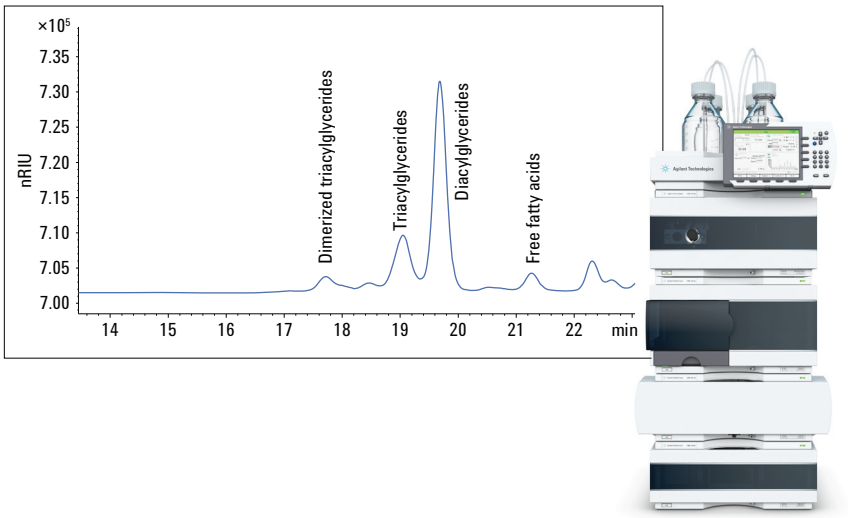
Quality analysis of virgin olive oils – Part 2

Thermal treatment analysis – determination of polymerized triglycerides with SEC/GPC using the Agilent 1260 Infinity LC System with Refractive Index Detection

Application Note

Food Analysis & Agriculture



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Abstract

This Application Note shows the analysis of polymerized triacylglycerides in olive oil using the Agilent 1260 Infinity LC with size exclusion chromatography/gel permeation chromatography (SEC/GPC) with refractive index detection (RID). The analysis of six virgin olive oils revealed only minor amounts of polymerized triacylglycerides (PTAGs) indicating no refining or other thermal treatment processes. The partly refined olive oil (a mix of refined and virgin olive oils) revealed substantial amounts of PTAGs. In addition, the amount of free fatty acids in extra virgin olive oil could be determined using the same chromatographic conditions as for the PTAG analysis with SEC and RI detection.

Verified for Agilent
1260 Infinity II LC

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Introduction

Virgin olive oil is produced only through mild, cold pressing; thermal or chemical treatment is not permitted in the procedure. After pressing, the virgin olive oils are only purified and filtered. The name, virgin olive oil, is only permitted if it is produced using 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 heat-treated olive oils. In addition to the determination of stigmastadienes[1] and chlorophyll degradation products[2], another important factor is the analysis of the concentration of polymerized triacylglycerides (PTAGs) in olive oil.

During different refining steps (for example bleaching, deodorization, degumming, and neutralization of free fatty acids), the oil components are chemically altered. This results, predominately, in isomerization, hydrolysis, oxidation, and polymerization[3]. Polymerized triacylglycerides, generically used for dimerized, trimerized, and polymerized triglycerides, are normally not present in crude vegetable oils. In virgin olive oils, the maximum concentration is below 0.05 g/100 g sample[4].

The level of polymerization, especially dimerization of triacylglycerides (Figure 1), increases after the single refining steps, shown in Table 1.

Dimer triacylglyceride formation starts at 90 °C, and, with elevating temperatures during refining process or other thermal treatments, the amount of dimerization increases correspondingly. In addition to many other degradation processes, PTAGs are also formed during deep frying food products. Therefore, the determination of PTAGs can be used to indicate the degradation state of deep frying oils.

Several methods are described for the analysis of PTAGs in vegetable oils. In this Application Note, the analysis presented by Gertz in 1977[5] (modified by Guhr *et al*. 1981[6]) was used. This method was adopted by the IUPAC, GDF and ISO. For minor amounts of PTAGs (<3%) in vegetable oils, the DGF C-III3d (02) method was used for sample preparation.

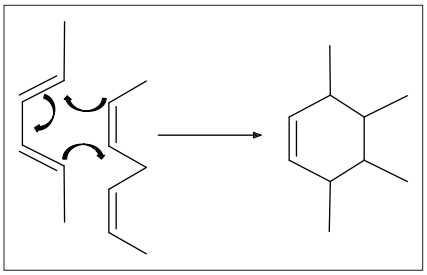

Figure 1
Cycloaddition.

Process	Range of applied temperature (°C)
Filtration/centrifugation	40 – 70
Degumming	50 – 80
Neutralization	90 – 95
Bleaching	90 – 120
Deodorizing	180 – 250

Table 1
Refining processes.

The polar fraction, containing the dimerized and other polymerized triacylglycerides, is extracted on silica columns using diethyl ether/petroleum ether. This fraction is then analyzed using size exclusion chromatography/gel permeation chromatography (SEC/GPC) with refractive index detection (RID), which allows quantification of the main groups of compounds formed during the thermal treatment of refining or deep frying process.

Experimental

The Agilent 1260 Infinity Quaternary LC System consisted of the following modules:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Refractive Index Detector (G1362A)

Sample

The standards of triacylglycerides (TAGs), diacylglycerides (DAGs), monoacylglycerides (MAGs), and free fatty acid methyl ester (FAME) (Supelco standards) were purchased from Sigma-Aldrich, St. Louis, MO, USA and were dissolved in tetrahydrofuran (THF). Several olive oils (virgin and partly refined olive oils) were purchased in local stores. The SPE extraction was carried out using Bond Elut SI cartridges 1 g, 6 mL (p/n 12256008). Sample preparation was carried out according to DGF C-III3d (02).

Solvents

All solvents used were LC grade. THF, petroleum ether, and diethyl ether were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Columns

2x Agilent PLgel 3 µm MIXED-E, 7.5 × 300 mm, 3 µm (p/n PL1110-6300) together with guard column MIXED Guard 7.5 × 50 mm, 3 µm (p/n PL1110-1320)

Software

OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]

Chromatographic conditions

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)

Table 2
Chromatographic conditions.

Results and Discussion

The injected standard mix containing TAGs, DAGs, MAGs, and FAME is shown in the following chromatogram (Figure 2). This was used for further peak identification based on retention time. In addition, the relative standard deviation (% RSD) for the retention times and area was determined using this standard mixture. The method showed high retention time and area precision with RSD values <0.041% and <1.9% respectively for all four compounds. Polymerized triglycerides would be expected to elute before the triacylglycerides due to the larger molecule size of the PTAGs.

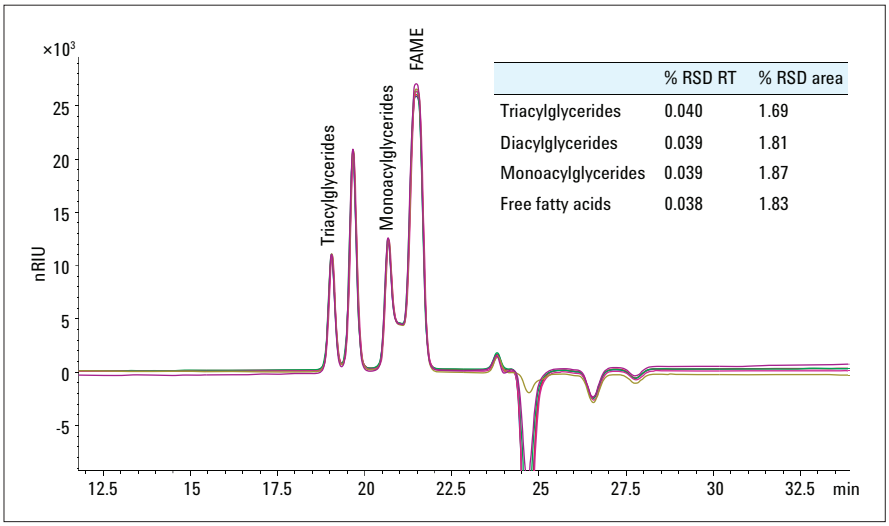

Figure 2
Standard mix containing TAGs, DAGs, MAGs, and FAME.

Seven olive oils were analyzed for PTAGs after solid phase extraction (SPE). Figure 3 shows an overlay of six chromatograms of six different virgin olive oil samples. Only very small peaks were detected in front of the triacylglyceride peak, pointing to insignificant amounts of PTAGs. Table 3 shows detailed numbers. The values were calculated using Formula 1. Extra virgin olive oil (diluted in THF) was used without SPE fractionation as the reference standard for the calculation of PTAGs.

The allowed maximum concentration of PTAGs in virgin olive oils is below 0.05 g/100 g sample[4]. All tested virgin olive oils were below the required concentration with the highest amount being 0.013 g/100 g sample. The partly refined olive oil sample, composed of a mixture of refined and virgin oils, had significantly higher amounts of PTAGs compared to the virgin oils (Table 3).

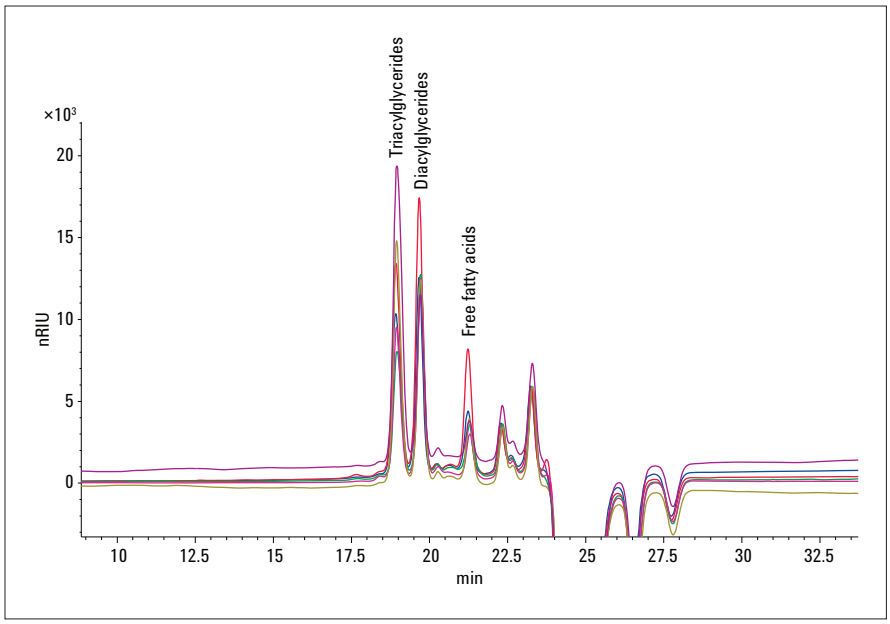

Figure 3
Virgin olive oil analysis after SPE. Overlay of six chromatograms of six different virgin olive oil samples.

Olive oil	1	2	3	4	5	6	Mix of refined and virgin oils
Content of PTAGs	0.005 g	0.013 g	0.002 g	0.005 g	0.004 g	0.004 g	0.224 g

Table 3
Content of PTAGs in g/100 g sample.

$$w = \frac{A_P \times E_V \times G_V}{F \times E_P \times A_{TG}}$$

w = Content of PTAGs in g/100 g sample
A_P = Peak area of PTAGs
E_V = Concentration of reference standard in g/mL
G_V = Percentage of TAGs in reference standard in % of all peak areas
F = Concentration factor
E_P = Sample concentration in g/mL
A_{TG} = Peak area of the TAGs in reference standard

Formula 1
Calculation of the amount of PTAGs in g/100 g sample[4].

Figure 4 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.

Extremely high amounts of PTAGs were found after a thermal treatment of the oils at 180 °C for over 20 hours. Not only dimerized and trimerized triacylglycerides were visible, as normally found in refined vegetable oils, but also many higher polymerized triglycerides, which cannot be separated into single peaks, resulting in a very broad peak, (Figure 5).

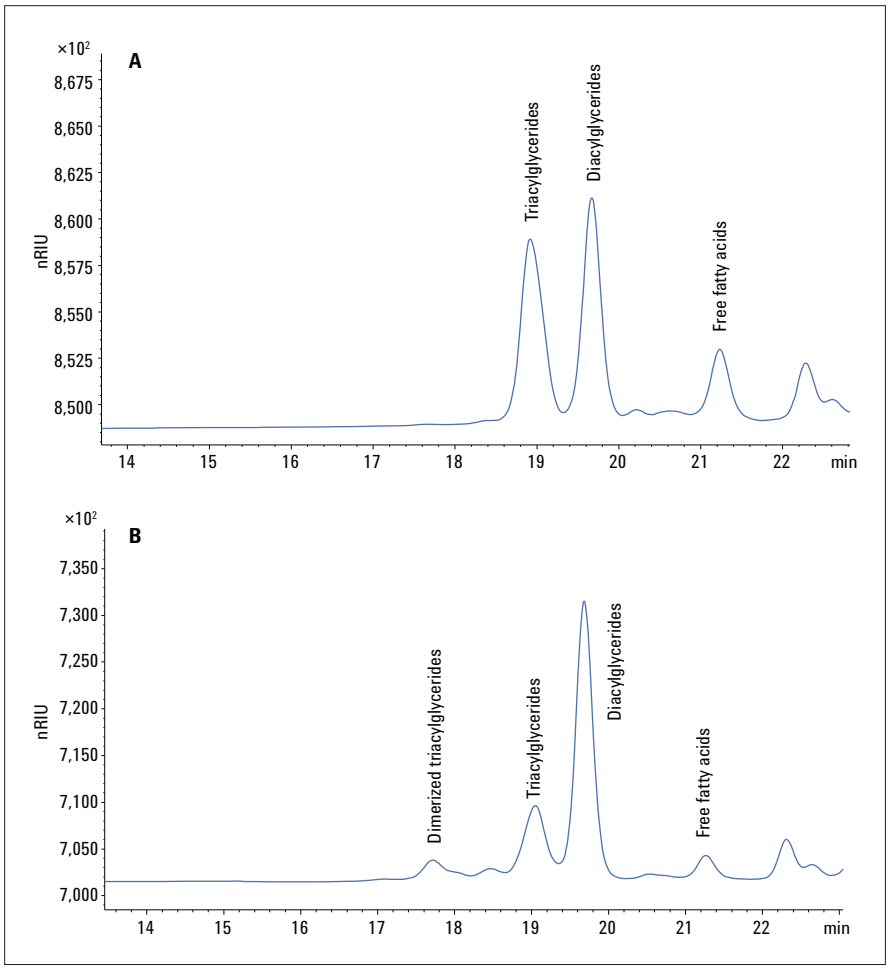

Figure 4
Virgin olive oil (A) versus partly refined olive oil (B).

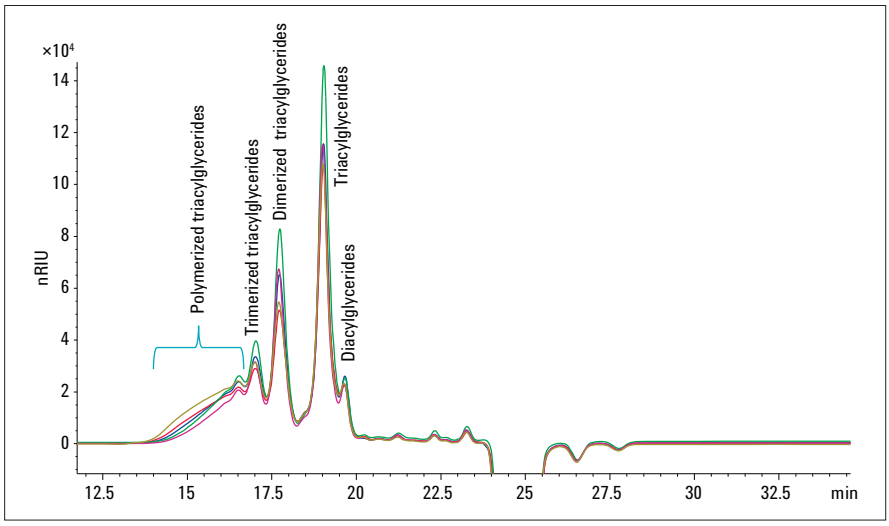

Figure 5
Thermally treated olive oils, containing high amounts of PTAGs.

With the combination of SEC and RI detection, the amount of free fatty acids could be determined. This can also be used as a quality marker for olive oils. Depending on the classification of the olive oil, different amounts free fatty acids are allowed in the oils according to EU regulations, for example, a maximum of 2% in virgin olive oil and 0.8% in extra virgin olive oil. Using Formula 1, the amount of free fatty acids in olive oil in g/100 g sample was calculated, replacing the values for PTAGs with the values for the free fatty acids. Sample preparation of the oils (using SPE) was not necessary because that would have led to a concentration of the free fatty acids, which would have adulterated the results to much higher amounts. Figure 6 shows the analysis of extra virgin olive oil without SPE (also used as reference standard in Formula 1 to calculate the amount of PTAGs). The amount of free fatty acids was calculated as 0.554 g in 100 g oil, which was in the expected range for extra virgin olive oil (max. 0.8%). For more exact results, a calibration using external standards is recommended.

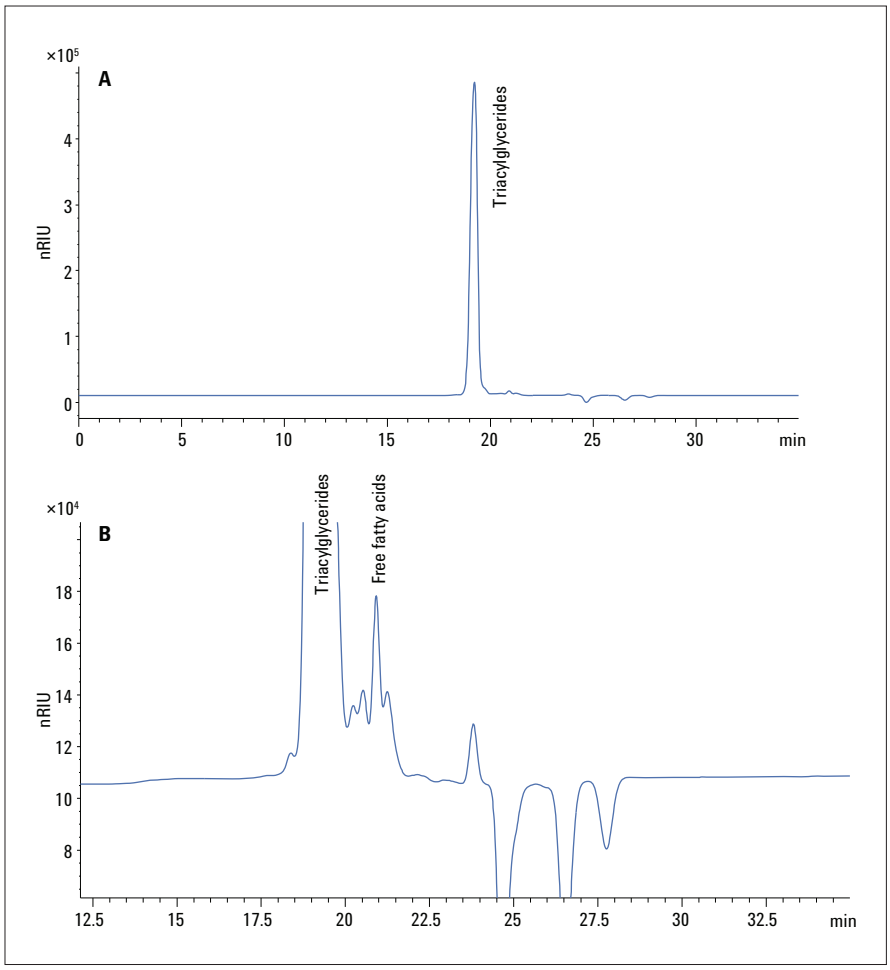

Figure 6
Analysis of extra virgin olive oil without SPE (A), zoomed (B), used as reference standard and for the determination of the amount of free fatty acids in g/100 g sample.

Summary and Conclusion

Seven olive oils were analyzed for the occurrence of PTAGs indicating refining processes or other thermal treatments according to DGF C-III3d (02). Only negligible amounts of PTAGs were detected in all six virgin olive oils. Amounts of PTAGs found in virgin olive oils were far below the maximum acceptable concentration of PTAGs in virgin olive oils of 0.05 g/100 g sample. Only the partly refined olive oil (a mix of refined and virgin olive oils) revealed substantial amounts of PTAGs. The standard mix of TAGs, DAGs, MAGs, and FAME showed highly precise retention times and areas indicating excellent identification and quantification capabilities for this method.

In addition, the amount of free fatty acids in extra virgin olive oil could be determined using the same chromatographic conditions as for the PTAG analysis with SEC and RI detection.

The Agilent 1260 Infinity LC for SEC/GPC with RI detection is an optimum solution for the determination of PTAGs in olive oils.

References

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