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

Guenter Boehm, Reto Bolliger, Hans-Joachim Huebschmann
CTC Analytics AG, Industriestrasse 20, 4222 Zwingen, Switzerland
email: gboehm@ctc.ch

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 chocolate 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 methanol 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:

<table>
<thead>
<tr>
<th>Sample</th>
<th>C11/ C14:1</th>
<th>FAME-9/ C14:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ISs w/o methoxide</td>
<td>1.19</td>
<td>0.99</td>
</tr>
<tr>
<td>3 ISs with methoxide</td>
<td>1.21</td>
<td>1.00</td>
</tr>
<tr>
<td>08 Mango_Budget Milchschokolade</td>
<td>2.4</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Instrumentation and chromatography:
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
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-citrate 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 another one is being analyzed (“prep ahead”).

Acknowledgments
The authors would like to acknowledge Maurus Biedermann, Kantonalabore Zürich, Switzerland for valuable information and the gift of cocoa butter reference samples, Beat Schilling, BGB Analytik, for valuable advice.

References

Experimental
Chemicals:
Reactant: 5 % Na-methoxide in methanol
IS solution: C11:1 alkene, FAME-9, triglyceride
Stop solution: 15 % Na-citrave in water

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

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

Figure 3: Chromatogramm of FAMEs from a milk chocolate sample

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

http://www.palsystem.com