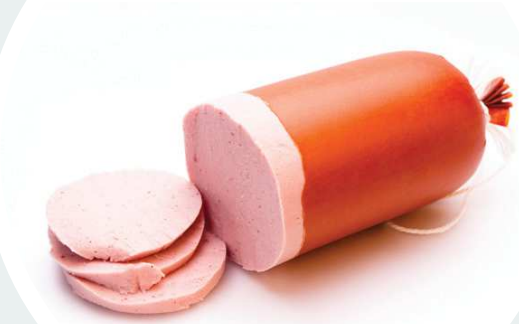
Application Note
No. 382/2019

Total fat determination in meat products

HydrolEx H-506, FatExtractor E-500:
Total Fat Determination in Meat Products by Hot Extraction



1. Introduction

A simple and fast procedure for fat determination is introduced. The sample is hydrolyzed with the HydrolEx H-506, followed by an extraction with the FatExtractor E-500 Hot Extraction. The total fat content is determined gravimetrically, after the extract has been dried to a constant weight. This application follows official methods (ISO 1444:1996, ISO 11085:2016, AOAC 2003.05, AOAC 991.36).

2. Equipment

- HydrolEx H-506
- Suction set with vacuum pump, BUCHI (Order No. 11068473)
- FatExtractor E-500 Hot Extraction, Standard Interface
- Mixer B-400
- Analytical balance (accuracy ± 0.1 mg)
- Microwave oven
- Drying oven / Vacuum drying oven
- Weighing support for hydrolysis vessels, BUCHI (Order No. 11067040)

3. Chemicals and Materials

Chemicals:

- Quartz sand, particle size 0.3-0.9 mm, BUCHI (Order No. 037689)
- Celite® 545, BUCHI (Order No. 11068920)
- Hydrochloric acid 4 mol/L, 4 L HCl 32% (Hänseler, 20-2000-5) are filled up to 10 L with deionised water
- Petroleum ether, Emsure® ACS, ISO, for analytical, boiling range 40-60 °C, 2.5 L, Merck Millipore (Order No. 1.01775.2500)
- Diethyl ether, AnalR NORMAPUR, ACS/Reag. Ph. Eur, 2.5 L, VWR (Order No. 23811.326)

For a safe handling please pay attention to all corresponding MSDS.

Sample:

- Cooked sausage, certified fat content of 27.46 g/100 g (+/- 0.595 g/100 g), LVU No. 16-01j.
- Cervelat sausage, declared fat content 20 g/100g. Purchased at a local supermarket.
- Minced beef meat, declared fat content 13 g/100g. Purchased at a local supermarket.

4. Procedure

The fat determination includes the following steps:

- Sample homogenization
- Hydrolysis of the sample with 4 M hydrochloric acid to break up the matrix
- Hot Extraction of the fat
- Calculation of fat content

4.1. Sample homogenization

1. Homogenize the sample with the Mixer B-400 once for 2 s.

4.2. Acid hydrolysis

4.2.1. Preparation of the glass sample tubes

2. Add approx. 20 g of quartz sand to the glass sample tube and compact the sand by gently tapping the glass sample tube onto the table
3. Add approx. 2 g Celite® 545 and spread it evenly using a spoon

The sand and the Celite® layer should not be mixed together. Otherwise the Celite® phase may break through the frit and affect the results either by increasing the recovery or by blocking the frit.

4.1.2. Hydrolyzing the sample matrix

4. Place 2 g Celite® 545 in the hydrolysis vessel
5. Add up to 10 g homogeneous sample[1] to the hydrolysis vessel and note the accurate weight of the sample
6. Add 50 mL hydrochloric acid (4 M) and form a suspension by gently swirling the vessel
7. Add another 50 mL hydrochloric acid (4 M) making sure to rinse any remaining sample off the glass wall
8. Preheat the HydrolEx H-506 for 10 min
9. Insert the samples into the unit and lower the vessels
10. Connect the aspiration tubes and start the vacuum pump
11. Reduce the heat to level 2.5 when one position is boiling

Violent foaming can be prevented by adding 4 M hydrochloric acid dropwise. The degree of foaming depends on the sample and on the preheating time of the unit. Do not extend preheating excessively.

12. Hydrolyze the sample for 30 min after constant boiling is observed in each position
13. Add 50 mL of warm (50 °C) deionised water to each hydrolysis vessel at the end of the hydrolysis time
14. Switch off the heating and lift the hydrolysis vessels to the top position in order to filter the hydrolysate
15. Wash each of the vessels by gradually adding a total of at least 400 mL warm deionised water, until a neutral pH is reached
16. Remove the glass sample tubes from the hydrolysis unit.
17. Check the pH with a pH paper on the bottom of the frit. The pH should be neutral.
18. Stir the Celite® layers (without touching the sand layer) with a spatula to loosen the pulp
19. Carefully wipe off the spatula with a piece of tissue and add it on the top of the sample
20. Dry the glass sample tubes in a vacuum oven (2 h at 100 °C/200 mbar), in a drying oven (4 h at 100 °C) or in a microwave oven

Using a microwave oven accelerates the drying process. However, its control is more delicate. This is due to the fact that the sample can easily overheat (> 105 °C) if an inappropriate heating power is chosen. The following suggestion is valid for the drying of six hydrolyzed samples at the same time. First step: 15 min 640 W, second step: 9 min 480 W, power of microwave oven 800 W (the optimal parameters may depend on the model of microwave).

Faster drying at higher temperatures is not recommended because fat may decompose at temperatures above 105 °C. Oxidized fat can result in an excessive recovery.

21. Allow the glass sample tubes to cool down to room temperature in a desiccator
22. Add another layer of quartz sand (20 g). This prevents the Celite® from being re-suspended in the condensed solvent.

---

[1] The sample weight has to be chosen according to the approximate fat content of the sample.
80-100 %: 0.7-1 g 20-50 % 1.5-3.5 g <10 %: 7- 10 g
50-80 %: 1-1.5 g 10-20 % 3.5-7 g

4.3. Hot Extraction of the fat

4.3.1. Preparation of the beakers

Always use dry and clean beakers for the Hot Extraction. Dry them for at least 30 min at 102 °C. Let them cool down to ambient temperature in a desiccator for at least 2 h. Record the exact weight prior to extraction.

4.3.2. Hot Extraction

Place the glass sample tubes containing the sample into the solvent beaker. Close the safety shield and lower the rack. Fill in the solvent through the condensers into the beakers. Activate the occupied positions, open the cooling water or switch on the connected chiller and start the extraction according to the parameters listed in Table 1.

Table 1: Parameters for the Hot Extraction with the FatExtractor E-500.

Step	Value	Heating level [-]
Solvent	Petroleum ether / Diethyl ether[2]	
Extraction	5 min	4-5[3]
Rinse	30 min	4-5[3]
Drain	3	
Dry	3	4-5[3]
Solvent volume [mL]	50	

4.3.3. Drying of the extract

Dry the beakers containing the extract in a drying oven at 102 °C until constant weight. Let the beakers cool down to ambient temperature for at least 2 h in a desiccator and record the weight.

Make sure that the cooling down time of the beakers in the desiccator is the same before and after extraction. Differences in beakers temperature falsify the results.

4.4. Calculation

The results are calculated as percentage of the fat according to equation (1).

$$\%\,\mathrm{Fat} = \frac{(m_{Total} - m_{Beaker})}{m_{Sample}} \bullet 100\% \qquad (1)$$

% Fat: Percentage of fat in the sample
m_{Total}: Beaker + extract [g]
m_{Beaker}: Empty beaker weight [g]
m_{Sample}: Sample weight [g]

5. Results

The determined fat content corresponds well with the specified value of the certified reference sample and with the expected values. Depending on the type of solvent used, minor differences in the fat content were observed. This can be explained as an effect of the solvent polarity which affects the mass transfer during the extraction. The results are shown in Tables 2 - 7.

[2] Please select the solvent used in the menu.
[3] Heating level proposed by the system depending on the selected solvent.

Table 2: Determined fat content of cooked sausage, LVU No. 16-01j (specification: 27.46 ± 0.60 g/100 g), extracted with petroleum ether 40/60.

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	3.0068	141.5270	142.3596	27.36
Sample 2	2.9878	141.0146	141.8406	27.65
Sample 3	3.1267	141.1662	142.0285	27.58
Mean value				**27.53**
sd				0.15
rsd [%]				**0.55**

Table 3 Determined fat content of cooked sausage, LVU No. 16-01j (specification: 27.46 ± 0.60 g/100 g), extracted with diethyl ether.

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	3.1658	140.5872	141.5609	27.60
Sample 2	3.5275	141.1714	142.1517	27.79
Sample 3	3.4133	142.3722	143.3150	27.62
Mean value				**27.67**
sd				0.10
rsd [%]				**0.38**

Table 4: Determined fat content of cervelat sausage (declared fat content: 20 g/100 g), extracted with petroleum ether 40/60.

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	4.9056	140.5882	141.6650	21.95
Sample 2	4.4599	141.1728	142.1647	22.24
Sample 3	4.7709	142.3734	143.4411	22.38
Mean value				**22.19**
sd				0.22
rsd [%]				**0.99**

Table 5: Determined fat content of cervelat sausage (declared fat content: 20 g/100 g), extracted with diethyl ether.

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	4.7807	141.5280	142.6003	22.43
Sample 2	5.6666	141.0167	142.2845	22.37
Sample 3	4.6880	141.1675	142.2144	22.33
Mean value				**22.38**
sd				0.05
rsd [%]				**0.22**

Table 6: Determined fat content of minced beef meat (declared fat content: 13 g/100 g), extracted with petroleum ether 40/60.

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	5.4698	140.9607	141.6518	12.63
Sample 2	4.9951	140.4051	141.0275	12.46
Sample 3	5.0042	140.7732	141.4003	12.53
Mean value				**12.54**
sd				0.09
rsd [%]				**0.70**

Table 7: Determined fat content of minced beef meat (declared fat content: 13 g/100 g), extracted with diethyl ether.

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	5.1286	140.8526	141.5042	12.71
Sample 2	5.0063	140.3594	140.9857	12.71
Sample 3	4.9812	141.2482	141.8919	12.92
Mean value				**12.78**
sd				0.12
rsd [%]				**0.97**

6. Conclusion

The determination of fat in a meat product using the HydrolEx H-506 and the FatExtractor E-500 provided reliable and reproducible results. The results correspond well to the certified and the expected values, with low relative standard deviations (rsd). With the FatExtractor E-500 Hot Extraction, the extraction time is reduced significantly. The total extraction time takes only 38 min with a solvent consumption of 50mL.

7. References

[1] ISO 1444:1996, Meat and meat products – Determination of free fat content
[2] ISO 11085:2016, Cereals, cereals-based products and animal feeding stuffs – Determination of crude fat and total fat content by the Randall extraction method
[3] AOAC 2003.05, Crude Fat in Feeds, Cereal Grains and Forages
[4] AOAC 991.36, Fat (crude) in Meat and Meat Products

Extraction Reports App
Operation Manual of HydrolEx H-506
Operation Manual of FatExtractor E-500