Application Note
No. 354/2019

Fat determination in bakery product and chocolate

HydrolEx H-506, FatExtractor E-500:
Fat Determination in Bakery Product and Chocolate according to Weibull-Stoldt

1. Introduction

A simple and reliable procedure for fat determination of bakery and chocolate products according to Weibull-Stoldt is introduced. The sample is hydrolyzed with the HydrolEx H-506. The Soxhlet extraction is performed with the FatExtractor E-500. Calculation of total fat content follows gravimetrically after the extract has been dried to a constant weight. This application follows official methods (EN 98/64/EG, AOAC 963.15, ISO 14156:2001, ISO 1443:1973, AOAC 945.16). The combination of the new HydrolEx H-506 and the FatExtractor E-500 increases the sample throughput.

2. Equipment

- HydrolEx H-506
- Suction set with vacuum pump, BUCHI (Order No. 11068473)
- FatExtractor E-500 Soxhlet, Standard Interface, no Analyte protection
- 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)
- Hexane, AnalR NORMAPUR, analytical grade, 2.5 L, VWR (Order No. 24608.321)
- Diethyl ether, AnalR NORMAPUR, ACS/Reag. Ph. Eur, 2.5 L, VWR (Order No. 23811.326)
- Chloroform, HiPerSolv CHROMANORM, 2.5 L, VWR (Order No. 83627.320)

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

Samples:

- Cookie with a certified fat content of 27.47 g/100 g (+/- 0.311 g/100 g), LVU No. 17-11
- Chocolate with a certified fat content of 30.93 g/100 g (+/- 0.356 g/100 g), LVU No. 17-13

The cookie sample was broken into small pieces with a pestile and mortar and the chocolate sample was grated with a kitchen grater.

4. Procedure

The fat determination according to Weibull-Stoldt includes the following steps:

- Sample homogenization
- Hydrolysis of the sample with 4 M hydrochloric acid to break up the matrix
- Filtration of the hydrolysis solution to separate the fat
- Drying of the filtered sample
- Soxhlet extraction of the fat
- Drying of the extract
- Weighing of the extract
- Calculation of fat content

4.1. Acid hydrolysis

4.1.1. Preparation of the glass sample tubes

1. 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
2. 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 breakthrough the frit and affect the results either by increasing the recovery or by blocking the frit.

4.1.2. Hydrolyzing the sample matrix

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

11. Hydrolyze the sample for 30 min after constant boiling is observed in each position
12. Add 50 mL of warm (50 °C) deionised water to each hydrolysis vessel at the end of the hydrolysis time
13. Switch off the heating and lift the hydrolysis vessels to the top position in order to filter the hydrolysate
14. Wash each of the vessels by gradually adding a total of at least 400 mL warm deionised water, until a neutral pH is reached
15. Check the pH with a pH paper on the bottom of the frit

For maximum efficiency, aspire aspirate all samples/rinsing water at the same time.

16. Stir the Celite® layers (without touching the sand layer) with a spatula to loosen the pulp
17. Carefully wipe off the spatula with a piece of tissue and add it on the top of the sample
18. 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.

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

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[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

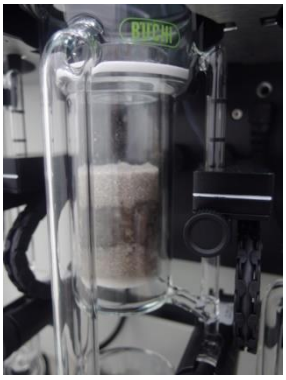
4.2. Fat extraction

4.2.1. Preparation of the beakers

Always use dry and clean beakers for the Soxhlet extraction. Dry them for at least 30 min at 102 °C. Let them cool down to ambient temperature in a desiccator for at least 1 h. Record the exact weight prior to extraction.

4.2.2. Soxhlet Extraction

Put the glass sample tubes into the extraction chamber and adjust the level sensor with the white line to the center of the upper sand layer. See Picture 1.



Picture 1: Adjusting the level sensor for the Soxhlet Extraction

Fill the solvent directly into the beakers and place them on their corresponding heating plate. Close the safety shield and lower the rack. Alternatively, fill in the solvent by the condensers after lowering the rack. 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 Soxhlet Extraction with the FatExtractor E-500

Step	Time [min] / No. of cycles [-]	Heating level [-]
Solvent	Petroleum ether / Hexane / Diethyl ether / Chloroform[2]	
Extraction	20 cycles	5 - 9[3]
Rinse	5 min	5 - 9[3]
SmartDrying	on[4]	-
Solvent volume [mL]	100	

4.2.3. Drying of the extract

Dry the beakers containing the extract in a drying oven at 102 °C until a constant weight is reached. Let the beakers cool down to ambient temperature for at least 1 h in a desiccator and record the weight.

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

4.3. Calculation

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

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[2] Please select the solvent used in the menu.
[3] Heating level proposed by the system depending on the selected solvent.
[4] Instead of using SmartDrying it is possible to use the following drying parameters. Then, SmartDrying is switched off:
Petroleum ether: 12 min Hexane: 12 min
Diethyl ether: 10 min Chloroform: 13 min

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

% 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

Determined fat contents for the certified reference materials are in line with the specified and labelled values, independent of the solvent used.
Depending on the type of solvent used, minor differences in the fat content are observed. This can be explained as an effect of the solvent polarity which affects the mass transfer during the extraction. The complete findings are summarized in Tables 2 and 3.

Table 2: Cookie, LVU No. 17-11 (Specification: 27.47 ± 0.311 g/100 g)

	Petroleum ether	Hexane	Diethyl ether	Chloroform
Sample 1	27.56	27.49	27.35	27.56
Sample 2	27.61	27.50	27.53	28.04
Sample 3	27.53	27.73	27.56	27.57
Average [%]	**27.57**	**27.57**	**27.48**	**27.72**
rsd [%]	0.16	0.50	0.41	0.99

Table 3: Chocolate, LVU No. 17-13 (Specification: 30.93 ± 0.356 g/100 g)

	Petroleum ether	Hexane	Diethyl ether	Chloroform
Sample 1	30.86	31.03	31.20	31.43
Sample 2	31.03	31.01	31.20	31.45
Sample 3	31.05	31.11	31.15	31.40
Average [%]	**30.98**	**31.05**	**31.18**	**31.42**
rsd [%]	0.34	0.17	0.09	0.08

6. Conclusion

The determination of fat in different food products using the HydrolEx H-506 and the FatExtractor E-500 provides reliable and reproducible results. These results correspond well to the labelled values, with low relative standard deviations (rsd). With the FatExtractor E-500 Soxhlet, the time per cycles is reduced significantly. The programmed 20 cycles are accomplished in approx. 70 min.

7. References

[1] EN 98/64/EG Commission Directive 98/64/EC Fat in feedingstuffs
[2] ISO 14156:2001 Milk and milk products -- Extraction methods for lipids and liposoluble compounds
[3] ISO 1443:1973 Meat and meat products -- Determination of total fat content
[4] AOAC 963.15 Fat in Cacao Products
[5] AOAC 945.16 Oil in Cereal Adjuncts

Extraction Reports App

Operation Manual of HydrolEx H-506
Operation Manual of FatExtractor E-500