ANALYTICAL CRITERIA FOR QUALITY AND PURITY EVALUATION OF OLIVE OIL.

By Efi Christopoulou, chemist of Ministry of Development-Athens-Greece.

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Quality criteria of olive oils

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   (applicable only to virgin olive oils)

Quality criteria
1. FREE ACIDITY

Definition :

Free acidity is the content of free fatty acids determined according to the procedure specified in the method.

Method : ISO 660 or AOCS Cd 3d-63

Causes :

The lipolytic action of lipase (specific enzyme, found in the olive fruit) produces free fatty acids which are responsible for the acidity of the oil.

Quality criteria
2. ULTRAVIOLET SPECTROMETRY

Definition :

Spectrophotometric examination in the ultraviolet is the absorption at the wavelengths specified in the method and expressed as specific extinctions.

Method: COI/T.20/Doc. no. 19 or ISO 3656 or AOCS Cg 5-91

Quality criteria
2. ULTRAVIOLET SPECTROMETRY

Causes :

1. The absorbency at 232nm is caused by :
   - ✓hydroperoxides (primary stage of oxidation) and
   - ✓conjugated dienes (intermediate stage of oxidation).
2. The absorbency at 270nm is caused by :
   - ✓carbonylic compounds(secondary stage of oxidation).
   - ✓conjugated trienes (technological treatments).
3. The index DK is a criterion of discrimination between a bad quality virgin olive oil and a virgin olive oil adulterated with refined olive oil.

Quality criteria
3. PEROXIDE VALUE

Definition :

Peroxide value is the quantity of those substances in the sample, which oxidize potassium iodide under the operating conditions described.

Method: ISO 3960 or AOCS Cd 8b-90

Causes :

The peroxide value is caused by hydroperoxides (primary stage of oxidation). The oxidation may be enzymatic or chemical.

Quality criteria
4. MOISTURE AND VOLATILE MATTER

Definition :

Moisture and volatile matter is the loss in mass undergone by the product on heating at $103^0C \pm 2^0C$ under the conditions specified in the method.

Method: ISO 662

Causes :

Oils can contain water and volatile compounds as a result of the extraction method (water from vegetable tissues, e.t.c.). Besides being a foreigh matter, water impair the quality of oils.

Quality criteria

5. INSOLUBLE IMPURITIES IN LIGHT PETROLEUM

Definition :

Insoluble impurities content is the quantity of dirt and other foreign matter insoluble in n-hexane or light petroleum under the conditions specified in the method.

Method: ISO 663

Causes :

The application of bad manufacturing practices during the production of olive oil are responsible for high insoluble impurities content.

Quality criteria
6. TRACE METALS

Definition :

Trace metals is the amount in µg/Kg of copper and iron in all types of olive oils, including contaminants from bleaching earth and/or catalysts.

Method: ISO 8294

Causes :

Traces of iron and copper may originate from the soil and fertilizers or from contamination from the processing equipment and storage.

Quality criteria
7. ORGANOLEPTIC ASSESSMENT

Definition :

Organoleptic assessment is the detection and the description of both qualitative and quantitative flavour characteristics of virgin olive oil using human senses and the classification of this virgin olive oil according to its flavour characteristics.

Method: COI/T.20/Doc. no. 15:

The method directly use a group of tasters selected and trained as panel and it is only applicable for the classification of virgin olive oils according to the perceived intensity of the predominant defect and the presence or not of the fruity attribute.

Quality criteria
7. ORGANOLEPTIC ASSESSMENT

Causes :

The minor components of olive oils included in the unsaponifiable matter are significant for the olfactory and gustatory attributes of olive oils.

The compounds which are responsible for the negative organoleptic attributes are not found in good quality olive fruit.

Quality criteria
Factors affecting the quality criteria

Good quality characteristics are obtained :

- ✓ Applying good practices in the cultivation, harvesting, storage and processing the olives and so, avoiding the enzymatic oxidation.
- ✓ Protecting the olive oil from light, temperature, air and traces of metallic elements and so, avoiding the chemical oxidation.
- ✓ Separating olive oil from muddy sediment
- ✓ Avoiding the lengthily storage of olive oil.
- ✓ Packing the olive oil in suitable materials and under controlled conditions.

Quality criteria

QUALITY CRITERIA ACCORDING TO THE INTERNATIONAL STANDARDS

Criterion	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil	Refined olive oil	Olive oil
ORG. ASS. Mef. Med.	>0 =0	>0 ≤ 2,5	- ≤ 6,0	- > 6,0	- -	- -
ACIDITY	≤ 0,8	≤ 0,22	≤ 3,3	>3,3	≤ 0,3	≤ 1,0
K270	≤ 0,22	≤0,25	≤ 0,30	-	≤ 1,10	≤ 0,90
K232	≤ 2,50	≤ 2,60	-	-	-	-
ΔK	≤ 0,01	≤ 0,01	≤ 0,01	-	≤ 0,16	≤ 0,15

Quality criteria

QUALITY CRITERIA ACCORDING TO THE INTERNATIONAL STANDARDS

Criterion	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil	Refined olive oil	Olive oil
PER. VALUE	≤ 20	≤ 20	≤ 20	-	≤ 5	≤ 15
Moist. and v.m.	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,3	≤ 0,1	≤ 0,1
Insob. Impur.	≤ 0,1	≤ 0,1	≤ 0,1	≤ 0,2	≤ 0,05	≤ 0,05
Trace metals Iron Copper	 ≤ 3,0 ≤ 0,1	 ≤ 3,0 ≤ 0,1	 ≤ 3,0 ≤ 0,1	 ≤ 3,0 ≤ 0,1	 ≤ 3,0 ≤ 0,1	 ≤ 3,0 ≤ 0,1

Quality criteria

Each of the quality criteria evaluates a different part of oil quality.

So, all quality criteria are necessary for the correct evaluation of the quality of olive oils.

Purity criteria

A. Detection of olive oils adulteration with other vegetable oils

B. Detection of olive oils adulteration with olive-pomace oils.

C. Detection of virgin olive oils adulteration with refined oils.

Purity criteria
Detection of other vegetable oils

1. Unsaponifiable matter
2. Sterols (total and individual)
3. Fatty acids
4. Trans fatty acids
5. ΔECN42
6. Saturated fatty acids at the 2-position in the triglycerides.

Purity criteria
Detection of other vegetable oils

A.1. Unsaponifiable matter

It gives the total amount of the most important nonglycerides constituents (sterols, aliphatic alcohols, pigments, hydrocarbons).

It is generally low in natural fats and lies between 0,3-1,5%.

Method : **ISO 3596**

Limits : for olive oils ≤ 15 g/Kg

Purity criteria
Detection of other vegetable oils
A.2. Sterols (total and individual)

Sterols make up sizeable part of the unsaponifiable matter.

In olive oils the most abundant sterol is b- sitosterol (75-90%) followed by d5-avenasterol, campesterol, stigmasterol.

Determination of a vegetable oil's sterolic composition is an important criterion for identifying its botanical origin.

The use of individual sterols (brassicasterol, campesterol, stigmasterol, apparent b-sitosterol and Δ7-stigmasterol) combined with the total sterols are very effective in the detection of olive oil adulteration with other vegetable oils.

Purity criteria
Detection of other vegetable oils
A.2. Sterols (total and individual)

Method :COI/T.20/Doc. no. 10 or ISO 12228 or AOCS Ch 6-91

Limits for olive oils:

Cholesterol ≤ 0,5 % of total sterols

Brassicasterol ≤ 0,1 % of total sterols

Campesterol ≤ 4,0 % of total sterols

Stigmasterol ≤ campesterol in edible oils

Apparent b-sitosterol ≥93,0 % of total sterols

Δ7-stigmasterol ≤ 0,5 % of total sterols

Total sterols ≥ 1000 mg/Kg

Purity criteria
Detection of other vegetable oils
A.2. Sterols (total and individual)

Kind of oil	brassic asterol	campest erol	Apparent b-sito	Δ7-stigmas terol	Total sterols
Rapeseed	8,8-13,5	30-37	49-54	<0,2	3300-8000
Soyabean	<0,2	17,5-24,6	50-65	<4	2500-4500
Corn	<0,1	18,0-21,5	64-73	<0,6	7000-11000
Sunflower		8,0-10,0	58-68	10-18	2500-4000
Cotton		7,0-8,0	80-85	0,2-0,3	3800
Peanut	<0,1	12,9-16	80	0,2-0,3	1000-1900

Purity criteria
Detection of other vegetable oils
A.2. Sterols (total and individual)

Kind of oil	brassicasterol	campesterol	Apparent b-sito	Δ7-stigmasterol	Total sterols
Mustard	6,5	27,9	64	0,20	8090
Sesame	<0,1	16-20	69	0,3-0,5	3300-5900
Safflower	<0,1	13,8-15,1	52	4,0-18	3400-4100
Hazelnut	<0,15	3,8-5,6	87-94	0,9-3,7	1000-2000
Almond		2,8-4,0	93	0,1-0,7	1900-2700

Purity criteria
Detection of other vegetable oils
A.3. Fatty acids (a)

The fatty acids which exhibit the higher values in olive oils in descending order are : oleic, linoleic, palmitic, stearic and palmitoleic.

The most important fatty acids for the detection of olive oils adulteration with other vegetable oils are linolenic, myristic, arachidic, gadoleic, behenic and lignoceric.

Nowadays, the production of vegetable oils with high oleic acid (sunflower, safflower, rapeseed) decreased the importance of fatty acids determination.

Purity criteria
Detection of other vegetable oils
A.3. Fatty acids (b)

Method :

COI/T.20/Doc. no. 24 : Preparation of the fatty acids methyl esters from olive oil and olive-pomace oil.

Method A. Methylation with cold methanolic solution of potassium hydroxide

Method B. Methylation by heating with sodium methylate in methanol followed by heating in acidic medium.

ISO 5508 : Analysis by gas chromatography of methyl esters of fatty acids

Purity criteria
Detection of other vegetable oils
A.3. Fatty acids (c)

Category of oil	Method A	Method B
Virgin olive oil with acidity <3,3 %, Refined olive oil Olive oil Refined olive pomace oil Olive pomace oil	⇒ *Fatty acids* ⇒ *trans fatty acids* ⇒ *ΔECN42 (after passage through silica-gel column)*	
Virgin olive oil with acidity >3,3 %, Crude olive pomace oil	*1. trans λιπαρά οξέα* *2. ΔECN42 (after passage through silica-gel column*	*fatty acids*

Purity criteria
Detection of other vegetable oils
A.3. Fatty acids (d)

Limits for olive oils (% m/m methyl esters):

Myristic acid ≤ **0,5 %**

Linoleic acid ≤ **1,0 %**

Arachidic acid ≤ **0,6 %**

Gadoleic acid ≤ **0,4 %**

Behenic acid ≤ **0,2 %**

Lignoceric acid ≤ **0,2 %**

Purity criteria
Detection of other vegetable oils
A.3. Fatty acids (e)

Kind of oil	Linoleic C18:2	Linolenic C18:3	Arachidic C20:0	Gadoleic C20:1	Behenic C22:0	Erucic C22:1	Lignoceric C24:0
rapeseed	16-24	7-11	0,4-0,7	1,9-4,5	< 0,5	< 5	<0,5
soya	48-58	5-9	<1	< 0,5			
peanut	15-48	<0,3	1-2,5	0,9-1,5	2-4,5		1-2,5
mustard	17,2	8-15	1,5	8,8	1,2	37	0,6
corn	41-63	0,6-1,1	0,2-0,7	0,2-0,5	< 0,2		
cotton	55	0,2	0,2	0,1	0,2		
sunflower	40-74	< 0,3	< 0,5	< 0,5	0,5-1		
hazelnut	6-14	< 0,6	< 0,3	< 0,3	< 0,2		

Purity criteria
Detection of other vegetable oils
A.4. Trans fatty acids (a)

The alteration from cis to trans form takes place during the refining.

Trans unsaturated fatty acids may be present at specific concentrations in natural vegetable oils and in such oils that have undergone refining.

So, the determination of trans fatty acids is a purity criterion of olive oil used for the detection of refining oils or for the detection of other oils.

Method : COI/T.20/Doc. No. 17

Purity criteria
Detection of other vegetable oils
A.4. Trans fatty acids (b)

Limits

Caterogory of oil	Trans C18:1 %	Sum trans C18:2 + C18:3 %
Edible virgin olive oil	max 0,05	max 0,05
Lampante virgin olive oil	max 0,10	max 0,10
Refined olive oil	max 0,20	max 0,30
Olive oil	max 0,20	max 0,30

Purity criteria
Detection of other vegetable oils
A.5. ΔECN42 (a)

The method of **ΔECN42** is applicable to the detection of the presence of small amounts of seed oils (rich in linoleic acid) in every category of olive oils.

It is based in the 1,3 random distribution followed in the biosynthesis of triglycerides in olive oil.

A difference larger than the values adopted for each category of olive oil points out that the oil contains seed oils.

Purity criteria
Detection of other vegetable oils
A.5. ΔECN42 (b)

Method : COI/T.20/Doc. No. 20 or AOCS 5b-89:

This method includes three phases :

- determination of fatty acids composition by capillary gas chromatography
- calculation of theoretical composition of triacylglycerols with ECN42
- HPLC determination of triacylglycerols with ECN42

Purity criteria
Detection of other vegetable oils
A.5. ΔECN42 (c)

Limits :

Caterogory of oil	ΔECN42
Edible virgin olive oil	max 0,2
Lampante virgin olive oil	max 0,3
Refined olive oil	max 0,3
Olive oil	max 0,3

Purity criteria
Detection of other vegetable oils

A.6. Saturated fatty acids at the 2-position in the triglycerides.

This method is suitable for the detection of esterified oils.

It is based on the fact that small amounts of saturated fatty acids are found in the two position of triglycerides in olive oils in comparison to the esterified oils.

Method : ISO 6800 or AOCS Ch 3-91 :

Another proposed method : determination of the percentage of palmitic acid at the 2-position of triglycerides.

Purity criteria
Detection of other vegetable oils
A.6. Saturated fatty acids at the 2-position in the triglycerides.

Limits:

Caterogory of oil	Palmitic + stearic acid in the 2-position
Virgin olive oil	max 1,5
Refined olive oil	max 1,8
Olive oil	max 1,8

Purity criteria
Detection of olive pomace oils

1. Erythrodiol and uvaol
2. Aliphatic alcohols
3. Waxes

The compounds erythrodiol+uvaol, aliphatic alcohols and waxes are present in small quantities in pressed oils whereas these compounds exhibit very high values in extracted oils.

Purity criteria
Detection of olive pomace oils
B.1. Erythrodiol and uvaol

Erythrodiol and uvaol are the two main triterpenic dialcohols of olive oil.

The major component of this group is erythrodiol, also called homo-olestranol, which is the glycol derived from oleanolic acid by reducing the carboxyl group to alcohol.

Method : IUPAC no. 2.431

Purity criteria
Detection of olive pomace oils
B.2. Aliphatic alcohols

Total aliphatic alcohols are components of the unsaponifiable matter and they do not usually exceed 350ppm in pressed olive oils.

Dry climatic conditions and high temperatures may cause high alcanol content of olive oil.

This method has been deleted from the international standards because certain olive oil producing countries exhibit high alcanol content.

Since 2002, this method is only applicable to the category lampante virgin olive oil.

Method : COI/T.20/Doc. no. 26

Purity criteria
Detection of olive pomace oils
B.3. Waxes

Waxes are esters of fatty alcohols with fatty acids.

The determination of waxes has substituted the determination of aliphatic alcohols, because of the high alcanols content of certain countries.

Method: COI/T.20/Doc. no. 18

Deviations from the established limits of waxes:

Refined olive oils and lampante virgin olive oils after lengthily storage.

Proposals : absolute erythrodiol and alcoholic index.

Purity criteria
Detection of olive pomace oils

The parameter alcoholic index is calculated by the components present in the alcoholic fraction isolated from olive oil.

$$\text{alcoholic index} = \frac{\text{C22 * total alcanols}}{\text{Cx * (cycloartenol + 24-methylocycloartenol)}}$$

Where : Cx = geranylgeraniol (acyclic diterpenoid)

Purity criteria
Detection of olive pomace oils

Statistical data on Greek edible virgin olive oils (n=600)

	mean	min	max	range	median
Cx (ppm)	23,61	7,30	102,30	95,00	22,85
C22 (ppm)	43,66	4,10	160,40	156,30	41,00
Total alcanols (ppm)	223,6	61,2	593,0	531,8	213,0
Cycloart.+24-methyl-cycl. (ppm)	861,2	154,6	3591,8	3437,2	711,0
Alcoholic index	0,71	0,03	3,84	3,81	0,57

Purity criteria
Detection of olive pomace oils

Limits:

Caterogory of oil	Erythrodiol + uvaol	Waxes mg/Kg	Alcanols mg/Kg
Edible virgin olive oil	≤ 4,5	≤ 250	-
Lampante virgin olive oil	≤ 4,5	≤ 300	When 300 < Waxes ≤ 350 then alcanols ≤ 350mg/Kg or E+U ≤ 3,5%.
Refined olive oil	≤ 4,5	≤ 350	-
Olive oil	≤ 4,5	≤ 350	-

Purity criteria
Adoption of aliphatic alcohols for the category lampante

Lampante virgin olive oil is protected by the addition of second centrifugation oil.

The authentic virgin olive oils, which exhibit high alcanols content, are classified in their real category.

Purity criteria
Second centrifugation oil

Second centrifugation oil or repasso is the oil obtained by centrifuging the olive paste from twin phases decanters.

This oil is of bad quality virgin olive oil and it could be classified as:

- ✓ Lampante olive oil, in case that the olive paste is centrifuged just after the first centrifugation.
- ✓ Olive pomace oil, in case that the olive paste is centrifuged after a long time.

Purity criteria
Identification of second centrifugation oil

The risk of adulteration with repasso oil is more evident in the category lampante virgin olive oil.

Lampante virgin olive oils could be classified in two types :

- One with low erythrodiol and high alcanols content (Greek and Italian oils), and
- the other with high erythrodiol and low alcanols content (Spanish oils)

Consequently, the adopted proposal is the most suitable solution for the identification of second centrifugation oil.

Purity criteria
Identification of second centrifugation oil

The adopted proposal is strict, because oils which exhibit

300 mg/Kg ≤ waxes ≤350 mg/Kg,

3,5 %≤E+U ≤4,5 % and

alcanols >350 mg/Kg

are classified in the category crude olive pomace oil.

However, if lampante virgin olive oils are produced applying good manufacturing practices, these oils will be classified as virgin as they are.

Purity criteria
Detection of refined oils

1. Absorbency in ultraviolet
2. Trans fatty acids
3. Stigmastadiene content

Purity criteria
Detection of refined oils

C.1. Absorbency in ultraviolet

- ✓ When the absorbency at 270nm is caused by conjugated trienes, then it could be used as purity criterion.
- ✓ Similarly, the index ΔK could be used for the detection of refined oils.

C.2. Trans fatty acids

The determination of trans fatty acids is effective in the detection of the presence of oils that have undergone refining in virgin olive oils.

Purity criteria
Detection of refined oils
C.3. Stigmastadiene content

The hydrocarbon stigmastadiene is formed by dehydration of sterols (especially b-sitosterol) during refining.

So, this method is particularly suited to detect the presence of refined oils in virgin olive oils.

Method : COI/T.20/Doc. no. 11

Purity criteria
Detection of refined oils
C.3. Stigmastadiene content

Limits :

Caterogory of oil	Stigmastadiene content mg/Kg
Edible virgin olive oil	≤ 0,15
Lampante virgin olive oil	≤ 0,50

Detection of adulteration of olive oil with the most common vegetable oils

Kind of vegetable oil	Used parameter for the detection of adulteration	% of detectable vegetable oil
Sunflower	ΔECN42	≅ 1%
	Appar. b-sitosterol	≅ 4%
	Δ7-stigmastenol	≅ 2%
Soyabean	Linolenic	≅ 2%
	ΔECN42	≅ 1%
	campesterol	≅ 6%
	Appar. b-sitosterol	≅ 6%
Cotton	ΔECN42	≅ 1%
Corn	ΔECN42	≅ 1%
	Campesterol	≅ 4%
Walnut	Linolenic acid	≅ 1%
	ΔECN42	≅ 1%
Sesame	ΔECN42	≅ 1,5%
	Campesterol	≅ 8%
	Appar. b-sitosterol	≅ 8%

Detection of adulteration of olive oil with the most common vegetable oils

Kind of vegetable oil	Used parameter for the detection of adulteration	% of detectable vegetable oil
Safflower	ΔECN42	≅ 1%
	Appar. b-sitosterol	≅ 3,5%
	Δ7-stigmastenol	≅ 2,5%
Rapeseed	Linolenic acid	≅ 2%
	Erucic acid	≅ 1%
	Campesterol	≅ 5%
	Brassicasterol	≅ 1%
	ΔECN42	≅ 4 %
Peanut	Behenic acid	≅ 3%
Mustard	Linolenic acid	≅ 1 %
	Erucic acid	≅ 1 %
	Campesterol	≅ 3 %
Hazelnut	None	
Almond	None	

Purity criteria
Detection of hazelnut oil

No one from the official parameters can detect the presence of percentages lower or equal to 5% of hazelnut oil in olive oil.

Since the characteristics of hazelnut oil are very close to those of virgin olive oil, it is very difficult to find a parameter able to detect this type of oil.

Purity criteria
Detection of hazelnut oil

Methods proposed by the IOOC expert chemists

- Global method : it is based on the determinations of fatty acids and triglycerides (propionitrile method). The data from these determinations are processed by a special computer program and the final conclusion is genuine or not genuine.
- Exempla method : it is based on the determinations of fatty acids and triglycerides. The data from these determinations are processed by a different from the above special computer program and the final conclusion is genuine or not genuine.
- Free and esterified sterols method : This method is based on the determinations of free and esterified sterols and using a formula, we conclude to the characterisation of a sample as genuine or not genuine.

Purity criteria
Detection of hazelnut oil

Comments on the three methods

The methods result in the identification and not in the quantification of the hazelnut oil.

The methods do not produce repeatable results because the final characterization depends on parameters calculated by formulas.

A decision concerning the effectiveness of the methods, presupposes :

- **work on the methods**, in order to achieve repeatable results.
- **data on samples of virgin olive oil from all olive oil producing countries.**

Purity criteria
Detection of hazelnut oil

FEDELI, CORTESI, ROVELLINI METHOD FOR THE DETECTION OF OF EXTRANEOUS OILS IN OLIVE OILS (EXEMPLA METHOD)

Modifications of the method

		conditions	conclusion	application of the method on Greek virgin olive oils: false negative results	no of exam. samples	% of false nagativeresults
1st version	1	IF THEOR ECN42<HPLC ECN42	NG	37	150	25
	2	IF THEOR ECN42<0,90 and L2ECN42<18 genuine	G			
	3	IF THEOR ECN42<0,90 and L2ECN42>18 and RL2>0,90 genuine	G			
	4	IF THEOR ECN42<0,90 and L2ECN42>18 and RL2<0,90	NG			
2nd version	1	if O/L <=5 and R<=0,95	G	33	150	22
	2	IF 5< O/L <=7 and R<=1	G			
	3	IF O/L >7 and R<=1,05	G			
3rd version	1	if O/L <=5 and R<=0,95	G	3	150	2
	2	IF 5< O/L <=15 and R<=1,05	G			
	3	IF O/L >15 and R<=1,10	G			

Purity criteria
Detection of hazelnut oil

METHODS FOR THE DETECTION OF EXTRANEOUS OILS IN OLIVE OILS

TABLE 1. Application of the GLOBAL and EXEMPLA methods on Greek virgin olive oils.

A/A	AREA	number of examined samples	FINAL RESULTS (EXEMPLA)		FINAL RESULTS (GLOBAL)	
			GENUINE	NOT GENUINE	GENUINE	NOT GENUINE
1	PELOPONISSOS	47	3	1	3	1
2	STEREA	19	5		5	1
3	THESSALIA	8	4		4	1
4	IPIROS	10	8		7	
5	MAKEDONIA	4	10		11	
6	EPTANISSA	4	16		16	1
7	KRETA	38	4	2	4	
8	DODEKANISSA	15	15		14	
9	THRAKI	5	8		7	
	SUM	**150**	**73**	**3**	**71**	**4**

Conclusion

The methods included in the International Standards for controlling olive oil authenticity are numerous, costly and time-consuming.

Every now and again, a new method is being added in the list of the already existing methods for the detection of fraud.

However, all the methods are essential for the protection of olive oil and sufficient information for the quality and the nature of an olive oil is obtained only in case that all quality and purity criteria are taken into account.