AUTOMATED WORKFLOW FOR THE DETERMINATION OF FATTY ACID METHYL ESTERS (FAME) IN FAT AND FAT CONTAINING FOOD SAMPLES USING A 90 SEC. TRANSESTERIFICATION

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Introduction

The analysis of oils, fat and fat containing food via fatty acid methyl esters (FAME) is a common task in governmental, quality control (QC) or contract research laboratories (CRO). In most cases the samples are processed manually, which is labor intensive and exposes the lab personnel to potentially hazardous chemicals [1,2].

This work presents a fully automated workflow using a workstation with robotic tool change (RTC, Fig. 1) based on a method using sodium methoxide in methanol as reactant [3]. The workflow improves process safety, optimizes throughput and minimizes handling errors. The PALworkstation was equipped with a Dilutor to dispense the liquids for the reactions, the extraction and the cleaning steps, a Vortex module to provide fast mixing and extraction and a tool for a 10 µl syringe to inject the sample into the GC.
The software of the workstation allows overlapped sample processing, which increases sample throughput.
The method allows the determination of the total fat content, quantitative analysis of saturated and unsaturated cis- and trans-fatty acids. Three internal standards are used to control extraction, transesterification and undesired saponification. The method was applied to a number of different vegetable oils and water containing animal fats such as butter, cheese and salami.

Concept of the Method using three Internal Standards (IS)

Sodium methoxide transesterifies triglycerides within a very short time at ambient temperature. In the presence of water, methoxide also forms hydroxide, which may saponify the triglycerides 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.

Three IS are used:

1. **Alkane C_{14}:1**, non reactive, to check for complete reaction.
2. **Triglyceride of C_{11} fatty acid**, to check for complete transesterification.
3. **FAME-9**, to check whether saponification occurred.

Peak areas of the three ISs are checked for every analysis. If the C_{11}-FAME/alkane peak ratio is < 0.75, transesterification was not complete e.g. through lack of the reactant (Fig. 4), or the FAMEs were saponified already. If the FAME-9/alkane peak ratio is < 0.67 saponification occurred already. In the work of Grob et al. [3] the use of a fourth IS was proposed when injecting into a SSL injector to check for thermal peak discrimination. Nowadays, thermal discrimination due to solvent evaporation in the syringe needle can be avoided by performing fast injections.

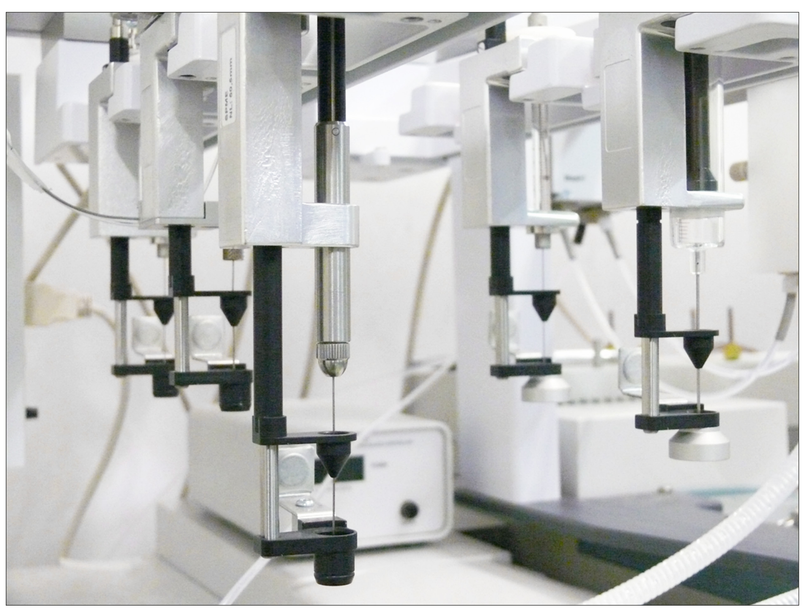

Figure 1: Robotic Tool Change: Tools in parkstation

Experimental

The following solutions were used:
Reactant: 5 % Na-methoxide in methanol
IS solutions: C_{14}:1 Alkane, FAME-9, Triglyceride C_{11}, @ 1 mg/mL in dioxane
Solution to stop the reaction: 15 % Na-citrate in Water

Instrumentation and Chromatography:

PALworkststation:	PAL RTC with two Park Stations, multi solvent Dilutor, Vortex Mixer, Fast Wash module, LS Tool (for Liquid handling)
GC:	Agilent 6890
Injector:	SSL @ 250 °C, split flow 5 mL/min.
Column:	25 m x 0.25 mm ID, 0.25 µm BGB-WAX
Oven:	40 °C @ 25 °/min → 180 °C @ 15 °/min → 250 °C → 3 min. hold
Detection:	FID @ 300 °C
Data processing:	Clarity (Dataapex)

A weighed amount of fat or fat containing food sample (e.g. 15.3 mg oil) was dissolved in the corresponding amount of dioxane containing the three Internal Standards (1.53 mL). 100µl of this solution was transferred to a 2 mL vial. The sequence of preparation steps is shown below. The phase separation occurs usually in less than 30 s. For some food samples, such as chocolate cream containing emulsifiers, more time is needed, in some cases even centrifugation. A prototype centrifuge for the PALsystem was used in theses cases. For some samples e.g. salami a pre-treatment with DMF is necessary to make the fat extractable from the cells. In this case about 100 mg of sample was heated up to 120 °C with 100 µL DMF for 10 min. before processing the samples. Dioxane has been chosen as a good solvent mediator between water and the fat containing sample and the reactant solution containing methanol.

Workflow

Accurate weighing of sample (e.g. 1 drop = 15.3 mg)
Addition of 1.53 mL dioxane incl. the 3 Internal Standards
Transfer of 100 µL to a 2 mL vial
Addition of 100 µL 5 % Na-methoxide in methanol
Vortexing 10 sec
Reaction time 90 sec
Addition of 1 mL n-heptane
Vortexing 10 sec
Addition of 300 µL Na-citrate (15 % in water)
Vortexing 10 sec
Wait for 60 sec (phase separation)
Injection of 1 µL extract to the GC

Conclusion

Transesterification of fatty acid esters with Na-methoxide is a fast, efficient and very robust method for fat analysis in food samples. With the use of three ISs the completeness of the transesterification as well as the extent of undesired saponification can be checked.
The PALworkstation allows to fully automate the FAME preparation, including injection into the GC. A Dilutor module was used to dispense Na-methoxide, heptane and Na-citrate. It was also used for intermediate washing steps with methanol and water (Fig. 6). The Vortex Mixer ensured rapid mixing. The Fast Wash module is required for efficient cleaning of the Dilutor Tool and the syringe including washing of the outside of the needle. No carry-over was detected (Fig. 5).
The described setup can prepare and analyze 50 samples fully automatically in 18 h 30 min. This is possible because the PAL Sample Control software allows to process one sample while another sample is being analyzed (“prep ahead”).
The good chromatographic separation achieved for all FAMEs enables robust quantitation. GC peak shapes remained perfect even after 75 injections (Fig. 3). Contamination of the injector liner or the column inlet was not observed.

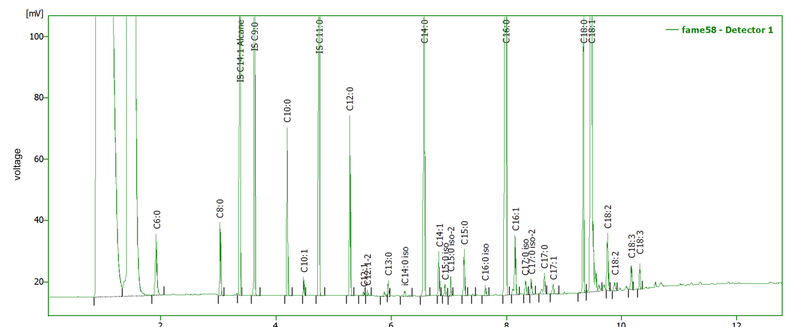

Figure 2: Typical result of butter FAMEs. Complete separation within 11 minutes.

Coconut Oil	%	Peanut Oil	%	Safflower Oil	%	Olive Oil	%	Sunflower Oil	%
C8:0	7.5	C16:0	8.9	C16:0	6	C16:0	12.3	C16:0	4.7
C10:0	5.8	C18:0	3.2	C16:1	0.1	C16:1	0.7	C16:1	0.1
C12:0	45.8	C18:1	68.8	C18:0	2.5	C17:0	0.1	C18:0	1.9
C14:0	18.5	C18:2	16.3	C18:1	17.1	C17:1	0.2	C18:1	13.3
C16:0	9.3	C18:3	0.1	C18:2	73.2	C18:0	2.4	C18:2	57.1
C18:0	2.9	C20:0	1.3	C18:3	0.3	C18:1	74.5	C18:3	0.2
C18:1	8.2	C20:1	1.4	C20:0	0.4	C18:2	8.2	C20:0	0.3
C18:2	21			C20:1	0.2	C18:3	0.8	C20:1	0.2
						C20:0	0.5		
						C20:1	0.4		

Table 1: Typical result of the FAME composition of vegetable oils.

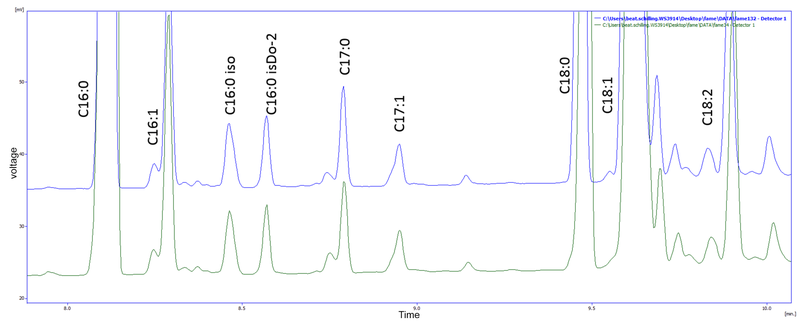

Figure 3: Detail of butter FAMEs before (blue) and after (green) 75 injections.

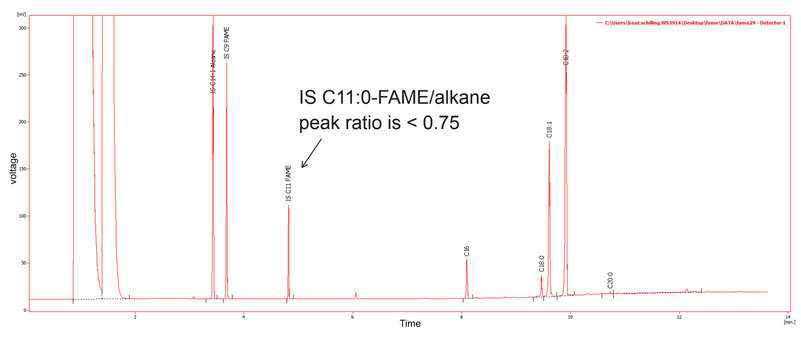

Figure 4: Example for incomplete esterification due to lack of reactant (sunflower oil)

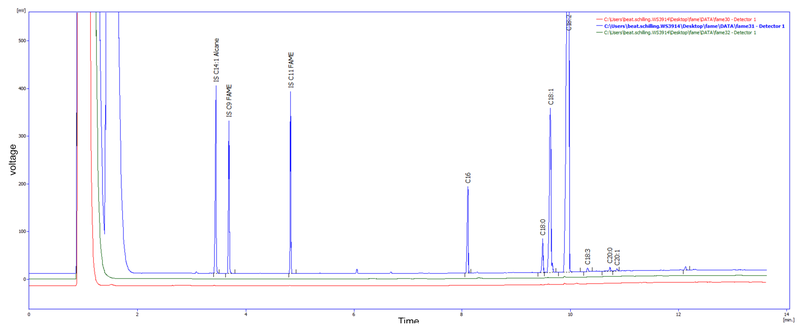

Figure 5: Blank before (red) and after (green) analysis of sunflower oil (blue)

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References

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