Chemical-physical characteristics of olive oils

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Introduction

Before beginning to examine the chemical-physical characteristics of the olive oils which are the object of EC Regulations, it may be desirable to explain the meaning of some of the terms which will appear later and to give a brief outline of the principal constituents of olive oil. Table 1 summarizes some of the limits of the characteristics of olive oils established by the EC.

However, these limits are subject to variations and additions published in the Official Journal of the European Communities. The detailed description of the analytical methods and of the legal limits is contained in the text of the EEC Regulation n. 2568/91 dated 11-7-91, L248 and in its successive modifications. The new definition of the various Categories of olive oils is to be found in the EC Regulation n. 1513/01 dated 23-7-01, L201 and is illustrated in Table 2.

ELIDINA O S 4 EL SCIENCIEL	Olive oil characteristics Type	Acidity (%)		K232	w	K270 ith Alumir Q	na
1	Extra virgin olive oil	≤0,8	≤20	≤2,50	≤0,20	≤0,10	≤0,01
2	Virgin olive oil	≤2,0	≤20	≤2,60	≤0,25	≤0,10	≤0,01
3	Lampante olive oil	>2,0	>20	≤3,70	>0,25	≤0,11	
4	Refined olive oil	≤0,5	≤5	≤3,40	≤1,20		≤0,16
5	Olive oil	≤1,5	≤15	≤3,30	≤1,00		≤0,13
6	Crude olive residue oil	>0,5					
7	Refined olive residue oil	≤0,5	≤5	≤5,50	≤2,50		≤0,25
8	Olive residue oil	≤1,5	≤15	≤5,30	≤2,00		≤0,20
		Per (r	oxide va nEqQ2/kq	lue j)	6 K270		o Delta-K

Tab.2

VIRGIN OLIVE OILS	Oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil, which have not undergone any treatment other than washing, decantation, centrifugation or filtration, to the exclusion of oils obtained using solvents or using adjuvants			
	naving a chemical or biochemical action, or by re- esterification process and any mixture with oils of other kinds.			
a) Extra virgin olive oil	Virgin olive oil having a maximum free acidity, in terms of oleic acid, of 0,8 g per 100 g, the other characteristics of which comply with those laid down for this category.			
b) Virgin olive oil	Virgin olive oil having a maximum free acidity, in terms of oleic acid, of 2 g per 100 g, the other characteristics of which comply with those laid down for this category.			
d) Lampante olive oil	Virgin olive oil having a free acidity, in terms of oleic acid, of more than 2 g per 100 g, and/or the other characteristics of which comply with those laid down for this acterized			
	this category.			

Fatty acids

These are molecules which carbon atoms normally numbering between 4 and 30 (Fig. 1).





These carbon atoms are linked in a kind of chain. When two carbon atoms are linked together with two links, a <u>double bond</u> is said to be present (the yellow ring in Fig. 1). If there are no double bonds, the acid is said to be saturated (e.g. palmitic acid, stearic acid). If a double bond is present, it is said to be monounsaturated (e.g. oleic acid); if there are two or more double bonds, the fatty acid is said to be polyunsaturated (e.g. linoleic acid, linolenic acid). In oils, fatty acids are mainly combined in the triglycerides (see Glycerides), whereas the free ones confer acidity. If two double bonds are separated by only one simple bond, there are two double conjugated bonds (conjugated diene).

If three double bonds alternated with simple bonds are present, there are three double conjugated bonds (conjugated triene). Symbols such as C18:3 refer to a fatty acid with 18 carbon atoms and 3 double bonds.

Glycerides

These are formed from the combination of a glycerol molecule with one of a fatty acid (a monoglyceride) or with two (a diglyceride) or with three (a triglyceride, Fig. 2).





Figure 2

In natural fats, the usual form is that of the triglyceride. The fatty acids present in the same molecule of di- or triglyceride may show differences. The three carbon atoms of the glyceride are numbered 1,2,3 with n. 2 being the central carbon atom. In good quality oils of recent production small quantities of 1,2 diglycerides are present; these are partly intermediates in the biosynthesis of the triglycerides. As time passes, the 1,2 diglicerides tend to change into 1,3 diglicerides. Thus, an increasing content of the latter is an indication of old and/or badly preserved oils (e.g. exposed to light, heat or in unsuitable receptacles etc.).Usually the ratio 1,2-/1,3-diglycerides is considered, in good quality oils of recent production, to be greater than 1. The ratio descends with the ageing of the oil. As lipolysis proceeds, free acidity must increase. Consequently oils with low acidity but with a ratio of 1,2-/1,3-diglycerides less than 1, a fraudulent deacidification may be suspected.

Sterols

These are complex compounds which carry out biochemical functions within cellular membranes. Their composition is typical of the botanic species from which the oil originates. As we shall explain further on, this specific quality can be used to test the purity of the oils in question.

Erythrodiol and Uvaol

These are two compounds (triterpenic dialcohols) naturally present also in pressed olive oils (Fig. 3). They are to be found in the greatest concentration in the skins and kernels of the drupe. In residue oils their concentration is normally higher because of the drastic extractive action carried out by the solvent. This fact can be used to look for residue oil in olive oils obtained by pressing.



Uvaol

Figure 3

Wax esters

The wax ester of greatest analytical interest are esters made up of a combination of one aliphatic alcohol molecule with one of a fatty acid. Waxes may be found on the epicarp (skin) of the drupe and carry out various functions, among which is that of reducing the loss of water by evaporation from the underlying tissues, the partial waterproofing of the fruit and also that of repelling some insect parasites. Wax esteres also prevent pathogenous micro-organisms from developing and penetrating into the fruit.

Phenolic compounds

These compounds present in olive oil in appreciable quantities (50 - 500 mg/Kg) have an important role in the stability of the oil to oxydation. *Moreover some of them confer the bitter-pungent organolectic note*. The process of refining removes them almost totally. Phenolic composition varies from one oil to another and varies in the course of time according to the conditions of preservation (fig.5).

ORGANIZZAZIONE NAZIONALE ASSAGGIATORI OLIO DI OLIVA										
	Tyrosol;	2-(4-hydroxyphenyl)-ethanol	CsH10O2	CH2-CH2-OH OH						
	Hydroxytyrosol;	2-(3,4-dihydroxyphenyl)-ethanol	CsH10O3	CH2-CH2-OH OH						
	Caffeic acid;	3,4-dihydroxy-cinnamic acid	C9H8O4	СН-СН-СООН ОН						
	Ferulic acid;	4-hydroxy-3-methoxycinnamic acid	C10H10O4	CH=CH-COOH						

Figure 5

Aroma components

These are substances (over 150) belonging to 9 groups of chemical compounds (aldehydes, ketones, esters etc). Although their concentration is relatively low, *it is to these compounds that we owe the principal characteristics of the aroma and flavour* by which different oils are distinguished (Fig 6).



Figure 6

Tocopherols

These compounds (Fig.7) are present in olive oil in concentrations of about 150 - 250mg/Kg, Normally present are the forms α , β , γ and δ of which α (vitamin E) is the most abundant (90 – 95%). Tocopherols carry out an *anti-oxydant action* in oils exposed to "light" (especially ultraviolet radiation).



Figure 7

Hydrocarbons

These are composed exclusively of carbon and hydrogen. Amongst the most important hydrocarbons present in olive oil is *squalene* (C30), which constitutes an important intermediary for the biological synthesis of sterols, and is present in concentrations of about 1500 – 2000 mg/Kg; β -carotene (C40), precursor of vitamin A, which gives the oil its yellow-orange colour (Fig. 8). Its concentration is about 300 – 400 mg/Kg. Also present are small quantities of Polycyclic Aromatic Hydrocarbons (PAH) which may be contaminating, but some of them could perhaps be natural metabolites (Fig. 9).



Xenobiotics

It is considered that these substances should not be found in oils. Among these are the *aromatic hydrocarbons* (benzene, toluene, xylene etc) which may result from environmental contamination like the *Polycyclic Aromatic Hydrocarbons (PAH)* but may be residues of extractions with solvents; *halogenated solvents*, which will be mentioned later; *pesticides*, used for example to combat the olive fly (*Bactrocera oleae Gmel.*), which refining processes remove almost completely. It is for this reason that extra virgin oils may contain a greater quantity of these than olive oils since the law permits certain processes only (see EC Regulation 1638/98, 20-07-98. L210), which, however, do not remove the content.

Unsaponifiable matter

It is well-known that if a fatty substance is heat-treated with an alkaline solution (e.g. caustic soda), soap is formed. After such treatment, all that can be extracted with a suitable solvent (e.g. diethyl ether) is called unsaponifiable. It contains sterols, alkanols, tocopherols, hydrocarbons etc. In olive oil this constitutes about 1 - 1.5% in weight. As we will see later, it contains compounds of great interest for the ascertainment of the purity of an olive oil.

Chemical analysis

In this paragraph we will give only a brief account of the most common analysis techniques in the field of oil. They may or may not be instrumental. Amongst the not instrumental techniques there is titration, of which the best-known example is the determination of acidity. Instrumental determinations have been used on oils more and more since the 1960s. Gaschromatography (GC) was the first to find an application. It can be schematized as follows (Fig. 10):



Figure 10

A device (injector) vaporizes a small amount of the solution (a few microlitres) containing the substance under analysis. The solution is introduced into the instrument by means of a special syringe. Due to the flow of a suitable gas (carrier gas: nitrogen, helium, hydrogen etc.) these vapours are conveyed into a spiral tube (column) which has, on its internal surface, a fine layer of a special liquid which is very viscous and has a high boiling point (stationary phase). The length of the gaschromatographic column may vary from only a few decimeters to over 100 m and have a diameter varying from a few tenths to a few mm. The chamber in

which the column sits, is kept at relatively high temperatures which, in the case of oil analysis, are generally between 150 and 350°C. In the stationary phase the various substances contained in the injected solution and which are to be separated, are retained with greater or lesser efficiency. Amongst them, those which are retained less run through the column, carried by the gas, in less time than the others. On exit, and with each substance separate, they pass through a device (detector) which recognises them. The detector generates an electric signal which is recorded on a time scale. The intensity of the electric signal varies in relation to the quantity of substance which generates it. The trace obtained is called gaschromatogram.

Another instrumental technique of more recent application in the oil sector is the <u>High Pressure Liquid Chromatography (HPLC)</u> (Fig. 11). The general principles are the same as those for GC. The main differences consist in the fact that instead of using a gas as a carrier, a liquid is used (mobile phase) which is pushed through the analytical column by a pump which can reach pressures of over 400atm. The column is much shorter and stronger than that for GC. This technique is more suitable for substances which are difficult to vapourize, mainly because of their high boiling point.





Finally, another technique of which we would like to remind our readers is Spectrophotometry (Figs 12) which consists of measuring the differences in intensity of the electromagnetic radiation before and after going through the sample under examination. If the sample contains substances able to absorb this radiation, the variation in intensity will be greater as their concentration increases. In the most common case of oils, the radiation is ultraviolet and the presence of conjugated dienes and trienes (see Fatty acids paragraph) increases the absorbences in proportion to their concentration.







The aims of the EC Regulations

Before studying the aims of the EC Regulations, it may be useful to consider the meaning of the words **Quality** and **Purity**.

The intended meaning of <u>**Quality**</u> is "the totality of requisites and functions of the aliment which can satisfy the consumer's needs". Hereunder we can place sensory characteristics, stability to oxydation, absence of xenobiotics, nutritional values (e.g. essential fatty acids, relationship between saturated fatty acids, mono- and polyunsaturated fatty acids etc.), natural antioxidants etc.

The <u>**Purity**</u> of an aliment refers to the fact that "it has not been subjected to technologies different from those traditionally used, nor has any substance extraneous to its nature been added".

It is evident that a large part of the aims of the EC Regulations is dedicated to ascertaining the purity of olive oils which, due to their high cost, are the object of illegal practices. Finally we feel it necessary to emphasize that, as demonstrated by the number of tests necessary, one single analysis is not yet available which can establish unequivocably the purity of an oil.

Tests on quality

Acidity

The acidity expresses the percentage content (in weight) of the free fatty acids in the oil under examination. Free fatty acids are normally present also in oils obtained from sound olives: when the triglycerides are formed, there is a progressive increase in acidity due to the action of enzymes (lipase) naturally present in the fruit, which help the fatty acids to detach from the molecule of triglyceride (lipolisis). The same lipolitic phenomenon can be caused by enzymes produced by micro-organisms which grow on the fruit. Thus, in order to obtain a product which is organoleptically better and which has lower acidity, it is necessary to preserve the olives well in the store.

Acidity is determined by means of titration:

To a known quantity of oil a suitable solvent is added along with a substance which in the example is phenolphtalein (indicator). Measured volumes of a potash solution, at a known concentration, are added. The indicator turns red as soon as all the free fatty acids have reacted with the potash. When this happens, the amount of potash used is measured and acidity may be calculated simply.

The peroxide value

Peroxides (Fig. 13) are formed because of the oxygen dissolved in the oil and other factors present at the same time like pigments (chlorophyll and pheophytin) or metals which catalyze their formation. In particular, two types of oxydation can be distinguished: *auto-oxydation* and *photo-oxydation*. In both cases, at a certain point in the succession of reactions which are activated, a free radical is formed from an unsaturated fatty acid which reacts with an oxygen molecule and gives rise to a peroxidic radical. This reacts with another molecule of fatty acid and eventually forms a hydroperoxide (auto-oxydation). In the case of photo-oxydation, ultraviolet radiation activates a molecule of pigment (e.g. chlorophyll) which initiates the process of oxydation by using oxygen.



Metals also favour oxydation (auto-oxydation), but without the contribution of ultraviolet radiation. The presence of substances like phenols, tocopherols, β -carotene etc. oppose the spread of oxydation.



Figure 13

The content of peroxides in the oil under examination is expressed by the peroxide value. The higher the number, the greater is the degradation due to oxydation of the oil. In their turn the peroxides are subject to further oxydation which gives rise to the formation of other compounds which are determinable in different ways (aldehydes, ketones etc.) These compounds, called compounds of secondary oxydation, are responsible for making the oil rancid. Because of oxydation and due to the enzymes present in the tissue of the fruit (lipoxygenases), a certain concentration of peroxides is already present in the fruit before pressing. Particular natural circumstances (e.g. temperatures below freezing, dacic infestations, drought etc.), or olives incorrectly harvested and preserved may encourage a further formation of peroxides. Even during milling peroxides can increase greatly through bad processing or due to incorrect hygiene in the olive-press and/or of the vessels. Finally, prolonged exposure of the oil to light or heat sources is another cause of the increase of peroxides. They are determined through titration.

Spectrophotometric investigation in ultraviolet.

This test consists of measuring three parameters (K232, K270, Δ K) determined during the same analytic procedure. The greater the value of K232, the greater the concentration of conjugated dienes, whereas K270 is proportional to the concentration of conjugated trienes. However, compounds of oxydation of the

conjugated dienes contribute to K232 while compounds of secondary oxydation (aldehydes, ketones etc.) contribute to K270.



Figure 14

It is for this reason that if the value of K270 exceeds the limit of the category to which the oil is believed to belong, EC regulations provide for a particular pretreatment of the sample (with alumina) before a second spectrophotometric test. If the new value exceeds that limit, the oil must be declared not pure. The ΔK results from a calculation which we will omit. Its geometric meaning is more clearly shown in Fig. 14. Since the process of refinement favours an increase of it, it was in the past considered to be a parameter by which mixtures of processed oils and virgin oils could be revealed. Nowadays more suitable examinations exist and it is known that the "ageing" of the oil, with its phenomena of oxydation, increases the value of the spectrophotometric indices. For this reason we prefer to include this kind of determination among those of quality.

Sensory evaluation of virgin olive oil (Panel test)

Please see the relative notes.

Tests on purity

Determination of the composition and content of sterols

Sterols are compounds which are normally found in oils and natural fats, in concentrations and compositions which vary depending on the origin of the fatty matter. Olive oil has quantities of around 1200 - 1800 mg/Kg (= *sterol content*). Vegetable oils contain roughly the same type of sterols but in different relationships and the characteristics for each are different (*=sterol composition*). This specificity should allow the mixture of olive oils with foreign oils to be recognized. In fact, the addition of appreciable quantities of foreign oil to an olive oil will alter its natural sterolic composition. This makes it possible to recognize fraud. However, the careful choice of foreign oils and the amount used may make it difficult to discover fraud. This analysis is carried out by means of gaschromatography (Fig. 15).



Figure 15

Determination of erythrodiol and uvaol

High values of these two substances may be an indication of the addition of residue oil to the olive oil. The solvent used for the extraction of residue oils (hexane) also dissolves these compounds, which are more abundant in the skins and kernels than in the pulp. However, the so-called "green" oils are high in erythrodiol and uvaol content, even though they have not been obtained through the use of solvents. This fact is due to the repressing of the pastes from the first pressing ("remilling") and to the great pressure to which they are subjected or to new centrifugation. They have a deep green colour from which they get their name. This analysis is carried out through gaschromatography, usually contextually to sterols (Fig. 15).

Determination of saturated fatty acids in position 2 in the triglyceride.

This analysis allows us to have an indication of the presence of any esterified oils in pressed oils. The saturated fatty acids present in the molecule of the triglyceride of an olive oil are linked, for the greater part, to the glicerol in positions 1 and 3, while position 2 is preferably occupied by unsaturated fatty acids. This "uneven" distribution in the three positions depends on the particular pathway of the biosynthesis of the triglycerides during the oiling of the fruit. On the other hand, the industrial process of chemical synthesis of the triglyceride (esterification) does not discriminate between saturated and unsaturated fatty acids (i.e., it "has no preferences"): this means that the quantity of saturates in position 2 will be greater than that present in a natural oil. This analysis determines this content by means of the use of enzymes (lipase) commonly in commerce.

In suitable environmental conditions, lipase reacts with the molecules of triglyceride, causing the separation of the fatty acids from positions 1 and 3 (Fig. 16). The resulting glycerides (2-monoglycerides) have therefore only one remaining fatty acid in position 2. The 2-monoglycerides are then analyzed to find out the percentage of saturated fatty acids present. This percentage is precisely what we wish to know. This analysis is carried out by means of gaschromatography.



Figure 16

Determination of the difference: ECN42 (HPLC) and ECN42 (theoretic calculation)

The aim of this determination is to ascertain the presence of seed oils added to olive oil. Since it is known the rule according to the fatty acids are distributed in the three positions of the triglyceride when this is formed due to biochemical synthesis in the olive fruit, it is possible to <u>calculate</u> the composition in triglycerides of the oil under examination by starting from the composition of its fatty acids. This calculation is easily made on a computer and with a suitable calculation programme. The composition in triglycerides must also be determined through analysis (by means of HPLC). A particular group of successive peaks, labelled ECN42, is compared for quantity with the corresponding value determined through calculation. In principle, the two values should be identical. In practice, however, there may be differences which must not exceed the legal limits. In fact, the presence of foreign oils, in particular of seed oil, increases this value greatly, thus revealing fraud.

This analysis is carried out by means of HPLC, as recommended by EC regulations.

Gaschromatographic analysis of methyl esters of fatty acids (and trans isomers)

The aim of this determination is to establish the percentage composition of fatty acids in olive oil, more commonly known as acidic composition. Since, as we know, fatty matters foreign to olive oil have acidic compositions which may be totally different, any mixture may be revealed by this means. In reality this analysis has nowadays lost a great deal of importance even though it was the first gaschromatographic detrmination carried out on olive oils. As we have seen, there are more efficient ways of reaching the same objectives. At present, EC Regulations give limits of concentration only for a few fatty acids which are considered "tracing", that is, typical of oils other than olive. They are miristic (C14:0; coconut oil), linoleic (C18:3; linseed oil), arachidic (C20:0; peanut oil), eicosenoic (C20:1; rapeseed oil), beenic (C22:0; peanut oil), lignoceric (C24:0; peanut oil). EEC Regulation n. 1429/92 dated 26/5/92, L150, introduces limits for trans oleic isomer content and for trans linoleic and trans linolenic (commonly known as trans isomers) (Fig. 17). Illicit industrial procedures which tend to mask a seed oil in order to enable its use in mixtures with olive oil (e.g. de-sterolization, i.e. removal of sterols), cause some modifications in the structure of the fatty acids: in particular, they generate trans isomers.





In olive oil, they are normally present in very low concentrations. Higher levels are an indication of unjustified industrial practices. The determination of trans isomer content is carried out contextually to the acidic composition, in particular analytic conditions (Fig. 18).



Figure 18

Determination of the wax ester content through gaschromatography with capillary column.

The EEC Regulation n. 183/93 dated 29-1-93, L 22, introduces the determination of wax esteres and gives limits for their concentration. Wax esteres are compounds naturally present in olives (these are non gliceridal esters, that is they do not contain glicerol). In particular, they are more abundant on the epicarp of the drupe and, during pressing, some of them are transferred to the oil. The solvent used in the extraction of residue oil also dissolves a certain quantity of wax esteres which, after the removal of the solvent, are abundant in the oil. The aim of this determination is therefore to seek out mixtures of pressed olive oils and residue oils. As wax esters are compounds which contain, combined in the molecule, aliphatic alcohols (alkanols), the latter are present in much greater amounts in residue oils than in pressed oils. Before the introduction of this analysis, the law provided for the determination of the

content of aliphatic alcohols. However, when the method was approved, it was already obsolete, since the means of reducing alkanol content was already known (and perhaps in use) (cold treatment in suitable solvents), thus nullifying the efficacity of the analysis.



Figure 19

Later, the possibility of performing the determination directly on the wax esters with 40,42,44 and 46 carbon atoms was studied. In fact, their content remains more or less constant even after the fraudulent treatments mentioned above (Fig.19).

Determination of the quantity of volatile halogenated solvents in olive oil

solvents The presence of halogenated (e.g. Freon. trichloroethylene, perchloroethylene, chloroform etc.), may derive either from the use of oils extracted with solvents or from environmental contamination. The presence of these compounds even in oils which are certainly pure has been the object of a great deal of research. It was discovered that in olive mills, the use of drinkable water for extractions by pressing, may cause the formation of halogenated compounds (especially chlorides and bromides) which are extremely soluble in oils, and which thus become more concentrated. Moreover, the contamination of the water strata caused by used industrial waters, in addition to the pollution of the atmosphere caused by halogenated solvents (one may think of the quantity of perchloroethylene

used in dry-cleaning) which then penetrate the water strata in rainfall, are factors which influence greatly the levels of concentration of these compounds in oils. This analysis is carried out by gaschromatography.

Determination of stigmastadienes in vegetable oils.

These compounds are formed during refinement from free sterols present in the oil being processed. The EC Regulation n. 656/95 dated 28-3-95, L 69, recommends its determination and sets the limits of its concentration in the various virgin oils. The method, formulated in its first version in 1989, before its insertion among Community Regulations, had a long and painful gestation, with experiments and improvements on the way. This method is more suitable for ascertaining mixtures of processed oils with pressed oils than the spectrophotometric method. Nonetheless it must be remembered that refinement, carried out with suitable precautions, can reduce enormously the presence of these compounds. This analysis is carried out by gaschromatography.

Once again it must be emphasized that until now it has not been possible to reveal the presence of foreign oils by means of one single analysis.