Application Note

088/2012

SpeedExtractor E-916

Fat determination in Mayonnaise and Chocolate using the SpeedExtractor E-916

088/2012

SpeedExtractor E-916

SHORT NOTE

Fat determination in Mayonnaise and Chocolate using the SpeedExtractor E-916

The work presented is the result of collaboration between BUCHI and Nestlé. The purpose of this study was to compare Pressurized Liquid Extraction (PLE) results with those gathered by the application of the classical Mojonnier and Weibull-Stoldt (Soxhlet) methods. The described method has been validated in-house amongst other samples for mayonnaise and chocolate and can be extended to other type of foods as well. The results of the extraction with the SpeedExtractor E-916 are equivalent to the results achieved by the classical extraction methods (i.e. Mojonnier and Weibull-Stoldt).

Introduction

Pressurized Liquid Extraction (PLE) is a well-established alternative for extracting lipids and/or various components faster and cheaper, but less frequently used for the verification of fat labels in food products. A reliable and fast procedure using PLE to determine the fat content in samples with easily accessible fat is presented below. The extraction was carried out using the SpeedExtractor E-916 in combination with Multivapor™ P-12 for solvent evaporation in parallel. The fat content was determined gravimetrically after the extract has been dried to a constant weight.

Figure 1: Mayonnaise

Experimental

Instrumentation: SpeedExtractor E-916, Multivapor™ P-12 with Vacuum pump V-700 and Controller V-855, drying oven

Samples: Mayonnaise and chocolate (Round robin test MCL-3/2011)

The samples were ground with diatomaceous earth in a mortar to a homogeneous powder and quantitatively transferred into a 20 ml extraction cell. The extraction was carried out with the SpeedExtractor E-916 using the parameters shown in Table 1. The samples were extracted in triplicate. Per Position (sample) about 30 ml of solvent was used.

Table 1: Extraction method for fat determination with SpeedExtractor E-916

Temperature	100 °C
Pressure	100 bar
Solvent	n-Hexane 90 %; Ethanol 10 %
Cells	20 ml
Vials	60 ml
Cycles	2
Heat-up	1/1 min
Hold	10/10 min
Discharge	2/2 min
Flush with solvent	1 min
Flush with gas	3 min
Total time	42 min

During the extraction the extracts of each sample were collected simultaneously in 60 ml vials. The solvent was evaporated to dryness using the Multivapor™ P-12 with the pressure gradient shown in Fig 2.

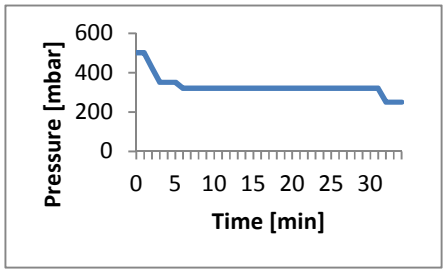

Figure 2: Pressure gradient for the evaporation of the solvent using Multivapor™ P-12

The extracts were dried to a constant weight in a drying oven (102 °C) and the fat contents were calculated.

Results

The fat content of mayonnaise and chocolate are shown in Table 2. The results correspond to the reference values.

Table 2: Determined fat contents in food samples, fat in g/100 g (relative standard deviation for the experimental results in brackets), n = 3

Sample	Expected fat contents	Experimental results
Chocolate	40.62	40.84 (0.26 %)
Mayonnaise	79.03	79.29 (0.30 %)

Conclusion

The determination of the fat content in food samples by PLE using SpeedExtractor E-916 provides reliable and reproducible results that correspond to the expected values. This method increases the sample throughput and is more environmental friendly due to less solvent consumption compared to Soxhlet extraction.

Acknowledgement

We sincerely thank Nestlé Research Center, Switzerland for their support in developing this application note.

References

SpeedExtractor E-916 operation manual

For more detailed information refer to Application note 088/2012

1 Introduction

The classical extraction procedures for the determination of the total fat amount in food products are time and solvent consuming. Pressurized Liquid Extraction (PLE) is a well-established alternative for extracting lipids and/or various components faster and cheaper, but less frequently used for the verification of fat labels on food products. At elevated temperatures, penetration of the matrix, diffusion rates as well as the ability to disrupt matrix-analyte interactions are increased, thus enhancing the extraction efficiency. The elevated pressure is mainly used to keep the solvent in a liquid state and to make sure the extraction cell is quickly filled with fresh solvent. In this study PLE was performed with BUCHI's SpeedExtractor E-916 in combination with Multivapor™ for solvent evaporation in parallel.

The work presented is the result of a collaboration between BUCHI and Nestlé. The purpose of this study was to compare PLE results with those gathered by the application of the classical Mojonnier and Weibull-Stoldt (Soxhlet) methods and to skip acid hydrolysis prior to extraction for selected products. The method was tested as an alternative procedure for total fat content determination (aiming to verify of food label) in order to speed-up the process, reduce solvent consumption and limit operator exposure to solvents.

The described method has been validated in-house amongst other samples for mayonnaise and chocolate and can be extended to other type of foods as well. Additionally the described extraction method is also suitable for the determination of the fat composition (e.g. fatty acids).

Two different sample preparation procedures are employed. One procedure is applied to dry products containing easily accessible fat (also called free fat), the second one is applied to wet products (moisture above 50%) containing easily accessible fat.

The results of the extraction with the SpeedExtractor E-916 are equivalent to the results achieved by the classical extraction methods (*i.e.* Mojonnier and Weibull-Stoldt).

This study indicates that time and solvent consumption using Soxhlet extraction (or other techniques) can be significantly reduced using PLE technology (e.g. SpeedExtractor) for the determination of fat content (e.g. fat labelled) in different type of foods. We concluded that SpeedExtractor E-916 is a designed solution for laboratories aiming to increase their analysis throughput and also protect their staff against solvents exposure.

2 Equipment

- SpeedExtractor E-916 with 20 ml cells
- Multivapor™ P-12
- Vacuum Pump V-700 with Vacuum Controller V-855
- Recirculating Chiller F-108
- Analytical balance (accuracy +/- 0.1 mg)
- Drying oven / vacuum drying oven
- Desiccator

3 Chemicals and Materials

- n-Hexane, for analysis, Merck (104391)
- Ethanol, for analysis, Merck (100983)
- Diatomaceous earth, BUCHI (053201)
- Mortar and pistil
- Weighing boat, BUCHI (053202)

4 Samples

- **Mayonnaise à la française**, Thomy, fat declaration: 79.03 g/100 g
- **Chocolate MCL-3/2011**, round robin test 2011, fat declaration: 40.62 g/100 g (drs: 0.603 %)

5 Procedure

The determination of fat in food samples includes the following steps:

- Homogenization of the sample
- Sample preparation
- Preparation of the cells
- Preparation of the vials
- Extraction of the fat, using SpeedExtractor E-916
- Evaporation of the solvent, using Multivapor™ P-12
- Drying the extracts to a constant weight
- Gravimetric determination of the extract and calculating the fat content

5.1 Homogenization of the sample

- Mayonnaise: direct use from the tube
- Chocolate: Break in smaller pieces and cut with a spatula

5.2 Sample preparation

- Place a weighing boat onto the balance
- Weigh approx. 3 g of diatomaceous earth onto the weighing boat and make a small dell into it
- Tare the balance
- Weigh 1.0 +/- 0.1 g mayonnaise or 2.0 +/- 0.1 g chocolate on top of the diatomaceous earth
- Record the sample amount
- Add approx. 0.5 g diatomaceous earth on top of the sample and transfer the content of the weighing boat into a mortar
- Grind the sample and the diatomaceous earth to a homogeneous powder. If necessary add a small amount of diatomaceous earth

5.3 Preparation of the cells

- Place a glass fibre filter (11055932), a metal frit (049568) and close with a plug (053209) at the bottom of the extraction cell
- Turn the extraction cell around and transfer the ground sample quantitatively into the 20 ml extraction cell using a funnel (053396)
- Use a brush to wipe the residue sample from the mortal into the extraction cell
- Fill any void volume of the cell with diatomaceous earth until about 1 cm below the cell's rim
- Place a cellulose filter (049572) on top using the plunger

5.4 Preparation of the vials

- Prior to extraction, dry the vials in a drying oven to constant weight at 102°C for 30 +/- 5 min
- Allow the vials to cool down to ambient temperature for at least 45 min a desiccator and record the weight.

5.5 Extraction of the fat using SpeedExtractor E-916

Carry out the extraction using the parameters of Table 1. Each sample was extracted in triplicate.

Table 1: Extraction method of Speed Extractor E-916

Parameter	Value
Temperature	100 °C
Pressure	100 bar
Solvent	Hexane 90 % Ethanol 10 %
Cells	20 ml
Vials	60 ml
Cycles	2
Heat-up	1/1 min
Hold	10/10 min
Discharge	2/2 min
Flush with solvent	1 min
Flush with gas	3 min
Total extraction time	42 min

The total time for the extraction is 42 min, and approx. 30 ml of solvents are used per position (sample).

5.6 Evaporation of the solvent, using Multivapor™ P-12

- If necessary remove the lower aqueous phase using a long glass Pasteur pipette without aspirating the top organic layer
- Evaporate the hexane-ethanol mixture in the vials using the following parameters on the Multivapor™ P-12 (Table 2).

Table 2: Parameters for Multivapor™ P-12

Temperature 45°C Rotation Level 8			
Step	Pressure start	Pressure end	Time
1	500 mbar	500 mbar	1 min
2	500 mbar	350 mbar	2 min
3	350 mbar	350 mbar	2 min
4	350 mbar	320 mbar	1 min
5	320 mbar	320 mbar	25 min
6	320 mbar	250 mbar	1 min
7	250 mbar	250 mbar	1 min
Total time			35 min

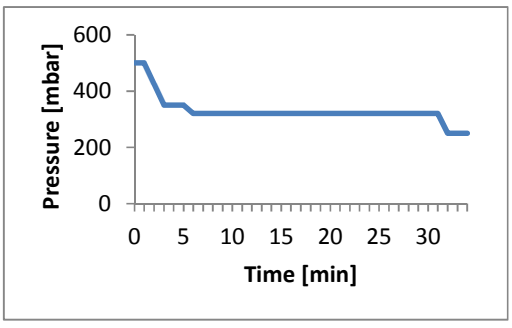

Figure 1: Pressure gradient for evaporating the solvents

5.7 Drying the extracts to a constant weight

- After the evaporation of the solvent to dryness, remove last traces of solvent by placing the vials in a drying oven to constant weight at 102°C for 30 +/- 5 min
- Allow the vials to cool down to ambient temperature for at least 45 min a desiccator and record the weight. Make sure that the cooling down time of the vials in the desiccators is the same prior and after extraction. Differences in the vial's temperature taint the result.

5.8 Gravimetric determination of the extract and calculating the fat content

- Calculate the result as percentage of fat using equation (1).

$$\%\,\mathrm{Fat} = \frac{(m_{total} - m_{vial})}{m_{sample}} \bullet 100\,\% \qquad (1)$$

%Fat : percentage of fat in the sample

m_{total} : empty vial weight + extract [g]

m_{vial} : empty vial weight [g]

m_{sample} : sample weight [g]

6 Results

The result of the fat determination in mayonnaise using the SpeedExtractor E-916 is presented in Table 3.

Table 3: Results of fat extraction of mayonnaise; expected fat content: 79.03 g/100 g (Weibull-Stoldt)

	m_{sample}	m_{vial}	m_{total}	% Fat
Position 1	1.0371	30.9875	31.8115	79.45
Position 2	0.9688	30.5780	31.3473	79.41
Position 3	1.0989	32.5874	33.4557	79.02
Mean value				**79.29**
rsd [%]				0.30

The result of the fat determination in chocolate using the SpeedExtractor E-916 is presented in Table 4.

Table 4: Results of fat extraction of chocolate; expected fat content: 40.62 g/100 g (Mojonnier)

	m_{sample}	m_{vial}	m_{total}	% Fat
Position 1	1.9711	30.5596	31.3627	40.74
Position 2	2.0610	32.8292	33.6707	40.83
Position 3	2.0611	32.7127	33.5569	40.96
Mean value				**40.84**
rsd [%]				0.26

7 Comparison of fat determination with Soxhlet extraction and Pressurized Liquid Extraction (PLE)

The fat determination in food samples like chocolate or mayonnaise can be performed with a Soxhlet extraction. In relation to time and solvent consumption Table 5 shows a comparison between Soxhlet extraction and PLE using the SpeedExtractor E-916.

Table 5: Comparison between Soxhlet and Pressurized Liquid Extraction

	Soxhlet extraction (Extraction Unit E-816 SOX)	PLE (SpeedExtractor E-916)
Extraction	120 min	42 min
Rinse	5 min	
Drying	25 min	~35 min (performed with a Multivapor™ P-12, 12 samples in parallel)
Final drying in drying oven	30 min	30 min
Cooling down to ambient temperatures in desiccator	60 min	45 min
Total time	240 min	152 min
Solvent consumption	130 ml	30 ml

8 Conclusion

The fat determination by Pressurized Liquid Extraction using the SpeedExtractor E-916 gives reliable and reproducible results which correspond to the reference values of the samples mayonnaise and to the result of the round robin test for the chocolate sample. This application note describes the procedure applied to samples with easily accessible fat and low moisture content.

Compared to Soxhlet extraction the procedure of the fat determination using Pressurized Liquid Extraction is faster (Cuts down the total analysis time by 1.5 hours) with less solvent consumption (Solvent consumption is reduced by 100 ml per sample). Using SpeedExtractor E-916 the sample throughput can be increased while causing less impact on the environment.

9 Acknowledgements

We greatly acknowledge Mrs. Elisabeth Gouézec and Dr. Petra Kopeckà from the Quality & Safety Department – Authenticity & Adulteration Group, Nestlé Research Center, Switzerland for their support in developing this application note.

10 References

Operation manual SpeedExtractor E-916

Operation manual Multivapor™ P-12

Nestlé Labour Instruction (LI-00.017)

BÜCHI Labortechnik AG
CH-9230 Flawil 1/Switzerland
T +41 71 394 63 63
F +41 71 394 65 65
www.buchi.com

Quality in your hands