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Olive Oil

CAS RN®: 8001-25-0.

DEFINITION

Olive Oil is the refined fixed oil obtained from the ripe fruit of *Olea europaea* L. (Fam. Oleaceae). It may contain suitable antioxidants.

IDENTIFICATION

• **A. IDENTITY BY FATTY ACID COMPOSITION**

Analysis: Proceed as directed in the test for [Fats and Fixed Oils \(401\)](#), [Fatty Acid Composition](#).

Acceptance criteria: Meets the composition profile of fatty acids in [Table 1](#)

• **B. IDENTITY BY TRIGLYCERIDE PROFILE**

Analysis: Proceed as directed in the test for [Identification of Fixed Oils by Thin-Layer Chromatography \(202\)](#).

Acceptance criteria: Meets the requirements in the chapter

IMPURITIES

• **ALKALINE IMPURITIES**

Sample: 10 mL of Olive Oil

Analysis: Mix 10 mL of freshly opened acetone and 0.3 mL of water, and add 0.05 mL of bromophenol blue TS. Add the *Sample*, shake, and allow to stand. Titrate with 0.01 N hydrochloric acid VS to change the color of the upper layer to yellow.

Acceptance criteria: NMT 0.1 mL of 0.01 N hydrochloric acid is required.

SPECIFIC TESTS

• [FATS AND FIXED OILS, Acid Value \(Free Fatty Acids\) \(401\)](#): NMT 0.3. [NOTE—Petroleum ether with a 100°–120° boiling range can be used to replace ether in the test.]

• [FATS AND FIXED OILS, Peroxide Value \(401\)](#): NMT 10.0

• [FATS AND FIXED OILS, Fatty Acid Composition \(401\)](#): Olive Oil exhibits the composition profile of fatty acids shown in [Table 1](#), as determined in the chapter.

Table 1

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
<16	0	≤0.1
16	0	7.5–20.0
16	1	≤3.5
18	0	0.5–5.0
18	1	56.0–85.0
18	2	3.5–20.0
18	3	≤1.2

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
20	0	≤0.7
20	1	≤0.4
22	0	≤0.2
24	0	≤0.2

• **ABSENCE OF SESAME OIL**

Sample: 10 mL of Olive Oil

Analysis: Mix the *Sample* with a mixture of 0.5 mL of a 0.35% (v/v) solution of furfural in acetic anhydride and 4.5 mL of acetic anhydride, and shake the mixture for about 1 min. Pass through a filter paper previously wetted with acetic anhydride. Add 0.2 mL of sulfuric acid to the filtrate.

Acceptance criteria: No bluish-green color develops.

- **FATS AND FIXED OILS, *Unsaponifiable Matter* (401):** NMT 1.5%. [NOTE—Petroleum ether with a 40°–60° boiling range can be used to replace ether in the test.]

• **ULTRAVIOLET ABSORBANCE**

Sample solution: Dissolve 1.0 g of Olive Oil in cyclohexane, and dilute with cyclohexane to 100 mL.

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: UV-Vis

Wavelength: 270 nm

Path length of the cell: 1 cm

Analysis: Determine the UV-Vis absorbance using the *Instrumental conditions* described above.

Acceptance criteria: The absorbance is NMT 1.20.

- **WATER DETERMINATION, *Method Ic* (921):** NMT 0.1%

• **STEROL COMPOSITION**

2 M Alcoholic potassium hydroxide solution: Dissolve 12 g of potassium hydroxide in 10 mL of water, and dilute with alcohol (ethanol) to 100 mL.

Sample A: Accurately weigh 5 g of Olive Oil into a 150-mL flask fitted with a reflux condenser. Add 50 mL of 2 M Alcoholic potassium hydroxide solution, and heat on a water bath for 1 h, shaking frequently. Add 50 mL of water through the top of the condenser, shake, and allow to cool. Transfer the contents of the flask to a separating funnel. Rinse the flask with several portions totaling 50 mL of petroleum ether with a 40°–60° boiling range, and add the rinsings to the separating funnel. Shake vigorously for 1 min. Allow to separate, and transfer the aqueous layer to a second separating funnel. If an emulsion forms, add small quantities of alcohol or a concentrated solution of potassium hydroxide. Shake the aqueous layer with two 50-mL quantities of petroleum ether with a 40°–60° boiling range. Combine the petroleum ether layers in a third separating funnel and wash with three 50-mL quantities of 50% alcohol. Transfer the petroleum ether layer to a tared 250-mL flask. Rinse the separating funnel with small quantities of petroleum ether with a 40°–60° boiling range, and add to the flask. Evaporate the petroleum ether on a water bath and dry the residue at 100°–105° for 15 min, keeping the flask horizontal. Allow to cool in a desiccator and weigh.

Reference A: Accurately weigh 5 g of sunflower oil into a 150-mL flask fitted with a reflux condenser. Proceed as directed for *Sample A*, beginning with “Add 50 mL of 2 M Alcoholic potassium hydroxide solution”.

Separation of the sterol fraction by LC

Mobile phase: Isopropyl alcohol and *n*-hexane (1:99)

Sample solution A: Transfer *Sample A* with three 4-mL quantities of petroleum ether with a 40°–60° boiling range to a 15-mL test tube.

[NOTE—Ether can be used to replace petroleum ether if *Sample A* is not well soluble in petroleum ether.] Evaporate to dryness under a stream of nitrogen. Dissolve *Sample A* in *Mobile phase* to obtain a solution with an approximate concentration of 40 mg/mL. Add a few drops of isopropyl alcohol to improve the solubility. [NOTE—3 drops are normally sufficient to ensure complete solubilization.] Pass through a membrane filter (nominal 0.45-μm pore size).

Reference solution A: Prepare as directed for *Sample solution A*, except use *Reference A* instead of *Sample A*.

Chromatographic system

(See [Chromatography \(621\)](#).)

Mode: LC