Determination of the fat content profile of different chocolate products using an automated workflow for the generation of fatty acid methyl esters (FAME)

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PAL SYSTEM
Ingenious sample handling

Introduction

The composition of the fat in chocolate is an important factor influencing the quality of chocolate. Specifically the replacement of cocoa butter by replacements or CBEs (cocoa butter equivalents) is of interest to food quality laboratories [1].
This work presents a fully automated workflow for the generation and analysis of FAME from chococolate samples, using an autosampler with robotic tool change (PAL RTC, fig. 1). The automated workflow improves process safety and minimizes handling errors. The method allows the determination of total fat content and the quantitative analysis of saturated and unsaturated fatty acids. Nine chocolate samples from Japan, Switzerland and the USA were analyzed, as well as cocoa butter and palm oil reference samples.

Rapid transesterification using methylate and three internal standards (IS)

Sodium methoxide transesterifies triglycerides within a very short time (here 120 s) at ambient temperature. In the presence of water, methoxide also forms hydroxide, which may saponify the tri-glycerides directly or via the methylesters of the fatty acids. This reaction is about thousands times slower. Saponification is undesired but can be detected and quantified via the internal standard FAME-9 [ref.3].

Three IS are used:

1. **Alkene C14:1**, non reactive, to check for complete reaction
2. **Triglyceride of C11 fatty acid**, to check for complete transesterification.
3. **FAME-9**, to check whether saponification occured.

The peak area ratios for different samples are listed below:

	C11/ C14:1	FAME-9/ C14:1
3 ISs w/o methoxide	1.19	0.99
3 ISs with methoxide	1.21	1.00
08_Migros_Budget Milchschokolade	1.24	1.02

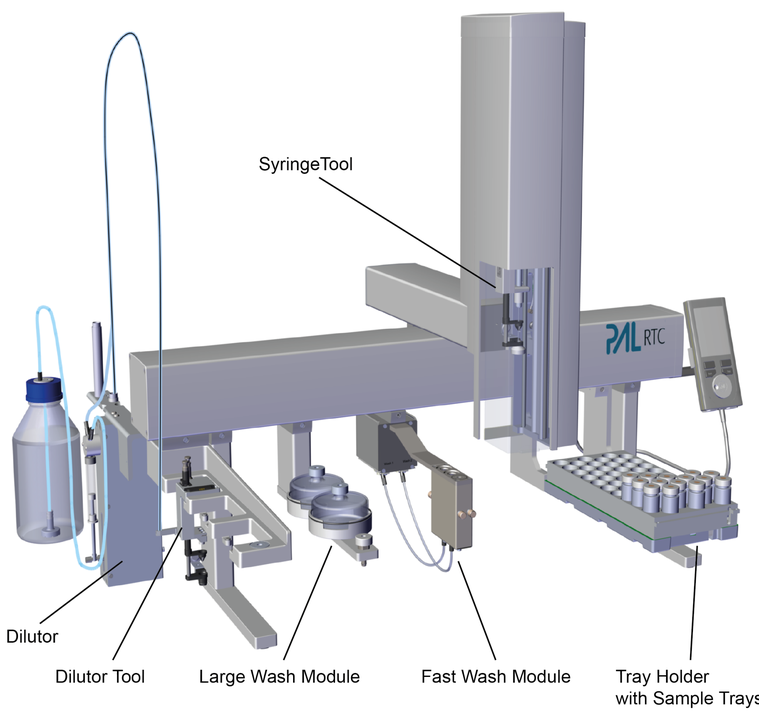

Figure 1: PAL RTC for FAME prep with tool parkstation, syringe & dilutor tools

Experimental

Chemicals:

Reactant:	5 % Na-methoxide in methanol
IS solution:	C14:1 alkene, FAME-9, triglyceride C11, @ 100 mg/mL in dioxane
Stop solution:	15 % Na-citrate in water

Instrumentation and chromatography:

Sample prep:	PAL RTC with Multisolvent Dilutor, Vortex Mixer, Large Wash Module, Fast Wash Module, Syringe Tools
GC:	Shimadzu GC-2010 Plus
Injector:	SSL @ 250°C, split ratio 300/1
Column:	Restek Rtx-Wax 30m, 0.25 mm x 0.25 µm
T-program:	80°C 1 min hold; @ 25°/min→180°C, 3 min hold; @ 10°/min→250°C; 3 min. hold
MS:	Shimadzu GCMS-QP 2010 SE scan range 60-300 m/z
Data analysis:	Shimadzu GCMS Solutions v. 4.30

Workflow

Weigh sample, about 15 mg (offline)

Add 1.0 mL dioxane to a 2 mL vial containing the chocolate sample [dilutor tool]
Add IS solution (@ 1 µL/mg sample) to the sample vial [liquid tool 1]
Vortex 60 s
Transfer 200 µL to a 2 mL vial [dilutor tool]
Add 200 µL 5% Na-methoxide in MeOH
Vortex 30 s
Reaction time 120 s
Add 200 µL 15% Na-ctirate in H2O
Add 500 µL n-heptane
Vortex 30 s
Wait 60 s for phase separation
Inject 1 µL extract into the GC [liquid tool 2]

Total sample prep time/sample is 14 min. GC runtime is 20 min.

Conclusions

– Transesterification of fatty acid esters with Na-methoxide is a fast, efficient and very robust method. With the three ISs the completeness of the transesterification as well as the extent of undesired saponification can be checked [refs. 3, 4]

– The PAL RTC allows to fully automate the FAME preparation, including injection into the GC. This improves process safety and minimizes handling errors.

– The described setup can prepare and analyze 50 samples automatically in 16 h 55 min (= GC runtime). This is possible because the system can process one sample while an other sample is being analyzed (“prep ahead”).

– In total 250 samples were processed, without failures. The good chromatographic separation achieved for all FAMEs enabled robust quantitation.

– The precision (peak area) for the processed standards ranged from 2.7-3.0% RSD, for processed chocolate samples from 2-11% RSD.

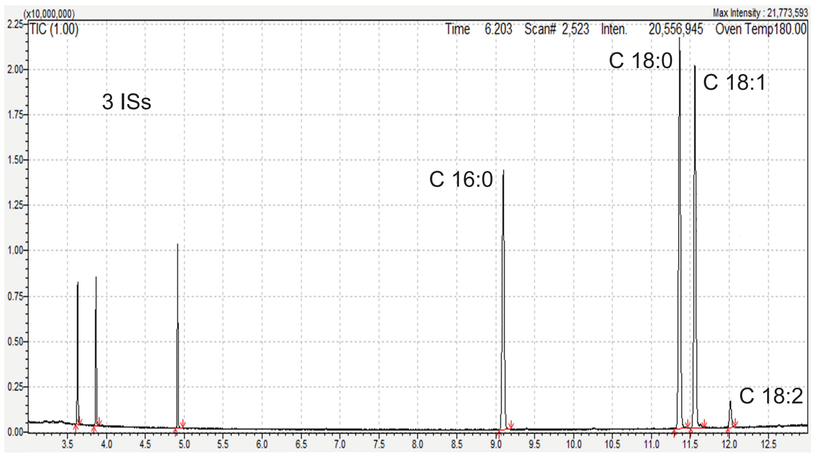

Figure 2: Chromatogramm of FAMEs from a cocoa butter reference sample

Figure 3: Chromatogramm of FAMEs from a milk chocolate sample

Acknowledgments

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References

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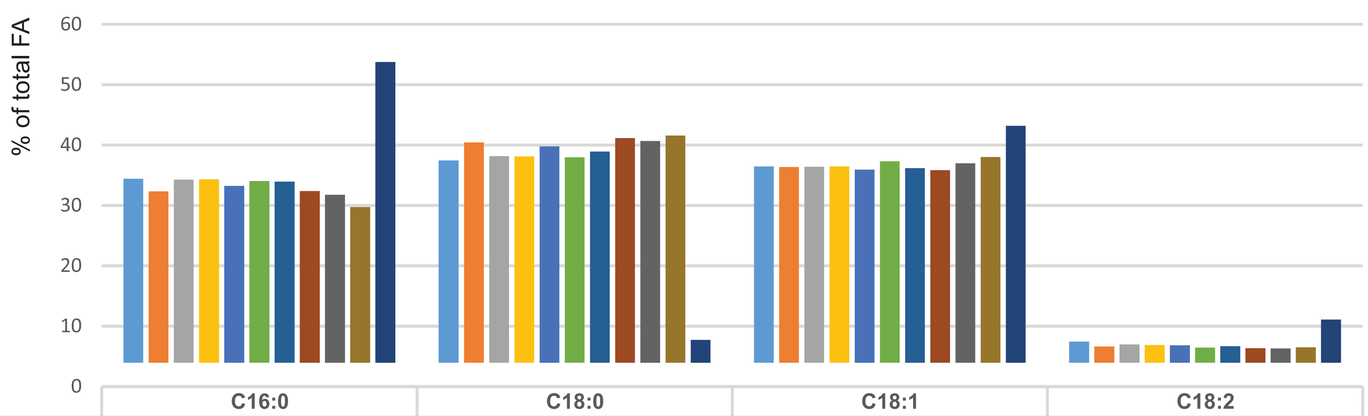

	C16:0	C18:0	C18:1	C18:2
01_Meiji_black chocolate	30.5	33.5	32.5	3.5
02_Meiji_milk chocolate	28.4	36.5	32.4	2.7
03_Meiji_himilk chocolate	30.3	34.2	32.5	3.0
04_Meiji_white chocolate	30.4	34.2	32.5	2.9
05_Lotte_Ghana milk chocolate	29.3	35.9	32.0	2.9
06_Lotte_Zero	30.1	34.0	33.3	2.5
07_Hersheys_milk chocolate	30.0	35.0	32.3	2.8
08_Migros_Budget Milchschokolade	28.4	37.2	31.9	2.4
09_Migros_Noir Special 72%	27.8	36.7	33.1	2.4
Kakaobutter Referenz KL ZH	25.8	37.6	34.1	2.5
Aldrich_Palmoil standard	49.8	3.8	39.3	7.1

Figure 4: Fatty acid profile of different chocolate samples,as well as cocoa butter and palm oil reference samples.

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