Chemicals for Fat analysis

a) Petroleum Ether 40°-60°
b) HCL Solution - 25:11 (Acid: Water) dilution
c) Ethyl Alcohol 95% or Ethanol absolute
d) Anhydrous Ethyl Ether

Fat Determination (Extruder Animal Feed)

1- Grind the dried sample through a 1mm sieve.

2- Weigh 1 gram ground sample into a 50 ml glass screw-top test tube → W0

3- Wet sample with 1 ml of ethanol, saturating it

4- Add 5 ml HCl Solution to glass screw-top test tube

5- Place in preheated water bath (75.5 °C) for 40 minutes. Shake occasionally every 8 minutes

6- Glass screw-top test tube is carried from water bath and allows cooling to room temp

7- Add 5 ml ethanol to glass screw-top test tube and mix

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8- Add 12 ml anhydrous ethyl ether to glass screw-top test tube and orbital shake for 1 minute

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9- Add 12 ml petroleum ether to glass screw-top test tube and orbital shake for 1 minute

↓

10- Let ether and residue separate (Vortexing samples may aid in effecting a better separation)

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11- Pull off top layer from glass screw-top test tube into a dried and tared 150 ml empty conical Flask via a Pasteur pipette, where the top layer is white layer and leave the black layer in the bottom

↓

12- Weigh empty conical Flask → W1

↓

13- Repeat steps 7-11 with 8 ml portions of ethers three more times.

↓

14- Evaporate ether and any water contained in conical Flask. Evaporation on a hot plate at low temperature works best. Approximately 1 hour is required.

or

Evaporation on a water bath at75° C

↓

15- Place conical Flask in a 135° C oven for 10 minutes.

↓

16- Transfer conical Flask to desiccator and allow cooling to room temperature

↓

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17- Weigh conical Flask plus fat to +/-0.01g → W2

Calculation

$$\text{Fat \%} = \frac{W2 - W1}{W0} \times 100 = \quad \%$$

W2 = Weight of conical Flask and fat

W1 = empty conical Flask

W0 = Sample Weight

References

- Fat by Acid Hydrolysis JUNE 2007
- AOAC, 1980 Official Methods of Analyses. p.132.
- Budde, E. F. 1952 J. Assoc. Off. Agric. Chem. 35.799.

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Chemicals for Fat analysis

a) HCL 37%
b) Ethyl Alcohol 95% or Ethanol absolute
c) Diethyl Ether
d) Distilled Water

Fat Determination (Extruder Animal Feed)

(Applicable Work instruction)

1- Grind the dried sample through a 1mm sieve.

2- Weigh 1.6 : 1.8 gram ground sample into a 250 ml Glass Bottle → W0

3- Add 8 ml Distilled Water in glass bottle with Sample

4- Mix glass bottle to good homogeneity and Add 10 ml HCl (37 %)

5- Place in preheated water bath (75 °C) for 50 minutes. Shake occasionally every 8 minutes

6- Glass Bottle that is carried from water bath and allows cooling to room temperature

7- Add 10 ml ethanol to glass bottle and mix well

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8- Then Leave the bottle to cool well

↓

9- Add 25 ml Diethyl ether to glass bottle and orbital shake for 1 minute at the Second speed

↓

10- After Shaking open Cover of glass bottle and put it on the Bench of Laboratory for 12 min

↓

11- Add 10 ml Diethyl ether to glass bottle and orbital shake for 1 minute and wait for 10 min in constant state

↓

12- Pull off top layer from glass bottle into a dried and tared 150 ml empty conical Flask via a Pasteur pipette, where the top layer is white layer and leave the black layer in the bottom

↓

13- Weigh empty conical Flask → W1

↓

14- Repeat steps 11 and 12

↓

15- Evaporation on a water bath at75° C

↓

16- Dry Sample contained in conical Flask. Evaporation on a hot Air Oven at 103° C. Approximately 2 hour is required.

↓

17- Transfer conical Flask to desiccator and allow cooling to room temperature

↓

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18- Weigh conical Flask plus fat to +/-0.01g → W2

Calculation

$$\text{Fat \%} = \frac{W2 - W1}{W0} \times 100 = \quad \%$$

W2 = Weight of conical Flask and fat

W1 = empty conical Flask

W0 = Sample Weight

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Chemicals for Fat analysis

e) Petroleum ether (40°-60° C)

Fat Determination (Animal Feed)

1- Grind the dried sample through a 1mm sieve.

2- Weigh 2.5 to 3 gram ground sample into a extraction thimble → W0

3- Weigh empty extraction beaker of Soxchelt → W1

4- Add 60 ml of petroleum ether into the extraction beaker

5- 30 minutes with thimble immersed in boiling petroleum ether at 110° C

60 minutes of reflux washing at 110° C

6- The extraction beaker with the extract are placed in an oven at 105° C for 30 minute to remove the last traces

7- Then the extraction beaker is placed in Desiccator to cool at room temperature for 15 min

8- Weigh the extraction beaker plus fat to +/-0.01g → W2

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Calculation

$$\text{Fat \%} = \frac{W2 - W1}{W0} \times 100 = \quad \%$$

W2 = Weigh extraction beaker plus fat

W1 = empty extraction beaker

W0 = Sample Weight

Reference

[1] AOAC 1990 method 920.39)

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Chemicals for Fat analysis

a) Petroleum ether (40°-60° C)

Fat Determination (Dry Animal Feed)

1- Hydrolysis Procedures for Sample of dry animal feed by HU 6 Hydrolysis unit

Or you can do Hydrolysis Procedures for Sample Manually

a) Chemicals

1) Hydrochloric acid 4 mol/L: 4 Litre HCl 32% are filled up to 10 L with deionized or distilled water
2) Petroleum ether (40°-60° C)

b) Hydrolysis Procedures

1) feed is homogenized in the mixer
2) Work in a fume hood. Preheat the heating plate with a low heating level.
3) Place 4-5 dried boiling stones in 250 mL beakers and weigh up to 6 g of homogeneous sample W0
4) Add 50 mL 4 M HCl and stir gently. Add another 50 ml 4 M HCl rinsing the walls of the beaker.
5) Place the beaker on the heating plate; cover it with a watch glass. Note you should keep the low heating level, to prevent excessive splashing of the sample to the walls.
6) Watch the solution until a stable gentle boil is reached.
7) After boiling occurs, let the sample be hydrolyzed for 30 minutes.
8) Prepare a rack with digestion tubes, glass funnels and Filter papers. Wet the filters with boiling water. Wear thermal protection gloves
9) After the hydrolysis time is up, use a Tongs to carefully remove the beakers from the heating plate. Prepare at least 500 mL of boiling water for each sample.
10) Rinse the watch glass with boiling water into the beaker. Dilute the solution with 100 mL of boiling deionized or distilled water.

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11) **Filtrate the solution through the filter and wash the beaker with 100 ml of boiling water until the sample is transferred completely. Three rinses should be enough,**

12) **Rinse thoroughly each filter paper with a volume of at least 300 mL of boiling water until the filtrate reaches neutral water pH. Check the pH, Note you should Rinse the filter paper. Make sure to rinse all the filter so there are no acidic zones left.**

13) **Place the filter in the cellulose thimble. By folding the filter paper**
14) **the filter in the cellulose thimble is placed in a clean extraction beaker.**
15) **Dry the thimble and the beaker in a drying oven for 1.30 hour at 102 ± 2 °C.**
16) **Allow the thimble and beakers to cool down to ambient temperature in a desiccator (for at least 15 : 20 minutes).**
17) **Once the dried beakers have cooled down, (pulling up the thimble contained sample and place it in Soxchelt)**

2- **Weigh empty clean extraction beaker of Soxchelt → W1**

3- **Add 70 ml of petroleum ether into the extraction beaker**

4- **60 minutes with thimble immersed in boiling petroleum ether at 110° C**

60 minutes of reflux washing at 110° C

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5- The extraction beaker with the extract are placed in an oven at 103° C for 30 minute to remove the last traces

6- Then extraction beaker is placed in Desiccator to cool at room temperature for 15 min

7- Weigh extraction beaker plus fat to +/-0.01g → W2

Calculation

$$\text{Fat \%} = \frac{W2 - W1}{W0} \times 100 = \quad \%$$

W2 = Weigh of extraction beaker plus fat

W1 = Weigh of Empty extraction beaker

W0 = Sample Weight

References

[1] AOAC official method of analysis .Arlington, Virginia, usa, method 7.0

[2] ISO 1443:1973 Meat and meat products -- Determination of total fat content

[3] AOAC 945.16 Oil in cereal adjuncts

[4] § 64 LFGB Nr. L 06.00-6: 2014-06 Bestimmung des Gesamtfettgehaltes in Fleisch und Fleischerzeugnissen

[5] Application Note 773/2021 - Fat determination in manually hydrolyzed samples

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