AOAC Official Method 989.05
Fat in Milk
Modified Mojonnier
Ether Extraction Method
First Action 1989
Final Action 1992

IDF–ISO–AOAC Method

A. Principle

Fat is extracted with mixture of ethers from known weight of milk. Ether extract is decanted into preweighed dry weighing dish, and ether is evaporated. Extracted fat is dried to constant weight. Result is expressed as % fat by weight.

B. Apparatus

(**a**) *Flask.*—Mojonnier-style ether extraction flask with volume of 21–23 mL in lower bulb plus neck at bottom of flask. Flask should have smooth, round opening at top that will seal when closed with cork.

(**b**) *Weighing dishes.*—Metal, 8.5–9.5 cm diameter and 4.5–5.5 cm tall; or 250 mL glass beakers.

(**c**) *Calibration weights.*—Class S, standard calibration weights to verify balance accuracy within weight range to be used for weighing empty flasks and flask plus test portion and weighing empty dishes and dish plus fat.

(**d**) *Analytical balance.*—Readability 0.0001 g. Accuracy on verification within 0.0002 g. Check periodically and whenever balance is moved or cleaned. Keep record of balance calibration checks.

(**e**) *Desiccator.*—Room temperature. For cooling weighing dishes after preliminary and final drying. Use coarse desiccant (mesh size 6–16) that contains minimum of fine particles and that changes color when moisture is absorbed.

(**f**) *Tongs.*—For handling weighing dishes.

(**g**) *Hot plate.*—Steam bath or other heating device. For evaporation of ether at 100°C. Carry out evaporation in hood.

(**h**) *Corks.*—High quality natural cork stoppers (size 5) for flasks. Soak corks in H_2O several hours to improve seal.

(**i**) *Vacuum or forced air oven.*—Vacuum oven maintaining temperature of 70 –75°C at 50.8 cm (20 in.) of vacuum, or forced air oven maintaining temperature of 100 1°C.

(**j**) *Water bath for tempering test samples prior to weighing.*—With thermometer and device to maintain milk temperature of 38 1°C.

C. Reagents

(**a**) *Ethyl ether.*—ACS grade, peroxide free. No residue on evaporation.

(**b**) *Petroleum ether.*—ACS grade, boiling range 30 –60°C. No residue on evaporation.

(**c**) *Ammonium hydroxide.*—Concentrated, ACS grade, specific gravity 0.9.

(**d**) *Ethyl alcohol.*—95%. No residue on evaporation.

(**e**) *Distilled water.*—Free of oil and mineral residue.

(**f**) *Phenolphthalein indicator.*—0.5% (w/v) in alcohol.

D. Determination

(**a**) *Weighing test portion.*—Prepare by tempering milk to 38°C as in **925.21** (*see* 33.2.02). Weigh empty flask with clean, dry cork stopper. Remove stopper. Pipet ca 10 g milk into flask. Place stopper in flask. Weigh to nearest 0.1 mg. Check balance zero between test portions.

(**b**) *Weighing dishes.*—Number clean weighing dishes and predry under same conditions that will be used for final drying after fat extraction. Be sure that all surfaces where weighing dishes will be placed (i.e., hot plate, desiccator, etc.) are clean and free of particulates. At end of oven drying, place dishes in room temperature desiccator and cool to room temperature. On same day as fat extraction, (**c**), weigh dishes to nearest 0.1 mg and record weights. Check balance zero after weighing each dish. Protect weighed dishes from contamination with extraneous matter.

(**c**) *Fat extraction.*—To test portion in flask add 1.5 mL NH_4OH and mix thoroughly. NH_4OH neutralizes any acid present and dissolves casein. Add 3 drops of phenolphthalein indicator to help sharpen visual appearance of interface between ether and aqueous layers during extraction. Add 10 mL 95% alcohol, stopper with H_2O-soaked cork, and shake flask 15 s. For first extraction, add 25 mL ethyl ether, stopper with cork, and shake flask very vigorously 1 min, releasing built-up pressure by loosening stopper as necessary. Add 25 mL petroleum ether, stopper with cork, and repeat vigorous shaking for 1 min. Centrifuge flasks at ca 600 rpm for 30 s to obtain clean separation of aqueous (bright pink) and ether phases. Decant ether solution into suitable weighing dish prepared as in (**b**). When ether solution is decanted into dishes, be careful not to pour over any suspended solids or aqueous phase into weighing dish. Ether can be evaporated at 100°C from dishes while conducting second extraction.

For second extraction, add 5 mL 95% alcohol, stopper with cork, and shake vigorously 15 s. Next, add 15 mL ethyl ether, replace cork, and shake flask vigorously 1 min. Add 15 mL petroleum ether, stopper with cork, and repeat vigorous shaking for 1 min. Centrifuge flasks at ca 600 rpm for 30 s to obtain clean separation of aqueous (bright pink) and ether phases. If interface is below neck of flask, add H_2O to bring level ca half way up neck. Add H_2O slowly down inside surface of flask so that there is minimum disturbance of separation. Decant ether solution for second extraction into same weighing dish used for first extraction.

For third extraction, omit addition of 95% alcohol and repeat procedure used for second extraction. Completely evaporate solvents in hood on hot plate at 100°C (avoid spattering). Dry extracted fat in weighing dish to constant weight in forced air oven at 100 1°C (30 min) or in vacuum oven at 70 –75°C at >50.8 cm (20 in.) of vacuum for 7 min. Remove weighing dishes from oven and place in desiccator to cool to room temperature. Record weight of each weighing dish plus fat.

Run pair of reagent blanks each day tests are conducted. To run reagent blank, replace milk test portion with 10 mL H_2O and run test as above. Record weight of any dry residue collected and use value in calculation. Reagent blank should be <0.0020 g residue. If reagent blanks for set of tests are negative use negative number in calculation. (*Note*: To subtract negative number [average weight blank residue] in equation below, add it to [(weight dish + fat) weight dish].) Negative blank usually indicates that dishes were not completely dry at start of determination or that balance calibration shifted between weighing of empty pans and pans plus fat. Cause of negative blanks should be identified and corrected.

E. Calculations

$$\text{Fat, \%} = 100 \frac{[(\text{weight dish} \quad \text{fat}) \quad (\text{weight dish})] \quad \text{average weight blank residue}}{\text{weight test portion}}$$

Maximum recommended difference between duplicates is <0.03% fat.

At 3.6% fat: $s_r = 0.015\%$; $RSD_R = 0.512\%$; $s_R = 0.020\%$; r value = 0.044%; $RSD_r = 0.396\%$; R value = 0.056%

Reference: *JAOAC* **71,** 898(1988).

Revised: March 1996