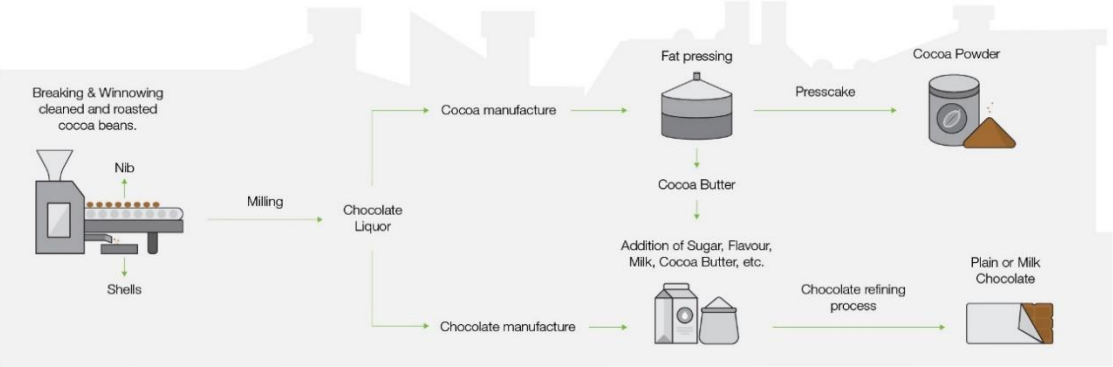
Application Note
No. 769/2021

Fat determination in cacao products: from the bean to the chocolate bar

HydrolEx H-506, FatExtractor E-500:
Fat determination in cacao products according to Weibull-Stoldt: from the bean to the chocolate bar

1. Introduction

A simple and reliable procedure for fat determination of cacao products according to Weibull-Stoldt is introduced. Many steps are involved in the production of chocolate. Therefore, a quality control of the intermediate products is necessary to ensure a high quality of the end product. In this application note, the samples are hydrolyzed with the HydrolEx H-506. The Soxhlet extraction is performed with the FatExtractor E-500. Calculation of total fat content follows gravimetrically after the extract has been dried to a constant weight. This application follows the AOAC 963.15 official method with slight adaptations.



2. Equipment

- HydrolEx H-506
- Suction set with vacuum pump, BUCHI (Order No. 11068473)
- FatExtractor E-500 Soxhlet, Standard Interface, with Analyte protection
- Analytical balance (accuracy ± 0.1 mg)
- Microwave oven
- Drying oven / Vacuum drying oven
- Weighing support for hydrolysis vessels, BUCHI (Order No. 11067040)
- Mixer B-400

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 mol/L, 4 L HCl 32% (Hänseler, 20-2000-5) are filled up to 10 L with deionised water
- Petroleum ether, Emsure® ACS, ISO, for analytical, boiling range 40-60 °C, 2.5 L, Merck Millipore (Order No. 1.01775.2500)

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

Samples:

- Cacao nibs 1 & 2, expected fat content: 50%
- Cacao extract powder, low fat, expected fat content: 6%
- Cacao extract powder, high fat, expected fat content: 16%
- Cacao powder, expected fat content: 25%
- Chocolate dark with a certified fat content of 39.93 g/100 g (+/- 0.15 g/100 g), DRRR, RM CP L SL S 4.

The cacao nibs were mixed using the Mixer B-400. The certified chocolate sample was melted in a drying oven at 50 °C.

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 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 increasing the recovery or by blocking the frit.

4.1.2 Hydrolyzing the sample matrix

3. Place 2 g Celite® 545 in the hydrolysis vessel
4. Add up to 10 g homogeneous sample[1] to the hydrolysis 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 vessel
6. Add another 50 mL hydrochloric acid (4 M) making sure to rinse any remaining sample off the glass wall
7. Preheat the HydrolEx H-506 for 10 min
8. Insert the samples into the unit and lower the vessels
9. Connect the aspiration tubes and start the vacuum pump
10. Reduce the heat to level 2.5 when one position is boiling

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.

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. 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.

16. Stir the Celite® layers (without touching the sand layer) with a spatula to loosen the pulp
17. Carefully wipe off the spatula with a piece of tissue and add it on the top of the sample
18. 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

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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

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.

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

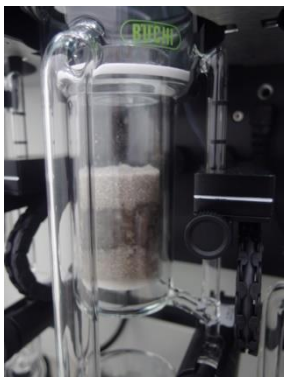
4.2 Fat extraction

4.2.1 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.

4.2.2 Soxhlet Extraction

Put the glass sample tubes into the extraction chamber and adjust the level sensor with the white line to the center of the upper sand layer. See Picture 1.



Picture 1: Adjusting the level sensor for the Soxhlet Extraction

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[2]
Rinse	5 min	6[2]
SmartDrying	on[3]	-
Solvent volume [mL]	100	

[2] Heating level proposed by the system depending on the selected solvent.
[3] Instead of using SmartDrying it is possible to use the following drying parameters. Then, SmartDrying is switched off:
Petroleum ether: 12 min Hexane: 12 min
Diethyl ether: 10 min Chloroform: 13 min

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.

4.3 Calculation

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

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

% 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

All the cacao products are analysed in duplicates and triplicates. The determined fat content corresponds well with the expected and certified values with low relative standard deviations (rsd). The results are shown in Table 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	Expected
Cacao nibs	1.0428	110.2719	110.8208	52.64	52.91% rsd : 0.61%	50%
	1.0809	111.0426	111.6136	52.83		
	1.0074	111.6964	112.233	53.27		
Cacao extract powder low fat	10.0418	109.7679	110.4848	7.14	7.21% rsd: 1.38%	6%
	10.0361	109.6236	110.3542	7.28		
Cacao extract powder high fat	5.0084	108.3738	109.2052	16.60	16.50% rsd: 0.51%	16%
	5.0662	108.6441	109.4776	16.45		
	5.1022	109.5814	110.4211	16.46		
Cacao powder	1.9951	111.0398	111.5367	24.91	25.16% rsd : 0.88%	25%
	2.0877	110.7323	111.2603	25.29		
	2.0808	108.0054	108.5316	25.29		
Chocolate DRRR	1.4932	110.6651	111.2622	39.99	40.00% rsd : 0.50%	39.93% +/- 0.15%
	1.5263	111.6593	112.267	39.82		
	1.5364	110.6334	111.2512	40.21		

6. Conclusion

The determination of fat in different cacao products using the HydrolEx H-506 and the FatExtractor E-500 provides reliable and reproducible results. These results correspond well to the expected and certified values, with low relative standard deviations (rsd).

7. Acknowledgements

We gratefully thank Mr. Claudio Keel and Mr. Joel Pfadenhauer from Oro de Cacao AG for the fruitful cooperation, for providing the samples and for sharing their expertise for this application note. Oro de Cacao AG is a swiss chocolate brand, utilizing an innovative manufacturing technique to (r)evolutionize the making of chocolate.

8. References

[1] AOAC 963.15 Fat in Cacao Products

Extraction Reports App

Operation Manual of HydrolEx H-506
Operation Manual of FatExtractor E-500