

1 Olive oil mixtures. Part one: decisional trees or how to verify the olive oil  
2 percentage in declared blends.

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19 ABSTRACT

20 The commercialization of declared blends of olive oil and seed oil is something long approved by the  
21 European Union. There, the olive oil percentage must be at least 50 % if the producer aims to advertise  
22 its presence on the front label, i.e., somewhere other than in the ingredients list. However, the  
23 Regulation did not propose any method to verify such proportion. For this purpose, we recommend  
24 the use of decisional trees, being the parameters under study those in which the greatest differences  
25 between olive and seed oils are shown: triacylglycerols, acyclic saturated hydrocarbons, free sterols,  
26 and tocopherols. In this way, to guarantee the presence of olive oil at 50 % : i) palmitodiolein must be  
27 above 11-15 %; ii) the  $\beta/\gamma$ -tocopherol ratio must be below 2.4; iii) the alkane sum C21-C25 should  
28 be higher than 3.5-6 %; and iv) the total sterol content cannot surpass 2400 mg/kg.

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30 *Keywords:* decisional tree, declared blends, OLEUM Project, olive oil percentage, oil labelling,  
31 seed oil.

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33 *Chemical compounds studied in this article:*

34 Beta-tocopherol (PubChem CID: 6857447); Gamma-tocopherol (PubChem CID: 92729);  
35 Heneicosane (PubChem CID: 12403); Palmitodiolein (PubChem CID: 25240174); Pentacosane  
36 (PubChem CID: 12406); Sterols (PubChem CID: 12303662); Tricosane (PubChem CID: 12534)

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## 38 **1. Introduction**

39 According to the International Olive Council (IOC) ‘virgin olive oils are oils obtained from the fruit  
40 of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions,  
41 particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone  
42 any treatment other than washing, decantation, centrifugation, and filtration’ (IOC, 2016).

43 The paramount importance of olive oil in the global market derives from three phenomena: First of  
44 all, the role of the European Union as the most important producer and consumer of this kind of oil  
45 in the world. Actually, it accounts for 66 % of the production according to the IOC predictions for  
46 season 2017-18 (IOC, 2018). Secondly, the fact that more than 20 non-EU countries (many of them  
47 out of the Mediterranean area) are developing a certain olive oil culture, increasing their domestic  
48 production and rising the competitiveness of the global olive oil market. Thirdly, the role of olive oil  
49 as a pool of healthy constituents which has no comparison with other edible fats and oils thanks to  
50 both, its beneficial fatty acid composition, where oleic acid can be present at concentrations as high  
51 as 83 % (IOC, 2016), and to its minor compound profile responsible for its antioxidant activity,  
52 sensory characteristics, and overall complexity (Gómez-Coca, Pérez-Camino, and Moreda, 2015).

53 It is then justified that olive oil price and reputation had boosted over the years and so had its  
54 attractiveness as a target for adulteration, either by illegal blending with vegetable oils other than  
55 olive oil or by deliberate mislabeling. In this context, the European Parliament pointed out that olive  
56 oil was one of the foods which was most at risk of suffering fraudulent activities (European  
57 Parliament, 2014). This situation was of high concern due to the potential impact on the market’s  
58 confidence. As a consequence, the European Commission was requested to give it full attention which  
59 finally ended up in the so-called OLEUM Project (2016). The Project’s global goal evolves around

60 olive oil fraud detection by both improving the existing analytical methods and developing new  
61 strategies of analysis. It has been organized in seven work packages distributed in a number of tasks.  
62 This work focuses on Work Package 4 ('Analytical solution addressing olive oil authentication  
63 issues'), specifically on the study of legal blends between olive oils and other vegetable oils.

64 The concept of 'legal blends' arises from the authorization of the European Commission to market  
65 blends of olive oil with other vegetable oils and to highlight the presence of olive oil on the labelling,  
66 and not just on the ingredient list, only if it accounts for at least 50 % of the blend (European  
67 Commission, 2012). This last requirement evidenced a major problem: The lack of analytical methods  
68 to control the percentage of olive oil in declared mixtures. Truly, when conducting a bibliographical  
69 search through the SciFinder database using terms like 'olive oil legal blends', and 'olive oil  
70 commercial blends' as research topics, it is unmistakable that most of the work focuses on the  
71 detection of olive oil adulterations with low quality olive oils or with vegetable oils other than olive  
72 (Ou, Hu, Zhang, Li, Luo, and Zhang, 2015; Santos, Kock, Santos, Lobo, Carvalho, and Colnago,  
73 2017), and on authentication issues such as that of monovarietal oils (Agrimonti, Vietina, Pafundo,  
74 and Marmiloli, 2011; Da Ros, Masuero, Riccadonna, Brkic Bubola, Mulinacci, Mattivi, Lukic, and  
75 Vrhovsek, 2019) or geographical origin confirmation (Gertz, Gertz, Matthäus, and Willenberg, 2019;  
76 Vera, Jiménez-Carvelo, Cuadros-Rodríguez, Ruisánchez, and Callao, 2019; Quintanilla-Casas,  
77 Bertin, Leik, Bustamante, Guardiola, Valli, Bendini, Gallina Toschi, Tres, and Vichi, 2020).  
78 Although some of these approaches can be applied with semi-quantitative purposes, like the use of  
79 DNA-based methodologies for the detection of olive oil in commercial products and plant oils  
80 (Ramos-Gómez, Busto, Albillos, and Ortega, 2016; Alonso-Rebollo, Ramos-Gómez, Busto, and  
81 Ortega, 2017), very few of them really focus on verifying the percentage of olive oil in fraudulent  
82 blends with other vegetable oils. Such is the case of Santos and coworkers (Santos et al., 2017) who  
83 used Time-Domain NMR Relaxometry (TD-NMR) to detect olive oil adulteration with seed oils  
84 present at high concentrations. Specifically, they tested this approach on olive oil samples mixed with  
85 soybean oil at different concentrations and were able to separate them according to the adulteration

86 level (i.e. 25, 50, and 75 % adulterant) applying Principal Component Analysis to the TD-NMR  
87 results. Those results are promising but the technique is an expensive one and has not been tried with  
88 controlled blends (e.g. blends prepared in a laboratory and whose composition is actually well  
89 known). Other approaches to quantify EVOO in commercial blends have been proposed by Aroca-  
90 Santos and colleagues, but the 50:50 proportion was not included (Aroca-Santos, Lastra-Mejías,  
91 Cancilla, and Torrecilla, 2019). So far, and to the best of our knowledge, the only lines of research  
92 developed *ad hoc* for declared blends were those of de la Mata (de la Mata, Domínguez-Vidal,  
93 Bosque-Sendra, Ruiz-Medina, Cuadros-Rodríguez, and Ayora-Cañada, 2012) and of Monfreda  
94 (Monfreda, Gobbi, and Grippa, 2012). De la Mata applied attenuated total reflection Fourier  
95 transform infrared spectroscopy, together with chemometric analysis, and obtained very promising  
96 results: They tested 76 mixed samples, some of them with olive oil at 50 %, and were able to classify  
97 them correctly, although the model has some limitations -as the authors pointed out- that make it to  
98 be considered as semi-quantitative. Monfreda used the fatty acid composition of the samples followed  
99 by chemometric tools to classify the oil blends under study. The advantage of this proposal is clear,  
100 since they used an official method for olive oil analysis highly established in laboratories devoted to  
101 this matter. However, although they took into account the possible variability among olive oil  
102 composition, their main limitation relies in the fact of having used a single sunflower oil sample,  
103 whereas de la Mata took into account the unevenness encountered by the differences between olive  
104 cultivars and by the dissimilar seed oils (although, again, including just one kind sunflower oil).  
105 Accordingly, it is the purpose of the present work to design an analytical strategy in order to confirm  
106 if the amount of olive oil in a label-claimed blend is at least 50 %, using two of the most representative  
107 seed oils: normal type and high oleic sunflower oils. Our hypothesis is that there is actually no need  
108 of developing new methods of analysis, neither of applying chemometric, metabolomic or genomic  
109 strategies -the latter usually expensive-, all of them of great help either themselves or in combination  
110 with other approaches in olive oil authentication and purity assessment (Agrimonti et al., 2011;  
111 Bosque-Sendra, Cuadros-Rodríguez, Ruiz-Samblás, and de la Mata, 2012; de la Mata, et al. 2012;

112 Gómez-Caravaca, Maggio, and Cerretani, 2016; Avramidou, Doullis, and Petrakis, 2018; Da Ros et  
113 al., 2019). Yet, it would be enough if one combines properly some of the official purity parameters  
114 described in the legislation (IOC, 2016). In order to test it we decided to focus on the parameters in  
115 which the greatest differences between olive and seed oils were to be observed and ended up in the  
116 fact that not only one, but four different determinations were needed to discern olive oil concentration,  
117 as was to be expected due to the complexity of olive oil chemical composition. In this sense, we  
118 considered triacylglycerols (TAG), acyclic saturated hydrocarbons (SHC), free sterols (FS), and  
119 tocopherols (TCPH), and we organized them in the form of decisional trees in a way that the blend  
120 who claims to be composed of at least 50 % olive oil must comply not just with one but with four  
121 terms. We must point out that we decided to use sunflower oils as representative of seed oils, since it  
122 is the edible oil most used in this kind of mixtures. Actually, other studies pondering the same problem  
123 than we consider here take sunflower as model seed oil too (Monfreda, et al., 2012).

124

## 125 **2. Materials and methods**

### 126 **2.1 Analytical materials and reagents**

127 All reagents and solvents were super purity or HPLC grade unless otherwise stated. Anhydrous  
128 pyridine, 2',7'-dichlorofluorescein solution, dichloromethane, and hexamethyl disilazane were from  
129 Honeywell (Fisher Scientific SL, Madrid, Spain). Acetone, ethanol, ethyl acetate, ethyl ether,  
130 methanol, n-hexane, n-heptane, propan-2-ol, propionitrile and trimethylchlorosilane were supplied  
131 by VWR International, LLC (West Chester, Pennsylvania, USA), anhydrous sodium sulfate and  
132 potassium hydroxide by Panreac Quimica, S.A.U. (Castellar del Valles, Barcelona, Spain). The  
133 standards 5 $\alpha$ -cholestan-3 $\beta$ -ol,  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol, and n-eicosane (C20) were purchased at  
134 Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Silica gel 60 for column chromatography, 70-  
135 230 mesh (Merck KGaA, Darmstadt, Germany) was used directly from the container. Glass  
136 chromatography columns (50 cm length x 1.5 cm i.d.) were supplied with Teflon stopcocks; a plug  
137 of glass wool fiber was placed at the bottom and everything was washed with n-hexane before use.

138

## 139 **2.2 Instrumentation**

140 The TAG determination was carried out according to the IOC method (IOC 2017a) using 1 g SPE  
141 silica gel cartridges (Varian, Inc., Harbor City, CA) for the intact analyte separation (i.e. TAG are  
142 isolated as such and with no chemical transformation), and reverse phase high-resolution liquid  
143 chromatography (RP-HPLC) with propionitrile as mobile phase at 0.6 mL/min flow rate in order to  
144 get trilinolein elution at around 12.5 min. These RP-HPLC analyses were carried out by dissolving  
145 the purified extract in 2 mL acetone and injecting 10  $\mu$ L onto an HPLC system equipped with a  
146 Beckman Gold 508 autosampler (Beckman-Coulter, Fullerton, CA, USA) and a Lichrosphere 100  
147 RP-18 phase column (4  $\mu$ m particle size, 25 cm x 4 mm i.d.). The system was set with a Beckman  
148 Gold 126 pumping component (Beckman-Coulter, Fullerton, CA, USA), a Perkin Elmer 200  
149 refractive index detector (RID) (Perkin Elmer, Norwalk, CT, USA), and a Beckman Mistral Peltier  
150 thermostat oven (Beckman-Coulter, Fullerton, CA, USA) in order to keep the column temperature at  
151 20 °C (Moreda, Pérez-Camino, and Cert, 2003). The peaks on the HPLC chromatogram were  
152 integrated by the data acquisition system, grouped according to their equivalent carbon number  
153 (ECN) and identified after the pattern given in the official method (IOC, 2017a).

154 The FS, profile and content were determined as it is described by Cert, Moreda, and García-  
155 Moreno (1997). In short: after sample saponification with a 2 M ethanolic potassium hydroxide  
156 solution and extraction of the unsaponifiable matter with diethyl ether, the analytes are separated  
157 using a Beckman Coulter HPLC system (Beckman Coulter Inc., CA, USA) provided with a System  
158 Gold 125P Solvent Module, a System Gold 166P Detector, and a System Gold 508 Autosampler (200  
159  $\mu$ L injection). The separation was done through a LiChroCART 250-4 Superspher Si 60 cartridge (5  
160  $\mu$ m particle size; Merck KGaA, Darmstadt, Germany), using as mobile phase a hexane:diethyl ether  
161 50:50 (v/v) mixture at 1 mL/min. The FS fraction was collected using a Gilson FC 203B fraction  
162 collector (Gilson Inc., WI, USA). The FS were analyzed as trimethylsilyl ethers by capillary column  
163 gas chromatography (GC) with a flame ionization detector (FID). The GC analyses were carried out

164 with an Agilent 6890N Gas Chromatograph (Agilent Technologies, Santa Clara, California) equipped  
165 with an Agilent 7683B Automatic Liquid Sampler and a FID. Acquisition of data was done with the  
166 Agilent ChemStation for GC System program. The conditions for the GC assays were: TRB-5HT  
167 column (5 % diphenyl-95 % dimethylpolysiloxane; 30 m x 0.25 mm ID x 0.25  $\mu$ m film; Teknokroma,  
168 Sant Cugat del Vallés, Barcelona, Spain), 1.0  $\mu$ L injection volume, hydrogen carrier gas at 1.9  
169 mL/min, and 10:1 split injection. The oven worked isothermally at 255  $^{\circ}$ C during 40 min. The detector  
170 temperature was 300  $^{\circ}$ C. The quantitative evaluation was carried out using  $\alpha$ -cholestanol as internal  
171 standard considering that the response factor for all sterols equaled 1.

172 The method of analysis of the SHC fractions was based on the official method for the analysis of  
173 stigmastadienes in vegetable oils (Cert and Moreda, 1998; IOC 2017b) which uses column  
174 chromatography (CC) on silica gel to separate alkanes from steroidal hydrocarbons. Summarizing,  
175 this arrangement required 15 g of silica gel to be suspended in a container with 40 mL n-hexane, and  
176 then poured into a column prepared with a plug of glass wool fiber at the bottom. The packing was  
177 allowed to settle, a small amount of anhydrous sodium sulfate was added on top, and the excess of n-  
178 hexane eluted. The silica bed was cleaned with 60 mL n-hexane and the oil together with the C20 IS  
179 transferred to the top of the column. Linear SHC were then eluted with 35 mL n-hexane. The  
180 equipment for the GC analysis was identical to that described for FS analysis. The GC conditions  
181 were: DB-5HT column (5 % diphenyl-95 % dimethylpolysiloxane; 15 m x 0.32 mm ID x 0.10  $\mu$ m  
182 film; Agilent Technologies, Santa Clara, California), 1.0  $\mu$ L injection volume, hydrogen carrier gas  
183 at 5 mL/min and cool on column injection. The initial oven temperature was set at 70  $^{\circ}$ C for 2 min,  
184 then raised at 12  $^{\circ}$ C/min until 280  $^{\circ}$ C, and finally at 7  $^{\circ}$ C/min until 340  $^{\circ}$ C, holding for 5 min. The  
185 detector temperature was 380  $^{\circ}$ C. The quantitative evaluation was carried out using C20 as internal  
186 standard considering that all alkanes had the same response coefficient.

187 Finally, TCPH measurements were carried out by HPLC analysis on silica gel column and  
188 fluorescence detection (FLD) setting the excitation and emission wavelengths at 290 and 330 nm,  
189 respectively. The analytical procedure consists on dissolving 10 mg oil in 1 mL n-hexane and

190 injecting it into an HPLC system set with a Superspher Si 60 column (4  $\mu\text{m}$  particle size, 25 cm x 4  
191 mm i.d.) and equipped with a Hewlett Packard 1050 Series pumping component and a RF-10AXL  
192 Shimadzu detector. The quantitative evaluation was carried out by external standardization using each  
193 standard to build the respective calibration curve (concentrations from 4 to 6  $\mu\text{g}/\text{mL}$  in n-hexane).  
194 This procedure is based on the IUPAC Standard Method 2432 (IUPAC, 1988).

195

### 196 **2.3 Samples**

197 Samples were provided by Fera (Fera Science Ltd, Sand Hutton, York) within the frame of the  
198 OLEUM Project. Individual (not blended) oils arrived at the laboratory in August 2017; these were:  
199 extra virgin olive oil (EVOO), olive oil (OO), refined high oleic sunflower oil (HOSO) and refined  
200 normal type sunflower oil (NTSO). In this way, we would prepare our own blends after the  
201 instructions depicted on the Project's analytical plan (e.g. controlled temperature conditions). Those  
202 blends consisted on binary mixtures of each of the olive oils with every sunflower oil at 60:40, 50:50,  
203 and 40:60 v/v proportions. Lastly, in November 2018 we were provided with a batch of blind  
204 commercial samples collected in the Swedish market (#1-#8) which consisted of EVOO, virgin olive  
205 oil (VOO), or OO, and one oil other than olive such as rapeseed oil (RSO), NTSO, or a non-identified  
206 vegetable oil (VGO). The composition of sample #8 was not given.

207 All samples had been prepared and bottled under a headspace of nitrogen to increase their stability,  
208 and stored at 4 °C until their dispatch. Once in the laboratory, they were kept at analogous conditions  
209 protected from light, until we were ready to perform the experimental work. At that moment, samples  
210 were taken from the cold storage, left to equilibrate at room temperature a minimum of 6 hours and  
211 shaken vigorously before extracting any aliquot.

212

### 213 **3. Results and discussion**

214 TAG, FS, SHC, and TCPH were investigated in order to use them as markers for the determination  
215 of the correct proportion of olive oil in legal blends. They were chosen because it is there where the



216 greatest differences between seed oils and olive oils appear, and have been often selected to detect  
217 small amounts of vegetable oils other than olive oil in genuine olive oils (Christopoulou et al., 2004).  
218 TAG are the main components of olive oil and they are mostly responsible for its principal features.  
219 In fact, their usefulness becomes patent when approaches such as that of Chemometrics in  
220 combination with TAG composition are used for authentication purposes (Bosque-Sendra et al., 2012;  
221 Gertz et al., 2019) or in the discrimination between oil blends according to their olive oil percentage,  
222 this latter one actually through the use of spectroscopic techniques (de la Mata et al., 2012). From a  
223 more classical point of view, chromatography methods are utilized in TAG analysis and even if  
224 traditionally the only compounds considered in an HPLC chromatogram were trilinolein (LLL) first,  
225 and the equivalent carbon number (ECN) 42 TAG group later (Christopoulou et al., 2004; Aparicio,  
226 Conte, and Fiebig, 2013), we decided to study the whole TAG profile. Table 1 shows the results of  
227 the analysis of the four different mixtures of olive and sunflower oils at the three different proportions  
228 under study. Although sunflower oil and olive oil normally exhibit considerably different fatty acid  
229 and TAG composition (Christopoulou et al., 2004), if one compares the content of the various TAG  
230 among the different oil proportions within a given blend it is clear that, on the one hand, except for  
231 the OOO+PoPP pair (eluted as one chromatographic peak within the ECN48 group) and for POO, all  
232 the species can be considered to maintain a constant value within the error limits -i.e. mean+standard  
233 deviation (SD)- regardless the percentage of (EV)OO added. Besides, the SD is too high for some of  
234 the TAG present at the lowest concentration such as OLL<sub>n</sub> or PLL<sub>n</sub>, which is usual when small  
235 chromatographic peaks have to be -manually- integrated. On the other hand, the OOO+PoPP pair  
236 showed one of the highest shifts from one blend to another, but such changes were not consistent,  
237 that is to say, the OOO+PoPP concentration does not just decrease by decreasing the presence of olive  
238 oil in the mixture, but it is also highly influence by the kind of sunflower oil added, in a way that in  
239 blends with NTSO the lower the amount of olive oil, the lower the percentage of the OOO+PoPP  
240 pair, whereas this trend reverses if HOSO is included. Actually, one can observe that in those latter  
241 blends the lower the amount of olive oil, the *higher* the OOO+PoPP concentration, which is due to

242 the fact that HOSO can form more POO than NTSO, presumably, thanks to the oleic acid availability.  
243 However, if we observe the results in the case of POO (emphasized in bold letters), significant  
244 differences among mixtures at dissimilar proportions appeared, indicating that such species is actually  
245 affected by the presence of (EV)OO at different quantities and by the kind of sunflower oil (normal  
246 type or high oleic) used, although in the same sense. In fact, in every case the higher the amount of  
247 (EV)OO in the sample, the higher the percentage of POO, and on each blend the POO concentration  
248 is higher if HOSO, and not NTSO, is the second component of the mixture. One could argue that in  
249 the TAG chromatograms POO and SOL elute together in a single peak, but the amount of SOL in  
250 olive oil is generally very low (below 0.4 %), since the presence of stearic acid is also scarce (between  
251 0.5 and 5 %).

252 A more clear effect of the blend composition on the POO concentration may be observed in Table  
253 S1 (Supplementary material), where the differences ( $\Delta$ ) between the mean values corresponding to  
254 the 50:50 and 40:60 (EV)OO:(NT/HO)SO blends are given, together with the respective SD.  
255 According to our results, a 10 % increment in olive oil (i.e., from 40:60 to 50:50 olive oil:sunflower  
256 oil mixtures) rises the percentage of POO around 8-9 % in mixtures with HOSO, whereas the increase  
257 is around three times higher (23 % and 30 %) in blends with NTSO. These obvious differences  
258 demonstrate that even if HOSO can form much more POO than NTSO, it is not able to form as much  
259 as olive oils, probably due to the differences in the substrate specificity and selectivity of the  
260 acyltransferase enzymes involved in the TAG the biosynthetic pathway. Besides, according to our  
261 experience, low POO concentrations are to be expected for seed oils. In the case of POO, such  
262 differences are clearly above the method's uncertainty (i.e. there is a clear differentiation regardless  
263 the error limit) for each of the cases under study. In view of such results, we may postulate that in  
264 order to ascertain that (EV)OO is at least at 50 % in a mixture with sunflower oil, the percentage of  
265 POO must be above 11 % if NTSO has been added, whereas it must go beyond 15 % in the case of  
266 HOSO. Really, if we calculate the lower limit of the possible percentage range applying one SD (the  
267 most conservative option in this case), the POO concentration for both 50:50 blends (with NTSO and

268 HOSO) are still above 11 % (actually it would be 11.6 % and 11.12 %, respectively), whereas the  
269 calculation of the upper range for the 40:60 mixtures considering this time 3SD (9.96 % and 14.5 %,   
270 respectively) would still be below the set limits, supporting our decision of taking 11 % and 15 % as  
271 cut-off limits.

272 The second group of analytes under considerations were sterols (4-desmethyl sterols). To this  
273 respect our results are shown in Table 2. Sterols are of utmost importance when analyzing vegetable  
274 oils since they are known to be their analytical fingerprint. Countless efforts have been made to  
275 unravel the many forms of sterols that one may encountered in vegetable oils in general, and in olive  
276 oil in particular (Gómez-Coca, Fernandes, Aguila-Sánchez, Pérez-Camino, and Moreda, 2014).  
277 Actually, these species are of special importance in the case of olive oil (Barjol, 2013) since their  
278 application in its authentication goes back to the eighties (Brumley, Sheppard, Ridolf, Yasaei, and  
279 Sphon, 1985). It was demonstrated, for instance, that the addition of sunflower or soybean oils boosts  
280 the stigmasterol, campesterol and, in the case of sunflower oil,  $\Delta^7$ -stigmastenol percentages above  
281 those found in pure olive oil, whereas brassicasterol is often useful to detect the addition of rape seed  
282 oil (Grob, Giuffré, Leuzzi, and Mincione, 1994; Alonso, Fontecha, Lozada, and Juarez, 1997).  
283 According to these statements, when we determined the sterol composition and content of the blends  
284 under study the first hint of the presence of seed oil was the high campesterol concentration which,  
285 as expected, was above the legal limit for any virgin olive oil (IOC, 2016). Actually, the higher the  
286 share of sunflower oil in the blend, the higher the campesterol level. Nonetheless, when pondering  
287 on taking this free sterol as indication for the presence of seed oil above the 50 % limit (and therefore,  
288 that of olive oil below the threshold set by the European authorities), its concentrations for the 50:50  
289 and 40:60 olive oil:seed oil samples (v/v) were the same within the error limits (one SD). The  
290 respective ranges overlapped with each other, evidencing that a 10 % difference in the (EV)OO  
291 presence did not have a clear enough effect on the campesterol percentage. The same held for other  
292 seed-oil-characteristic sterols like stigmasterol and  $\Delta^7$ -stigmastenol. Apparent  $\beta$ -sitosterol, on the  
293 other hand, was *below* the expected limit for virgin olive oils but again, not in a way that allowed us

294 to use it as a marker (data not shown). Nevertheless, the fact of not being able to use individual sterols  
295 as indicators to assess the presence of olive oil does not turn the total sterol content in a useless  
296 parameter. On the contrary, a maximum limit of 2400 ppm can be set to assure that the proportion of  
297 (EV)OO in a blend with sunflower oil is at least 50 %. This is supported by the data shown in Table  
298 2. According to our results, the lower limit for the FS concentration to be found in 40:60 olive oil:seed  
299 oil blends is always above 2400 ppm, whereas the upper one for 50 % olive oil blends is in half of  
300 the cases below 2400 ppm. The fact that the OO mixtures display upper limits slightly above the  
301 proposed cut-off values is a clear hint that more determinations are needed. In the worst case, this  
302 would drive to discard some of the correctly declared 50:50 blends but never to admit as good those  
303 blends in which olive oil is below 50 %.

304 Our third set of compounds was that of tocopherols, which comprise four different forms:  $\alpha$ -,  $\beta$ -  
305 ,  $\gamma$ -, and  $\delta$ -TCPH-, and whose separation and analysis has been long ago described (Kofler, 1947).  
306 Table 3 shows the results on their analysis. Lines 3 to 6 shows data on the individual oils, whereas  
307 from lines 7 to the end the outcomes of the different mixtures are displayed. All four oil types gave  
308 results according to our expectations: Absence of  $\delta$ -TCPH, olive oil  $\alpha$ -TCPH (vitamin E) content  
309 lower than that of sunflower oils, and  $\beta$ -TCPH concentration lower than  $\gamma$ -TCPH (the one that exerts  
310 the highest antioxidant activity in vitro) in the cases of olive oils, whereas it is in the other way round  
311 for sunflower samples. If we analyze the individual TCPH profiles for the blends under study the first  
312 hint of the presence of seed oil is that the  $\beta$ - vs  $\gamma$ -TCPH relationship typical of olive oils ( $\beta$ -TCPH <  
313  $\gamma$ -TCPH) flips in all of them regardless the kind of seed oil. Besides, the  $\beta$ -TCPH / $\gamma$ -TCPH ratio  
314 increases in direct relationship with the sunflower oil presence. Also, the average value for the total  
315 TCPH concentration in all twelve mixtures affected by with three times the SD (3SD) displays an  
316 upper limit equal to or below 2.5 in 75 % of the 50:50 blends, whereas the lower limit for the 40:50  
317 olive oil:seed oil blends is always above the 2.5 cut-off value. Again, just one of the blends (i.e.  
318 OO:NTSO, 50:50 v/v) behaves in an unexpected way. Therefore, a  $\beta$ -TCPH / $\gamma$ -TCPH ratio equal to  
319 or lower than 2.4 will assure the presence of olive oil at least at 50 %.

320 The last parameter under consideration was the alkane fraction (Table 4). Generally speaking,  
321 hydrocarbons have been used as fingerprint of certain seed oils, to characterize varietal virgin olive  
322 oils, in geographical traceability, to detect irradiated food, or in the study of mineral oil  
323 contaminations (Lesgards, Raffi, Pouliquen, Chaouch, Giamarchi, and Prost, 1993; Moreda, Pérez-  
324 Camino, and Cert, 2001; Ju, Huynh, Gunawan, Chern, and Kasim, 2012; Gómez-Coca, Pérez-  
325 Camino, and Moreda, 2016; Quintanilla-Casas et al., 2020). In our case, the analysis of the n-alkane  
326 series showed natural hydrocarbon profiles consisting of odd C-atom number compounds mainly,  
327 between C21-C35 in the cases of olive oils, and between C27-C41 (there is a very small amount of  
328 C25) for both types of sunflower oils, although in all circumstances centered at C29. Since the n-  
329 alkane range from C21-C25 was virtually absent in sunflower oils, we decided to check on that in our  
330 quest for markers since it was clear than in any mixture practically all C21-C25 linear saturated  
331 hydrocarbons would come from olive oil. Subsequently, according to our results in mixtures with  
332 EVOO, percentages of  $\Sigma$ C21-C25 above 6 % would guaranty the presence of at least 50 % olive oil,  
333 whereas in mixtures with OO this must be above 3.5 %.

334 All these limits and their application have been summarized in the form of two decision trees  
335 combined in one (Figure 1), differentiating EVOO mixtures from mixtures with OO, and at the same  
336 time considering the possibility of using NTSO or HOSO.

337 Additionally, in order to test the effectiveness of this method a number of declared blind commercial  
338 samples collected in the Swedish market were tested and the decision trees applied (Table 5). The  
339 identity and composition of these samples were initially unknown, being disclosed once the test  
340 results have been reported. On the front label of each of the original bottles it was clearly revealed  
341 the fact that they contained olive oil, therefore one would expect to detect it at least at 50 %. However,  
342 the results of applying the decision trees let us conclude that none of these packers have complied  
343 with the official regulation: POO was below the minimum percentage in all cases. The limit for such  
344 TAG had been set at 11 % when NTSO was mixed with olive oils. Strictly speaking only samples #1,  
345 #2, and #8 contained that kind of oil, but the same limit was considered for every one of them since

346 it was set on the basis of a non-high oleic acid oil such as those at hand. Thus, when observing the  
347 analysis outcomes, results on the POO concentration ranged between 1.70 and 8.71 %, showing one  
348 of the lowest values in the case of sample #4 which turned out to be the one with the highest declared  
349 EVOO content. Regarding the global FS content, just one out of six determinations was below the  
350 2400 mg/kg maximum threshold. In the case of the SHC percentages (which include species with odd  
351 C-atom number from C21-C25) two minimum limits were established depending on the kind of olive  
352 oil used: 3.5 % for OO (exclusively in sample #7) and 6 % for EVOO (for the purpose of the present  
353 study, VOO and EVOO can be considered to be of the same kind since, generally speaking, from a  
354 chemical point of view the main difference between them and OO is the presence of refined oil in the  
355 sample). Results showed that 50 % of the samples (#2, #3, #5, and #7) would fulfill this limit. Lastly,  
356 the  $\beta$ -/ $\gamma$ -TCPH ratio was calculated, being results below the 2.4 threshold in five out of eight samples,  
357 and surprisingly high in the case of sample #5. Therefore, according to our results none of these  
358 commercial blends contained olive oil in percentages equal or above 50 %, hence, their highlighting  
359 of the presence of olive oil on the label was against Regulation No 29/2012 (European Commission,  
360 2012). Actually, checking over the list of ingredients samples #1-#4 contained between 20 and 35 %  
361 EVOO, samples #5 and #6 contained 20 % VOO, and sample #7 contained 25 % OO. The characters  
362 on sample #8's list of ingredients were too small to be readable and on the front label it was just  
363 indicated that the blend consisted of EVOO and NTSO. This means that in no case the fact of  
364 containing olive oil should have been highlighted on the main label.

365 We are aware that even if these decision trees have been designed on the bases of results obtained  
366 from blends with two types of sunflower oils (normal type and high oleic kinds), this is a pilot  
367 approach, therefore it will be interesting to observe how they also work with mixtures with other  
368 relevant seed oils such as rapeseed and soybean which are the first and third most produced seed oils  
369 in the last ten years in the EU (FEDIOL, 2019). For both matrices a comprehensive characterization  
370 is being carried out.

371 Finally, our use of sunflower oil is justified, on the one hand, by the place that it keeps within the  
372 ranking of seed oil production in the EU, which drives to understand why it is the most commonly  
373 utilized oil in blends with olive oil: According to FEDIOL, sunflower oil has been the second most  
374 consumed oil in the European Union in the last eleven years. Besides, it plays an important role  
375 regarding exports, imports and, as pointed out, production, this latter one amounting to more than  
376 39000000 tones in 2018 (FEDIOL, 2019). On the other hand, sunflower gave us the possibility of  
377 getting both normal type and high oleic oils easily and thus of including this possibility in the study.

378

#### 379 **4. Conclusions**

380 Our hypothesis has been confirmed: The combination of four of the official purity parameters,  
381 arranged as decision trees, may be enough to check if olive oil is at least at 50 % in commercial  
382 mixtures with seed oils. The effectiveness of the decision trees has been tested with positive results  
383 by applying them to blends prepared ad hoc and composed of olive oils and sunflower oils.

384 So far, we can state that to make sure that olive oil is present at least at 50 %:

385 a) The concentration of POO must be at least 11 % for mixtures with NTSO or 15 % for blends with  
386 HOSO.

387 b) The total sterol content must lie below 2400 ppm.

388 c) The  $\beta$ -TCPH/ $\gamma$ -TCPH ratio must not exceed 2.4.

389 d) The percentages of the n-alkanes of the  $\Sigma$ C21-C25 series must be above 6.0 % or 3.5 % in mixtures  
390 with EVOO or OO, respectively.

391 This is a preliminary study where data were generated from a limited number of samples (two olive  
392 oils and two sunflower oils), all corresponding to the first stages of the project. However,  
393 determinations with blind samples were later on carried out and the utility of the decision trees  
394 ratified. Further studies will consider olive oil climate and seasonal variability and its dependence on  
395 olive variety, specific soil and climatic conditions, geographical origin, possibility of mixing with  
396 edible vegetable oils other than sunflower oils, etc.

397 Presently, we have decided to treat all four parameters as complementary of each other in a way that  
398 all together will assure the presence of olive oil at least at 50 %, even if by doing so some genuine  
399 50:50 OO:seed oil samples may not be considered as compliant. We think that this could be taken as  
400 a ‘worse-case scenario’ and that such situation would be better than open the margins to  
401 counterfeiters. In any case, it remains to be seen if a certain flexibility regarding the application of  
402 the decision trees (e.g. allowing one of the fours parameters to fall outside the established interval in  
403 the cases of blends with OO, but being more restrictive with another) will increase the likelihood of  
404 fraud by permitting OO:sunflower oil mixtures at proportions of 40:60 v/v to be labelled as containing  
405 OO at 50 %. This is something that will also be addressed in the future.

406

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419

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**TABLE 1** Triacylglycerol (TAG) composition of the oil blends under study, consisting of extra virgin olive oil (EVOO) and normal type or high oleic sunflower oil (NTSO and HOSO, respectively), at three different proportions (60:40, 50:50, and 40:60, v/v). Measurements were done in duplicate. The standard deviation (SD) is also given; italics have been used when the SD is higher than the mean value, and bold to emphasize the usefulness of POO in the decision trees.

Blend	EVOO:NTSO						EVOO:HOSO					
	60:40		50:50		40:60		60:40		50:50		40:60	
Proportion, v/v	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD
TAG*	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD
LLL	10.41	1.45	13.45	1.44	17.04	0.35	0.66	0.01	0.81	0.01	1.08	0.03
OLL <sub>n</sub>			0.06	<i>0.09</i>	0.07	<i>0.10</i>	0.13	0.04	0.12	0.04	0.08	<i>0.11</i>
PoLL												
PLL <sub>n</sub>	0.10	<i>0.10</i>	0.06	<i>0.09</i>	0.08	<i>0.11</i>	0.03	0.01	0.03	0.01	0.03	0.01
OLL	10.95	1.37	13.76	1.37	17.46	0.45	1.45	0.01	1.52	0.01	1.62	0.01
OOL <sub>n</sub> +PoOL	0.98	0.10	0.82	0.08	3.32	<i>3.64</i>	0.90	0.03	0.75	0.03	0.64	0.01
PLL+PoPoO	3.74	0.44	4.70	0.44	2.95	<i>4.17</i>	0.46	0.01	0.46	0.01	0.49	0.01
POL <sub>n</sub> +PPoPL+PPoL	0.49	0.10	0.42	0.01	0.35	0.03	0.44	0.01	0.36	0.01	0.30	0.01
OOL+LnPP	9.47	0.06	9.54	0.06	9.61	0.18	7.72	0.01	7.33	0.01	6.98	0.02
PoOO	3.13	0.06	3.51	0.06	3.97	0.17	1.29	0.02	1.19	0.02	1.14	0.08
SLL+PLO	4.66	0.07	4.75	0.07	4.96	0.07	2.85	0.01	2.51	0.01	2.23	0.08
PoOP+SPoL+SOL <sub>n</sub> +SPoPo	0.63	0.10	0.48	0.09	0.35	0.02	0.69	0.03	0.59	0.03	0.61	0.16
PLP	0.51	0.08	0.55	0.08	0.62	0.07						
OOO+PoPP	27.68	1.68	23.83	1.68	19.14	0.01	51.20	0.22	53.40	0.22	55.76	0.33
SOL	1.74	0.10	1.94	0.10	2.12	0.06	0.94	0.01	0.86	0.01	0.87	0.11
<b>POO</b>	<b>14.98</b>	<b>1.00</b>	<b>12.58</b>	<b>1.00</b>	<b>9.69</b>	<b>0.09</b>	<b>17.75</b>	<b>0.04</b>	<b>16.30</b>	<b>0.04</b>	<b>14.90</b>	<b>0.02</b>
POP	2.68	0.24	2.38	0.24	2.01	0.04	2.43	0.06	2.11	0.06	1.78	0.01
SOO	4.64	0.30	3.95	0.32	3.22	0.05	7.14	0.02	7.19	0.02	7.23	0.04
POS+SLS	1.25	0.05	1.12	0.05	0.94	0.04	1.22	0.07	1.10	0.07	1.04	0.04
SSO	0.57	0.04	0.52	0.04	0.41	0.02	0.79	0.04	0.86	0.04	0.81	0.01

\*L (linoleic acid), Ln (linolenic acid), O (oleic acid), P (palmitic acid), Po (palmitoleic acid), S (stearic acid)

TABLE 1 (cont.)

Blend	OO:NTSO						OO:HOSO					
	60:40		50:50		40:60		60:40		50:50		40:60	
Proportion, v/v	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD
TAG*	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD	Mean, %	SD
LLL	11.84	0.17	14.01	0.17	16.86	0.01	0.95	0.02	0.94	0.02	1.06	0.02
OLL <sub>n</sub>	0.53	0.01	0.17	0.01	0.08	0.12	0.21	0.01	0.19	0.01	0.17	0.02
Poll							0.03		0.02		0.05	
PLL <sub>n</sub>	0.12	0.01	0.15	0.01	0.08	0.12	0.07	0.01	0.05	0.01	0.05	0.01
OLL	13.34	0.19	15.05	0.19	17.57	0.06	2.54	0.05	2.30	0.05	2.27	0.06
OOL <sub>n</sub> +PoOL	0.99	0.05	0.85	0.05	0.70	0.03	0.95	0.03	0.82	0.03	0.68	0.04
PLL+PoPoO	4.74	3.40	2.93	3.40	6.13	0.01	0.98	0.02	0.87	0.02	0.81	0.02
POL <sub>n</sub> +PPoPL+PPoL	0.47	0.02	0.41	0.02	0.33	0.03	0.47	0.02	0.38	0.02	0.30	0.02
OOL+LnPP	11.31	0.10	11.09	0.10	10.76	0.06	9.61	0.20	8.87	0.20	8.29	0.23
PoOO	3.56	0.03	3.75	0.03	4.12	0.07	1.51	0.01	1.40	0.01	1.25	0.03
SLL+PLO	6.17	0.01	6.02	0.01	5.85	0.03	4.36	0.03	3.74	0.03	3.14	0.08
PoOP+SPoL+SOL <sub>n</sub> +SPoPo	0.53	0.03	0.42	0.03	0.33	0.02	0.64	0.09	0.62	0.09	0.46	0.02
PLP	0.80	0.04	0.80	0.04	0.79	0.01	0.47	0.07	0.42	0.07	0.00	
OOO+PoPP	23.22	0.22	21.10	0.22	17.71	0.08	48.24	0.59	51.67	0.59	54.85	0.35
SOL	1.78	0.02	1.98	0.02	2.16	0.03	0.98	0.08	0.89	0.08	0.78	0.03
<b>POO</b>	<b>12.66</b>	<b>0.03</b>	<b>11.15</b>	<b>0.03</b>	<b>9.05</b>	<b>0.05</b>	<b>16.79</b>	<b>0.14</b>	<b>15.27</b>	<b>0.14</b>	<b>14.14</b>	<b>0.12</b>
POP	2.49	0.01	2.28	0.01	2.03	0.05	2.51	0.05	2.10	0.05	1.69	0.03
SOO	3.00	0.02	2.80	0.02	2.46	0.01	5.68	0.05	6.07	0.05	6.43	0.03
POS+SLS	0.83	0.01	0.83	0.01	0.78	0.01	0.98	0.01	0.89	0.01	0.84	0.02
SSO	0.39	0.14	0.46	0.14	0.33	0.01	0.63	0.01	0.65	0.01	0.70	0.72

\*L (linoleic acid), Ln (linolenic acid), O (oleic acid), P (palmitic acid), Po (palmitoleic acid), S (stearic acid)

**TABLE 2** Total sterol content (mg/kg) in the oil blends under study, which consisted of either extra virgin olive oil (EVOO) or olive oil (OO), and normal type or high oleic sunflower oil (NTSO and HOSO, respectively), at three different proportions. Measurements were done in duplicate. The standard deviation (SD) and the corresponding lower (mean value – SD) and upper (mean value + SD) limits are also given.

Blend, proportion (v/v)	Mean	SD	Sterol content (mg/kg)	
			lower limit	upper limit
<b>EVOO:NTSO, 60:40</b>	2014	113		
<b>EVOO:NTSO, 50:50</b>	<b>2296</b>	<b>104</b>		<b>2399</b>
<b>EVOO:NTSO, 40:60</b>	2575	121	2454	
<b>EVOO:HOSO, 60:40</b>	2092	60		
<b>EVOO:HOSO, 50:50</b>	<b>2259</b>	<b>79</b>		<b>2338</b>
<b>EVOO:HOSO, 40:60</b>	2488	41	2447	
<b>OO:NTSO, 60:40</b>	2301	38		
<b>OO:NTSO, 50:50</b>	<b>2409</b>	<b>68</b>		<b>2477</b>
<b>OO:NTSO, 40:60</b>	2579	8	2572	
<b>OO:HOSO, 60:40</b>	2121	83		
<b>OO:HOSO, 50:50</b>	<b>2330</b>	<b>133</b>		<b>2463</b>
<b>OO:HOSO, 40:60</b>	2481	32	2450	



**TABLE 3** Total tocopherol content and contents of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -Tocopherol (TCPH), in the initial oils and in the oil blends under study, which consisted of either extra virgin olive oil (EVOO) or olive oil (OO), and normal type or high oleic sunflower oil (NTSO and HOSO, respectively), at three different proportions. Measurements were done in duplicate. The three times the standard deviation (3SD) and the corresponding lower (mean value – 3SD) and upper (mean value + 3SD) limits are also given.

Sample	$\alpha$ -TCPH	$\beta$ -TCPH	$\gamma$ -TCPH (mg/kg)	TOTAL	$\beta$ -TCPH/ $\gamma$ -TCPH ratio			
					Mean	3SD	Upper limit	Lower limit
OO	256.20	2.61	13.23	272.03	0.20			
EVOO	332.72	3.19	18.65	354.56	0.17			
HOSO	460.76	36.77	2.65	500.18	13.89			
NTSO	518.49	46.98	3.40	568.88	13.81			
EVOO:NTSO, 60:40 (v/v)	350.00	10.44	8.37	368.82	1.21	0.18		
EVOO:NTSO, 50:50 (v/v)	432.72	15.25	10.44	458.41	1.46	0.18	1.6	
EVOO:NTSO, 40:60 (v/v)	470.99	21.03	7.04	499.06	2.99	0.18		2.8
EVOO:HOSO, 60:40 (v/v)	441.47	13.68	9.86	465.01	1.36	0.10		
EVOO:HOSO, 50:50 (v/v)	481.96	16.81	9.16	507.92	1.87	0.14	2.0	
EVOO:HOSO, 40:60 (v/v)	521.51	19.70	7.46	548.67	2.64	0.01		2.6
OO:NTSO, 60:40 (v/v)	477.87	15.78	7.84	501.49	1.93	0.34		
OO:NTSO, 50:50 (v/v)	331.71	11.75	4.95	348.41	2.28	0.39	2.7	
OO:NTSO, 40:60 (v/v)	520.49	18.69	5.83	545.01	3.27	0.27		3.0
OO:HOSO, 60:40 (v/v)	443.82	13.61	8.14	465.57	1.66	0.07		
OO:HOSO, 50:50 (v/v)	515.82	16.84	7.13	539.79	2.31	0.20	2.5	
OO:HOSO, 40:60 (v/v)	526.60	18.99	5.92	551.51	3.15	0.24		2.9

**TABLE 4** Acyclic saturated hydrocarbons or n-alkanes (SHC) composition (%) in the oil blends under study, consisting of either extra virgin olive oil (EVOO) or olive oil (OO), and normal type or high oleic sunflower oil (NTSO and HOSO, respectively), at three different proportions. SHC are named according to their C-atom number from C21 to C45.

					EVOO:NTSO (v/v)			EVOO:HOSO (v/v)			OO:NTSO (v/v)			OO:HOSO (v/v)		
	EVOO	OO	NTSO	HOSO	60:40	50:50	40:60	60:40	50:50	40:60	60:40	50:50	40:60	60:40	50:50	40:60
<b>Alkanes,</b>	%				%			%			%			%		
<b>C21</b>	<b>0.84</b>	<b>0.20</b>			<b>0.35</b>	<b>0.32</b>	<b>0.15</b>	<b>0.38</b>	<b>0.39</b>	<b>0.19</b>		<b>0.71</b>	<b>0.08</b>	<b>0.11</b>	<b>0.11</b>	<b>0.06</b>
<b>C23</b>	<b>5.44</b>	<b>1.85</b>			<b>2.18</b>	<b>1.91</b>	<b>1.05</b>	<b>2.20</b>	<b>1.89</b>	<b>1.16</b>	<b>0.64</b>	<b>0.76</b>	<b>0.60</b>	<b>0.90</b>	<b>0.76</b>	<b>0.68</b>
<b>C25</b>	<b>13.57</b>	<b>6.37</b>	<b>0.66</b>	<b>0.56</b>	<b>5.65</b>	<b>4.96</b>	<b>3.06</b>	<b>5.01</b>	<b>4.22</b>	<b>3.14</b>	<b>3.05</b>	<b>2.94</b>	<b>2.21</b>	<b>3.33</b>	<b>2.79</b>	<b>2.44</b>
C27	15.71	18.26	8.59	7.13	11.35	10.77	9.92	10.26	9.50	8.90	12.46	12.36	11.28	12.51	11.38	10.51
C29	21.33	25.70	44.44	41.89	35.10	35.88	39.87	34.08	35.29	37.49	34.83	36.45	38.26	33.75	35.22	36.23
C31	14.87	18.34	32.05	35.54	25.30	25.52	28.69	27.83	29.05	30.95	25.38	26.41	28.06	27.46	29.05	30.32
C33	6.91	11.03	1.93	2.15	3.86	3.48	2.87	3.79	3.41	3.07	5.96	5.62	4.42	6.24	5.50	4.82
C35	1.79	3.43	0.53	0.50	0.97	0.80	0.81	0.98	0.78	0.79	1.80	1.61	1.45	1.83	1.52	1.47
C37	0.28		0.50	0.65	0.32	0.36	0.60	0.36	0.23	0.29	0.18	0.76	0.38	0.22	0.30	0.29
C39			0.37	0.34	0.20		0.31	0.23	0.33	0.34	0.23	0.64	0.35	0.22	0.24	0.32
C41			0.20	0.27	0.13		0.24	0.20	0.37	0.27	0.17		0.20	0.16	0.31	0.29
C43			0.18				0.12	0.10		0.12	0.10		0.25	0.08	0.13	0.14
C45										0.12					0.09	0.11
<b>ΣC21-C25</b>	<b>19.86</b>	<b>8.43</b>	<b>0.66</b>	<b>0.56</b>	<b>8.18</b>	<b>7.19</b>	<b>4.26</b>	<b>7.59</b>	<b>6.49</b>	<b>4.49</b>	<b>3.70</b>	<b>4.41</b>	<b>2.90</b>	<b>4.33</b>	<b>3.66</b>	<b>3.18</b>

**TABLE 5** Dioleoyl palmitin (POO) percentage, total sterol content, saturated aliphatic hydrocarbon sum from C21 to C25, and  $\beta/\gamma$ -tocopherol ratio in commercial blends. The original blend compositions are also given, where extra virgin olive oil (EVOO), virgin olive oil (VOO), olive oil (OO), and normal type or high oleic sunflower oil (NTSO and HOSO, respectively), unspecified vegetable oil (VGO), and rapeseed oil (RSO).

Sample No	Blend composition according to the label	POO (%)	Sterol content (mg/kg)	$\Sigma$ C21-C25 (%)	$\beta/\gamma$ -Tocopherol	Compliance with EU R 29/212
#1	25% EVOO:75% RSO	6.03	5837	2.00	0.01	No
#2	20% EVOO:80% RSO	6.08		9.60	0.00	No
#3	20% EVOO:80% RSO	5.83		9.20	0.01	No
#4	35% EVOO:65% NTSO	2.65	1904	4.20	2.81	No
#5	20% VOO:80% NTSO	3.29	2587	9.20	31.08	No
#6	20% VOO:80% VGO	3.41	3004	4.30	1.09	No
#7	25% OO:75% RSO	8.71	6536	4.20	0.01	No
#8	EVOO:NTSO	1.70	3262	3.50	4.31	No

**Figure 1. Gómez-Coca, R. B.** Decision tree for mixtures of extra virgin olive oil (EVOO) or olive oil (OO) with normal type or high oleic sunflower oils (NTSO or HOSO, respectively), where POO stands for dioleoyl palmitin, and  $\Sigma C_{21-C_{25}}$  corresponds to the group of alkanes that includes those with odd C-atom number from C<sub>21</sub> to C<sub>25</sub>.

