CITRIC AND FATTY ACID ESTERS OF GLYCEROL

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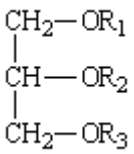
SYNONYMS Citric acid esters of mono- and di-glycerides, citroglycerides, CITREM; INS No. 472c

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

Test for fatty acids (Vol. 4) Passes test

Test for citric acid (Vol. 4) Passes test

Test for glycerol (Vol. 4) Passes test

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/k Determine using an AAS (electrothermal atomization 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

Principle: Determined by oxidation of glycerol by sodium periodate in a strongly acid medium and subsequent periodate titration.

Procedure:
Weigh to the nearest 0.1 mg 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 99 ml ± 0.2 ml (from a buret) of chloroform.

Add 25 ml of glacial acetic acid (using a graduated cylinder). 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 further, and shake vigorously for about 1 min. Dilute to volume with water, stopper, 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 layer into one of the 400-ml 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 200 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 iodine-starch complex colour from the aqueous layer. Read the buret to the nearest 0.01 ml. Treat the blanks in the same way as the sample.

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

where
B is volume (ml) of 0.1 N sodium thiosulfate used for the blank
S = is volume (ml) of 0.1 N sodium thiosulfate used for the sample
N = exact normality of 0.1 N sodium thiosulfate
W = weight of sample used for the analysis

i.e. W = [a x b]/900

where a = weight in g of sample, b = volume of aqueous sample layer used

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.*

Reagents
Tartaric acid, Citric acid, potassium hydroxide TS, ethanolic, hydrochloric acid, heptane, pyridine, trimethyl-chlorosilane, hexamethyl-disilazane, N-methyl-N-trimethylsilyl-tri-fluoroacetamide

Preparation of solutions
Internal Standard solution: 1mg/mL Tartaric acid solution
Standard Stock solution: 3 mg/ml Citric acid solution in water

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

Extraction of sample
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 of sample
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), or equivalent. Recommended conditions are: oven temperature, 165º; injection block temperature, 240º; detector block temperature, 240º; nitrogen carrier gas flow rate, 24 ml/min, injection volume, 5 µl.

Procedure
Inject a 5 µl sample of the TMS derivative of sample. Measure each peak area by a suitable method, and calculate the percentage of citric acid in the sample taken. The retention time for tartaric acid is about 12 min. and the retention time for citric acid is about 27.6 min.
Repeat the procedure of saponification, extraction and derivitization as described above for sample using 1 ml of the standard stock solution (3 mg/ml citric acid) instead of 1 ml of sample solution. Perform the same Gas chromatography procedure.

Calculation
Measure each peak area by a suitable method.

$$\% \text{ Total citric acid} = R_s \times 100 \times R_o \times 100 \times (W_o/W_s)Rs$$

here
R_s = peak area ratio of citric acid and tartaric acid for sample solution
R_o = peak area ratio of tartaric acid and citric acid for standard solution
W = sample weight, g
Wo = weight (g) of citric acid in standard solution

Total fatty acid

Principle: This method measures total fatty acids by extracting with diethyl ether.

Procedure
Weigh accurately 5.000 g of the sample into a 250-ml round-bottomed flask, add 50 ml of potassium hydroxide, ethanolic, TS, 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 indicator solution.

Cautiously add 50% hydrochloric acid until the colour of solution changes to a red methyl orange end point. Add 1 ml of excess acid after the end point is reached. Shake well to mix the contents and separate the fatty acids.

Cool to room temperature and 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. This is the weight of the total fatty acids.

Calculation:

$$\text{Total Fatty acids, \%} = \frac{\text{mass of fatty acids, g} \times 100}{\text{mass of sample, g}}$$