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Fat by Acid Hydrolysis

1. Application

This procedure is applicable for the determination of crude fat in dried forages and mixed feeds. It is not applicable for oilseeds, baked and/or expanded products (pet foods), liquid feeds, sugar products, and feeds containing dairy products.

2. Summary of Methods

A dried, ground sample is extracted with diethyl ether which dissolves fats, oils, pigments, and other fat soluble substances. The ether is then evaporated from the fat solution. The resulting residue is weighed and referred to as ether extract or crude fat. Both the ether and the samples must be free of moisture to avoid co-extraction of water-soluble components in the sample such as carbohydrates, urea, lactic acid, glycerol, etc. If water-soluble components are present in large amounts in the sample, they are washed out of the sample prior to drying. Low temperatures are used to evaporate the ether and remove residual moisture to prevent oxidation of the fat.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

This procedure is extremely sensitive to variations in technique. Use tongs in handling beakers, and wear gloves throughout the procedure. Keep the beakers in a desiccator when not in use. Be sure the tubes are well cooled before doing ether extraction.

5. Sample Collection, Preservation, and Handling

All samples are dried at 55^{o}C in a cabinet-type forced air dryer for 12-18 hrs. After drying the sample is ground to pass through a 1 mm Wiley mill.

6. Apparatus and Materials

6.1 50 ml screw-top test tubes
6.2 Automatic dispenser
6.3 Water Bath set to 75.5^{o}C

6.4 Orbital Shaker
6.5 Pasteur pipettes
6.6 Cotton plugs
6.7 Long stem funnels
6.8 150 ml beakers
6.9 Hot plate
6.10 Dessicator
6.11 Laboratory Oven (135^{o}C)

7. Reagents

7.1 Ethyl Alcohol – 95%
7.2 Anhydrous Ethyl Ether
7.3 Petroleum Ether
7.4 Hydrochloric Acid – 25:11 (Acid:Water) dilution

8. Methods

8.1 Grind the dried sample through a 1mm sieve.
8.2 Weigh 1 gram ground sample into a 50 ml screw-top test tube.
8.3 Wet sample with 1 ml of ethanol, saturating it.
8.4 Add 5 ml HCl.
8.5 Place in preheated water bath (75.5^{o}C) for 40 minutes. Shake occasionally.
8.6 Remove and allow cooling to room temp.
8.7 Add 5 ml ethanol and mix.
8.8 Add 12 ml anhydrous ether – orbital shake for 1 minute.
8.9 Add 12 ml petroleum ether – orbital shake for 1 minute.
8.10 Let ether and residue separate. (With feces samples, a portion of the ether layer may get trapped under a mat of particulate matter in the tube. Vortexing samples may aid in effecting a better separation.
8.11 Pull off top layer into a dried and tared 150 ml beaker via a Pasteur pipette, pouring through a filter paper in a long stem funnel.
8.12 Repeat steps 7-10 with 8 ml portions of ethers three more times.
8.13 Evaporate ether and any water contained in beaker. Evaporation on a hot plate at low temperature works best. Approximately 1 hour is required.
8.14 Place beakers in a 135^{o}C oven for 10 minutes.
8.15 Transfer to desiccator and allow cooling to room temperature.
8.16 Weigh beaker plus fat to +/-0.01g.

9. Calculations

9.1 % Fat = ((Weight of beaker and fat – tared beaker weight) / Sample weight)) * 100

10. Quality Control

An in house standard is run with each batch to assure method accuracy.

11. Reporting

Results are reported as % of Dry Matter.

12. References

12.1 AOAC, 1980 Official Methods of Analyses. p.132.
12.2 Budde, E. F. 1952 J. Assoc. Off. Agric. Chem. 35.799.