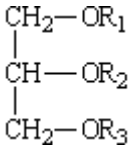
THERMALLY OXIDIZED SOYA BEAN OIL

Prepared at the 39th JECFA (1992), published in FNP 52 Add 1 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI of 0-3 mg/kg bw was established at the 39th JECFA (1992)

SYNONYMS TOS

DEFINITION Obtained by oxidation of refined soya bean oil with air at 190 - 250°, until the refractive index has reached a value of 1.475 - 1.485; consists of a complex mixture of substances formed during the oxidation.

Structural formula (principal component)



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

DESCRIPTION Brown, sticky gel

FUNCTIONAL USES Release agent, emulsifier

CHARACTERISTICS

IDENTIFICATION

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

PURITY

Refractive index (Vol. 4) n (40, D): 1.475 - 1.485

Saponification value (Vol. 4) Not more than 220

Unsaponifiable matter Not more than 1 % w/w
See description under TESTS

Total fatty acids 91 - 97 % w/w
See description under TESTS

Fatty acids, insoluble in petroleum ether Not more than 40 % w/w of total fatty acids
See description under TESTS

Fatty acid methyl esters, not forming adduct with urea Not more than 60 % w/w of total fatty methyl esters
See description under TESTS

Peroxide value Not more than 5

See description under TESTS

Epoxides

Not more than 0.5 % w/w as 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

Unsaponifiable matter

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}). Combine the organic extracts in a separating funnel and transfer the aqueous solution to a clean 1000-ml round bottomed flask (this solution is used for the determination of Total fatty acids).

Wash the organic extract with 3 100-ml portions of 50 % v/v of ethanol and transfer the extract to a dry and previously tared 500-ml round bottomed flask. Evaporate using a rotary evaporator at 60^{o}. When all visible solvent has been removed, empty the receiver and evaporate under full vacuum at 80^{o} for 15 min. Place the flask in a vacuum oven at 70^{o} for 30 min, cool in a desiccator and weigh.

Add 20 ml of 99% v/v of ethanol to the flask and dissolve the residue. Titrate the solution with a 0.1 N sodium methylate solution using phenolphthalein as an indicator.

Calculation

$$Unsaponifiable\ matter\ (\%) = \frac{(B - A) - 0.28 \times V \times N}{W}$$

where
A = weight of empty flask (g)
B = weight of flask and residue (g)
W = weight of sample (g)
V = volume of sodium methanolate used for titration (ml)
N = normality of sodium methylate

Total fatty acids

Use the aqueous solution obtained in the test for Unsaponifiable matter for this test.

Reduce the volume of the 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.

Extract the aqueous layer with further 2 100-ml portions of ether. Discard the aqueous solution. Combine all 3 ether extracts in a separating funnel. Wash the combined ether extracts with 3 100-ml portions of water. 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^{o} to 70^{o}. Add 100 ml of acetone and evaporate to dryness. Empty the receiver and continue to evaporate at full vacuum at 100^{o} for further 20 min.

Place the flask in an oven at 110±5^{o} 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)

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^{o}) and reflux for 30 min. at 55^{o} on a water bath. Cool, close the flask with a glass stopper and leave over-night.

Heat the flask under reflux to 55^{o} 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^{o}. Add 50 ml of petroleum ether. Heat to 55^{o} 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^{o} at a water bath. Continue to evaporate for further 15

min. at full vacuum. Leave the flask in an oven at 105±5^{o} for 1 hour. 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^{o} 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^{o}.

Place the flask now containing the fatty acid methyl esters in an oven at 105±5^{o} 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 separatory 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^{o}, 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^{o}. 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^{o} for 45 min. Place the flask in an oven at 105±5^{o} 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

Carry out the test on an oil/gel solution, and calculate back to the content in pure gel; determine the blank value of the oil before mixing with the gel. 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

Carry out the test on an oil/gel solution, and calculate back to the content in pure gel; determine the blank value of the oil before mixing with the gel. 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:

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

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)