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Olive oil quality and authenticity: A review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future

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Olive oil quality and authenticity: a review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future

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1 **Olive oil quality and authenticity: a review of current EU legislation, standards,**  
2 **relevant methods of analyses, their drawbacks and recommendations for the future**

3  
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## Abstract

### *Background*

The physical, chemical and organoleptic characteristics of olive oil (OO) are regulated by the European Union (EU) by Reg. (EEC) 2568/91 as amended, which also establishes methods for their analysis.

Despite the fact that the OO sector is highly regulated, it is acknowledged that there are still problems; fats and oils, including OOs, are ranked third, after meat and meat products and fish and fish products, in the 2016 EU Food Fraud report on non-compliances per product category.

For this reason, EU legislation, among the most advanced in the field, continuously chases after the emerging frauds. The process of proposing new methods or reviewing those current is constantly in progress, to ensure the robustness and the clarity required by official standardized procedures.

### *Scope and Approach*

This review will identify current gaps in EU legislation and discuss drawbacks of existing analytical methods with respect to OO. Suggestions for replacement of specific steps within the present EU methods with more efficient analytical solutions to reduce time and/or solvent consumption will be proposed.

### *Key Findings and Conclusions*

This review critiques existing regulatory methods and standards, highlights weaknesses and proposes possible solutions to safeguard the consumer and protect the OO market.

**Keywords:** Authenticity, olive oil; fraud; quality; analytical method; normative.

### **Highlights**

- Normative and standard sources for olive oil quality and purity
- Analytical methods for olive oil: drawbacks and limitations
- Normative failures and suggestions for improvements

## 52 Introduction

### 53 Normative and standard sources for olive oil quality and purity: a global framework

54 OOs have to comply with different rules and standards depending on where they are traded: three of  
55 the most important standards are those specified by the EU, the International Olive Council and the  
56 Codex Alimentarius. Within the EU, all OO legislation is comprised of regulations, i.e. mandatory rules.  
57 The early Regulation by EEC where olive oil has been mentioned was Reg (EEC) 136/66, a regulation  
58 for the establishment of a common organisation of the market of fats and oils that posed the basis for  
59 the descriptions and definitions of olive oils and residue olive oils marketed within the Member States  
60 and third countries. It, however, just established descriptions and definitions of different types of olive  
61 oils and did not report a detailed list of analytical parameters and related analytical methods. Further  
62 Regulations were later published: Reg (EEC) 177/66, repealed by Reg (EEC) 618/72, subsequently  
63 repealed by Reg (EEC) 1058/77, which was finally repealed by Reg (EEC) 2568/91. This latter is the  
64 cornerstone of all EU legislation on OO, establishing four important issues:

- 65 - the parameters that can be used to check for OO quality and purity, also indicating that no  
66 other parameter can be used for this purpose when an official control is carried out;
- 67 - limits for each parameter and commercial category of OO;
- 68 - descriptions of analytical methods that have to be used to assess if a sample of OO fits the  
69 limits (specification) of the commercial category for all the parameters in the regulation;
- 70 - only methods reported within this regulation, as amended, can be used for official control.

71 The EU Regulations are valid within the EU area, while outside this, International Olive Council (IOC)  
72 standards apply. The IOC, formerly the International Olive Oil Council, established in 1963 a "Trade  
73 standard for olive oils and olive pomace oils" that is a reference for any country which is a member of  
74 IOC. Member countries are obliged to apply it in the frame of international trade and, in the meantime,  
75 are encouraged to approximate their legislation to IOC Trade standard. After EEC turned to EU, the  
76 latter became a member of IOC (while, earlier, it was an observer and single countries were members),  
77 so that EU must harmonize its regulation to IOC Trade Standard. If OOs are considered in a worldwide  
78 scenario, different rules must be considered: in such a case, edible OOs, as most of foods, undergo to  
79 the standard developed by Codex Alimentarius whose Commission has at now more than 180  
80 Members made up of 188 Member Countries and 1 Member Organization (The European Union) (i.e.  
81 within the frame of FAO-OMS), whose mission is to facilitate the international trade of foods and to  
82 reach harmonization, that is a very hard mission due to the high number of countries involved. The OO  
83 standard is CODEX STAN 33-1981, reviewed in 2017. The Codex standard has a unique structure:  
84 some essential purity and quality characteristics are fixed as mandatory, while for a number of further  
85 characteristics adoption by a member is voluntary.

86

**87 Standard establishment procedure**

88 Figures 1a, 1b and 1c summarise the basic procedure for standard elaboration in EU, IOC and Codex,  
89 respectively. The ultimate approval by Codex is made by a plenary session that is held alternatively in  
90 Rome and Geneva; the process of revision usually takes about four years.

91

**92 Mandatory and voluntary standards**

93 EU Regulations are mandatory within the EU area and must be applied without any further procedures  
94 by member countries; however, outside of the EU, the same regulations cannot be applied. The IOC is  
95 in charge of developing standards that can also be applied outside of the EU, by a large number of  
96 countries as for the Codex Standard. Unlike EU Regulations, the use of these standards is not strictly  
97 mandatory: if an EU operator does not respect an EU regulation, he can undergo a penalty, while the  
98 use of IOC and Codex standards is not mandatory. These latter two standards are adopted based on a  
99 consensus, so that it should be expected that anyone who signs it would respect it; however, the IOC  
100 and Codex are not in a position to apply penalties if a member does not apply their standards.

101

**102 Analytical methods: drawbacks, inappropriateness, eventual normative failures and  
103 suggestions for improvement**

104 Analytical methods adopted within EU Regulations and IOC Standards have often originated from  
105 methods developed previously in individual Member States. These methods underwent some updates,  
106 taking account of improvements in analytical instrumentation (e.g. replacement of packed columns with  
107 capillary columns in gas chromatography), to considerations about solvent toxicity or through the  
108 possibility to improve the method. Table 1 reports a list of official methods, their current drawbacks and  
109 possible improvements.

110

**111 Determination of the peroxide value (PV)**

112 PV is probably one of the oldest analytical tests used for quality evaluation of fats and oils and dates to  
113 the “chemistry of indexes” (a term used to identify a number of tests developed before separative  
114 techniques, mainly gas chromatography, were widely used). PV is related to only the primary oxidation  
115 products therefore it does not give an exhaustive representation of the oxidative status of an oil (e.g.  
116 secondary compounds are also formed during the oxidative process). Briefly, the method (Reg. (EEC)  
117 2568/91) is based on a redox reaction between peroxide and an iodine ion, the latter being oxidised to  
118 molecular iodine. In the original method the oil is dissolved in a mixture of chloroform (that dissolves the  
119 oil) and acetic acid (to provide the acid reaction medium necessary for the redox reaction to take

120 place). Successively, a saturated solution of potassium iodine is added and in the original method the  
121 reaction takes place for 5 minutes in the dark. Later, ISO (ISO, 2003) amended the test by substituting  
122 the toxic chloroform with isooctane and limiting the reaction time to 1 minute. The main drawback when  
123 isooctane is used, instead of chloroform, is the fact that the addition of water produces an inversion of  
124 phases with the titratable one remaining at the bottom of the Erlenmeyer flask, necessitating the need  
125 for very efficient mixing to be applied in order for accurate titration to be carried out. For this drawback,  
126 the IOC, also, after some collaborative tests, returned to the use of chloroform as solvent. A further  
127 problem is that PV evaluation is strongly influenced by the amount of sample used for determination;  
128 thus it is particularly important to take care of the right amount of sample to be weighted (depending on  
129 the expected peroxide value, as recommended in the Annex III of the Reg. (EEC) 2568/91).

130

### 131 ***Determination of the ethyl esters of fatty acids (FAEE)***

132 Oil quality depends by both agricultural aspects and manufacturing practices. The content of FAEE is  
133 related to low quality fruits that may have undergone fermentative processes in the case of ethanol,  
134 while hydrolytic processes linked to pectin esterase activity releases methanol. Since fermentation  
135 processes are thought to be only related to FAEE, the parameter was changed in 2014 to these  
136 compounds with a limit of 40 mg/kg, which was then reduced to 35 mg/kg after discussion within the  
137 IOC expert chemist group. Low quality OO with slight organoleptic defects may be subjected to illegal  
138 practices, such as neutralisation and/or soft deodorisation at low temperature, to conceal their negative  
139 attributes. This practice is difficult to detect and several methods have been proposed, although most  
140 have produced unreliable results due to the fact that different technologies are used, leading to the  
141 formation of a variety of different marker compounds. In an attempt to solve this problem, the  
142 determination of the content of FAEE was proposed (Pérez-Camino, Cert, Romero-Segura, Cert-Trujillo  
143 & Moreda, 2008). It is believed that the soft deodorisation used by fraudsters does not remove FAEE,  
144 thus remaining as indirect markers of soft deodorization, when this process is applied on oils sensory  
145 defected for fermentative reasons (Biedermann, Bongartz, Mariani, & Grob, 2008). On the other hand,  
146 not all oils subjected to soft deodorisation (e.g. rancid oils) have a high amount of FAEE, so the FAEEs  
147 are obviously markers for some and not all the oils subjected to soft deodorization (Gómez-Coca,  
148 Fernandes, Pérez-Camino, & Moreda, 2016).

149 The method was adopted by IOC in 2010 (IOC, 2010) and by the EU (amending Reg (EEC) 2568/91).  
150 The method has been modified several times: for example, as reported in the IOC website, a reduction  
151 of the amount of silica, as well as the use of *n*-hexane to eliminate the hydrocarbon fraction, was  
152 proposed. The latter is an important issue, since hydrocarbons can elute in the same region of the  
153 chromatogram as FAEE, and the purity of solvent is a key point in the analysis. As stated before, the

154 amount of FAEE was thought to be stable over time, but this was shown to not be true since the  
155 amount of FAEE increased over time (Gómez-Coca, Fernandes, Pérez-Camino, & Moreda, 2016),  
156 (Mariani & Bellan 2013). Possible suggestions to improve the official procedure concern two main  
157 issues:

158 i) the separation of the fraction containing ethyl esters, by a preparative liquid chromatography on a  
159 hydrated silica gel column, that is particularly laborious and time consuming and requires large volumes  
160 of solvents (about 30 mL of *n*-hexane and 220 mL of a *n*-hexane/diethyl ether mixture per replicate) and  
161 quantity of silica gel (around 15 g per replicate)

162 ii) the use of an on-column injector for the final GC analysis, that is not a widespread system in the  
163 laboratories all over the world due to its very limited used in analytical methods for the quality control.

164 For the first task, an alternative could be the fractionation by SPE or HPLC to reduce solvents and  
165 speed the preparative step, while for the second a PTV (Programmed Temperature Vaporization) could  
166 be considered a promising alternative, as one of the most versatile sample introduction devices for GC.  
167 A revision of the actual official method for the determination of the ethyl esters of fatty acids in virgin  
168 olive oils could take into consideration these ways of improvements.

169

#### 170 ***Sterol composition***

171 Since genetic improvement of edible seed oils deeply modifies their fatty acid composition, attention  
172 has been given to sterol composition, and determination of these compounds has become a powerful  
173 tool to assess their purity. The official EU method for determining sterols implements the IOC method  
174 by the use of GC capillary column instead of packed column. The method involves a saponification step  
175 with KOH solution in methanol, followed by liquid-liquid extraction with diethyl ether, clean-up of the  
176 unsaponifiable matter by thin layer chromatography (TLC), preparation of trimethylsilyl derivatives and  
177 GC-FID analysis. As reviewed by Panagiotopoulou and Tsimidou (Panagiotopoulou & Tsimidou, 2002)  
178 different approaches, mainly based on the use of solid phase extraction, have been investigated to  
179 speed up sample preparation. The ISO 12228:2009 (included in Codex standard) purposed a method  
180 circumvents solvent-extraction, replacing it with solid-phase extraction (SPE) on aluminum oxide.  
181 However, a footnote reports that results obtained by this method can lead to different results from those  
182 obtained by liquid-liquid extraction: because of this, part 2 of the method (ISO, 2014) was later  
183 published, devoted to OO and olive pomace oils only. With the aim to save time, some modifications of  
184 the saponification method have also been proposed. Moreda suggested to carry out unsaponifiable  
185 fractionation by HPLC instead of TLC (Cert, Moreda, & García-Moreno, 1997). Earlier results of this  
186 approach have been presented at the IOC Olive Chemists group and the method's characteristics  
187 seem very promising.

188 On-line HPLC-GC determination of sterols have improvements in terms of time and solvent, increased  
189 sensitivity, avoiding sample contamination, but require dedicated instrumentation and skilled operators.  
190 In 1993, a method of this kind was proposed by Biedermann et al. (Biedermann, Grob, & Mariani,  
191 1993). By optimising HPLC conditions, it was possible to separately analyse the individual classes of  
192 minor compounds, or to send them to the GC as a single fraction. Determination of sterols and other  
193 minor components of the oil were later achieved by employing HPLC-GC techniques with PTV based  
194 interface (Toledano, Cortés, Andini, Vázquez, & Villén, 2012). The use of reverse phase HPLC allowed  
195 to avoid the backflush of the LC column for eliminating any retained lipids, that is a step requested  
196 when using the normal phase. More recently, a fully automated method (Nestola & Schmidt, 2016),  
197 including a saponification and extraction step (performed by the autosampler), followed by injection into  
198 the LC-GC without previous derivatization, was developed. When applied to OOs, the quantitative  
199 results were fully comparable with the ISO method.

200 Besides being time consuming, the official method (ISO, 2014) only evaluates the whole sterol  
201 composition, with no possibility to distinguish between free and esterified ones, despite the fact that  
202 such information can be useful to distinguish oils that present slight differences in whole sterol  
203 composition. A number of papers have highlighted that sterols in plant and vegetable oils extracted  
204 from both seeds and fruits may be in a free or esterified form and that the ratio between the two forms  
205 is not constant, and varies for different oils (Grob, Lanfranchi, & Mariani, 1989). Mariani et al. (Mariani,  
206 Bellan, Lestini, & Aparicio, 2006) proposed the evaluation of free and esterified sterols to highlight the  
207 presence of hazelnut oil in OO.

208

### 209 ***Determination of stigmastadienes***

210 In 1975, Niewiadomski elucidated the structures of hydrocarbons deriving from the dehydration of  
211 sterols. Later, in 1989, Lanzon et al. (Lanzon, Cert & Albi, 1989) proposed the use of the determination  
212 of these compounds to detect oils that had been refined. Cert et al. (Cert, Lanzon, Carelli, Albi, &  
213 Amelotti, 1994) identified stigmasta-3,5-diene, derived from  $\beta$ -sitosterols as the main compound of  
214 interest, and in 1995 it was adopted within the trade standards (Reg (EEC) 656/95, IOC, 2001); the limit  
215 was fixed at 0.15 mg/kg for edible virgin OOs and 0.50 mg/kg for lampante OOs. Later the limit was  
216 amended to 0.10 and then to 0.05 mg/kg for edible virgin oils. IOC split the method into two parts: when  
217 the expected concentration is between 0.01 and 4.0 mg/kg, saponification of 20 g of oil is carried out,  
218 followed by LC on silica column to isolate the sterene fraction, while when the concentration is more  
219 than 4 mg/kg (refined oils), direct fractionation of the oils on silver ion silica is carried out.

220 One of the suggested purposes of this analytical determination was the possibility to check for the  
221 presence of seed oils depleted in sterol concentration in refined OO. The starting point was the

222 consideration that the ratio between selected sterols should also be maintained in the sterene fraction.  
223 However, the dehydration reaction takes place at different speeds depending on whether it involves  
224 free or esterified sterols, the latter being slower. Attempts to improve the method e.g. by saving time  
225 were published by Grob et al. (Grob, Artho, & Mariani, 1992; Grob, Biedermann, Artho, & Schmid,  
226 1994), Biedermann et al (Biedermann, Grob, & Bronz, 1995) and Amelio et al. (Amelio, Rizzo,&  
227 Varazini, 1998).

228

### 229 ***Waxes composition and triterpenic dialcohols content***

230 The analytical determination of fatty acid and sterol composition made it possible to detect the  
231 presence of seed oils (when sterols were not removed). Another possible fraud is the blend between  
232 pomace oils and refined OOs, so that no problems with the presence of fatty acids *trans*-isomers and  
233 stigmastadienes occur.

234 Since early '70s, erythrodiol and uvaol, two triterpenic dialcohols, whose concentration is very high in  
235 pomace as they are mainly present in the fruit skin, have been used as potential markers of pomace oil.  
236 A limit of 4.5% (calculated on the sum of sterols and erythrodiol plus uvaol) was established for any  
237 non-pomace OO. Due to the fact that fraudsters started to remove these compounds, the evaluation of  
238 the content of waxes, suitable to detect the presence of pomace oils, was proposed by Mariani and  
239 Fedeli (Mariani & Fedeli, 1986). The scientific basis was that waxes concentration is about tenfold in  
240 solvent extracted oils with respect to pressure extracted oils and that any attempt of remove them  
241 would lead to a significant loss of oil. The method was adopted as the EU and IOC official one in 1997.  
242 It had been widely and successfully applied for a number of years, and was also approved as the ISO  
243 method. In 2007 Ceci and Carelli (Ceci & Carelli, 2007) noted that when applying the method to  
244 selected authentic Argentinian extra virgin OO anomalous results were produced. It was discovered  
245 that the main problem was the measurement of the area of C40 peak, as this is split into two peaks that  
246 are not well resolved, one of which (the smaller) comprises the linear esters C40, while the other one is  
247 phytol behenate. A satisfactory separation of the two peaks, mainly when high concentrations of phytol  
248 behenate are present, may be problematic for less skilled laboratories, so that the proposed and  
249 adopted solution was to delete the C40 peak area in the sum for extra virgin and virgin oils.

250 Some improvements in the method need to be found, on one hand in enhancing the separation of GC  
251 peaks (e.g. by using a slightly more polar stationary phase), and on the other hand by reducing the  
252 need for the large volumes of solvents that are currently used. Nota et al. (Nota et al., 1999) applied a  
253 silica SPE ,and Amelio et al. (Amelio, Rizzo, & Varazini, 1998) were able to separate waxes by a single  
254 HPLC run. LC-GC also remains a suitable approach.

255

## 256 **Triacylglycerol Analysis**

257 Triacylglycerols (TAGs) consist of a glycerol moiety with each hydroxyl group esterified to a fatty acid  
258 (FAs). Twenty TAGs have been identified and independently quantified in olive oil, but only five are  
259 present in significant proportions (León-Camacho, Morales, & Aparicio, 2013).

260 The analytical evaluation of TAGs is carried out with different purposes:

- 261 1. To evaluate the botanical origin of the oil: the pathway for biosynthesis of TAGs, the so-called  
262 “Kennedy pathway”, is the same in seeds and fruits. The flux of the pathway and, thus, the final  
263 amount of TAG synthesized, is mainly under the control of the first step of FA synthesis in  
264 about 27-31% in seeds, while in the case of fruits it is about 57% in olive and 61-65% in palm.  
265 Therefore, based on this knowledge, it is not possible to check if an oil is extracted from fruits  
266 or from seeds.
- 267 2. To discriminate TAGs from plant biosynthesis from those of chemical synthesis, the latter  
268 obtained by a reaction between FAs (e.g. from oil refining) and glycerol (e.g. from biodiesel  
269 production): this is possible because the lysophosphatidic acyltransferase is selective to oleyl-  
270 CoA and has no activity to saturated fatty acids. The result is that for the 2-position of  
271 triacylglycerol molecule, no more than 1% of palmitic acid can be detected in most authentic  
272 vegetable oils (with the exception of palm oil), and does not depend on seed or fruit oils.

273 The International Olive Council trade standard (IOC, 2016), and Reg. (EEC) 2568/91 established  
274 methods for the control of the authenticity of OO; among them, the determination of FA composition in  
275 relationship with triglycerides seems to be very useful (Synouri, Frangiscos, Christopoulou, & Lazaraki,  
276 1995). Within this context, trilinolein was identified as a possible and powerful marker for detecting the  
277 presence of other vegetable oils. However, the incomplete separation of trilinolein peak, and therefore  
278 the difficulty of accurate quantification using the refractive index, have prompted the scientific  
279 community to replace trilinolein content with the use of the equivalent carbon number (ECN) 42. The  
280 experimental and the calculated values of ECN42 are compared to assess the degree of correlation  
281 between FA composition and ECN42 TAGs. A limit of  $|0.20|$  as an absolute value in extra virgin OO had  
282 been established. An improvement of this method was obtained with the so-called global method (IOC,  
283 2001), which uses the same principle of the ECN42 but extended to some TAG of ECN44 and ECN46.  
284 With this method, the limit of detection of hazelnut oil in OO was set at 8–10%. The analysis of TAG to  
285 apply this method was also improved using propionitrile as HPLC eluting solvent (Moreda, Pérez-  
286 Camino & Cert, 2003). Other approaches are being studied to compare reliability with the official  
287 method (Beccaria et al., 2016). Some drawbacks have arisen in terms of false positive results, with the  
288 result that in 2015 the method was deleted from the EU legislation.

289 In light of this, two main linked strategies for improving the TAGs analysis in OO can be considered:

290 1) the use of columns packed with sub-2  $\mu\text{m}$  particles or with fused-core particle to avoid high  
291 backpressure; 2) the use of detectors that can accommodate gradients. Regarding the first point, it is  
292 well known that reduction of particle size down to sub-2  $\mu\text{m}$  (compared to conventional columns  
293 packed with 3 or 5  $\mu\text{m}$  particles), as well as the use of columns packed with superficially porous  
294 particles, allows speeding up of the analytical process by a factor of 9 while maintaining similar  
295 efficiencies or a theoretical 3-fold increase in efficiency for a similar column length (Núñez, Gallart-  
296 Ayala, Martins, & Lucci, 2012). C18 phase, which is the most common, can provide satisfactory results  
297 in gradient elution even if other promising stationary phases such as C30 can also be explored to  
298 further improve the separation efficiency of TAGs. With regard to the second point, the use of universal  
299 detection in gradient elution is another crucial aspect. Two possible alternative methods to RI detection  
300 are represented by evaporative light scattering detection (ELSD) and charged aerosol detection (CAD).  
301 ELSD, however, provides a non-linear response, thus preventing accurate measurements of TAGs. On  
302 the contrary, CAD has been shown to provide linear response as well as homogenous response factors  
303 for the main TAGs in OO. Another option is analysis by HPLC-MS because MS can identify partially  
304 resolved HPLC peaks (Cozzolino & De Giulio, 2011), giving much more information on the position of  
305 the three FA molecules in TAGs. Although few ionisation techniques can be coupled to HPLC, the  
306 identification of positional isomers can be carried out by atmospheric pressure chemical ionisation  
307 (APCI) coupled to HPLC, whereas the identification of individual acyls cannot be done without  
308 electrospray ionisation (ESI) and reference materials. An interesting alternative is the use of  
309 atmospheric pressure photoionisation (APPI), which has been recently introduced for the ionisation of  
310 non-polar compounds that are insufficiently ionised by either APCI and ESI sources. In APPI, MS  
311 spectra TAG ions are present mainly as  $[\text{M}+\text{H}]^+$ . However, other fragment ions present in APPI-MS are  
312 those to acylium ion  $[\text{RCO}]^+$  and  $[\text{RCO}-\text{H}_2\text{O}]^+$  (Gomez-Ariza et al. 2006). This latter ion, which is absent  
313 in ESI mass spectra, provides valuable complementary information for identification of TAG molecular  
314 species. However, mass spectrometry is an expensive instrument that also requires skilled personnel.  
315 Therefore, it probably does not represent the best choice for routine TAG analysis.

316 Finally, GLC is scarcely applied today even though it also offers attractive possibilities as an efficient  
317 separation method, good quantitative recovery and reproducibility, adequate time for analysis and the  
318 availability of a flame ionisation detector (FID), a simple but universal linear response detector.  
319 However, the GLC technique is not free of problems such as, for example, the injection system and  
320 column deterioration. Selectivity in GLC depends on the length and chemical nature of the column  
321 stationary phase. For example, columns with phenyl-methyl-silicone phase can reach temperatures of  
322 about 360–370  $^{\circ}\text{C}$  for a long period and separate TAGs by carbon atom number; unsaturated positional

323 isomers cannot be separated by this phase unless sample derivatisation followed by a reduction is  
324 previously performed (León-Camacho, Morales, & Aparicio, 2013).

325 As stated above, the determination of FA composition in position 2 of TAG molecule gives very  
326 important information; this analysis applies enzymatic hydrolysis with pancreatic lipase, which is  
327 selective for positions 1 and 2, and in the original method the reaction mixture made by TAGs, DAGs,  
328 MAGs and FFA was fractionated on silica gel TLC plates, MAGs were recovered, submitted to  
329 transmethylation and finally GLC analysis of fatty acids was carried out (IOC, 2006). The method was  
330 very time consuming and cumbersome and several artefacts were detected, mainly depending on silica  
331 plate constituents. Thanks to the development of capillary GC, Motta et al. (Motta, Brianza, Stanga &  
332 Amelotti, 1983) were able to skip all the procedures after hydrolysis and directly inject the reaction  
333 mixture after silylation; the method was adopted in Italy and later by the IOC and EU.

334

### 335 ***Method for organoleptic assessment of OO***

336 A virgin OO, obtained from olives only by mechanical-physical processes (crushing, malaxation,  
337 centrifugation, filtration) and without additional refining, has a sensory profile strongly linked to the  
338 quality of raw material, namely olives. In fact, any damage to drupes and subsequent activity of  
339 microorganisms and enzymes, which can trigger triacylglycerol hydrolysis, FA oxidation, sugar  
340 fermentation and amino acid degradation, produces molecules that affect the compositional profile of  
341 the virgin OO obtained, mainly in terms of phenolic and volatile compounds (Angerosa et al., 2004;  
342 Morales, Luna, & Aparicio, 2005; García-González, Tena, & Aparicio, 2007; Cayuela, Gomez-Coca,  
343 Moreda, & Perez-Camino, 2015).

344 In the context of food regulation, there is no food other than virgin OO whose quality categories are  
345 defined with different international standards (e.g. Codex Alimentarius, International Olive Council, and  
346 European Union) including sensory assessment. The organoleptic assessment of virgin OOs by the  
347 "IOC Panel test" methodology (IOOC/T.20/Doc. no. 3, 1987) has been applied with a legal purpose  
348 since the early nineties in Europe (Reg. (EEC) 2568/91), and specifically to classify a sample within a  
349 commercial category. Over the years, it has undergone many revisions, as a result of continuous study  
350 of its performance. In fact, since the most important result for sensory analysis of a virgin OO is to  
351 define its quality grade, identification of the main perceived defect and evaluation of its intensity, as well  
352 as of the fruity attribute, are the main outputs. Keeping in mind this objective, the methodology has  
353 been amended exhaustively starting from the Reg. (EEC) 2568/91 Annex XII:

354 *i)* The updated profile sheet requests evaluation of descriptors using a continuous 10-cm scale instead  
355 of the original score from 0 to 5; moreover, the median value related to the intensity of each attribute is  
356 calculated, thus replacing the overall score from 1 to 9 (introduced by amending Reg. (EC) 796/2002).

357 The sensory data, collected by the panel leader, can be elaborated by an excel program that permits to  
358 calculate for each attribute: median value (indicates the 50th percentile of a distribution of numbers  
359 arranged in increasing order), the robust coefficient of variation % (useful for checking the reliability of  
360 the panel assessors), the 95% confidence intervals (represents the interval of variability of the test  
361 under operating conditions, assuming it is repeated many times) (introduced by amending Reg. (EC)  
362 796/2002).

363 *ii)* More emphasis has been given to the list of defects (fusty/muddy sediment, musty-humid-earthy,  
364 winey-vinegary-acid-sour, rancid, frostbitten olives-wet wood, others), which is now present in the upper  
365 section of the profile sheet. Specifically, a unique negative attribute named “fusty/muddy sediment” has  
366 been inserted (by amending Reg. (EC) 640/2008) instead of the two separated ones. This merge was  
367 aimed to limit the variability of tasters in the identification of these two single defects; even if fusty and  
368 muddy are elicited by slightly different qualitative-quantitative volatile profiles (Angerosa et al. 2004;  
369 Procida, Giomo, Cichelli & Conte, 2005; Aparicio, Morales, & Garcia-Gonzalez, 2012; Bendini, Valli,  
370 Barbieri & Gallina Toschi, 2012; Cayuela, Gomez-Coca, Moreda, & Perez-Camino, 2015), because  
371 they are produced by specific microorganisms, but during different steps of olive processing (“fusty”  
372 when olives are stored in piles, while “muddy” when the olive oil is in contact with the sediment); these  
373 two defects were often confused by assessors, thus causing difficulties in the calculation of the main  
374 perceived defect.

375 *iii)* By the Reg. (EU) 1348/2013, the defect named “frostbitten olives-wet wood” already included among  
376 the “other negative attributes” according to Reg. (EC) 640/2008, has been moved to the list of the main  
377 defects and a IOC reference standard has been made available. This sensory defect, unusual until a  
378 few decades ago, has become a common defect today and little is known about its related volatile  
379 markers. The origin of this defect has been linked to the change in weather conditions during the  
380 autumn-winter period that can cause extracellular or intracellular ice formation in olives by a gradual  
381 drop of temperature or a rapid freezing repeated in several freeze–thaw cycles, respectively. The final  
382 effect due to the forming of ice crystals is cell dehydration and parenchyma destruction as well as an  
383 increased contact between enzymes and their respective substrates that bring about the formation of  
384 several volatile compounds (Morello, Motilva, Ramo, & Romero, 2003; Romero, García-González,  
385 Aparicio-Ruiz, & Morales, 2017). The identification and quantification of the most important markers of  
386 this defect would be highly desirable to understand better its origin and to define accurately its sensory  
387 notes. A recent study has highlighted the sensory importance of some volatile compounds, such as  
388 some esters with ripe fruity attributes, typically found in samples with this defect (Romero, García-  
389 González, Aparicio-Ruiz, & Morales, 2017).

390 iv) The list of positive attributes has been restricted only to fruity, bitter and pungent, with the possibility  
391 of using a specific optional terminology for labelling purposes according to the frequency (green or ripe  
392 fruitiness, in Reg. (EC) 640/2008), and by specifying the intensity of these attributes (e.g. robust,  
393 medium, delicate, well balanced, mild oil in the Reg. (EC) 640/2008, amended by Reg. (EU)  
394 1227/2016).

395 v) The organoleptic assessment is both a qualitative and quantitative method, since its application  
396 results in the classification of samples based on the median of the predominant defect and the  
397 presence or not of the fruity attribute. Consequently, tasters must be supervised for correct  
398 classification of samples and for correct recognition of the intensities of perceived attributes. With this  
399 aim in mind, it is strictly recommended that the IOC member laboratories performing sensory analysis  
400 of virgin OO apply the guidelines for the accomplishment of the Norm ISO 17025 requirements  
401 (COI/T.28/Doc. No 1, 2007 – revised in 2017), in order to work in compliance with the characteristics of  
402 a quality system (drawing up, implementing and maintaining of procedures, identifying possible minor,  
403 major or critical nonconformities) and also with the laboratory organisation and technical conditions of  
404 analysis requirements (COI/T.20/Doc. No 15/Rev. 8, 2015). In agreement with these guidelines,  
405 accredited laboratories should demonstrate that they obtain equivalent results within defined limits in  
406 terms of precision (repeatability and reproducibility) by calibration and testing activities (interlaboratory  
407 tests), adopting the correct methodology to monitor the panel proficiency. The periodic participation of  
408 laboratories in proficiency tests (recommended at least once a year) permits detection of possible  
409 systematic errors and to check the validity of the entire quality system. Since current reference  
410 materials are “natural” virgin olive oils selected for being representative of a single sensory defect, they  
411 can be slight different year by year in sensory properties and intensity of the defect. On the contrary, a  
412 perfect reproducibility of each defect would be extremely useful to align all the panels. The availability  
413 of certified reference materials (e.g. samples from inter-laboratory tests conducted by the IOC or other  
414 accredited suppliers), having intensity ranges for specific attributes that cover different classes of virgin  
415 OO, can assure correct training of sensory assessors, and is useful to determine the trueness of the  
416 evaluation carried out by tasters (closeness to the accepted reference value is a measure of accuracy).  
417 It is particularly important to improve the sensory skills of the panel through the adoption of new  
418 formulated reference materials (RMs) built with a specific mixture of sensory relevant volatile molecules  
419 and appropriately combined in defined concentrations, also considering their odour thresholds.

420 In consistency with decisions taken at IOC level, the Reg. (EU) 1348/2013 recommends the number of  
421 oils to be assessed by the sensory panels, fixing a maximum number of four samples at each session.  
422 Moreover, a maximum of three sessions per day is specified, to leave enough time between a session  
423 and another, thus avoiding the contrast effect that could be produced by immediately tasting sequences

424 of samples. These specifications strongly limit the number of samples (namely 12) that can be  
425 assessed by one panel per day; the establishment of instrumental methods for a rapid screening could  
426 represent a solution for supporting the sensory panels (particularly for the large private industries) in the  
427 discrimination of sample far away from the boundaries (EVOO/VO and VO/LO). Actually, the need to  
428 support organoleptic analysis was also reported in a specific call of the Horizon 2020 EU program  
429 (H2020-SFS-14a-2014) and is one of the main objectives of the OLEUM project (Horizon 2020, Grant  
430 Agreement No. 635690). With this purpose, some encouraging examples of analytical instrumental  
431 techniques, of which many are based on the determination of volatile markers, have been proposed in  
432 the literature.. These, thanks to the application of appropriate multivariate analysis tools, try to find  
433 relationships by targeted and untargeted approaches between instrumental signals (fingerprints) and  
434 sensory quality attributes (Morales, Luna & Aparicio, 2005; Procida, Giomo, Cichelli & Conte, 2005;  
435 Vichi, Romero, Tous, López Tamames, & Buxaderas, 2008; Lerma-García et al., 2010; Sinelli,  
436 Cerretani, Di Egidio, Bendini, & Casiraghi, 2010; Aparicio, Morales, & García-González, 2012; Morales,  
437 Aparicio-Ruiz, & Aparicio, 2013; Borràs et al., 2016). Due to its rapidity, one of the most promising  
438 screening method under testing to support the sensory analysis is the flash gas-chromatography  
439 electronic nose) used in tandem with chemometrics (Melucci, et al., 2016).

440 The volatile compounds, as molecules strongly dependent on the OOs sensory profiles, should be  
441 considered as relevant quality markers for OOs; the determination of these compounds could support  
442 the sensory analysis, especially within the so-called “boundary zones” between virgin OOs designations  
443 (e.g. extra virgin OO vs. virgin OO). In particular, during the last years researchers are working hard for  
444 the setting up of robust analytical methods for evaluating the quali-quantitative profiles of volatile  
445 compounds in OOs (Romero, García-González, Aparicio-Ruiz & Morales, 2015; Fortini et al., 2017);  
446 further research efforts should be done in focusing on a low number of volatile compounds, previously  
447 selected as relevant markers of the sensory defects, to be determined by possibly using less expensive  
448 instruments, such as SPME-GC-FID.

449

#### 450 **Normative failure and inappropriateness**

451 Despite the fact that OO is highly regulated, some critical aspects of the OO sector as i) the lack of  
452 proper analytical methods for identification of specific frauds ii) the lack of a defined method for specific  
453 markers, remain. There is therefore an urgent need to resolve these gaps and limitations in regulatory  
454 methods and frameworks and to identify appropriate analytical solutions for specific fraud and marker  
455 detection, as well as to provide relevant information required from international markets. Specific cases  
456 of the above-mentioned lacks are discussed in the following paragraphs: the assessment of the amount  
457 of olive oil in mixtures with seed oils and the assessment of deodorised oils as examples of i) whereas

458 the methods to assess polyphenol health claims, to estimate OO freshness and to verify geographical  
459 origin as examples of ii).

460

#### 461 ***Health claims related to selected polyphenol content***

462 Consumers are cautious about the nutritional and health claims provided on food labelling, which are  
463 expected to assist them in making purchase decisions. To increase confidence in the market and  
464 ensure a high level of consumer protection, the European Food Safety Authority (EFSA) works for the  
465 approval of clear, accurate and corroborated nutritional and health claims (EFSA, 2011). Substantiation  
466 of a nutritional or health claim is often a time-demanding procedure that involves approval of several  
467 evaluation steps. For example, a health claim for “olive oil polyphenols” was made only very recently  
468 after many years of discussion. The health claim stated: “Olive oil polyphenols contribute to the  
469 protection of blood lipids from oxidative stress.” The claim may be used only for OO that contains at  
470 least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of OO.  
471 Furthermore, information is given to the consumer that “the beneficial effect is obtained with a daily  
472 intake of 20 g of olive oil”. This health claim presents some weaknesses regarding terminology  
473 interpretation and analytical methodology. The term “olive oil polyphenols” is not entirely clear and  
474 accurate, considering that only fresh virgin OO of high quality contains considerable amounts of  
475 oleuropein/ligstroside aglycons and derivatives. “Olive oil” is a generic term for all types of oils extracted  
476 by mechanical means from olives. Moreover, the term “polyphenols”, probably derived decades ago  
477 from terminology used for wine phenolic compounds, does not match the basic structure of the  
478 secoiridoids present in virgin OO for which the claim was assigned (i.e., hydroxytyrosol and its  
479 derivative, e.g. oleuropein complex and tyrosol). Beyond concerns for ambiguous interpretation of the  
480 terminology, there is a lack of a standardised analytical methods that allow quantitative determination of  
481 unequivocally identified individual phenolic compounds belonging to the group of hydroxytyrosol/tyrosol  
482 and its derivatives. The latter comprises more than 10 identified compounds. This lack has an impact  
483 on the reliability of the lower limit set (5 mg/20 g oil) for the health claim.

484 A candidate protocol could be that recommended by the International Olive Council (IOC, 2009).  
485 However, difficulties in complete separation of all types of phenolic compounds in a single  
486 chromatographic run and limitations in the choice of standards for accurate quantification in the UV  
487 region or using other detection means, repeatedly discussed over the past 20 years, does not support  
488 its adoption for standardisation. To address such a challenge, any experienced analytical chemist  
489 would reach simplify the analytical protocol. Simplification in this case would involve hydrolysis of the  
490 bound forms of hydroxytyrosol and tyrosol, and quantification of their total free forms (Mulinacci et al.,  
491 2006; Romero & Brenes, 2012; Purcaro, Codony, Pizzale, Mariani, & Conte, 2014).

492

493 ***Estimate (predict) an appropriate “best before date”***

494 According to the Reg. (EU) 1169/2011, the label of any food must report the “use by” date or the “best  
495 before” date; this regulation also very clearly describes the differences between these two phrases: the  
496 “best before” date is used in the case of foods that can undergo chemical, physical or sensory  
497 modifications without any prejudice for consumers’ health, while the “use by” is used for foods whose  
498 modifications involve an health risk for consumers health.

499 Despite this mandatory rule, no method had been validated and no shared method is available to  
500 calculate the best before date for foods. From a scientific and technological point of view, these dates  
501 are called “shelf life” (McGinn, 1982) which is a very important aspect for managing companies (Stone  
502 & Sidel, 2004). As a preliminary step, the difference between “freshness” evaluation that estimates *ex*  
503 *post* the (residue) quality of an oil and “shelf life” that try to predict *ex-ante* the behaviour of the quality  
504 of the oil during its commercial life must be very clear.

505 The OO shelf life can be described as the period of time during which, in correct storing conditions, no  
506 off-flavours or defects arise and any quality parameters remain inside the limit established for the  
507 category the oil belongs to (Guillaume & Ravetti, 2016).

508 Although there are many studies describing the effects of selected environmental factors affecting OO  
509 quality in the scientific literature, no studies are available dealing with information that can be used by  
510 companies to predict shelf life, even bearing in mind the time and costs of the test. Del Nobile et al. (Del  
511 Nobile, Bove, La Notte & Sacchi, 2003) developed a model by studying the effects of material and  
512 dimension of packaging on oxidative degradation. The model, however, did not consider light and  
513 temperature changes during storage. This aspect had been considered by Coutelieris and Kanavouras  
514 (Coutelieris & Kanavouras, 2005) who evaluated improvement in the concentration of hexanal to  
515 estimate the loss of oil quality by the activation energy of oxidative reactions. Later, the same authors  
516 (Coutelieris & Kanavouras, 2006; Kanavouras & Coutelieris, 2006), used the same approach to study  
517 additional aspects of the degradation of oil during storage. Mancebo-Campos et al. (Mancebo-Campos,  
518 Fregapane, & Salvador, 2008) developed a kinetic study to estimate the potential shelf life of OO, while  
519 Aparicio-Ruiz et al. (Aparicio-Ruiz, Aparicio, & García-González, 2014) used the degradation of  
520 pyropheophytines as a marker of OO ageing. An empiric model had been proposed by Guillaume and  
521 Ravetti (Guillaume & Ravetti, 2016), which uses induction time, 1,2-diacylglycerols, pyropheophytin A,  
522 and free FAs to predict the shelf life. On the other hand, Tena et al., (Tena, Aparicio, & García-  
523 González, 2017) also demonstrated the importance of light at moderate conditions during storage when  
524 measuring hydroperoxides by infrared spectroscopy and the utility of spectroscopy in this task.

525 The availability of a method that is reliable, fast and relatively inexpensive are a priority for companies in  
526 order to avoid legal problems related to oil degradation once its trade begins.

527

### 528 ***Assessment of the amount of olive oil in mixtures with seed oils***

529 Olive oils, of any edible category, can be mixed with seed oils. When the presence of olive oils is  
530 mentioned in the labelling, outside of the list of ingredients, by words, images or graphic  
531 representations, the following trade description has to appear on that blend: "Blend of vegetable oils (or  
532 the specific names of the vegetable oils concerned) and olive oil", directly followed by the percentage of  
533 olive oil in the blend. The presence of olive oil may only be highlighted by images or graphics on the  
534 labelling of the mixtures referred, in the case that it accounts for more than 50% (Reg. (EU) 29/2012).  
535 Since the year of its publication, four amendments have been made to this regulation. All modifications  
536 are important, but perhaps the most significant is related to the possibility for Member States to prohibit  
537 the production in their territory of blends of olive oil and other vegetable oils for internal consumption.  
538 However, they may not prohibit the marketing in their territory of such blends coming from other  
539 countries, as well as the production in their territory of such blends for marketing in another Member  
540 State or for exportation. It is noteworthy that there is no mention about the values of the analytical  
541 parameters that these oils should comply with to ensure that the blend contains the percentage of OO  
542 that is established in the regulation, meaning, there is no analytical protocol to ensure the percentage of  
543 oils in the admixture. Moreover, it cannot be confirmed if OO is present or not in the blend. It is  
544 important to keep in mind that blends can be made with any type of OO; for this reason, the analytical  
545 parameters of virgin OO such as volatile compounds, triterpenic acids, or polyphenols, which are not  
546 present in the refined ones, should be discarded and work should be focused on compounds that  
547 remain after a refining process. There are several compounds at high enough concentration in OO (e.g.  
548 TAGs profile, sum of saturated aliphatic hydrocarbons,  $\beta/\gamma$ -tocopherol ratio, total sterol amount) that  
549 can be detected after blending if the blend is more than 50%.

550

### 551 ***Assessment of deodorised oils***

552 The origin of soft deodorised olive oils and evaluation of ethyl esters as possible markers for their  
553 detection has been already discussed in a previous paragraph. Nowadays, it seems that more reliable  
554 analytical approaches have not been found; other parameters have been proposed such as  
555 diacylglycerols (DAGs) and pyropheophytin (PPP), but none is used since they are not unequivocal and  
556 also change during aging of oils. The high content of PPP or DAGs could mean the oil was either  
557 subjected to soft deodorisation or that the oil is old or was poorly stored. Possible interesting results  
558 could be obtained studying in-depth the differences between experimental DAG content and theoretical

559 DAG content (this latter calculated from free acidity) of genuine OO and corresponding samples  
560 subjected to the soft deodorization process.

561

### 562 ***Verification of geographical origin of olive oils***

563 The behaviour of consumers when purchasing foods is oriented towards a greater preference for  
564 products whose geographical origin is declared since this information clearly increases their  
565 confidence. The importance of geographical declaration on the label mainly concerns extra virgin OO  
566 since consumers perceive information on the provenance as an additional warranty of their quality and  
567 authenticity. For this reason, the European Union implements a quality system of geographical  
568 indications, such as Protected Designation of Origin (PDO) (EU, 2012). In addition to the PDO  
569 information on the label, the consumer can also demand information on the provenance in the case of  
570 non-PDO oils. However, the complex regulation on PDO and labelling does not specify an analytical  
571 procedure to verify the information reported on the label. This fact has raised the interest of analysts  
572 and researchers to develop a reliable method for authentication purposes. After extensive research on  
573 the chemical characterisation of OOs from different locations, sometimes even from the same cultivar,  
574 there are now sufficient chemical and mathematical backgrounds to suggest that the chemical  
575 compositions of oils are partially associated with their provenance. A recent review (Valli et al., 2016)  
576 summarises the most interesting and innovative solutions (e.g. using optical techniques, measurement  
577 of electrical characteristics, instruments equipped with electronic chemical sensors) with a potential and  
578 realistic application for the development of rapid, easy-to-use, environmentally friendly instruments to  
579 be used in monitoring of the geographical origin of virgin OO.

580 The methodologies that have been proposed are based on chemical fingerprint of the oils by  
581 chromatographic analysis, spectroscopic methods and genetic studies (Aparicio & García-González,  
582 2013; Gallina Toschi, Bendini, Lozano-Sánchez, Segura-Carretero, & Conte, 2013). Concerning the  
583 former approaches, Forina and Tiscornia (Forina & Tiscornia, 1982) were among the first researchers  
584 to apply FA composition to discriminate the geographical origin of extra virgin OOs from several areas  
585 of Italy. Later, a pioneering study called SEXIA (Spanish acronym for Expert System for Identification of  
586 Oils) was developed in the 1990s in addition to later research on the same topic (Aparicio & Alonso,  
587 1994; Aparicio, Alonso & Morales, 1994; García-González, Luna, Morales, & Aparicio, 2009). In the  
588 same years, the FA and the TAG profiles were used to discriminate Greek and French samples,  
589 respectively (Tsimidou, Macrae, & Wilson, 1987; Tsimidou & Karakostas, 1993; Ollivier, Artaud, Pinatel,  
590 Durbec, & Guérère, 2003; Bajoub et al., 2016). Furthermore, some studies have focused on the  
591 influence of the geographic area of origin on the positional distribution of FAs in the structure of  
592 triacylglycerols (Damiani et al., 1997; Vichi, Pizzale & Conte, 2007).

593 Differences in volatile compounds has also been attributed to geographical origin (García-González,  
594 Romero & Aparicio, 2010). Melucci et al. (Melucci et al., 2016) highlighted the potential of flash gas  
595 chromatography E-nose for rapid control of the compliance of information on geographic origin declared  
596 in the label ("100% Italian" vs "non-100% Italian" as specific case of oils originating from one Member  
597 State) using non-targeted chromatographic signals of the volatile fraction of virgin OOs as variables for  
598 multivariate analysis using more than 250 samples. An interesting investigation based on the  
599 simultaneous analysis of mono- and sesquiterpene compounds in virgin OO by HS-SPME-GC-MS  
600 (Vichi, Guadayol, Caixach, Lopez-Tamames, & Buxaderas, 2006) showed that this fraction may be  
601 used to distinguish samples from different cultivars grown in different geographical areas). The profile in  
602 terpenic hydrocarbons strictly depends on the variety and growing conditions of the olive trees and is  
603 not influenced by technological factors.

604 To better understand the variability that depends on geographical origin, it is desirable to identify the  
605 factors (e.g. temperature) that cause alterations in specific compounds, so that samples can be  
606 classified (Aparicio & García-González, 2013).

607 In addition to chromatographic methods, spectroscopic techniques can be proposed as a rapid  
608 alternative to obtain chemical profiling of oils without laborious lab work. Some of the techniques  
609 applied are inductively coupled plasma (ICP) coupled to atomic emission spectrometry or mass  
610 spectrometry, and atomic absorption spectrometry (AAS) to determine the presence of chemical  
611 elements (Benincasa, Lewis, Perri, Sindona, & Tagarelli, 2007; Beltrán, Sánchez-Astudillo, Aparicio &  
612 García-González, 2015). Multi-isotope ratio analysis ( $^2\text{H}/^1\text{H}$  or  $\text{D}/\text{H}$ ,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{18}\text{O}/^{16}\text{O}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{34}\text{S}/^{32}\text{S}$ ,  
613  $^{87}\text{Sr}/^{86}\text{Sr}$ ) has been also applied to geographical origin studies of olive oils (Camin et al., 2017).

614 A different strategic approach to verify geographical origins of OO is based on the genomics of olives  
615 and OOs. Thus, the molecular markers characterising cultivars have also been applied to 'olive oil  
616 fingerprinting' (Banilas & Hatzopoulos, 2013).

617 Regardless of the strategy followed for geographical identification, all approaches require a large  
618 database. The representativeness of oils selected for such a database according to market reality and  
619 genuineness in terms of geographical provenance is of paramount importance to develop a reliable  
620 method. This database would allow building an 'olive oil map' including both chromatographic and  
621 spectroscopic information from the most relevant cultivars and all approved PDOs (Aparicio & García-  
622 González, 2013).

623

## 624 **Conclusions and future trends**

625 Despite the OO analysis remains a cornerstone in terms of diagnostic possibilities of fraud in the field of  
626 food analytics and the constant and substantial efforts towards the development of new procedures to

627 be used for assessment of OO quality and authenticity, some specific and proper analytical solutions  
628 (e.g. detection of selected blends of OOs with other vegetable oils, of soft-deodorised OOs, methods  
629 for supporting the organoleptic assessment of OOs, etc.) have not yet been found. It is therefore urgent  
630 to identify and/or improve analytical solutions that are able to detect both common and emerging frauds  
631 and to provide all the information required by the international market.

632 This review has highlighted weaknesses in the regulatory framework as well as some critical points of  
633 existing analytical methods adopted in OO quality and purity control. Suggestions for replacement of  
634 specific steps of analytical protocols, especially with more advanced analytical solutions to reduce time  
635 or solvent consumption, have also been proposed.

636 Once the weaknesses of the regulatory framework and the lack of proper analytical methodologies has  
637 been overcome, the next step should be an extensive and significant work towards global  
638 harmonisation of parameters, limits and analytical protocols in order to establish a worldwide- system of  
639 fraud protection, providing a unique framework that is unaffected by misunderstanding and  
640 misconceptions. Such action will definitively ensure fair trade as well as the safety and consumer  
641 protection of the entire OO sector.

642

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647

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653

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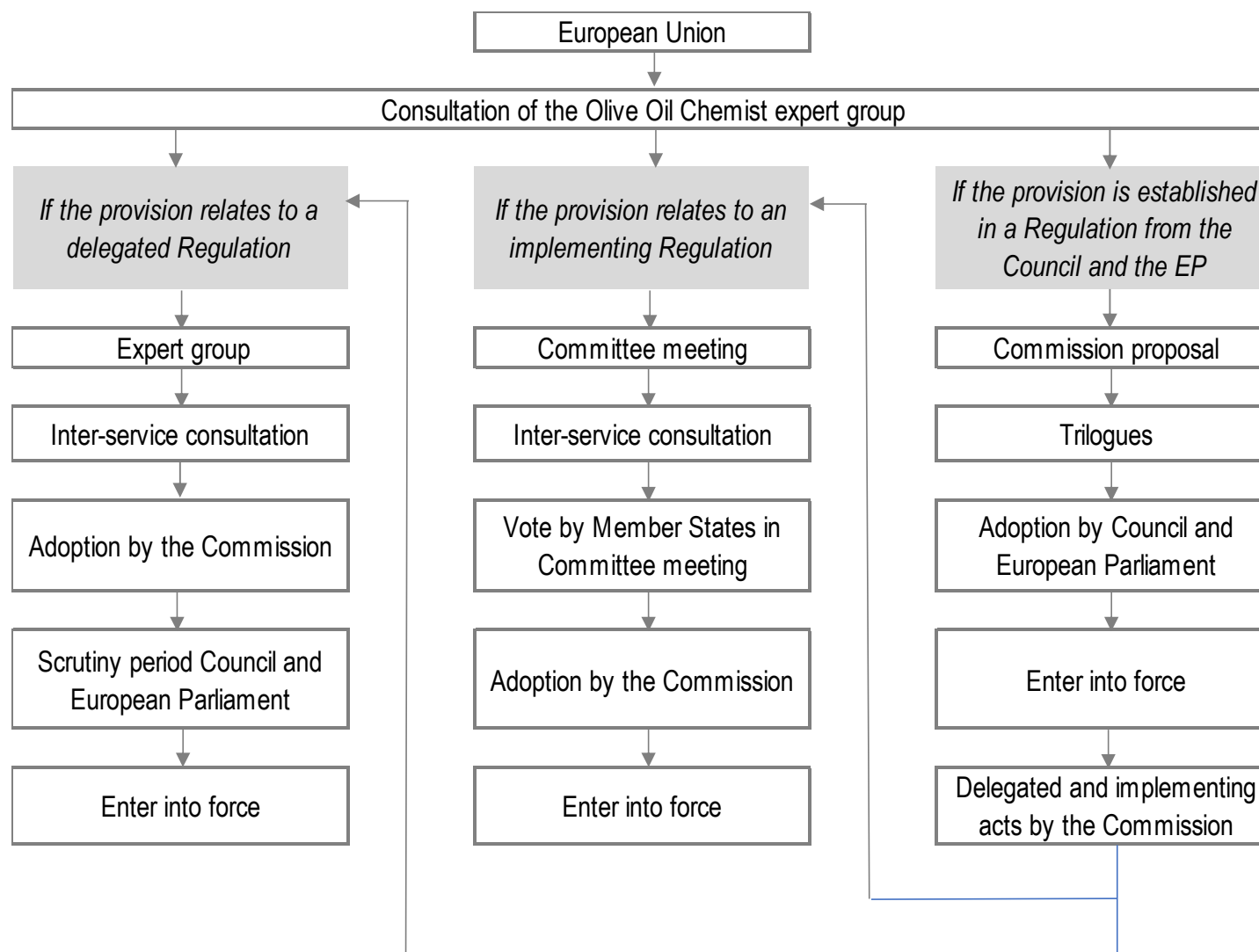
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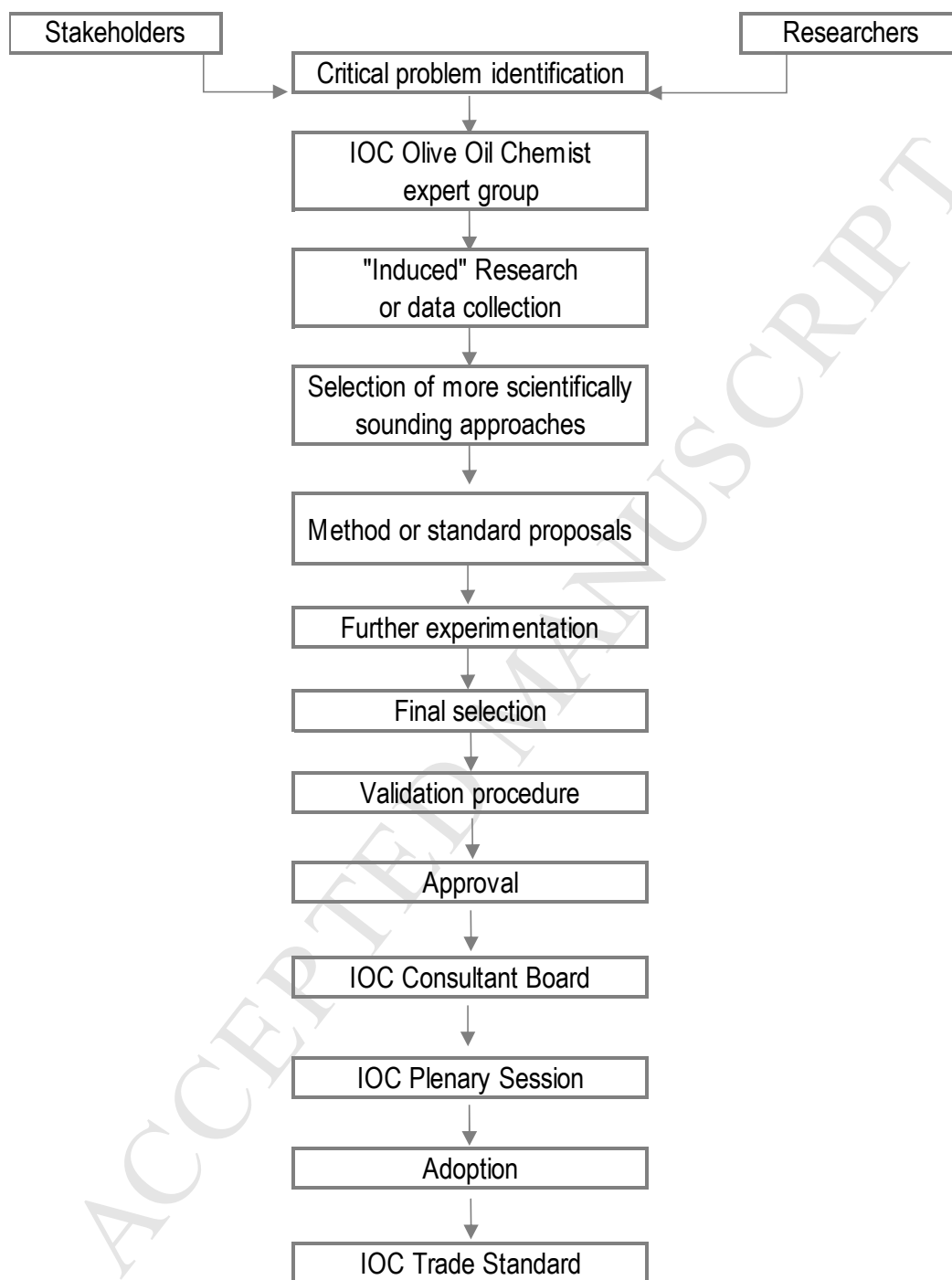
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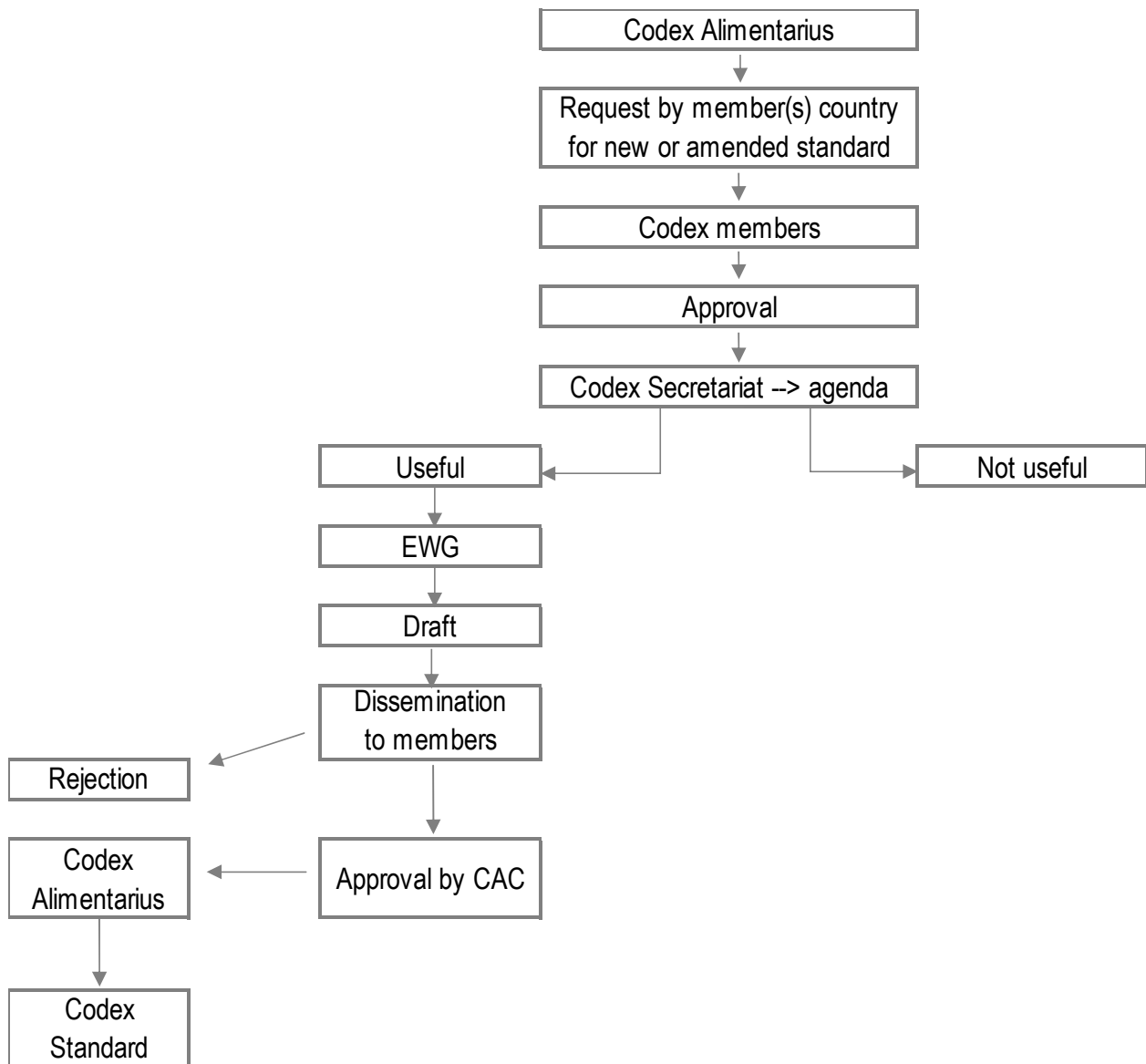
**Table 1.** Methods for quality and purity evaluation of olive oils applied in official controls, related drawbacks and possible improvements.

	Purpose	Method	Drawbacks	Possible improvements
Quality assessment	Quality of raw matter (fruits)	Acid Basic titration Reg (EU) 2016/1227 Annex II	Use of toxic phenolphthalein.	Possibility to substitute phenolphthalein with alkali blue 6B or thymolphthalein.
	Oxidation status	Peroxide value Reg (EEC) 2568/1991 Annex III	Use of toxic solvent (chloroform).	Already experimented replacement of chloroform with isooctane: poor results depending on problem of solvent miscibility. ISO validate a potentiometric end point determination. Measurement of optical density of an emulsion between the oil sample and ferrous ion oxidation with xylenol orange reagent.
	Oxidation status	UV Absorption Reg (EU) 2015/1833 Annex IX	None.	Indication to check the response of the photocell and photomultiplier (wavelength and absorbance scales) by reference materials.
	Lower quality olive oils	Ethyl esters Reg (EU) 1348/2013 Annex XX	Sample preparation time lengthy. Need to use an on-column injector for GC analysis.	Shorten the time of the preparative step by proposing HPLC or SPE as an alternative to the traditional liquid chromatography. Use of PTV injector instead of the on-column injector.
	Quality grade of virgin olive oils	Sensory assessment Reg. (EU) 1348/2013 Annex XII	Low number of samples assessed per day, accuracy.	Reduce the number of samples to be assessed by panellists thanks to predictive models built using instrumental techniques based on the volatile compounds analysis. Formulate new reference materials for improving the training of assessors.
Purity assessment	Presence of extraneous oils	Fatty acids composition Reg.(EU) 2015/1833 Annex X	Not effective in case of blends with specific oils (e.g. high oleic sunflower), lack of sensitivity for some other blends.	None.
	Presence of extraneous oils	Sterols composition Reg. (EU)1348/2013 Annex V	Sample preparation time lengthy.	Save time: i) Unsaponifiable fractionation by HPLC instead of TLC ii) Microwave Assisted Saponification (MAS) Improvement of information on free and esterified sterols
	Presence of extraneous oils	$\Delta$ ECN42 –TAGs Analysis Reg. (EC) 2472/1997 Annex XVIII	ECN42 instead of LLL depending on poor separation due to isocratic elution that is mandatory because of refractive index detector.	Improve HPLC separation of LLL e.g. by UHPLC-CAD. GC TAGs analysis suitable to detect small amounts of selected oils (HOSO, palm olein).
	Presence of extraneous oils	Global method–TAGs Analysis Reg. (EU) 1348/2013 Annex XX bis	False positive results?	Improvement of method performances.
	Presence of refined oils	Stigmastadienes Reg. (EEC) 656/1995 Annex XVII	Sample preparation time lengthy.	Direct analysis even if concentration is < 4 mg/kg.
	Presence of esterified oils	2 glyceril monopalmitate Reg. (EC) 702/2007 Annex VII	Need to use an on-column injector for GC analysis.	Use of PTV injector instead of the on-column injector.
	Presence of pomace oils	Waxes determination Reg. (EEC) 183/93 Annex IV	Sample preparation time lengthy. Need to use an on-column injector for GC analysis. GLC analysis: poor separation of selected compounds (e.g. C40).	Shorten the time of the preparative step by proposing SPE as an alternative to the traditional liquid chromatography. Use of PTV injector instead of the on-column injector.

**Figure 1a.** Flow chart of standards development procedures within EU.



**Figure 1b.** Flow chart of standards development procedures within IOC.

**Figure 1c.** Flow chart of standards development procedures within Codex Alimentarius.

**Highlights**

- Normative and standard sources for olive oil quality and purity
- Analytical methods for olive oil: drawbacks and limitations
- Normative failures and suggestion for improvements

ACCEPTED MANUSCRIPT