Application Note
No. 414/2020

Fat content in plant-based milk alternatives

HydrolEx H-506, FatExtractor E-500:
Total fat determination in plant-based milk alternative samples according to Weibull-Stoldt

1. Introduction

A simple and reliable procedure for determination of fat content in different plant-based milk alternatives according to Weibull-Stoldt is introduced. Vegan nutrition and sustainable plant-based protein sources are increasingly demanded by consumers. The global revenue is expected to increase by 16.7% per year, forecasting to double every 4.5 years [1]. The most popular products are soy-, almond-, and rice-milk. However, these alternatives must also fulfil the same food laws regarding safety and labelling as any other types of food. To meet this requirement, a compliant fat determination is of great interest, also for the quality control.
In the presented application, the samples are hydrolyzed using the HydrolEx H-506 and followed by an extraction using with the FatExtractor E-500 Soxhlet. Gravimetric determination of the total fat content follows the drying of the extract to a constant weight, resulting in highly repeatable fat contents. This application note complies with official methods- (eg.) EN 98/64/EG [2], AOAC 963.15 [3].

2. Equipment

- HydrolEx H-506
- Suction set with vacuum pump (BUCHI, Order No. 11068473)
- FatExtractor E-500 Soxhlet, with Standard interface and Analyte protection sensor
- 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, 4.44 mol/L (273.49 g HCl 32% (Hänseler, 20-200-5) filled up to 540 mL with distilled water, corresponds to 4.44 M HCl[1]
Petroleum ether, boiling range 40-60 °C (Sigma Aldrich, Order No. 32299-2.5L)

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

Samples:

- Oat drink, labelled fat content: 1.4%
- Almond drink, labelled fat content: 3.3%
- Coconut drink, labelled fat content: <0.5%
- Rice drink, labelled fat content: 1.0%
- Soy drink, labelled fat content: 1.8%

The samples were purchased in a local supermarket. These samples cover a wide variety and the best-selling plant-based milks.

[1] Higher concentrated hydrochlorid acid to compensate for the diluting effect of the samples

4. Procedure

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

- Sample homogenization
- Hydrolysis of the sample with 4.44 M hydrochloric acid to break up the matrix
- Soxhlet extraction of the fat
- Calculation of fat content

Homogenization of the sample

1. Homogenize the samples by shaking it well.

Acid hydrolysis

4.1.1.Preparation of the glass sample tubes

2. Fill approx. 20 g of quartz sand into the glass sample tube and compact the sand by gently tapping the glass sample tube onto the table.
3. Add approx. 2 g of Celite® 545 and spread it evenly.

The sand and the Celite® layer should not be mixed together. Otherwise the Celite® phase may break through the frit and affect the results either by increasing the recovery or by blocking the frit.

4.1.2.Hydrolyzing the sample matrix

4. Place 2 g of Celite® 545 in the hydrolysis vessel.
5. Add approximately 10 g homogeneous sample[2] to the hydrolysis vessel and note the accurate weight of the sample.
6. Add 50 mL of hydrochloric acid (4.44 M) and form a suspension by gently swirly the vessel.
7. Add another 50 mL of hydrochloric acid (4.44 M) making sure to rinse any remaining sample off the glass wall.
8. Preheat the HydrolEx H-506 for 10 min.
9. Insert the samples into the unit and lower the vessels. Reduce the heating power to the level 2.5.
10. Connect the corresponding aspiration tubes and start the vacuum pump.

Violent foaming can be prevented by adding 4.44 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. Remove the glass sample tubes from the hydrolysis unit.
16. Check the pH with a pH paper on the bottom of the frit. The pH should be neutral.
17. Stir the Celite® layers (without touching the sand layer) with a spatula to loosen the pulp.
18. Carefully wipe off the spatula with a piece of tissue and add it on the top of the sample.

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[2] 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
Note: A higher sample weight must be compensated by a higher concentrated HCl (diluting effect of the sample). A final concentration (including the sample) of 4 M HCl is recommended. Total volume of sample + HCl must not exceed 110 mL.

19. 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. 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.

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

Extraction of the fat with the FatExtractor E-500 Soxhlet

4.1.3. 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.

4.1.4. Soxhlet Extraction

22. Place the glass sample tubes containing the sample into the extraction chamber and adjust the level sensor to the samples height (see Figure 2).
23. Fill the solvent into the beakers and place them on their corresponding heating plate.
24. Close the protection shield and lower the rack. Alternatively, fill the solvent through the condensers after lowering the rack. Activate the occupied positions and open the cooling water tap or switch on the connected chiller.
25. Start the Soxhlet extraction according to the parameters listed in Table 1.

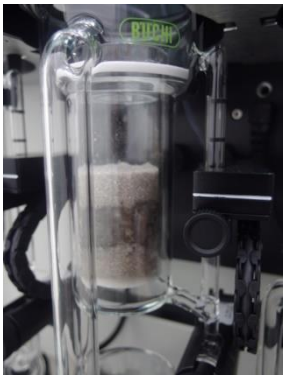

Figure 1: Adjusting the level sensor for the Soxhlet Extraction

Table 1: Parameters for the Soxhlet Extraction with the FatExtractor E-500 SOX.

Step	Value	Heating level
Solvent	Petroleum ether	
Extraction	20 cycles	6[3]
Rinse	5 min	6[3]
Drying	SmartDrying[4]	
Solvent volume [mL]	100	

[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. Time 12 min, level 6. (for petroleum ether)

4.1.5. Drying of the extract

Dry the beakers containing the extract in a drying oven at 102 °C until constant weight. 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 extraction. Differences in beakers temperature falsify the results.

Calculation

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

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

% Fat:	Percentage of fat in the sample
m_{Total}:	Beaker + extract [g]
m_{Beaker}:	Empty beaker weight [g]
m_{Sample}:	Sample weight [g]

Results

All the plant-based milk alternatives are analysed either in dublicates or in triplicates. The determined fat content corresponds well with the labelled values with low relative standard deviations (rsd). The results are shown in Tables 2.

Table 2: Results for the total fat determination of all samples, determined with FatExtractor E-500 Soxhlet.

Sample	m_{sample} [g]	m_{beaker} [g]	m_{total} [g]	Fat [%]	Mean value	Labelled
Oat 1	10.0921	110.9547	111.1109	1.55	1.56% rsd : 1.33%	1.4%
Oat 2	10.1262	116.7055	116.8652	1.58		
Almond 1	10.0518	110.9485	111.2588	3.09	3.08% rsd : 0.34%	3.3%
Almond 2	10.0487	111.4228	111.7315	3.07		
Coconut 1	10.3879	111.2967	111.3220	0.24	0.24% rsd : 0.58%	<0.5%
Coconut 2	10.3839	111.1310	111.1565	0.25		
Rice 1	10.0428	110.9474	111.0535	1.06	1.07% rsd : 1.08%	1.0%
Rice 2	10.0296	111.4218	111.5300	1.08		
Rice 3	10.5861	110.9551	111.0687	1.07		
Soy 1	10.1690	116.7068	116.8813	1.72	1.70% rsd : 1.18%	1.8%
Soy 2	10.2746	111.2962	111.4686	1.68		
Soy 3	10.1626	109.7672	109.9408	1.71		

5. Conclusion

The determination of total fat content in different plant-based milk alternatives by use of the HydrolEx H-506 and the FatExtractor E-500 provides reliable and repeatable results. These results correspond well to the labelled values with low relative standard deviations (rsd).

6. References

[1] https://www.vegansociety.com/news/market-insights/plant-milk-market, 11.11.2020
[2] EN 98/64/EG Commission Directive 98/64/EC Fat in feeding stuffs
[3] AOAC 963.15 Fat in Cacao Products
Application note 348/2019 - Fat determination in dairy products.
See www.buchi.com/application

Extraction Reports App
Operation Manual of the HydrolEx H-506
Operation Manual of the FatExtractor E-500