Method to estimate Total Polar Compounds in Edible Oils and Fats

(AOAC Official Method 982.27)

A. Principle

The method determines the extent to which fats and oils deteriorate when used for frying. These fats and oils can be separated by the process of Silica Gel based column chromatography into polar and non polar components.

Note: Polar components include polar substances such as monoglyerides, diglycerides, free fatty fatty acids that occur in unused fats, as well as polar transformation products formed during frying of foodstuffs and/or during heating and these components of fats can be determined by column chromatography under specified conditions. Nonpolar components are mostly unaltered triglycerides.

B. Equipment

(a) Column - Glass, 2.1 cm id x 45 cm. with Teflon Stopcock and ground-glass joint

(b) TLC plates – Pre coated silica gel (without fluorescence indicator), 20 x 20 cm, layer thickness = 0.25 mm

C. Chemicals & Reagents

(a) Silica gel 60 (Adsorbent) - particle size 0.063-0.200 mm (70-230 mesh ASTM), adjust to H_2O content of 5% as follow: Dry silica gel≥4 h in porcelain dish in 160˚C oven; cool in desiccator to room temperature. Adjust H_2O content to 5%, e.g., weigh 152gm silica gel and 8gm H_2O in 500mL round-bottom flask with ground-glass stopped and mechanically shake 1 h.

(b) Petroleum ether (bp 40°-60°C) - ether (87+13) [Eluting solvent mixture]
(c) Sea-sand - Analytical reagent grade; purified by acid and calcined.
(d) Spray reagent- Molybdophosphoric acid, 10% in alcohol.

D. Procedure

(i) Preparation of Sample

Semi liquid and solid fats are warmed to temperature slightly above melting point and mix thoroughly such as to avoid overheating. Visible impurities are removed by filtration. Hydrophobic filter are to be used, if water is present.

(ii) Preparation of Column

(a) Column is to be prepared using 30 mL (approx.) of petroleum ether-ether (87+13). Also place wad of cotton wool in bottom of column and remove air by pressing with glass rod.
(b) Prepare slurry of 25gm silica gel and approx 80 mL petroleum ether-ether (87+13) in 100 mL glass beaker. Pour the slurry into column using 8 cm glass funnel. Beaker, funnel and sides of column are to be rinsed with same solvent. Open stopcock and drain solvent to 10cm above silica gel. Silica gel is leveled by tapping the column.
(c) Approx 4gm of sea-sand is added through funnel into column. Solvent is drained to sand layer.

(iii) Chromatography

Only nonpolar fraction is used to determine polar components by difference. However, if separation is controlled by TLC, both polar and nonpolar fractions are required. Separation may also be controlled by checking recovery of analytes. But for products containing substantial

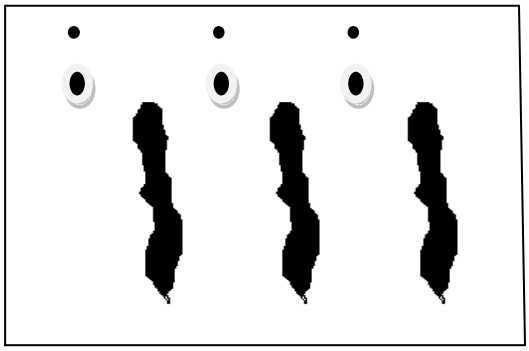
amounts of polar material, recovery may be incomplete because small amounts of highly polar material, generally 1-2%, are not eluted under conditions specified.

2.5±0.1 gm (to 0.001 gm) test portion is accurately weighed into 50 mL volumetric flask, and dissolved in approximately 20 mL petroleum ether-ether (87+13) while warming slightly. Let it cool to room temperature and dilute to volume with same solvent. 20 mL aliquot is transferred to column using volumetric pipet, without disturbing surface.

Two 250 mL round-bottom flasks are dried in 103° ± 2°C oven, cool to room temperature, and accurately weigh to 0.001 gm. One flask is placed under column, stopcock is opened, and solution is drained to level of sand layer. Nonpolar components are eluted with 150 mL petroleum ether-ether (87+13) contained in 250 mL dropping funnel. Flow rate is adjusted such that 150 mL passes through column within 60-70 min. After elution, wash any substance adhering to outlet of column into round-bottom flask with petroleum ether-ether (87 + 13).

In same manner, polar components are eluted into second 250 mL round-bottom flask with 150 mL ether. Silica gel is discarded.

Solvent is removed from each fraction with a rotary evaporator and 560°C water bath or with N_2 Stream in 250 mL flask on steam bath.



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FRACTION

Figure1. Evaluation of efficiency of fractionation by TLC separation of polar and nonpolar fraction:
Fraction 1 contains nonpolar components, and
Fraction 2 contains polar components.

Avoid losses due to foaming. If rotary evaporator is used, shortly before end of evaporation, introduce N_2 into system. Cool residue to ambient temperature and introduce N_2 into flask. Weigh flasks.

(iv) Calculations

Calculate polar components, as percent (w/v) with formula:

Polar components, % $=\frac{E-A}{A}x100$

Where A = nonpolar fraction (in gm); E= test portion (in gm) in 20 mL aliquot (ca 1 g). Report result to one decimal place.

(v) Check of Column Chromatography Efficiency by Thin-Layer Chromatography

Dilute polar and nonpolar fraction (1+9) in $CHCI_3$. Apply 2µL spots using capillary dispensing pipet. Develop plate with petroleum ether-ether-CH_3COOH (70+30+2) in tank lined with filter paper for approximately 35 minutes (ca 17 cm). Remove plate and let solvent evaporate.

Spray plate with 10% molybdophosphoric acid. After evaporation of alcohol, heat plate in 120˚-130˚C drying oven. Fraction 1 (nonpolar) should be free of polar substances (see Figure 1).