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Quality Control of Olive Oils in EEC: Origins, Evolution and Recent Trends

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Summary

Oils obtained from olives represent a very important field of the agro-food industry and economy of the Mediterranean countries of the EEC. Until September 1991, every country had national laws and national official methods of analysis. Starting from September 1991, EEC published a regulation (No 2568), that by its nature superseded the national laws.

The composition of fatty acids and sterols, with respect to genuineness, is analyzed by capillary gas chromatography, so that ample information can be obtained by one analysis: in the sterol fraction, up to 16 compounds can be observed, against the reduced numer separated by packed column GLC.

If the determination of fatty acid composition is carried out by capillary GLC, the presence of trans isomers above 0.05% in a virgin oil can be detected, thus revealing its being mixed with refined oil.

If sterols are destroyed in seed oils so that they can be mixed with virgin olive oils, dehydration products of sterols, named sterenes, are formed: the new EEC regulation No 656, published in March 1995, introduced the determination of stigmastadienes.

COI has also included the determination of ratios between the dehydration products of single sterols, with the aim of detecting the mixture of seed oils in refined olive oils. Waxes, too, are determined, as their high value is a consequence of mixing with olive pomace oil.

Among the analytical parameters, for the first time sensory analysis has been used by law: a panel test is one of the parameters prescribed by the EEC. COI is developing a new method based upon continuous scales.

Keywords: olive oils, quality control, analytical methods, EEC, COI

Oils obtained from olives represent a very important field of the agro-food industry and economy of the Mediterranean countries of the European Economic Community (EEC). Until September 1991, every country had national laws and national official methods of analysis. Starting from September 1991, EEC published a regulation (No 2568) *(1)*, that by its nature superseded the national laws.

We speak of »oils« obtained from olive fruit since there are different kinds of products. In Fig. 1, a schematic description of their classification is shown, based on the declarations of the last IOOC trade norm (November 24, 1995) *(2)*.

Oils obtained from olive fruits are subject to three sources of rules: EEC, International Olive Oil Council (IOOC – COI) and, as any food, Codex Alimentarius (FAO – OMS). A different number of parameters are considered by each. Those pertinent to extra virgin olive oil are compared in Table 1.

As it can be seen, IOOC and EEC do not consider the same number of parameters. This fact has led to the first classification of parameters. They can be divided into parameters used to assess the genuineness and those used to assess quality (Fig. 2).

The evaluation of genuineness is based on the determination of botanical origin, which can be verified by determining the composition of fatty acids, sterols and triglycerides. Since olive oil is a natural product, several sources of variation can influence its composition, as

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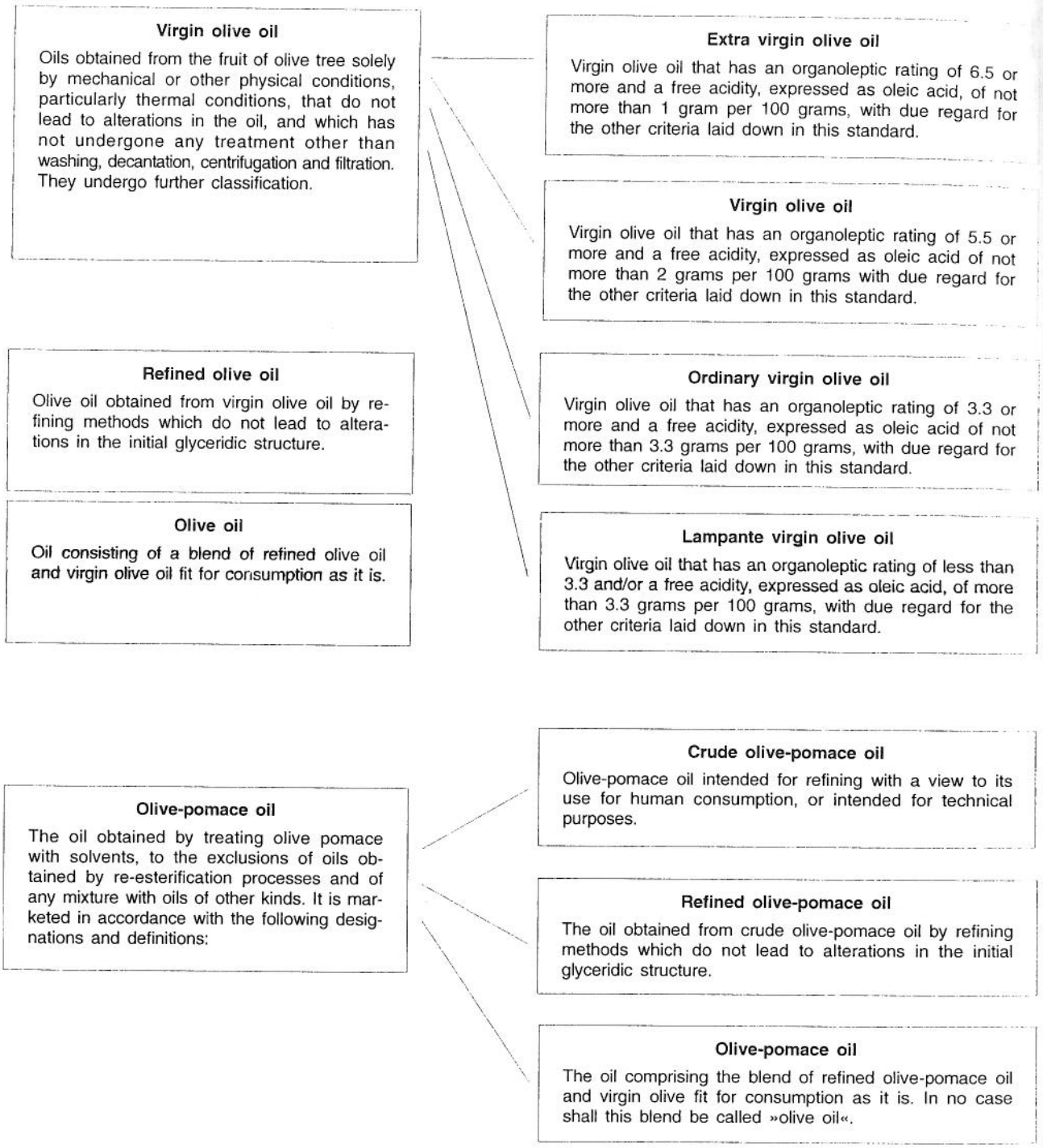

Fig. 1. Classification of olive oils, according to EEC Reg. 2568/91 and to IOOC trade standard

shown in Fig. 3 (Montedoro *et al.* modified) *(3)*; nevertheless, it has been possible to establish a number of regulation limits, used to control the genuineness and the quality characteristics.

The analysis of fatty acid composition has been one of the early topics in gas chromatographic analysis the introduction of capillary gas chromatography allows the separation of a higher number of fatty acids, for some of which a regulation limit has been stated (*e.g.*, linolenic, arachidic, behenic). Capillary gas chromatography is used to obtain information concerning the mixture with seed oils.

For a number of years, the determination of fatty acid composition results were used to certify the genuineness of olive oils, in consideration of their peculiar fatty acids composition, as seed oils usually contain higher amount of polyunsaturated fatty acids in addition to oleic acid. The availability of genetically modified

Table 1. Comparison of characteristics of extra virgin olive oils established by EEC, IOOC and Codex Alimentarius

Parameter	EEC	IOOC	Codex Alimentarius
Acidity/(% oleic acid)	M 1.0	M 1.0	M 1.0
Peroxide value	M 20	M 20	M 20
K 270	M 0.20	M 0.25	M 0.25
ΔK	M 0.01	M 0.01	M 0.01
w (C 14:0)/%	M 0.05	M 0.05	0.0–0.1
w (C 16:0)/%	–	7.5 - 20	7.5–20
w (C 16:1)/%	–	0.3 - 3.5	0.3–3.5
w (C 17:0)/%	–	M 0.3	M 0.3
w (C 17:1)/%	–	M 0.3	M 0.3
w (C 18:0)/%	–	0.5 - 5	0.5–5
w (C 18:1)/%	–	55.0 - 83.0	55.0–83.0
w (C 18:2)/%	–	3.5 - 21.0	3.5–21.0
w (C 18:3)/%	M 0.9	M 0.9	M 1.5
w (C 20:0)/%	M 0.6	M 0.6	M 0.6
w (C 20:1)/%	M 0.4	M 0.4	–
w (C 22:0)/%	M 0.2	M 0.2	M 0.2
w (C 24:0)/%	–	M 0.2	M 1.0
w (cholesterol)/%	M 0.5	M 0.5	M 0.5
w (brassicasterol)/%	M 0.1	M 0.1	M 0.1
w (campesterol)/%	M 4.0	M 4.0	M 4.0
w (stigmasterol)/%	< Campesterol	< Campesterol	< Campesterol
w (β-sitosterol)*	m 93.0	m 93.0	m 93.0
w (Δ^7-stigmasterol/%	M 0.5	M 0.5	M 0.5
w (total sterols)/ppm	m 1000	m 1000	m 1000
w (waxes)/ppm	M 250	M 350	M 300
w (saturated fatty acids in position 2 of triacylglicerol/%	M 1.3	M 1.5	M 1.5
w (eritrodiol + uvaol/%	M 4.5	M 4.5	M 4.5
w (ECN 42)/HPLC-calculated	–	M 0.4	M 0.4
w (trilinolein)/%	M 0.5	–	–
w (tigmastadienes/ppm	M 0.15	M 0.10	–
R1		> 15	–
w (C 18:1 trans)/%	M 0.05	–	–
w (C 18:2 trans + C 18:3 trans)/%	M 0.05	–	–
w (water + volatiles/%	–	M 0.2	M 0.2
Flame point	–	–	m 120 °C
w (iron)/ppm	–	M 3.0	M 5.0
w (copper)/ppm	–	M 0.1	M 0.4
w (lead)/ppm	–	–	M 0.1
w (arsenic/ppm	–	–	M 0.1
w (halogenated solvents)/(ppm each one)	M 0.10	M 0.1	M 0.1
w (halogenated solvents)/(ppm sum)	M 0.20	M 0.2	M 0.2
Saponification number	–	–	184–196
Iodine number	–	–	75– 94
w (unsaponifiable)/(g/kg)	–	–	15
Bellier index	–	–	M 17
w (Aliphatic alcohols/ppm	–	–	M 300
nD 20 °C	–	–	1.4677–1.4705

Note: M = maximum; m = minimum

* (sum: clerosterol + β-sitosterol + sitostanol + $\Delta^{5,24}$-stigmastadienol + Δ^5-avenasterol)/%

seed oils, with fatty acid composition similar to the olive oil increases the importance of determination of sterol composition, since for a number of seed oils, characteristic sterols were recognized (*e.g.* brassicasterol for rapeseed, Δ^7-stigmasterol for sunflower, and so on) by introduction of capillary chromatography which allows the separation of up to 17 peaks instead of four to five as with packed GC colums.

Sterols appear to be the fingerprint of oils. It was though that if sterols were removed from seed oil it would be possible to mix seed oil with olive oil. The sterols of seed oil can be destroyed by applying hard refining conditions, mainly in bleaching and deodorizing steps. During this operation structural modifications of the aliphatic chain of fatty acids take place, with formation of *trans*-isomers of unsaturated fatty acids. Capillary gas chromatographic analysis realized by means of stationary phases, made by bis-cyanopropyl-phenyl-cyanopropyl polysiloxane, enables a very good separation of *trans*-isomers, which are eluted shortly before the correspondent *cis*-isomers. Traditional polyester-based stationary phases (*e.g.* Carbowax^R) are able to separate them, but in this case some *trans*-isomers are eluted after the correspondent *cis*-form, which may cause some mistakes in peaks identification *(4)*.

As in virgin oil no *trans* fatty acids are usually present, the regulation limit should be 0. Nevertheless, a limit of 0.05% of total fatty acids was fixed, bearing in

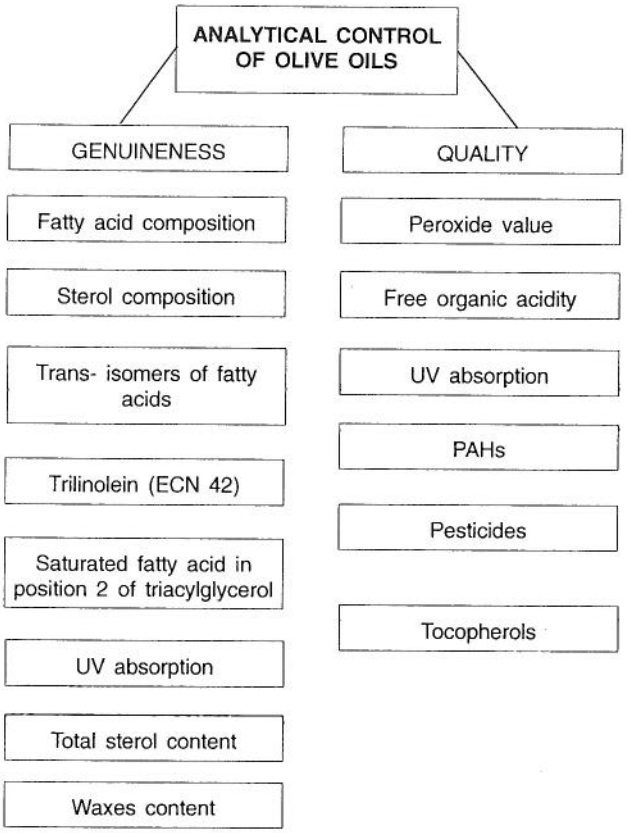

Fig. 2. Genuineness and quality parameters for olive oils

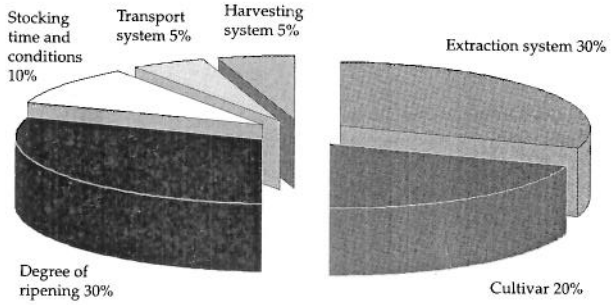

Fig. 3. Parameters influencing oil quality *(3)*

Table 2. The regulation limits for *trans* isomers in olive oil

	w(*trans* fatty acids)/(% of fatty acids)	
	C18:1	C18:1 + C18:2
Extra virgin olive oil	≤ 0.05	≤ 0.05
Virgin olive oil	≤ 0.05	≤ 0.05
Ordinary virgin olive oil	≤ 0.05	≤ 0.05
Lampante virgin olive oil	≤ 0.10	≤ 0.10
Refined olive oil	≤ 0.20	≤ 0.30
Olive oil	≤ 0.20	≤ 0.30
Crude olive-pomace oil	≤ 0.20	≤ 0.10
Refined olive-pomace oil	≤ 0.40	≤ 0.35
Olive-pomace oil	≤ 0.40	≤ 0.35

mind that such a low peak can sometimes be confused with noise signal; at first a limit of 0.03% had been established by the Italian Technical Committee for Oils and Fats that proposed the method, but it was not in agreement with the characteristics of replicability and reproducibility of the method, so the Group of Chemistry Experts for Olive Oil of the EEC modified it last year, in agreement with IOOC. The regulation limits for *trans*-isomers in olive oil are summarized in Table 2.

Refined olive oils contain certain amounts of *trans* fatty acids, so this chemical indicator could not be used to determine if desterolized seed oil has been added to this class of oils.

Lanzon and Cert *(5)*, studying the modifications of unsaponifiable fraction during refining processes, with particular attention to the already noted reactions that cause losses of the –OH group with the formation of unsaturated hydrocarbons named sterenes, supposed that the ratio between single native sterols should be the same as in their dehydration products. From this hypothesis they demonstrated that as the ratio β-sitosterol/campesterol and β-sitosterol/stigmasterol are constant in the particular kind of oil, the same should happen for their dehydration products. They proposed the determination of the ratios:

R1 = Stigmastadienes/Campestadienes

R2 = Stigmastadienes/Stigmastatrienes

as a method of determining the presence of desterolized seed oils in refined olive oils.

IOOC accepted these ratios as provisional within its trade standard characteristics, but EEC did not as no agreement about their validity was reached. EEC only accepted the determination of the presence of stigmastadienes as a means of detecting the mixture of refined oils with crude olive oils, with the regulation limit of 0.15 ppm for extra virgin and virgin oils, and 0.50 ppm for lampante virgin olive oils *(6)*.

Even if there is no reason to admit the existence of any trace of sterenes in crude oils, considerations about the possibility of accidental contamination suggested the need to fix these regulation limits, as virgin oils may be contaminated during refining in the bottling plants while lampante can be contaminated by refined oils before refining, during storage or transportation.

IOOC in fact recognized only the validity of *R1* ratio, whereas EEC did not. Further experimentation is under way with the aim of reaching an agreement about these methods.

Recently, at the Department of Food Science of the University of Udine, some other sterenes were found, that by GLC-MS were identified as dehydrogenated compounds. For this reason, we hypothesized that they originate from oxidated products of sterols: it was confirmed by experimentally produced oxisterols submitted to soft bleaching processes. These findings could perhaps lead to a new important use of sterenes composition, related to the quality of crude oil before refining.

As not only desterolized seed oils can be used to realize fraud, but olive pomace oil as well (the new name for the husk oils), in 1972 Minguzzi and Capella *(7)* and then other researchers *(8–11)*, found in pomace olive oils two compounds, named erythrodiol and uvaol, and their

Table 3. The regulation limits for waxes content in olive oil

	w (waxes)/(mg/kg) (C40 + C42 + C44 + C46)
Edible Virgin oils	≤ 250
Lampante virgin olive oil	≤ 350
Refined olive oil	≤ 350
Olive oil	≤ 350
Olive-pomace oil	≥ 350 *

* for EEC only, not for IOOC

presence and amount became the limit (maximum 4.5% for virgin oils). Their origin was related with the presence of oleanoic and ursolic acids, studied in skin lipidic fraction of olive drupes by Jacini and Fedeli *(12)*. Unfortunately, erythrodiol and uvaol can be easily removed by oxidation with potassium dichromate, with the aim of obtaining an oil that can be used to produce a fraud, mixing it with virgin oils.

Skin lipids contain also waxes that release aliphatic alcohols after saponification. Several researchers *(13–17)* proposed the determination of aliphatic alcohols as a tool to detect the presence of the lipidic fraction of olive drupe skin, characterized by waxes.

This method was adopted at first, but further research, carried out with new instrumentation based on hyphenated techniques liquid chromatography – capillary gas chromatography (LC-GC) demonstrated that some oils may contain high amounts of free aliphatic alcohols *(18–20)*.

Mariani and Fedeli *(21)* proposed the determination of waxes content to detect the presence of solvent extracted oils in pressure extracted oils. Waxes determination is actually part both of the EEC regulations and of the IOOC trade standard. The regulation limits are shown in Table 3.

Waxes determination is a good example of a method that became possible because of the availability of capillary chromatography; a new approach to fatty substances analysis is linked to the evaluation of high molecular weight molecules, without breaking them into simpler molecules. A similar approach led to the evaluation of the triglycerides composition. A IUPAC method was already available and it has been adopted for olive oil control. It is an HPLC method with refractive index detector and shows clearly the trilinolein peak. Genuine olive oils never contain more than 0.5% of this triacylglycerol, higher values being possible only for mixtures with seed oils or, within a lower range (0.7–0.8%), for olive oils from Tunisia.

As the reverse phase HPLC determination does not distinguish between the different kinds of triacylglycerol, with regard to unsaturation level and carbon atom numbers, it appears somewhat imprecise to speak of »trilinolein«: the term »ECN« (equivalent carbon number) was proposed and accepted, even if in fact, from a strictly chromatographic point of view, the term »partition number« would be more correct *(22)*. The ECN is defined as: $ECN = N_C - 2 \cdot \Delta$, where N_C is the number of carbon atoms, and Δ represents the number of double bonds.

Therefore, we will speak of ECN 42 instead of trilinolein (LLL).

In addition, the determination seems not to give exact information, as several fatty acids can give a similar carbon number for triacylglycerol. Fedeli *(23)* proposed a calculation method to evaluate the theoretical ECN 42 content: a comparison between these data and the experimental ones should give more detailed information. IOOC soon adopted this calculation method and the maximum difference admitted between calculated and experimental data was set at 0.4

EEC had some difficulties with accepting this method in its original form, as it was furnished on computer disk only, while for EEC needs it had to be published in the EEC Official Journal. The Group of Chemical Experts for Olive Oils had many meetings addressing this problem, and eventually two of the members – Fiebig *(24)* and Staphylakis *(25)* – proposed a written form of the calculation method. Preliminary trials show substantial agreement between the data obtained by Fedeli method and the ones obtained by these two calculation methods and at present a ring test about ECN 42 determination is in progress at a number of EEC laboratories. As some problems may occur, especially for olive pomace oils, because of the presence of oxidated substances that show similar chromatographic behavior as ECN 42, a purification step was introduced, essentially based on the IUPAC method for determination of polar compound in frying oils. This step, too, will be tested in the cited ring test.

A number of other chemical parameters are contained in the EEC Regulation 2568/91, modified by the No 656/95, and are listed in the Annex I. Many of them are less innovative, as UV absorption, free organic acidity, fatty acid composition in the position 2 of triacylglycerol, and so on. Peroxide value, even though one of the traditional methods, is actually submitted to revision, in order to harmonize it with the IUPAC directive of eliminating the use of chloroform in laboratories. For this reason the peroxide method was modified by substituting chloroform with isooctane. A ring test was carried out by IOOC and the result obtained with the old method are in good agreement with those obtained by the new one. EEC will next consider the possibility of adapting its method to this one.

Among the analytical parameters, for the first time sensory analysis has been used by law: a panel test has become one of the EEC parameters. Organoleptic evaluation of olive oils had been already carried out by expert tasters; in 1978, the fundamental studies of Olias *et al. (26–28)* in Spain and of Montedoro *et al (29)*, Camurati *et al. (30)* and of Solinas *et al. (31–33)* in Italy, established a scientific approach to olive oil sensory analysis.

IOOC adopted the method for organoleptic evaluation of olive oil in 1987 and the same method was adopted by EEC in 1991. The method was named panel test: samples are scored on a nine-point scale, by a standardized profile sheet. This kind of profile sheet, albeit very detailed, compelled EEC to introduce a bonus system of 1.5 units of score, for three years, scaling 0.5 point each year, since a number of genuine and quality olive oils did not agree with this scoring method. At the end of three years, EEC had to maintain the 0.5 bonus. In the meantime, IOOC has worked out a new method for organoleptic assessment of olive oils. At first, a new profile

sheet was adopted for the panelist use and another one for the panel coordinator. The proposed method, however, was not in good agreement with the ISO rules for sensory analysis and used the calculation mean for data obtained on a noncontinuous scale. This is incorrect, as nonparametric elaboration should be applied. Furthermore, this method yielded widely distributed data. As a result of co-operation between IOOC and the Department of Food Science of the University of Udine, a small group was formed that recently (COI meeting of the working group, April 18–19, 1996) proposed a new method, which would restrict the range of data distribution among the panels of different countries. The main difference between this method and the old one, besides the use of a continuous scale, is the use of median calculation, instead of mean, which makes the method unaffected by outlier values.

On the basis of a notched box plot, a range of confidence for defects was established, in a provisional form, to discriminate among extra virgin, virgin, ordinary virgin and lampante oil. On the same basis it is possible to fix some ranges for the fruity flavor. This could be very useful in the application of the method for more detailed evaluations, such as the ones for the Officially Guaranteed Typical Names (DOC).

Further developments in the quality control of olive oils are now in progress, essentially dealing with assessment of analytical methods for determination of xenobiotic substances, mainly organophosphorus residues and policyclic aromatic hydrocarbons (PAHs). IOOC and Italian Technical Committee are at present working on the first topic.

Some years ago, several Italian researchers worked on PAHs content of olive oils *(34–37)* and they concluded that a minimum content of PAHs could be considered physiologically acceptable for olive oils. Recent experiments were carried out in Italy by Istituto Superiore di Sanità *(38)* and by the Department of Food Science of the University of Udine *(39–40)*. It was found that the maximum content of PAHs in virgin olive oils was less than 10 ppb. Only the phenantrene content was higher than 40 ppb. As the phenantrene presence was observed in oils with a low level of other PAHs, and in developing olive drupes isolated from the environment, a first hypothesis could be that it is a natural occurence, perhaps linked to some metabolic pathway.

A number of other chemical parameters are important and should be considered in olive oil quality definition. However, while for some of them a standardized method is already available and ring tests are being carried out (*e.g.* tocopherols), for others, albeit very important ones (*e.g.* polyphenols), no standardized method is available, in spite of a number of papers in the literature which deal with their determination.

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Kontrole kakvoće maslinovih ulja u EEZ: Današnje stanje, razvoj i tendencije

Sažetak

Ulja dobivena od maslina važno su područje poljoprivredno- prehrambene industrije i ekonomije sredozemnih zemalja EEZ-a. Do rujna 1991. svaka je zemlja imala svoje zakone i nacionalne službene postupke za analizu; nakon rujna 1991. EEZ je objavila Pravilnik (br. 2568) koji je po svojoj prirodi prešao nacionalne granice.

Sastav masnih kiselina i sterola povezanih s izvornosti ulja utvrđuje se na kapilarnoj koloni tako da se jednom analizom može dobiti veliki broj informacija: u sterolnoj frakciji može se utvrditi do 16 spojeva za razliku od ograničenog broja spojeva koji se mogu odijeliti na običnim kolonama.

Ako se određivanje sastava masnih kiselina provodi kapilarnom GLC, može se utvrditi prisutnost trans-izomera već iznad 0,05% u prirodnom djevičanskom maslinovom ulju, što upućuje na miješanje s rafiniranim uljima.

Ako su u biljnim uljima razgrađeni steroli, tada bi se takva ulja mogla pomiješati s djevičanskim (prirodnim) maslinovim uljima. Razgradnjom sterola nastaju dehidratacijski produkti nazvani sterini: u novom Pravilniku EEZ (broj 656) objavljenom u ožujku 1995. unesen je način određivanja stigmastadiena.

Međunarodni savjet za maslinovo ulje (IOOC) uvrstio je određivanje odnosa između dehidratacijskih produkata pojedinih sterola sa svrhom utvrđivanja smjese biljnog ulja i rafiniranih maslinovih ulja. Utvrđeni su i postupci određivanja voskova čiji je veliki udjel posljedica miješanja s maslinovim uljem iz komine.

Po prvi put među analitičke pokazatelje uvrštene su ovim zakonom i senzorne analize: jedan od pokazatelja EEZ je panel-test, a sada IOOC razrađuje novi postupak koji se zasniva na kontinuiranim bodovnim skalama.