

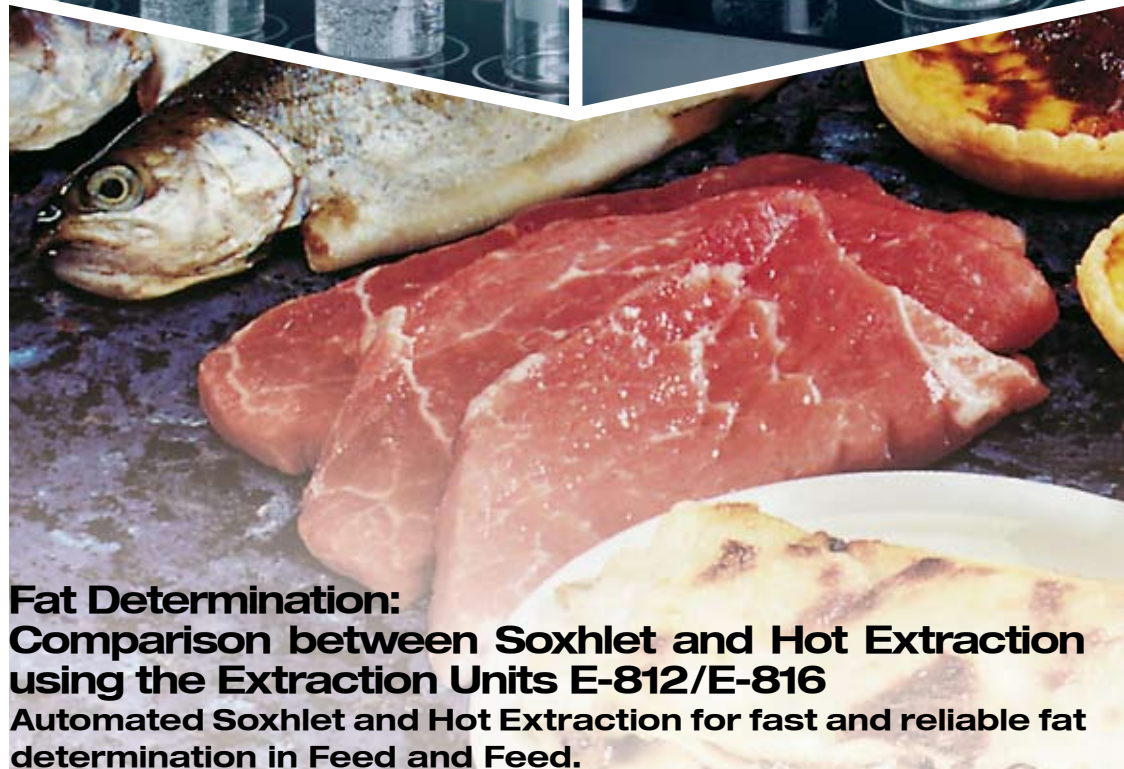
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**Fat Determination:
Comparison between Soxhlet and Hot Extraction
using the Extraction Units E-812/E-816**
Automated Soxhlet and Hot Extraction for fast and reliable fat
determination in Feed and Feed.

Fat determination, automated extraction, Soxhlet, hot extraction

Abstract

Fat determination is one of the key analyses used for food labelling and quality control. Büchi's Extraction Units E-812/E-816 perform either classic Soxhlet or hot extraction. The two principles were compared with respect to practicality and reliability of the results. The extraction units meet the requirements of daily routine analyses and are suitable for a broad range of samples. The results with both procedures are excellent: the determined fat content of certified references material corresponded to the specifications. The relative standard deviation was very low for all analyzed samples, below 1%.

Introduction

Extraction procedures for sample preparation have been established in many areas of analytical chemistry. The separation and reclamation of analytes from solid matrices represent a broad application base for solid-liquid extractions. Fat determination is one of the key analyses performed in food industry and official food monitoring for controlling the production process, labelling and quality control. The Extraction Units E-812/ E-816 from Büchi offer two different methods for fat extraction: automated Soxhlet or hot extraction. This paper will point out the benefits of the extraction units and compare the two different extraction principles.

Theoretical Basis of Extraction

The basis of all extraction procedures is described in the Nernst's distribution law [1]. A substance distributes itself between two immiscible solvents so that the ratio of this concentration in the two solvents is constant. The system's specific constant ratio is described as the Nernst distribution coefficient K_d (1). Using this equation, the more meaningful extraction efficiency we can be derived (2).

$$K_d = \frac{C_u}{C_l} \quad (1)$$

$$w = 1 - \left[\frac{1}{1 + K_d * V} \right]^n \quad (2)$$

K_d Nernst's distribution coefficient

C_u/C_l Concentration of the analyte in the upper/lower phase

w Extraction efficiency

V Ratio volume upper/lower phase

n Number of cycles

The step which determines the speed of the liquid-solid extraction is the suspension of the equilibrium state of the analyte between the solid and the liquid phase, where the following parameters are most influential:

- The amount and surface of the sample. Acid hydrolysis prior to extraction denatures starch and proteins, which makes the lipids more accessible to the solvent and thus enhances extraction efficiency.
- The amount and the polarity of the solvent have a major influence on the extraction results.
- The turn-over (number of cycles per time) of the solvent, as well as the time until the equilibrium is reached, depend directly on the temperature. As a rule of thumb, in reaction kinetics, the reaction speed doubles with an increase in reaction temperature of 10°C (equation of Arrhenius). A reduction in extraction time can therefore be achieved through the use of hot extraction, where the sample is heated up, because it sits in the boiling solvent.

Extraction Unit E-816 Soxhlet



Figure 1: E-816 Soxhlet

The Soxhlet extraction and the specific extraction chamber were invented by Franz Soxhlet in 1879 [2]. The Extraction Unit E-816 Soxhlet follows the original principle: The sample is placed in an extraction chamber and solvent is heated to reflux. The Soxhlet extraction chamber is emptied when the set level is reached, with the solvent flowing to the heated beaker. During each cycle, a portion of the non-volatile compound (fat) dissolves in the solvent. At the end of the extraction, the desired compound is concentrated in the beaker. Automated systems have an optical level sensor instead of a siphon, which allows to execute more cycles per hour and thus makes the extraction more efficient and faster. The Extraction Unit E-816 Soxhlet and the Extraction System B-811 from Büchi are the only automated Soxhlet systems on the market.

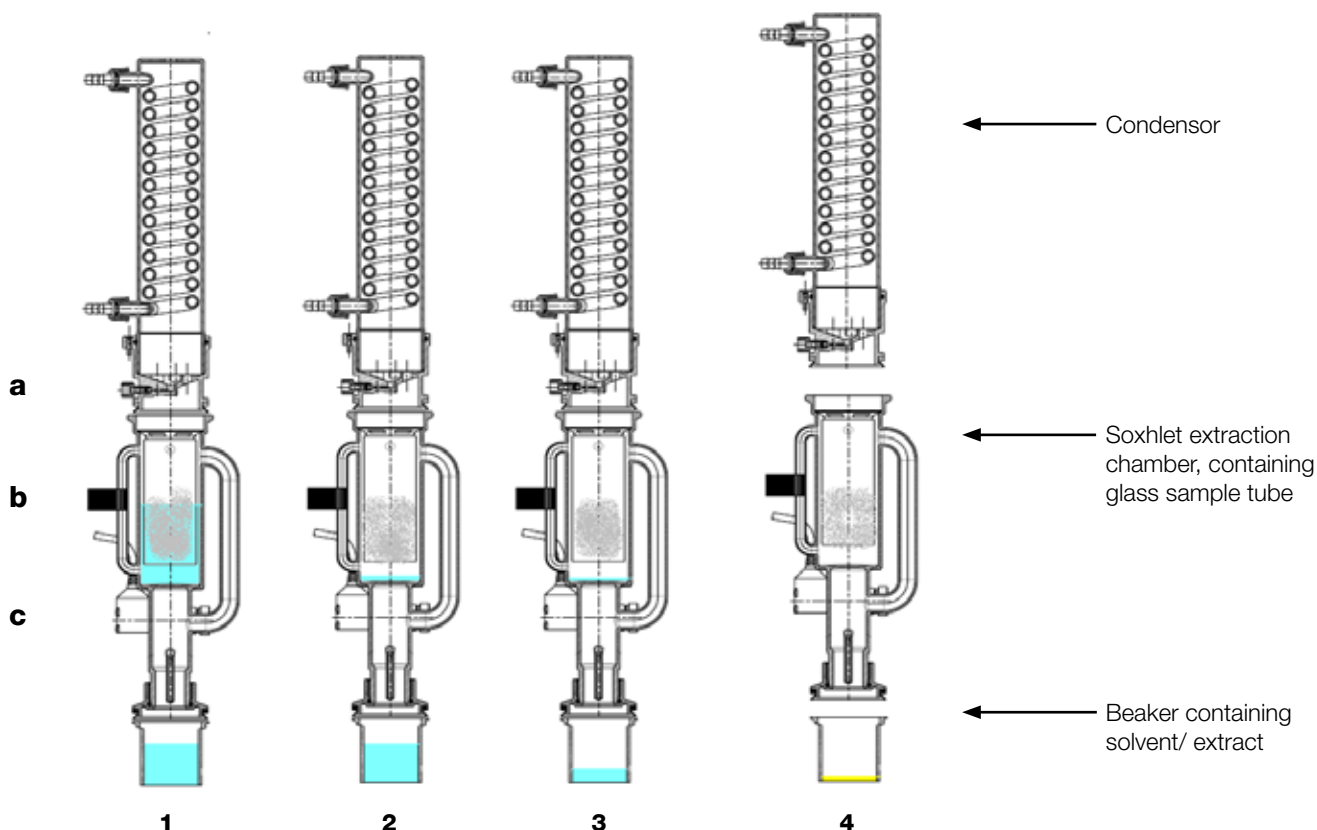


Figure 2: Schematic diagram of a Soxhlet extraction. **a:** Tank valve, **b:** Level sensor, **c:** Valve unit.

- 1) Extraction:** The extraction chamber is filled up with condensed solvent. As soon as the solvent reaches the optical sensor, the chamber is emptied by opening the valve unit.
- 2) Rinse:** The valve unit is always open, which allows the condensed solvent to rinse the sample.
- 3) Drying:** The solvent is evaporated and collected in the water-cooled tank at the back of the instrument, which allows it to be re-used for further extractions. The tank valve is open.
- 4) After completion of the extraction, the beakers containing the extract have to be removed and dried to constant weight.**

Extraction Unit E-816 Hot Extraction



Figure 3: E-816 Hot Extraction

The hot extraction procedure was developed to speed up the classic Soxhlet extraction; examples are the extraction according to Randall and to Goldfish.

In hot extraction, the sample is placed directly in the beaker containing the boiling solvent. The extraction time is reduced because of the higher temperatures. There are no distinct cycles as in Soxhlet. The sample is first heated up, the fat dissolved in the solvent, and subsequently washed out during the rinse step.

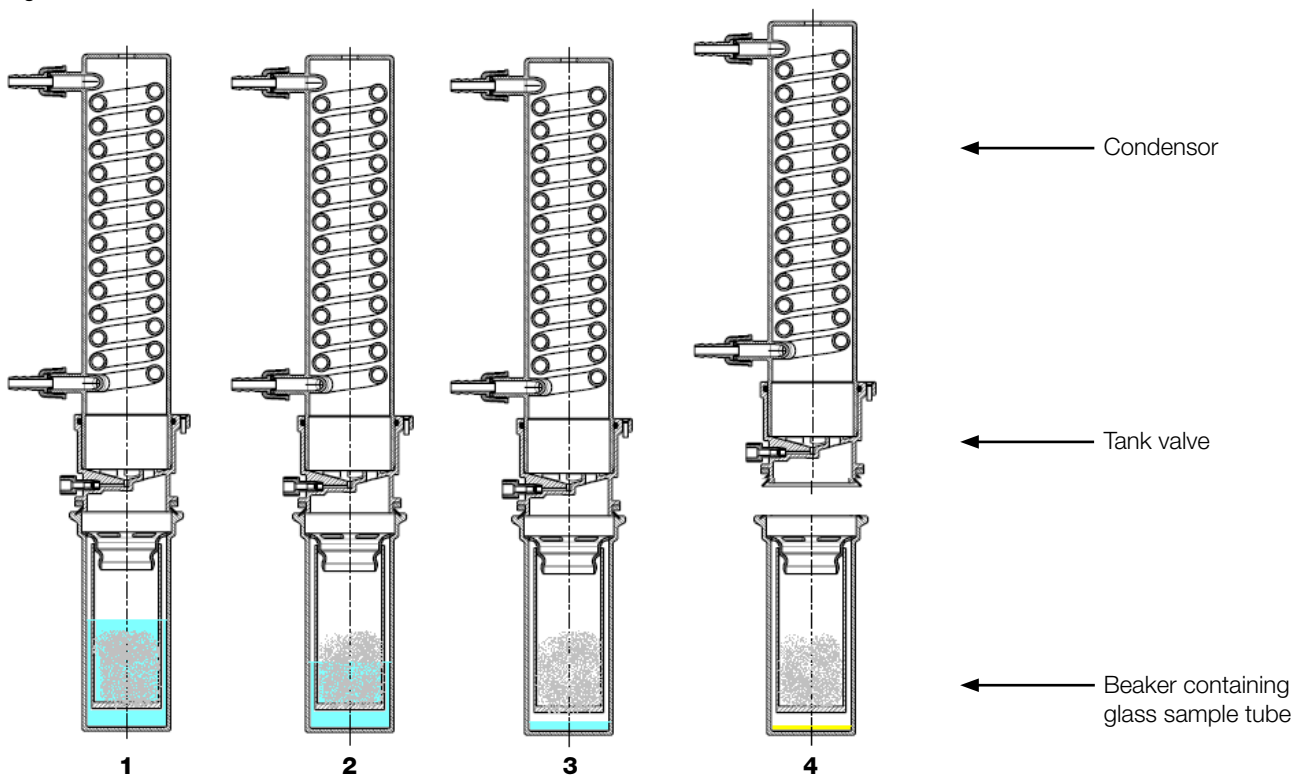


Figure 4: Schematic diagram of a hot extraction.

1) Extraction: The sample sits in the boiling solvent; the sample and the glassware are heated up.

2) Rinse: The tank valve opens and closes according to the chosen drain interval and decreases the solvent level

3) Drying: The solvent is evaporated and collected in the water cooled-tank at the back of the instrument, which allows it to be re-used for further extractions. The tank valve is open.

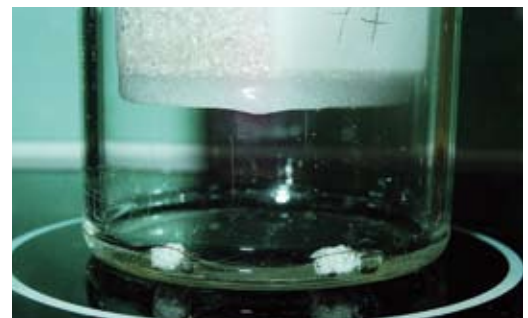
4) After completion of the extraction, the beakers containing the extract have to be removed and dried to a constant weight.



Extraction



Rinse



Dry

Benefits of Extraction Units E-812/816

The units are operated with an intuitive software. Several safety features and functions allow for a safe and environmental friendly workflow. The solvent can be filled at the top of the condensers to minimize exposure to the operator. The z-seal-system ensures an absolute tight connection, so no unpleasant solvent smell is present, even when not working in the fume hood. The collection of the solvent directly after the condensing (at the bottom of the condensers) guarantees clean and re-usable solvent for further extractions. The status of the extraction process can be constantly monitored. The units allow for a fully automated and unattended operation. The glassware is easy to assemble and disassemble. Both types of units are available as a 2 or 6 place instrument, giving the operator a maximum of flexibility due to the individual heating of each position.

Materials and Methods

The analyzed samples were all certified reference materials:

- Madeira cake LGC 7107
- Chocolate LGCQM 1003
- Milk powder (spray dried) MUVA RM-55
- Processed meat LGC 7152
- Canned pet (cat) food LGC 7176

All samples were purchased at LGC Promochem, Wesel, Germany. The AOAC developed a nine-sectored fat-protein-carbohydrate triangle, in which all food can be classified according to their major components (carbohydrates, protein, fat) [3]. The samples chosen in this study cover a broad range of food matrices, positioned in 5 different sectors, as demonstrated in Figure 5.

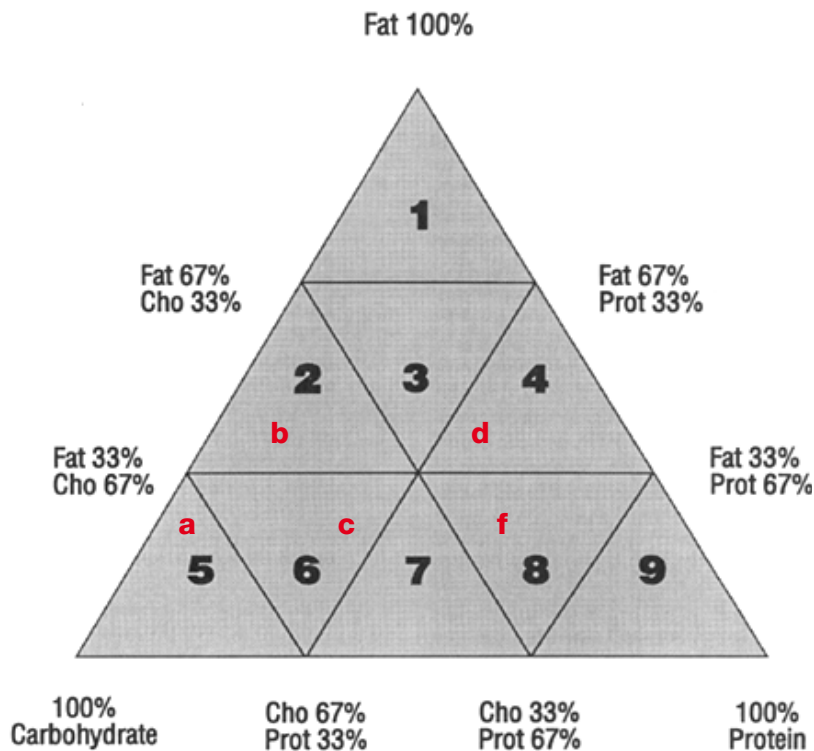


Figure 5: AOAC Triangle of Food Matrix (**a:** Madeira cake; **b:** Chocolate, **c:** Milk powder; **d:** Processed meat, **f:** Pet food)

No. 1-9: The nine different sectors. Cho: Carbohydrates, Prot: Proteins

The homogenized samples were hydrolyzed using the Hydrolysis Unit E-416 according to the standard procedure [4] and [5]. They were hydrolyzed with 4 N HCl for 30 min, filtered, washed to a neutral pH, and dried before they were extracted using the parameters in Table 1.

Table 1: Extraction parameters; for more details consider standard procedure [4, 5]

	E-816 Soxhlet	E-816 Hot Extraction
Extraction step [min]	120	5
Rinse step [min]	5	30
Drain interval [min]	-	15
Dry step [min]	20	5
Total time	145 min	40 min
Solvent volume [ml]	110	80
Heaters (all steps)	100%	100%

Petroleum ether 40-60°, diethyl ether, chloroform, and hexane were used as solvents.

Results and Discussion

Comparison between Soxhlet and hot extraction

The processes of Soxhlet extraction and hot extraction were compared in the following table:

Table 2: Comparison between Soxhlet and hot extraction

	Soxhlet	Hot Extraction
Total time	-	+
Solvent volume	(-)	(+)
Official method	+	+
Optimization	+	-
Handling	+	+

Hot extraction has two impressive advantages. It is far less time consuming (40 min vs. 145 min) and requires a smaller amount of solvent (80 ml vs. 110 ml). However, since the solvent can be re-used for further extraction, the consumption is the same for both types of extractions.

Soxhlet is a well-known and established method for more than 100 years and is required for many standard methods such as ISO, EN, AOAC, etc. Hot extraction, however, is also part of an official method (Commission Directive 96/64/EC).

For regular, hydrolyzed samples, the standard application in Table 1 can be used. For un-hydrolyzed samples, or other unusual or difficult samples, the application has to be optimized. The optimization of the Soxhlet extraction is straight forward: Only the extraction time, the number of cycles, and the turn-over (by adjusting the heating power) have to be considered. The optimization of the hot extraction is more challenging, because more parameters (turn-over, extraction and rinse time, drain interval, solvent volume) influence the extraction performance. These parameters are interdependent and have to be optimized together.

With respect to the work-flow and daily routine requirements, both principles are outstanding.

Extraction Results

The results of the extraction of the samples are shown in Table 3 and in Figures 6-9. The results demonstrate the reliability and the robustness of the fat determination using Hydrolysis Unit E-416 and the Extraction Units E-816. Although different solvents and two different extraction procedures were used, the extraction results are comparable and are within the specifications of the certified reference materials. The relative standard deviations are very low (< 1% for all tested samples).

Table 3: Extraction results, (mean values, n=6)

	Soxhlet Fat g/100g	rsd %	Hot Extraction Fat g/100g	rsd %
Pet Food 4.46 +/- 0.17 g/100g				
<i>Petroleum ether</i>	4.48	0.39	4.43	0.66
<i>Diethyl ether</i>	4.57	0.19	4.58	0.81
<i>Chloroform</i> ¹	4.64	0.64	4.65	0.58
<i>Hexane</i>	4.60	0.20	4.58	0.30
Madeira Cake 13.4 +/- 0.7 g/100g				
<i>Petroleum ether</i>	12.96	0.74	13.00	0.42
<i>Diethyl ether</i>	13.20	0.34	13.43	0.89
<i>Chloroform</i>	13.46	0.54	13.25	0.39
<i>Hexane</i>	13.26	0.59	13.14	0.65
Processed Meat 15.0 +/- 0.7 g/100g				
<i>Petroleum ether</i>	15.20	0.84	15.08	0.43
<i>Diethyl ether</i>	15.35	0.45	15.25	0.87
<i>Chloroform</i>	15.45	0.55	15.37	0.52
<i>Hexane</i>	15.27	0.41	15.25	0.69
Whole Milk powder 26.48 +/- 0.36 g/100 g				
<i>Petroleum ether</i>	26.51	0.22	26.34	0.27
<i>Diethyl ether</i>	26.47	0.53	26.52	0.27
<i>Chloroform</i>	26.71	0.21	²	-
<i>Hexane</i>	26.34	0.32	26.28	0.23
Chocolate 29.5 +/- 1 g/100g				
<i>Petroleum ether</i>	29.49	0.61	29.59	0.26
<i>Diethyl ether</i>	30.08	0.42	29.82	0.41
<i>Chloroform</i>	30.35	0.46	29.92	0.57
<i>Hexane</i>	29.81	0.21	29.43	0.14

¹ Values are not within the specifications. Using chloroform as a solvent often leads to higher amounts of extract compared to other solvents.

² Sample was no longer available. Therefore, this analysis could not be carried out

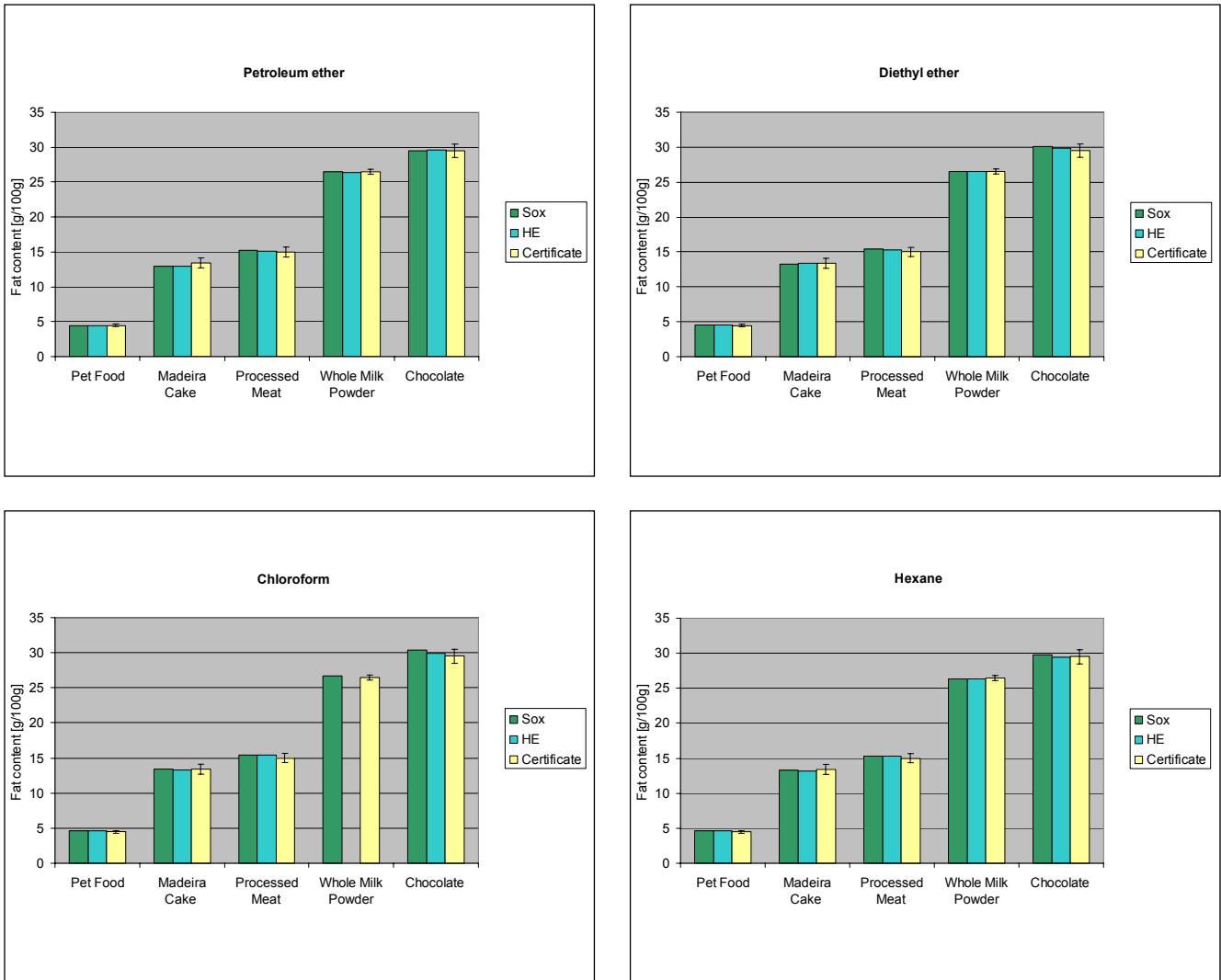


Figure 6-9: Comparison of the Results obtained by Soxhlet and by Hot Extraction

Summary and Conclusion

Five certified reference materials with different matrices, representing different product groups, have been analyzed using the Buchi's Extraction Units E-816. All samples have undergone an acid hydrolysis and were either extracted with Soxhlet or hot extraction, using the four most commonly used solvents (petroleum ether, diethyl ether, chloroform and hexane).

The Extraction Units E-812/E-816 have many features that make them well suited for daily routine analyses. The determined fat contents were all within the specifications, and the results had a low variation (rsd < 1 %).

References

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- [2] Soxhlet, F. Die gewichts-analytische Bestimmung des Milchfettes. Dinglers Polytechnisches Journal, 1879, 232: 461.
- [3] Sullivan, D.M., Carpenter, D.E. Methods of Analysis for Nutritional Labeling, AOAC International, Gaithersburg, US, 1993, 115-120.
- [4] Fat Determination by Hot Extraction-Standard Application, No. E-416-E-816-HE-001
- [5] Fat Determination according to Weibull-Stoldt-Standard Application, No. E-416-E-816-Sox-001

Examples of official methods:

AOAC 963.15 Fat in Cacao Products, Soxhlet Method
AOAC 948.22 Fat (Crude) in Nut and Nut Products
Commission directive 98/64/EC

Analysis for the determination of amino-acids, crude oils and fats, and olaquinox in feeding stuffs.

More Literature:

Soxhlet extraction of Fat from Food, Best@Büchi, 21 (2002)

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