Fat determination in fish products

Extraction Unit E-816 ECE:
Fat determination in fish products using Twisselmann extraction
1. Introduction

An effective procedure for fat determination in fish products with low and higher fat content is presented. The sample is hydrolyzed with the Hydrolysis Unit E-416. The Twisselmann extraction is performed using the Extraction Unit E-816 ECE (Economic Continuous Extraction). With this extraction technique the sample is constantly kept in hot vapor whilst being efficiently rinsed with freshly distilled solvent. After the extract has been dried to a constant weight the total fat content is determined gravimetrically.

2. Equipment

- Mixer B-400
- Extraction Unit E-816 ECE
- Hydrolysis Unit E-416
- Analytical balance (accuracy ± 0.1 mg)
- 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 % (analytical reagens, Beijing Chemical Works) to 10 L with deionised water
- Petroleum ether, boiling range 40-60 °C, analytical grade, ACS, Fluka (34491-2.5L)

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

Samples:
- Fried mackerels, declared content:
  - 7.2 % fat
  - 9.2 % carbohydrates
  - 16.5 % protein

- Albacore tuna, declared content:
  - 1.6 % fat
  - 0 % carbohydrates
  - 25.0 % protein

The samples were purchased at a local supermarket.
4. Procedure

The determination of fat includes the following steps:

- Homogenization of the sample by grinding, using the Mixer B-400
- Acid hydrolysis of the sample, using the Hydrolysis Unit E-416
- Fat extraction of the sample following Twisselmann extraction, using the E-816 ECE
- Calculation of the fat content

4.1. Homogenization of the sample, using the Mixer B-400

1. Fill approx. 50 g of samples to the beaker (not higher than the BUCHI logo)
2. Place the beaker in the beaker holder
3. Close the guard door
4. Keep the rocker button pressed to the right (IN) to start the mixing cycle for 1-2 s
5. Keep the rocker button pressed to the left (OUT) to move the beaker away from the cutter, loosening the contents by shaking or stirring
6. Let the sample be cooled

4.2. Acid hydrolysis of the sample, using the Hydrolysis Unit E-416

4.2.1. Preparation of the glass sample tubes

7. 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
8. 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.

4.2.2. Hydrolyzing the sample matrix

9. Place 2 g Celite® 545 in the digestion vessel
10. Add up the appropriate amount of sample to the digestion vessel and note the accurate weight of the sample
11. Add 50 mL hydrochloric acid (4 M) and form a suspension by gently swirling the tube
12. Add another 50 mL hydrochloric acid (4 M) making sure to rinse any remaining sample off the glass wall
13. Preheat the Hydrolysis Unit for 10 min
14. Insert the samples into the unit and lower the digestion vessels
15. Insert the glass sample tubes into the Hydrolysis Unit
16. Connect the digestion tubes and the glass sample tubes with the aspiration tubes, reduce the heat to level 3

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.

17. Hydrolyze the sample for 30 min after constant boiling is observed in each position and start the water-jet pump after boiling begins and the foaming stop
18. Add 100 mL of warm (40-50 °C) deionised water to each digestion vessel at the end of the hydrolysis time
19. Switch off the heating and lift the digestion vessels to the top position in order to filter the hydrolyzate
20. Wash each of the digestion vessels by gradually adding a total of at least 400 mL warm deionised water, until a neutral pH is reached
21. Check the pH with a pH paper on the bottom of the frit

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

22. Stir the Celite® layers (without touching the sand layer at the bottom) with a spatula to loosen the pulp
23. Carefully wipe off the spatula with a piece of tissue and add it on the top of the sample
24. 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 (maximum power rating 800 W) 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 at 640 W, second step: 9 min at 480 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.

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

4.3. Fat extraction of the sample following Twisselmann extraction, using the Extraction Units E-816

4.3.1. 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.

4.3.2. Twisselmann extraction

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

![Figure 1: Twisselmann extraction chamber with sample before starting the extraction.](image)

Fill the solvent 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.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Petroleum ether²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction step</td>
<td>60 min (Heater 100 %)³</td>
</tr>
<tr>
<td>Drying step</td>
<td>10 min (Heater 100 %)⁴</td>
</tr>
<tr>
<td>Total time</td>
<td>70 min</td>
</tr>
<tr>
<td>Solvent volume</td>
<td>70 mL</td>
</tr>
</tbody>
</table>

4.4. 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.

4.5. Calculation

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

\[
\% \text{Fat} = \left( \frac{m_{\text{Total}} - m_{\text{Beaker}}}{m_{\text{Sample}}} \right) \times 100\% \quad (1)
\]

- \( \% \text{Fat} \): Percentage of fat in the sample
- \( m_{\text{Total}} \): Beaker + extract [g]
- \( m_{\text{Beaker}} \): Empty beaker weight with boiling aid [g]
- \( m_{\text{Sample}} \): Sample weight [g]

² Please select the solvent, which is the default in the menu.
³ Choose the heater power between 100 – 120 % so the boiling is sufficient.
⁴ Choose the same parameter for the heater power that was selected for the extraction step.
5. Results and Discussion

The fat contents of the fish products obtained with the Twisselmann extraction method are listed in Tables 2-3. The measured fat content was slightly higher but close to the labelled value. Since the samples are natural products which were purchased in a supermarket, deviations from the declared content can occur and are accepted by law. Importantly, the results also revealed low relative standard deviations. Hence, the presented method is well applicable to analyze fish products with low and higher fat contents. The results are summarized in Tables 2-3.

Table 2: Fat content of fried mackerel samples (declared fat content 7.2 g/100 g).

<table>
<thead>
<tr>
<th>m_sample [g]</th>
<th>m_beaker [g]</th>
<th>m_total [g]</th>
<th>Fat [g/100 g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>10.0750</td>
<td>98.6233</td>
<td>99.3746</td>
</tr>
<tr>
<td>Sample 2</td>
<td>9.9418</td>
<td>98.4597</td>
<td>99.2046</td>
</tr>
<tr>
<td>Sample 3</td>
<td>10.0525</td>
<td>99.6959</td>
<td>100.4424</td>
</tr>
<tr>
<td>Mean value</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rsd [%]</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Fat content of albacore tuna samples (declared fat content 1.6 g/100 g).

<table>
<thead>
<tr>
<th>m_sample [g]</th>
<th>m_beaker [g]</th>
<th>m_total [g]</th>
<th>Fat [g/100 g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>9.9898</td>
<td>98.4547</td>
<td>98.6760</td>
</tr>
<tr>
<td>Sample 2</td>
<td>10.0085</td>
<td>98.5895</td>
<td>98.6760</td>
</tr>
<tr>
<td>Sample 3</td>
<td>10.0225</td>
<td>99.3046</td>
<td>99.5282</td>
</tr>
<tr>
<td>Mean value</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rsd [%]</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

6. Conclusion

The determination of fat in different fish products using the Hydrolysis Unit E-416 and the Extraction Unit E-816 ECE according to Twisselmann yields results that correspond well with the labelled fat content and have low relative standard deviations (rsd). With the Extraction Unit E-816 ECE the time to results is significantly reduced (70 min) when compared to extractions using classical glassware.