ADPI Analytical Method #004
Determination of Milkfat: Mojonnier Method

1.0 Purpose

This Analytical Method defines the standard operating procedure for determination of milkfat content in dairy powders.

2.0 Scope

This SOP is applicable to determination of milkfat content in dry powder dairy products. Note that product additives which are soluble in ethyl ether and/or petroleum ether (e.g., lecithin) will also be extracted along with milkfat and will cause inaccurate, higher results.

3.0 Definitions

3.1 **Milkfat** is the term applied to the fatty fraction of dairy. Milkfat is a complex mixture of fats comprised of many fatty acids, along with phospholipids, fat-soluble vitamins, and other constituents. Milkfat is nutritionally and economically important. Some dairy products are required by established standards to contain a minimum level of milkfat (e.g., butter) while others are limited to a maximum level (e.g., nonfat dry milk).

3.2 **Miscibility** is the degree to which two liquids are soluble in one another. Liquids are said to be **miscible** when they are mutually soluble in any proportion; conversely, they are described as **immiscible** if they are entirely insoluble in one another. Oil and water are the classic example of immiscible liquids, and they will readily separate into distinct layers when mixed in a single container. Ethyl alcohol and water are miscible; when combined, they form a homogeneous solution where the two liquids are no longer visually distinguishable from one another.

3.3 **Liquid-liquid extraction** is a preparative technique by which the analyte(s) of interest (in this case, milkfat) is/are recovered from a sample by first dissolving that sample in one solvent (in this case, water) and then recovering the analyte from that solution by use of one or more additional, immiscible solvents (in this case, ethyl ether and petroleum ether).

3.4 The **Mojonnier method** for fat determination is named for the eponymous dairy chemist brothers who invented the technique. It relies on ammonium hydroxide to disrupt and dissolve casein micelles; ethanol to help mitigate the interaction between the aqueous phase and subsequent organic solvents; and then a combination of ethyl ether and petroleum ether to dissolve and sequester the milkfat constituents. Numerous other analytical methods exist for milkfat, but the

Mojonnier method is widely accepted as the standard, especially in the U.S. dairy industry; the Gerber method is an alternative method more commonly accepted outside the U.S.

3.5 The immiscible solvents utilized in a liquid-liquid extraction are called often called **phases** (or **layers**), typically with one water-based portion which is called the **aqueous phase (layer)**, and one solvent-based portion which is often called the **organic phase (layer)**. Because water is more dense than most organic solvents typically used in liquid-liquid extraction, the lower layer tends to be the aqueous phase, while the upper layer tends to be the organic phase.

3.6 **Decanting** is the process by which one phase or layer is carefully drawn off such as with a pipette, or simply poured away from another phase or layer, such as on completion of a liquid-liquid extraction.

4.0 Principle

Milkfat is extracted from reconstituted dairy powder via liquid-liquid extraction, using ammonium hydroxide and ethyl alcohol to solubilize the powder, followed by ethyl ether and petroleum ether as extraction solvents. The solvents are then evaporated from the combined extracts, and the quantity of milkfat recovered from the sample is measured gravimetrically. A blank preparation tested in parallel ensures that extraction solvent residues conform to a maximum limit, and such residues are accounted for in the results calculation.

5.0 Reagents and Materials

In general, ether-based extraction methods are on the decline due to the handling dangers posed by ethers. Methods which employ different extraction solvents should be expected to yield potentially different results. *Standard Methods for the Examination of Dairy Products* endorses a specific grade of pentane for use in lieu of petroleum ether, for example, but ethyl ether is still specified.

Adhere to the following requirements carefully for consistent and accurate results.

5.1 Laboratory balance, with capacity of approximately 200 grams and with sensitivity of ± 0.0001 grams or better;

5.2 Spatula, or equivalent, suitable for weighing the samples;

5.3 Glazed paper or equivalent, suitable for weighing the samples;

5.4 Weighing dish, aluminum, flat-bottomed, or equivalent, suitable for collecting and drying the ether extracts;

5.5 Tongs or similar utensil, suitable for handling the weighing vessels;

5.6 Dessicator, suitable for holding the weighing vessels, with color-changing calcium sulfate dessicant or equivalent;

5.7 Laboratory oven, vacuum or convection type, capable of maintaining temperature as described in weighing dish preparation below;

5.8 Fat extraction flask, Mojonnier® G3, Nelson-Jameson part #1563437, or equivalent;

5.9 Stopper, No. 1, neoprene, or equivalent, suitable for use with the fat extraction flask;

5.10 Distilled water, or higher purity, warmed for use (approximately 60°C is recommended);

5.11 Pipette, 9.0 mL, Class A, calibrated to deliver, or equivalent apparatus, suitable for measuring the water addition below;

5.12 Ammonium hydroxide solution, ACS reagent grade or better, specific gravity 0.9;

5.13 Pipette, or equivalent apparatus, suitable for measuring the ammonium hydroxide addition;

5.14 Ethyl alcohol, 95% (v/v), either undenatured or Specially Denatured Alcohol (SDA) Formula No. 1 or No. 3-A, formulated with ACS reagent-grade ethanol or better, leaves no residue upon evaporation;

5.15 Pipette, or equivalent apparatus, compatible with the ethyl alcohol additions as described below;

5.16 Phenolphthalein indicator solution, ethanolic, 0.5% (m/v);

5.17 Pipette, dropper type, or equivalent apparatus, suitable for adding the phenolphthalein indicator solution;

5.18 Ethyl ether, peroxide-free, purified for fat extraction purposes;

5.19 Petroleum ether, boiling point range 30-60°C;

5.20 Pipette, or equivalent apparatus, compatible with the ether additions as described below;

5.21 Steam bath or hot plate, suitable for evaporating the ether extraction solvents as described below (steam bath apparatus is preferred because its temperature is limited to the boiling point of water, while a hot plate may develop contact temperatures substantially greater and may pose a greater risk when evaporating the extraction solvents);

5.22 *Optional:* Mojonnier Dairy Tester®, model A, D, F, H, or equivalent (manufacture of these has been discontinued, though some laboratories own and continue to operate them);

5.23 *Optional:* centrifuge, Garver, or equivalent, equipped with baskets designed to accommodate the Mojonnier® fat extraction flasks, only used if also utilizing the Mojonnier Dairy Tester®.

6.0 Personal Safety Precautions

In all cases, the practitioner's company's internal policies and procedures regarding personal safety supersede the following ADPI recommendations:

6.1 Milk (dairy) is globally classified as an allergen and should be properly handled with personal safety needs in mind.

6.2 Read and understand all precautions for safe handling and disposal shown in the Safety Data Sheets (SDS) for all the reagents required by this method, including use of any prescribed Personal Protective Equipment (PPE).

6.3 Dairy ingredients are foods and as such are exempt from U.S. requirements regarding Safety Data Sheets (SDS), where ingredient-specific safe handling instructions would be provided.

Despite this exemption, many dairy ingredients are manufactured and marketed in powder form, and powders should be recognized as potential physical irritants, such as to the eyes, nose, and if inhaled.

6.4 Some testing apparatus described above may be susceptible to breakage, therefore be aware of associated personal risks. Inspect apparatus before use and replace any items which are compromised.

6.5 Vacuum apparatus develops stored energy in use. Read and understand the manufacturer's warnings and instructions for safe use.

6.6 Exercise care when using lab ovens, steam tables, hot plates, and heated testing materials. Read and understand the equipment manufacturer's warnings and instructions for safe use and handle any heated materials with tongs or other suitable utensil.

6.7 Ethyl alcohol, the ethanolic phenolphthalein indicator solution, ethyl ether, and petroleum ether are extremely flammable. Utilize caution when handling these reagents.

6.8 Ethers may form peroxides over time, such as when in storage. Such deterioration renders these solvents especially dangerous, and they may even detonate spontaneously when disturbed. Special care must be taken when using ether reagents under any circumstances.

6.9 Evaporation of the organic extraction solvents should be performed under conditions that provide adequate ventilation of vapors, such as in a suitably rated fume hood; with the use of a fume extractor apparatus; or equivalent.

7.0 General Considerations

Rapid methods, such as mid-infrared (MIR) or other methods, may be used for routine determination of milkfat, such as for process control. Where possible, rapid methods should be calibrated against the Mojonnier method, especially if rapid methods are utilized for official purposes such as release testing of finished products.

General procedural considerations:

7.1 Confirm that any dessicant media are not exhausted prior to engaging in this procedure, in all locations where used, if applicable.

7.2 It is advisable that the means for oven temperature indication (e.g., separate thermometer, electronic thermocouple, built-in temperature controller) be traceable to a recognized third-party standard (e.g., NIST), maintained according to a calibration policy/procedure, and that oven operating temperatures be documented during testing.

7.3 Avoid transferring any measurable quantities of residues onto the weighing vessels that could result from direct contact with the hands. Use utensils or equivalent suitable handling technique.

7.4 Manage the weighing vessels very carefully throughout testing, given that measurements are being made to the nearest 0.1 mg. Utilize a method for marking the weighing vessels that will not affect the validity of weight measurements obtained during testing.

7.5 Weighing vessels may be prepared in advance of testing, provided that they can be held under conditions that preserve their dry condition and that they are allowed to equilibrate to ambient temperature before use.

7.6 Samples should be homogeneous, representative, and equilibrated to ambient temperature before handling, in a manner which will not compromise their suitability for moisture testing.

7.7 Follow Good Laboratory Practices (GLPs) wherever applicable.

8.0 Weighing Dish Preparation

8.1 If using the Mojonnier Dairy Tester®, prepare the weighing dishes as follows:

a. Condition the weighing dishes in the vacuum oven at 135°C for 5 minutes;
b. Place dried weighing dishes into the cooling dessicator for 7 minutes before use.

8.2 If not using the Mojonnier Dairy Tester®, prepare the weighing dish as follows:

a. Dry the weighing dishes in a convection oven at 102 ± 2°C for 1 hours, **OR** in a vacuum oven at 100°C with pressure below 50 mm of mercury for 10 minutes;

b. Place dried weighing dishes into a dessicator and allow them to equilibrate to ambient temperature before use.

9.0 Sample Determination

9.1 Accurately weigh between 1.00 and 1.25 grams of sample powder, recording the sample weight to the nearest 0.1 mg, and transfer quantitatively to a clean, dry fat extraction flask.

9.2 Add 9.0 mL of warm water to the flask; stopper, shake until the contents are homogeneous, then allow to cool to ambient temperature.

9.3 Add 1.5 mL of ammonium hydroxide solution to the flask; stopper and shake thoroughly.

9.4 Add 3 drops of phenolphthalein indicator solution to the flask; stopper and mix. Contents will immediately turn a bright pink color at this alkaline pH. The bright color will help sharpen the visual distinction between this aqueous phase and the organic phase in the subsequent solvent extraction steps, aiding in decanting the organic phase to the weighing dish.

9.5 Add 10 mL of ethyl alcohol; stopper and shake thoroughly.

9.6 Add 25 mL of ethyl ether; stopper and shake vigorously for 1 minute, periodically venting the pressure in the flask as necessary by loosening the stopper.

9.7 Add 25 mL of petroleum ether; stopper and shake vigorously for 1 minute.

9.8 Allow the flask to stand until the upper organic phase is practically clear (this typically takes approximately 20 minutes); **OR** centrifuge the flask at 600 rpm for approximately 1 minute if using the Mojonnier Dairy Tester®.

9.9 If the interface between the lower aqueous phase and the upper organic phase is still not clearly defined, gently swirl the flask to break any emulsion which may have formed.

9.10 Record the tare weight of a clean, dry weighing dish to the nearest 0.1 mg.

9.11 Carefully decant as much of the upper organic phase as possible into the weighing dish, using a small amount of petroleum ether to rinse the lip of the extraction flask into the dish.

9.12 Place the weighing dish onto the steam bath and carefully evaporate the organic solvent, avoiding spattering or boiling during the evaporation process.

9.13 Add 4-5 mL of ethyl alcohol to the flask; stopper and mix. (Not necessary in the final repetition of the extraction steps.)

9.14 Repeat steps 9.6 through 9.13 as follows:

a. **Two times** for dry whole milk / whole milk powder, nonfat dry milk / skimmed milk powder, or dry buttermilk / buttermilk powder (for a total of three solvent extractions); OR

b. **One time** for all other powders (for a total of two solvent extractions).

9.15 The weighing dish now contains the milkfat which has been extracted from the powder sample. Take the weighing dish to constant weight as follows:

a. Dry the weighing dishes in a convection oven at 102 ± 2°C; **OR** in a vacuum oven at 70-75°C with pressure below 50 mm of mercury;

b. Place dried weighing dishes into a dessicator and allow them to equilibrate to ambient temperature.

9.16 Record the final weight of the dish plus extracted milkfat, to the nearest 0.1 mg.

10.0 Blank Determination

10.1 Proceed as described in section 9.0, but simply omitting the sample powder.

10.2 On completion, the blank weighing dish will contain any trace residues contributed by the reagents used in sample preparation. Record the final weight of the blank dish plus reagent residues, to the nearest 0.1 mg.

11.0 Results Calculations

11.1 Calculate the reagent residues weight as follows. Replace or purify reagents if the calculated result is greater than 0.5 mg:

residue (mg) = (final wt. of blank dish, g) - (tare wt. of blank dish, g) x 1000 mg/g

11.2 Calculate the milkfat content of the sample as follows:

$$\textbf{milkfat (\%)} = \frac{\text{(final wt. of sample dish, g) - (tare wt. of sample dish, g) - (residue wt., g)}}{\text{(initial wt. of sample, g)}} \times 100\%$$

12.0 External References

12.1 *Standard Methods for the Examination of Dairy Products* ("SMEDP"), 17th edition, Ch. 15 – Chemical and Physical Methods, section 15.086 – Mojonnier Method, Milk and Cream (Class A1); Other Products (Class O);

12.2 *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOAC), monograph 989.05;

12.3 IDF Provisional Standard 1D:1996.

13.0 ADPI Document Linkages

Analytical Method #001: *Sampling Dry Powders*

14.0 Revision History

Version	Effective Date	Notes
1.0	???	First officially approved version of this Standard Operating Procedure.
2.0	08/31/2023	Migrated this analytical method to the new modernized Standard Operating Procedure format as established by the ADPI Vice President of Technical Services.