Determination of the fatty acid composition in refined oils and fats by alkaline transesterification

by the FAME Workstation

Introduction

Animal and vegetable fats play a key role as components of our daily diet and as greases or lubricants. According to the EU food information regulation from December 2016 manufacturers are obligated to declare the fatty acid composition on their packagings. Thus, not only the fatty acid composition is analyzed but also the content of cis and trans fats. Moreover, the degree of purity is determined.

Naturally, the triglycerids of fatty acids cannot be analyzed by gas chromatography. A preliminary splitting and derivatization must be performed. Ester bounds are broken and the free fatty acids are converted into their corresponding fatty acid methyl esters (FAMEs). Unlike fatty acids the FAME are nonpolar, moderately volatile and therefore suitable for GC analysis.

There are various specifications that describe the analytics of FAME in oleaginous samples. For this application the specification Ce 2-66 by the American Oil Chemists' Society (AOCS) is applied. It provides several methods for the preparation of fat samples. Here, method 3 and 4 (figure 1) are used for automation. Method 4 is realized for fat samples with a number of acids <2 whereas method 3 is a general, but complex and time-consuming approach.

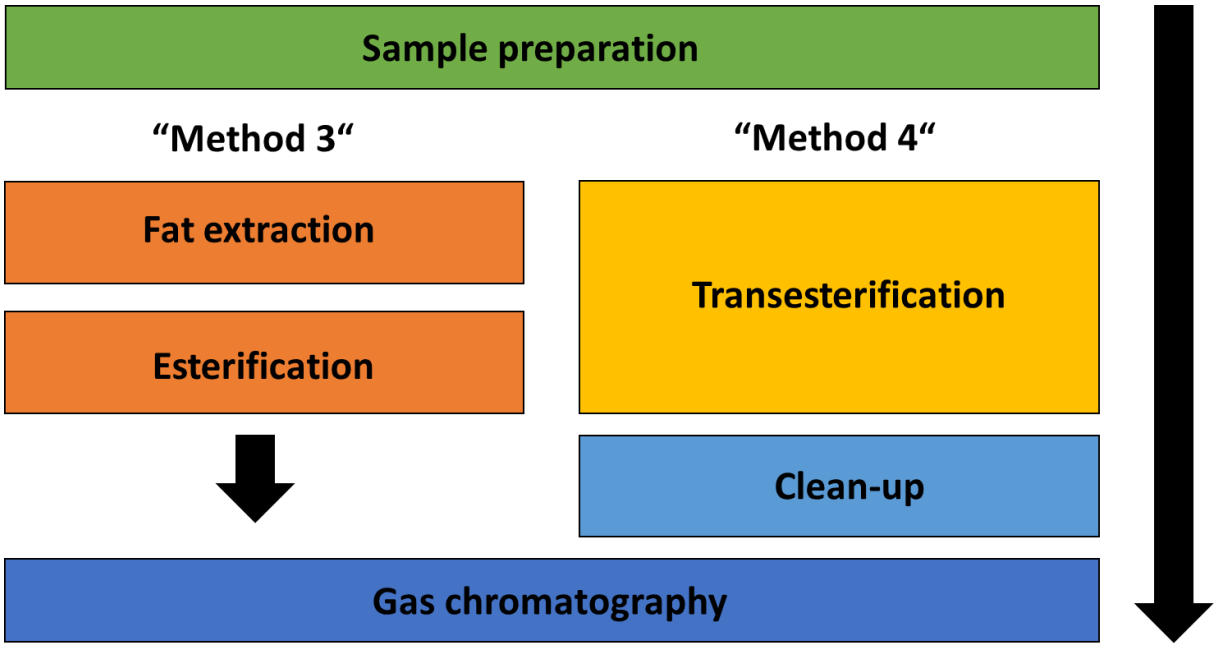

Figure 1: Schematic sample preparation of an oleaginous sample according to standard specification AOCS Ce 2-66.

Configuration

The fatty acid analysis was realized by an application which performs sample preparation and analysis fully automated. Therefore, a 7890A GC-FID system by Agilent with a S/SL-injector by SIM was utilized. For the sample preparation the PAL RTC autosampler by CTC Analytics was applied. Figure 2 displays the configuration of the modules within the x-axis of the autosampler.

Device setup

- Agilent 7890 GC with an FID-detector, S/SL-injector and BPX-70 column 60 m
- PAL RTC 120 cm with Agitator Module, 2x Park Station, 2x Tray Holder with 3x VT-54 racks and 3x VT-15 racks, Vortex Mixer, Wash Station and Solvent Module

Software

- CHRONOS software by Axel Semrau®
- DataApex Clarity

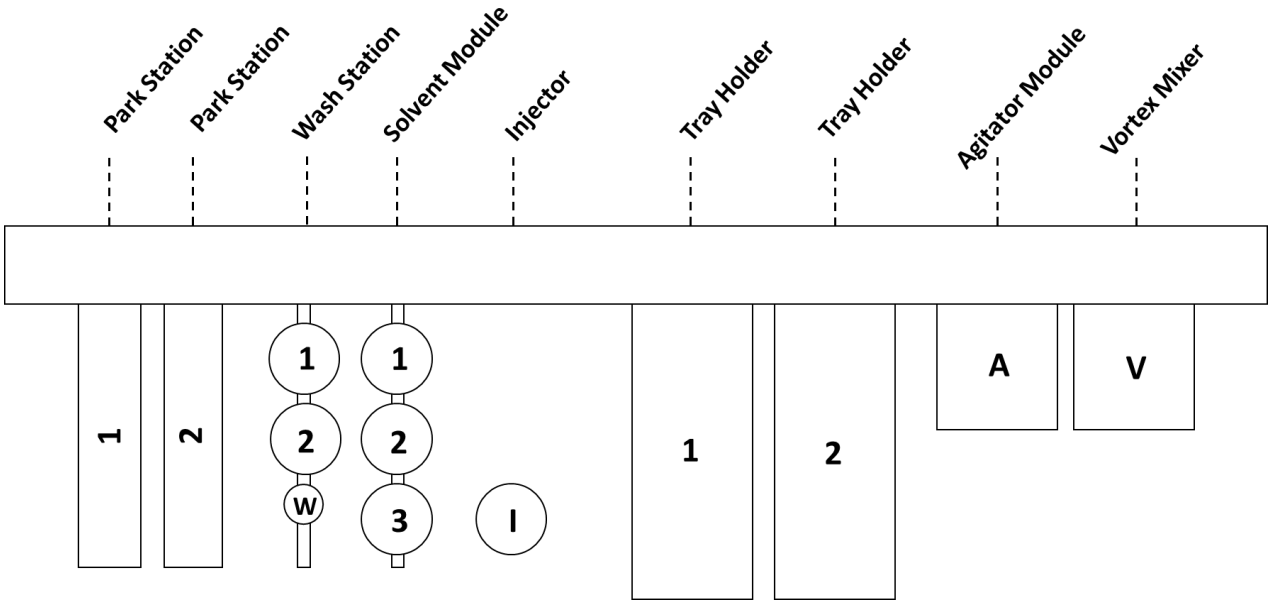

Figure 2: Module configuration on a PAL 3 RTC on an Agilent GC 7890 FID.

Measuring parameters and results

The realization of a solution for the sample preparation according to AOCS Ce 2-66 with two different preparation procedures requires a flexible system for automation. A PAL RTC autosampler including automated tool exchange allows this degree of flexibility. Featuring two Park Stations up to six different needle tools may be utilized for various tasks. This provides a high degree of efficiency, flexibility and helps avoiding carryovers.

In combination with the software platform CHRONOS a high-performance automation application is created including intelligent time management, interlacing and an optimal capacity utilization of the entire system. Providing interfaces to all common chromatography evaluation programs this CHRONECT Robotic solution is suitable for analytical systems of almost every manufacturer. Consequently, the presented automation is not bound to certain hardware configurations. All measuring parameters for the gas chromatography were retrieved from AOCS Ce 1f-96 (table 1). This specification describes only the chromatographic setting for FAME analytics.

Table 1: Parameters of the gas chromatograph.

Heating rate [°C/min]	Final temperature [°C]	Holding time [°C]	Total time [min]
	70	0	0
5.00	220	15	45
Injector	250 °C, 1 mL/min, Split 1:30		
Detector	250 °C, 25 mL/min N_2 makeup		
Carrier gas	H_2		
Separating column	BPX 70, 60 m, 250 µm i.d. und 0.25 µm film		

Method 3 specifies the sample preparation by alkaline esterification using methanolic sodium hydroxide (meth. NaOH) and the subsequent methylation using methanolic boron trifluoride (BF_3). Next, the sample is heated to a boil and diluted by n-heptane. This is followed by another boiling and precipitation using saturated sodium chloride. Two phases are formed of which the upper organic phase is dried upon sodium sulfate and subsequently injected into the GC-FID. 50 mg of an oleaginous fluid in a 10 mL vial with a crimp lid serve as a starting material for the automated preparation.

Transesterification is entirely performed in this 10 mL vial. The subsequent drying over sodium sulfate takes place in a 2 mL vial. A PAL RTC with automatic tool change is the optimal solution for prevention of carryovers using such samples during a full automation.

The results of the fully automated analysis of an oleaginous sample are presented in table 2.

Table 2: Fatty acid composition of an olive oil compared to literature data.

Component	% Area	% Area literature[1]
C16:0 (Palmitic acid)	11.4	7.5 – 20.0
C18:1 (Oleic acid)	76.0	55.0 – 83.0
C18:2 cc	6.1	3.5 – 21.0
Other farty acids	6.4	0.0 – 10.0

Using CHRONOS in combination with PAL RTC 40 oleaginous samples may be prepared and analyzed by GC-FID within 24 h (figure 3). In the process, aggressive reagents as meth. BF_3 are required. In order to avoid corrosion of the PAL tools a periodical maintenance of the system should be considered.

The magnets for the vial transport are especially sensitive to corrosion. Therefore, those should be, if permanently used, cleaned on a regular basis (~ once per month). This kind of sample preparation is particularly suitable for solid state samples.

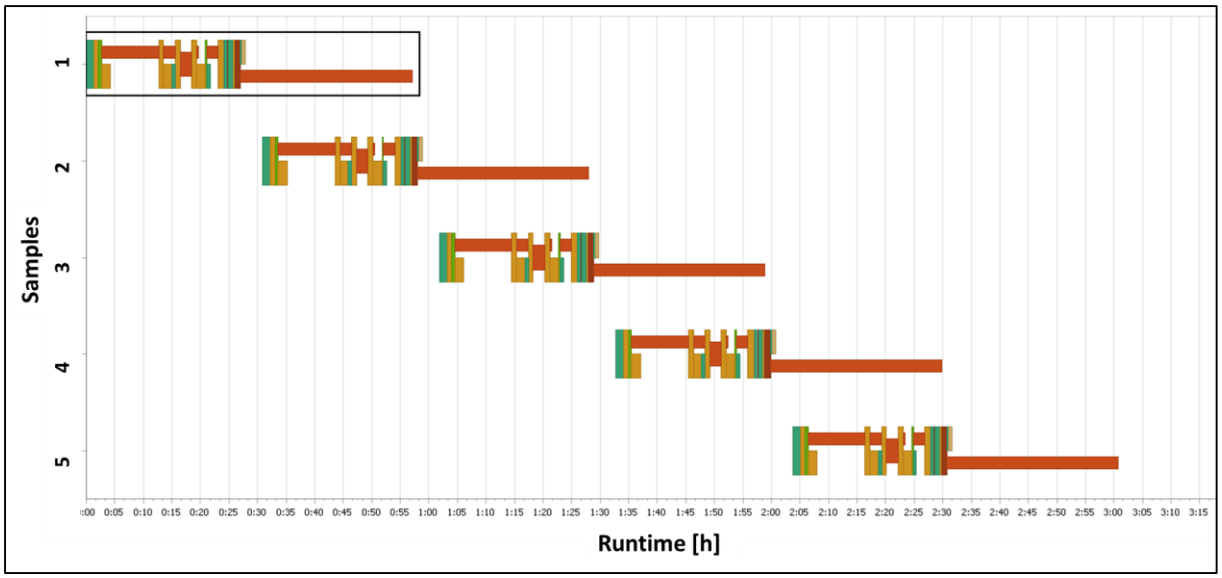

Figure 3:. Example of an interlaced CHRONOS sequence using five samples according to AOCS Ce 2-66 method 3 in 3 h.

[1] Data represent the Trade Standard Applying to Olive Oil and Olive Pomace Oil by the International Olive Council (Madrid) from 1998.

According to method 4 of AOCS specification Ce 2-66 transesterification of ~ 50 mg sample is performed using methanolic potassium hydroxide (meth. KOH). First, the sample is solved in n-heptane. After the phase separation the organic phase is washed by water and dried on sodium sulfate. The dry extract is taken up with n-heptane and injected into the GC-FID system, directly from the vial (table 3).

A process for sample preparation is shown in table 4 and includes all important steps.

This kind of sample preparation is especially suitable for oleaginous samples in a short period of time. If required, the sample preparation may be accelerated up to 40 minutes per sample by adding a centrifuge to the application. This allows the fast phase separation. Doing that, the throughput is increased from 32 up to ~ 70 samples in 24 hours. The results of such an analysis are listed in table 5.

Table 3: General steps during sample preparation according to method 3 from the CHRONOS method.

Task	Description
Transfer	Add 0.5 M methanolic NaOH with LS2
Transfer	Move to Agitator
WaitOverlapped	Heat until dissolved
Transfer	Add methanolic BF_3 with LS3
WaitOverlapped	Boil Time 1
Transfer	Add heptane with LS2
WaitOverlapped	Boil Time 2
Transfer	Add saturated sodium chloride with LS3
WaitOverlapped	Shake for 15 sec
Transport	Remove vial from Agitator vial on tray
WaitOverlapped	Wait for phase separation
Transfer	Transfer 1 mL with LS2 to destination vial with sodium sulfate

Table 4: General steps during sample preparation according to method 4.

Task	Description
Transfer	Add n-heptane
Transfer	Add 2 M methanolic KOH
VortexVial	Shake sample well
WaitOverlapped	Wait for phase separation
	Transfer 0.5 mL water into next vial with LS2
Transfer	Transfer 0.5 mL of upper phase to next vial with LS2
VortexVial	Shake sample well
WaitOverlapped	Wait for phase separation
Transfer	Transfer 2 drops of upper phase onto sodium sulfate
Transfer	Dilute with heptane LS2
VortexVial	Shake sample well

Table 5: Fatty acid composition of a real oil from foodstuffs industry.

Component	Actual % Area	Target % Area[2]
C14:0	0.06	0.07
C16:0	4.30	4.29
C16:1	0.22	0.27
C18:0	1.94	1.97
C18:1 c	63.66	63.86
C18:2 ct	0.02	0.04
C18:2 cc	18.26	18.42
C18:3 t	0.16	0.15
C18:3 ccc	8.15	8.25
C20:0	0.56	0.56
C20:1	1.03	1.02
C22:0	0.28	0.27
C24:0	0.12	0.13
C24:1	0.13	0.13

[2] Data based on a single measurement by the oil manufacturer with manual sample preparation.

The Automation of sample preparation represents the focus of this application. Here, an important criterion is a sufficient rinsing of all needles to prevent carryovers between samples and achieve an optimal phase separation during the precipitation of triglycerids. A good separation results in an accurate transfer of the organic phase after methylation. Washing by water (1:1) and drying over sodium sulfate assures the injection of a sample as clean as possible.

Aside from the development of methods for sample preparation the parameters of the Agilent GC-FID system were adjusted to achieve well separated fatty acid methyl esters. Therefore, a FAME standard including 11 components from C14:0 to C24:0 was injected and the temperature gradient matched.

During method development, standard solutions consisting of various fatty acid methyl esters were measured. Figure 4 shows a chromatogram of the used standard. With the GC parameters utilized there, a good separation of all analytes was achieved; even C18:3 and C20:0 reveal an appropriate degree of separation.

Furthermore, the Restek FAME 37 standard solution with 37 fatty acid methyl esters in total was injected to aim for a more accurate calibration of the retention times for different fatty acids. The corresponding chromatogram is shown in figure 5 and reveals the area from C14:0 to C24:1 with, as expected, more signals in that region.

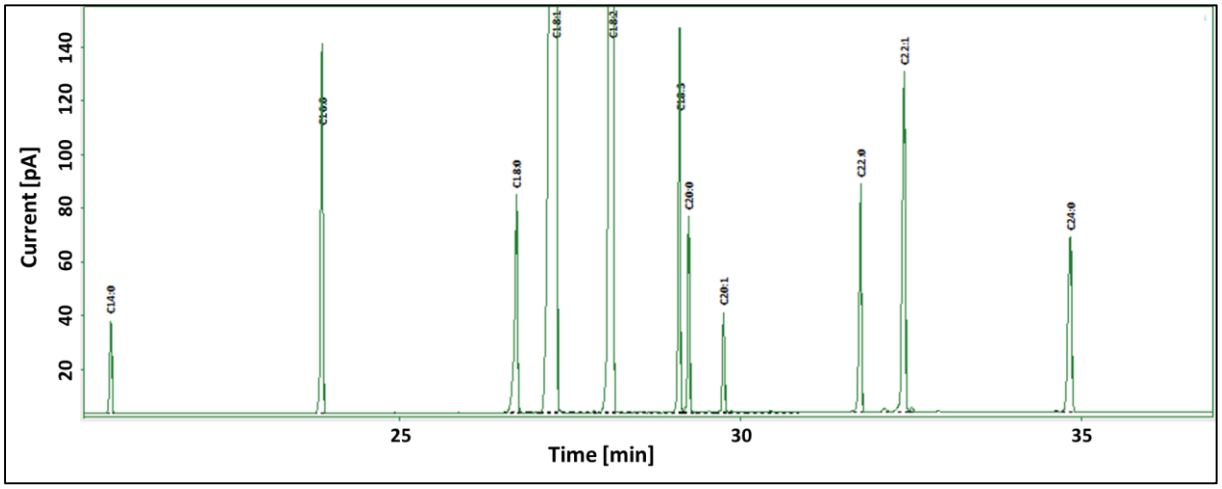

Figure 4: Chromatogram of a separation of 11 FAME analytes from C14:0 to C24:0.

For validation of the entire method, a real sample with known features was analyzed (figure 6). Comparing the results of the measurement with the data for the fatty acid composition that was provided by the manufacturer, only few variances were observed (table 5).

A fully automated analysis of any oleaginous sample therefore provides data comparable to manual sample preparation. The few variances to the data from the manufacturer are all occurring within the processing variance (up to 20 %) and therefore close to the signal/noise ratio.

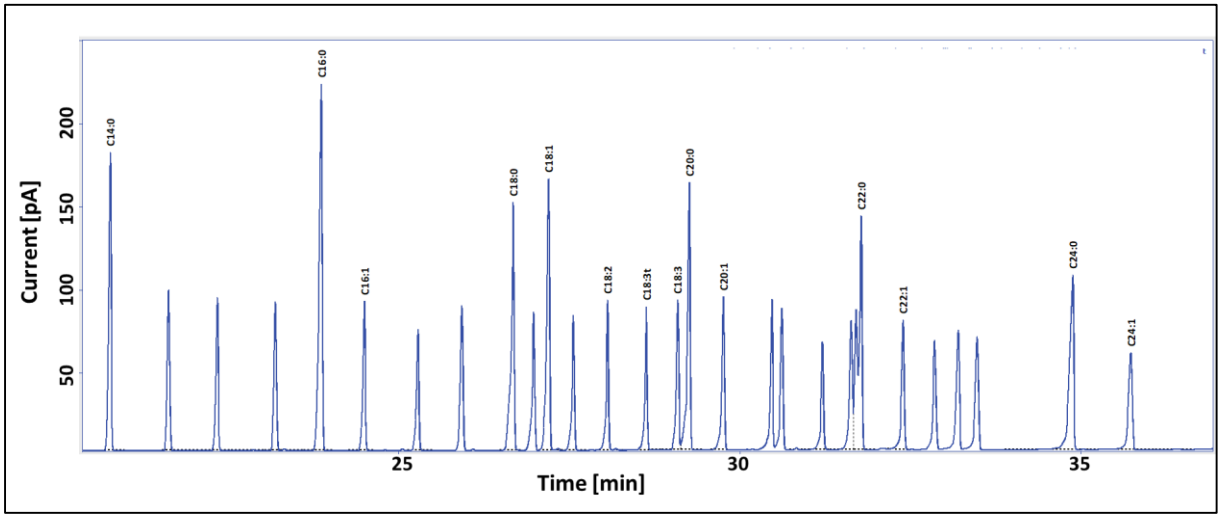

Figure 5: Section from the chromatogram of the Restek FAME 37 standard.

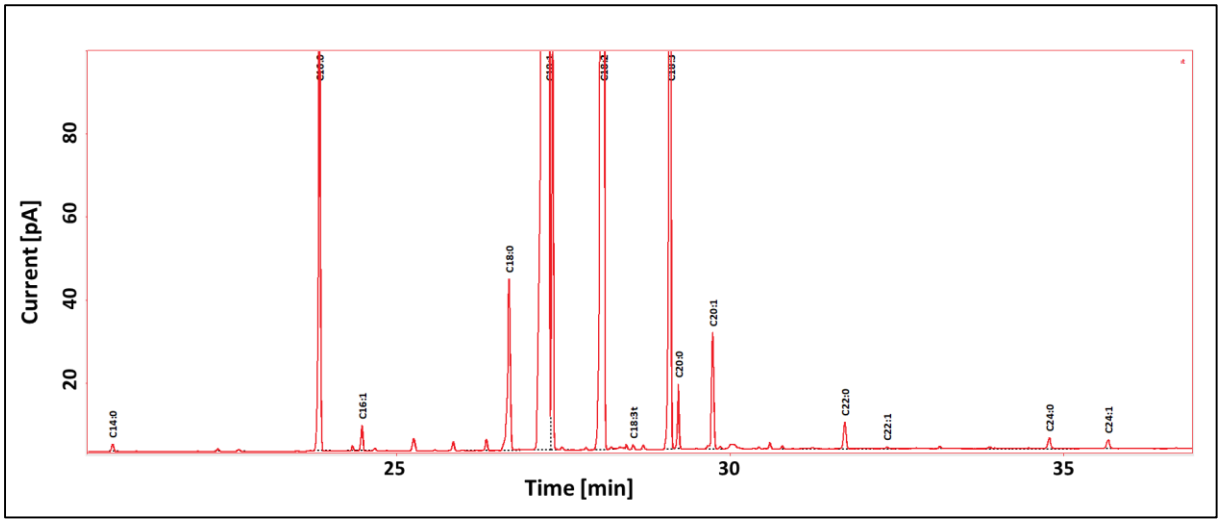

Figure 6: Measurement of fatty acids in a real oleaginous sample.

Summary

The presented automation of FAME analytics according to AOCS specifications allows the entire fully automated analysis of an oleaginous sample. Manual sample preparation is limited to the exchange of solvents and weighing of the sample. Handling with the sample and chemicals as well as injection into the GC-FID system is completely carried out by the autosampler.

At the same time the amount of sample and chemicals is reduced to few milliliters per sample. Additionally, hazard potential is considerably decreased as they rarely have to handle hazardous chemicals. Chemicals need to be exchanged once daily. The procedure includes only a refill of washing reagents and other reagents as needed. Solely complex matrices as feeding stuff and other solid matter require further preparation.

This kind of automation assures the user on the one hand a carryover-free handling and on the other hand a high sample throughput. Sample preparation via CHRONECT Robotic is robust and applicable on a 24 h basis. The total throughput is significantly increased compared to manual handling. Depending on chromatographic conditions 27 to 40 samples may be processed in 24 hours.

Subject to technical changes

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