CRUDE FAT IN FEED – SPECIAL METHOD FOR FOAMING SAMPLES

1. Principle

The sample is heated up with hydrochloric acid to digest protein and free bound lipids. The digestion solution is filtered and the fat remaining in the filter is extracted with petroleum ether after the drying process. The solvent is distilled and the dried residue is weighed. The fat content is calculated from the difference between the initial sample weight and the weight at the end of the analysis.
This document describes the special parameters for the hydrolysis of samples showing excessive foaming or heavy delays in boiling.

2. Method

Based on
Method book III, Feed Research, Determination of Crude Fat, Chapter 5.1.1, Governmental Method, Analysis B

3. Product Table

Feed Regulations, commented texts, Product Table 1 "Single feed" and
Product Table 2 "Mixed feed"

4. Chemicals

Quality: p.a.
Water: demineralized or distilled
4.1. Petroleum ether 40/60
4.2. Hydrochloric acid HCl, c = approx. 4 mol/l
4.3. pH-indicator paper

5. Instruments

5.1. Analytica balance (0,0001g)
5.2. Desiccator with drying agent, e. g.: Blaugel
5.3. Universal Mixer e.g. Moulinex 'Moulinette'
5.4. HYDROTHERM
5.5. Extraction unit SOXTHERM micro / macro with MULTISTAT, cat. no. 13-0011
or SOXTHERM Manager, cat. no. 13-0012
5.6. Electric drying chamber with natural aeration and automatic control of the temperature with a precision of ± 2 °C
5.7. Cotton wool, chemically clean and fat-free
5.8. HT Weighing Paper for HYDROTHERM, cat. no. 1004939
5.9. Folded Filter FF240, cat. no. 1004092

6. Sample Preparation

About 3 - 5 boiling stones are put into the extraction beakers and placed into a drying chamber for about 1 hour at 100 °C ± 3 K. After cooling down to room temperature in the desiccator, they are weighed to 1 mg accuracy. The sample has to be conserved in order to avoid spoilage and any changes of the composition. Prior to the analysis, the sample should be at room temperature. It has to be thoroughly mixed to ensure an even distribution of the fat. The analysis should be run immediately after the mixing.

7. Procedure

7.1. Hydrolysis

The weight of the sample should be determined by aiming to achieve a target of extracting 0.5 – 1.5 g fat; so up to 10 g sample is weighed into a weighing paper (5.8.) and put into the digestion beaker (± 0.1 mg precision).
The digestion beaker containing the sample is inserted in the HYDROTHERM and is locked.
For further information regarding the sample weight please see section 9 (Product table).

Start use of the HYDROTHERM unit following the instruction manual.
A dry folded filter (5.9.) is placed into the respective position of the instrument. Then the apparatus is closed and the program can be started.

The hydrochloric acid (4.2.) is added automatically. The liquid is quickly brought to boil and simmered with reduced heating capacity for about 1 h. At the end of the hydrolysis the digestion mixture is diluted with hot water to the double amount and is then immediately filtered through a pleated filter, which has been moistened automatically by the system with water. The beaker, the condenser and the filtration components are rinsed several times with hot water. The filter is rinsed with hot water till the backwash water has a neutral reaction. HYDROTHERM performs all these steps automatically.

Recommended Settings and Parameters: HYDROTHERM Soft Method

Parameter	Setting	Unit	Comment
Fill levels			
HCl amount	100	ml	
H_2O amount / dilution	100	ml	
Heating / cooling phases			
Heat up phase	6	min	
Boiling phase I	20	min	Boiling phase power 50 %
Boiling phase II	30	min	Boiling phase power 30 %
Cool down phase - duration	15	min	
Filter moisture			
Number of moisture cycles	3		
Moisture amount per cycle	30	ml	
Filter phase:			
Filter wait time	5	sec	
Rinsing cycles	16		
Pipe opening time	200	ms	
Sample rinse time	10	sec	
Sample shower - amount	40	ml	
Cooling shower - amount	20	ml	
Filter shower - amount	40	ml	

After the program has finished, the filter is placed on a watch glass and dried for up to 1.5 h at 103 °C ± 2 K in a drying oven. Since humidity may have an impact on the result, the drying time might have to be prolonged.

7.2. Extraction

After the cooling period, the filter is put into an extraction thimble and covered with cotton wool (5.7.). Any remaining fat traces on the watch glass have to be taken up with the cotton (5.7.), soaked with the extraction agent, and put into the extraction thimble as well. After adding 150 ml of extraction agent in case of SOXTHERM macro and 100 ml of extraction agent (4.1.) respectively in case of SOXTHERM micro, the sample is extracted using the following program:

Solvent:	Petroleum ether
Boiling Range:	40 to 60 °C
Amount of Solvent:	100 ml SOXTHERM micro / 150 ml SOXTHERM macro
Size of Extraction Beakers:	micro, cat. no. 13-0051, macro, cat. no. 13-0050
Type of Sealing :	Viton, cat. no. 1000578
Extraction Thimbles:	Type SE33A, 33 x 80 mm, cat. no. 13-0054
	Type SE33B, 33 x 94 mm, cat. no. 13-0057
Extraction Baskets:	SHK2, cat. no. 13-0062
Boiling Stones:	cat. no. 1000774
Air Pressure / Compressor:	min. 4.5 bar or cat. no. 13-0010
Water Pressure or	min. 0.5 bar or
Recirculating Cooler	FL 601, cat. no. 10-0046 or FL1201, cat. no. 10-0045

Program Step	Program parameter	Comment
Safety Temperature	200 °C	
Extraction Temperature	150 °C	
Reduction Interval	4 min	
Reduction Pulse	2 s	
Hot Extraction	30 min	
Evaporation A	4-5 x Interval	After A the level of solvent should be at least 10 mm below the thimble
Rinsing Time	60 min	
Evaporation B	3-4 x Interval	After B the extraction beaker should be nearly empty
Evaporation C	4 min	

After the program is run, the extraction beakers are dried in the drying chamber for 1.5 hours at 100°C ± 2 K. Then, the are put into the desiccator, left to cool down to room temperature and weighed with a precision of +/- 1 mg. In order to check the weight consistency, the samples are dried for another 30 minutes and weighed again after cooling down. This procedure is repeated as long until two successive weighings show no more difference than 0.1 % of the initial sample weight. Should the weight increase then the previous lower value should be taken. Extraction, drying, and weighing have to be effected consecutively.

8. Evaluation

8.1. Calculation

w: Crude fat content in g/100 g (equivalent %) of the sample to be calculated as follows:

$$w = \frac{(m_2 - m_1) * 100}{m_0}$$

m1: Weight (g) of the empty extraction beaker with boiling stones
m2: Weight (g) of the extraction beaker with fat after the drying process
m0: Sample weight (g)
The result is given rounded off to one fractional digit.

8.2. Reproducibility

The difference in results of double-determinations should not exceed:

At a crude fat content of:
- less than 5 % - 0.2 % absolute
- 5 % - 10 % - 4.0 % of the higher result
- more than 10 % - 0.4 % absolute

8.3. Comment

Possibly, no hydrolysis is necessary for the crude fat determination in single feedstuffs - this depends on the fat type.

9. Product Table

9.1. Single Feed

Sample Type	Sample weight [g] +/- 10 %	Crude fat content [%]
Babssu extraction coarse meal	10.000	max. 4
Babssu extraction coarse meal, partly husked	10.000	max. 4
Babassu cake	10.000	min. 5
Coarse cotton seed extraction meal from husked seed	10.000	max. 4
Coarse cotton seed extraction meal from husked seed, fattened	10.000	min. 4
Coarse cotton seed extraction meal from partly husked seed treated with formaldehyde, for cattle, sheeps and goats	10.000	max. 4
Coarse cotton seed extraction meal from unpeeled seed	10.000	max. 4
Coarse cotton seed extraction meal from unpeeled seed, fattened	10.000	min. 4
Coarse cotton seed cake from husked seed	10.000	min. 5
Coarse cotton seed cake from partly husked seed	10.000	min. 5
Ecuador palm kernel cake	10.000	max. 4
Peanut, peeled, husked	10.000	max. 4
Peanut extraction coarse meal from husked seed	10.000	max. 4
Peanut extraction coarse meal from partly husked seed	10.000	max. 4
Peanut extraction coarse meal from partly husked seed, fattened	10.000	min. 4
Peanut extraction coarse meal from non-husked seed	10.000	max. 4
Peanut bran	10.000	min. 10
Peanut cake from husked seed	10.000	min. 5
Peanut cake from partly husked seed	10.000	min. 5
Fish liver meal	10.000	max. 10
Fish liver meal, partly hydrolysed	10.000	max. 2,5
Fish meal type 55	10.000	max. 12
Fish meal type 60	10.000	max. 12
Fish meal type 64	10.000	max. 12
Meat-and-bone meal	10.000	max. 14
Bone coarse	10.000	max. 5
Poultry waste, dried	10.000	max. 12
Poultry waste, rich in fat, dried	10.000	min. 12
Hemp extraction coarse meal	10.000	max. 4
Hemp cake	10.000	min. 5
Cacao extraction coarse meal	10.000	max. 4
Kapok extraction coarse meal	10.000	max. 4
Kapok extraction coarse meal, fattened	10.000	min. 4
Kapok cake	10.000	min. 5
Coconut extraction coarse meal	10.000	max. 4
Coconut extraction coarse meal, fattened	10.000	min. 4
Coconut cake	10.000	min. 5
Copra, dried	3.500	min. 60
Lin extraction coarse meal	10.000	max. 4
Lin extraction coarse meal, fattened	10.000	min. 4
Lin cake	10.000	min. 5
Macoya palm kernel extraction coarse meal	10.000	max. 4
Macoya palm kernel extraction coarse meal, fattened	10.000	min.4
Macoya palm kernel cake	10.000	min. 5
Maize germ	10.000	min. 20
Coarse maize germ meal (maize milling industry)	10.000	max. 4
Coarse maize germ meal (starch industry)	10.000	max. 4
Maize germ bran, extracted	10.000	max. 4

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Sample Type	Sample weight [g] +/- 10 %	Crude fat content [%]
Maize germ cake (maize milling industry)	10.000	min. 4
Maize germ cake (starch industry)	10.000	min. 4
Almond cake	10.000	min. 5
Milk powder, partly skimmed	10.000	min.10
Poppy seed extraction coarse meal	10.000	max. 5
Poppy seed cake	10.000	min. 5
Murumuru Palm kernel extraction coarse meal	10.000	max. 4
Niger seed extraction coarse meal	10.000	max. 4
Nut cake	10.000	min. 4
Olive extraction coarse meal	10.000	max. 4
Palm kernel extraction coarse meal	10.000	max. 4
Palm kernel extraction coarse meal, fattened	10.000	min. 4
Palm kernel cake	10.000	min. 5
Rape extraction coarse meal	10.000	max. 4
Rape extraction coarse meal, fattened	10.000	min. 4
Rape extraction coarse meal with reduced goitrin content	10.000	max. 4
Rape extraction coarse meal with reduced goitrin, fattened	10.000	min. 4
Rape seed cake	10.000	min. 5
Rice germ	10.000	min. 17
Rice germ extraction coarse meal	10.000	max. 4
Rice germ extraction coarse meal, fattened	10.000	min. 4
Rice germ cake	10.000	min. 5
Safflower extraction coarse meal from husked seed	10.000	max.4
Safflower extraction coarse meal from partly husked seed	10.000	max.4
Safflower extraction coarse meal from husked seed	10.000	min. 5
Silk worm cocoon coarse meal, extracted	10.000	max. 4
Sesame extraction coarse meal	10.000	max. 4
Sesame cake	10.000	min. 5
Soy extraction coarse meal	10.000	max. 4
Soy extraction coarse meal, steamed	10.000	max. 4
Soy extraction coarse meal, steamed, fattened	10.000	min. 4
Soy cake	10.000	min. 5
Sunflower extraction coarse meal from husked seed	10.000	max. 4
Sunflower extraction coarse meal from husked seed, fattened	10.000	min. 4
Sunflower extraction coarse meal from partly husked seed	10.000	max. 4
Sunflower extraction coarse meal from partly husked seed, fattened	10.000	min. 4
Sunflower extraction coarse meal from unpeeled seed	10.000	max. 4
Sunflower extraction coarse meal from unpeeled seed, fattened	10.000	min. 4
Sunflower seed cake from husked seed	10.000	min. 5
Sunflower seed cake from partly husked seed	10.000	min. 5
Tengkawang coarse meal, extracted	10.000	max. 4
Meat-and-bone meal	10.000	max. 11
Meat-and-bone meal, rich in fat	10.000	min. 11
Tucum extraction coarse meal	10.000	max. 4
Uricuri extraction coarse meal	10.000	max. 4

7.2. Mixed Feed

Sample Type	Sample weight [g] +/- 10 %	Crude fat content [%]
Milk replacer for growing calves (complete feed))	8.000	5 – 30
Milk replacer I for fattening calves (complete feed)	8.000	8 – 30
Milk replacer II for fattening calves from approx. 80 kg on (complete feed)	8.000	15 – 30
Supplementary feed rich in energy for skimmed milk for fattening calves	4.000	30 – 60
Dairy concentrate I (supplementary feed for dairy cows)	10.000	max. 6
Dairy concentrate II (supplementary feed for dairy cows)	10.000	max. 6
Dairy concentrate III (supplementary feed for dairy cows)	10.000	max. 8
Dairy concentrate IV (supplementary feed for dairy cows, rich in protein)	10.000	max. 8
Cattle feed I (supplementary feed for fattening cattle)	10.000	max.6
Cattle feed II (supplementary feed for fattening cattle, rich in protein)	10.000	max. 6
Milk replacer for piglet (complete feed)	10.000	min 4
Piglet starter I (complete feed) up to approx. 20 kg	10.000	max. 7
Piglet starter II (complete feed) up to approx.35 kg	10.000	max. 7
Complete feed I for fattening pigs up to approx. 50 kg	10.000	max. 8
Complete feed II for fattening pigs from approx. 50 kg on	10.000	max. 10
Complete feed for fattening pigs from approx. 35 kg on	10.000	max. 9
Complete feed for lactating sows	10.000	max. 8
Supplementary feed for suckling piglet	10.000	max. 6
Supplementary feed I for fattening pigs	10.000	max. 12
Supplementary feed II for fattening pigs	10.000	max. 12
Supplementary feed I for stock pigs	10.000	max. 12
Milk replacer for ewe lambs (complete feed)	8.000	min. 20