerides, CITREM; INS No. 472c

DEFINITION Obtained by esterification of glycerol with citric acid and edible fatty acids or by reaction of a mixture of mono- and diglycerides of edible fatty acid with citric acid; consists of mixed esters of citric acid and edible fatty acids with glycerol; may contain minor parts of free fatty acids, free glycerol, free citric acid and mono- and diglycerides; may be wholly or partially neutralized with sodium hydroxide or potassium hydroxide (as declared on the label).

Structural formula



Where at least one of R_1, R_2 or R_3 represents a citric acid moiety, one represents a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen.

DESCRIPTION White to ivory coloured, oily to waxy material.

FUNCTIONAL USES Stabilizer, emulsifier, dough conditioner, antioxidant synergist

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water; dispersible in hot water; soluble in oils and fats; insoluble in cold ethanol

Tests for fatty acids (Vol. 4) Passes tests

Test for citric acid (Vol. 4) Passes tests

Test for glycerol (Vol. 4) Passes tests

PURITY

Sulfated ash (Vol. 4) Not neutralized products: not more than 0.5%
Partially or wholly neutralized products: not more than 10%
Test 2 g of the sample (Method I)

Free glycerol (Vol. 4) Not more than 4%

Total glycerol 8-33%
See description under TESTS

Total citric acid

13-50%
See description under TESTS

Total fatty acid

37-81%
See description under TESTS

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

Total glycerol

Weigh accurately about 2 g of the sample into a saponification flask, add 50 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min.
To a 1-liter volumetric flask add exactly 99 ml (from a buret) of chloroform and 25 ml of glacial acetic acid. Quantitatively transfer the content of the saponification flask to the 1-liter volumetric flask, using three 25 ml portions of water. Add about 500 ml of water, and shake vigorously for about 1 min. Dilute to volume with water, mix thoroughly, and set aside for separation of layers.

Pipet 50 ml of acetic periodic acid TS into a series of 400-ml beakers. Prepare two blanks by adding 50 ml of water to each. Pipet 50 ml of the aqueous sample solution into one of the beakers containing 50 ml of acetic periodic acid TS, shake gently to mix, cover with watch glass, and allow to stand 30 min. but not longer than 1.5 h. Add 20 ml of 15% potassium iodide solution, shake gently to mix, and allow to stand at least 1 min. but not more than 5 min. Do not allow to stand in bright or direct sunlight. Add 100 ml of water and titrate with 0.1 N sodium thiosulfate. Use a variable speed electric stirrer to keep the solution thoroughly mixed. Continue the titration to the disappearance of the brown iodine colour from the aqueous layer. Add 2 ml of starch TS and continue the titration to the disappearance of iodine from the tiny chloroform layer separated during titration and the disappearance of the blue iodo-starch colour from the aqueous layer.

Calculation
% total glycerol = [(B - S) x N x 2.302]/W

where
B = titration of blank containing 50 ml of water
S = titration of sample
N = exact normality of 0.1 N thiosulfate
W = weight of sample represented by aliquot pipetted for test
i.e. W = [a x 50]/900
where a = weight in g of sample

Total citric acid

Principle
The sample is saponified with alcoholic potassium hydroxide solution and the fatty acids removed by extraction. The citric acid is converted to trimethylsilyl

(TMS) derivatives and analyzed by *gas liquid chromatography.*

Saponification
Weigh accurately about 1 g of the sample into a round bottomed flask, add 25 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min. Acidify the mixture with hydrochloric acid and evaporate in rotary evaporator or by other suitable method.

Extraction
Quantitatively transfer the content of the flask to a separator, using not more than 50 ml of water and extract with three 50-ml portions of heptane, discarding the extracts. Transfer the aqueous layer to a 100-ml volumetric flask, neutralize, dilute to volume with water, and mix.

Derivatization
Pipette 1 ml of this solution, and 1 ml of tartaric acid solution (1 mg/ml in water) into a 10 ml cappable round bottom flask and evaporate to dryness. Add 1 ml of pyridine, 0,2 ml of trimethyl-chlorosilane (TMCS), 0.4 ml of hexamethyl- disilazane (HMDS), 0.1 ml of N-methyl-N-trimethylsilyl-tri-fluoroacetamide (MSTFA). Cap the flask tight and swirl carefully to obtain total dissolution. Heat the flask in an oven at 60º for 1 h.

Gas chromatography
Any suitable gas chromatograph may be used fitted with a flame ionization detector and a column (glass 1.8 m x 2 mm i.d.) packed with 10% DC-200 on chromosorb Q (80/100 mesh). Recommended conditions are: oven temperature, 165º; injection block temperature, 240º; detector block temperature, 240º; nitrogen carrier gas flow rate, 24 ml/min.

Procedure
Inject a 5 µl sample of the TMS derivatives. The retention time for tartaric acid is about 12 min. and the relative retention time citric acid/tartaric acid is about 2.3.

Repeat the procedure as described above under Derivatization and Gas chromatography using 1 ml of a reference citric acid - solution (3 mg/ml in water) instead of 1 ml of sample solution.

Calculation
Measure each peak area by a suitable method.
% total citric acid = $[A_{CS} \times A_{TR} \times W_{CR} \times 100 \times 100]/[A_{TS} \times A_{CR} \times W]$

where
A_{CS} = peak area of citric acid (sample solution)
A_{TS} = peak area of tartaric acid (sample solution)
A_{TR} = peak area of tartaric acid (reference solution)
A_{CR} = peak area of citric acid (reference solution)
W_{CR} = weight (g) of citric acid in 1 ml of the reference solution
W = weight (g) of sample of citric and fatty acid esters of glycerol

Total fatty acid

Weigh accurately about 5 g of the sample into a 250-ml round-bottomed flask, add 50 ml of 1 N ethanolic potassium hydroxide, and reflux for 1 h on a water bath.

Quantitatively transfer the content of the saponification flask to a 1,000-ml separating funnel, using three 25-ml portions of water, and add 5 drops of methyl orange TS.

Cautiously add concentrated hydrochloric acid until the colour of solution changes clearly red, and shake well to separate fatty acids.

Extract the separated fatty acids with three 100-ml portions of diethyl ether. Combine the extracts, and wash with 50-ml portions of 10% sodium chloride solution until the washed sodium chloride solution becomes neutral.

Dry the ether solution with anhydrous sodium sulfate. Then evaporate off ether on a steam bath, leave additional 10 min on the steam bath, and weigh the residue.