Application Note
No. 773/2021

Fat determination in manually hydrolyzed samples

FatExtractor E-500
Fat determination in Food Products according to Weibull Stoldt after manual hydrolysis

1. Introduction

The determination of fat in food and feed is a routine procedure in quality control and labelling. This Application Note describes a procedure for total fat determination according to Weibull-Stoldt in certified food samples. The samples are hydrolyzed manually. The Soxhlet extraction is performed with the FatExtractor E-500. Calculation of total fat content follows gravimetrically after the extract has been dried to constant weight. The fat contents correspond well with the certified values of the reference materials.

This application follows official methods AOAC 963.15, ISO 1443:1973, AOAC 945.16, § 64 LFGB Nr. L 06.00-6: 2014-06.

2. Equipment

- Mixer B-400
- FatExtractor E-500 Soxhlet, Standard Interface, with Analyte protection
- Heating plate, Ceran GK T
- Analytical balance (accuracy ± 0.1 mg)
- Drying oven / Vacuum drying oven

3. Chemicals and Materials

Chemicals:

- Hydrochloric acid 4 mol/L: 4 L HCl 32% (Hänseler, 20-2000-4) are filled up to 10 L with deionised water
- Petroleum ether, GPR RECTAPUR, boiling range 40-60 °C, 5 L, VWR (Order No. 23826.360)

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

Materials:

- 250 mL beakers
- Glass watches
- Glass funnels
- Prepleated filter paper, 150 mm diameter, e.g. Whatman qualitative filter paper 595 ½
- Paper thimbles, 33 x 94 mm, BUCHI (order No. 11058983)
- Digestion tubes for Kjeldahl or measuring cylinders
- Thermal protection gloves
- Boiling stones
- pH strips

Reference material samples:

- Buttercookies with a certified fat content of 15.67 g/100 g (± 0.219 g/100 g), LVU No. 20-11
- Boiled sausage with a certified fat content of 13.90 g/100 g (± 0.391 g/100 g), LVU No. 20-01ab
- Fruit ice cream mixture with a certified fat content of 5.07 g/100 g (± 0.065 g/100 g), LVU No. 18-41

Commercial samples:

- Buttercookies, labelled fat content 15%
- Breakfast cereal, labelled fat content 12%
- Pet food pellets, labelled fat content 14%
- Vanilla icecream, labelled fat content 13%

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 Homogenization

1. The buttercookies samples were broken into small pieces and homogenized twice for 2 seconds with mixer B-400.
2. The boiled sausage was homogenized for 5 seconds with the mixer B-400.
3. The ice cream mixture was brought to ambient temperature and then homogenized by stirring for 2 minutes.
4. The vanilla ice cream was melted at 40 °C and then stirred for 2 minutes.
5. The cereal and the pet food were homogenized for 5 seconds in the mixer B-400.

Figure 1 shows the samples after homogenization.

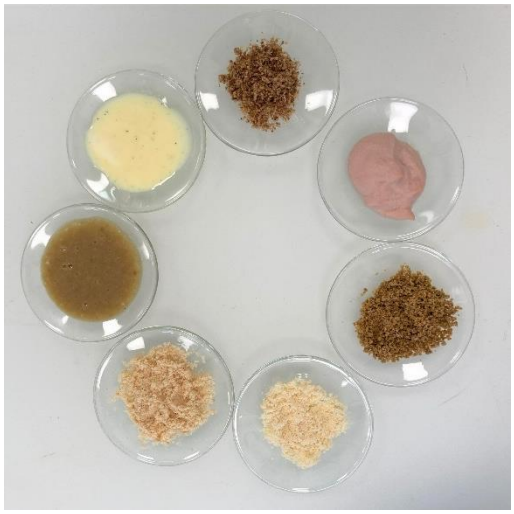

Figure 1: homogenized samples. From the top and in clockwise sense: breakfast cereal, boiled sausage, pet food, 2 samples of cookies, 2 samples of icecream.

4.2 Acid hydrolysis

6. Work in a fume hood. Preheat the heating plate with a low heating level.
7. Place 4-5 dried boiling stones in 250 mL beakers and weigh up to 6 g of homogeneous sample. For foamy samples it is recommended to use a smaller amount of sample.
8. Add 50 mL 4 M HCl and stir gently. Add another 50 ml 4 M HCl rinsing the walls of the beaker.
9. Place the beaker on the heating plate; cover it with a watch glass. Keep the low heating level, to prevent excessive splashing of the sample to the walls.
10. Watch the solution until a stable gentle boil is reached.
11. After boiling occurs, let the sample be hydrolyzed for 30 minutes.
12. Prepare a rack with digestion tubes, glass funnels and folded filters. Wet the filters with boiling water. Wear thermal protection gloves. See Figure 2.

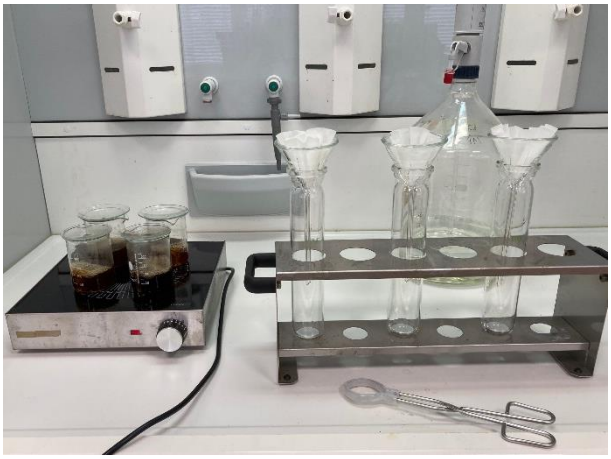

Figure 2: set up for manual hydrolysis

13. After the hydrolysis time is up, use pliers to carefully remove the beakers from the heating plate. Prepare at least 500 mL of boiling water for each sample**.**

During rinsing, keep water as close as possible to the boiling point. If the water cools down, the filtering will be affected.

14. Rinse the watch glass with boiling water into the beaker. Dilute the solution with 100 mL of boiling deionized water.
15. Filtrate the solution through the filter and wash the beaker with boiling water until the sample is transferred completely. Three rinses should be enough.
16. Rinse the filter paper. Make sure to rinse all the filter so there are no acidic zones left. See Figure 3.
17. Rinse thoroughly each filter paper with a volume of at least 300 mL of boiling water until the filtrate reaches neutral water pH. Check the pH with a paper strip. See Figure 4.

Check for any residues stuck on the walls of the beaker.

If there is a high amount of solids in the filter, it is recommended to use small portions of boiling water, so as not to fill the funnel. If the funnel is filled up to the top with water, the solids will go up to the top of the filter paper, at a risk of losing some of them.

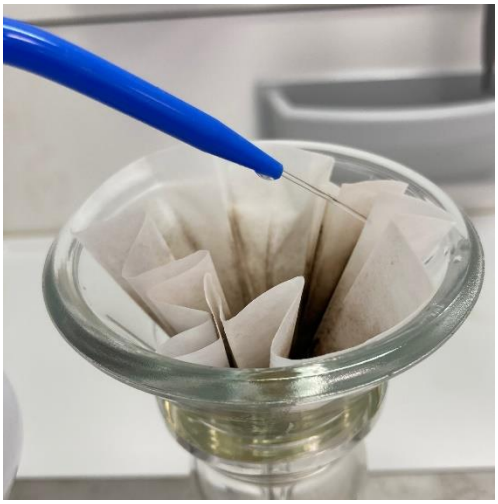

Figure 3 (left): Rinsing of the filter paper. Figure 4 (right): sample in the filter after the hydrolysis.

18. With the help of tongs, place the filter paper in the thimble by folding it in half. Place the filter in the cellulose thimble. By folding the filter paper, the level sensor can be placed at a lower position, resulting in faster Soxhlet cycles.
19. The thimble is placed in a clean extraction beaker.
20. Dry the thimble and the beaker in a drying oven for 1:30 hour at 102 ± 2 °C.
21. Allow the sample and beakers to cool down to ambient temperature in a desiccator (for at least 1 hour).

4.3 Soxhlet Extraction

22. Once the dried beakers have cooled down, record their exact weight (pulling up the thimble).
23. Add a glass wool plug over the filter.
24. Place the thimble in the thimble holder. Put the thimbles into the extraction chamber and adjust the level sensor according to the filter paper height (marked with pencil). See figure 5.

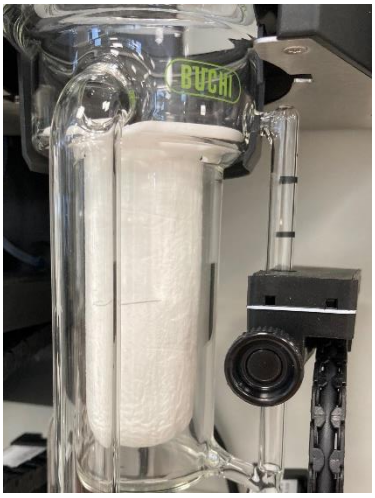

Figure 5: placing the level sensor according to the pencil mark at filter paper's height.

25. 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	
Extraction	20 cycles	6
Rinse	5 min	6
SmartDrying	on[1]	-
Soxhlet valve open time	mid	
Solvent volume	110 mL	

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 desiccator is the same before and after the extraction. Differences in beakers' temperature alter the results.

4.5 Calculation

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

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

% 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

Determined fat contents for the certified reference materials are in line with the certified and labelled values. The achieved rsd values are low or very low for most of the samples. A higher rsd was achieved for the boiled sausage sample. This is not a major concern, given that this reference material sample can be considered as challenging. The values obtained from the 42-participants of the ring test varied from 13.10 g/100 g to 15.26 g/100 g. The certificate indicates that the results were considerably scattered. Because of this, the statistical characteristic data determined in the round robin tests could not be used to evaluate the results, but only the z-scores. The complete findings are summarized in Tables 2 to 7.

[1] Instead of using SmartDrying it is possible to set a drying time of 12 minutes and heating level 5 for petroleum ether.

Table 2: Butter cookie, LVU No. 20-11 (Specification: 15.67 ± 0.219 g/100 g)

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	3.9165	111.1064	111.7202	15.67
Sample 2	3.7940	111.6894	112.2898	15.82
Sample 3	3.8938	110.5847	111.1946	15.66
Average [%]				**15.72**
rsd [%]				0.58

Table 3: Molten icecream, LVU No. 18-41 (Specification: 5.07 ± 0.065 g/100 g)

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	6.0242	111.0890	111.3919	5.03
Sample 2	5.0175	109.5584	109.8080	4.97
Sample 3	5.5548	111.0990	111.3793	5.05
Average [%]				**5.02**
rsd [%]				0.74

Table 3: Boiled sausage, LVU No. 20-01ab (Specification: 13.90 ± 0.391 g/100 g)

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	1.9412	111.0877	111.3469	13.35
Sample 2	2.4239	110.4904	110.8291	13.97
Sample 3	2.2712	108.5882	108.8905	13.31
Average [%]				**13.55**
rsd [%]				2.74

Table 4: Butter cookies, labelled fat content 15%

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	3.9637	110.9956	111.5826	14.81
Sample 2	4.0057	108.1162	108.7097	14.82
Sample 3	4.1792	107.9537	108.5717	14.79
Average [%]				**14.80**
rsd [%]				0.10

Table 5: Vanilla icecream, labelled fat content 13 %

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	4.7926	108.3418	108.9372	12.42
Sample 2	4.7953	110.8977	111.4853	12.25
Sample 3	4.8577	111.2684	111.8706	12.40
Average [%]				**12.36**
rsd [%]				0.74

Table 6: Pet food, labelled fat content 14 %

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	4.0390	110.5914	111.1873	14.75
Sample 2	4.0464	109.7933	110.3983	14.95
Sample 3	4.1111	110.8960	111.5085	14.90
Average [%]				**14.87**
rsd [%]				0.69

Table 7: Breakfast cereal, labelled fat content 12 %

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Sample 1	5.1174	108.4071	109.0081	11.74
Sample 2	5.0153	111.2364	111.8273	11.78
Sample 3	5.1584	110.8943	111.4874	11.50
Average [%]				**11.67**
rsd [%]				1.32

6. Conclusion

The determination of fat content in food products following a manual hydrolysis procedure and Soxhlet extraction with BUCHI FatExtractor E-500 provides reliable and reproducible results. The results correspond well to the labelled values, with low relative standard deviations (rsd) for most samples and an acceptable rsd for the boiled sausage.

The manual hydrolysis method is laborious and time-consuming. For 3 samples in parallel, an average time of 60 minutes of thorough rinsing is required after the hydrolysis. Special attention must be paid to avoid loss of sample by splash when transferring from beaker to filter. The HydrolEx H-506 is a good and safe alternative for manual hydrolysis process (HydrolEx H-506 | Buchi.com).

With the FatExtractor E-500 Soxhlet, the time per cycle is reduced significantly. The programmed 20 cycles are accomplished in approximately 150 min.

7. References

[1] ISO 1443:1973 Meat and meat products -- Determination of total fat content
[2] AOAC 963.15 Fat in Cacao Products
[3] AOAC 945.16 Oil in cereal adjuncts
[4] § 64 LFGB Nr. L 06.00-6: 2014-06 Bestimmung des Gesamtfettgehaltes in Fleisch und Fleischerzeugnissen

Extraction Reports App

Operation Manual of FatExtractor E-500