Fat Determination in Bakery and Dairy Products according to the Weibull-Stoldt Method

1. **Summary**

A simple and fast procedure for fat determination of bakery and dairy products according to Weibull-Stoldt is introduced. The sample is hydrolyzed with the BÜCHI Hydrolysis Unit B-411, Soxhlet Extraction is performed with the BÜCHI Extraction Unit B-811. Calculation of total fat content follows gravimetrically after drying the extract.

2. **Introduction**

The Acid Hydrolysis Method (Weibull-Stoldt) is one of the official methods (AOAC 963.15, SLMB 36A6...) to determine the total fat content of bakery and dairy products. It can be subdivided in the following steps:

- sample homogenization
- hydrolysis of the sample with 4 M hydrochloric acid to breakup the matrix (compounds)
- filtration of the hydrolysis solution to separate the fat
- drying of the filtered sample
- Soxhlet extraction of the fat with petroleum ether
- drying of the extract
- weighing of the extract
- calculation of fat content

3. **Instruments**

- BÜCHI Hydrolysis Unit B-411
- BÜCHI Extraction Unit B-811
- Büchi Vac V-503 with secondary condenser and Vacuum Controller V-800
- Analytical balance (tolerance ± 0.1mg)
- Equipment for sample homogenization: ball mill, oven
- Vacuum oven for drying
4. Reagents/Chemicals

- Quartz sand, particle size 0.3-0.7 mm  
  Büchi 037689, Fluka 84878
- Celite 545, particle size 20-45 µm  
  Fluka 22140
- Petroleum ether p.a., fraction 40/60 °C  
  Fluka 77379
- Hydrochloric acid, 4 M  
  Add 350ml hydrochlorid acid 32% (HCl) to 500ml deionized water. Dilute to one liter with deionized water

5. Experimental
With the introduced method absolute fat contents up to a mass of m = 1.5 g can be determined.

Sample
- Lemon Cake  (21-23% Fat, Acid Hydrolysis Method (Weibull-Stoldt))
- Biscuits  (20-24% Fat, Acid Hydrolysis Method (Weibull-Stoldt))
- Chocolate  (35-37% Fat, § 35 LMBG¹ Nr. L 44.00-4, Soxhlet Method)
- Chocolate Bar  (25-30% Fat, AOAC² 963.15 (1990), Soxhlet Method)
- Powdered Milk  (22-24% Fat, Acid Hydrolysis Method (Weibull-Stoldt))

Prior sample preparation
- the biscuits and cake were grounded with a ball mill
- the chocolate was melted in a water bath at 50°C and well homogenised with a spatula
- all other samples were directly weighed without preparation

Preparation of the glass sample tubes
Add approx. 50 g quartz sand to the frit and compact the sand by tapping it gently on the table. Add approx. 5 g Celite 545, spreading evenly by shaking the tubes carefully.

⚠️ The sand and the celite layers must not be mixed. Otherwise the celite phase may break through the frit and falsely influence the results.

Hydrolyzing the sample matrix
Place 1.5-10 g homogeneous sample and 5 g celite in the digestion vessel. Add 50 ml hydrochloric acid (4 M). Suspend carefully by gently swirling. Add another 50 ml hydrochloric acid (4 M) making sure to rinse any remaining sample off the glass wall. Preheat the Hydrolysis Unit for 10 min. Insert the samples into the unit, lower the vessels, reduce the heat output (step 3) and start the water jet pump (after boiling begins to avoid prematurely evacuating the samples).

1 LMBG Amtliche Sammlung für Lebensmittel- und Bedarfsgegenständegesetz
2 AOAC Official Methods of Analysis
Violent foaming can be prevented by adding 4 M hydrochloric acid drop by drop.

Hydrolyze the samples for 30 min after boiling has been observed. At the end of the hydrolysis time, 70 ml warm (40-50°C), distilled water is added to each digestion vessel. Switch heating off. Lift digestion vessels so that the digested solution is filtered. Wash each of the vessels by gradually adding a total of 250 ml warm water (40-50°C).

Before adding a new portion of water, wait until the previous portion of water was aspirated though the frit. Otherwise this will lead to irregular aspiration.

Stir the hydrolysate and the celite layer (without touching the sand layer) with a spatula to loosen the pulp. Dry the glass sample tubes in a vacuum oven (≤ 4 h at 100°C/200mbar) or in an oven (≤ 8 h at 100°C) to constant weight.

Faster drying at higher temperatures is not recommended because fat is decomposed by temperatures T greater than 105°C.

6. Extraction of the fat

Always use dry solvent beakers (drying for at least 30 min at 105°C, cool down the beakers in a dessicator) and record exact weight prior to extraction.

Allow the sample tubes to cool to room temperature. Loosen the dried pulp with the spatula to get a powdery hydrolysate; try to avoid mixing up the bottom layer of quartz sand. Shake off the spatula from remaining sample and add another layer of quartz sand (20 g). This prevents the celite from being re-suspended by condensed solvent and distributed on the upper tube wall where it will be prevented from a sufficient extraction.

The parameters of the extraction program are shown in table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>solvent (S)</td>
<td>S: Petroleum ether, fraction 40-60°C</td>
</tr>
<tr>
<td>solvent volume (V)</td>
<td>V = 140ml</td>
</tr>
<tr>
<td>extraction mode</td>
<td>Soxhlet Standard</td>
</tr>
<tr>
<td>extraction time (t)</td>
<td>t = 140min</td>
</tr>
<tr>
<td>heating program</td>
<td>step 1: l: 8, t = 120min, step 2: l: 8, t = 10min, step 3: l: 4, t = 10min</td>
</tr>
<tr>
<td>external drying</td>
<td>Vacuum oven</td>
</tr>
</tbody>
</table>

Dry the samples in a vacuum oven (1h at 100°C/200mbar) to constant weight. Let the samples cool to room temperature for 30 min in a dessicator and record an accurate weight.
7. Calculation
The results are calculated using the following equations.

\[
\% \text{ fat} = \left( \frac{m_{\text{total}} - m_{\text{beaker}}}{m_{\text{sample}}} \right) \times 100
\]

- \(m_{\text{sample}}\): sample weight [g]
- \(m_{\text{total}}\): beaker weight after fat extraction [g]
- \(m_{\text{beaker}}\): empty beaker weight [g]

8. Results
The following samples, determined in the Büchi laboratories, are not certified reference materials.

<table>
<thead>
<tr>
<th>Number of extractions</th>
<th>Sample weight</th>
<th>% Fat</th>
<th>Sample weight</th>
<th>% Fat</th>
<th>Sample weight</th>
<th>% Fat</th>
<th>Sample weight</th>
<th>% Fat</th>
<th>Sample weight</th>
<th>% Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate (35 – 37% Fat)</td>
<td>4.0075</td>
<td>22.06</td>
<td>4.0938</td>
<td>24.07</td>
<td>3.2077</td>
<td>35.46</td>
<td>2.9988</td>
<td>26.92</td>
<td>3.0474</td>
<td>26.88</td>
</tr>
<tr>
<td>Chocolate Bar (25 – 30% Fat)</td>
<td>4.0086</td>
<td>22.03</td>
<td>4.0662</td>
<td>24.05</td>
<td>3.2706</td>
<td>35.83</td>
<td>3.0474</td>
<td>26.88</td>
<td>3.2080</td>
<td>22.94</td>
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<tr>
<td></td>
<td>3.9973</td>
<td>23.60</td>
<td>3.9973</td>
<td>23.60</td>
<td>3.9863</td>
<td>21.69</td>
<td>3.9767</td>
<td>23.90</td>
<td>3.9643</td>
<td>24.08</td>
</tr>
</tbody>
</table>

Mean 21.79 23.74 35.89 26.91 22.94
SD [%] 0.20 0.24 0.34 0.02 0.01
RSD [%] 0.92 0.99 0.96 0.09 0.03
Fig. 1 Comparison of the determined and the expected total fat values

9. Conclusion
Figure 1 shows that the results obtained by fat determination of bakery and dairy products according to the Weibull-Stoldt Method with the Büchi Instruments are comparable to the expected value.