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Almond Oil

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CAS RN®: 8007-69-0.

DEFINITION

Almond Oil is the refined fixed oil obtained by expression from the kernels of varieties of *Prunus dulcis* (Miller) D.A. Webb (formerly known as *Prunus amygdalus* Batsch) (Fam. Rosaceae), except for *Prunus dulcis* (Miller) D.A. Webb var. amara (De Candolle) Focke. 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⟩, Procedures, 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 *Identification of Fixed Oils by Thin-Layer Chromatography ⟨202⟩, Identification, Method I* or *Method II*.

  Acceptance criteria: Meets the requirements in the chapter

SPECIFIC TESTS

- **Fats and Fixed Oils ⟨401⟩, *Procedures, Acid Value*:** NMT 0.5
- **Fats and Fixed Oils ⟨401⟩, *Procedures, Fatty Acid Composition*:** Almond Oil exhibits the composition profiles of fatty acids in *Table 1*.

Table 1

Carbon-Chain Length	Number of Double Bonds	Percentage (%)
<16	0	≤0.1
16	0	4.0–9.0
17	0	≤0.2
18	0	≤3.0
20	0	≤0.2
22	0	≤0.2
24	0	≤0.2
16	1	≤0.8
17	1	≤0.2
18	1	62.0–76.0
18	2	20.0–30.0
18	3	≤0.4
20	1	≤0.3
22	1	≤0.1

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- **Fats and Fixed Oils ⟨401⟩, *Procedures, Peroxide Value*:** NMT 5.0
- **Fats and Fixed Oils ⟨401⟩, *Procedures, Unsaponifiable Matter*:** NMT 0.9%
- **Specific Gravity ⟨841⟩:** 0.910–0.915
- **Sterol Composition**

Separation of the sterols fraction

Reference solution A: 5% (w/v) of cholesterol in chloroform

Developing solvent system: Toluene and acetone (19:1) or hexane and ether (13:7)

Sample solution A: Weigh 5 g of Almond Oil into a 250-mL flask. Add 50 mL of 2 N alcoholic potassium hydroxide, and heat to gentle boiling with continuous vigorous stirring until saponification takes place (the solution becomes clear). Continue heating for an additional 20 min, and add 50 mL of water from the top of the condenser. Cool the flask to approximately 30°. Transfer the contents of the flask to a 500-mL separating funnel with several rinses of water, amounting in all to 50 mL. Add approximately 80 mL of ether, shake vigorously for approximately 30 s, and allow to settle.

[Note—Any emulsion can be destroyed by adding small quantities of ethyl or methyl alcohol by means of a spray.]

Separate the lower aqueous phase, and collect it into a second separating funnel. Perform two further extractions on the water–alcohol phase in the same way, using 60–70 mL of ether on each occasion. Pool the ether extracts into a single separating funnel, and wash with water, 50 mL at a time, until the wash water is no longer alkaline to phenolphthalein. Dry the ether phase with anhydrous sodium sulfate, and filter on anhydrous sodium sulfate into a previously weighed 250-mL flask, washing the funnel and filter with small quantities of ether. Distill the ether down to a few milliliters, and bring to dryness under a slight vacuum or in a stream of nitrogen. Completely dry at 100° for approximately 15 min, and then weigh after cooling in a desiccator. Dissolve the unsaponifiables so obtained in chloroform to prepare a solution having a concentration of approximately 5%.

Sample solution B: Treat 5 g of canola oil in the same way as prescribed for Almond Oil in *Sample solution A*, beginning with "Add 50 mL of 2 N alcoholic potassium hydroxide".

Sample solution C: Treat 5 g of sunflower oil in the same way as prescribed for Almond Oil in *Sample solution A*, beginning with "Add 50 mL of 2 N alcoholic potassium hydroxide".

Analysis: Precondition the thin-layer chromatographic plate (see *Chromatography ⟨621⟩*), 20-cm × 20-cm silica gel on polyester with a layer thickness of 200 µm and particle size of 5–17 µm, by immersing completely in the 0.2 N alcoholic potassium hydroxide for 10 s, then allow to dry in a fume cupboard for 2 h, and finally place at 100° for 1 h.

Remove from the validated heating device, and keep the plate in a desiccator until required for use. The plates must be used within 15 days. [Note—Thin-layer chromatographic plates without requiring the preconditioning are also commercially available.] Use a separate plate for each *Sample solution*.

Place the *Developing solvent system* in the chamber to a depth of approximately 1 cm. Close the chamber with the appropriate cover, and leave for at least 30 min. Strips of filter paper dipping into the eluant may be placed on the internal surfaces of the chamber. The *Developing solvent system* should be replaced for every test to ensure reproducible elution conditions.

Apply 0.3 mL of *Sample solution A* approximately 2 cm from the lower edge in a streak that is as thin and as uniform as possible. In line with the streak, place 2–3 µL of *Reference solution A* at one end of the plate. Develop the chromatograms in an equilibrated chamber with a *Developing solvent system* until the solvent front reaches approximately 1 cm from the upper edge of the plate. Remove the plate from the developing chamber, and evaporate the solvent under a current of hot air (avoid excessive heat), or by leaving the plate for a short while under a hood. Spray the plate with a 0.2% alcoholic solution of 2,7-dichlorofluorescein, and examine in UV light at 254 nm.

[Note—The plates pretreated with UV indicator are also commercially available and used equivalently.]

In each of the plates, mark the limits of the sterol band identified through being aligned with the stain of *Reference solution A* along the edges of the fluorescence, and additionally include the area of the zones 2–3 mm above and below the visible zones corresponding to *Reference solution A*. Remove the silica gel in the marked area into a filter funnel with a G3 porous septum. Add 10 mL of hot chloroform, mix carefully with the metal spatula, filter under vacuum, and collect the filtrate in the conical flask attached to the filter funnel. Wash the residue in the funnel three times with ether, 10 mL each time, and collect the filtrate in the same flask attached to the funnel. Evaporate the filtrate to a volume of 4–5 mL, transfer the residual solution to a previously weighed 10-mL test tube with a tapering bottom and a sealing stopper, and evaporate to dryness by mild heating in a gentle stream of nitrogen. Dissolve the residue in a few drops of acetone, and evaporate again to dryness. Place at 105° for approximately 10 min, allow to cool in a desiccator, and weigh.

Treat *Sample solution B* and *Sample solution C* the same way as prescribed for *Sample solution A*.

Determination of the sterols

Sample solution D: To the test tube containing the sterol fraction separated from Almond Oil by thin-layer chromatography add a freshly prepared mixture of anhydrous pyridine, hexamethyldisilazane, and chlorotrimethylsilane (9:3:1) in the ratio of 50 µL for every mg of sterols, avoiding any uptake of moisture.

[Note—This reagent is also commercially available and used equivalently.]

Stopper the test tube, and shake carefully until the sterols are completely dissolved. Allow it to stand for at least 15 min at ambient temperature, and centrifuge for a few minutes if necessary. Use the supernatant. The slight opalescence that may form is normal and does not cause an anomaly. However, the formation of a white floc or the appearance of a pink color is indicative of the presence of moisture or deterioration of the reagent. If these occur, repeat the test.

Reference solution E: To 9 parts of the sterols separated from canola oil by thin-layer chromatography add 1 part of cholesterol. Treat the mixture in the same way as prescribed in *Sample solution D*.

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