Thermal treatment analysis of virgin olive oils

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Introduction

Virgin olive oil can be created only by mild, cold pressing of the olives (Olea europea L.). Thermal or chemical treatment is not allowed in the procedure. After pressing, the virgin olive oils are only purified and filtered. The name, virgin olive oils, is only given to those oils produced by physical techniques such as pressing, filtration, decantation, and centrifugation (Regulation (EG) Nr. 1234/2007, Appendix XVI). There are different analytical methods to differentiate virgin from refined or thermally-treated olive oils. In addition to the determination of stigmastadienes[1] (Figure 1) and chlorophyll degradation products[2] (Figure 2), the analysis of the concentration of polymerized triacylglycerides (PTAGs)[3] (Figure 3) in olive oil is another important factor. The quality of olive oils (especially regarding nutritive value) can be further described in the amount of tocopherols, squalen, and fatty acid composition.

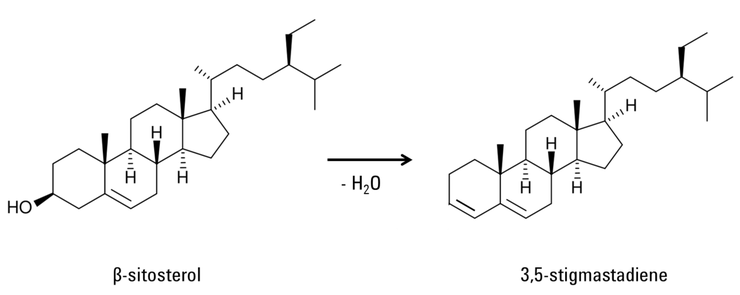
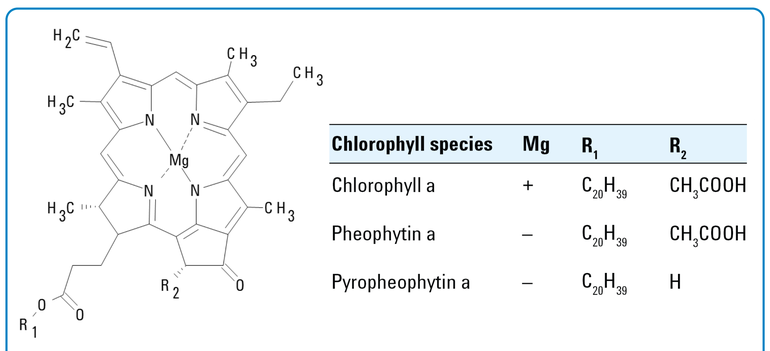


Figure 1 Formation of β-sitosterol to 3,5-stigmastadiene by dehydration.



Chlorophyll species	Mg	R_1	R_2
Chlorophyll a	+	$C_{20}H_{39}$	CH_3COOH
Pheophytin a	–	$C_{20}H_{39}$	CH_3COOH
Pyropheophytin a	–	$C_{20}H_{39}$	H

Figure 2 Structure of chlorophyll and its degradation products

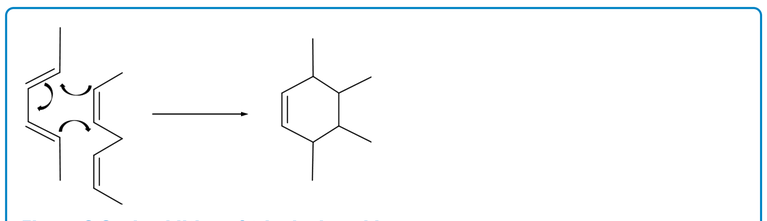


Figure 3 Cycloaddition of triaclyglycerides

During different refining steps, the oil components are chemically altered. This results, predominately, in isomerization, degradation, hydrolysis, oxidation, and polymerization. Stigmastadienes, chlorophyll degradation products or polymerized triglycerides, are normally not present in crude vegetable oils. The determination of stigmastadienes and PTAGs in olive oils also detects minor amounts of refined oils in virgin olive oils and is, therefore, an important quality characteristic for virgin olive oils.

In addition, the amount of chlorophyll degradation products provides information about the storage conditions. Chlorophyll degradation products are generated during storage in different amounts depending on the conditions to which the oil has been exposed. This reaction is boosted by heat treatments during refining processes.

Agilent 1220 Infinity Mobile LC Solution

The Agilent 1220 Infinity Mobile LC Solution is a robust and rugged system for on-site measurement. It is resistant against shocks or vibrations during transportation. The system can be installed in a vehicle and measurements can be performed at different test sites, e.g. olive farms.

Due to the UV detection of the stigmastadienes analysis method, the Agilent 1220 Infinity Mobile LC Solution can be used in a mobile laboratory as a starting point for olive oil quality analysis before further quality analyses are applied in a stationary lab.

Experimental

Systems

1. Agilent Infinity 1220 LC System with Diode Array Detector
2. Agilent 1260 Infinity Quaternary LC system with Refractive Index and Fluorescence detection

Software

OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
OpenLAB CDS 3D UV Add-On software.

Sample preparation

- Stigmastadienes: sample preparation was carried out according to EN ISO 15788-3:2004 (D).
- Chlorophyll degradation products: sample preparation was carried out according to ISO 29841:2009(E).
- Polymerized triglycerides: sample preparation was carried out according to DGF C-III3d (02).

Several olive oils (virgin and partly refined olive oils) were purchased in local stores.

Chromatographic conditions

Table 1 Chromatographic conditions: stigmastadienes

	Long run	Short run
Mobile phase:	ACN/methyl tert-butyl ether (70:30)	
Flow rate:	1 mL/min	
Isocratic run:	Stop time – 30 minutes	Stop time – 5 minutes
Injection volume:	10–50 µL	20 µL
Temperature TCC:	RT	
DAD:	235 nm/4 nm Ref.: off	
Peak width:	>0.05 minutes (1.0 seconds response time) (5 Hz)	

Columns: Agilent LiChrospher C18, 4 × 250 mm, 5 µm, Agilent ZORBAX Extend-C18 RRHT, 4.6 × 50 mm 1.8 µm

Table 2 Chromatographic conditions: chlorophyll degradation products

	Long run	Short run
Mobile phase:	Water:methanol:acetone (4:36:60)	
Flow rate:	1 mL/min	1.2 mL/min
Isocratic run:	Stop time – 30 minutes	Stop time – 8 minutes
Injection volume:	20 µL	
Temperature TCC:	RT	20 °C
FLD:	Ex: 430 nm, Em: 670 nm	
Peak width:	> 0.05 min (1 s resp. time) (9.26 Hz)	

Columns: Agilent ZORBAX SB-C18, 4.6 × 250 mm, 3.5 µm, Agilent ZORBAX RRHT SB-C18, 4.6 × 50 mm, 1.8 µm

Table 3 Chromatographic conditions: polymerized triglycerides

Mobile phase:	THF
Flow rate:	0.8 mL/min
Isocratic run:	Stop time – 35 minutes
Injection volume:	40 µL
Temperature TCC:	35 °C
RID:	35 °C
Peak width:	>0.2 minutes (4 seconds response time) (2.28 Hz)

Columns: 2x Agilent PLgel 3 µm MIXED-E, 7.5 × 300 mm, 3 µm together with guard column Agilent MIXED Guard 7.5 × 50 mm, 3 µm

Results and Discussion

In contrast to virgin olive oils, 3,5-stigmastadienes were detected in partly refined olive oil (mix of refined and virgin oils), (Figure 4). Table 1 shows an overview for six virgin and one partly refined olive oil sample.

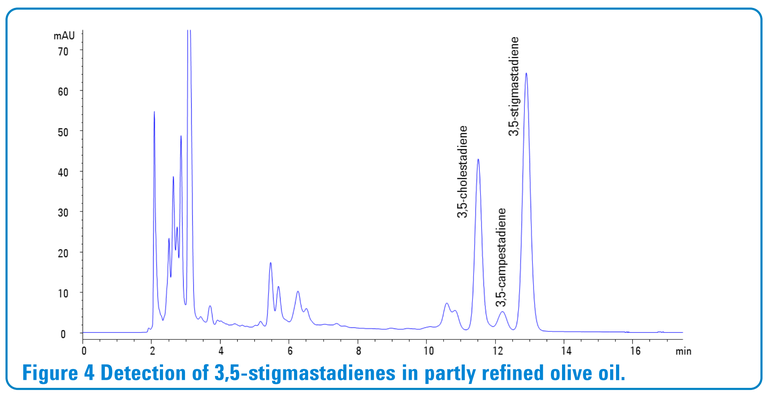


Figure 4 Detection of 3,5-stigmastadienes in partly refined olive oil.

Table 1 Relative content of pyropheophytin in seven olive oils.

Olive oil	1	2	3	4	5	6	Mix of refined and virgin oils
Content of 3,5-stigmastadiene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.63 mg/kg

Figure 5 shows the comparison between chromatograms obtained with samples from virgin olive oil (A) and partly refined olive oil (B). In contrast to the partly refined olive oil chromatogram, the virgin olive oil sample showed only a negligible peak in front of the triglyceride peak.

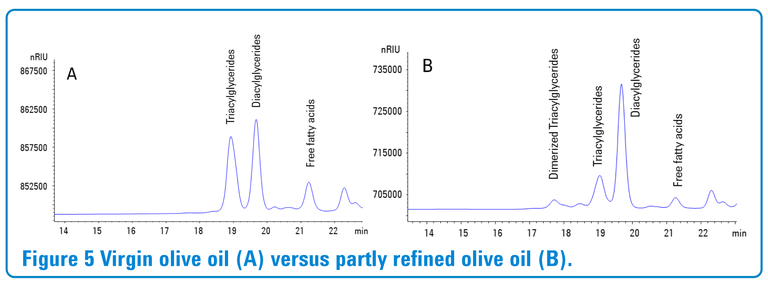


Figure 5 Virgin olive oil (A) versus partly refined olive oil (B).

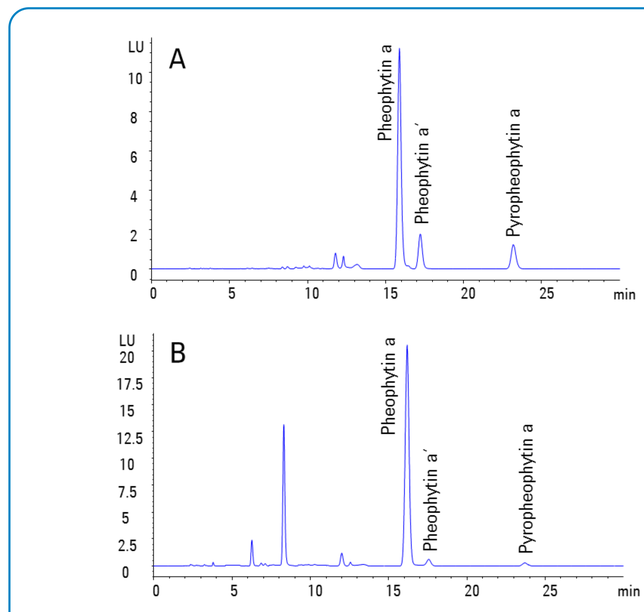


Figure 6 Separation of chlorophyll a and its degradation products in virgin olive oil of the same brand, harvest 2011 (A) and harvest 2012 (B).

Table 2 Relative content of pyropheophytin in seven olive oils.

Harvest	2011	2011	2011	2011/2012	2011/2012	2012	n.k.
Olive oil	Virgin olive oil 1	Virgin olive oil 2	Virgin olive oil 3	Virgin olive oil 4	Virgin olive oil 5	Virgin olive oil 6	Partly refined olive oil
% pyropheophytin	13.54	11.50	13.10	14.76	8.12	1.46	18.03

Figure 6 shows the separation of chlorophyll degradation products in virgin olive oil (same brand) from olives from the same region, but from two different harvesting years, 2011 and 2012. The percentage of pyropheophytin is substantially higher (11.5%) in the virgin olive oil from 2011 compared to the one from 2012 (1.5%), (Table 2). Pyropheophytin is not present in freshly extracted oils, but its concentration increases during storage depending on conditions such as light and temperature.

To accelerate the analysis time of the stigmastadienes and chlorophyll degradation products in olive oil, short 50-mm columns with sub-2 µm particles were used. Figure 7 shows a short run of the analysis of the fresh virgin olive oil in 8 minutes, providing great resolution between the pheophytins and pyropheophytin a. The stigmastadiene analysis could be shortened down to 5 minutes.

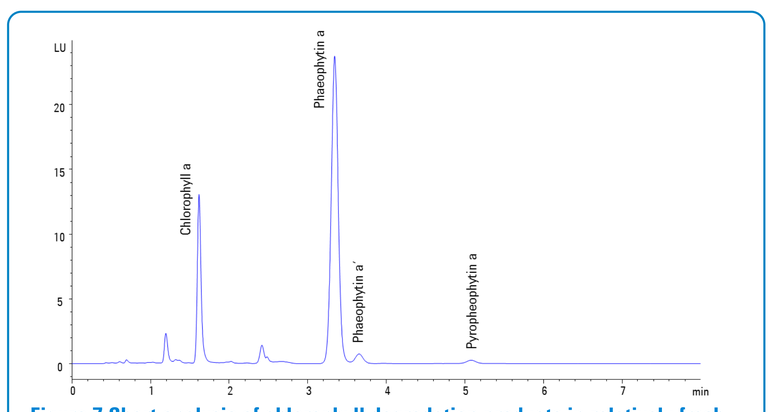


Figure 7 Short analysis of chlorophyll degradation products in relatively fresh virgin olive oil.

Conclusions

Seven olive oil samples were analyzed regarding thermal treatment processes during olive oil production:

- 3,5-stigmastadienes were analyzed using the Agilent 1220 Infinity Mobile LC..
- Polymerized triacylglycerides (PTAGs) were analyzed using the Agilent 1260 Infinity LC with size exclusion chromatography/gel permeation chromatography (SEC/GPC) with refractive index detection (RID).
- Chlorophyll degradation products pheophytin and pyropheophytin were analyzed using the Agilent 1260 Infinity LC with fluorescence detection.

References:

1. *Quality Analysis of Virgin Olive Oils – Part 1, Application Note, Agilent Publication Number 5991-1894EN, 2013.*
2. *Quality Analysis of Virgin Olive Oils – Part 1, Application Note, Agilent Publication Number 5991-1894EN, 2013.*
3. *Quality Analysis of Virgin Olive Oils – Part 3, Agilent Application Note, Publication Number 5991-1896EN, 2013.*