Total Fat by Acid Hydrolysis Filter Bag Technique using the ANKOM^{HCl} Hydrolysis System

Definition

Using Filter Bag Technology, this method determines Total Fat by releasing complexed lipids using acid hydrolysis. The fat is then capable of being extracted by fat solvents. The compounds extracted are predominantly triacylglycerides. Small amounts of other lipids having some solubility in fat solvents are also extracted.

Scope

This method is applicable to foods and feeds with 0-100% fat content.

Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Dryer—forced air dryer that neutralizes acid fumes and capable of maintaining a temperature between 60-120°C (ANKOMRD Dryer, ANKOM Technology).
3. Hydrolysis instrument—capable of performing the hydrolysis at 90 ± 2°C. The instrument must also be capable of creating a uniform flow pattern around each sample to ensure uniformity of digestion (ANKOM^{HCl} Hydrolysis System, ANKOM Technology).
4. ANKOM Extractor capable of performing extractions under pressure at 90 ± 2°C (XT10, XT15, XT20, ANKOM Technology).
5. Filter Bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 1 micron particles while permitting solution penetration (XT4, ANKOM Technology).
6. Heat sealer—sufficient for sealing the Filter Bags closed to ensure complete closure (HS, ANKOM Technology).
7. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the Filter Bags (MoistureStop weigh pouch, ANKOM Technology).
8. Diatomaceous earth (DE)—(Item # DE1 or DE2, ANKOM Technology).
9. Marking pen—Solvent and acid resistant (F08, ANKOM Technology).

Reagents

1. Fat solvent used is based on the comparative method targeted. When comparing to ISO 6492 use petroleum ether (B.P. 35-65° C). When comparing to Mojonnier method use a mixture of 45% petroleum ether, 45% diethyl ether and 10% ethanol.

   Caution: Most fat solvents are extremely flammable. Use a fume hood when handling exposed solvents and use the appropriate procedures to avoid static electricity discharge.
2. 3N HCl (*Note* – Some strongly bound samples will need 5N HCl)

Sample Preparation *(see the Hydrolysis Procedure section of the Operator's Manual for more detail)*

1. Label the filter bags using a solvent resistant marker.
2. Record the weights of all the filter bags prior to filling them.
3. Place an empty filter bag in the Bag Holder in an open position.
4. Tare the weight of the empty filter bag and the holder together.
5. Add the necessary DE and sample to the filter bag for Dry/Granular, Moist, and Liquid samples. (See the "Sample Categorization" section for help in determining how much DE to add to the filter bag.)

   Note: The total weight of DE and sample **dry matter** must NOT exceed 1.5g. Too much volume inside the bags will make it difficult for the acid to completely saturate the sample during hydrolysis. This may reduce the number of bags that can be processed in the Hydrolysis Vessel at one time. For liquid samples, the total weight of DE and sample can exceed 1.5g.

Sample Preparation (continued)

Dry/Granular Samples

a. Add the full amount of DE to the filter bag.
b. Record the weight of the DE and tare.
c. Add an appropriate amount of sample to the filter bag. Keep all particles away from the sealing area of the bag.
d. Record the weight of the sample (W_1).

Moist Samples

a. Add ½ of the amount of DE to the filter bag.
b. Using a spatula, make a valley in the DE for the sample.
c. Record the weight and tare.
d. Place an appropriate amount of sample into the DE valley in the filter bag, being careful that the sample does not touch the inside walls of the filter bag so the sample can be fully covered with DE.
e. Record the weight of the sample (W_1) and tare.
f. Add the rest of the DE to the filter bag to completely cover the sample.
g. Record the weight of the DE.

Liquid Samples

a. Add the full amount of DE to the filter bag.
b. Using a pencil eraser (or similar sized utensil), make an indentation or a space in the DE into which the liquid sample can be added using a pipette.
c. Record the weight and tare.
d. Pipette an appropriate amount of sample into the indentation in the DE in the filter bag.
e. Record the weight of the sample (W_1).

6. Set the Heat Sealer dial to 6. (The setting may vary from sealer to sealer.)
7. Seal the filter bag within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag. If the seal is not strong, re-seal the bag.
8. For Dry/Granular or Moist samples, squeeze the edges of the filter bag then shake it to mix or cover the sample with the DE. Flatten the bag after shaking.
9. If the sample is liquid or clumpy (for example ground beef) and makes the filter bag bulge, gently squeeze the bag at the bulge location to flatten it.
10. Repeat steps 3 – 9 for all filter bags that will be used in the ANKOM^{HCl} Hydrolysis System. (Up to 15 bags can be processed during one procedure.)
11. Include two blank bags (C1 and C2) filled with approximately 0.5 – 0.75g of DE.
12. Insert filter bags into the Multi-bag Holder and snap the handle in place. Samples are now ready for the hydrolysis procedure.

Calculation (all weights in grams)

$$\% \text{ Total Fat} = \frac{[W_2 - (W_3 + (C_1 - C_2))]}{W_1} \times 100$$

Where:

- W_1 = Original sample weight (g)
- W_2 = Weight of dried sample, filter bag, and Diatomaceous Earth (DE) after hydrolysis (g)
- W_3 = Weight of dry extracted sample, filter bag, and DE (g) ***(determined after using the ANKOM^{XT15} or ANKOM^{XT10} instruments)***
- C_1 = Blank filter bag dry weight after hydrolysis (g)
- C_2 = Blank filter bag weight after extraction (g)

HCl Procedure *(see the Hydrolysis Procedure section of the Operator's Manual for more detail)*

1. Turn the cold water supply on.
2. Using the Lid Latch, open the Vessel Lid.
3. Place the Multi-bag Holder into the Hydrolysis Vessel.
4. Pour 500ml of 3N HCl into the Hydrolysis Vessel.
5. Roll up a piece of paper towel and place it on the Vessel Lid just above the Hinge. This will help prevent drips of liquid from getting on the Hinge after a run.
6. Using the Lid Latch, close the Vessel Lid.
7. Press ENTER on the Keypad.
8. Use the UP and DOWN arrows on the Keypad to enter the hydrolysis time (suggested time is 60 minutes).
9. Press ENTER on the Keypad.
10. Use the UP and DOWN arrows on the Keypad to enter the hydrolysis temperature (suggested temperature is 90°C).
11. Press ENTER on the Keypad.
12. Use the UP and DOWN arrows on the Keypad to enter the rinse time (suggested time is 20 minutes).
13. Press ENTER on the Keypad.
14. Make sure the Vessel Lid is closed (the screen will remind you to do this).
15. Press START on the Keypad. The ANKOM^{HCl} Hydrolysis System will automatically heat and maintain the set temperature. After hydrolysis is complete, the samples are automatically rinsed with cold water.
16. When the process is complete, the instrument will show "Process Complete" on the Display. Open the Lid Latch and immediately wipe the Teflon pad on the underside of the lid with paper towel. This will prevent moisture from corroding the Lid Latch hinge.
17. Remove the Multi-bag Holder.
18. Remove the individual filter bags from the Multi-bag Holder and place them on four layers of paper towel.
19. Place two layers of paper towel on top of the filter bags. Using the ANKOM Blotter, apply uniform pressure to the top of the paper towels for two minutes to gently press the water out of the filter bags. Blotting helps to reduce the drying time and the amount of acid residue in the bags.
20. Repeat the blotting process for two minutes before drying.
21. Completely dry the samples in the ANKOMRD Dryer or an oven resistant to HCl fumes. (ANKOM recommends drying for three hours at 100 - 105°C.) Residual acid left in the samples due to incomplete drying will cause corrosion in the extractor and artificially high fat values.
22. Remove samples from the dryer/oven and place them directly into a Desiccant Pouch containing pH paper.
23. Allow samples to cool to room temperature and check the pH paper. If the presence of acid shows on the paper, continue drying. If the pH paper is neutral, record weight (W_2) of samples.

    Note: If your hydrolysis results are low, it is possible that the acid was not able to completely saturate and hydrolyze the sample. In this case, it may be necessary to use no more than 1.0g of the combination of sample and DE in your filter bags. A possible combination could be 0.5g of sample and 0.5g of DE.
24. Your samples are now ready for fat extraction in an ANKOM^{XT15} or an ANKOM^{XT10} Extractor. See the specific extractor Operator's Manual for instructions.

    Note: When doing a fat extraction, use the fat solvent for the comparative method targeted. For ISO 6492, use petroleum ether (B.P. 35-65° C). For ISO 1735 (the Mojonnier method), use a mixture of 45% (v/v) petroleum ether, 45% (v/v) diethyl ether and 10% (v/v) ethanol.