AppNote 2/2009

Simple, Fast and Reliable Determination of Fat in Food According to the Caviezel® Method using a Turnkey Fat Determination System

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Abstract
Fat determination is one of the key analyses performed in food industry and in public food monitoring programs in order to control production processes, to ensure accurate labelling, and to monitor food safety. Therefore, a fast and reliable procedure for routine determination of fat is in great demand. Using the Cavieziel® Method, based on the fat definition given by the FDA [1], sample extraction and saponification are performed in one step, followed by gaschromatographic analysis of the resulting free fatty acids. This sample preparation method was developed to enable higher sample throughput for routine fat determination without sacrificing data quality. The total extraction time of 4 samples in parallel was reduced to 30 minutes. A predefined GC method allows the fully automated subsequent determination of total fat content and butyric acid/milk fat in one single GC run.

INTRODUCTION

Consumer protection regarding the quality of food as well as accuracy in nutritional labelling are requirements that are increasingly gaining importance. Therefore, producers, contract laboratories and public health departments need analytical solutions that enable them to accurately determine the nutritional value of foods in order to meet labelling criteria or to control that these are being met.

One important parameter to be determined in various food sample matrices is the fat content. It can be determined as total fat content, often in addition differentiating between saturated, mono-, and polyunsaturated fat content as separate sum parameters.

Since the type of fat added to or naturally present in food can be value-determining, such as milk fat, the possibility to qualify and quantify this type is also of great importance.

The 30-minute sample preparation method (Caviezel method) described in this paper is based on extraction of fat from the matrix with simultaneous saponification, subsequent conversion into free fatty acids followed by analysis with gas chromatography – flame ionization detection.

The analysis is performed in a single chromatographic run of 12 minutes and provides a free fatty acid profile including omega 3-fatty acids. Determination of the total fat content as well as the milk fat content is part of the integrated reporting tool.

EXPERIMENTAL

Instrumentation. The fat determination system consists of a sample preparation unit (B-815, Büchi, Switzerland, figure 1) with four digestion positions with integrated optimized heating and magnetic stirring program, and an analysis unit for subsequent analysis of the extract (GC 7890, Agilent, Autosampler MPS 2, GERSTEL). A GERSTEL MAESTRO software integrated reporting tool tailored for fat analysis provides detailed sample information.

Figure 1. Büchi extraction unit B-815 for simultaneous extraction and saponification of fat according to the Caviezel method.

Analysis conditions.

Injector:	MPS 2, 1 µL
S/SL inlet:	250°C, split 1:20
Column:	15 m FFAP (Phenomenex)
	d_i = 0.25 mm d_f = 0.25 µm
Pneumatics:	H_2, constant flow = 4 mL/min
Oven:	100°C; 20°C/min; 140°C; 40°C/min; 240°C (5 min)
Detector:	FID, 260°C

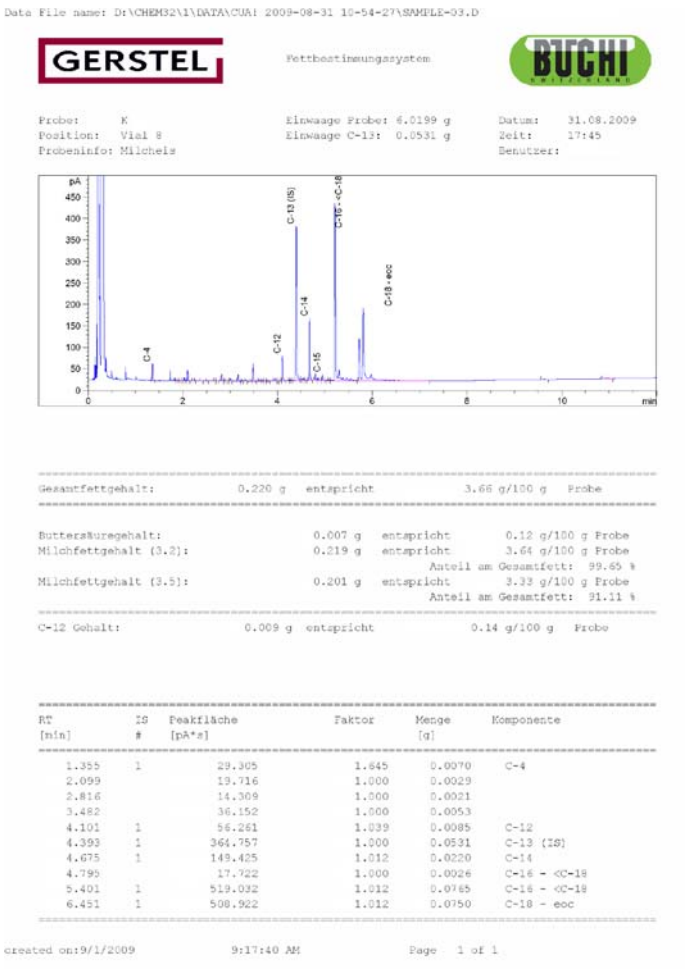
Sample preparation. The homogenized sample is weighed into a reaction vessel. Two internal standards are added: valeric acid for milk fat determination and tridecanoic acid for total fat determination. 1.5 g potassium hydroxide and 45 mL n-butanol are added and the entire mix is stirred and boiled under reflux for 30 minutes (figure 2).

Figure 2. Simultaneous extraction and saponification in the Büchi B-815 Extraction Unit.

Following extraction and saponification, the saponifiable part of the fat is present as glycerol and as potassium salts of the fatty acids. Adding a formic acid solution of dihydrogenphosphate dihydrate to the hot extract while stirring for another two minutes, releases the free fatty acids into the organic phase. After stirring has ended and phase separation has taken place, an aliquot of the organic supernatant is taken (figure 3) and transferred into an empty vial for further GC-analysis.

Figure 3. Taking a sample aliquot for GC analysis.

Reporting. The analysis report created contains the following data and results:



Data File name: D:\CHEM32\1\DATA\CUA1 2009-08-31 18-54-27\SAMPLE-03.D

GERSTEL — Fettbestimmungssystem — BÜCHI

Probe:	K	Einwaage Probe: 6.0199 g	Datum: 31.08.2009
Position:	Vial 8	Einwaage C-13: 0.0531 g	Zeit: 17:45
ProbenInfo:	Milchels		Benutzer:

Gesamtfettgehalt:	0.220 g	entspricht	3.66 g/100 g	Probe
Butters%uregehalt:	0.007 g	entspricht	0.12 g/100 g	Probe
Milchfettgehalt (3.2):	0.219 g	entspricht	3.64 g/100 g	Probe
		Anteil am Gesamtfett:	99.65 %	
Milchfettgehalt (3.5):	0.201 g	entspricht	3.33 g/100 g	Probe
		Anteil am Gesamtfett:	91.11 %	
C-12 Gehalt:	0.009 g	entspricht	0.14 g/100 g	Probe

RT [min]	IS #	Peakfläche [pA*s]	Faktor	Menge [g]	Komponente
1.355	1	29.305	1.645	0.0070	C-4
2.099		19.716	1.000	0.0029	
2.816		14.309	1.000	0.0021	
3.482		36.152	1.000	0.0053	
4.101	1	56.261	1.039	0.0085	C-12
4.393	1	364.757	1.000	0.0531	C-13 (IS)
4.675	1	149.425	1.012	0.0220	C-14
4.795		17.722	1.000	0.0026	C-16 - <C-18
5.401	1	519.032	1.012	0.0785	C-16 - <C-18
6.451	1	508.922	1.012	0.0750	C-18 - eoc

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Results and Discussion

Calibration is performed by determining the response of a known amount of lard (99.9% fat) versus an internal standard (tridecanoic acid) and using this response factor for the individual fatty acids. The total fat content is calculated as sum of the individual peak areas. For milk fat calibration a second standard (valeric acid) together with a known amount of butyric acid is added. Figure 4 is showing a chromatogram of such a calibration run.

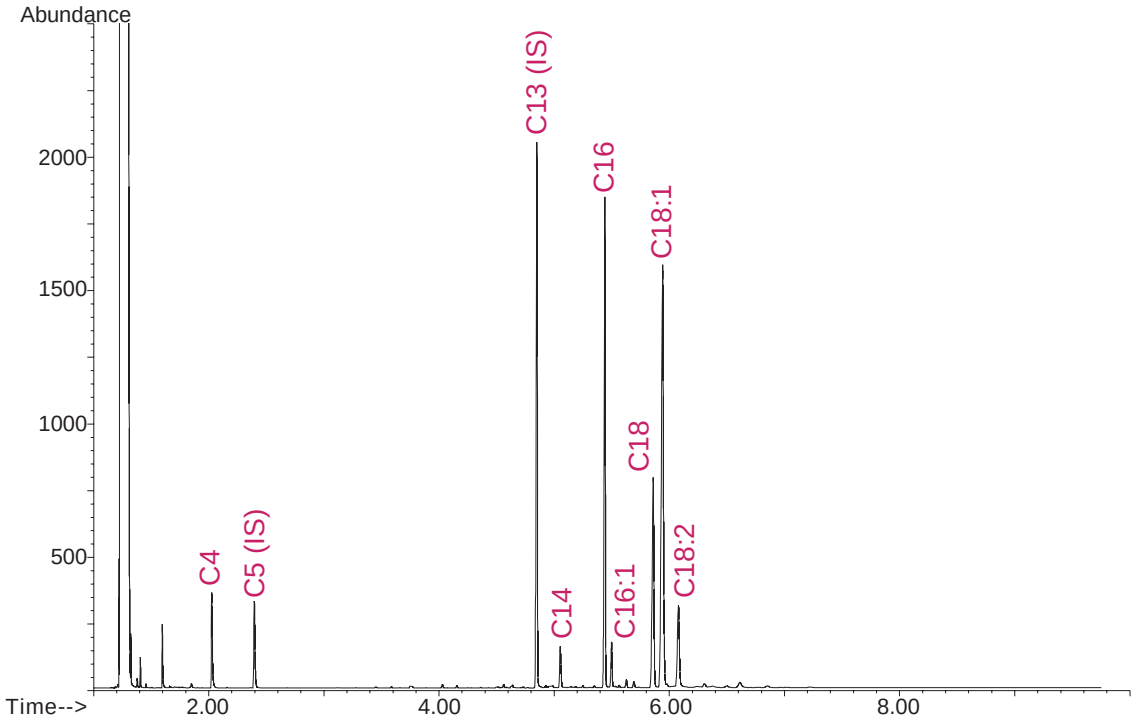

Figure 4. Calibration run.

Figure 5 shows the chromatogram of a goat cheese, the advantage of simultaneous detection of total fat and milk fat in a single analysis run can be seen.

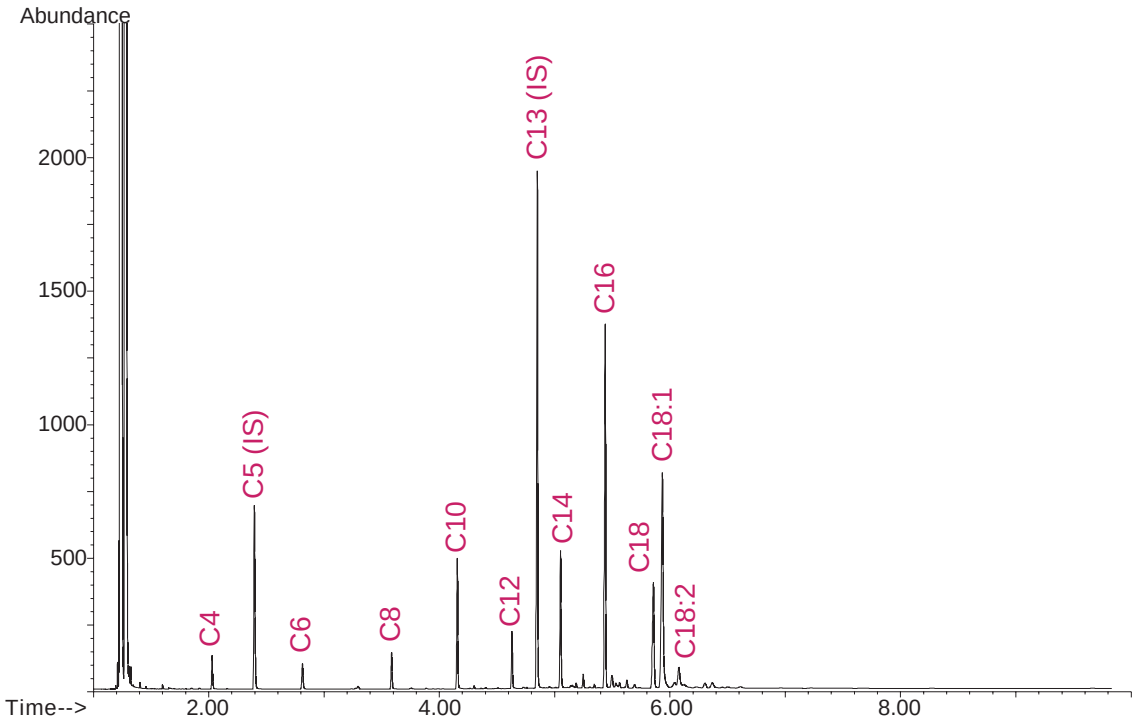

Figure 5. Chromatogram of fatty acids in a goat cheese, total fat content = 28.3%, milk fat content = 26.3%.

Figure 6 shows the chromatogram of a food product that needs differentiation between milk- and total fat, an apple cake with cream content.

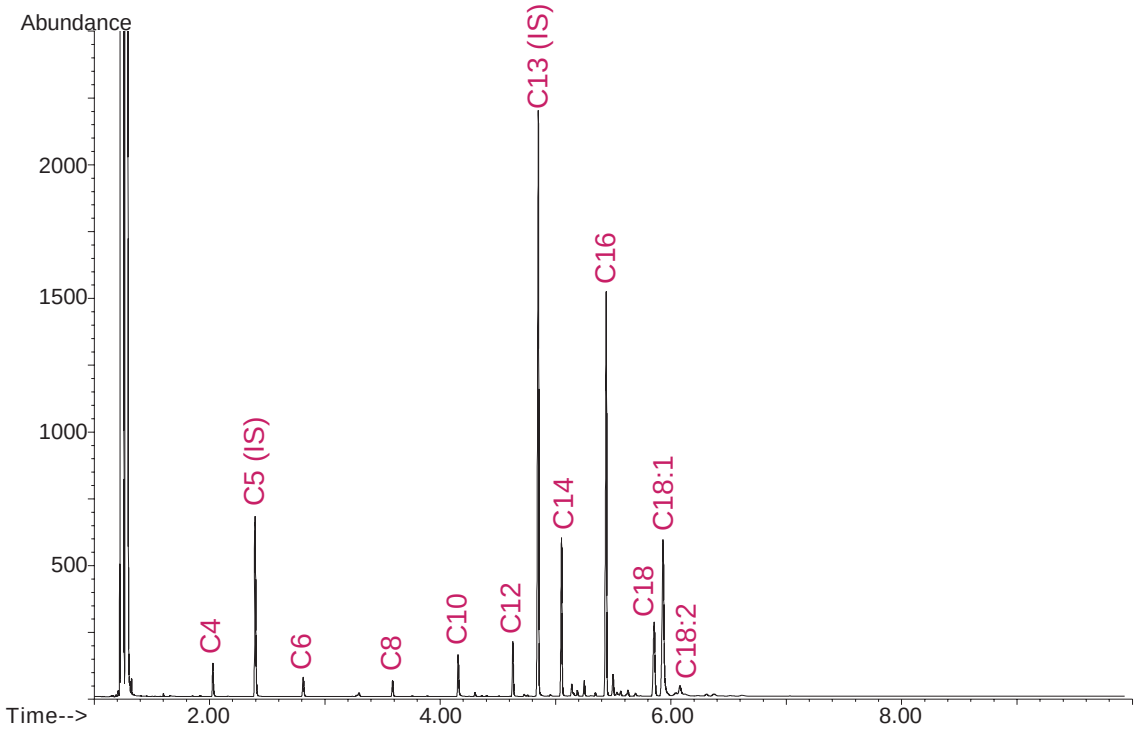

Figure 6. Chromatogram of fatty acids in an apple and cream cake, total fat content = 26.1%, milk fat content = 28.9%.

Figure 7 shows the chromatogram of a shrimp cocktail, where only the amount of milk fat contained in the dressing was of interest.

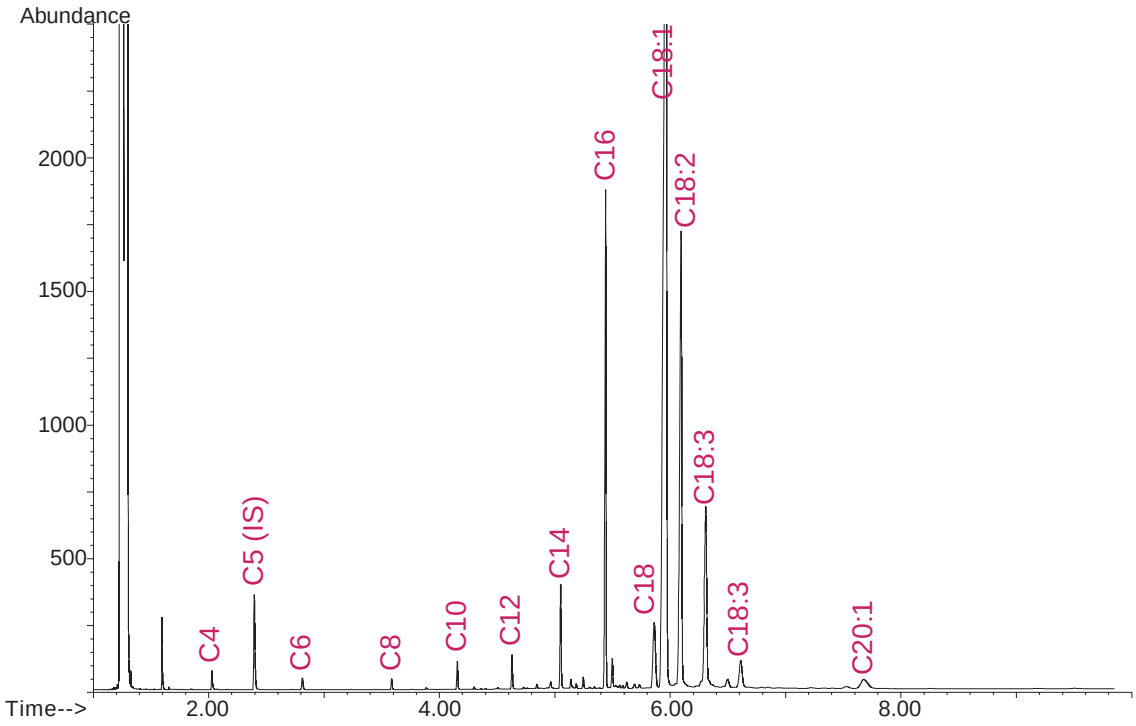

Figure 7. Chromatogram of fatty acids in a shrimp cocktail, milk fat content = 3.9%.

Figure 8 shows the chromatogram of a “matjes herring” (pickled young herring).

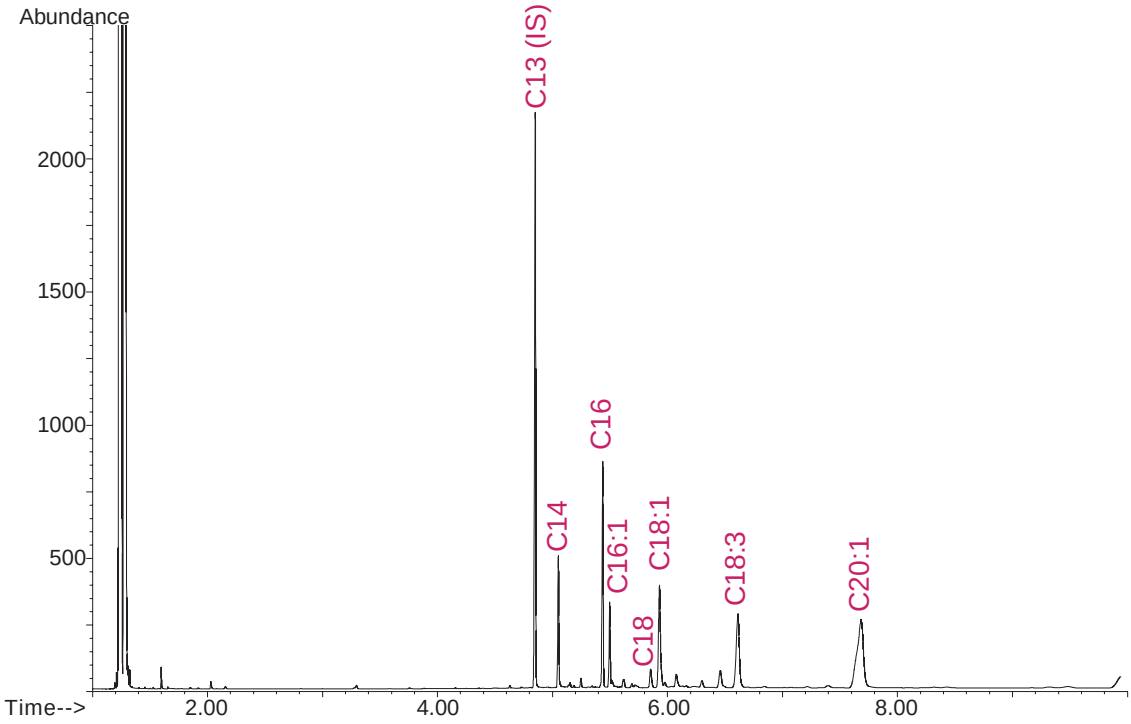

Figure 8. Chromatogram of fatty acids in a matjes herring, total fat content = 21.0%.

Conclusion

The single step extraction and saponification Caviezel method followed by GC analysis combined with a novel reporting tool presents a very powerful turnkey fat determination system. The presented analyzer provides information about fat content and fat composition in food and feedstuff, within a significantly shorter period of time compared with existing methods. Especially the incorporation of milk fat determination makes this method a very interesting alternative to other analysis methods.

References

[1] Fed. Reg. 58 (3), January 6, 1993, 631 - 2964

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