FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

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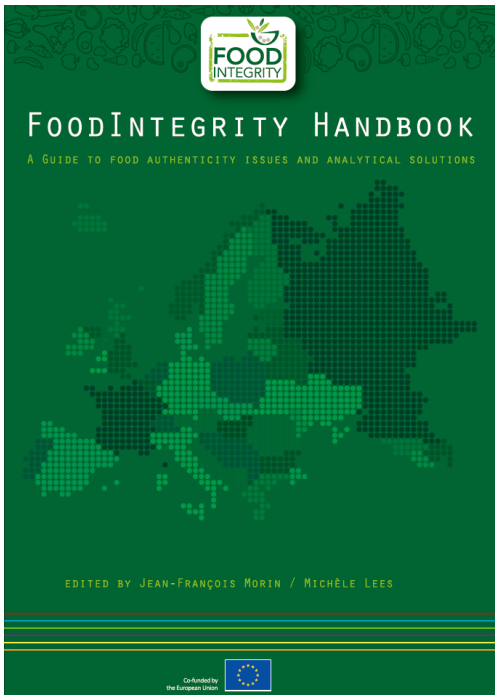

ISBN *print version* *978-2-9566303-0-2*
electronic version *978-2-9566303-1-9*

https://doi.org/10.32741/fihb

Olive oil

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General overview of the product

Olive oil has gained in popularity in counties where it was a relatively underused commodity in the past. Not least among the reasons for the increased popularity of olive oil are its potential health benefits, its delicious taste and aroma and its culinary and nutritional advantages over other edible oils [1]. Since countries that were only importers a few decades ago have started to produce olive oil, it has become another daily oil for cooking for consumers from these countries.

Olive oil represents only around 2 %, or even lower, of the worldwide production of oils and fats [2,3] and it is a foodstuff cherished by the consumers of the Mediterranean countries where it is of enormous economic importance for their farmers. Thus, 20 % of farms in Spain are devoted to olive cultivation compared with 25 % in Greece and 19 % in Italy. These countries produce around 70 % of the world production, Spain and Italy being the main producers (Table 1).

The International Olive Council (IOC) has clearly defined the different categories of olive oil and olive-pomace oil [4]. The most popular category is virgin olive oil. This is the oil obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration. In the course of this technical procedure, olives are washed, milled, and the resulting paste is malaxed. The purpose of malaxation is to facilitate the separation of the oil and water phases. The paste is then slightly warmed in order to accelerate the merging of the oil drops. It has been found that the lower the temperature, the better the sensory quality but the lower the yield [5]. Next, a centrifugation process that produces two fractions (wet olive cake and oil) is widely implemented in olive mills all over the world. Prior to bottling, the oil is submitted to a vertical centrifuge or decanter.

Olive being a fruit, its chemical composition depends not only on the action of enzymes involved in the biochemical pathways but also on the extraction process and external parameters, such as the weather. Consequently, there are different categories of olive oil that differ significantly in their quantitative chemical composition and price. Extra-virgin olive oil commands a high price on the oils and fats market due to its sensory characteristics, the demand for it and its production costs. It is therefore the main focus of attention of fraudsters. Adulterations, which were very common in the past – like the addition of refined edible oils – are easily detected, and have been substituted by sophisticated ones, like the addition of soft-deodorized virgin olive oils or the use of oils with tailored composition to meet the legal limits. Thus, the kind of adulterant is not a cheaper edible

https://doi.org/10.32741/fihb.18.oliveoil

oil in the market anymore but a formulation of different cheaper edible oils that can avoid detection when using trade standards. This procedure is harmful for emerging virgin olive oil markets whose local consumers buy olive oil for its potential health benefits and they would be concerned if they receive an adulterated oil instead that does not have these benefits.

Hence, effective control of olive oil adulteration requires tighter controls by exporting countries, clear definitions for olive oil products and uniform labelling regulations. As regards Analytical Chemistry, the best solution probably lies in multi-disciplinary studies involving instrumental methods of chemical and sensory analysis, and mathematical procedures.

Table 1: World production and consumption of olive oil (2015/16) of olive oil by country

Country	Production (1000 tm)	World production (%)	Consumption (1000 tm)	World consumption (%)
Spain	1403.3	44.18	494.5	16.60
Italy	474.6	14.94	598.1	20.07
Greece	320.0	10.07	140.0	4.70
Portugal	109.1	3.44	70.0	2.35
France	5.4	0.17	113.4	3.81
EU	2324.0	73.16	1660.4	55.73
Turkey	150.0	4.72	116.0	3.89
Tunisia	140.0	4.41	35.0	1.18
Syria	110.0	3.46	104.0	3.49
Morocco	130.0	4.09	120.0	4.03
Australia	20.0	0.63	42.0	1.41
USA	14.0	0.44	321.0	10.77
Chile	17.5	0.55	5.5	0.19
Argentina	24.0	0.76	7.5	0.25
China	17.5	0.56	39.0	1.31
TOTAL	3176.5		2979.5	

Source: www.internationaloliveoil.org.

1. Product Identity

1.1. Definition of the product and manufacturing process

Olive oil is the oil obtained from the fruit of the olive tree (*Olea europaea sativa* L.) to the exclusion of oils obtained by solvents or re-esterification procedures and of any mixture with oils of other kinds [4]. Olive oil is defined in three categories: virgin olive oil, refined olive oil and olive oil.

Virgin olive oil is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration of the oil. The result of the process is an oil that is chiefly a mixture of glycerides, which are esters of glycerol with fatty acids. In addition, olive oil contains small quantities of many chemical compounds (Table 2) that are commonly used in its characterisation and authenticity [6,7]. The generic concept of virgin olive oil contains four different types: extra-virgin olive oil, virgin olive oil, ordinary virgin olive oil and lampante virgin olive oil although the category ordinary virgin olive oil is not accepted by all the regulatory bodies (as for example in the EU).

Table 2: Ranges of the chemical components of virgin olive oil [8]. Information from fatty acid, triacylglycerides and squalene are given in percentages while the rest is given in mg/kg. Note: tr, traces; nd, not detected; [a], values exclusively circumscribed to some major Spanish and Italian cultivars

Component	Range	Component	Range
Fatty acids		*Triglycerides*	
Myristic	Not detected	POP	2.16 - 5.73 %
Palmitic	6.3 - 16.9 %	PXO-PLP	0.13 - 2.66 %
Palmitoleic	0.3 - 1.6 %	POS	0.39 - 2.30 %
Margaric	0.002 - 0.3 %	POO	19.54 - 30.57 %
Margaroleic	0.02 - 0.4 %	PLO-XOO	2.76 - 12.31 %
Stearic	1.02 - 3.9 %	PLL	tr - 2.43 %
Oleic	65.4 - 86.6 %	SOS	tr - 1.04 %
Linoleic	2.7 - 18.3 %	SOO	3.17 - 8.39 %
Linolenic	0.2 - 1.1 %	OOO	27.75 - 53.34 %
Arachidic	0.15 - 0.7 %	OLO	4.24 - 17.46 %
Gadoleic	0.09 -0.6 %	OLL	tr - 4.43 %
Behenic	0.01 - 0.2 %	AOO	0.25 - 1.09 %
Aliphatic alcohols and diols		GOO	tr - 1.06 %
Docosanol	0.77 – 56.27	*Hydrocarbons*	
Tetracosanol	17.79 - 60.63	α-Copaene	0.12 - 4.77
Hexacosanol	26.88 – 93.81	Calarene	tr - 0.26
Octacosanol	10.53 – 44.94	Muurolene	tr - 1.51
Phytol	35.97 – 364.58	Eremophylene	tr - 2.63
Erythrodiol +uvaol	8.07 – 112.51	Heptadecene	tr - 0.45
4,4'-dimethylsterols		Heneicosane	tr - 0.72
Taraxerol	4.14 – 12.94	Tricosane	0.65 - 16.35
Dammaradienol	5.14 – 34.94	Tetracosane	0.47 - 14.93
β-Amyrin	10.78 – 121.17	Pentacosane	2.51 - 28.8
Butyrospermol	17.7 – 80.91	Hexacosane	0.74 - 3.26
24-methylene-lanost-8-en-3-β-ol	6.33 - 20.46	Heptacosane	3.61 - 13.69
Cycloartenol	83.49 – 652.84	Octacosane	0.81 - 2.28
24-methylene-cycloartanol	144.67 – 1464.06	Nonacosane	3.07 - 9.93
4-Desmethylsterols		Triacontane	0.46 - 1.95
Campesterol	31.11 -108.37	Hentriacontane	1.89 - 8.83
Δ^5-avenasterol	52.43 – 575.04	Dotriacontane	0.16 - 1.09
β-sitosterol	681.41 – 2872.06	Tritriacontane	0.70 - 5.52
Stigmasterol	4.24 – 41.32	Pentatriacontane	0.12 - 1.33
Cholesterol	0.79 – 18.02	α -Farnesene	tr - 32.59
24-methylen cholesterol	0.63 – 7.01	Squalene	0.125 - 0.7 %
Campestanol	0.79 - 7.96	α-Tocopherol	125 – 200
Δ^7-campesterol	0.15 – 8.09	β-carotene	0.11 - 16.27 [a]
Chlerosterol	1.99 – 32.44	Lutein	1.20 - 4.49 [a]
Sistostanol	4.63 – 60.14	Violaxantin	10^{-3} - 0.77 [a]
$\Delta^{5,24}$-stigmastadienol	3.04 – 30.61	Neoxantin	70^{-3} - 0.79 [a]
Δ^7-stigmastenol	1.38 – 15.71	Antheraxanthin	nd - 0.64 [a]
Δ^7-avenasterol	2.81 – 26.93	β-cryptoxanthin	nd - 0.62 [a]
4-monomethylsterols		Luteoxanthin	90^{-3} - 0.80 [a]
Obtusifoliol	8.29 – 29.29	Mutatoxanthin	30^{-3} - 0.11 [a]
Gramisterol	6.54 – 20.71	Chlorophyll a	nd - 1.55 [a]
Cycloeucalenol	9.43 – 68.43	Chlorophyll b	nd - 0.80 [a]
24-Etillophenol	6.04 – 18.86	Pheophytin a	0.98 - 25.04 [a]
Citrostadienol	50.27 – 228.19	Pheothytin b	nd - 2.92 [a]
Oleanolic aldehyde	3.17 – 17.36	Pheophorbide a	nd - 0.57 [a]

The free acidity, expressed as oleic acid, and the organoleptic characteristics have been the parameters used to define these categories according to the trade standard of the International Olive Council [4]. Extra-virgin olive oil, a gourmet oil highly prized for its delicious flavour, tops all olive oil categories in terms of the strictest quality parameters.

Refined olive oil is obtained by refining virgin olive oil under conditions which do not lead to alteration of the initial glyceridic structure. Olive oil is the oil consisting of a blend of virgin and refined olive oil fit for consumption. Olive pomace oil is obtained by solvent extraction of the olive residue that remains after mechanical extraction of the virgin olive oil, made edible by refining methods. There are three different olive-pomace oils: olive-pomace oil, crude olive-pomace oil and refined olive-pomace oil. The first one is the oil comprising a blend of refined olive-pomace oil and virgin olive oil. The second is olive-pomace oil intended for refining while the last is the oil obtained from crude olive-pomace oil by a refining process which does not lead to alterations in the initial glyceridic structure [4].

Table 3 shows the limits of the parameters for olive oil designations according to the European Union. This information describes the characteristics of each designation that are not fully accepted by all the institutions involved in the olive oil business; in fact, there are notable disagreements [9]. Thus, Australian and South African standards propose values for palmitic, oleic and linolenic which are different from those of the IOC and the EU whereas the difference with Codex Alimentarius is in linoleic and gadoleic acids. The limit values for some 4-desmethylsterols (i.e. campesterol and stigmasterol) differ between IOC trade standards and standards from other institutions because the concentrations of those compounds are influenced by the latitude and altitude of olive tree orchards [10]. These scientific explanations have increased the debate about how olive oils from new producing regions (mostly in the Southern Hemisphere) can be classified as genuine without compromising the control of adulteration that a change in limits for these sterols would mean for the rest of world production. Thus, IOC has included decision trees for olive oils with percentages of campesterol between 4.0 and 4.5. The objective is to classify those oils as genuine oils, because they are, but without, however, comprising the fight against olive oil fraud; although no certainty value is associated to the decision tree yet. In addition, some regulations such as Australian and South African standards have even established a limit higher than 4.5 while they do not include any limit for total sterols (Australia and South Africa) and erythrodiol plus uvaol. With the aim of having a single regulation, a harmonisation program between the IOC, the EU and Codex Alimentarius is under progress.

The content of waxes is another source of disagreement between IOC/EU and the other institutions. IOC and EU assign different contents of waxes according to the olive oil designation (extra, virgin, ordinary, lampante, etc.) while the remaining institutions – Codex, USA, California, Australia and South Africa – give a value (≤ 250 mg/kg) whichever the designation. The maximum content of stigmastadienes, which can be used to determine the presence of any refined edible oil in virgin olive oil, is another source of disagreement among IOC/EU and the other institutions. Thus, the IOC and the EU recently lowered the limit from 0.10 to 0.05 due to modern analytical instruments have higher sensitivity with excellent values of precision while Codex and USA standards have values of 0.15 mg/kg.

Table 3: Limits of the chemical compounds used as parameters for protecting virgin olive oil designations against potential adulterations with edible oils [11]

Designations	(1)	(2)	(3)	(4)	(5)	(6[c])	(7)	(8)	(9[d])
Extra-virgin olive oil	≤0.05	≤0.05	≥1000	≤4.5	≤150	≤0.05	≤|0.2|	*B*	≤2.50
Virgin olive oil	≤0.05	≤0.05	≥1000	≤4.5	≤150	≤0.05	≤|0.2|	*B*	≤2.60
Lampante virgin olive oil	≤0.10	≤0.10	≥1000	≤4.5[a]	≤300[a]	≤0.50	≤|0.3|	*C*	-
Refined olive oil	≤0.20	≤0.30	≥1000	≤4.5	≤350	-	≤|0.3|	*C*	-
Olive oil	≤0.20	≤0.30	≥1000	≤4.5	≤350	-	≤|0.3|	*B*	-
Crude olive pomace oil	≤0.20	≤0.10	≥2500	>4.5[b]	>350[b]	-	≤|0.6|	≤1.4 %	-
Refined olive pomace oil	≤0.40	≤0.35	≥1800	>4.5	>350	-	≤|0.5|	≤1.4 %	-
Olive pomace oil	≤0.40	≤0.35	≥1600	>4.5	>350	-	≤|0.5|	≤1.2 %	-

Designations	(10[d])	(11[d])	(12)	(13)	(14)	(15)	(16)	(17)	(18[e])	(19)
Extra-virgin olive oil	≤0.22	≤0.01	≤0.8	≤20	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Virgin olive oil	≤0.25	≤0.01	≤2.0	≤20	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Lampante virgin olive oil	-	-	>2.0	>20	≤0.5	≤0.5	≤0.1	≤4.0	-	≥93.0
Refined olive oil	≤1.25	≤0.16	≤0.3	≤5	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Olive oil	≤1.15	≤0.15	≤1.0	≤15	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Crude olive pomace oil	-	-	no limit	no limit	≤0.5	≤0.5	≤0.2	≤4.0	-	≥93.0
Refined olive pomace oil	≤2.00	≤0.20	≤0.3	≤5	≤0.5	≤0.5	≤0.2	≤4.0	<Camp	≥93.0
Olive pomace oil	≤1.70	≤0.18	≤1.0	≤15	≤0.5	≤0.5	≤0.2	≤4.0	<Camp	≥93.0

Designations	(20[d])	(21[d])	(22[d])	(23)	(24)	(25)	(26)	(27)	(28)	(29)
Extra-virgin olive oil	Mf>0	Md=0	≤35	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Virgin olive oil	Mf>0	0<Md≤3.5	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Lampante virgin olive	-	Md>3.5[(f)]	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Refined olive oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Olive oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Crude olive pomace oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.3	≤0.2	([2])
Refined olive pomace	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.3	≤0.2	([2])
Olive pomace oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.3	≤0.2	([2])

Note: (1): *trans*-oleic fatty acid (%); (2): Sum of *trans*-linoleic & linolenic fatty acids (%); (3): Total sterol content (mg/kg); (4): Erythrodiol and uvaol content (% total sterols); (5): Wax content: C42+C44+C46 for extra virgin and virgin designations and C40+C42+C44+C46 for the rest of designations (mg/kg); (6): Stigmastadiene content (mg/kg); (7): Difference between the actual and theoretical ECN42 triacylglycerol content; (8): Content of 2-glyceryl monopalmitate (2P); B, 2P≤0.9 if total C16:0≤14.0 % or 2P≤1.0 if C16:0>14.0 %; *C*, 2P≤0.9 if C16:0≤14.0 % or 2P≤1.1 if C16:0>14.0 %; (9): Absorbency in ultra-violet at K_{232}; (10): Absorbency in ultra-violet at K_{270}, if cyclohexane is used, and K_{268} if iso-octane is used; (11): Absorbency in ultra-violet (ΔK); (12): Free acidity (%m/m expressed in oleic acid); (13): Peroxide value (in milleq. peroxide oxygen per kg/oil); (14): Δ^7-Stigmastenol (%); (15): Cholesterol (%); (16): Brassicasterol (%); (17): Campesterol (%); (18): Stigmasterol (%); (19): The value of β-Sitosterol is calculated as : $\Delta^{5,23}$-Stigmastadienol + Clerosterol + β-Sitosterol + Sitostanol + Δ^5-Avenasterol + $\Delta^{5,24}$-Stigmastadienol; (20) Organoleptic assessment: median of fruity attribute (Mf); (21) Organoleptic assessment: median of defect (Md); (22) Fatty acid ethylesters (FAEEs); (23) Myristic acid (% m/m methylesters); (24) Linolenic acid (% m/m methylesters); (25) Arachidic acid (% m/m methylesters); (26) Eicosenoic acid (% m/m methylesters); (27) Behenic acid (% m/m methylesters); (28) Lignoceric acid (% m/m methylesters); (29) Other fatty acids (% m/m methylesters). [a], when the oil has a wax content of between 300 mg/kg and 350 mg/kg it is considered a lampante olive oil if the total aliphatic alcohol content is ≤ 350 mg/kg or if the erythrodiol + uvaol content is ≤ 3.5 %; [b], when the oil has a wax content of between 300 mg/kg and 350 mg/kg it is considered a crude olive pomace oil if the total aliphatic alcohol content is > 350 mg/kg and the erythrodiol + uvaol content is >3.5 %; [c], Total isomers which could (or could not) be separated by capillary column; [d], quality characteristics; [e], Camp, campesterol (%); [f], or where the median defect is less than or equal to 3,5 and the fruity median is equal to 0; ([2]), Palmitic: 7.5-20.0; Palmitoleic: 0.3-3.5; Heptadecanoic: ≤ 0.4; Heptadecenoic: ≤ 0.6; Stearic: 0.5-5.0; Oleic: 55.0-83.0; Linoleic: 2.5-21.0.

The presence of re-esterified oils in olive oils is detected by the quantification of 2-glyceryl monopalmitate, for which maximum admitted percentages depend on the designation. Values proposed by IOC/EU are lower than those described in the standards supported by Codex Alimentarius, California, Australia and South Africa. The free acidity and the peroxide value associated with olive oil designations are stricter in the standards of California than in the rest of institutions. Finally, virgin olive oil is widely regulated by IOC as regards sensory assessment by a complete set of documents, which have been copied by all the other institutions. Differences once again concern the limits for the medians of defects and fruity attribute associated to extra-virgin and virgin olive oil designations. Thus, the median of defects for VOO has been raised to 3.5 – to take into account the uncertainty in the classification of the boundaries of virgin and ordinary/lampante – in the IOC/EU trade standard/regulation whereas this value has not been changed in the other standards. Another source of disagreement is the fact that the Californian, Australian and South African standards also consider these values of medians of defects and fruity attribute for olive pomace and refined oils.

International regulatory bodies have designed their standards with the information supplied by their delegates though a high percentage of the parameters qualifying the olive oil designations and the limits for determining their genuineness were initially proposed by the IOC. The limits for some parameters are, as already described, at the core of the disagreements among international regulatory bodies because climate conditions affect the chemical and biochemical pathways that are responsible for quantitative changes in olive oil chemical composition, and today there is an increasing number of orchards that are not located at the Mediterranean basin as was the case in the past.

Harmonisation among international institutions is being developed and this activity has been identified as a priority objective for the present [9]. The harmonisation should come from the collaboration among regulatory bodies in order to achieve an agreement for some specific parameters that are currently the subject of debate. Other actions, such as reducing the number of standard parameters and methodologies for example, would be beneficial for facilitating international trade as well. Most of the methods were proposed by the IOC specifically for olive oils although there are alternatives proposed by other institutions (i.e. AOCS, ISO, IUPAC, FOSFA).

2. Authenticity issues

2.1. Identification of current authenticity issues

It has been proved that fraud has been part of commercial transactions, in one manner or another, since they were practised in the remote past, and today olive oil is still considered a vulnerable product in terms of authenticity [6,9]. Fraud can mean ruin for many actors in the olive oil market like farmers and sellers although the consumer is the ultimate one affected by this dishonest attitude. Mass media, in fact, do not usually distinguish among food actors that intentionally carry out this illegal and dishonest activity and those that are simply affected by a one-off unintentional fail in quality control. Thus, the product's authenticity of the entire food market is called into question when the mass media publicise news on fraud, with the real risk that consumers might decide not to consume olive oil any more even though the potential fraud does not pose a threat to public health. Consumer perception of the product may be affected negatively despite the strict controls that are imposed on this product today.

Authenticity has many aspects, from adulteration and mislabelling to characterisation of protected designations of origin (PDOs). With so many potential issues to be studied, the great number of olive oil designations, and the large variety that can be used in adulteration, a questionnaire was prepared in order to obtain a broad opinion of producers, wholesalers, retailers, researchers, analysts etc. A first survey was collected in 1996, inside FAIM - a European funded project -, and a second survey was launched in 2016 inside FoodIntegrity –another European funded project- with updated questions about olive oil authenticity. Table 4 shows how the priorities of olive oil actors have evolved in the last twenty years. The importance of protecting virgin olive oil designations has not decreased and there is a great interest in determining the presence of soft-deodorized virgin olive oil in extra-virgin ones, and in knowing the traceability of the extra-virgin olive oils. The authenticity of extra-virgin olive oils is still linked to the classification by means of the sensory assessment ("Panel Test") [7], the results of which are questioned by some olive oil actors up to the point that objective methods based on the quantification of volatiles responsible for sensory descriptors are being studied as a potential alternative or a complementary action to sensory assessment. Consumer interest in a reliable geographical declaration of extra virgin olive oil (EVOO) has increased over the last years but not in the expected percentage. The importance of 'Typicality' (distinctive production) is revealed in the surveys that were carried out with information from consumers. However, a PDO may show or not clear differences in characteristics compared with other PDOs or non-PDOs.

Table 4: Percentages of the importance of authenticity issues according to answers of olive oil actors to surveys launched in 1996 – European funded project FAIM – and 2016 – European funded project FoodIntegrity

Issues	Sub-issues	FAIM 1996	FoodIntegrity 2016
Authenticity	Categories of olive oil	91	95
	VOO spiked with ROO	78	28
	VOO/ROO spiked with hazelnut	83	67
	EVOO spiked with soft-deodorised VOO	-	96
	VOO/ROO spiked with genetically engineered oils	87	63
	ROO spiked with desterolised oils	64	47
	ROO spiked with refined seed oils	93	53
	ROO spiked with pomace oil	37	48
	Olive oil spiked with esterified edible oils	58	49
	VOO spiked with other vegetable oils	26	11
Mislabelling	Declared mixtures (olive oil spiked with seed oils)[1]	15	-
Characterisation	Olive oil varieties	62	58
	Designation of Origin, Countries, etc.	69	77
Miscellany	Characterisation of sensory quality of olive oil varieties	66	68
	Addition of flavour and colour to ROO	8	36
	Authentication of Organic Virgin Olive Oil	11	43
	Characterisation of extraction systems	21	-

Legend: VOO, Virgin Olive Oil; EVOO, Extra Virgin Olive Oil; ROO, Refined Olive Oil; [1], this market is banned inside producer countries but it was an increasing market in some non-producer countries, e.g. Holland, Germany; Source: FAIM, FoodIntegrity project.

2.2. Identification of potential issues

As soon as certain rough adulterations (e.g. virgin olive oil mixed with refined oils) have been practically solved with efficient methods, the fraudsters have focused on developing new adulterations that are more sophisticated and difficult to detect since they are based on selecting

oils that after being mixed cannot be detected with regular methods. However, to commit this fraud, the fraudster needs to have an advanced knowledge of olive oil chemistry [12]. On the other hand, these new adulteration issues are described in terms of feasibility from a chemical/analytical point of view. In other words, in many cases reliable information is not available about the actual incidence of these frauds and their importance in the market and they are considered, among other reasons, because they are included in those malpractices that from a theoretical point of view would pose difficulties in their detection. In order to identify potential issues in olive oil authentication, those adulteration cases that are described to prove the potential of a new method/technology but which do not exist in the real world because as they are not economically viable should be omitted to avoid confusion. That would be the case of mixtures with more expensive oils or with oils that are easily detected with existing methods (e.g. mixture of virgin with seed oils).

A new possible adulteration is the addition of soft-deodorised virgin olive oil to extra virgin olive oils, that are more difficult to be detected and new strategies are needed [13]. Thus, when soft-deodorisation at low temperatures (<100 °C) is carried out in a virgin olive oil to remove slight sensory defects, the resulting soft-deodorised oil is the so-called ‘‘deodorato” or ‘‘deodorato soft”. After undergoing this thermal process, it can no longer be considered ‘virgin’ according to the legal definition for ‘‘virgin olive oil” [4]. For that reason, any mixture of a VOO with a ‘‘deodorato” is considered to be a fraud. The proposed chemical parameters for their detection (pyropheophytins, alkyl esters) has demonstrated not to be infallible so far, so new analytical strategies are needed.

Another relevant authenticity issue that is gaining importance is the authentication of geographical provenance. Since production today is moving beyond the Mediterranean countries (USA, Australia, Argentina, Chile, South Africa) etc., and consumers are aware about commercial transactions between countries, they demand more information about geographical origin. The fact that provenance is sometimes presented as an additional value to the product (regardless of actual quality) within a marketing strategy has resulted in an authenticity issue related with mislabelling. Thus, today, if the declared origin on the label does not match with the new origin, then it is considered that the oil clearly fails in its integrity. No standard methods exist in this regard. However, building a large database with major and minor compounds and the implementation of an expert system have been suggested for geographical characterization. That was the case of the SEXIA project [10,14]. Today, new alternatives based on non-targeted techniques are being developed [15].

Despite the strict regulations in force, advanced knowledge of the chemical composition of olive oil and other edible oils has brought to the table the possibility of building tailored oils designed to pass all the controls. This possibility has led researchers to consider other authentication strategies other than those based on existing methods. Since some compounds have been studied on olive oils and are not included in the standards, they are being tested for potential authentication purposes.

Other complex authenticity issues are related to the current use of olive oil as an ingredient to be incorporated in more complex food formulations. Thus, once the olive oil is mixed with other ingredients (e.g. canned foods in olive oil), the current methods are difficult to apply since the mixing changes the natural composition of the lipid fraction. Since the addition of virgin olive oil is claimed on the label as an additional value in the food formulation to attract consumers, the authentication of the olive oil content is perceived today as an emerging authenticity issue. Sometimes, even the highest quality designation of virgin olive oil (“extra virgin”) is mentioned on the label. In this case, evaluating the quality of virgin olive oils in mixtures with other ingredients is also difficult considering the migration of compounds between ingredients.

2.3. Potential threat to public health

All the adulterations that are considered today in oils in the regular market do not pose a direct serious threat to public health. The administration actively fights against fraud because it negatively affects consumer confidence with respect to a product that has a solid cultural background and is the centre of the Mediterranean Diet. In terms of toxic effects of fraud, only hypothetical rough adulterations in clandestine oils being sold outside the regular commercial circuits are concerned. That was the case of the Toxic Oil Syndrome (TOS) in the early 80s where the oil was not distributed in the regular food supply chain. For that reason, traceability and control of the food chain is considered as an essential authenticity tool that complements the analytical methods for fraud detection. Public administration at different levels is aware of the importance of this additional control and they implement regular inspections at retail outlets and in the food service sector.

3. Analytical methods used to test for authenticity

Currently, there is a proliferation of proposals trying to demonstrate that adulterants in olive oil can be easily detected. Advances in knowledge and technology have undoubtedly led to greater success in the fight against adulteration over the years. However, it is equally true that the same techniques and knowledge have been also used by fraudsters to invalidate the usefulness of some standard methods. Such competition has required not only a considerable investment on perfecting classical techniques or developing new ones, but also that the pace of R&D for detection of malpractice has to be rapid enough to counteract the fraudster's actions.

Numerous methods have been used to detect olive oil adulteration, but most of them can detect only adulterations greater than 10 %. This scarcely represents any advantage over the standard methods, the latter being described in Table 5.

Current tests and methods can be naturally divided into two groups: those based on the determination of signals related with almost all the possible analytes in the oil sample or a large group of them - the so-called "non-targeted" methods - and those that rely on measurement of more definite information obtained from fractionation of olive oil components - the so-called "targeted" methods. The latter, which identify and quantify series of chemical compounds, analyte by analyte, ideally have the objective of looking for compounds that do not appear, or only at trace levels, in genuine olive oil but appear in adulterated oils. Since these techniques give information about how these compounds came to be present in the adulterated food, this information can also be used to remove or diminish the amount of these analytes during adulteration, e.g. the use of desterolised oils.

The other group of techniques is based on the analysis of the total chemical make-up of the oil, using a spectroscopic technique for instance. Here, fraudsters may have no clues to how to manipulate composition such that the results comply with genuine oils, but the analysts in control labs can also have problems in the interpretation of the information with plausible chemical explanations. The utility and applicability of this group of techniques can be increased by applying multivariate statistical techniques. Even then, the conclusions should be supported by chemical or biochemical explanations to rule out noise or random effects in the samples.

3.1. Officially recognised methods

The methods in the international regulations and trade standards for the detection of olive oil adulteration are mainly based on LC (liquid chromatography) and GC (gas chromatography (Table 5).

These official methods [4] have enabled the control of virgin olive oil adulteration, but have led to some particular situations in which genuine extra-virgin olive oils are classified outside their natural category applying IOC Trade Standards and other national and international regulations [12]. They are usually olive oils from certain olive tree varieties cultivated outside the Mediterranean basin that do not comply with the limits of some criteria for authenticity in official trade standards and regulations (Table 3) even when they are carefully extracted, stored and delivered. Some traditional but minor cultivars, even harvested in regions inside the Mediterranean basin, also have values of their chemical compounds that do not comply with the limits described in Table 3. The paradigm might be the Spanish cultivar *var.* Verdial de Huévar, for which limits of erythrodiol exceed those defined for the extra virgin olive oil designation simply due to its particular biochemical pathways [16]. However, if large virgin olive oil databases and multivariate statistical algorithms had been applied in the past, this and other problems would no longer exist, and *var*. Verdial de Huévar, for instance, would not have just disappeared from Andalusian olive oil orchards. The highest interest for minor cultivars today is the possibility to maintain gene diversity (Olive Germplasm Bank) and to tackle chemical singularities when establishing legal limits.

Table 5 shows the methods that can be used for the quantification of parameters for which the limits, described in Table 3, are markers for the authenticity of the different olive oil designations. The methods are provided by different institutions (AOCS, EU/EC, FOSFA, IOC, ISO, IUPAC) although those provided by the IOC (named COI/T.20) have particularly been designed to analyse olive oils. This is, for instance, the case of the method for the detection of refined oils in virgin olive oil by means of the quantification of stigmastadienes [17,18], which is still one of the most powerful methods.

The methods described in Table 5 are not exempt from required improvements, comments and useful tips. Thus, the high diversity of available chromatographic columns for the determination of fatty acid composition can produce differences in the results. Columns characterized by the highest polarity are recommended for a better separation of PUFA while lowest polarity columns are better for saturated and monoenoic compounds. A good separation of *trans* fatty acids is much better with a 50 m column with a cross-linked stationary phase of cyanopropylsiloxane [9]. The determination of sterols and triterpene dialcohols is easier with a previous HPLC separation – instead of TLC – though this kind of separation, widely used in the laboratories, is not included in some official methods. As regards the determination of actual and theoretical ECN42, the IOC recommends a method based on the use of propionitrile solvent in the determination of triacylglycerides which adds a supplementary complication with no clear advantage. The determination of the content in stigmastadienes should be implemented by determining the concentration of sterenes (campestadienes and stigmastadienes) if the concentration of stigmastadienes is higher than 4 mg/kg. The presence of re-esterified oils in olive oils is detected by the quantification of 2-glyceryl monopalmitate, a lengthy and tedious method that requires previous knowledge such as neutralising the sample if its acidity is higher than 3 %, the readjustment of the pH to 8.3, and a strict control of pancreatic lipase that may lose activity easily.

Table 5: Summary of the relevant methods proposed in the international regulations supported by the International Olive Council (IOC), Codex Alimentarius, EU, USDA, California State (USA), Australia, and South Africa (Source: [9])

Determination	Method
Fatty acid composition	(EU) 1833/2015 Annex IV; COI/T20/Doc No 33; AOCS Ce 1f-96 Methyl ester preparation: ISO 5509:2000; AOCS Cc 2-66; COI/T20/Doc No 24 Gas Chromatography: ISO 5508:1990; AOCS Ch 2-91
Trans fatty acid content	(EU) 1833/2015 Annex IV; COI/T20/Doc No 33; COI/ T20/Doc No 17 Rev 1; ISO 15304:2002; AOCS Ce 1f-96; AOCS Ch 2a-94 (Rev 2002)
Sterol and triterpene dialcohols composition	(EU) 1348/2013 Annex IV; COI/T20/Doc No 30; COI//T20/ Doc No 10 Rev 1; ISO 12228:1999; AOCS Ch 6-91 -Erythrodiol + uvaol: COI/T20/ Doc No 30 ; IUPAC 2431
Wax content	COI/T20/Doc No 18 ; AOCS Ch 8-02; (EC) 702/2007 Annex IV
Aliphatic and triterpenic alcohol content	COI/T20/Doc No 26 Rev1; (EU) 2015/1833 Annex VI
Difference between the actual and theoretical ECN 42 triacylglycerol content	COI/T20/Doc No 20 rev 3; COI/T20/ Doc No 23; AOCS Ch 5b-89; (CE) 2472/97 Annex XVIII
Stigmastadiene content	COI/T20/Doc No 11/Rev2; COI/T20/Doc No 16/Rev1; ISO 15788-1:1999; AOCS Cd 26-96; ISO 15788-2:2003(EC) 656/95 Annex XVII
Content of 2-glyceryl monopalmitate	COI/T20/Doc No 23; (EC) 702/2007 Annex VII
Unsaponifiable matter	ISO 3596:2000; ISO 18069:2000; AOCS Ca 6b-53
Organoleptic characteristics	(EU) 1348/2013 Annex V; Amended by (EU) 2016/1227; COI/T20/Doc No 15
α-tocopherol	ISO 9936
Waxes and alkyl esters	COI/T20/Doc No 28; COI/T.20/Doc. No 33; (EU) No 61/2011 Annex II
Biophenols	COI/T20/Doc No 29
Free acidity	COI/T20/Doc No 34; (EU) 2016/1227 Annex I; ISO 660(03); AOCS Cd 3d-63; AOCS Ca 5140
Peroxide value	COI/T20/Doc No 35; ISO 3960; (EU) 2016/1784 Annex III; AOCS Cd 8b-90
Absorbency in ultra-violet	COI/T20/Doc No 19 Rev 3/Rev 2; ISO 3656; AOCS Ch 5-91; (EU) 2015/1833 Annex III
Moisture and volatile matter	ISO 662; AOCS Ca 2c-25
Pyropheophytins	ISO 29841:2009
Insoluble impurities in light petroleum	ISO 663; AOCS Ca 3a-46
Flash point	FOSFA Int. method; ISO 15267:1998
Trace metals copper, iron and nickel	ISO 8294
Traces of heavy metals	Lead ISO 12193; AOCS Ca 18c-91; AOAC 994.02 Arsenic AOAC 952.13; AOAC 942.17; AOAC 985.16
Traces of halogenated solvents	COI/T20/Doc No 8; (EEC) 2568/91 Annex XI
Waxes fatty acid methyl esters and fatty acid ethyl esters by GC using 3g of silica gel	COI/T20/Doc No 31 *provisional*
Composition of triaclyglycerols and diacylglycerols by GC in vegetable oils	COI/T20/Doc No 32 *provisional;* ISO 29822
Refractive Index	ISO6320:2000; AOCS Cc 7-25
Iodine value	(EEC) 2568/91 Annex XVI
Saponifiable value	ISO 3657:2002; AOCS Cd 3-25
Fatty acid in the 2-position of triglycerides	ISO 6800:1997; AOCS Ch 3-91
Relative density	IUPAC 2101[1]
Oxidative stability index	AOCS Cd 12b-92

Note: [1], with the appropriate conversion factor; EU, European Union; EC, European Commission; AOCS, American Oil Chemists Society; ISO, International Organization for Standardization; COI, International Olive Council; FOSFA, Federation of Oils, Seeds and Fats Associations Ltd; IUPAC, International of Union of Pure and Applied Chemistry.

Sources: IOC: www.internationaloliveoil.org, Codex: www.fao.org/fao-who-codexalimentarius/codex-home/es/, EU: ec.europa/agriculture/olive-oil_en, USDA: www.ams.usda.gov/grades-standards/olive-oil-andolive-pomace-oil-grades-and-standards, California State (USA):www.cdfa.ca.gov, Australia: www.aph.gov.au, and South Africa (SANS) www.sabs.co.za/.

The determination of pyropheophytins (PPP) is another major point of discrepancy between the IOC and the associations of new olive oil producing countries (Australia, California, South Africa, New Zealand). The increment in PPP is associated with the presence of energy in terms of light and/or temperature during the extra virgin olive oil (EVOO) shelf-life, which provides information on EVOO freshness, a concept that is not accepted for producer countries structured around the IOC. The analytical method based on the reverse-phase solid-phase extraction (RP-SPE) has a critical point in the collection of the analytes in 0.2-0.3 mL of acetone because of its high volatility which suggests that the injection in the HPLC instrument should be as rapid as possible. The method allows two kinds of elution, with petroleum ether (40-60 °C) or with petroleum ether (40-60 °C): ethyl ether (9:1) for removing the lipids.

The concentration of ethyl esters of fatty acids (FAEEs) is among the parameters that have been recently approved by IOC/EU for determining EVOO quality though there is no causal relationship between the concentration of these compounds and the sensory assessment, which is the official method for determining whether a virgin olive oil is or is not extra-virgin. The role of FAEEs is not accepted by international associations other than the IOC.

The development of standard methods is normally a consequence of industrial or commercial needs and they are established as standards after their validations by collaborative studies. The lobbying that groups of chemists carry out in the implementation of methods is becoming increasingly irrational, so the usefulness of some new methods gets more and more preposterous. However, the requirement of validation as a prerequisite may prevent standard methods providing unsatisfactory results being put forward.

3.2. Other used methods

Most of the methods described in Table 5 are based on chromatography, which is a time-consuming technique that needs several steps to carry out quantification, uses polluting solvents and is impracticable for on-line control, the latter being a common demand from farmers and co-operative societies in the fight against adulteration. Alternatives must come from techniques that have simple or no sample preparation or pre-treatment as those described in Table 6. Such techniques have been thought most likely to be spectroscopic though unfortunately their methods have not been widely applied in olive oil authentication yet. There have been numerous attempts, however, such as the procedure that combined artificial neural networks and Curie-point PyMS (Pyrolysis Mass Spectrometry) [19] for a rapid assessment of adulteration of extra-virgin olive oil or the application of ^{13}C-NMR to distinguish virgin olive oil from refined olive oils and olive-pomace oil [20]. The comparison of these, and other techniques, with methods based on the detection of stigmastadienes by gas chromatography showed the superior behaviour of chromatographic methods in terms of time of analysis and false positives, which has led to the delayed implementation of spectroscopy in olive oil authenticity. Table 7 shows the application of some alternative methods in authenticity issues, mostly based on these spectroscopic techniques.

Table 6: Main characteristics of alternative techniques proposed for the authentication of olive oils

Characteristics	Techniques
Structural & Pattern Recognition	NMR, MS, NIR, FTIR, FT-Raman, DSC, TG, SF.
Stable Isotope Analysis	IRMS.
Trace Element Analysis	ICP-AES, AAS, FAAS, ETA-AAS.
In-tandem	GC-MS, HPLC-MS, ICP-MS, CG×GC, LC×LC, SFC, δ^2H-EA-Py-IRMS, δ^2H-GC-Py-IRMS.

Note: Nuclear magnetic resonance (NMR); near infrared spectroscopy (NIR), Fourier transform infrared spectroscopy (FTIR) and Fourier transform Raman spectroscopy (FT-Raman); isotope ratio mass spectrometry (IRMS); inductive coupled plasma-atomic emission spectroscopy (ICP-AES); atomic absorption spectroscopy (AAS); flame absorption spectroscopy (FAAS); electrothermal atomization-AAS; mass spectrometry (MS), GC-MS, LC-MS and ICP-MS; elemental analyser-pyrolysis-isotope ratio mass spectrometry (δ^2-H-EA-Py-IRMS) and δ^2-H-GC-Py-IRMS; bidimensional chromatography (GC×GC, LC×LC); supercritical fluid chromatography (SFC); synchronous fluorescence (SF); differential scanning calorimetry (DSC) and simultaneous thermogravimetry (TG).

3.2.1. Vibrational Spectroscopy *

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Vibrational spectroscopy methods, based on NIR, MIR or Raman spectroscopy technique, are part of the fingerprinting methods used in authenticity, which regroup all the analytical protocols that provide a full physical or chemical pattern of the samples [21]. Spectroscopic techniques have been considered as promising tools for rapid sample screening over a number of years. However, the fact that they need large datasets in order to calibrate any given instrument and to provide a chemical interpretation of spectra has limited their application in olive oil authentication beyond the determination of classical values and oil indices; i.e. *trans*/*cis* double bonds, free fatty acids, unsaturation degree, oxidation state and moisture content among others [22].

Considering near infrared spectroscopy (NIR), in the last years, more and more applications have been developed in at-line and on-line quality control. Regarding specifically the authentication of olive oil, NIR spectroscopy has been used to detect the hypothetical adulteration of olive oil with vegetables oils like sunflower seed, corn, walnut, soya and hazelnut [23,24].

The application of Mid Infrared Spectroscopy (MIR) to the detection of extraneous edible oils in olive oil in 1990's [22] has led to a period where infrared spectroscopy was used in olive oil traceability with success [25,26]. Thus, for example, MIR has been used to detect the adulteration of extra virgin olive oil with a corn-sunflower binary mixture (5 % (v/v)), cottonseed and rapeseed oils (5 % (v/v)) [27,28]. Baeten and collaborators [29] also proposed the use of MIR spectroscopy in combination with Raman spectroscopy to determine the presence of hazelnut oil in olive oil.

Several studies have also described the use of Raman spectroscopy for detecting and quantifying the adulteration of olive oil [30-32]. The method is suitable for the analysis of compounds rich in unsaturated functional groups and has proved to be useful in studies involving olive oil. Based on the intensity ratio of the cis (=C-H) and cis (C=C) bonds normalised by the band at 1 441 cm-1 (CH_2), Zou and collaborators [33] demonstrates the interest of Raman spectroscopy for the authentication or the detection of fake olive oil. El-Abassy and collaborators [34] tested a dispersive Raman system using a 514 nm laser to discriminate olive oils from different types of sunflower oils in only a few seconds.

Fatty acids are the most abundant biomolecules in olive oil, and they do not allow vibrational spectroscopy to get information from minor compounds due to the barrier effect that is exerted by saponifiable matter in the spectra acquisition [30]. Thus, one of the problems in vibrational spectroscopy is caused by the interferences of the saponifiable matter (fatty acids and TAGs) when determining unsaponifiable compounds (i.e. sterols) [9]. One solution is to perform a previous transesterification of the oil [30]. Unfortunately, those minor compounds are the most informative compounds for detecting habitual and sophisticated adulterations of oils nowadays.

3.2.2. ^{1}H and ^{13}C -NMR spectroscopy

The first application of high resolution proton nuclear magnetic resonance (^{1}H-NMR) in the field of oils and fats was the determination of global unsaturation (corresponding to the classical iodine number value) made on the basis of the integral of olefinic protons at 5.3-5.4 ppm. In addition to this application, several researchers have proposed NMR as a suitable technique for analysing different components in olive oil [35]. Thus, ^{1}H-NMR methods can be applied to obtain structural and quantitative information on a wide range of organic metabolites. NMR can be applied to quantitate fatty acids, although the determination of individual fatty acids is not possible. Thus, saturated fatty acids (SFAs), monounsaturated oleic acid (MUFA), and polyunsaturated linoleic and linolenic acids (PUFAs) can be obtained by determining several signal intensities. With respect to ^{13}C-NMR, it enables almost all analyses performed by ^{1}H-NMR and it is the preferred technique to obtain information about the positional distribution of the saturated, oleyl, linoleyl, and linolenyl chains on the glycerol moiety [35]. Whatever the kind of NMR used, this technique requires the application of multivariate statistical analysis of ^{1}H or ^{13}C signal intensities of the oil samples or suitable chemical parameters determined by NMR. Assuming that a fraudulent addition of an extraneous oil changes slightly the chemical composition of the oil, NMR spectra can point out changes in the profile that can be highlighted with statistical analysis. Thus, the appearance of a resonance in the carbonyl region ascribed to saturated fatty acids at the *sn* -2 position of glycerol and slight differences in the chemical shifts of the saturated and unsaturated acids is associated with fraudulent oils [35]. However, in the case of real adulterated oils, where low adulteration percentages and oils with similar composition are used, it is more difficult to highlight slight differences in the NMR spectra.

Table 7: Basic characteristics of non-standard (in-house) methods proposed for some analytical challenges of the olive oil authenticity issues

Issue	**Addition of cheaper oils to olive oils**
Objective:	Detection of the presence of any edible oil (crude or refined) in virgin or refined olive oil.
Analyte/Signal:	Selected ^{13}C- & ^{1}H-NMR bands of the spectrum.
Technique:	^{13}C-NMR and ^{1}H-NMR spectroscopies.
Level of applicability:	Universal although has been checked with only a few adulterants.
Official method?:	No, but the adulteration with hazelnut oils have been validated with blind trials.
Time of analysis[a]:	Pre-treatment: No; measurements: 4 h for ^{1}H-NMR and 1.45 h for ^{13}C-NMR; data analysis: 20 min applying procedures of Artificial Neural Networks (ANN).
Limit of detection[b]:	>10 % using bands from ^{13}C-NMR and ^{1}H-NMR for adulterations with hazelnut oils. ~15 % using bands from ^{13}C-NMR or from ^{1}H-NMR for adulterations with hazelnut oils.
Advantages:	Good repeatability.
Disadvantages:	Time-consuming. Poor reproducibility. False positives. Hyper-optimist models.
References:	[36-38]
Objective:	Detection of the presence of any edible oil (crude or refined) in virgin or refined olive oil.
Analyte/Signal:	Infrared or Raman bands.
Technique:	FTIR or FT-Raman.
Level of applicability:	Universal although has been checked with only a few adulterants.
Official method?:	No, some kinds of adulteration have been validated with blind samples.
Time of analysis[a]:	FTIR: Pre-treatment: 5min[c]; measurement: 5min; data analysis: 5min applying ANN. FT-Raman: Pre-treatment: nil[c]; measurement: 10min; data analysis: 5min applying ANN.
Limit of detection[b]:	>10 %
Advantages:	Rapid and easily implementable method.
Disadvantages:	Full checked with hazelnut oils only. A large set of spectra is required. Unstable mathematical equations.
References:	[22,29,39-40]
Issue	**Addition of refined oils to virgin olive oils**
Objective:	Detection of the presence of any refined edible oil in virgin olive oils.
Analyte/Signal:	*cis*/*trans* FTIR or FT-Raman bands.
Technique:	Spectroscopy by FTIR or FT-Raman.
Level of applicability:	Universal.
Official method?:	No, but the method has been validated with blind samples.
Time of analysis[a]:	Pre-treatment: Nil; measurement: 10min; data analysis: 10min.
Limit of detection[b]:	>8 %
Advantages:	Rapid method.
Disadvantages:	Limit of detection. The method does not work properly with less unsaturated oils.
References:	[25,29,31]
Issue	**Geographical traceability of VOOs**
Objective:	Determination of the geographical provenance (country, region, county, PDO, PGI) of VOOs.
Analyte/Signal:	Several: fatty acids, alcohols, sterols, hydrocarbons etc.
Technique:	Gas chromatography for chemical analysis and expert system (SEXIA®) for data analysis[e].
Level of applicability:	Whole Spain and partially the other EU producer countries.
Official method?:	No, but SEXIA® has been validated with hundreds of samples for years.
Time of analysis[a]:	Pre-treatment: 180min; measurement: 300min; Data analysis: 10min using expert system.
Correct classification[b] (%):	Average certainty factors (CF): 92 % for Andalusian PDOs, 95 % for Spanish regions, and 96 % for the identification of major EU producing countries/varieties among others.
Advantages:	Results are associated to high CFs. It based on the largest VOO database.
Disadvantages:	Time-consuming. Several different chemical analyses. It constantly needs to be updated.
References:	[14,41,42]

Table 7 (follow-up)

Objective:	Determination of the geographical provenance of VOOs.
Analyte/Signal:	δ^2H, $\delta^{13}C$ or $\delta^{18}O$.
Technique:	EA-IRMS or NMR.
Level of applicability:	Universal.
Official method?:	No.
Time of analysis[a]:	Pre-treatment: nil; measurement: few minutes; data analysis: 5 min.
Correct classification[b] (%):	Not reported by authors.
Advantages:	Rapid method.
Disadvantages:	Reproducibility. Need of a previous large database. Harmonisation of calibration procedure.
References:	[43-45]
Objective:	Determination of the geographical provenance of VOOs.
Analyte/Signal:	Multi-elements.
Technique:	ICP-MS or ICP-AES.
Level of applicability:	Universal.
Official method?:	No.
Time of analysis[a]:	Digestion (in microwave): 75-90 min; measurement: 3-5min; data analysis: 15 min using ANN.
Correct classification[b] (%):	Not reported by authors.
Advantages:	Causal relationship between soil and oil. A large number of variables (elements). Repeatability.
Disadvantages:	Low concentration of elements in the oils. Need of information of soils for training the model. Interference of fertilizers and fungicides[d].
References:	[46-49]

Note: [a], checked by the authors at their labs and in the course of collaborative analyses of European funded projects. [b], the best figure reached in the course of collaborative analyses with blind samples. [c], the measurement is carried with the entire oil but if the measurement is of the unsaponifiable matter, 60 min has to be added to the total analytical procedure. [d], foliar fertilizers can contain K, Fe, Mg, Mn, P and Zn in different proportions, together with other elements (i.e. B, Ca), which can be presented complexed with amino acids such in the cases of Ca, Fe, Mg, Mn and Zn. Fungicides can contain Cu among other elements. [e], other authors have proposed the study of particular geographical production zones by diverse series of compounds, and data are analysed by an umpteen different number of statistical procedures, either unsupervised (e.g. PCA, MDS) or supervised (e.g. LDA, PLS).

3.3. Looking to the future

Today both the production and the consumption of olive oil are moving slowly but inexorably beyond the Mediterranean countries, and olive trees are being planted in countries as far from the Mediterranean basin as New Zealand, Australia, Argentina and Chile with an agricultural technology that has increased up to 16 MT of olives per hectares with adequate sensory and nutritional properties. These practices overcome the negative benefit balance of traditional agriculture while maintaining the prestige of olive oil as a tasteful and health promoting oil.

This revolution in agricultural techniques is not, however, exempt from challenges and even problems from the chemical viewpoint. Traditional orchards were planted with diverse and autochthonous cultivars and used rainfed water supply, but the new orchards demand large quantities of water and the diversity of their cultivars is fewer than one dozen. Questions emerge beyond the classical issues concerning olive oil purity and nutritional benefits [58]. How does the water demand of the new orchards fit into sustainable agriculture? How does the water quality (i.e., salinity) influence olive oil chemical composition? Are the current techniques ready to treat and make use of the increasing tons of by-products? How much is the olive oil chemical composition affected by the latitude of new orchards? Are we going to lose the great diversity of olive tree germplasm with the unstoppable new monocultivar plantations? Are the numerous virgin olive oil Protected Designations of Origin (PDOs) and Protected Geographical Indications

(PGIs) safeguarded from fraudulent labelling? Is the authentication of the geographical origin of olive oil the great forthcoming challenge? How are the proposed non-targeted techniques managed in a legal framework (in court proceedings)? How can olive oil authenticity benefit from the new approaches on big data and data management? Should the olive oil market move toward a common commercialization as daily oil instead of delicatessen marketing?

These are some questions that highlight the current problems in the field of olive oil research and compel food scientists to bring continue their efforts to solve them and to find new methods. The solutions to the current problems of olive oil may come from a high level of chemical characterization. International institutions, led by the IOC, are tackling the influence of climate and geographical provenance in the chemical composition of some genuine olive oils by means of a mathematical algorithm so-called Decision Trees. Although the already accepted Decision Trees [4] do not have mathematical support to their conclusions yet - it is a matter of time that they have a statistical probability associated to their conclusions -, the results seem to be acceptable.

4. Overview of methods for authenticity testing

The combined action of methods and trade standards that regulate the limit values of some analytical parameters results in a procedure that allows determining the presence of extraneous edible oils in olive oils. Figure 1 shows the minimum detectable percentage of some edible oils when they are mixed with olive oil. On the other hand, Table 8 shows the chemical parameters that are used for detecting these oils and the information on authenticity that is derived from them. Thus, some vegetable oils are characterized by relatively high concentration of some compounds, so the latter may indicate their presence in olive oil. However, it is advisable to check the concentration of all the compounds to extract conclusions. Tables 9 and 10 show the methods used to quantify these chemical parameters and the basic characteristics of the analytical procedures.

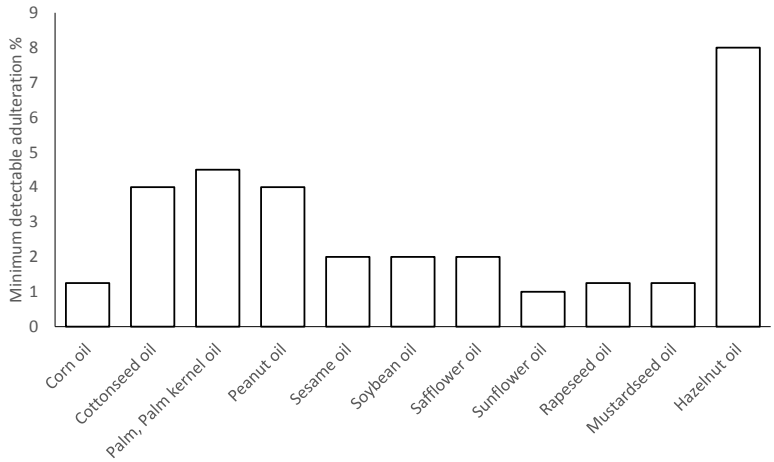

Figure 1: Minimum detectable percentage of some edible oils from different vegetable origins when they are mixed with virgin olive oil by applying the methods described in Table 8

Table 8: Methods and their analytical parameters to be quantified with the objective of detecting the presence of extraneous edible oils in olive oils.

Parameter	Compounds	Authenticity information (presence of…)
Sterols	Brassicasterol Brassicasterol; β-sitosterol Campesterol; β-sitosterol Cholesterol Stigmasterol	Brassicaceae oils Rapeseed oils Mustards seed oils Fractionated palm oils Palm kernel oil Peanut oils
ECN42	ΔECN42 + apparent-β-sitosterol + campesterol + stigmasterol ΔECN42 + Apparent-β-sitosterol ΔECN42 + Campesterol + Stigmasterol ΔECN42 + Apparent-β-sitosterol+ Δ7-stigmastenol	Corn oils Safflower, sesame and soybean oils Cotton oils Sunflower oils
Fatty acid methyl esters	Myristic acid Linolenic, eicosanoic and behenic acids Lignoceric acids	Fractionated palm oils Soybean and canola oils Peanut oils
Trans isomers of fatty acids	tC18:1 & t(C18:2+C18:3)	Refined oils
Stigmastadienes	Stigma-3,5-diene Campestadiene and stigmastadiene	Refined seed oils Desterolised oils
Triterpene dialcohols	Erythrodiol + Uvaol	Olive-pomace oil Seed oils (e.g. grapeseed oil)
Waxes	C40+C42+C44+C46	Olive-pomace oils
2-Glyceryl monopalmitate	Palmitic acid at the 2-position of the triacylglycerols	Oils synthesized by means of FFA esterification with glycerol

Note: The minimum detectable percentage of adulteration with some of these oils are shown in Figure 1.

Table 9: The standard methods for quantifying acyl lipids and fatty acids

Compounds	Technique	Sample preparation	Chromatographic characteristics
Triacylglycerols	HPLC-RI	0.12 g oil in 0.5 mL hexane is charged into SPE-cartridge (1 g of Si) and solution pulled through and, then, eluted with 10mL hexane-diethylether (87:13 v/v).	Mobile phase flow-rate (0.6 to 1.0 mL/min) Oven temperature: 20 °C Mobile phase: propionitrile Column: RP-18 (4 µm) Detector: RI
	HPLC-RI	0.5 g oil in 10 mL acetone or acetone/chloroform (1:1 v/v).	Mobile phase flow-rate (0.6 to 1.0 mL/min) Oven temperature: 25 °C Mobile phase: acetone/acetonitrile (1:1 v/v) Column: RP-18 (4 µm) Detector: RI
2-glyceryl monopalmitate (%)	GC-FID	Hydrolysis with pancreatic lipase. Separation by LC or SPE. Require silanisation.	Column: Capillary (12m×0.32mm×0.10-0.30 µm) Phase: methylpolysiloxane or 5 % phenylmethylpolysiloxane. Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: on-column
Fatty acids	GC-FID	Total Fatty acids: Methylation with cold methanolic solution of KOH or double methylation in a methanolic medium with alkaline and acid catalysis.	Column: Capillary (25-100m×0.2-0.8mm×0.1-0.2µm) Stationary phase: polyglycol, polyester or cyanopropylsilicone Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split
	GC-FID	*Trans* fatty acids: Methylation with cold methanolic solution of KOH.	
	GC-FID	Fatty acid in the 2-position: Hydrolysis with pancreatic lipase previously and methylation in a methanolic medium with alkaline and acid catalysis.	
Waxes	GC-FID	Isolation on LC Si-column.	Column: Capillary (12-15m×0.25-0.32mm×0.1-0.3µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split or on-column

Note: GC, Gas Chromatography; FID, Flame Ionisation Detector; HPLC, High Performance Liquid Chromatography; RI, Refractive Index detector; SPE, Solid Phase Extraction.

Table 10: The standard methods for determining minor compounds

Chemical series	Technique	Sample preparation	Chromatographic characteristics
Sterols	GC-FID	Unsaponifiable-matter isolation TLC or HPLC. Requires silylation.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Isothermal Injection mode: split
Erythrodiol+uvaol	GC-FID	Unsaponifiable-matter isolation TLC or HPLC. Requires silylation.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Isothermal Injection mode: split
Aliphatic alcohols	GC-FID	Unsaponifiable-matter isolation TLC or HPLC. Requires silylation.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split
Aliphatic hydrocarbons and sterenes	GC-FID	Unsaponifiable-matter isolation on LC Si-column.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split

Note: GC, Gas Chromatography; FID, Flame Ionisation Detector; HPLC, High Performance Liquid Chromatography; TLC, Thin Layer chromatography.

5. Conclusion

The criteria defining the authenticity, purity or genuineness of a food product are numerous and vary from one foodstuff to another although many generic definitions have been proposed. Concerning virgin olive oil, authenticity issues may be associated with adulteration with other edible oils but also with designation of origin, olive varieties and with oils that do not meet the requirements of integrity and good practices in labelling. Trade standards, either at national or international level, define the chemical characteristics of a genuine oil with much more detail compared to other vegetable oils. However, considering that consumers expect olive oil to be a foodstuff endowed with reputed sensory and healthy properties, today the authenticity issues of extra virgin olive oil also reach the sensory properties of the oil, which would be inside the declared designation.

The great interest of researchers in virgin olive oil authentication shown in the last few years, mostly analysing the chemical/sensory results by mathematical procedures, has led to an improvement in the control of virgin olive oil adulteration although the new adulterations - e.g. oils with similar chemical composition (hazelnut oil) - represent a new challenge for researchers. Regardless the endless discussion on olive oil authenticity over the decades, the continuous achievement of solutions from analytical chemistry has posed serious problems to the fraudster to commit adulteration and it can be concluded that only the most sophisticated authenticity issues are challenges for the future (olive oils spiked with soft-deodorised oils or tailored oils).

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