Application Note
No. 402/2020

Determination of fat content in avocado

FatExtractor E-500: Fat determination in avocado by Soxhlet extraction

1. Introduction

A simple and reliable procedure for determination of fat content in avocado is introduced. Traditionally, avocados (Persea americana) are mainly used in the latin american diet, where it is an important part of any dish or used for the famous Guacamole sauce. Nowadays, avocados are increasingly popular all around the world. One reason behind the increasing demand is its unique composition. Hardly any other fruit provides as many important nutrients as the avocado. It is a particularly good source of healthy unsaturated fatty acids as well as for many vitamins and minerals [1].
After homogenization, the avocado was mixed with sodium sulfate, dried and extracted using the Soxhlet method. The presented application gives reliable and highly repeatable results.

2. Equipment

- Mixer B-400
- FatExtractor E-500 Soxhlet, with Standard interface and Analyte protection sensor
- Recirculating chiller F-308, Temperature = 10 °C
- Analytical balance (accuracy ± 0.1 mg)
- Drying oven / Vacuum drying oven
- Weighing support for hydrolysis vessels (BUCHI, Order No. 11067040)

3. Chemicals and Materials

Chemicals and materials:

- Sodium sulfate, anhydrous, granular, free-flowing, Redi-DriTM, ACS reagent, ≥99 %, Sigma Aldrich (Order No. 798592)
- Petroleum ether, boiling range 40-60 °C (Sigma Aldrich, Order No. 32299-2.5L)
- Cellulose thimbles, 25x100, BUCHI (Order No. 018105)
- Glass wool, BUCHI (Order No. 033701)

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

Samples:

Avocado, ready-to-eat ripeness. Purchased in a local supermarket.
Expected fat content approx. 15 % [1,2]

4. Procedure

The determination of the fat content includes the following steps:

- Sample homogenization
- Mixing of samples with Na_2SO_4
- Drying of the sample
- Soxhlet extraction of the fat
- Calculation of fat content

4.1 Homogenization of the sample

1. Peel the fresh avocado, remove the seed and cut the flesh into pieces.
2. Homogenize the samples with the Mixer B-400 once for 2 s.

Figure 1: Fresh, cutted and homogenized avocado

4.2 Drying of the samples

3. Add 15 g[1] of Na_2SO_4 to the cellulose thimble.
4. Weigh in approx. 2.5 g of homogenized avocado into the thimble, note the exact weight.
5. Mix the Avocado well with the Na_2SO_4 using a spatula.
6. Clean the spatula with a small piece of tissue and place it into the paper thimble, too.
7. Dry the thimble in a drying oven at 103 °C for 1 h.
8. Cool down the samples in a dessicator
9. Mix up the sample- Na_2SO_4 -mixture until it is a fine powder. Clean the spatula with a small piece of tissue and place it into the paper thimble, too.
10. Close the thimble using glass wool.

4.3 Extraction of the fat with the FatExtractor E-500 Soxhlet

Preparation of the beakers

Always use dry and clean beakers for the Soxhlet extraction.

11. Dry the extraction beakers for at least 30 min at 102 °C. Let them cool down to ambient temperature in a desiccator for at least 1 h.
12. Record the exact weight prior to extraction.

4.1.1. Soxhlet Extraction

13. Place the cellulose thimble containing the sample into the extraction chamber and adjust the level sensor to the samples height.
14. Fill the solvent into the beakers and place them on their corresponding heating plate.
15. 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.
16. Start the Soxhlet extraction according to the parameters listed in Table 1.

[1] The amount of Na_2SO_4 needed to absorb the moisture of the sample can be calculated as follows: 1 g of Na_2SO_4 can absorb 0.127 g of water (1 mol Na_2SO_4 absorbs 1 mol H_20). The water content of avocado is approx. 75 %.

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

Step	Value	Heating level
Solvent	Petroleum ether	
Extraction	20 cycles	6[2]
Rinse	5 min	6[2]
Drying	SmartDrying[3]	
Soxhlet valve opening time	medium	
Solvent volume [mL]	120	

4.1.2. 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).

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

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

5. Results

The avocado was extracted in triplicate. The results are presented in Table 2.

Table 2: Determined fat content in avocado.

	m_{Sample} [g]	m_{beaker} [g]	m_{total}[g]	% Fat
Replicate 1	2.1667	110.5499	110.8991	16.12
Replicate 2	2.188	111.1368	111.4881	16.06
Replicate 3	2.2718	110.9095	111.2777	16.21
Mean value				**16.13**
sd				0.08
rsd [%]				**0.47**

6. Comparison to free fat determination (without acid hydrolysis)

There are two main types of fat extraction, total and crude fat extraction. Crude fat extraction is a direct extraction that is limited to determine only free fat. Total fat extraction is used to detect free fat and fat enclosed by other components of the sample matrix.

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

In the current application note, the avocado was extracted with and without the prior hydrolysis step. For the fat determination without hydrolysis the samples were mixed with sodium sulfate and dried (see Chapter 4.1). For the total fat determination, the avocado was hydrolysed with 4 M HCl prior to extraction. For the detailed procedure, please refer to BUCHI application notes, e.g. 354/2019 Fat determination in bakery products and chocolate [3])
The results of the determination of the free and total fat contents were compared (see Table 3). The determined contents of the total fat were only a little higher than the values determined for free fat. Therefore, it can be concluded that a prior acid hydrolysis is not mandatory for the determination of fat in avocado. Lastly, it is noted that the fatty acid composition of avocado, with its high content of unsaturated fatty acid [2], can change during the hydrolysis step.

Table 3: Comparison of free and total fat (with and without acid hydrolysis, respectively) determined in avocado. Fat content in g/100g.

	Free fat, without hydrolysis	Total fat, with acid hydrolysis
Replicate 1	16.12	16.58
Replicate 2	16.06	16.57
Replicate 3	16.21	16.39
Mean value	**16.13**	**16.51**
sd	0.08	0.10
rsd [%]	**0.47**	**0.64**

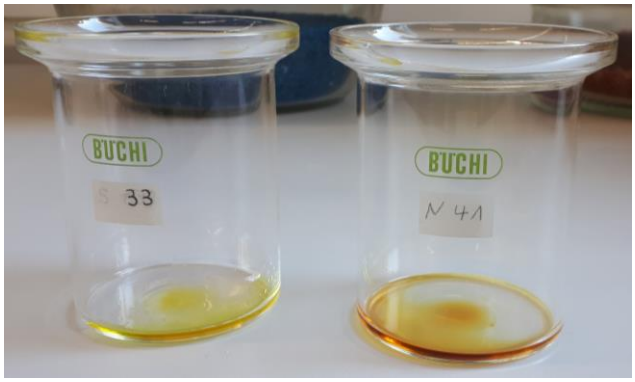

Figure 2. Extracted fat without hydrolysis (left) and with hydrolysis (right). The color of the hydrolyzed sample has changed to brownish, which can indicate a change in the fat quality.

7. Conclusion

The determination of fat content in avocado using the FatExtractor E-500 Soxhlet provides reliable and repeatable results. The determined fat content corresponds well to the expected value with a low relative standard deviation (rsd).

8. References

[1] Dreher, M.L. and Davenpoort, A. J. 2013. Hass Avocado Composition and Potential Health Effects. Critical review in Food Science and Nutrition, 53:738–750.
[2] Food data central, data base for nutrient and food composition, U.S. Department of Agriculture https://fdc.nal.usda.gov/
[3] Application note 354/2019 -Fat determination in bakery product and chocolate- See https://www.buchi.com/en/content/fat-determination-bakery-product-and-chocolate-soxhlet

Extraction Reports App
Operation Manual of the Mixer B-400
Operation Manual of the FatExtractor E-500