

# Quality Analysis of Extra Virgin Olive Oils – Part 8

## Nutritive Benefits—Triacylglycerol Composition in Virgin Olive Oils

### Application Note

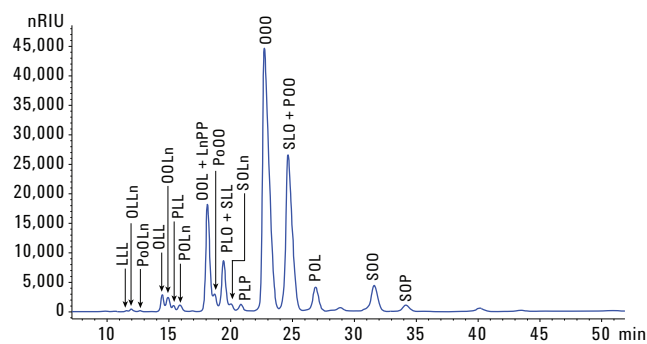
Food Testing & Agriculture

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#### Abstract

The triacylglycerol (TAG) profile of extra virgin olive oil can be used as a fingerprint to determine the variety or the production origin of monovarietal oils (oils produced from a single olive variety). This Application Note describes the TAG profile analysis of olive oil after solid phase extraction (SPE) according to the protocol from the Commission Regulation (EEC) No 2472/97, adapted to Agilent BondElut cartridges. The isocratic reversed phase analysis with subsequent refractive index (RI) detection allowed the quantification of 17 different TAGs, which were used for the classification of the olive oils. The precision of the method was very good with respect to retention time and area.



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## Introduction

Virgin olive oil is obtained from the fruits of the olive tree (*Olea europaea* L.) by mechanical procedures only, without the use of any thermal or chemical treatment. In the last two decades, numerous methods have been developed for the evaluation of vegetable oils to ensure authenticity of virgin olive oil with respect to adulteration, mislabeling, characterization, or misleading origin<sup>1</sup>. The analysis of thermally treated olive oils has been shown in previous Application Notes<sup>2,3,4</sup>. With respect to nutritive benefits, virgin olive oil is a good source of several bioactive components related to highly chemoprotective effects on human health.

Olive oil is primarily composed of triacylglycerols, and contains small amounts of highly valuable antioxidants such as vitamin E<sup>5</sup>, squalene<sup>6</sup>, phytosterols, carotenoids, and phenols<sup>7,8</sup>. Triacylglycerols are molecules derived from the esterification of three fatty acids with one glycerol molecule, as shown for triolein (Figure 1), a triacylglycerol containing three oleic acid molecules, representing the most abundant (up to 83 %) fatty acid present in olive oil<sup>9</sup>.

The TAG composition in olive oils can be used as a factor to determine the origin of monovarietal oils (oils produced from a single olive variety). In addition, the presence of trilinolein (LLL) indicates the presence of seed oils in olive oil. However, the latter is usually analyzed by determining the differences between experimental and theoretical values of TAGs with an equivalent carbon number equal to 42, the  $\Delta ECN_{42}$  method<sup>10</sup>. In this method, the oil is previously purified (solid phase extraction over a silica-gel column) before the TAG composition is

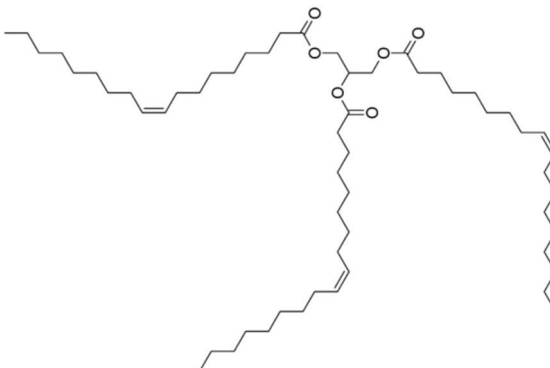


Figure 1. Triolein (OOO), triacylglycerol containing three oleic acids.

determined by isocratic normal phase high performance liquid chromatography (HPLC) followed by refractive index (RI) detection. The TAGs are separated according to their equivalent carbon number (ECN), which is defined by following formula:

$$ECN = CN - 2n$$

CN = acyl carbon number

n = number of double bonds in fatty acids constituting the triacylglycerols

The triacylglycerols, which are mainly found (between 3 and 59 %) in olive oil, are OOO, POO, OOL, POL, and SOO, in addition to other TAGs, present in smaller amounts (O – Oleic acid, P – Palmitic acid, S – Stearic acid, L – Linoleic acid, and Ln – Linolenic acid).

This Application Note shows the analysis of TAGs after sample preparation according to Commission Regulation (EEC) No 2472/97 for 10 different olive oils. The results were used to classify the oils according to different olive varieties.

## Experimental

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

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity High Performance Autosampler (G1367E)
- Agilent 1290 Infinity Thermostat (G1330B) for sample cooling
- Agilent 1290 Thermostatted Column Compartment (G1316C)
- Agilent 1260 Infinity Refractive Index Detector (G1362A)

## Sample

Triacylglycerol standards (triolein and glyceryl trilinoleate) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Several olive oils were purchased directly from Italian olive oil farms or in local stores. All solvents were LC grade.

Sample preparation was carried out according to the protocol from the EEC No 2472/97, adapted to Agilent BondElut cartridges 10 g, 60 mL (p/n 14256034). One gram of oil was dissolved in 10 mL BE-solvent (petroleum ether : diethylether 87:13).

The cartridge was washed twice with 10 mL of BE-solvent, and the sample was applied onto the BondElut cartridge. Elution was carried out with 2 × 30 mL of BE-solvent into a 100-mL round bottom flask (tared and weighed). The solvent was evaporated to dryness in a rotary evaporator (~ 90 % recovery was expected). For analysis, a 5 % solution of the sample was prepared in acetone (0.5 g in a 10-mL volumetric flask).

### Columns

Agilent Superspher 100 RP-18, 4.0 × 250 mm, 4 μm (p/n 7992518-484) together with LiChrospher 100 RP-18 Guard, 4.0 × 4 mm, 5 μm (799250D-504)

### Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems Rev. C.01.05 [38]

### Results and Discussion

Figure 2A shows the analysis of the standards glyceryl trilinoleate (LLL) and triolein (OOO), whereas Figure 2B shows the separation of the TAG content of an extra virgin olive oil after solid phase extraction according to the protocol described above. The analysis allowed the quantification of 17 different triacylglycerols. The method showed very good precision of retention time (RT) and area. The relative standard deviation (RSD) for RT was < 0.1 % for most of the analyzed TAGs except for POL, SOO, and SOP with RSDs < 0.15 %. The area RSD was < 1 % for most of the analyzed TAGs except for SLO + POO with RSDs < 2 %. Only POL showed a higher RSD of 4.4 %.

Ten different olive oils were analyzed for their TAG profile, nine extra virgin olive oils and one refined olive oil. Seven olive oils consisted of blends of oils from different olive varieties, whereas three olive oils were produced from a single olive variety (monovarietal oils).

### Chromatographic conditions

Table 1. Chromatographic conditions.

Isocratic run with acetone : ACN 60:40	
Stop time	55 minutes
Flow rate	1 mL/min
Injection volume	1 μL
Thermostat autosampler and FC	18 °C
Temperature TCC	30 °C
RI	
Temperature	30 °C
Peak width	> 0.2 minutes (4 seconds response time) (2.28 Hz)

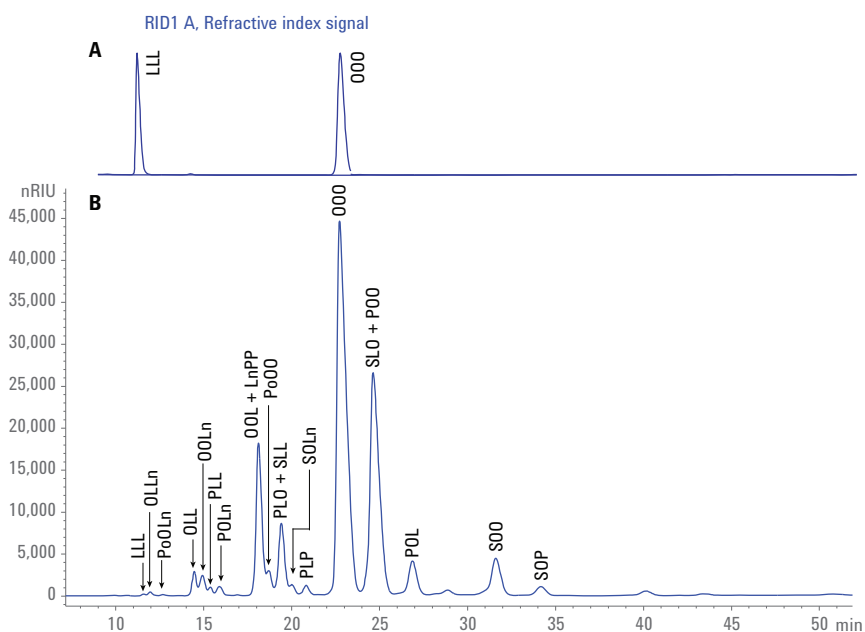


Figure 2. A) Analysis of standard TAGs LLL and OOO; B) analysis of extra virgin olive oil after solid phase extraction.

Table 2 shows the TAG profiles of 10 different olive oils, where the amount of the single TAGs is expressed in %, so that the total amount of all TAGs detected equals 100 %. Triolein (OOO) represents the TAG with the highest concentration in olive oil, ranging from ~ 38 % up to ~ 46 % in the analyzed oils. Using this TAG profile with the TAG amount in %, a Principal Component Analysis (PCA) was carried out to classify the different oils.

Using the PCA, monovarietal olive oils can be classified for their olive variety or their production origin (Figure 3). Here, the two principal components PC1 and PC2 account for approximately 68 % of the variance in the data. The Number 1 and Number 4 monovarietal olive oils were found in a group separated from the others. This was expected, as these oils are monovarietal olive oils from the same variety. In addition, the Number 9

monovarietal oil contains an olive oil variety that is not found in the other oils with the exception of a small amount in olive oil Number 10, which is proven by the closer grouping of Numbers 10 to 9. All the other oils are classified in different groups. The biplot in Figure 3 shows, in addition to the allocation of the olive oils, the underlying TAG profile used for the calculation of the olive oil classification.

Table 2. TAG profile of 10 different olive oils; the amount of TAGs is expressed in %. The sum of all 17 detected TAGs is 100 %.

	1 Monovarietal	2 (refined) Blend	3 Blend	4 Monovarietal	5 Blend	6 Blend	7 Blend	8 Blend	9 Monovarietal	10 Blend
LLL	0.07	0.23	0.07	0.15	0.22	0.05	0.08	0.12	0.06	0.07
OLLn	0.20	0.29	0.22	0.22	0.32	0.17	0.20	0.23	0.20	0.23
PoOLn	0.06	0.07	0.06	0.07	0.10	0.04	0.00	0.05	0.08	0.08
OLL	1.21	2.41	1.34	1.25	2.30	1.29	1.27	2.28	1.18	1.42
OOLn	1.22	1.16	1.24	1.21	1.16	1.14	1.21	1.09	1.12	1.14
PLL	0.28	0.88	0.24	0.25	0.49	0.22	0.22	0.60	0.53	0.35
POLn	0.46	0.25	0.40	0.44	0.35	0.40	0.37	0.22	0.30	0.44
OOL+ LnPP	10.93	12.20	12.03	10.23	14.16	12.66	9.74	13.45	10.68	11.61
PoOO	1.44	1.77	0.92	1.43	0.93	0.48	1.32	1.39	1.28	1.47
PLO + SLL	5.42	7.20	4.98	4.81	6.56	4.80	4.13	7.15	6.15	6.23
SOLn	0.55	0.91	0.37	0.57	0.40	0.21	0.51	0.75	0.57	0.62
PLP	0.58	1.03	0.48	0.53	0.70	0.43	0.50	1.02	0.70	0.67
OOO	43.32	38.61	45.56	43.31	38.67	46.17	45.13	37.93	38.64	39.44
SLO + POO	25.22	23.52	22.98	25.71	22.86	23.31	24.25	23.77	28.42	26.52
POL	3.46	3.70	3.19	3.45	3.35	2.95	3.25	3.86	4.88	4.17
SOO	4.50	4.85	4.77	5.15	5.92	4.64	6.26	4.84	3.95	4.35
SOP	1.07	0.93	1.14	1.21	1.52	1.04	1.57	1.25	1.27	1.20

## Conclusion

Ten olive oils were processed with solid phase extraction according to the protocol from the Commission Regulation (EEC) No 2472/97, adapted to Agilent BondElut cartridges. Seventeen different TAGs could be quantified after isocratic analysis on an Agilent Superspher column with subsequent RI detection. The precision of the method was very good with respect to retention time and area. The resulting TAG profiles were subsequently used for the variety and origin classification of monovarietal olive oils using principle component analysis. Monovarietal oils from the same variety could be grouped together, and be differentiated from the other varieties.

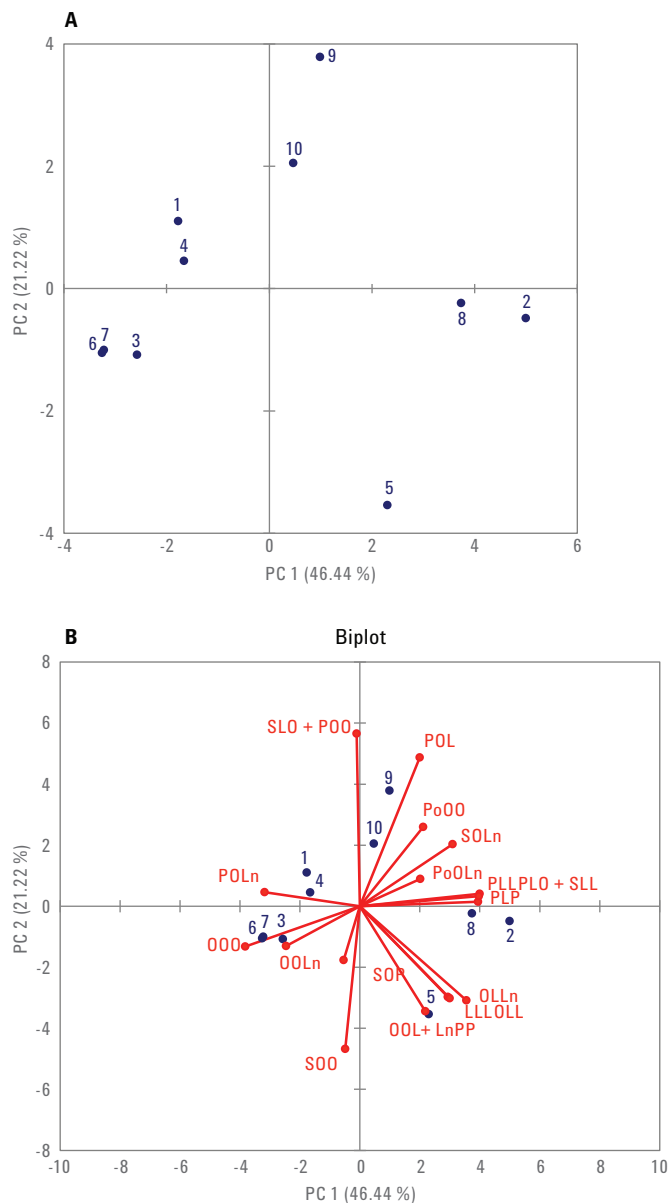


Figure 3. Principal component analysis of 10 different olive oils. The two principal components PC1 and PC2 account for approximately 68 % of the variance in the data. A) The grouping of the different oils represent the origin of variety, the biplot (B) shows both, the component profile (red) and the consequential allocation of the olive oils (blue).

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