THERMALLY OXIDIZED SOYA BEAN OIL interacted with MONO- and DIGLYCERIDES of FATTY ACIDS

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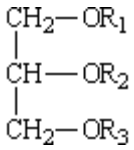
SYNONYMS INS no. 479, TOSOM

DEFINITION A complex mixture of esters of glycerol and fatty acids found in edible fat and fatty acids from thermally oxidized soya bean oil; produced by interaction and desodourization under vacuum at 130° of 10 % w/w of thermally oxidized soya bean oil (thermally oxidized soya bean oil is obtained by oxidation of refined soya bean oil with air at 190 - 200°) and 90 % w/w of mono- and diglycerides of food fatty acids.

Structural formula (principal component)



where R_1, R_2 and R_3 variously may be a:
- normal fatty acid
- oxidized fatty acid (e.g. hydroxyl and/or carbonyl compound of fatty acid)
- hydrogen
- short chain fatty acid
- di- and polymer of oxidized fatty acids

The product may contain small quantities of free fatty acids and free glycerol.

DESCRIPTION Pale yellow to light brown with a waxy or solid consistency.

FUNCTIONAL USES Emulsifier, antispattering agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in hot fats and oils

PURITY

Melting range (Vol. 4) 55 ° - 65°

Free fatty acids (Vol. 4) Not more than 1.5 % w/w calculated as oleic acid
Proceed as directed under *Free Fatty Acids* using the equivalence factor e = 28.2.

Free glycerol (Vol. 4) Not more than 2 % w/w

Total fatty acids 83 - 90 % w/w

	See description under TESTS
Total glycerol	16-22% w/w See description under TESTS
Fatty acids, insoluble in petroleum ether	Not more than 2 % w/w of total fatty acids See description under TESTS
Fatty acid methyl esters, not forming adduct with urea	Not more than 9.0 % w/w of total fatty acid methyl esters See description under TESTS
Peroxide value	Not more than 3 See description under TESTS
Epoxides	Not more than 0.03 % w/w oxiran oxygen 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 fatty acids

Weigh accurately about 12 g of the sample into a 500-ml conical flask, add 100 ml of 2 N alcoholic potassium hydroxide solution and reflux for 1 hour on a sand bath. It is necessary to swirl the flask every 5 to 10 min during the reflux period.

Transfer quantitatively the hot content of the saponification flask to a 500-ml separating funnel, using 100 ml of water and set aside to cool. Extract the aqueous solution by vigorously shaking for 1 min with 3 100-ml portions of petroleum ether (40-60^{o}). Transfer the aqueous solution to a clean 1000-ml round bottomed flask and discard the organic extracts. Reduce the volume of the aqueous solution by evaporation till about 200 ml, using a rotatory evaporator under vacuum at 70^{o} (the smell of ethanol has disappeared). Add cautiously 75 ml of 4 N hydrochloric acid and shake vigorously. Transfer the content of the flask to a 500-ml separating funnel, using 2 25-ml portions of water. Set aside to cool to 30-35^{o} and then add 2 50-ml portions of ether. When the mixture has cooled to room temperature, shake vigorously for about 1 min. Set aside for separation of layers.

Transfer the aqueous layer to a 500 ml separation funnel and extract with further 2 100-ml portions of ether. Transfer the aqueous solution to the 1000 ml round bottomed flask and combine all 3 ether extracts in a separating funnel. Wash the combined extracts with 3 100-ml portions of water and combine the washings with the aqueous solution in the round bottomed flask. This solution is used for the test for Total glycerol. Transfer the ether fraction to a dry and previously tared 500-ml round

bottomed flask. Evaporate to dryness using a rotatory evaporator at vacuum and slowly increasing the temperature from 40° to 70°. Add 100 ml of acetone and evaporate to dryness. Empty the receiver and continue to evaporate at full vacuum at 100° for further 20 min.
Place the flask in an oven at 110±5° for 1 hour. Cool in a desiccator and weigh the flask, now containing the isolated fatty acids.

Calculation:

$$Total\ fatty\ acids\ (\%) = \frac{(B - A) \times 100}{W}$$

where
A = weight of empty flask (g)
B = weight of flask and fatty acids (g)
W = weight of sample (g)

Total glycerol

Use the aqueous solution obtained by the test for Total fatty acids. Transfer quantitatively the aqueous solution to a 1000-ml volumetric flask. Dilute to mark with water. Pipet 50 ml of periodic acid into a 400 ml beaker and then 15 ml of the sample solution. Shake gentle to affect thorough mixing. Cover with a watch glass and allow to stand for 30 min. Add 20 ml of potassium iodide solution (150 g/l), mix by gentle shaking and allow to stand 1 min (never more than 5 min) protected from light. Add water to approximately 200 ml and titrate with 0.1 N sodium thiosulfate TS solution using Starch solution TS as indicator. Carry out a blank using 15 ml of water instead of sample solution.

Calculation:

$$Total\ glycerol\ (\%) = \frac{(B - S) \times 2.302 \times 1000}{W \times 15}$$

where,
B = ml of sodium thiosulfate used for the blank
S = ml of sodium thiosulfate used for the sample
N = normality of sodium thiosulfate
W = weight of sample (g)

Fatty acids, insoluble in petroleum ether

Weigh accurately about 5 g of the isolated fatty acids, obtained by the test for Total fatty acids, into a 250-ml round bottomed flask (flask I). Add 100 ml of petroleum ether (40-60°) and reflux for 30 min. at 55° on a water bath. Cool, close the flask with a glass stopper and leave over-night.

Heat the flask under reflux to 55° and decant and discard the organic solution. Wash the flask and its content with 2 25-ml portions and 1 10-ml portion of petroleum ether. Discard the washings.
Add 30 ml of 96 % v/v solution of ethanol to flask I and dissolve the content at low heat. Filter the solution into a dry and previously weighed 100-ml round bottomed flask (flask II). Wash flask I and the filter thoroughly with 3 10 ml-portions of 96 % v/v solution of ethanol.

Evaporate the content of flask II to dryness using a rotatory evaporator under vacuum at 70°. Add 50 ml of petroleum ether. Heat to 55° at a water bath under reflux for 30 min. Cool and decant and discard the petroleum ether solution. Wash the content of flask II with further 2 25-ml portions of petroleum ether. Discard the washings.

Evaporate the content of flask II to dryness using a rotatory evaporator under vacuum at 70° at a water bath. Continue to evaporate for further 15 min at full vacuum. Leave the flask in an oven at 105±5° for 1 h. Cool in an desiccator and weigh the flask.

Calculate the Fatty acids, not soluble in petroleum ether (% w/w of total fatty acids, from:

$$\frac{(B - A) \times 100}{W}$$

where
A = weight of empty flask II (g)
B = weight of flask II with content (g)
W = weight of sample of fatty acids (g)

Fatty acid methyl esters, not forming adduct with urea

Weigh accurately about 5 g of the isolated fatty acids, obtained by the test for Total fatty acids, into a dry previously weighed 250-ml round bottomed flask. Add 10.0 ml of methanol, 1.0 ml of conc. hydrochloric acid and 25 ml of dimethoxypropane (mix after each addition). Close the flask using a glass stopper, swirl if necessary to dissolve and leave for reaction at room temperature for 1 hour.

Add 50 ml of toluene and evaporate to dryness under vacuum at 60° at water bath using a rotatory evaporator. Dissolve the residue in 50 ml of petroleum ether and evaporate to dryness under the same conditions as before. Continue to evaporate for further 15 min under full vacuum at 100°.

Place the flask now containing the fatty acid methyl esters in an oven at 105±5° for 1 hour. Cool in a desiccator.

Introduce in small portions 250 g of urea into a 30 x 2 cm glass column with a fritted glass disk at bottom tapping the column to assure optimal packing. Connect a separatory funnel to the top of the column through a stopper. Add to the separator y funnel, 150 ml of methanol, previously saturated with urea at room temperature. Introduce the methanol through the stopcock of the separatory funnel at a flow rate of approximately 10 ml/min.

Weigh accurately about 5 g of the fatty acid methyl esters into a 250-ml conical flask and dissolve in 100 ml of methanol. Transfer quantitatively the solution to the separatory funnel using 2 25-ml portions of methanol, previously saturated with urea at room temperature. Elute the solution through the stopcock of the separatory funnel at a flow rate of approximately 10 ml/min. Collect the eluate in a 500-ml roundbottomed

flask. Add to the separatory funnel when empty, 200 ml of methanol, previously saturated with urea at room temperature and continue elution until the flow from the column stops.

Evaporate the eluate, using a rotatory evaporator under vacuum at 60°, until crystals accurately appear in the liquid. Add 200 ml of water to the flask and diluted hydrochloric acid till pH less than 3.

Transfer quantitatively the solution to a 1000-ml separatory funnel using 2 25-ml portions of water and 1 50-ml portion of ether. Shake vigorously and set aside to separate. Repeat the extraction with 3 50-ml portions of ether further, collecting the ether fractions in a 500-ml separatory funnel. Discard the water fraction. Wash the combined ether fractions with 2 50-ml portions of water. Discard the washings.

Transfer quantitatively the ether solution to a dry previously weighed 500-ml round bottomed flask using a small quantity of acetone. Evaporate to dryness using a rotatory evaporator under vacuum at 40-50°. Add 50 ml of acetone and dissolve the residue. Evaporate to dryness under the same conditions. Add further 50 ml of acetone, dissolve and evaporate to dryness. Continue evaporation under full vacuum at 100° for 45 min. Place the flask in an oven at 105±5° for 1 hour, cool in a desiccator and weigh the flask with content.

Calculate Fatty acid esters not forming adduct with urea (% of total fatty acid esters) from

$$\frac{(B - A) \times 100}{W}$$

where,
A = weight of empty flask (g)
B = weight of flask with content (g)
W = weight of fatty acid esters (g)

Peroxide value

Weigh accurately about 5 g of the sample into a 200-ml conical flask. Add 30 ml of a 2:3 solution of chloroform and acetic acid TS and close the flask with a stopper. Heat with warm water and swirl to dissolve the sample. Cool to room temperature and add 0,5 ml of saturated potassium iodide solution. Close the flask with the stopper and shake vigorously for 60±5 sec.

Add 30 ml of acetic acid TS and titrate immediately with 0.01 N Sodium thiosulfate using Starch TS as indicator.

Carry out a blank determination without sample.
Calculation:

$$Peroxide\ value = \frac{(a - b) \times N \times 1000}{W}$$

where

a = amount of sodium thiosulfate used for the sample (ml)
b = amount of sodium thiosulfate used for the blank (ml)
N = normality of the sodium thiosulfate
W = weight of sample (g)

Epoxides

Accurately weigh about 3 g of the sample into 250-ml round bottomed flask, add 10 ml of monochlorobenzene and dissolve the sample. Dilute with 40 ml of 2-propanol, add 10 ml of 0.1 N 2,4,6-trimethylpyridin hydrochloride solution and reflux for 1 hour at a warm sand bath. Let cool to room temperature and add 25 ml of water. Measure the temperature of the solution and determine the excess of 2,4,6-trimethylpyridin hydrochloride by potentiometric titration with 0.1 N sodium methylate solution. Carry out a blank without sample.

Calculation:

$$\%\ of\ oxiran\ oxygen = \frac{(a - b) \times N \times 16}{10 \times W}$$

where
a = amount of sodium methanolate solution used for the sample (ml)
b = amount of sodium methanolate solution used for the blank (ml)
N = normality of sodium methanolate solution
W = weight of sample (g)