Extraction Unit E-816 ECE:
Fat Determination in Food using Twisselmann Extraction

The determination of fat in food is a routine procedure used for quality assurance and labelling. Below, a simple and easy procedure for fat determination in food, according to §64 LFGB L 17.00-4 and ISO 1444:1996 is introduced. The sample is hydrolyzed with hydrochloric acid using the Hydrolysis Unit E-416, followed by a Twisselmann extraction with the Extraction Unit E-816 ECE (Economic Continuous Extraction). The determined fat contents correspond well to the labelled values.

1. Introduction

Fat determination is one of the key analysis performed in the food industry. The samples require a hydrolysis step with hydrochloric acid to break the chemically bound and naturally encased fat from the matrix. Afterwards, the fat is extracted with a suitable solvent according to Twisselmann. With this extraction technique the sample is constantly kept in hot vapor whilst efficiently rinsed by fresh distilled solvent. After the extract has been dried to a constant weight the total fat content is determined gravimetrically.

2. Experimental

Equipment: Hydrolysis Unit E-416, Extraction Unit E-816 ECE

Samples: Madeira Cake LGC7107 with a labelled fat content of 13.4 ± 0.7 g/100 g; Processed meat ERM®-BB501b, labelled fat content 11.57 ± 0.44 g/100 g.

Determination: 20 g of quartz sand was added to a glass sample tube and 2 g Celite 545 was placed on top. The homogenized samples were weighed into a digestion vessel containing 2 g of Celite. After adding 2 x 50 mL hydrochloric acid (4 M) into each vessel the samples were hydrolyzed for 30 min using the E-416. The hydrolyzate was transferred and the vessels washed with warm (40-50 °C) deionised water, until a neutral pH was obtained. The glass sample tubes were dried in a vacuum oven, drying oven or microwave oven. After cooling down in a desiccator another layer of quartz sand (20 g) was added to the sample tube. The extraction was performed using the E-816 ECE (Figure 1) applying the parameters specified in Table 1.

Table 1: Parameters for the extraction with the Extraction Unit E-816 ECE

Method parameters	
Solvent	Petroleum ether / Diethyl ether / Hexane / Chloroform
Extraction step	50 min (Heater 100 %)
Drying step	10 min (Heater 100 %)
Solvent volume	70 mL

The samples were extracted sixfold. The extracts were dried to a constant weight in a drying oven at 102 °C and the total fat content was calculated.

Figure 1: Extraction Unit E-816 ECE (Economic Continuous Extraction)

3. Results

The determined fat contents are presented in Table 2. The results correspond well to the certified values of the reference materials. The determinations show low relative standard deviations.

Table 2: Determined fat content in food samples, fat in g/100g (relative standard deviation in brackets), n=6

Solvent	Maderia Cake	Processed meat
Petroleum ether	12.82 (1.13)	11.22 (1.70)
Diethyl ether	13.24 (1.36)	11.62 (0.42)
Hexane	12.97 (0.51)	11.36 (1.42)
Chloroform	13.16 (0.56)	11.68 (1.70)

4. Conclusion

The determination of fat content in different hydrolyzed food samples using the Twisselmann extraction on the E-816 ECE provides reliable and reproducible results that correspond well to the labelled values of the certified reference materials.

5. References

§64 LFGB L 17.00-4:1982-05 Bestimmung des Gesamtfettgehaltes in Brot einschliesslich Kleingebäck aus Brotteigen
ISO1444:1993 Meat and meat products – Determination of fat content

- Operation Manual of Hydrolysis Unit E-416
- Operation Manual of Extraction Unit E-816 ECE

For more detailed information and safety conside-rations please refer to the Application Note no. 173/2014.

Application Note
No. 173/2014

Extraction Unit E-816 ECE:
Fat Determination in Food using Twisselmann Continuous Extraction

1. Introduction

An effective procedure for fat determination in food according to §64 LFGB L 17.00-4 and ISO 1444:1996 is presented [1, 2]. The sample is hydrolyzed with the Hydrolysis Unit E-416. The Twisselmann extraction is performed with the Extraction Unit E-816 ECE (Economic Continuous Extraction).This allows the sample to be constantly kept in hot solvent vapor whilst efficiently rinsed with freshly distilled solvent. The total fat content is determined gravimetrically after the extract has been dried to a constant weight.

2. Equipment

- Hydrolysis Unit E-416
- Extraction Unit E-816 ECE
- Analytical balance (accuracy ± 0.1 mg)
- Mixer, Retsch Grindomix GM 200
- Microwave oven
- Drying oven / Vacuum drying oven

3. Chemicals and Materials

Chemicals:

- Quartz sand, particle size 0.3-0.9 mm, BUCHI (037689)
- Celite 545, Macherey-Nagel (815560)
- 10 L of 4 M Hydrochloric acid (HCl) are prepared by dilution of 4 L HCl 32 % (Hänseler 20-2000-5) to 10 L with deionised water
- Petroleum ether, boiling range 40-60 °C, analytical grade, ACS, EGT (ET0093005M)
- Diethyl ether, puriss. meets analytical specification of Ph. Eur. BP ≥ 99.5 % (GC), Sigma-Aldrich (24004)
- Hexane, puriss. p.a. ACS reagent, reag. Ph. Eur, ≥ 99 %(GC), Sigma-Aldrich (32293)
- Chloroform, Chromasolv ® Plus, for HPLC, ≥ 99.9 %, contains amylenes as stabilizer, Sigma-Aldrich (650498)

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

Samples:

- Madeira Cake, certified reference material LGC7107, specified fat content: 13.4 ± 0.7 g/100 g
- Processed meat, certified reference material ERM®-BB501b, specified fat content: 11.57 ± 0.44 g/100 g

The samples were purchased from LGC Standards GmbH (Wesel, Germany).

4. Procedure

The determination of fat includes the following steps:

- Homogenization of the sample by grinding
- Acid hydrolysis of the sample, using the E-416
- Filtration of the hydrolysed solution to separate the fat
- Drying of the filtered sample
- Extraction of the sample, using the E-816 ECE
- Drying of the extract
- Weighing of the extract
- Calculation of the 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 a increasing the recovery or by blocking the frit.

4.1.2. Hydrolyzing the sample matrix

3. Place 2 g Celite 545 in the digestion vessel
4. Add up to 10 g homogeneous sample[1] to the digestion 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 tube
6. Add another 50 mL hydrochloric acid (4 M) making sure to rinse any remaining sample off the glass wall
7. Preheat the Hydrolysis Unit for 10 min
8. Insert the samples into the unit and lower the vessels
9. Connect the aspiration tubes, reduce the heat to level 3 and start the water-jet pump after boiling begins

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.

10. Hydrolyze the sample for 30 min after constant boiling is observed in each position
11. Add 100 mL of warm (40-50 °C) deionised water to each digestion vessel at the end of the hydrolysis time
12. Switch off the heating and lift the digestion vessels to the top position in order to filter the hydrolyzate
13. Wash each of the vessels by gradually adding a total of at least 400 mL warm deionised water, until a neutral pH is reached
14. 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.

15. Stir the Celite layers (without touching the sand layer) with a spatula to loosen the pulp
16. Carefully wipe off the spatula with a piece of tissue and add it on the top of the sample
17. Dry the glass sample tubes in a vacuum oven (≤ 4 h at 100 °C/200 mbar), in a drying oven (≤ 8 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.

18. Allow the glass sample tubes to cool down to room temperature in a desiccator
19. 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 Twisselmann extraction. Add a boiling aid (e.g. boiling stones) to each beaker and 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. Twisselmann extraction

Put the sample tubes into the extraction chamber using the pliers. See Figure 1.

Figure 1: Twisselmann extraction chamber before start

Fill the solvent directly into the beakers and place them on their corresponding heating plate. Close the safety shield and lower 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 extraction with the Extraction Unit E-816 ECE

Method parameters Extraction Unit E-816 ECE	
Solvent	Petroleum ether / Diethyl ether / Hexane / Chloroform[2]
Extraction step	50 min (Heater 100 %)[3]
Drying step	10 min (Heater 100 %)[4]
Solvent volume	70 mL

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.

[2] Please select the solvent used in the menu.
[3] Choose the heater between 100 – 120 % so the boiling is sufficient.
[4] Choose the same parameter for the heater as in the extraction step.

4.3. Calculation

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

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

% Fat : Percentage of fat in the sample
m_{Total} : Beaker + extract [g]
m_{Beaker} : Empty beaker weight with boiling aid [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. The relative standard deviations (rsd) are low, i.e. < 2 % for all samples.
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. With chloroform and diethyl ether – being more polar than petroleum ether and hexane –slightly more fat is extracted. The complete findings are summarized in Tables 2 and 3.

Table 2: Maderia Cake LGC7107 (Specification 13.4 ± 0.7 g/100 g)

	Petroleum ether	Diethyl ether	Hexane	Chloroform
Sample 1	12.80	13.21	12.93	13.19
Sample 2	12.63	13.35	13.02	13.24
Sample 3	13.00	13.32	12.97	13.09
Sample 4	12.99	13.03	12.90	13.26
Sample 5	12.75	13.03	12.92	13.09
Sample 6	12.77	13.47	13.07	13.13
Mean value [g/100g]	12.82	13.24	12.97	13.16
rsd [%]	1.13	1.36	0.51	0.56

Table 3: Processed Meat ERM®-BB501b (Specification 11.57 ± 0.44 g/100g)

	Petroleum ether	Diethyl ether	Hexane	Chloroform
Sample 1	11.11	11.67	11.58	11.88
Sample 2	11.21	11.56	11.29	11.49
Sample 3	11.23	11.55	11.15	11.44
Sample 4	11.12	11.61	11.44	11.79
Sample 5	11.06	11.66	10.73[5]	11.57
Sample 6	11.59	11.63	11.33	11.88
Mean value [g/100g]	11.22	11.62	11.36	11.68
rsd [%]	1.70	0.42	1.42	1.70

[5] This value is an outlier according to the statistical test of Dean-Dixon for outliers (α = 5 %).

6. Conclusion

The determination of fat in different food products using the Hydrolysis Unit E-416 and the Extraction Unit E-816 ECE provides reliable and reproducible results. These results correspond well to the labelled values, with low relative standard deviations (rsd).
With the Extraction Unit E-816 ECE the time to results is significantly reduced by 75 % compared to the use of classical glassware, and it offers an unattended automated process.

7. References

[1] §64 LFGB L 17.00-4:1982-05 Bestimmung des Gesamtfettgehaltes in Brot einschliesslich Kleingebäck aus Brotteigen
[2] ISO1444:1993 Meat and meat products – Determination of fat content

Operation Manual of Hydrolysis Unit E-416
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