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Title: Determination of Fat		
Revision: 03	Replaces: CLG-FAT.02	Effective: 08/10/2009

Contents

A. INTRODUCTION

1. Theory

This method involves a partial drying of a weighed sample prior to a Soxhlet extraction. The extracted fat is weighed and the fat content calculated. It is important that sand be incorporated with the sample before drying. The purpose of the sand is to create a greater surface area, necessary to remove moisture and prevent entrapment of fat.

2. Applicability

This method is applicable to the determination of fat in meat, poultry, and processed meat products at level ≥ 0.12%.

B. EQUIPMENT

Equivalent apparatus may be substituted.

1. Apparatus

a. Thimbles - 33 x 80 mm, Cat. No. 2800-338, Whatman.

b. Soxhlet extraction apparatus - Extraction tube id 40 mm, Cat. No. 09-551 B, Fisher.

c. Heating mantles - 6 units, Cat. No. 33749-324, VWR with steam bath or water bath.

d. Filter paper - 9 cm, Cat. No 541, Whatman.

e. Aluminum dishes - Disposable, 60 mm diameter x 18 mm depth, Cat. No. 08-732, Fisher.)

f. Glass beads - Hollow, perforated, 4 mm diameter or carborundum chips #12 granules.

g. Oven - Mechanical convection oven.

h. Analytical balance - Capable of weighing 0.1 mg

i. Robot Coupe - Robot Coupe U.S.A., Inc.

j. Aluminum weighing paddles - L-shaped, approximately 25mm long, 12.5mm wide.

C. REAGENTS AND SOLUTIONS

Equivalent reagents may be substituted.

1. Reagents
   a. Petroleum ether - High purity, CAS 8032-32-4, Burdick & Jackson.
   b. Sand - Washed and ignited, CAS 74808-60-7, J. T. Baker

D. STANDARDS

None

E. SAMPLE PREPARATION

Process samples until homogeneous.

F. ANALYTICAL PROCEDURE

1. Drying

Note: Either Thimble procedure or Aluminum Dish procedure can be used.

Note: Sand is incorporated with the sample before drying to create a greater surface area, which is necessary to remove moisture and prevent entrapment of the fat

a. Thimble Procedure
   i. Accurately weigh by difference 3 - 4 g of sample into a thimble lined with a circle of filter paper and containing a small amount of sand.
   ii. Mix sand and samples with a glass rod, wipe glass rod with filter paper strips, and place strips in thimble.
   iii. Place thimble and contents in 50 mL beaker and dry in a mechanical convection oven for 6 hours ±10 minutes at 100 - 102 °C, or for 1½ hours ± 10 minutes at 125 ± 1 °C.
   iv. Proceed to F.2.

b. Aluminum Dish Procedure
   i. Using a weighing paddle, accurately weigh, 3 - 4 g of sample into a small disposable aluminum dish.
   ii. 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. Leave the paddle in the dish.

iii. Roll edges of dish. 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.

iv. After cooling, insert into extraction thimble.

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

Note: Sample can be cooled in a desiccator if the extraction is not performed that day.

v. Proceed to F.2.

2. Extraction

a. Accurately weigh an extraction flask containing a few glass beads or boiling chips (carborundum), and then add approximately 85 mL of petroleum ether.

b. Extract the sample contained in the thimble from F.1.a. or F.1.b. with petroleum ether for at least 80 cycles in a minimum of 4 hours in a Soxhlet extraction apparatus. (If sample has been dried as in F.1.a., rinse the 50 mL beaker with three 10 mL portions of petroleum ether and add rinsings to the extraction tube.)

c. Upon completion of the extraction, 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. Take apart the Soxhlet unit and place flask on a steam bath to evaporate the remaining petroleum ether. Swirl flask initially to avoid boil-over.

d. Dry flask and its contents in a mechanical convection oven at 100 - 102 °C for time required to obtain constant weight. Cool to room temperature.

Note: Excessive drying may oxidize the fat and give high results.

Note: 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.

G. CALCULATIONS

1. Procedure

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

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

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment - Safety glasses, laboratory coat, protective gloves, and heat resistant gloves.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
After extraction use steam bath or water bath to evaporate petroleum ether	Petroleum ether is a flammable liquid	Allow evaporation to continue several minutes beyond the point where no ether is observed in order to minimize personal exposure.
Petroleum ether	Flammable liquid and vapor. Harmful or fatal if swallowed. May cause irritation to skin, eyes, and respiratory tract. Harmful if inhaled, may affect central nervous system.	Work in a hood in well ventilated area. Keep away from sources of ignition.

3. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Extracted meat waste and extraction thimbles	Residual petroleum ether vapor	Store in cool, well ventilated area awaiting disposal. Clean thimbles can be used again
Waste ether	Petroleum ether is a flammable liquid	Dispose of properly following local, state, or federal guidelines. Petroleum ether can be filtered and reused as long as the blank is equivalent to that of a new unopened bottle (see I.2.c.).

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	*Analytical Range*	*Repeatability Standard Deviation*	*Reproducibility Standard Deviation*
Fat	[1]	< 0.63[2]	< 0.66[2]

[1]Limit may vary due to sample and aliquot sizes and sample type.

[2]One standard deviation based on historical data.

2. Critical Control Points and Specifications

	Record	*Acceptable Control*
a.	Sample Size	3.5 ± 0.5 g
b.	Pre-dry	In oven 6 hours ± 10 minutes at 100 - 102 ^{o}C for 1.5 hours ± 10 minutes at 125 ± 1 ^{o}C.
c.	Solvent extractables	< 0.004 g per 100 mL; Record in “Log Book”
d.	Sand extractables	< 0.004 g per 5 g sample; Record in “Log Book”
e.	Extraction time and rate	At least 4 hours via Soxhlet (minimum of 80 cycles/in a minimum of 4 hours; ~ 1 cycle/3 minutes)
f.	Drying time and temperature after extraction	101 ± 1 ^{o}C for time required to obtain constant weight.(Data must be available to prove validity of time used)
g.	Thermometer	NIST certified or traceable to a NIST certified thermometer.

3. Readiness To Perform

   a. Familiarization

      i. Phase I: Standards - Not Applicable

      ii. Phase II: Fortified samples or random replicates of previously analyzed samples.

      iii. Phase III: 15 check samples for analyst accreditation.

         (a) Samples submitted by the Quality Assurance Manager (QAM), Accredited Laboratory Program (ALP) or Supervisor.

         (b) Authorization from the Quality Assurance Manager (QAM) and Supervisor are required to commence official analysis

b. Acceptability criteria.

Refer to I. 1.

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: One check sample per week per analyst when samples are analyzed.

ii. Records are to be maintained

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability

a. Matrix: Meat, poultry, and processed products

b. Condition upon receipt: Unspoiled and sealed from air

c. Sample storage:

i. Time and Condition: 3 weeks refrigerated

6. Sample Set

a. A batch size of 1 - 20 samples. A Quality Control sample will be run with each batch set.

7. Sensitivity

a. Minimum Detection Level (MDL): 0.12%.

J. WORKSHEET

None

K. APPENDIX

1. Reference

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

L. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.