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

with the CHRONECT Workstation FAMEs

Application note 1602

CHRONECT Workstation FAMEs
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Introduction

Animal and vegetable fats play an important role as a component of our daily diet and as lubricants and lubricating agents. According to the EU Food Information Regulation, food manufacturers have been obliged to indicate the fatty acid composition on the packaging since December 2016. For the declaration, not only the fatty acid composition of fats and oils is examined, but also that of cis- and trans-fatty acids. In addition, the degree of purity is determined.

However, the triglycerides of the fatty acids cannot be analyzed by gas chromatography. This requires cleavage and derivatization of the fat. The ester bonds are broken and the free fatty acids are converted into their corresponding fatty acid methyl esters. In contrast to the corresponding fatty acids, the fatty acid methyl esters (FAMEs) are non-polar, moderately volatile and suitable for GC analysis.

There are different regulations describing an analysis of oil-containing samples for FAMEs. The American Oil Chemists' Society (AOCS) standard Ce 2-66 serves as a template for the application presented here. This standard contains several methods for the workup of grease samples, with Method 3 and Method 4 (Figure 1) of the standard being used for automation. Method 4 is used for the workup of fat samples with an acid number < 2. Method 3, on the other hand, is a comprehensive workup method. However, it is also more complex and time-consuming.

As an alternative, the analogous DGF unit methods C-VI 11(a) exist for sample preparation according to method 3 or the DGF unit method C-VI 11(d) according to method 4.

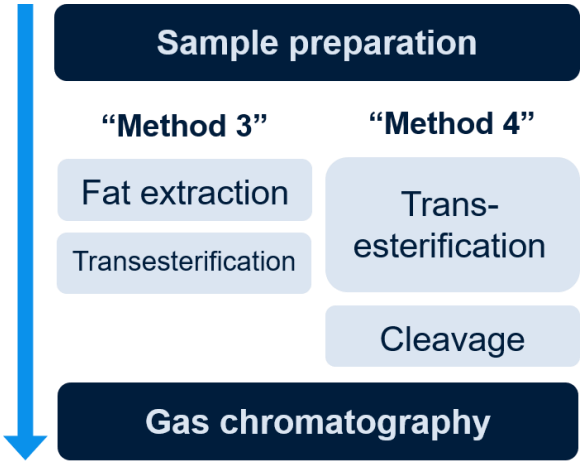

Figure 1: Schematic representation of the sample preparation of an oil sample using the AOCS Ce 2-66 standard.

Instrument configuration

The analysis of a fat for its fatty acids was realized on a system that can perform both sample preparation and analysis fully automatically. A 7890A GC-FID system from Agilent Technologies with an S/SL injector from SIM was used for this purpose. A CHRONECT Robotic RTC Autosampler was used for sample preparation. Figure 2 shows the configuration of the individual modules on the x-axis of the sampler.

Device setup

- Agilent 7890A GC with FID detector, S/SL injector and BPX-70 column 60 m
- CHRONECT Robotic RTC 120 cm with Agitator, 2x park stations, 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
- Clarity by DataApex

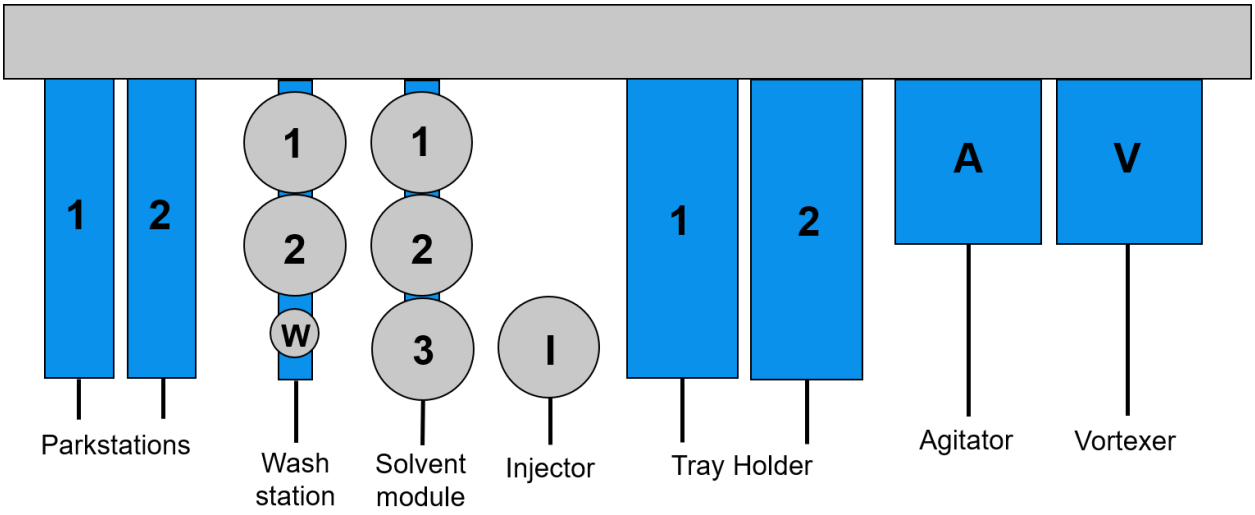

Figure 2: Configuration of the modules of a CHRONECT Robotic RTC on an Agilent 7890A GC with FID.

Measurement parameters and results

Setting up a solution for sample preparation according to AOCS Ce 2-66 with two different sample preparation variants requires a flexible system for automation. The CHRONECT Robotic RTC Sampler with automatic tool change enables exactly this. With two park stations it is possible to use up to 6 different syringe tools for different tasks on the system. This delivers the highest possible level of efficiency and flexibility and helps to avoid carryover.
Combined with the CHRONOS software platform, the result is a powerful automation with intelligent scheduling and stacking and optimal utilization of the entire system. Interfaces to almost all common chromatography evaluation systems open this CHRONECT Robotic solution for analytical systems of almost all manufacturers.

The automation presented here is therefore not bound to a specific instrument configuration. The measurement parameters of the gas chromatograph for the prepared samples were selected from the AOCS standard Ce 1f-96 (Table 1). This standard describes only the chromatographic conditions for FAME analysis.
Method 3 describes sample preparation by alkaline saponification using methanolic sodium hydroxide (meth. NaOH) followed by methylation with methanolic boron trifluoride (meth. BF_3). The mixture is then heated to boiling and diluted with *n*-heptane. This is followed by another boiling phase and then precipitation with saturated sodium chloride. Two phases are formed, and the upper organic phase is dried over sodium sulfate and then injected into the GC-FID. The template for sample preparation is 50 mg oil sample in a 10 mL vial with crimp cap.

Table 1: Parameters of the gas chromatograph.

Heating rate [°C/min]	Final temperature [°C]	Hold 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		
Separation column	BPX 70, 60 m, 250 µm i.d. and 0.25 µm film		

The transesterification takes place completely in the 10 mL vial and the subsequent drying of the sample over sodium sulfate is performed in a 2 mL vial. With the Robotic Tool Change (RTC), the autosampler provides the best solution for preparing these samples without carryover and at the same time fully automating the process. The analysis result of an oil sample prepared by this method is shown in table 2.
By using the CHRONOS platform in combination with the RTC, it is possible to prepare up to 40 oil samples within 24 h and analyze them by GC-FID (Figure 3). In this type of sample preparation, aggressive reagents such as meth. BF_3 are used. To avoid corrosion on the tools of the sampler, regular maintenance of the system is necessary. The magnets on the tools for transporting the vials are particularly susceptible to corrosion. These should be cleaned regularly. For continuous use, this should be done once a month. For samples that are present as solids, this sample preparation is very suitable.

Table 2: Fatty acid composition of an olive oil and comparison with literature data. 1: The values correspond to the Trade Standard Applying to Olive Oil and Olive Pomace Oil of the International Olive Oil Council (Madrid) of 1998.

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 fatty acids	6.4	0.0 – 10.0

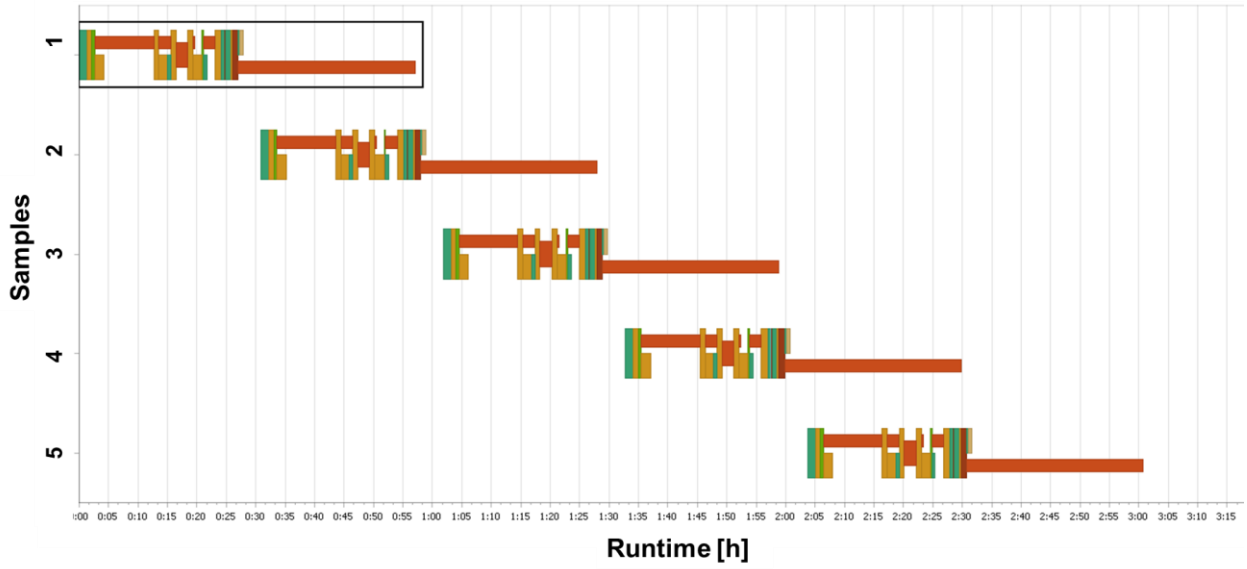

Figure 3: Example of a nested CHRONOS sequence of five samples according to AOCS Ce 2-66 Method 3 within 3 h.

In method 4 of AOCS standard Ce 2-66, transesterification of about 50 mg of sample is done using methanolic potassium hydroxide (meth. KOH). First, the sample is dissolved in n-heptane. After phase separation, the organic phase is washed with water and dried over sodium bisulfate. The dry extract is taken up in *n*-heptane and injected directly from the vial into the GC-FID system (Table 3).

A rough sample preparation procedure is shown in Table 4 and includes all major steps. This type of sample preparation is ideal for processing oil samples in a short time. If desired, sample preparation can be accelerated by up to 40 minutes per sample by using a centrifuge to achieve faster phase separation.

Table 3: General steps of 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~~Transfer	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 4: General steps of sample preparation according to method 3 from the CHRONOS method.

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

This increases the throughput of sample preparation from 32 to approximately 70 samples in 24 h. An analysis result according to this method is shown in Table 5. The processing time per sample can be reduced to a total of 15 minutes if larger vials (10 mL) are used. The amount of solvents, especially heptane, is increased so that a faster phase separation occurs.
The main focus of this application is the automation of the actual sample preparation. One criterion is sufficient rinsing of all syringes used to avoid carryover between different samples and to achieve good phase separation when precipitating the triglyceride.

This leads to a more accurate removal of the organic phase after methylation. In order to inject as clean a sample as possible, the prepared product is then washed with 1:1 water and the organic phase was dried over sodium sulfate.
In addition to developing the sample preparation method, the parameters of the Agilent GC-FID system were adjusted to obtain a sufficiently good separation of the fatty acid methyl esters. For this purpose, a FAME standard with 11 components from C14:0 to C24:0 was injected and the temperature program was adjusted accordingly.

Table 5: Fatty acid distribution of a real oil from the food sector. 2: The data are based on a single measurement of the oil manufacturer with manual sample preparation.

Component	Actual % area	Set % 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

For the development of the method, standard solutions consisting of different fatty acid methyl esters were analyzed. Figure 4 shows a chromatogram of the standard used. With the GC parameters from above, a good separation of all analytes could be achieved. Even C18:3 and C20:0 show an appropriate separation.

In addition, the Restek FAME 37 standard was injected with a total of 37 fatty acid methyl esters to obtain a more accurate calibration of the retention time for the different fatty acids. The chromatogram is shown in Figure 5 and also shows the range from C14:0 to C24:1 with correspondingly more signals in the range.

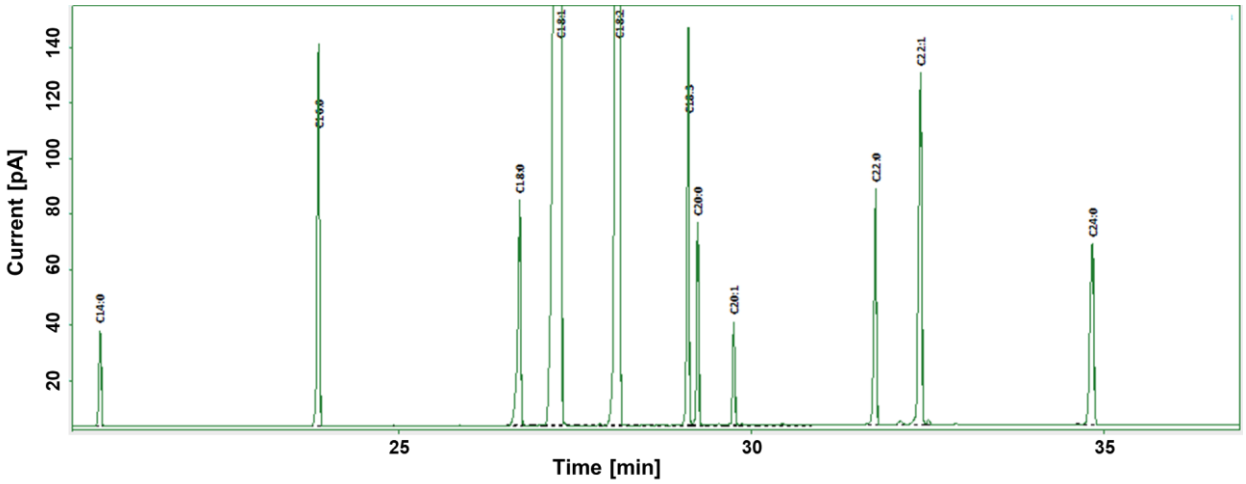

Figure 4: Chromatogram of a separation of 11 FAME analytes from C14:0 to C24:0.

For the validation of the complete method, a real sample was subsequently measured, where the characteristic data were known (Fig. 6). A comparison of the fatty acid composition with the characteristic data showed only slight deviations from the data supplied with the sample (Table 5).

The fully automated analysis of an oil sample thus provides comparable data to the manual sample preparation. The small deviations of the data from those of the oil manufacturer are clearly within the process deviation of partly up to 20 % for individual components close to the signal/noise ratio.

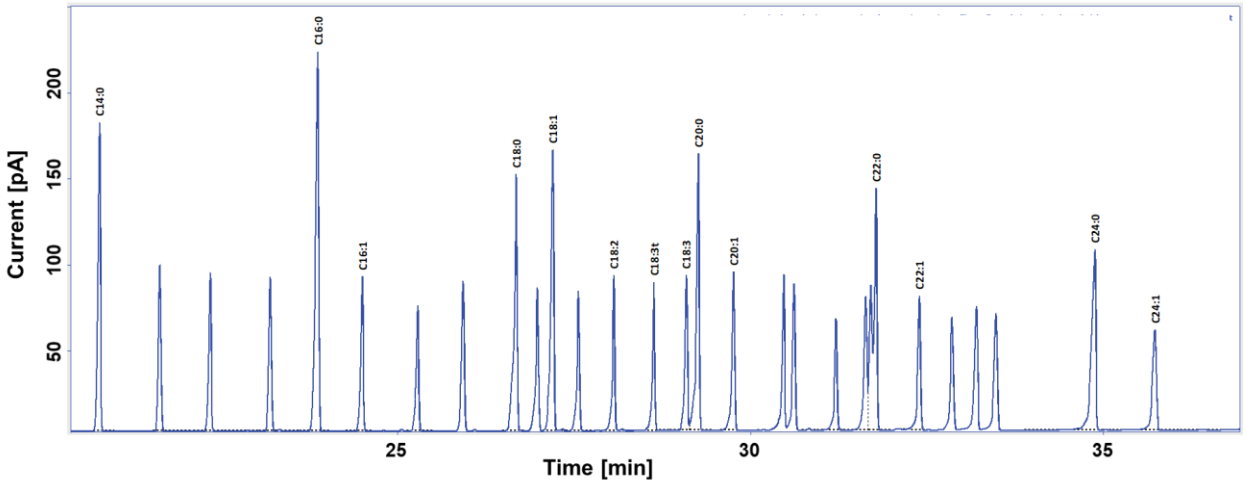

Figure 5: A section of the chromatogram of the Restek FAME 37 standard.

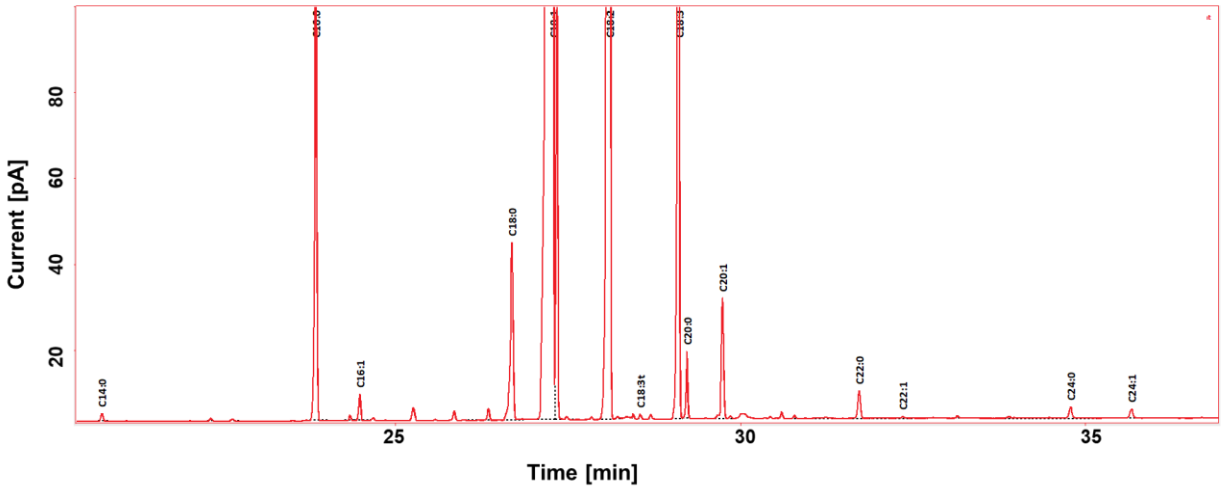

Figure 6: Measurement of fatty acids in a real oil sample.

Summary

The automation of FAME analysis according to the AOCS standard presented here makes it possible to perform the complete analysis of an oil sample fully automatically. The manual sample preparation is reduced to the changing of solvents and the manual weighing of the oil sample. The handling of the sample and the chemicals is completely taken over by the autosampler, as well as the automatic injection into the GC-FID system.
At the same time, the amount of sample and chemicals used is reduced to a few milliliters per sample. In addition, the potential danger for laboratory staff is significantly reduced, as they only come into contact with hazardous chemicals to a limited extent.
Changing the chemical supplies on the autosampler itself can be done once a day and amounts to refilling the wash solutions and possibly topping up the reaction reagents. Only more complicated matrices such as feeds and solids in general may require further pretreatment.

Automation ensures that the user can work without carryover and also achieves a high throughput. Sample preparation via CHRONECT Robotic is robust and can be operated 24 hours a day. The sample throughput can thus be increased compared to manual sample preparation. Depending on the chromatographic conditions, 27 to 40 samples can be processed in 24 hours.

The CHRONECT Workstation FAMEs is a development by Axel Semrau.

Subject to technical changes

Axel Semrau GmbH & Co. KG
Stefansbecke 42
45549 Sprockhövel
Germany
Tel.: +49 2339 / 12090
Fax: +49 2339 / 6030
www.axelsemrau.de
info@axelsemrau.de