Olive oil triglycerides separation by HPLC and on-line DAD and RID detection: a contribution to identify extra virgin oil blends with soft-deodorised olive oils

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Extra virgin olive oil is one of the healthiest vegetable oils and it is the best source of fats in the Mediterranean Diet. Olive tree cultivation and olive oil consumption spread all over the world and since extra virgin olive oil is also one of the most expensive oils, often undergoes fraudulent practices by mixing it with lower grade oils. Improved knowledge of olive oils and technology may give rise to a frequent extra virgin counterfeiting by mixing authentic extra virgin olive oils with the so-called soft- or mild-deodorised oils: these are virgin oils, deodorised in a soft way to distillate unpleasant compounds so that oils can be blended with real extra virgin oils and be illegally sold as if they were fully authentic. The aim of this paper is to describe an approach that takes into consideration the ultraviolet absorbency of each triglyceride in soft-deodorised oils or micro- or ultra-filtered oils and their blends with authentic extra virgin oils. Further data elaboration by principal component analysis allowed us to clearly distinguish false extra virgin oils from authentic. Furthermore, chromatographic separation enables us to calculate the ECN42 without performing a new HPLC separation according to the Official method, as required by the law in force.

Keywords: Soft/mild-deodorised olive oil, crossflow micro/ultra- filtered oil, HPLC, DAD, RID, PCA

List of abbreviations used:

IOC: International Olive Council
EU: European Union
GC-IMS: gas-chromatography ion mobility spectrometry
FGC-Enose: flash gas-chromatography electronic nose
NIR: Near infrared
MIR: Medium infrared
MF: Crossflow microfiltration
UF: Crossflow ultrafiltration
TDR: Time Domain Reflectometry
FAEE: Fatty acid ethyl ester
TAG: Triacylglycerol
DAD: Diode Array Detector
RID: Refractive Index Detector
ECN: Equivalent Carbon Number
ΔECN: Difference between calculated ECN and experimental ECN
SPE: Solid Phase Extraction
UV: Ultraviolet
PCA: Principal Component Analysis
EV: Extra virgin olive oil
Δ: it refers to a difference
NARP-HPLC-APCI-MS: Non aqueous reverse phase-high performance chromatography-atmospheric pressure chemical ionization-mass spectrometry

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1. INTRODUCTION

Olive oil is among the foods with a history that dates back thousands of years and is typical of the Mediterranean [1, 2], even if the olive tree seems to come from Asia Minor and, before, from the southern Caucasus, from the Iranian plateau, from the Mediterranean coasts of Syria and Palestine (IOC) [3] and, originally, from the "Fertile Crescent". It is recognised by numerous studies that olive oil is the preferable fat in human nutrition and has become perhaps the most characteristic ingredient of the Mediterranean diet [4]. Olive cultivation has spread from the Mediterranean to very distant areas such as South America (e.g. in Argentina), the United States (e.g. in California), South Africa (e.g. in the Cape Province), up to the Far East such as China and Japan, and even Australia and New Zealand. Appreciation for olive oil and its knowledge has naturally developed in these geographical areas. Where deemed necessary, the olive oil market has been regulated by laws aimed at guaranteeing its nature and authenticity. At an international level, the reference body is the International Olive Council (IOC) [5] which is based in Madrid (Spain). It currently includes 19 countries including the European Union as a single member. Although not all world markets interested in the production or trade of olive oil are part of it, this is the most important regulatory reference for international trade. In the case of the European Union, the matter is dealt with by ad hoc Regulations which comply with the requirements of the IOC. Among those in force, Reg. (EU) 1308/2013 [6] establishes the various olive oil Categories, Reg. (EU) 2104/2022 [7] their chemical-physical and sensory characteristics and the related analytical limits, and the Reg.(EU) 2105/2022 [8] the analysis methods to ascertain them (it refers to IOC Methods). Among the eight categories envisaged, the first is extra virgin olive oil which is the best for its chemical-physical and sensory properties. Among vegetable oils, olive oil has always been considered the most valuable and, therefore, also the subject of fraudulent attention aimed at marketing oils declared as olive, but containing foreign fats or, in the case of oils with chemical-physical characteristics of extra virgin, but with sensory defects, treated with processes aimed at removing those defects (e.g.: soft/mild deodorisation) and mixing them with authentic extra virgin olive oils and sold as such. The evolution of knowledge of olive oil and the progress of chemical-physical analysis techniques [9] have made it possible to increasingly refine the possibility of discovering frauds, but also applying advanced technological procedures, aimed at adulterating oils then sold as belonging to more valuable categories, such as virgin oils "transformed" into extra virgin. This transformation can be implemented, for example, through the so-called "soft deodorisation", also called "mild-deodorisation", performed under high vacuum, at much lower temperatures than for normal deodorisation of oils under refining. The aim is to remove by distillation those volatile components that give it sensory defects, without excessively altering the other chemical-physical parameters, so as to allow the oil to be mixed with authentic extra virgin olive oils and fraudulently placed on the market as entirely extra virgin olive oils, respecting the limits established for this Category. During the last twenty years or so, the problem of recognising deodorised oils mixed with extra virgin olive oils has become the subject of multiple research projects, sometimes supported by analytical checks conducted through appropriate interlaboratory proficiency testing. Below we will refer to only some of them, among the most significant. We remember the studies that considered the transformations of chlorophyll pigments combined with those of diglycerides [10, 11]. Various other research followed, among which the one that indicated a method for their determination intended to be included in the German Standard Methods [12]. Investigation on the content of fatty acid ethyl esters (FAEE), pyropheophytins and volatile compounds in oils subjected to soft-deodorisation conducted on a laboratory scale were also performed [13]. In [14] interesting results that require further investigation to be useful for the purpose are described. As already mentioned, diglycerides have been the subject of studies and research. In addition to those that studied the kinetics of transformation of 1,2- into 1,3-diglycerides [15, 16], we recall a recent work carried out within the European Oleum Project in the years 2016-2020 [17, 18], also based on diglycerides isomerisation kinetics and their relationship with the free acidity of the oil.

Among the methods aimed at finding markers of deodorisation, we recall the one that identified methyl 9(E),11(E)-octadecadienoate at trace level [19]. However, the markers which have been limited by an EU Regulation are the alkyl esters of fatty acids (methyl and ethyl). In fact, their presence is due to the formation of methyl and ethyl alcohols due to anaerobic fermentations that can occur in the olives during their storage before transformation, with consequent production of sensory defects in the oils from them, such as, for example, winey and, after oxidation in aerobic conditions, vinegary. Soft deodorisation allows the distillation of these alcohols and other compounds responsible for the defects but is less efficient in removing those alkyl esters. In this regard, among the numerous works, we remember those that use gas chromatography [20, 21, 22], while, with other analytical techniques, we recall the results obtained using TDR (Time Domain Reflectometry) [23] and others by means of gas chromatography-mass spectrometry with processing of the results via PCA [24]. The adoption by the European Union of Reg. (EU) 61/2011 [25] has introduced a limit to the content of methyl and ethyl esters of fatty acids in extra virgin olive oils. Their evolution over time has been the subject of various studies among which we mention just one [26]. Later, with Reg. (EU) 1348/2013 [27] that

limit was lowered and provided only for ethyl esters (FAEE). In fact, especially in unfiltered extra virgin oils, there may be the formation of methyl alcohol due to the degradation of the pectin present. Again, with the aim of preparing reliable methods for the recognition of mixtures of extra virgin oils with soft-deodorised oils, other studies have been conducted with different techniques. We refer to non-targeted methods, where high resolution mass detectors are used [28], and where the fingerprints of the volatile fractions are obtained with gas-chromatography ion mobility spectrometry (GC-IMS) and flash gas-chromatography electronic nose (FGC-Enose) techniques [29]. We also mention the use of NIR and MIR and chemometric analysis to process the data [30] and the use of near infrared spectroscopy (NIR) together with other traditional analytical parameters processed with a specific statistical approach [31]. Finally, we refer to the studies aimed at verifying the use of crossflow microfiltration (MF) and crossflow ultrafiltration (UF) for the purpose of removing the compounds responsible for off-flavours in oils. Among those, we mention one that dealt with the purification of lampante oils [32] and the study of the effect of membrane filtration on virgin olive oils to remove the compounds responsible for sensory defects [33].

The work presented in this publication illustrates the results obtained in the investigation on the possible variations of the specific extinctions at 270 nm (K270), increased by the conjugation of the trienes in single triglycerides (TAG), some of them perhaps more sensitive to soft-deodorisation treatments. Although these variations may be significant, the possible low concentration of the relevant TAGs may cause the effect on the specific extinction of the oil to be negligible. TAG separation was conducted by isocratic HPLC with on-line DAD and RID detectors. Furthermore, we wanted to compare the ECN42 values determined using this method under study with those obtained with the official method.

2. MATERIALS AND METHODS

2.1 CHEMICAL REAGENTS AND SOLVENTS

SPE-Si, 1g / 6mL (Strata®SI-1 Silica (55 µm, 70Å);
n-Hexane, ≥ 97.0%, ChromasolvTM for HPLC (Honeywell);
Diethyl ether, ≥ 99.8%, ACS Reagent, Reag. ISO, Reag. Ph. Eur., (Honeywell);
Acetone, ≥ 99.8%, HiPerSolv CHROMANORM® for HPLC, (VWR);
Propionitrile, ≥ 99.9%, for UV, HPLC, (PanReac AppliChem);
Nitrogen, Alphagaz 1 (Air Liquide).

2.2 SAMPLES

Oils (Table I): 56 oils were used of which: 6 extra virgin from the 2021-2022 campaign (n°10 to 15) and 9 from the 2022-2023 campaign (n°1 to 9) from Italy, Greece and Spain; 10 lampante olive oils from the 2022-2023 campaign (n°36 to 45) from Italy, Greece and Spain; 5 blends of extra virgin oils with the addition of refined olive oils at 1% (n°26 to 30) and 5 blends at 0.5% (n°31 to 35); 10 refined oils of which 5 from the 2021-2022 olive oil campaign (n°21 to 25) and 5 from the 2022-2023 campaign (n°16 to 20); 1 deodorised oil (100%, Spanish origin) (n°52) and 3 of its blends with 30%, 15% and 5% extra virgin olive oil (n°53 to 55); 1 oil, blend of extra virgin and 30% soft-deodorised (n°47); 1 blend oil (in unknown proportions) (n°46); 4 blend oils between extra virgin oils containing 30%, 20%, 10%, 4.6% of the latter in the list (n°48 to 51); 1 oil obtained from EV ultra filtered on membranes (n°56). All samples were stored in glass containers, in the dark at 18°C or some frozen at –20°C.

2.3 INSTRUMENTS AND SOFTWARE

Aspec XL Solid Phase Extraction Autosampler (Gilson, USA) with SW: 735 Sampler Software v.6.10 installed on PC with Microsoft Windows XP operating system.
HPLC 1260 Infinity with Degasser (1260 Degasser), Quaternary Pump (1260 Quat Pump VL), Autosampler (1260 ALS), Thermostated Column Chamber (1260 TCC) at 23°C, Diode Array Detector (1260 DAD VL) set at 270 nm, Refractive Index Detector (1260 RID) thermostated at 35°C (Agilent Technologies, USA);
HPLC columns: double column, InfinityLab Poroshell 120 EC-C18 (4.6 × 250 mm, 4 µm) (Agilent Technologies) thermostated at 23°C;
Vibrating shaker: Vortex mixer ZX3 (Velp Scientifica, Italy)
Micropipette: Eppendorf Research 10 - 100 µL (Eppendorf, Germany);
Vials: Chromacol 03-FIV with 300 µL fixed insert (Thermo Scientific, USA);
Vial closures: Ø 11 mm, with Silicone/PTFE septum (Microcolumn, Italy);
Common laboratory glassware;
Chromatogram acquisition and processing software: ChemStation for LC 3D system, Rev. B.04.03 (16) (Agilent Technologies, USA), installed on PC with Microsoft Windows 7 Professional operating system, Service Pack 1 (Microsoft);
Data collection and processing: Microsoft® Excel® 2019 MSO (Version 2307 Build 16.0.16626.20086) 64 bit (Microsoft Office 2019);
Principal Component Analysis (PCA): CAT (Chemometric Agile Tool) software, R version 3.1.2 [34] installed on PC with Microsoft Windows 10 Home operating system, Ver. 22H2;

2.4 METHODS

Oil clean-up.

Each aliquot of approximately 140 µL of oil was subjected to clean-up conducted automatically with As-

pec XL Solid Phase Extraction Autosampler in compliance with what is described in § 4.3.3 of the official method COI/T.20/Doc. No 20 /Rev. 4 2017 [35]. Once the eluate was collected, the solvent was evaporated in a stream of nitrogen. Approximately 50 µL of oil was added to the vial containing 70 µL of acetone, then closed and briefly vortexed.

Separation of TAGs.

Isocratic elution with propionitrile solvent at 1 mL/min, with the columns thermostated at 23°C. As already mentioned, we opted for the use of the double column to improve the separation of the peaks. DAD was set to signal recording at 270 nm (bandwidth = 4 nm) vs. 500 nm (bandwidth = 10 nm) as reference, while RID with Optical Unit Temperature set at 35°C. Injection volume = 5 µL. The chromatograph is first conditioned to a stable baseline, then the injection is performed. Run duration = 80 min. The RID signal is delayed by 0.2 min compared to that of the DAD, that is the first of the two detectors, due to the tube line connecting them. The same oils were also analysed according to the official method [35] for the determination of ECN42 to be compared with those determined with the method described here.

Integration of chromatograms.

The RID chromatogram shows, as expected, the separation of the TAGs according to the various ECNs and the use of the double column allows for better resolution (Figure 1).

In particular, ECN42 and ECN44 are extremely interesting for the purposes of this research, since they contain TAGs with triene fatty acids, namely linolenic acid (C18:3). As is known, because of the treatments to which the oil may have been subjected, part of those trienes conjugates, increasing the specific extinction at 270nm. In fact, it is in their correspondence that the greatest variations in the signals recorded by the DAD are detected, while for the higher ECN there is practically no response. The integration of the RID chromatograms was done by tracing the respective baselines underlying the ECN42 and ECN44. From ECN46 to ECN50 a single baseline was drawn and any peaks belonging to higher ECNs were integrated individually. Additionally, for ECN42 and ECN44, perpendiculars to the baseline were drawn at the valleys between incompletely resolved peaks. As already mentioned, the DAD chromatogram corresponds almost entirely to the first two ECN and was integrated by tracing the baselines under each of them as done for the RID signal, while the correspondence with the RID peaks was given by tracing the perpendiculars at the same times as the valleys of the relevant RID signal realigned for the 0.2 min time gap (Figure 2). The areas of the corresponding peaks were deduced from the integration reports and the A_{DAD}/A_{RID} ratios on which this work is based were calculated.

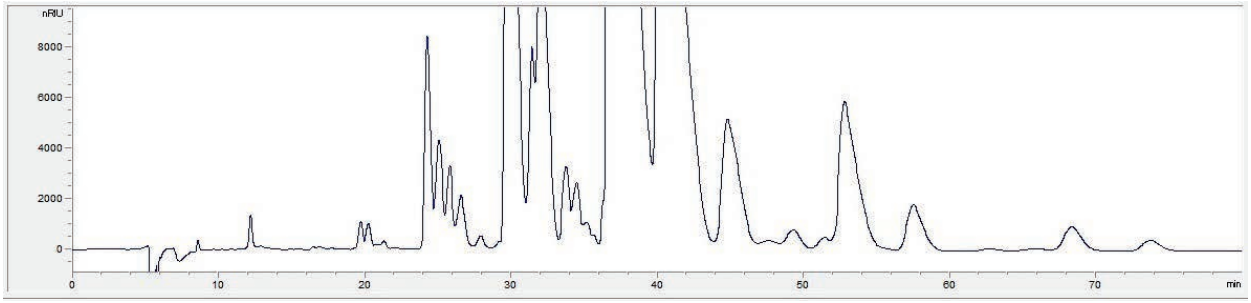

Figure 1 - olive oil triglyceride HPLC separation according to the method described in this research

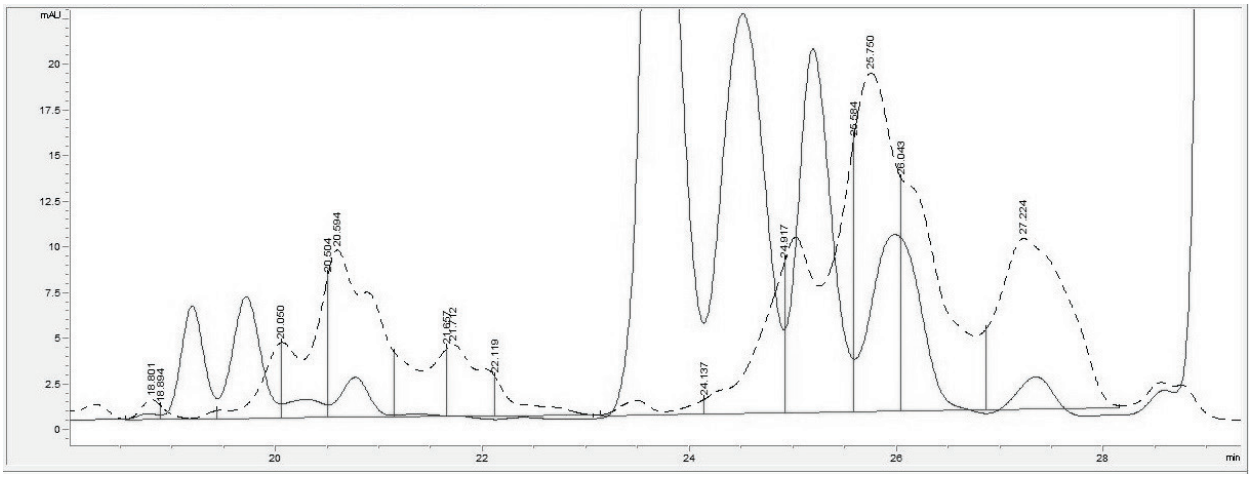

Figure 2 - DAD (dotted line) and RID (continuous line) signals integration of TGs belonging to ECN42 and ECN44

3. DISCUSSION

As already anticipated in the Introduction, the treatments to which an oil is subjected can increase the value of the specific extinction K, which is determined at 268 nm or 270 nm, according to the method requirements [36]. The reference spectrophotometric law is the Lambert-Beer Law which, as readers may remember, was first formulated in the 18th century, thanks to the studies of Bouguer in 1729 [37], of Lambert in 1760 [38] and of Beer in 1852 [39].
One of the most common formulations of this Law is taken from the official method:

$$E_\lambda = K_\lambda \times c \times s$$

where:
E_λ = extinction (or absorbance) measured at wavelength λ in nm; K_λ = specific extinction (or extinction coefficient) at wavelength λ; c = solution concentration, in g/100 mL; s = optical path of the measurement cell, in cm.

During the recording of the DAD signal due to the i^{th} compound eluted and completely resolved by the others, the absorbance Ei expressed by the Lambert-Beer law at wavelength λ, can be written in differential form, as follows:

$$dE_i = K_i \times N_i(t) \times dt$$

where $N_i(t)$ is the function that describes the elution trend of the moles of the i^{th} compound over the time t of passage through the detector. The integration between the start and end of the peak, ti_0 - ti_1, can be expressed:

$$E_i = \int_{ti0}^{ti1} Ki\, Ni(t)dt$$

Since the specific extinction K_i is a constant, it results:

$$E_i = Ki \int_{ti0}^{ti1} Ni(t)dt$$

The integral gives the number of total eluted moles of i:

$$E_i = K_i N_i \qquad (1)$$

Similarly, the integration of the RID signal of the same i^{th} compound (Ai) gives a value proportional to the number of moles eluted, Ni:

$$A_i = f_i \times N_i \qquad (2)$$

where f_i is a constant of proportionality. The ratio between the two relations (1)/(2), gives:

$$E_i / A_i = K_i \times f_i^{-1} \qquad (3)$$

In other words, the ratio between the DAD and RID signals gives a value proportional to the specific extinction coefficient of the compound considered, K_i. The constant f_i could have negligible variation with triene conjugation respect to the isolated triene compared to K_i changes. Thus, for our purposes we can consider it constant.

All data (E_i / A_i) were PCA processed by R-CAT software [34]. It is an R-based chemometric software that makes use of NIPALS algorithm. It opens a "RGui (32-bit)" window to load the file containing the data to be processed. In case of an Excel file: "Data Handling -> Load -> XLS/XLSX". Calculation is activated from the pull-down menu "PCA", then "Model Computation -> PCA". An "Input Choice" window opens to specify the "Matrix Name", the "Rows to be selected", the "Columns to be selected", the "Number of Components" and then "OK" to start the calculation. The file that collects and processes the relationships between the signals used in this work is Appendix I [40]: it is an Excel file made of 4 sheets: "DAD area pks" that collects the area of each peak of interest from the DAD signal integrated as described above; "RID area pks" that collects those corresponding from the RID signal; "DAD RID ratios" that calculates the E_i / A_i ratios that are multiplied by 10^5 to have all numbers not less than 0.1 and keeping unchanged each other proportion; "Sample C1 score calculation" that calculates the C1 score of a single new sample with two choices: including or not Refined olive oils in the original data base used for PCA. It must be underlined that this calculation is just a rough approximation of the new sample proper score, because its data are not included and processed with the full database to obtain a proper PCA calculation: this is just to have an idea about the sample position in the proper PCA score plot.

3.1 RESULTS

The parameters used in the processing of the experimental data presented in this work are the E_i / A_i ratio of each peak belonging to ECN42 or ECN44.
The relationships considered are 14 in total, of which 8 belong to ECN42 and 6 to ECN44. None of them, considered individually, allows us to unambiguously discriminate soft-deodorised oils or filtered with membranes and their blends with genuine extra virgin olive oils from the authentic ones. In contrast, their elaboration by PCA, which, as is known, also considers any existing relationships between the processed parameters belonging to the same sample, was much more efficient. In fact, the application of PCA to the entire set of results shows a clear discrimination of refined oils from others (Figure 3) with 96.7% of the total variance explained by the first two principal components (C1, C2) and as much as 94.4% explained by the component C1 along which samples are mainly separated.
If refined oils are excluded from the analysis, the score plot becomes the one shown in Figure 4, with 79.5% of the total variance explained by the first two component C1 and C2, with 70.8% by the C1 along which samples are mainly separated: those containing soft-deodorised oils (no. 46, 47, 53, 54, 55) or are entirely made up of it (n° 52), as well as the oil which has undergone membrane filtration (n° 56) are

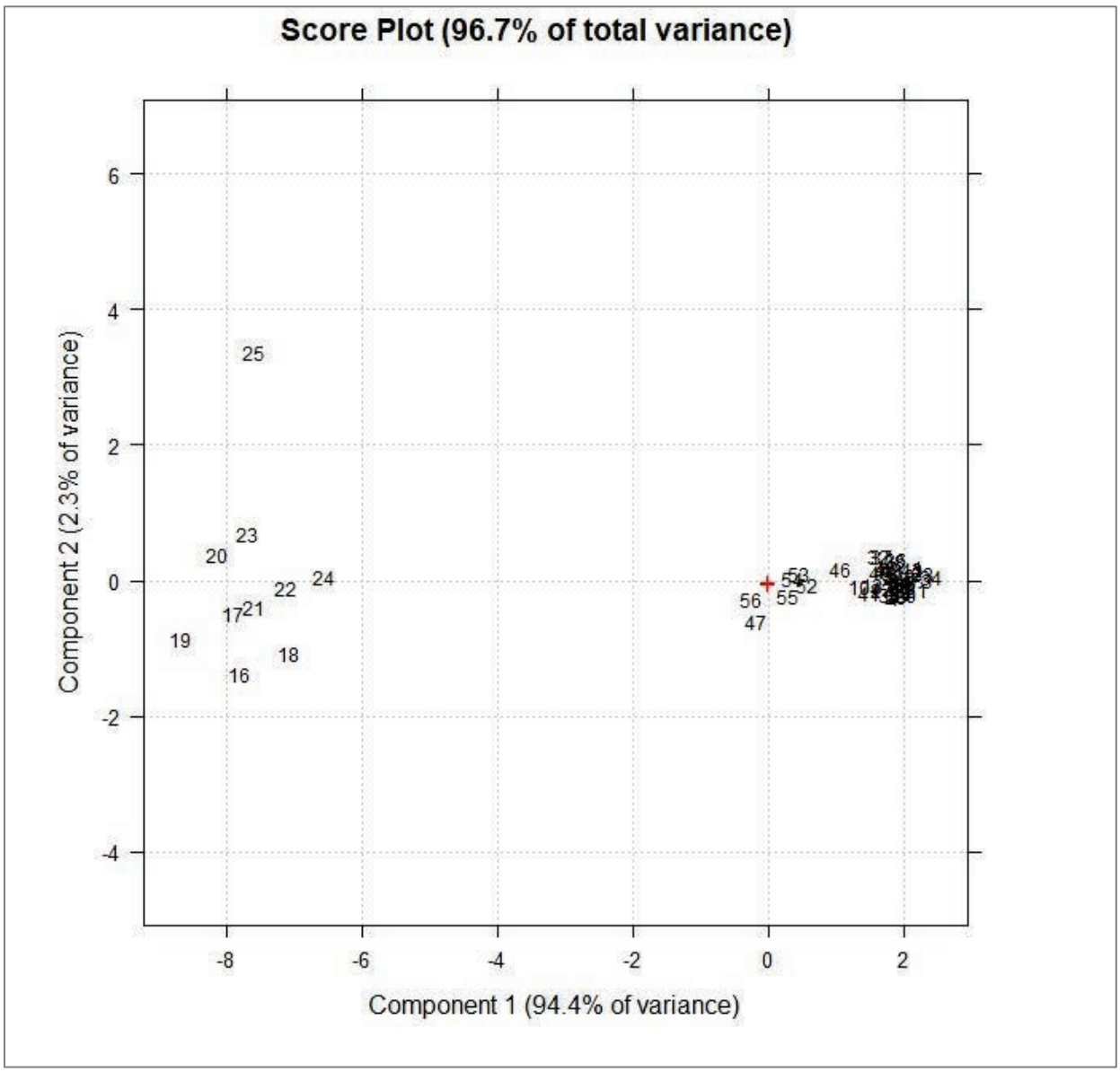

Figure 3 - PCA score plot of all samples. Samples from 16 to 25 are refined olive oils

well separate from the others. In particular, we remind the reader that samples 53, 54 and 55 are respectively dilutions of deodorised oil (n°52) in EV at 30%, 15% and 5%. N° 47 contains 30% of deodorised oil. However, neither EV, nor lampante oils, nor EV mixtures containing 1% or 0.5% of refined oil are distinct from each other, as well as n° 48, 49, 50 and 51 that are dilutions of n° 46, which is made of an unknown dilution of soft-deodorised oil in genuine extra virgin olive oil.

Because more than 70% of the total variance is explained by the component C1 in both cases (Refined olive oils included or not), it could be thought to find a sample data linear combination to detect blends with deodorised oils: Figure 3 shows all refined olive oil scores less than -6, while the other oils show scores greater than -1. Figure 4 shows oils containing deodorised or ultrafiltered oils with scores less than -3.60, while the others have scores greater than -1.50. This is why we included the fourth sheet "Sample C1 score calculation" into Appendix I. It makes use of C1 loadings from PCA calculation and, in order to z-standardise (mean=0, std.dev.=1) the new data, for each variable their mean value and standard deviation of data listed in "DAD RID ratios" sheet. The new sample "score" is calculated multiplying the new z-standardised data by the corresponding loading values. Again, we want to repeat that the "score" calculated in this way is a rough approximation of the real one, and just avoids the very basic and simple use of PCA that, on the contrary, we strongly advice to.

3.2 DETERMINATION OF ECN42

As anticipated in the Introduction, we also wanted to ensure that the quantitative results necessary to determine ΔECN42 could be obtained from the RID

chromatogram, as required by the current standard [7], without having to repeat the separation of the TAGs according to the official method [35]. The analyses conducted with the official method and by the one proposed in this work gave the results shown in Table I. This table also shows the differences between the results of the two methods (Δ (prop.-Offic.) for each sample. Among these, those differences of samples 1 and 3 are to be considered outliers according to the Grubbs test at both 95% and 99% level of confidence. The average value of these differences, excluding outliers, is equal to 0.02%. If we compare this result with the reproducibility value (R) reported in the official method for extra virgin oils, equal to 0.12% [35], it can be said that the two compared methods are consistent.

4. CONCLUSIONS

The method presented in this work is a preliminary one and gave encouraging results in detecting mixtures of extra virgin oils with soft-deodorised oils even to concentrations as low as only 5% of the latter. Membrane-filtered EV oil was also clearly discriminated. However, these results were achieved thanks to data processing via PCA, thus demonstrating that the effect of those treatments on the extinction coefficients of individual TAG is not nonspecific, but structured. In fact, no single E_i / A_i ratio allows us to clearly discriminate those samples. It was also observed that the inclusion of data related to refined olive oils in the PCA analysis produces a clear distinction between them from the others, with over 94% of the variance explained, described by the main component C1, along

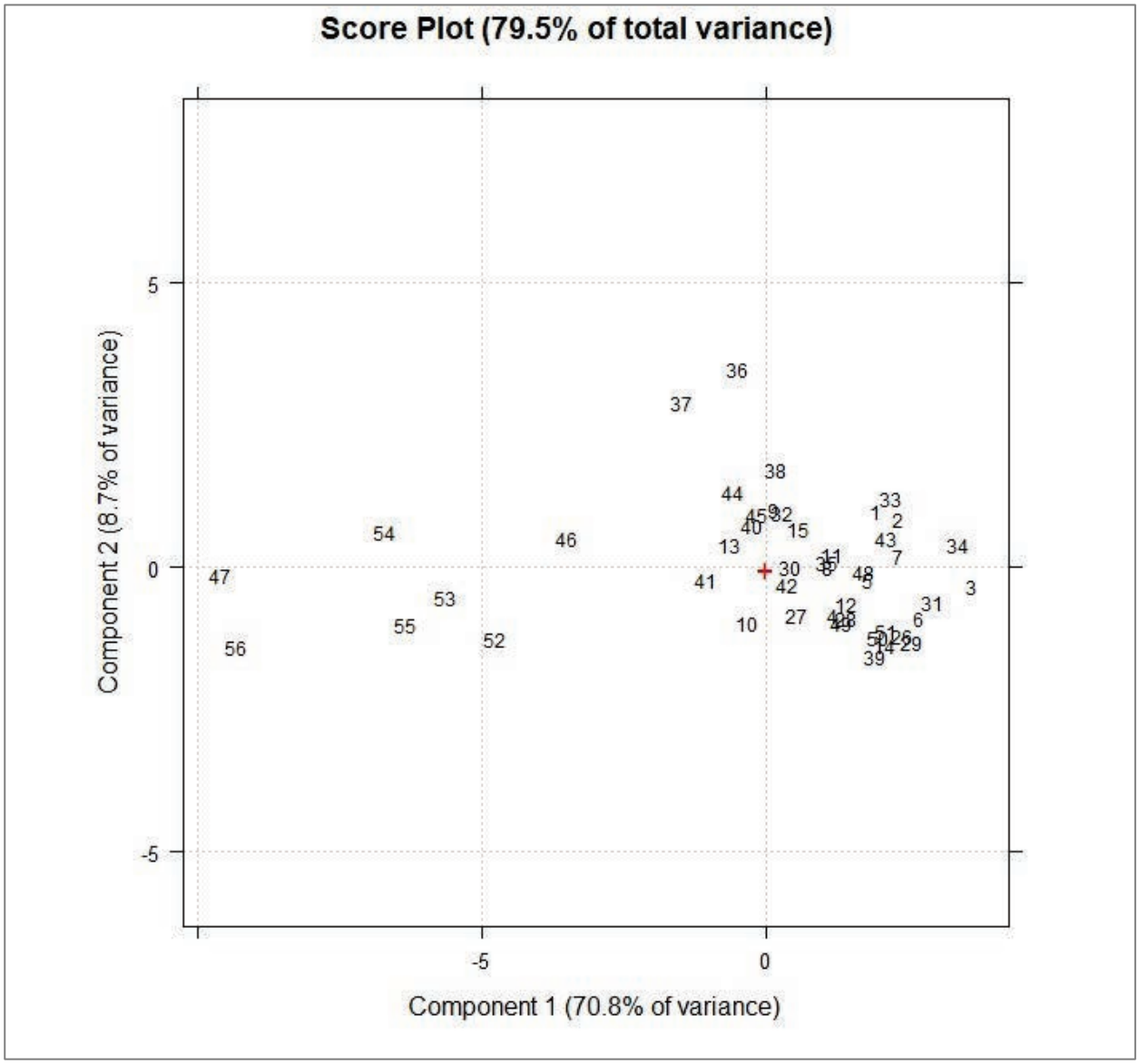

Figure 4 - PCA score plot after excluding refined olive oils from all sample set. Samples 46 and 47 are blends of EV with soft-deodorized oils; sample 52 is a soft-deodorized oil; samples from 53 to 55 are blends of EV with sample 52 at 30%, 15% and 5%; sample 56 is MF/UF oil

Table I - Comparison of ECN42 determined by the proposed method and by the Official Method

sample n°			Proposed method	Official Method	Δ (prop.-Offic.)	*sample n°*			Proposed method	Official Method	Δ (prop.-Offic.)
			%	%	%				%	%	%
1	Italy	EV 2022-2023	0,47	0,61	-0,14	29	EV n° 4 + 1% Ref n° 24		0,53	0,48	0,05
2	Italy	EV 2022-2023	0,37	0,31	0,06	30	EV n° 5 + 1% Ref n° 25		0,62	0,61	0,01
3	Italy	EV 2022-2023	0,38	0,64	-0,26	31	EV n° 1 + 0.5% Ref n° 21		0,36	0,37	-0,01
4	Greece	EV 2022-2023	0,34	0,34	0,00	32	EV n° 2 + 0.5% Ref n° 22		0,38	0,34	0,04
5	Greece	EV 2022-2023	0,29	0,29	0,00	33	EV n° 3 + 0.5% Ref n° 23		0,38	0,36	0,02
6	Italy	EV 2022-2023	0,37	0,36	0,01	34	EV n° 4 + 0.5% Ref n° 24		0,38	0,38	0,00
7	Spain	EV 2022-2023	0,38	0,37	0,01	35	EV n° 5 + 0.5% Ref n° 25		0,28	0,31	-0,03
8	Italy	EV 2022-2023	0,43	0,38	0,05	36	Greece	Lampante olive oil 2022-2023	0,38	0,30	0,08
9	Greece	EV 2022-2023	0,40	0,37	0,03	37	Italy	Lampante olive oil 2022-2023	0,37	0,37	0,00
10	Italy	EV 2021-2022	0,38	0,33	0,05	38	Italy	Lampante olive oil 2022-2023	0,39	0,36	0,03
11	Greece	EV 2021-2022	0,27	0,26	0,01	39	Italy	Lampante olive oil 2022-2023	0,37	0,36	0,01
12	Spain	EV 2021-2022	0,36	0,36	0,00	40	Greece	Lampante olive oil 2022-2023	0,30	0,29	0,01
13	Italy	EV 2021-2022	0,36	0,33	0,03	41	Italy	Lampante olive oil 2022-2023	0,70	0,70	0,00
14	Spain	EV 2021-2022	0,30	0,30	0,00	42	Italy	Lampante olive oil 2022-2023	0,51	0,44	0,07
15	Italy	EV 2021-2022	0,36	0,36	0,00	43	Spain	Lampante olive oil 2022-2023	0,46	0,51	-0,05
16	Refined olive oil	2022-2023	0,43	0,34	0,09	44	Spain	Lampante olive oil 2022-2023	0,83	0,79	0,04
17	Refined olive oil	2022-2023	0,31	0,26	0,05	45	Spain	Lampante olive oil 2022-2023	0,62	0,56	0,06
18	Refined olive oil	2022-2023	0,44	0,41	0,03	46	EV+soft deod.		0,52	0,52	0,00
19	Refined olive oil	2022-2023	0,47	0,44	0,03	47	EV+30% soft deod.		0,42	0,43	-0,01
20	Refined olive oil	2022-2023	0,37	0,36	0,01	48	EV n°3 + 30% n°46		0,48	0,48	0,00
21	Refined olive oil	2021-2022	0,45	0,42	0,03	49	EV n°3 + 20% n°46		0,70	0,67	0,03
22	Refined olive oil	2021-2022	0,57	0,50	0,07	50	EV n°3 + 10.5% n°46		0,64	0,60	0,04
23	Refined olive oil	2021-2022	0,52	0,43	0,09	51	EV n°3 + 4.6% n°46		0,21	0,24	-0,03
24	Refined olive oil	2021-2022	0,51	0,49	0,02	52	Soft deod.		0,25	0,21	0,04
25	Refined olive oil	2021-2022	0,54	0,49	0,05	53	EV + 30% n°52		0,34	0,32	0,02
26	EV n° 1 + 1% Ref n° 21		0,55	0,52	0,03	54	EV + 15% n°52		0,36	0,35	0,01
27	EV n° 2 + 1% Ref n° 22		0,58	0,52	0,06	55	EV + 5% n°52		0,35	0,35	0,00
28	EV n° 3 + 1% Ref n° 23		0,57	0,51	0,06	56	Ultra Filtered EV		0,37	0,35	0,02

Δ (prop.-Offic.) mean value, % = 0,02

which they are separated from other oils. Appendix I can be used as a database to which other data can be added to be processed as a single new set (up to row n° 1000). As regards the analytical part, in particular the chromatographic one, an UHPLC application of this method using appropriate DAD and RID detectors is considered desirable: a greater resolution of the TAG peaks would allow a more accurate determination of the relationship between the DAD and RID signals to the benefit of the analytical results and a possible verification of the proposed method. It is important to note that there is no full correspondence between the peaks of the DAD signal with those of the RID signal, despite the realignment of the two chromatograms for the 0.2 min gap already mentioned. The DAD plot maxima often do not match those of the RID plot. This is evidence of the incomplete resolution of the TAGs observed in the RID plot, whose peaks are however attributed to TAG as indicated by the official method [35]. In this regard, it is useful to refer to the research where the incomplete resolution of those peaks and their more correct identification is demonstrated through NARP-HPLC-APCI-MS [41]. As regards the integration of chromatograms, the choice of the method used to delimit the peaks of the DAD signal based on the integration of the RID chromatogram was explained in the "Method" part. It could be interesting to try the opposite, taking the integration of the DAD chromatogram as a reference, which constitutes an in-depth topic to be developed in the future. The method presented also proved to be accurate in the determination of ECN42, as proven by the comparison with the values obtained from the separation according to the official method. The possible control of the authenticity of an oil using the method presented would also allow us to have the data necessary to ascertain the value of ΔECN42 without any further HPLC separation according to the official method.

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Conflict of interest
The author declares that there is no conflict of interest

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