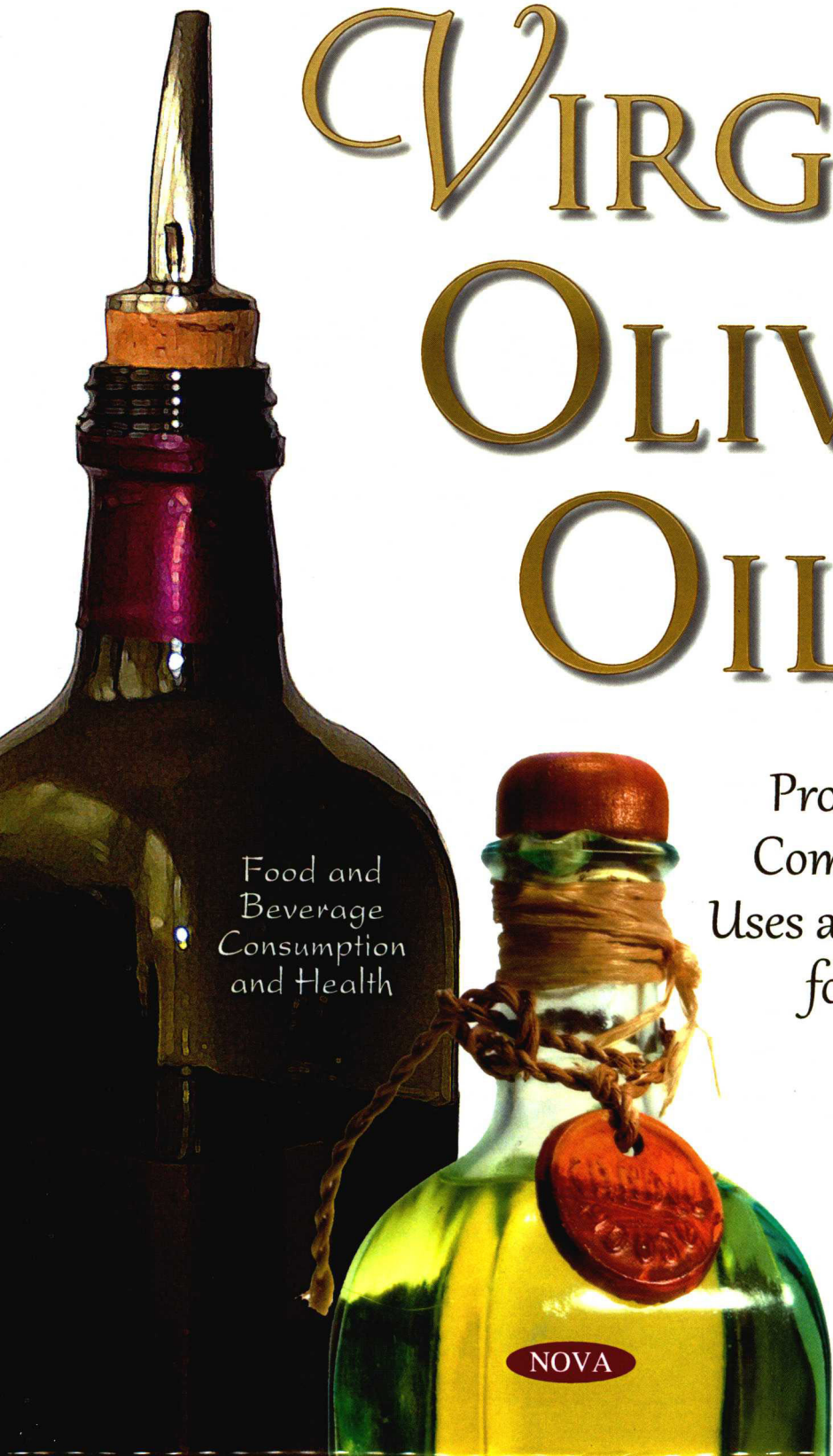
ANTONELLA DE LEONARDIS
EDITOR

VIRGIN OLIVE OIL



Food and Beverage Consumption and Health

Production, Composition, Uses and Benefits for Man

NOVA

FOOD AND BEVERAGE CONSUMPTION AND HEALTH

VIRGIN OLIVE OIL

PRODUCTION, COMPOSITION, USES AND BENEFITS FOR MAN

ANTONELLA DE LEONARDIS
EDITOR

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PREFACE

The population living in the countries bordering the Mediterranean Sea produce and consume olive oil from millennia. Recently, copious scientific literature has provided evidence that a regular consumption of olive oil is associated with longevity, healthier aging, cardiovascular health, prevention and protection against cancer.

Thus, while some decades ago olive oil was considered to be merely an ethnic food, today its worldwide recognition is rising given the fact that it is widely considered a functional food able to provide health and well-being.

Olive oil is considered to be a crucial component of the so-called 'Mediterranean Diet' that since 2010 it has been inscribed in the UNESCO's list of Intangible Cultural Heritage of Humanity.

Consequently, everything revolving around the olive tree attracts great interest from scientists, consumers, and producers alike. Moreover, in recent years, consumption and production of olive tree products, such as olive oil as well as olive fruits, food containing olive oils, leaves, bioactive extracts and single molecules, have become increasingly more popular in countries far from the Mediterranean area.

At the same time, scientific research on olive tree products are increasing exponentially involving academics of several disciplines, especially agronomy, arboriculture, engineering, economy, food technology, medicine and pharmacology.

This book is a collection of overviews and original research on various aspects relative to virgin olive oil as well as its well-known and innovative related products, with a special focus on the effects of such products on human health. Chapters are presented by contributors of international standing and leaders in the field.

The main topic of the book is, of course, virgin olive oil of which the latest findings on composition, extraction processes, varieties, growing, geographical characterizations, sensory qualities, culinary performance and medical activity are described. Table olives and other by-products are also addressed. However, in all the chapters, the benefits of olive tree products to human health are always emphasized and expanded.

Exceptional benefits of virgin olive oil derives mainly from both its typical composition and specific physical process of extraction (Chapters 1-3).

In Chapter 1 an accurate description of the chemical composition of virgin olive oil is given. Specifically, acidity, peroxide index, phenolic compounds, fatty acid and triglycerides composition, sterols, and other minor unsaponifiable compounds are discussed.

It emerges that the key elements that distinguish olive oil from other edible oils/fats are, above all, the high content of monounsaturated fatty acids (oleic acid) and the significant presence of natural antioxidants, such as tocopherols, carotenoids, and mainly the phenolic compounds.

Virgin olive oil is a fruit juice, produced exclusively by mechanical extraction processes.

In Chapter 2, after a brief historical presentation of the traditional process, an update on some recent processing techniques is reported. The effects of a few technical variants on the concentration of total phenolic compounds and anti radical activity is also discussed, focusing on how the new techniques could affect public health in the context of cardiovascular diseases, metabolic syndrome and cancers.

Extraction technology of virgin olive oil has remained substantially unchanged for centuries. In Chapter 3 the historical evolution of olive oil production with mechanical procedures is analyzed by using a novel industrial archaeological methodology. Specifically, the evolution of extraction mechanical procedures from the second half of the nineteenth century until the mid-twentieth century is reconstructed by studying the invention and patents implemented from the Historical Archive of the Spanish Office of Patents and Trademarks.

Another distinguishing feature of virgin olive oil is the fact that each oil can express its own identity. Indeed, olive oil composition is strongly affected by olive cultivars, the geographical origin and agronomical factors (Chapters 4-9).

In Chapter 4 the composition of fatty acids, sterols and polyphenols in most monovarietal oils of eastern Spain are presented. A detailed historical reconstruction of the olive tree is given in the introduction in order to show the origin of some Spanish varieties. Fatty acid composition has proven to be a good varietal indicator, especially the content of the major fatty acids (oleic, linoleic and palmitic acids) as well as the oleic/palmitic ratio. However, sterols, α-tocopherol, and other minor components are also able to group varietal virgin olive oils allowing, in a few cases, a clear and unequivocal differentiation.

Chapter 5 discusses a comparative study among a few Tunisian olive cultivars that is carried out by determining the volatile compounds polyphenols, *ortho*-diphenols and flavonoides in the leaves, fruits and stems. Significant differences among the cultivars studied have been found in function to the environmental conditions of growth.

Thus, variety and geographical origin may be used as the criteria for discriminating different commercial virgin olive oil. In order to guarantee the authenticity of the commercial virgin olive oil, the European Union has set up the labels of origin 'Protected Designation of Origin' and "Protected Geographical Indication".

Statistical procedures may be useful to prove the close relationship existing between quality and geographical origin of a virgin olive oil. An example is given in Chapter 6 in which Italian and Western Greek olive oils, analyzed by using both conventional analytical parameters and innovative instrumental techniques (^{1}H and ^{13}C-NMR), are compared by the application of multivariate statistical analysis. The application of chemometric techniques is essential, due to the huge number of chemical-physical variables that had to be analyzed simultaneously.

To respond to the increasing demand of olive oil and its related products, olive orchards are implanted in new areas and intensive olive cultivations are introduced with the aim of increasing production and limiting costs.

In Chapter 7 olive variety suitability and the training procedure for modern olive growing is reported. Research is carried out in Tunisia and specifically, a comparative trial has been

set to test different table olives cultivars cultivated with different tree-trainings and pruning systems (central leader form, open-vase form and free form). Both the free and open-vase forms have shown to be highly productive, easy to develop, received little or no pruning and the most economical training system for intensive tree conditions.

Olive oil quality and composition is mainly influenced by olive fruit characteristics, and, therefore, all aspects that influence their development have a crucial effect on olive products.

In Chapter 8 the influence of diverse agronomic factors on olive fruits and olive oil production, composition and quality is reviewed and discussed giving special importance to olive farming systems, fertilization and irrigation, as well as the incidence of olive pests and diseases. Specifically, different agricultural practices, production regimes, fertilization and irrigation systems are focalized. Moreover, the occurrence of pests (olive fruit fly, olive moth, olive black scale, among others), and diseases (olive anthracnose, and verticillium) are discussed in terms of chemical degradation caused in olive oils, loss of productivity, olive oils declassification, changes in chemical composition, and loss of bioactivity and stability, which altogether leads to unprecedented economic damage.

Chapter 9 describes the influence of agronomical and technological factors of production on both the quantitative and qualitative composition of phenolic compounds of virgin olive oil. Specifically, the influence of cultivar, fruit ripeness, climatic conditions and agronomical techniques are more widely considered. Moreover, regarding the technological aspects of production, phenolic compounds in virgin olive oil are related to the activity of endogenous enzymes present in the olive fruit.

In the fruits phenolic compounds are an important part of the fruit defense chemical system, where they exhibit antimicrobial activity and protection against the oxidative damages.

Although the olive's phenols are found in the virgin olive oil only in minimal part (at level of 100-400 mg/kg of oil), they are responsible for the oil oxidative stability and sensory properties.

The phenolic composition of olive oil is complex and includes hydroxytyrosol, tyrosol, the dialdehydic form of elenolic acid linked to either hydroxytyrosol or tyrosol, oleuropein aglycon, lignans and many others.

The current interest in olive oil polyphenols is based on their important nutritional and biological properties (Chapter 10-12).

In Chapter 10 the recent findings on the *in vitro* chemo-preventive activities of hydroxytyrosol are discussed. This compound has demonstrated to be able to counteract the main hallmarks of cancerogenesis acting as an antioxidant, anti-proliferative and an anti-inflammatory agent.

Chapter 11 summarizes the epidemiological studies and investigation trials focusing on the effects of virgin olive oil in the inflammatory process and/or inflammatory-related diseases. Also in this case, the cellular and molecular anti-inflammatory mechanisms of virgin olive oil have been particularly associated with its high amounts of phenolic compounds, as well as, to its composition in mono- and poly-unsaturated fatty acids.

Chapter 12 highlights the fact that olive-oil consumption is inversely correlated with the incidence of stroke. Specifically, diverse effects of olive oil and its chemical constituents on various molecular mechanisms that affect vascular cell function are discussed. Also in this context, oleic acid and the polyphenolic compounds appear to be the crucial factors that reduce the risk factors of cardiovascular disease.

Virgin olive oil also differentiates itself from all the other oil/fats for both its typical organoleptic characteristics and its flexibility to be used in culinary preparation (Chapter 13, 14).

Highly specialized tasting panels usually conduct the measurement of organoleptic properties of virgin olive oil. Traditional methods may confound sensory precision with other aspects related to the bias of the tasters, such as their beliefs, motivations, and preferences.

In Chapter 13 a new method in olive oil tasting based on 'Signal Detection Theory' is presented. The proposed procedure, based on a dissociative model, allows for the obtaining of independent measures of sensory and cognitive factors. From a practical point of view, this dissociation between sensory and decision process may contribute to an optimization of the evaluation of the quality of the olive oil, facilitating the comparison between evaluations of experts and regular consumers.

In several countries of the Mediterranean area, both in domestic and commercial appliances, virgin olive oil is preferred not only as a salad dressing, but also as one of the ingredients in the preparation of typical food.

In Chapter 14 the performance of olive oil as a cooking fat, as an ingredient in baked products, as a canning agent of vegetables and fish preserves and finally, as a component of various sauces is reported. In general, though sometimes contrasting results have been found, virgin olive oil seems to be a valid ingredient in the preparation of food, especially when the cooking time is not extensive and the temperature is not too high.

Monounsaturated oleic acid and the antioxidant substances contained in virgin olive oil are also present in the olive fruits that can also be eaten processed as table olives. Table olives contain further beneficial components as emerged from Chapter 15 in which the focus is on the dietary fibre content in olive fruit. The main components of the soluble fibre are pectic polysaccharides, such as homogalacturonans, rhamnogalacturonans and arabinans. These show many health benefits, such as hypocholesterolemic, hypoglycemic, prebiotic and anticancer activity.

Finally, as result of olive oil production a large amount of residue, such as mill wastewater, olive pomace, leaves and sediments are originated. These residual products are rich in bioactive compounds and so are considered very attractive as potential (source) of natural antioxidants (Chapter 16-18).

Chapter 16 focuses on chemical composition and health properties of olive tree by-products. Moreover, this chapter gives a description of the potential technologies that allow to recovery and purify the antioxidant polyphenols from olive by-products.

While, an innovative technology for the selective recovery of phenolic compounds from the olive oil mill wastewaters, called 'molecular imprinted technology', is presented in Chapter 17. This technique allows the recovery of target bioactive compounds with high purity and efficiency and at the same time, it also presents other interesting properties, such as low cost, physical resistance and reusing.

Although several techniques have been developed for olive-oil mill wastes, few industrial plants currently invest in purification and utilization of olive oil mill by-products that would prevent environmental pollution.

In Chapter 18 alternative use for the recycling olive oil mill wastewaters and solid wastes is presented. Solid wastes are low-cost lignocellulosic materials and can be used as an efficient fuel with low amounts of N and S. Instead, the wastewaters have favorable chemical properties (organic carbon, potassium and phosphorus content) and disposal on soils may be

considered as an appropriate option to solve management problems, restore soil fertility and promote productivity.

In conclusion, topics presented in this book show the versatility and usefulness of several olive tree products. Virgin olive oil is certainly the most important product and the most valuable. However, even other related products, including olive fruits and even the waste by-products have proven to be a source of beneficial substances for Man and for the environment.

The editor thanks all contributors for their careful and professional participation to the realization of the book.

Dr. Antonella De Leonardis
Editor
Università degli Studi del Molise
Via De Sanctis
86100 Campobasso, Italia
Tel: 0874-404641
antomac@unimol.it

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Chapter 1

CHEMISTRY AND BIOACTIVE COMPONENTS OF OLIVE OIL

Raquel de Pinho Ferreira Guiné*
CI&DETS Research Centre and Department of Food Industry,
Polytechnic Institute of Viseu, Portugal

ABSTRACT

Olive oil has been consumed since immemorial times and its production assumes a very important role in some countries, particularly in the Mediterranean surroundings. In fact this food product is one of the key components of the Mediterranean Diet, which, for its recognized properties and benefits, as well as cultural importance, has been inscribed by UNESCO in 2010 on the Representative List of the Intangible Cultural Heritage of Humanity.

Olive oil has proved to have many nutritional and medicinal effects. The health benefits of olive oil are extensive and new positive attributes are being discovered very frequently. In addition to bolstering the immune system and helping to protect against viruses, olive oil has also been found to be effective in fighting against diseases such as: cancer, heart disease, oxidative stress, blood pressure, diabetes, obesity, rheumatoid arthritis or osteoporosis.

These positive effects are due to the particular composition of olive oil, unique among the vegetables oils, and to the presence of compounds of nutritional importance and bioactive molecules. Therefore, the aim of this chapter is to focus on the chemical composition of the olive oil, the compounds present and their relation to effects produced.

Keywords: Chemical composition, fatty acids, phenolic compounds, bioactive components, triglycerides

* Department of Food Industry, ESAV, Quinta da Alagoa, Estrada de Nelas, Ranhados, 3500-606 Viseu, Portugal. Email: raquelguine@esav.ipv.pt.

1. INTRODUCTION

Olive oil is an integral ingredient of the Mediterranean diet. Its consumption dates back to biblical times, and the cultivation of the olive tree as well as the production of olive oil from the mature olives constitute an essential component of farming practices in the Mediterranean basin [1]. Olive cultivation is widespread throughout the Mediterranean region being important for the rural economy, local heritage as well as for the environment [2,3].

Virgin olive oil, obtained from the fruit *Olea europaea* L., is the only edible oil produced in large scale world-wide by mechanical or physical methods. Its consumption has been increasing in the past years due to its unique sensory characteristics, besides the nutritional and therapeutic properties reported [3]. In fact, olive oil is a very versatile product, long known to many generations in the Mediterranean areas as essential to the population's health and diet. However, at present its use is no longer limited to the Mediterranean countries, being widely appreciated around the world for its nutritional, health and sensory properties [2]. Virgin olive oil possesses singular sensory attributes, being characterized by a unique flavour, which represents one of its most important qualitative aspects, paying a major role in consumer approval [2].

The association of food with health is universal and well patent in the saying "you are what you eat". In the later decades, the importance of nutrition in the public's mind has grown and this tendency is still maintained. Surveys show that the protection/prevention against illness, tackling obesity, and the nutritional quality of foods is of even higher priority than the improvement to the taste, colour, and texture of food [4].

Over the years, the so-called Mediterranean diet has become widely associated with improved health and well-being as well as protection against cardiovascular diseases and colon, breast and skin cancers [5]. The Mediterranean diet includes an important consumption of fruits and vegetables complemented with a high intake of olive oil and other olive products.

Olive oil is a product of great importance due to its nutritional value, which has been acknowledged internationally [6]. Therefore, olive oil is an essential part of people's diet, because of its flavour and culinary value as well as its nutritional properties and biological effects on human health [7].

Virgin olive oil is one of the edible fats most highly valued by people in the Mediterranean area because it can be consumed without any refining process, since it is obtained from olives exclusively by mechanical processes, and thus retains its natural flavor and aroma. It also has highly appreciated nutritional characteristics [8]. Furthermore, olive oil appears to be a functional food with various biocomponents. Among these are monounsaturated fatty acids that may have special health benefits and also phytochemicals [9]. Some of these effects are associated with extra virgin olive oil content in phenolic compounds, high amounts of oleic acid, tocopherols and phytosterols [10]. Evidence showed that olive oil is a source of at least 30 phenolic compounds, which are strong antioxidants and radical scavengers. Recent findings confirm that olive oil phenols are powerful antioxidants, both *in vitro* and *in vivo*, besides possessing other potent biological activities that could in part account for the health benefits of the Mediterranean diet [9]. In fact, they have been suggested to play a preventive role in the development of cancer and heart disease [11].

Moreover, apart from the antioxidant activity, phenolic compounds from olive oil have other roles, namely relating to nutritional properties and sensory quality [11].

The beneficial effects are attributed to a favorable fatty acid profile and to the presence of some minor components that are also responsible for the unique flavor and taste of olive oil [5]. Accumulating evidence suggests that olive oil may have several health benefits that include a reduced risk of coronary heart disease, the prevention of several varieties of cancers and the modification of immune and inflammatory responses [9]. Furthermore, olive oil contains compounds with potent antimicrobial activities against bacteria, fungi, and mycoplasma [5]. In addition, olive oil has anti-inflammatory activities, having been demonstrated that newly pressed extra-virgin olive oil contains oleocanthal, with similar pharmacological activity as the drug ibuprofen [5].

The basic aspect that distinguishes olive oil from other vegetable oils is its high proportion of monounsaturated fatty acids, such as oleic acid which represents about two thirds of the total fatty acids content, as well as the modest presence of polyunsaturated fatty acids [7]. A healthy diet must contain a limited amount of saturated fatty acids so as to reduce the total cholesterol content and a high amount of monounsaturated fatty acids which prevent the risk of cardiovascular diseases, reduce the insulin body-requirement and decrease the plasma concentration of glucose. Moreover, there seems to be a relationship between the intake of olive oil, the richest food in the monounsaturated fatty acid oleic acid, and breast cancer risk and progression [12].

Olive oil contains natural antioxidants such as tocopherols, carotenoids, sterols and phenolic compounds that represent 27% of the unsaponifiable fraction. The main phenols identified in olive oil are gallic, caffeic, vanillic, p-coumaric, syringic, ferulic, homovanillic, p-hydroxybenzoic and protocateuric acids, tyrosol and hydroxytyrosol. Phenolic compounds of olive oil have multiple effects, including the stability to oxidation of extra virgin olive oil during storage. It has been claimed that hydroxytyrosol is the most active antioxidant compound in virgin olive oil. Furthermore, phenolic compounds have the capacity to inhibit or delay the growth rate of several bacteria and microfungi [7].

The unsaponifiable matter in olive oil constitutes about 1 to 2%, being the major portion represented by phytosterols, which are recognized by their biological effects, such as cytostatic activity, blood cholesterol control or cancer prevention [10].

The U.S. Food and Drug Administration permitted a claim on olive oil labels stating: "Limited and not conclusive scientific evidence suggests that eating about two tablespoons (23g) of olive oil daily may reduce the risk of coronary heart disease" [13].

2. Chemical Composition of Olive Oil

The chemical composition of virgin olive oil is influenced by genetic factors associated to the cultivar as well as environmental factors such as edaphological characteristics and climatic conditions. In this way, the characteristics of olive oil are greatly influence by the region of production [14,15]. Apart from these factors, the quality of olive oil is also strongly related to the physiological conditions of the fruit from which it was extracted, so that the stage of ripening may directly or indirectly affect the quality. Furthermore, there is an indirect effect provided by the action of external agents of deterioration which increase during fruit

ripening [16]. This is because as ripening advances certain metabolic processes take place which involve changes in the profile of certain compounds such as triglycerides, fatty acids, polyphenols, tocopherols, chlorophylls and carotenoids. These changes, besides influencing the oxidative stability and the nutritional value of the final product, also affect the sensory characteristics, with particular emphasis on aroma.

Gomez-Rico et al. [17] studied the influences of agronomic practices and found that irrigation positively affected both fruit and olive oil quality.

Mendoza et al. [15] studied 88 virgin olive oil samples original from Spain, from three successive crop seasons, produced from the mixture of two varieties of olives: Morisca and Carrasqueña. The results showed that 88.5% of selected original grouped cases are correctly classified according to the ripening stage (85.7% green, 80% spotted and 78.9% ripe) having this been based on the most discriminating variables: avenasterol, linolenic acid, beta-sitosterol and gadoleico.

Baccouri et al. [18] studied the influence of the olive ripening stage on the quality indices, the major and the minor components and the oxidative stability of the two main monovarietal Tunisian cultivars (Chétoui and Chemlali) virgin olive oils. Their results indicated a very good correlation between the oxidative stability and the concentrations of total phenols, practically secoiridoids and α-tocopherol.

Virgin olive oil is mainly composed of triacylglycerols (between 97 and 98%), minor variable amounts of free fatty acids and minor glyceridic compounds (partial glycerides, phospholipids and oxidized triacylglycerols) and finally around 1% of unsaponifiable constituents varying in structure and polarity [18,19].

The oxidative stability, sensory quality and health properties of virgin olive oil are possible due to a well-balanced chemical composition [20].

2.1. Acidity

One of the parameters that allow the classification of olive oils is the acidity. Extra-virgin olive oil comes from virgin oil production only and contains no more than 0.8% acidity, expressed per g oleic acid/100g olive oil [21] having a superior taste. Virgin olive oil comes from virgin oil production only too but the acidity is slightly higher, less than 2% and is judged to have a good taste. When the acidity of the olive oil exceeds 2%, and/or the sensory evaluation is lower the sample is graded as ''lampante virgin olive oil'' and is recommended to be refined prior to human consumption. Oils labeled as Olive oil are usually a blend of refined and virgin olive oils, and contain no more than 1% acidity, and commonly lack a strong flavor. *Olive pomace oil* is refined pomace olive oil often blended with some virgin oil. It has a more neutral flavor than virgin olive oil maintaining the same fat composition, thus giving it the same health benefits. Refined olive oil is the olive oil obtained from virgin olive oils by refining methods that do not lead to alterations in the initial glyceridic structure. It has a free acidity, expressed as oleic acid, of not more than 0.3 grams per 100 grams (0.3%) and its other characteristics correspond to those fixed for this category in this standard.

Table 1 shows some values of acidity obtained in some studies about olive oil composition.

Table 1. Acidity of olive oils

Cultivar	Acidity (g oleic acid/100 g)	Reference
Morisca and Carrasqueña	0.29 – 0.53	[15]
Chétoui and Chemlali	0.23 – 0.42	[18]
Cornicabra	0.08 – 0.55	[8]
Arbequina, Manzanilla, Nevadillo and Ascolana	0.12 – 1.03	[22]
Hor Kesra, Sredki, Chladmi, Betsijina and Aloui	0.19 – 0.31	[10]
Halhali, Egriburun, Hasebi, Karamani and Saurani	0.52 – 0.85	[23]

2.2. Peroxide Index

The established upper limit for the peroxide value in extra virgin olive oil and virgin oil is 20.0 miliequivalent O_2 per kg [21].

Table 2 shows some values of the peroxide index for different samples of olive oil, produced from different cultivars of olives.

Table 2. Peroxide index of olive oil samples

Cultivar	Peroxide index ($meqO_2/kg$)	Reference
Morisca and Carrasqueña	5.71 – 6.47	[15]
Chétoui and Chemlali	2.93 – 16.67	[18]
Cornicabra	1.9 – 19.1	[8]
Arbequina, Manzanilla, Nevadillo and Ascolana	7.84 – 11.0	[22]
Hor Kesra, Sredki, Chladmi, Betsijina and Aloui	4.19 – 5.83	[10]
Halhali, Egriburun, Hasebi, Karamani and Saurani	2.01 – 7.08	[23]

2.3. Phenolic Compounds

The amount of phenolic compounds present in extra virgin olive oil is a major factor influencing its quality because the natural phenols improve the resistance to oxidation and also because, to certain extent, they are also responsible for the characteristic taste [20].

Olive oil presents considerable amounts of natural antioxidants which have proven to be important in the prevention of many diseases [24].

Baccouri et al. [18] observed that the concentration of phenolic compounds progressively increased to a maximum at the ''reddish" and ''black" pigmentation stage of the olives used to make the oil, decreasing thereafter. This trend was also observed by Salvador et al. [25]. One other factor that proved to affect the amount of phenolic compound was irrigation of the fruits [17,18, 26].

Oleuropein belongs to a specific group of coumarin-like compounds, the secoiridoids, which are abundant in olives. The secoiridoids are present exclusively in plants belonging to the family of *Olearaceae* and are characterised by the presence of elenolic acid, either in the glucosidic or aglyconic forms. The most abundant secoiridoids of virgin olive oil are the