United States Department of Agriculture

Food Safety and Inspection Service

CLG-FAT.04

Quantitation of Fat

This method describes the laboratory procedure for quantitation of fat in meat, poultry, and processed meat products at a level ≥ 0.12%.

Notice of Change

This method has been modified for:

- The method is reformatted.
- Removed determination and added quantitation to the title and throughout the method.
- Removed the Thimble Procedure.
- Added Reagent Check.

Table of Contents

Safety Precautions

The personnel performing the analysis are to read the Safety Data Sheets for the standards and reagents used in this method. Follow all applicable federal, state, and local regulations regarding the disposal of chemicals listed in this method.

Introduction

Fat is an important constituent of the human diet. Monounsaturated and polyunsaturated fats are thought to lower disease risk, whereas saturated fats may have negative impacts on human health. The analysis of fat is used to ensure that food products are compliant with labeling requirements set by the Federal Meat Inspection Act (FMIA), the Poultry Products Inspection Act (PPIA), the Egg Products Inspection Act (EPIA), and Title 9 of the Code of Federal Regulations (CFR). The correct quantitation of the fat content of meat and poultry are important for the economic value of the food.

Method Overview

This method involves the drying of a weighed sample. Sand is incorporated with the sample prior to drying and provides the sample with a greater surface area that is necessary to prevent the entrapment of fat and moisture. Fat is then extracted through a Soxhlet extraction. The extracted fat is weighed, and the fat content is determined.

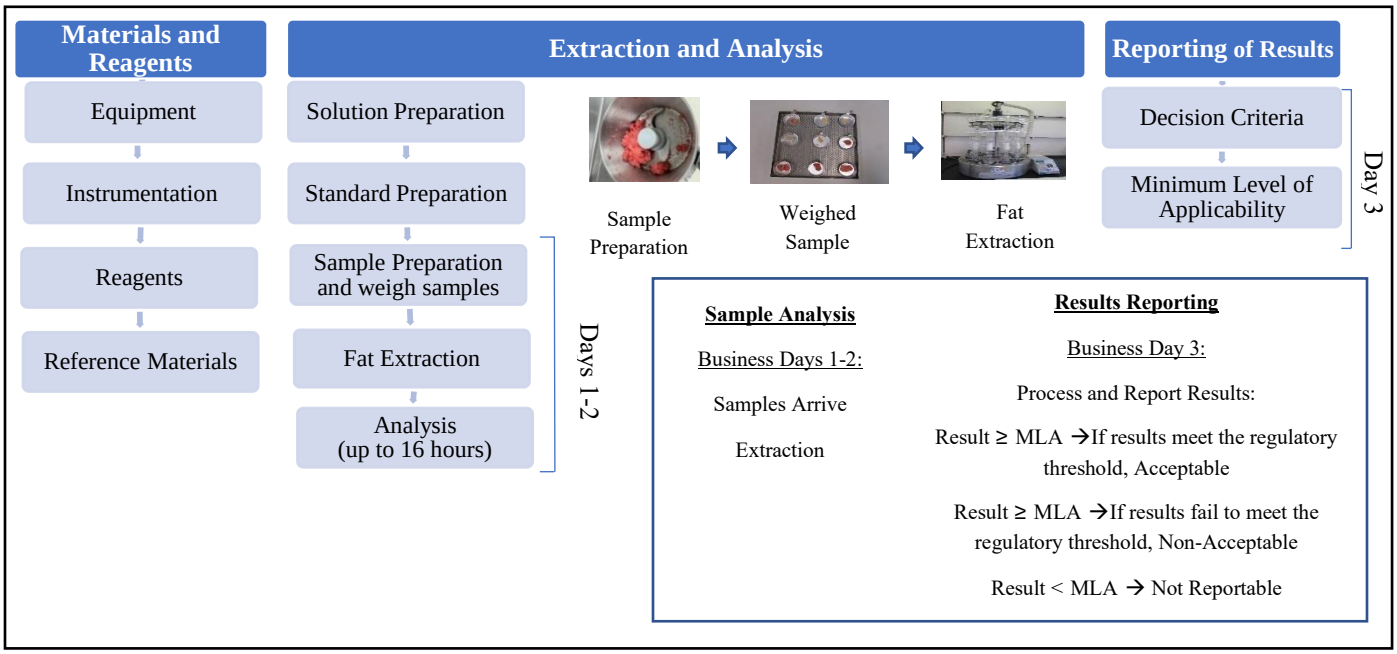

Figure 1: Overview and timeframe of fat extraction. Materials and reagents are obtained. Samples arrive at laboratory, are prepared into a homogenized mixture, weighed, and analyzed on business days 1-2. Results are reported on business day 3. This chart represents the best-case scenario, but analyses may take longer due to analytical testing circumstances. Pictures courtesy of Hue Quach and Killani Kadri USDA-FSIS.

Decision Criteria

Quantitative results are reported for all samples. Sample results are compared to the regulatory thresholds found in the 9 CFR for the particular product of interest and the MLA. A sample is considered "Acceptable" if the results meet the regulations. A sample is considered "Non-Acceptable" if the results fail to meet the regulations.

KEY DEFINITIONS

MLA: Lowest level at which an FSIS method has been successfully validated in each matrix. Full definition is on the CLG website here.

Disclosure Statement

FSIS does not specifically endorse any test products listed in this method. FSIS acknowledges that equivalent equipment, reagents, or solutions may be suitable for laboratory use. The FSIS laboratory system uses the method performance requirements when evaluating the equivalence of an alternative equipment, reagent, or solution for a given analyte and sample matrix pair. Significant equivalence changes would require FSIS laboratory leadership approval.

Materials and Reagents

Equipment

Table 1: Equipment Required to Perform CLG-FAT

Equipment	Supplier and Part Number	Purpose
Thimbles - 33 x 80 mm	General lab supplier	Contains sample and sand
Soxhlet extraction apparatus - Extraction tube id 40 mm,	Fisher Cat No. 09-551B	Extracts fat
TOT-X-TRACT-S extractor,	Organomation Cat No. 13308	Condensers to help extract fat.
Filter paper - 9 cm	General lab supplier	Filtration of recycled ether
Aluminum dishes - Disposable, 5760 mm diameter x 18 mm depth,	General lab supplier	Contains sample and sand
Glass beads - Hollow, perforated, 4 mm diameter or carborundum chips #12 granules.	General lab supplier	Helps with boiling
Oven - Mechanical convection oven	General lab supplier	Dry the sample and flasks.
Analytical balance	General lab supplier	Record weight of samples. Minimum accuracy ±0.0001g
Robot Coupe	General lab supplier	Homogenizes samples
Aluminum weighing paddles	General lab supplier	Flatten the sample in the weigh dish.
Freezer (-10 C)	General lab supplier	Samples storage

Instrumentation

None

Reagents

Table 2: Reagents

Reagent	Supplier and Part Number
Petroleum ether	General lab supplier
Sand - washed and ignited	General lab supplier

Reference Materials

None

Extraction and Analysis

Solution Preparation

None

Standard Preparation

None

Sample Preparation

Samples must be kept cold before and during shipping to the laboratory. An example of a sample is shown in Figure 2. Once received at the laboratory, samples must be frozen (≤ -10 °C) prior to grinding if they cannot be prepared on the day of receipt. Once frozen, temper (partially thaw) the sample while keeping it as cold as possible. As demonstrated in Figure 3, grind samples in a blender or vertical cutter-mixer until homogeneous. Store homogenized samples frozen (≤ -10 °C) prior to analysis. Equipment must be dry. Handle a sample in a way to avoid evaporation and condensation.

Figure 2: Processed meat product. Photo courtesy of Getty Images.

Figure 3: Homogenized sample. Photo courtesy of Hue Quach, USDA FSIS

Fat Extraction

Samples

1) Weighing and Drying Procedure:

a) As shown in Figure 5, using a weighing paddle, weigh 3.5 ± 0.5 g of sample into a small disposable aluminum dish.

Figure 5: Weighed samples in aluminum dish Photo courtesy of Killani Kadri, USDA FSIS.

b) As demonstrated in Figure 6, add a small amount of sand and with the aid of a small aluminum or glass paddle, spread the mixture across the bottom of the dish. After spreading, leave the paddle in the dish.

Figure 6: Sample with sand and paddle Photo courtesy of Killani Kadri, USDA FSIS.

c) As shown in Figure 7, roll the edges of the dish and dry the folded dish (on a metal mesh tray) in a mechanical convection oven for 6 hours ± 10 minutes at 100 - 102 °C or for 1½ hours ± 10 minutes at 125 ± 1 °C.

Figure 7: Dried folded dish Photo courtesy of Killani Kadri, USDA FSIS

d) After cooling, insert the folded dish into extraction thimble, as shown in Figure 8.

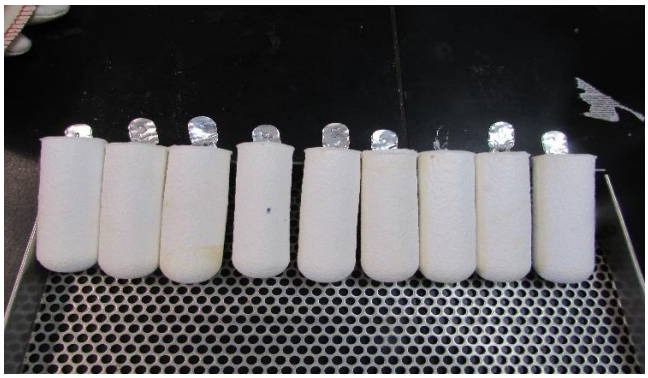

Figure 8: Dried samples in the extraction thimble/ Photo courtesy of Killani Kadri, USDA FSIS

e) Proceed to extraction.

Technical Notes:

Ensure that aluminum dish will allow sufficient contact of solvent with sample.

Sample can be cooled and stored in a desiccator if the extraction is not performed on the same day.

QUALITY CONTROL

1. Run a previously analyzed sample or a sample with a known reference value as a recovery per extracting unit.
2. Weigh one additional portion for an intra-laboratory check sample, if applicable.

KEY DEFINITIONS

Recovery: A sample with a known concentration of fat.

Reference value: The value of the known concentration of fat in the recovery.

2) Reagent Check

a) Petroleum Ether new lot number or recycled Petroleum Ether check
   i. Tare a fat flask with some boiling chips (carborundum) in it. Record flask weight.
   ii. Add 100 mL of Petroleum Ether to the flask.
   iii. Proceed to extraction.
   iv. Acceptability Criteria: < 0.004 g (keep a record of recovered mass)

b) Sand new lot number check
   i. In a labeled aluminum dish add 5g of sand
   ii. Fold Dish
   iii. Put it into a thimble.
   iv. Tare a fat flask with some boiling chips (carborundum) in it. Record flask weight.
   v. Proceed to Extraction
   vi. Acceptability Criteria: < 0.004 g (keep a record of recovered mass)

Extraction

1) As shown in Figure 9, accurately weigh an extraction flask containing a few glass beads or boiling chips (carborundum), and then add 85 mL of petroleum ether.

Figure 9: Extraction flasks, boiling chips and petroleum ether. Photo courtesy of Killani Kadri, USDA FSIS

2) Extract the sample contained in the thimble with petroleum ether for at least 80 cycles for a minimum of 4 hours in a Soxhlet extraction apparatus, as demonstrated in Figure 10. Measure the cycle times for one position per bath. If cycle times are > 3 mins, adjust extraction time to account for the difference. If the extraction flask becomes dry, rinse with three 10 mL portions of petroleum ether by adding it to the extraction tube.

Figure 10: Samples being extracted. Photo courtesy of Killani Kadri, USDA FSIS

3) Upon completion of the extraction, if condensation is present on the condensing unit and flask wipe dry before separating the unit.

4) Separate the unit and pour off the ether (and thimble) from the extractor into a large filter (to collect the thimbles) positioned on a container (such as a gallon bottle). Repeat until most of the ether is removed and the flask has very little ether left.

5) Take apart the Soxhlet unit and place the flask into a water bath to evaporate the remaining petroleum ether. Swirl flask initially to avoid boil-over.

6) As shown in Figure 11, dry the flask and its contents in a mechanical convection oven at 100 - 102 °C for an amount of time to obtain a constant weight. Cool to room temperature.

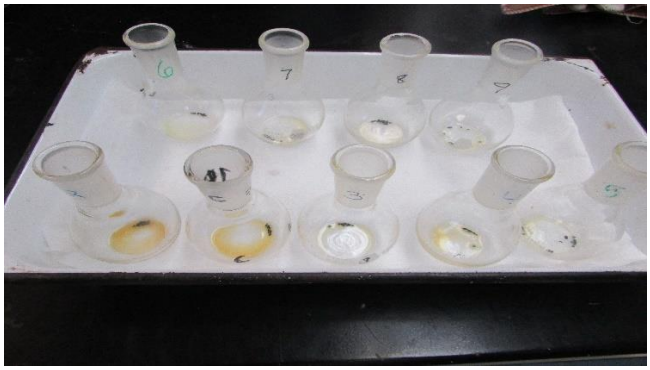

Figure 11: Dried Flask. Photo courtesy of Killani Kadri, USDA FSIS

Technical Notes:

Excessive drying may oxidize the fat and give high results.

The laboratory should have data available to support time used to obtain constant weight. If this data is available, analysts should use this time rather than remove, cool, and weigh several times for each sample.

Instrumental Analysis

Sample Set

The analysis sequence below can be modified, as needed, but must include required controls.

1) Recovery will be run on each extractor if using more than one.
2) Intra-laboratory check sample (if applicable).
3) Samples up to a maximum of 10. This will include nine samples and one recovery per extractor.

INTRA-LABORATORY CHECK SAMPLE

Defined on the CLG website here.

Reporting of Results

Decision Criteria

Calculations: $$\textit{Fat content, percent} = \frac{100(B - C)}{A}$$

where
A = Sample weight
B = Weight of flask after extraction
C = Weight of flask prior to extraction

QUALITY CONTROL

Quality Control Procedures

1) For set acceptance, the recovery/check samples must be within 0.91 % absolute fat content of the known reference value.

Intra-laboratory Check Samples (If applicable)

1) Acceptability criteria.
    a) For set acceptance, the recovery/check samples must be within 0.91 % of the known reference value.
    b) FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures when unacceptable values are obtained:
        i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.
        ii. If unacceptable values are obtained, refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Minimum Level of Applicability

Minimum Level of Applicability (MLA): 0.12%

References

9 CFR 439.1 for regulations involving food chemistry.

Official Methods of Analysis of the Association of Official Analytical Chemists, 960.39, 15th Edition

The Nutrition Source - Fats and Cholesterol. Harvard University School of Public Health. https://www.hsph.harvard.edu/nutritionsource/what-should-you-eat/fats-and-cholesterol/ Accessed 14 April 2023.

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

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This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

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