

Olive Oil
Chemistry and Technology
Second Edition

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Preface

A staple food for thousands of years for the inhabitants of the Mediterranean region, olive oil is now becoming popular among consumers all over the world. New consumers are looking to extract healthful benefits from the diet of the people living in the countries surrounding the Mediterranean Sea, since olive oil is a basic constituent of this diet.

Olive oil differs from other vegetable oils because it is used in its natural form and has unique flavor and other characteristics. In its production, technology, and tradition these two seemingly contradictory factors interplay successfully. On the issue of health, the news about olive oil get better every day, as more and more research confirms beneficial properties described even by Hippocrates and other doctors of the ancient world. In 2004, the U.S. Food and Drug Administration (FDA) announced the availability of a qualified health claim for monounsaturated fat from olive oil and reduced risk of coronary heart disease. Still, the secret of olive oil to combat disease is probably hidden not only in its fatty acids but also in the minor constituents, especially biophenols, as indicated by results of recent biochemical, pharmacological, and other studies.

The object of *Olive Oil: Chemistry and Technology, 2nd edition* is to provide a compact and readable text on most important aspects of chemistry, technology, quality, analysis, and biological importance of olive oil. Topics were selected that have been developing rapidly in recent years and are expected to provide the reader with a background to address more specific problems that may arise in the future. In this revised new edition there are many more contributors and chapters. This was dictated by the “avalanche” of publications since the first edition was published in 1996.

The book is organized in three parts and has also a [glossary](#) and index at the end.

Part 1 OVERVIEW AND ECONOMICS has three chapters: **1**—The Culture of the Olive Tree (Mediterranean World); **2**—Characteristics of the Olive Fruit; and **3**—Olive Oil in the World Market.

Chapter 1—The Culture of the Olive Tree (Mediterranean World) is a short presentation of the development of olive tree, the use of olive oil through the centuries, the myths, the habits, the bonds with religion, and generally the culture of the countries of the origin of the tree in the Mediterranean World.

Chapter 2—Characteristics of the Olive Fruit discusses briefly the characteristics of the olive tree and olive fruit (cultivation, varieties, maturity of olives, etc.).

Chapter 3—Olive Oil in the World Market is a presentation of olive oil economics with useful data on the production and consumption that is presented in figures and tables. Imports, exports, and national or international policies and trends in the

olive oil sector are also presented and critically analyzed.

Part 2—CHEMISTRY, PROPERTIES, HEALTH EFFECTS has five chapters: **4—Olive Oil Composition**; **5—Polar Phenolic Compounds**; **6—Olive Oil Quality**; **7—Analysis and Authentication**; and **8—Healthful Properties of Olive Oil Minor Components**.

Chapter 4—Olive Oil Composition constitutes the foundation of olive oil chemistry. It is the belief of the editor that an adequate understanding of the unique character of olive oil has to be based on a deep knowledge of its composition. Hence, the chapter has a broader coverage, with emphasis on both glyceridic and nonglyceridic compounds.

Chapter 5—Polar Phenolic Compounds is a broad discussion of phenols other than tocopherols. From the various classes of minor constituents, polar phenols seem to be the most important biologically and the literature is extensive. This is probably related to the message that the consumption of natural antioxidant phenolic compounds produce beneficial health effects; these substances were found to possess strong radical scavenging capacities and can play an important role in protecting against oxidative damages and cellular aging. In addition to their bioactivity, olive oil phenols are important for the flavor and the bitter taste of the oil.

Chapter 6—Olive Oil Quality covers important aspects related to stability and development of undesirable properties due to chemical and biochemical changes.

Chapter 7—Analysis and Authentication addresses adulteration and analytical methods that are applied to identify and check genuineness. In the last two decades a tremendous amount of analytical work has been produced and incorporated into *Codex Alimentarius* and European Union legislation. Pioneering work mainly with the use of hyphenated methods is also expected to contribute significantly to the revealing of fraudulent actions.

Chapter 8—Healthful Properties of Olive Oil Minor Constituents is the last chapter in Part 2. This chapter provides extensive coverage on the role of phenols in human health, their antioxidant activity, activity on enzymes, oxidative stress as well as bioavailability, and intake of bioactive compounds from olive oil.

Part 3—PROCESSING AND APPLICATIONS has four chapters: **9—Olive Oil Extraction**; **10—Treatments and Modifications**; **11—Storage and Packing**; and **12—Culinary Applications**.

Chapter 9—Olive Oil Extraction deals with harvesting and processing techniques; the importance of the quality of the olives to be crushed; the influence of extraction on the quality indices of the oil; and the newly developed processes to obtain better yields and better organoleptic characteristics and stability. Other topics covered are extraction and refining of olive pomace oils and good manufacturing practice.

Chapter 10—Treatments and Modifications is rather selective in its presentation of the material, since refining methods are more or less the same to those applied to

other vegetable oils. There is a more detailed discussion on some new proposals for mild purification and the changes in composition due to processing.

Chapter 11—Storage and Packing discusses conditions under which olive oil should be packed and stored; and includes International Olive Oil Council trade rules and U.S. labeling guidelines.

Chapter 12—Culinary Applications describes domestic and other uses, negative and positive flavor attributes, the behavior of olive oil during frying of food, and losses of phenols due to heating.

It is hoped that readers will find this new edition even more useful than the previous one and will appreciate the effort of our authors to include most of the new knowledge accumulated in the time since 1996. All the authors present updated information and have selected references covering almost every aspect of olive oil.

I am grateful to all the contributors, who share with me the enthusiasm for olive oil, and for their immense help. Without their expertise and cooperation such a book would not have been written. I am particularly indebted to my colleague Dr. Fani Mantzouridou for her valuable help in the corrections and the sifting through the manuscripts through the preparation of the final draft.

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Overview and Economics

1

The Culture of the Olive Tree (Mediterranean World)

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The olive tree is a familiar feature of the Mediterranean landscape. It may have originated in Syria, Asia Minor, Ethiopia, Egypt, or India. Since ancient times, it has contributed, in practical and symbolic terms, to the economy, health and *haute cuisine* of the inhabitants of the Mediterranean. Crete, the Peloponnese, the coastal regions of Greece, the islands of the Eastern Aegean, such as Lesbos, Samos and Thasos, and the Ionian islands all possess olive groves. Likewise the olive is found widely in Cyprus, the coasts of Turkey, Syria, Lebanon, Israel, the south of Spain, France, Italy, and the coast of North Africa. Spanish migrants spread the olive to Mexico, Argentina, and Uruguay in Latin America, and Italians took it to Australia. The significance of the olive tree rests upon the existence of these groves, or has done so over recent centuries. The culture of the olive tree has three aspects: the landscape itself, diet (consisting mainly of the use of oil), and the symbolic importance of the tree and its fruit. All these aspects have been the subject of intense discussion over recent decades. The culture of the olive tree is manifested in many different ways, in material objects, in the arts, and in various customs. It is also manifested in religious behavior, magical rituals, medical prescriptions, and cosmetics. Above all, the culture of the olive tree is manifested in a symbolism that transcends time and place.

Myths and the History of the Olive Tree

The great significance that the olive tree has had for the life and the economy of the ancient world in the eastern Mediterranean area is evident in the appearance of the olive tree in the myths of the people who lived there. In Hebrew mythology a dove brings an olive branch to Noah after the Great Flood, indicating that life has returned to earth. In the Old Testament, oil is often mentioned along with wheat and wine as one of the basic products in the land of Israel (Valavanis, 2004). Moses dreamt of the Promised Land as “*the land of olives and olive oil*” (2004). The dedication of the altar and various objects required for worship was performed with holy oil “*and Moses took the anointing oil, and anointed the tabernacle and all that was therein, and sanctified*

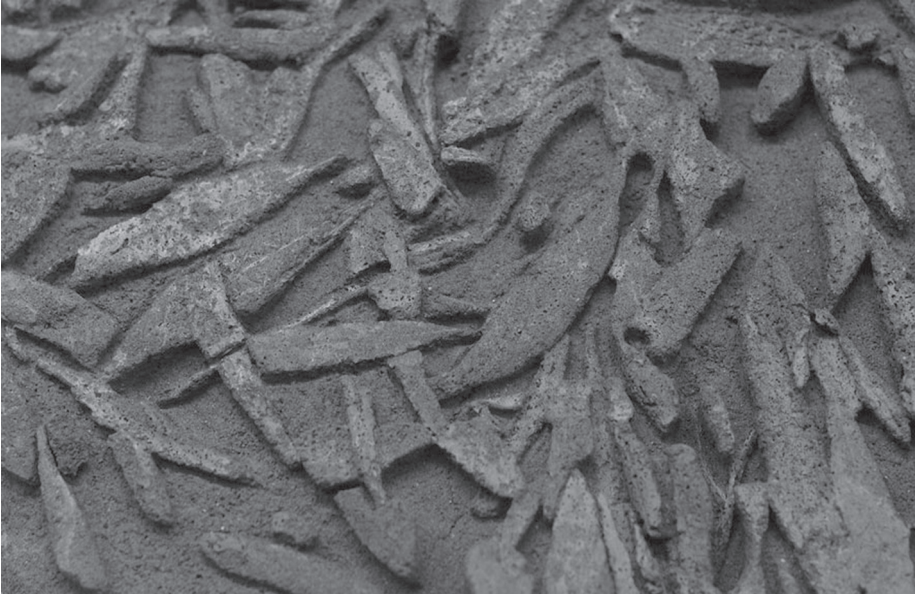


Fig. 1.1. Fossilized olive leaves recovered from the Caldera walls of Santorini, 6000 years old. From the book “Ode to the olive tree”, Hellenic Folklore Research Center of the Academy of Athens, General Secretariat for Olympic Games, Athens 2004.

them” (Leviticus, 8.10-12). According to Greek tradition, the bringing of the wreath to Olympia from the distant mythical Hyperborean countries was the initiative and deed of the demigod Heracles (Faklaris and Stamatopoulos, 2004). The ancient Egyptians crowned their dead with olive branches. The Phoenicians were possibly the first to produce olive oil.

The olive originated in the countries of south Asia and was carried by birds to the Mediterranean via the Middle East. The most ancient oleaster traces in Greece are fossilized leaves found in the caldera on the island of Santorini dating back some 50,000 – 60,000 years (Valavanis, 2004) (Fig. 1.1).

There is no evidence for the use of olive products by the inhabitants of the prehistoric Aegean. Nevertheless, it seems possible that at least since the Neolithic Age, namely since 8,000 B.C., the oleaster fruit would occasionally be collected, along with other wild edible fruit, to supplement the daily diet. Palynology, the relatively new science of the study of pollen, has revealed the presence of oleaster pollen towards the end of the Neolithic Age, about 3,200–3,100 B.C. in Kopais, Thessaly, and Crete.

The principles of cultivation of the olive were apparently discovered and formulated some time later, at some point in the third millennium B.C., at the beginning

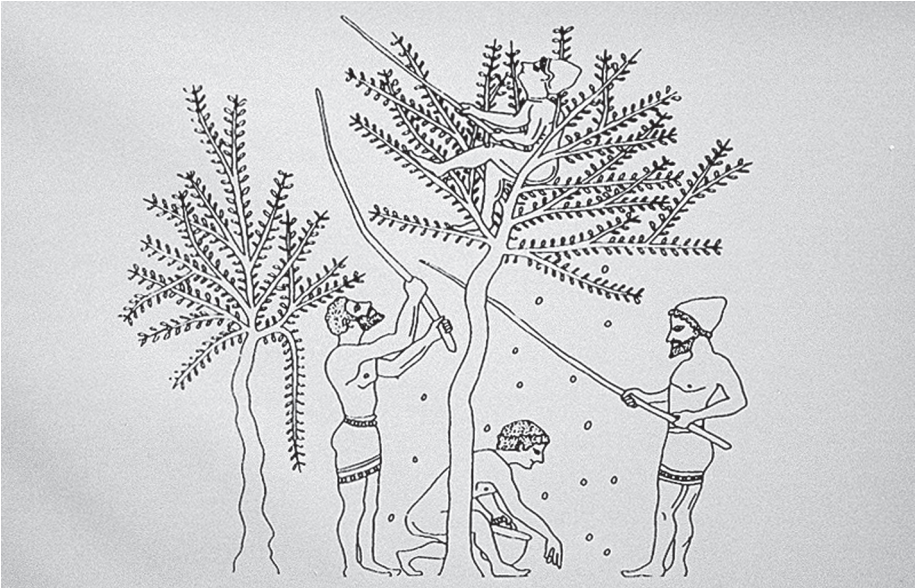


Fig. 1.2. Harvest by beating the branches. Drawing, amphora, 6th century B.C. (From the book "Ode to the olive tree"), Hellenic Folklore Research Center of the Academy of Athens, General Secretariat for Olympic Games, Athens 2004.

of the Bronze Age. The Cretans were in contact with the civilizations of the eastern Mediterranean and North Africa, where the olive had already been domesticated. This contact resulted in the spread of the knowledge of olive cultivation therefore in Crete.

Greek myth would seem to locate the origin of olive cultivation in North Africa. Aristaeus, a rural deity and the son of Apollo and the nymph Cyrene, was raised on the North African coast and subsequently spread the knowledge of olive cultivation. He, in his turn, had been taught by nymphs techniques of grafting to produce better fruit, and of pressing and extraction of oil. Aristaeus travelled to Greece and Sicily, spreading his knowledge of olive cultivation and being deified by the Greek inhabitants as the patron deity of cultivators of the olive (Valavanis, 2004) (Fig. 1.2.).

In another well-known myth, it is Athena who spreads knowledge of the cultivation of the olive. The myth is very probably the creation of the Athenian democracy, which very clearly discerned the importance of the olive for the economy of the Athenian state and olive oil which, after it had been produced in Athens, it was exported by Athenian ships.

Thanks again to palynology, olive pollen has been found spread throughout Greece from about the middle of the second millennium B.C. This suggests that the systematic spread of the cultivation of the olive and the exploitation of its products

begins with the construction of the palaces of the New Palace Period in Crete and of the palaces of Mycenaean civilization on the mainland of Greece. Linear B tablets from Messenia, deciphered in 1952, containing references to *e-ra-wa* ('olive tree') and *e-ra-wo* ('olive oil') give further proof of the existence of the olive tree in this period. A group of 51 tablets, from four different areas in the Mycenaean palace at Pylos, give an insight into various aspects of the use of the olive at the time. The oil, plain or scented, mentioned in these tablets was intended for purposes of worship. The tablets also mention the names of the gods who received this offering, for example, Poseidon ('*Po-se-da-o-ne*', which is the name of the god in the dative case) or Potnia ('*po-ti-ni-ja*', 'lady'). The presence of the names of these deities in the tablets also coincidentally indicates that many deities of the Greek pantheon are of Creto-Mycenaean origin. The tablets also make mention of various time parameters, including a month or reference to various festivals, which shows that some sort of sacred calendar was in operation at the time, to be used henceforward.

Perhaps the clearest proof of the strength and ubiquity of the Mycenaean olive trade is seen in the enormous numbers of stirrup-jars, used exclusively to transport scented oil throughout the Mediterranean, which bear witness to the importance of the production and trade of scented oil for the royal economy (Fig. 1.3.).



Fig. 1.3. Scent bottles (lekythoi) 6th century B.C. From the book "Ode to the olive tree," Hellenic Folklore Research Center of the Academy of Athens, General Secretariat for Olympic Games, Athens 2004.

There are many Greek and Latin sources for historical times and particularly the Greco-Roman world. Columella, a Spanish born Roman writer on agriculture, (1st century A.D.), characteristically calls the olive “the queen of plants” (Hadjisavvas, 2003).

Recent research in Cyprus has shown that all important late Bronze Age sites had olive presses and storage areas (Hadjisavvas, 2003). Various objects, such as the fragmentary relief depicting a bull before an olive tree, from the so-called “Treasury of Atreus,” ivory objects carved with representations of olive trees, a piece of a lady’s dressing table from Matmar in Egypt, of 13th c B.C., depicting a bull in front of an olive tree, a similar representation on a comb made from ivory from Megiddo in Palestine, of 13th c B.C., the naturalistic wall paintings from Crete, the landscapes depicted on the well-known cups from Vapheio, of 15th c B.C., show the prevalence of the olive in Mediterranean countries (Hadjisavvas, 2003).

The dominant spiritual and symbolic role of oil is closely connected with the geographical and cultural environment in which Christianity was born and prospered. The inhabitants of the Mediterranean adapted their needs to the productive capacities of their natural habitat, so making the olive and its oil a basic element of their daily nutrition. In addition, oil was valuable as the main source of light and as a healing medium, because of its calming qualities.

In the Byzantine Empire the areas with climatic conditions favorable to the growth of the olive tree were mainly Syria, Palestine and Cyprus in the East, Italy and Sicily in the West, and North Africa in the South (mostly the area of Carthage). Along the coastline of Greece and the Aegean, including the coastline of Asia Minor and the coastline of Pontus, olive cultivation was developed, albeit to a lesser extent. Production in Greece aimed mainly to cover local or family needs and less to export oil (Tsougarakis, 2004).

One of our most important sources relating to olive tree cultivation consists of the records of the monasteries. These give some idea of the presence of the olive tree in the country in certain areas. Although Corfu and Crete have been notable during recent centuries for intensive olive cultivation, they do not seem to have been such intensive producers in Byzantine times. In Crete especially there are reports in the writings of Al-Zuhri of shortages of olives in the 9th century and from the 12th century until the beginning of the 15th century in the writings of Buondelmonti. This necessitated importing of olive oil and olives from other areas (Tsougarakis, 2004).

As for the various aspects and uses of olive oil, such as its nutritional importance, its use in cooking, as a source of light, as a substitute for soap (which developed later) in cleansing the human body, there are numerous passages from Classical writers and numerous examples of ancient iconography that offer considerable information.

Nutrition

The nutritional habits of a society are an expression of its cultural level and their

study contributes to our understanding of that society. The changes in the nutritional code of a traditional agricultural society occur very slowly. In Greece, for example, all nutritional habits remained for the most part intact and the changes in the nutrition were very few. New nutritional habits and traditional foods from the Greeks of the East appeared when the refugees came from Asia Minor in 1922. In general, until the first half of the 20th century, food was plain, simple, and depended mainly on local production, the climate, occupations, and the professions, financial, and social status of the consumers and the degree of communication with other areas containing such things as commercial centers, weekly street markets and fairs. The nutritional attitude of the Greeks changed after World War II. Mass consumption of industrial and commercial goods increases day by day and foreign nutritional habits have invaded the country (Polymerou-Kamilakis, 2004).

Olive oil, a basic element in Greek nutrition, holds a predominant position in the nutritional code of the areas that produce it, even if it is not an area's main product. In addition, olives are nutritious, tasty, cheap, and they can be easily preserved. Thus they are widely used in modern Greek cuisine, even in areas that do not produce them. Over recent decades, there has been extensive research on the olive's beneficial qualities for consumers' health. This research has led to a more systematic recording of the traditional ways and methods of exploitation in order to incorporate the olives in today's nutritional code. People in Greece say: "We can just have bread and olives..."

Olives are considered suitable food for funeral feasts and are consumed during the Lenten fast, memorial services and funeral suppers. In Cyprus, slices of bread and black olives are offered, either at the cemetery or at home, to comfort the relatives of the deceased. In Thrace, a tablecloth is laid on the ground and Lenten food, such as beans, rice, olives and fresh hot bread, known as *makaria*, consumed. The deceased is believed to be present and participate in the funeral supper. In general, olives have always been the food of the poor because they are consumed with bread.

Oil is antiseptic and a disinfectant and it also has calming properties. It is, however, sensitive and difficult to preserve. Throughout the year, it should be decanted two or three times in order to separate out the dregs. This procedure takes place in factories. The oil containers are cleaned very carefully and care is taken to maintain a steady temperature in the storage room. At the beginning of this century, probably under the influence of agriculturists, olive oil producers poured pure sugar in the containers to avoid oil rancid. The oil storage vessels were often kept in dark storage rooms or buried in the soil to avoid changes caused by air and light.

Olive oil was used in almost every type of food conservation, but the fact that it was so rare made its frequent use as a preservative with antiseptic properties impossible. The loss of olive oil during decanting, transport or for any other reason was a bad omen for the owner, such a belief naturally expressing the importance of the oil.

Whenever sufficient quantities were produced, oil was used to preserve meat,

spiced cheese, vegetables, grapes, and olives, thus improving the taste of the product. Besides these basic uses in the area of food conservation, olive oil was, and still is, used for non-culinary purposes, such as coating wine vessel rims to avoid evaporation which spoils the wine. When the vessel is opened, the oil is poured out. It is said that even eggs remain fresh longer if they are coated with oil.

Heating and Lighting

Alongside light produced by fireplaces, torches, and spermaceti (cloth dipped in animal fat and wrapped around a stick), oil lamps for centuries provided the light necessary for those working at night, the oil lamp having one or more wicks.

Oil is still used to light the cresset in front of the icons in Orthodox Christianity. The same light was used in the past to illuminate rooms. The cresset oil is considered sacred and it should be pure. A family always deemed it necessary to use some of its oil to light the cressets.

Everything involved in the olive production process was of use in a traditional society. The by-product of oil production, the olive seed, was used in the past as food for domestic animals, as fertilizer, and as fuel. Since 1950, the seed has been almost exclusively exploited in olive seed factories for the production of seed-oil and seed-wood. Along with the products of the olive seed, an important soap industry developed making use of oil and oil dregs.

Treatment – Medicine

“Is any one of you sick? He should call for the leaders of the church people. They should talk to God about him and put oil on him in the name of the Lord...” (James 5:14-15).

Oil symbolism rests upon observation and the complex of beliefs concerning its therapeutic properties. In countries where Christianity spread beneficial effects of olive oil on the human body were known and as a result oil was used for worship and healing. It was also widely used in folk medicine and treatment, either as a treatment in itself, or as base for other preparations for internal or external use.

In particular, the *agourólado* (green olive oil produced by crushing the olives without the use of hot water) was, and still is, one of the most important natural medicines for various illnesses. Traditionally, oil was used as an antiseptic for curing small wounds and for dealing with skin irritations. It was also used as a pain killer for rheumatism, abdominal pains, and for earache. It was used as an embrocation, poison antidote and all-purpose antiseptic. It was also used in magic to remove the evil eye. The *Geoponicá*, of AD 10, notes that to avoid infection by scabies, sheep should be anointed around the tail with sulphur and oil after shearing (*Geoponicá* 18, 17, 5). Red oil in Crete, olive oil with poppy petals and sometimes spearmint balm was used as throat emollient and calmative for the joints. Threshed olive leaves and fruit were also used to soothe swollen glands.

Cosmetics

Dioskorides describes oil *as wetting and thermal at the same time*. In the past people would use oil to preserve skin moisture, to protect their body from the cold in winter and as sun protection during the summer.

Scented oils, for which olive oil was the basic ingredient, were mainly used by women. The most well-known ones were the *irinion* (with iris root extracts), the *storax balm* (aromatic tree) with vanilla flavor, the *melinion* with quince oil and the *rodion* with rose extracts, not to mention the extremely expensive and exotic scents from Egypt, Lydia, and Libya.

Writer Antiphanes informs us that the most elegant Greeks exaggerated so much that they used different scents for different parts of the body. The vessels used for the scented oils were small and delicate, made of clay, alabaster or glass, round or long with a special neck that allowed the oil to be poured in very small amounts. Of course, a vessel with scented oil was one of the most favorite love gifts.

Since ancient times, olive oil has been a basic ingredient for beauty treatment and body care, both for women and men, either on its own, or as base for the manufacture of cosmetics. It was used for hair care in everyday life and on special occasions, such as weddings. Hair is an important element of female beauty, and to cut it was considered shameful and disgraceful. So, after washing their hair, women would anoint it with oil to nourish it and make it shine. All of Greece abounds in beauty tips on how to produce lustrous black hair. In Archanes, in Crete, for example, a walnut tree root is placed inside a bottle with olive oil and buried in the soil for forty days. The oil is used to anoint the hair to make it full and wavy. In Lesbos, laurel seeds are crushed and browned in a pan with some olive oil and used for anointing hair. In Chios, crushed laurel seeds are placed in a bottle with olive oil. The bottle is kept in the sun for a few days to produce laurel oil and then used for washing to produce luxuriant hair.

Olive oil is used, along with other natural ingredients like vinegar, walnut tree, eucalyptus, and laurel leaves, to make hair stronger, shinier, and easy to comb.

Special care was taken with the dyeing and combing of a bride's hair, in a specific ritual procedure. One of the bride's brothers dropped some oil on her hair, while women sang:

*Mother, bring the olive oil, the oleaster olive oil
to anoint my hair, so that no one can cut it
and cast spells on me...*

In Thrace and Lesbos among the gifts that the groom sent to his bride before the wedding there were cosmetics for her bath. These contained olive oil and red dye for her hair. Olive oil is the basic ingredient for the preparation of dye for white hair.

They would use seven ingredients: candle from the church, mastic, olive oil, unsalted

fresh butter, lemon, mercury, resin and white lead (= white stone that was powdered). They mixed all the ingredients and applied them on the face during the night. To prevent the mixture from spoiling the woman who prepared it should tell lies.

*You used make-up and lipstick
And you made me crazy about you*

Religion and Worship

Olive oil was used to worship the Gods and to care for and honor the dead (Kambanis, 2004). Besides these vital spiritual functions, it also enjoyed prolific use in ancient medicine. But it also played an important role in ancient athletics (Stambolidis, 2004). The awareness that the olive tree and its oil are beneficial to the body contributed to the sanctity in which Mediterranean populations held the tree and its products. Its origin was connected with gods and the use of its products was under the jurisdiction of the clergy. In Christian religious rituals, olive oil holds an important position, from baptism to funeral. Moreover, the length of life is symbolized by the length of time the oil in the sanctuary lamp burns.

The Christian Church, although emancipating itself in a short period of time from the confines of the Jewish context, absorbed all of the latter's elements which served the Church's special identity and mission. Among these elements was the symbolic and ritual use of oil, which was, however, enriched with an intent, Christ-orientated, messianic and ecumenical character. In the New Testament oil continues to be evaluated as a precious source of lighting and as a healing medium. Christ combined this healing attribute of oil with the spiritual and liberating force of truth. For that reason He sent off His disciples to exorcise the evil spirits and to heal sick people by using holy oil. It was especially common for young men to anoint the body by using pure olive oil during exercise, training and racing. These activities were performed on a daily basis by large groups of citizens in ancient cities. Here we can see a scene at the ring, where a young man pours oil from an *arivalos* (earthenware vessel, wide at the bottom and narrow on top, used by athletes for anointing) to anoint his body, while the young slave on the left takes care of his master's foot. It is worth mentioning a special profession, the oil anointers, who used the oil to massage parts of the athletes' bodies that were sensitive from injuries.

Popular tradition assigns the olive tree a divine origin and the tree is associated with rituals and important religious locales. In modern worship, which retains elements from the ancient worship, elements from the Old Testament and Christianity are also incorporated. Olive oil holds a significant position in the official religious ceremonies. It is also important in secondary, quasi-magical ceremonies. So, when a new church is founded a lighted cresset full of olive oil is placed at the site of the altar and buried with rocks and soil "for the cresset to burn until the church is built." Near the cresset are written the names of the faithful, who will thereby obtain eternal

forgiveness.

Oil is used in other major ceremonies of the Church, such as baptism, confirmation and Holy Unction. Its initiatory, communicatory, and apotropaic significance is clear, in that it wards off evil from the Christian initiate and heals the wounds of the sinner. The baptized thus becomes an “athlete” of the Church and his body is to be anointed, so that he may fight the good fight (Karapidakis and Yfantis, 2004).

Apart from its ceremonial use, oil is used for lighting in every day routine in Christian churches, a habit inherited from ancient Greek, Jewish, and Christian religions. This role of oil as a means of illumination can be seen in many manifestations of popular religion, such as the eternally burning lamp in front of the altar, the other lamps in church, in cemeteries, or wayside shrines and the lamp that burns before the icon at home. From Messene, during the German occupation of 1941-44, a typical example that illustrates the role of oil as a metaphor for spiritual illumination in popular piety. While not using a single drop for cooking, a woman ensured that the lamp in front of the icon was always lit, offering up a prayer every day before departing for the fields. The symbolic importance of light, and the woman’s consciousness of it, is illustrated by her words, “How could I say this prayer if I left my icons in the dark?”

The habit of making an offering of olive oil, bread, and wine has existed since antiquity, as has the use of oil and olives in burial customs. The need of the deceased not to “end” with death explains the presence of a lamp on the grave. In a funeral song from Peloponnese, the dead brother and his living sister are compared to olive trees:

*The vineyard fence burnt, the garden burnt
The two olive trees burnt
Oh! My dear brother!
One of them burnt and fell and has no more worries
The other burnt and still stands and how will it survive?
My brother!*

A very characteristic phrase from a funeral song refers to the bad luck of the person who died away from his country and was thus not buried according to his country’s customs:

He was buried without olive oil on his eyes in a wild land.

Methods of Olive Oil Measurement

Galen, the medical writer (30-200 AD) gives some general information on methods of measuring liquids in the Greco-Roman World.

“Weights are evaluated based on their weight. Measures are evaluated based on the vessel’s capacity. The vessel measures either liquid or solid amounts. There are three different methods of measuring an amount; one by weights, one by solid substances like soil and one by liquid substances. There are various weights and measures depending on the country and the habits of the people that use them. We only mention the most common ones.”

Wine and olive oil are important products, especially in the Mediterranean, and methods of measuring quantity were naturally a matter of concern to Mediterranean communities that traded in these products. Various vessels, such as amphorae, skins, gallons, barrels, jars, wooden bowls, and other vessels, were used to define weight and volume of both wine and oil.

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2

Characteristics of the Olive Tree and Olive Fruit

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History of the Olive Tree

Olive is the common name for about 35 species of evergreen shrubs and trees of the genus *Olea* in the olive family, the *Oleaceae*, native to tropical and warm temperate regions. The name is especially used for *Olea Europaea*, the well-known olive which is grown for its edible fruits.

Olive trees are native to Greece, Italy, Palestine, and Syria, but different species are native to different areas. It is believed that cultivation of olives started around the fourth millennium B.C. in the area which is today Syria and Palestine. The inhabitants of Crete during the Minoan civilization cultivated olives as early as 2500 B.C. Pottery items such as jars found in Knossos Palace were probably intended for storing olive oil.

The botanical origin of the tree and the beginning of its cultivation have been a subject of dispute (Anon, 1983, Loukas and Krimbas, 1983, Blazquez, 1996). Archeologists tend to believe that the transformation to the cultivated tree should be placed in the early Bronze Age. During this period management of olive populations consisting of intentional and selective pruning have been probably applied by man to rejuvenate olive tree in order to favor flowering and fruit production.

From the 35 known species of the genus *olea* the one that is considered to be the ancestor of olive oil is *O. Chrysophylla*, found in Asia and Africa. There is, however, another theory according to which the progenitor is the Mediterranean wild olive, *olea oleaster* (Loukas and Krimbas, 1983). Others consider *olea oleaster* as an intermediate in the development from the wild olive tree *olea chrysophylla* to *olea europaea* (Blazquez, 1996, Lavee, 1996).

The spread of the olive tree to western places is due to Phoenicians who traded with other maritime centers. From the sixteenth century B.C., the tree began to reach the Greek islands and also Libya and Carthage. The Greeks extended olive farming and spread it through their colonies and routes taken by their seamen. The island of Samos was called "Elaeophytos," which means "planted with olives." The first sig-

nificant improvement of olive cultivation and a better organization occurred in the seventh century B.C. (Fiorino and Nizzi Griffi, 1992).

Later, the Romans discovered olive trees through their contacts with the Greek colonies in Italy. Although they were not admirers of olives and olive oil, the Romans expanded the tree throughout the huge empire. They used olive oil in their baths and as a fuel, but, for edible purposes, they considered it as a commodity of moderate quality. The rise of the Roman Empire and the conquest of Greece, Asia Minor, and Egypt increased the trading channels around the Mediterranean Sea and olive oil became far more important, not only as a staple food, but also as a pharmaceutical and a source of energy (Chazau-Gilling, 1994).

Expansion of olive growing continued until the fifth century A.D. and revived again when maritime cities began to grow. Between the twelfth and sixteenth century A.D., an impressive advance of olive oil orchards was observed in Italy (Fiorino and Nizzi-Griffi, 1992).

When America was discovered, missionaries and early settlers introduced wine and olive trees to the New World. Wine spread everywhere, but olive trees were cultivated only in restricted areas in Chile, Argentina, and California.

During the nineteenth century, olive farming reached a peak because lighting was still based on fatty substances, and oil seeds were not known enough to be exploited as sources of edible oils as they are today. The cultivation of the olive tree has now been extended to many regions of the world where climatic and other conditions are as favorable as those prevailing in the Mediterranean countries.

Until the advent of present-day propagation and cultivation techniques, olive trees usually began to crop after their eighth year. They could live to an age of several centuries and there are claims that olive trees exist with an age of more than one thousand years. One explanation for this longevity is its characteristic ability to send out shoots and roots from temporary buds which are abundant at the lower part of the trunk. The root system of the tree tends to spread horizontally rather than downwards. The tree is resistant to adverse conditions and adapts to all kinds of soils (even poor soils that cannot be used for other crops). It can sprout even if the part above the soil is seriously wounded. It can also tolerate dryness and lack of treatment better than most other fruit trees.

The olive tree is a broad evergreen tree. Depending on the subspecies and environmental conditions, its height may vary from 3 to 20 meters. The cost of modern cultivation and harvesting has made low shapes (4-5 meters in height) very popular to farmers in many olive-producing countries.

The trunk of the young tree is smooth with a green color, but later it becomes uneven and tends to twine and often hollow with the passing of time. The bark, greenish-gray in young trees, becomes dark gray as the tree grows older. The flowers are small, yellow-white, and appear in erect clusters. The leaves are narrow, lanceolate, leatherish, and persistent (they stay on the tree for 3 years). Their color is green above and silky white beneath.

The olive is widespread in many arid and semi-arid regions. It is traditionally dry-farmed but it can benefit from irrigation. In the Mediterranean area, climate conditions are favorable because rainfalls are frequent in the period from autumn to early spring. Thus, lack of humidity during the period of flower cluster formation is not normally observed. However, in dry years watering before blossoming is needed.

The olive tree has some tolerance to saline water. Researchers are experimenting with various cultivars to study the genotype response to sodium chloride salinity (Therios and Misopolinos, 1988) or to explore the potential use of brackish water for crop production (Briccoli et al, 1994).

The cultivation of the olive tree is heterogenous and varietal uniformity, even within restricted areas, is sometimes lacking. Thus, it is not always easy to develop cultural techniques to regulate production and quality of orchard harvests.

Normally the olive tree is very resistant to unfavorable conditions, but it is also a demanding crop if it is to produce well. Therefore, a suitable environment and proper cultural care (irrigation, control of harvesting, good nutritional conditions, pruning, and pest control) are necessary for the full development of the agronomic characteristics of the tree. Besides cultural care, other measures suggested to ensure a more steady fruit production are identity of cultivars (Sweeney, 2003) and pollinators. New cultivars can be used which have a reduced biennial bearing. Pollinators are suggested when the chief variety is incompatible or when an orchard is set up with a cultivar not widespread in the area. Foliar fertilization has also been tested as a means to encourage shoot growth, reduce alternate bearing and increase plant production (Cimato, 1994, Inglese, 2002, Connell, 2002). Recently, deficit irrigation strategies have been proposed in order to save water without reducing yields or modifying unfavorably color characteristics and phenolic composition (Fontanazza, 1996, Romero, 2002, Alegre, 2002).

Due to modern techniques of propagation and cultivation today's olive trees usually begin to crop after the 3rd year. Full productivity is developed between 11th and 12th year in dry groves and 7th and 8th year in irrigated groves (Fontanazza, 1996).

The oils produced in Tunisia often have a linoleic acid content higher than the level suggested in the olive oil norms and the European Union Regulations. To overcome this problem a lot of research work has been carried out (Fourati et al, 2002, Kamoun et al, 2002, Khlif et al., 2002) for better identification of varieties, cloning and genetic amelioration by crossing. Such techniques are expected to result in better chemical characteristics of the produced oil (less linoleic acid, better stability, higher phenolics content). Crossbreeding has also been applied in China for the creation of cultivars better adapted to local conditions (Fontanazza, 1996).

Varieties

The olive tree has many varieties which exhibit major or minor phenotypical and genetic differences. Today, most of the differences in size, color, oil content, fatty acid composition, and other properties have been recorded in the main olive growing

countries. The most important varieties have been discussed by Fontanazza (1996).

Some of them are only of local interest, others are more widely distributed. The same olive cultivars can be used for table olives and oil production but generally olives for oil production have a lower pulp to kernel ratio (4:1-7:1) in relation to the same ratio of olives for the preparation of table olives (7:1-10:1). As emphasized by Essadki and Ouazzani (2003), the task of identifying and classifying olive varieties is very complex.

The Olive Fruit. The fruit of the *Olea europea* is an oval-shaped drupe. It consists of a pericarp and endocarp (kernel, pit). It weighs from 2-12 g, although some varieties may weigh as much as 20g. The pericarp has two parts: the epicarp (skin) and the mesocarp (flesh, pulp) which accounts for about 65-83% of the total weight. The endocarp (kernel pit) may vary from 13% to 30%. The epicarp is covered with wax and turns from light green to black as the fruit ripens.

The fruit contains water (up to 70%) which is called "vegetable" water. The average chemical composition of the olive fruit is: water, 50%; protein, 1.6%; oil 22%; carbohydrates, 19%; cellulose, 5.8%; minerals (ash) 1.5%. Other important constituents are pectins, organic acids, pigments, and glycosides of phenols. Some of the components or their hydrolysis products are found in the vegetable water which is squeezed with the oil during processing and is separated by centrifugation.

Fruit weight increases in various phases until October or mid-November. Then, it begins to decrease, basically through loss of moisture. As a result, a rise in oil content is observed, usually from October to December. The oil accumulation starts in the period from late July to beginning of August. Through the autumn and winter, the fruit becomes black and the oil content reaches its maximum. Oil is mainly concentrated in the pericarp (96-98%). The formation and concentration of oil in the drupe, a rich reservoir of many classes of lipids, is possibly the reason why the oil has a unique flavor and fragrance.

The maturation of the olive is a slow and long process which lasts several months and varies according to the latitude of the growing area, variety, water availability, temperature, and cultural practices. In order to obtain a characteristically fragrant but delicately flavored oil, it is imperative that it is properly extracted from mature, undamaged olives. Therefore, the degree of ripeness is an important quality factor. From a scientific point of view, it is difficult to measure and express in mathematical terms the contribution of each factor to overall quality of the extracted oil. According to Montedoro and his co-workers (1986), the stage of maturity has a 30% contribution. Other factors contribute according to the following percentage: variety 20%; harvest 5%; transportation and pre-milling storage 15%; system of extraction 30%.

The first stage of ripening is known as the "green" stage. This corresponds to green mature fruits which have reached their final dimensions. Afterwards chlorophylls in the skin are gradually replaced by anthocyanins. This is the transition to a "spotted," "purple" and "black" stage. At the stage between the yellow green and purple skin

(veraison) the olives have the highest phenolic compounds content.

Various methods have been proposed for determining the stage of maturity of olives. Among them, the ratio of spectrophotometric absorbance of the olive paste in the visible region at two different wavelengths (665 nm and 525 nm). The assessment of volatiles, and the ratio maleic/citric acid are often reported. The International Olive Oil Council (1984) has suggested a simple technique which is based on the assessment of the color of 100 olives which are randomly drawn from 1 Kg of the sample. To calculate the maturation index the following formula is used:

$$\text{Maturation} = \frac{(0 \times n_0) + (1 \times n_1) + (2 \times n_2) \dots \dots \dots + (7 \times n_7)}{100}$$

where $n_0, n_1, n_2, \dots, n_7$, are the number of olives belonging to each of the following eight categories.

- 0 = Olives the skin of which is a deep or dark green color.
- 1 = Olives the skin of which is a yellow or yellowish-green color.
- 2 = Olives the skin of which is a yellowish color with reddish spots.
- 3 = Olives the skin of which is a reddish or light violet color.
- 4 = Olives the skin of which is black and the flesh is still completely green.
- 5 = Olives the skin of which is black and the flesh is a violet color halfway through.
- 6 = Olives the skin of which is black and the flesh is a violet color almost right through the stone.
- 7 = Olives the skin of which is black and the flesh is completely dark.

According to this approach, the best harvesting period is when the maturity value is 5. This index has a relative value and its use cannot be generalized because olive variety, growing area, climatic conditions and other minor parameters significantly affect the ripening index. Thus, the index has to be calculated in various producing countries on a case by case basis, by correlating the maturation value of olives from certain areas to quality of the oil that is produced and the level of phenolic compounds. Generally, the fruit polyphenols level reaches its peak at the turn point yellow-green-purple skin. Consequently, the decision to produce a more mellow product with a higher oil content or a more pungent oil, is highly dependent on harvest time, which is the most significant factor for the variations in composition and sensory qualities. The decision to produce highly pungent oil or alternatively, a mellow product is based on harvest time.

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3

Olive Oil in the World Market

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Introduction

As a commodity, olive oil has been and is still playing an important role in the world market of vegetable oils. Albeit a small share in world edible vegetable oils, less than 3.5 % (Tió, 1996), it has for centuries dominated the countries of the Mediterranean basin, which account for nearly 98% of the olive oil and table olives world production. The agronomic and climatic factors characteristic only to the Mediterranean Basin provided the necessary conditions for the development of such a significant olive oil and table olives sector.

The existence of the wild olive tree is lost in prehistoric times. Excavations in the Aegean Sea have unearthed fossilized olive leaves, dating more than 60,000 years. However, written evidence of the cultivation and production of olive oil are attested in the 3rd millennium B.C. in Evla (North Syria). The ideograms representing the olive tree, the olive fruit and the olive oil, found in the Cretan Linear A and B date from 6th millennium B.C. and are acknowledged to be of a high standard of pictography.

The Minoans, expert navigators and capable traders, spread the cultivation of the olive tree westwards across the Mediterranean. Over the ages it became a major type of cultivation and a significant source of agricultural income. Nutrition and cooking methods were shaped by this abundance of olive oil to what is nowadays known as the Mediterranean diet.

Olive oil gave wealth and health to the Mediterranean people and penetrated every aspect of their life. In myths, religious ceremonies, traditions, olive oil was ever-present.

High quality olive oil was used as nourishment and in the preparation of cosmetics and perfumes, where it was mixed with other ingredients. Inferior quality olive oil was equally valuable as it was used in lamps for lighting and to make soap.

With the expansion and development of olive oil production a whole range of occupations emerged, associated with storing, mixing, trading and the development of new products.

In later times, scientific and technological progress, such as the use of steam, and electricity, gave a boost to the olive mill business. Higher yields were achieved and of a much better quality of olive oil. Trade also increased and local societies flourished. In the 19th and 20th century the massive immigration of Italian, Greek and other Mediterranean people to the U.S.A., Canada, Australia, and Northern Europe brought the rest of the world in contact with their cuisines and nutritional habits. Nevertheless, olive oil remained, at all times, both in production and in consumption, a distinctively Mediterranean feature.

Only recently, an important but still vague change seems to be taking place. With the “Seven Countries Study” of Prof. Ancel Keys as a starting point, the Mediterranean Nutrition concepts have begun to command the respect of the medical world. Olive oil therefore became more and more popular among non-traditional consumers. At the same time fairly new countries such as Australia, Argentina, Chile, China, New Zealand, South Africa, the U.S.A., have begun to invest in the development of olive tree cultivation and the marketing of olive oil. Similarly, Egypt, Jordan, Lebanon and other North African, and Middle East countries have turned their attention to the development of the relatively insignificant olive oil sector they had.

What the future holds for this surge towards olive oil remains to be seen. Its extent will eventually settle whether the 21st century will bring to an end the specifically Mediterranean character of olive oil broadening it to cover the whole of the temperate zone around the world.

Olive Oil Facts and Figures

The available statistical data constitute a valuable source of information but two preliminary remarks should be taken into account. Firstly, the official data of production in the E.U. countries are determined according to the quantities eligible for the subsidy given to the producer. This official data do not automatically coincide with real market estimates, which, usually, fall short of the quantities officially specified as production. Official production data determine the quantities put down as consumption since after taking into account the import and export data of every country the balance must close. An additional difficulty, as far as the E.U. trade statistics are concerned, is that they are presented into two different ways causing difficulties when trying to isolate comparable quantities of exports and imports. The available statistical data, for a period of time, regarded the E.U. as a single state, in other words intra Community trade was not accounted for. Meanwhile, at different periods of time, each Member State of the E.U. was considered as a separate entity which means that intra Community as well as imports are accounted for.

World Olive Oil Production

The olive tree requires a mild winter and a warm and dry summer. Olive groves are

generally found where rainfall exceeds 60cm per annum, although they may be found in areas with only 40cm if the soil is particularly water retentive. Although the olive tree prefers light deep soils, it may be grown successfully on many types of soil, even in the most arid, stony, infertile and sloppy soils. On these marginal lands, olive growing may be the only feasible alternative to abandonment and desertification.

The “Mediterranean forest” that grew out of the extensive olive tree cultivation in the area is split into thousands of small holdings. These serve as a constant link between the product and all social and cultural life. In most Mediterranean countries, therefore, the prevailing type of holding is small, labor intensive, family run, with limited capital access, where olive growing is a supplementary but vital agricultural activity. This fragmentation, along with the family type holdings, explains why management is not always determined by strictly economic decisions.

The supply function is mainly determined by long-term and structural factors. The olive tree is a permanent cultivation, so growers’ decisions are slightly affected by yearly price fluctuations. The grower’s priority is to maximize the yields of his trees (volume of production) – keeping or improving the quality – and then to seek how to achieve the higher price. Hence, the price elasticity coefficient, in the short run, is less than the unit. In the long run the olive oil supply is affected by a number of structural factors:

- The future prices prospects
- The Common Agricultural Policy (in E.U.) and the national policies (in third countries) framework (i.e. subsidies and other incentives)
- The number of trees (new plantations)
- Investment in agronomic improvement
- Labor availability and costs

Labor is one of the most important factors. Depending on the yields, harvesting represents one third up to half of the total cultivation costs. The picking of olives is carried out during the winter months, a period of relative inactivity in farming. In this sense olive cultivation contributes significantly to the rural community’s overall income. The gradual drop in numbers of the agricultural population in the EU States and the continuing dwindling of the labor force have led to the wide use of economic immigrants arriving from North Africa or the Balkans.

The biennial phase of production is a very important characteristic that causes wide fluctuations in volume of yearly productions. The manifestation of this occurrence is palpably evident for each distinct area. When larger areas are considered, the forces of alternate bearing balance themselves out. Good farming practices, such as pruning, fertilizing and irrigation, may reduce but not eliminate these fluctuations. The volume of production, and quality, may also be affected by adverse climatic conditions, such as drought or frost and cause abrupt decrease of production (e.g. drought in Spain in 1995/96). In order to overcome those short-term variables it is better to examine the long-term economic and statistical tendencies by using the

TABLE 3.1
The World Olive Oil Production, in selected countries and groupings during 20
crop years 1985/86–2004/05 (Quantities in '000 tonnes)

1a. The 4 crop year averages

Countries	1985/86	1989/90	1993/94	1997/98	2001/02
	1988/89	1992/93	1996/97	2000/01	2004/05
Spain	505.03	601.58	593.65	877.93	1,154.25
Italy	498.28	462.70	489.50	566.88	683.93
Greece	278.05	289.48	348.50	424.50	375.08
European Union	1,315.83	1,392.20	1,472.13	1,910.63	2,248.75
Tunisia	94.50	168.75	166.25	162.00	123.75
Turkey	83.75	57.75	112.00	113.75	107.25
Syria	57.45	60.25	89.00	107.75	136.00
Morocco	35.75	47.25	57.50	52.50	63.75
The 4 Med	271.45	334.00	424.75	463.00	430.75
Rest of the World	78.35	89.45	103.38	105.38	132.88
World	1,665.63	1,815.65	2,000.25	2,452.00	2,812.38

Source: I.O.O.C., various documents

1b. The 20 year development

Countries	Change from 1985/86–1988/89 to 2001/02–2004/05		Annual Growth Rate (%)	Trend line
	(tn)	(%)		
Spain	649.23	228.55	4.49	$y = 37.958x + 347.93$
Italy	185.65	137.26	1.18	$y = 11.245x + 422.19$
Greece	97.03	134.89	1.61	$y = 8.6247x + 252.56$
European Union	932.93	170.90	2.45	$y = 57.921x + 1,059.7$
Tunisia	29.25	130.95	0.24	$y = 1.6098x + 126.15$
Turkey	23.50	128.06	3.83	$y = 3.1233x + 62.105$
Syria	78.55	236.73	8.49	$y = 5.575x + 31.552$
Morocco	28.00	178.32	1.17	$y = 1.4744x + 35.868$
The 4 Med	159.30	158.68	3.45	$y = 11.783x + 255.67$
Rest of the World	54.53	169.59	3.45	$y = 3.4772x + 65.374$
World	1,146.75	168.85	2.66	$y = 69.513x + 1,405.2$

Source: Table 1.a.

(Continued on next page)

four-year averages, instead of the simple yearly changes. The coefficient of biennial phase reveals higher fluctuations in the non-E.U. countries.

The biennial phase, together with the perennial nature, are not the only special characteristics of olive tree cultivation that make it significantly different from the other agricultural activities. Heterogeneity is another, since both yields and quality may differ from year to year in neighboring holdings as well (Lavee, 1996).

TABLE 3.1 (continued)
1c. The yearly fluctuations

Countries	min year	max year	mean year	Standard deviation	Biennial Phase ¹ (%)
Spain	337.60	1,412.00	746.49	298.97	37.00
Italy	163.30	760.00	540.26	151.71	55.00
Greece	170.00	473.00	343.12	75.56	24.00
European Union	993.70	2,463.70	1,667.91	415.98	23.00
Tunisia	35.00	280.00	143.05	73.21	86.00
Turkey	35.00	200.00	94.90	50.64	108.00
Syria	30.00	177.00	90.09	41.84	71.00
Morocco	30.00	110.00	51.35	21.12	47.00
The 4 Med	211.00	705.00	379.39	129.93	46.00
Rest of the World	67.00	154.40	101.89	26.46	26.00
World	1,442.10	3,164.50	2,149.18	489.76	17.00

Source: [Table 1.a.](#)

⁽¹⁾ Calculated on the basis of the formula

$$\begin{aligned}
 & t=N-1 \\
 _ & = 100 * (1 / (_ - 1)) * _ \log (X_t / X_{t-1}) \\
 & t=1
 \end{aligned}$$

During the last 20 years, world production increased by almost 70%, from 1.7 to 2.8 million tons. More than half of these additional quantities originated from Spain which increased its production from 505 to 1,154 thousand tons, reaching a record of 1,412 thousand tons in 2003/04. Italy is the second world producer with an average of 540 thousand tons, Greece the third (ø 343 thousand tons), Tunisia fourth (ø 143 thousand tons). Syria, which achieved the highest annual rate of growth (8.5%), slightly surpassed Turkey after the 2001/02 crop year.

The five E.U. Member States jointly share 74-80% of the world production and the group of the four other Mediterranean countries (Tunisia, Turkey, Syria, Morocco) have a share of 15-20%, leaving somewhat less of 5% for the rest of the world. After the recent enlargement of the E.U., three new producer countries (Cyprus, Slovenia and Malta) are added, but with a limited impact on Community olive oil production, of less than 0.5%. As far as the new producer countries are concerned the volume of product they contribute is still negligible. What is of special interest, is the geographical diversification they bring and their well organized efforts to increase volume, to improve quality and to enter the world market.

World Olive Oil Consumption

World consumption follows a parallel path with that of world production but with less marked fluctuations. The approach focuses on five different groups of countries.

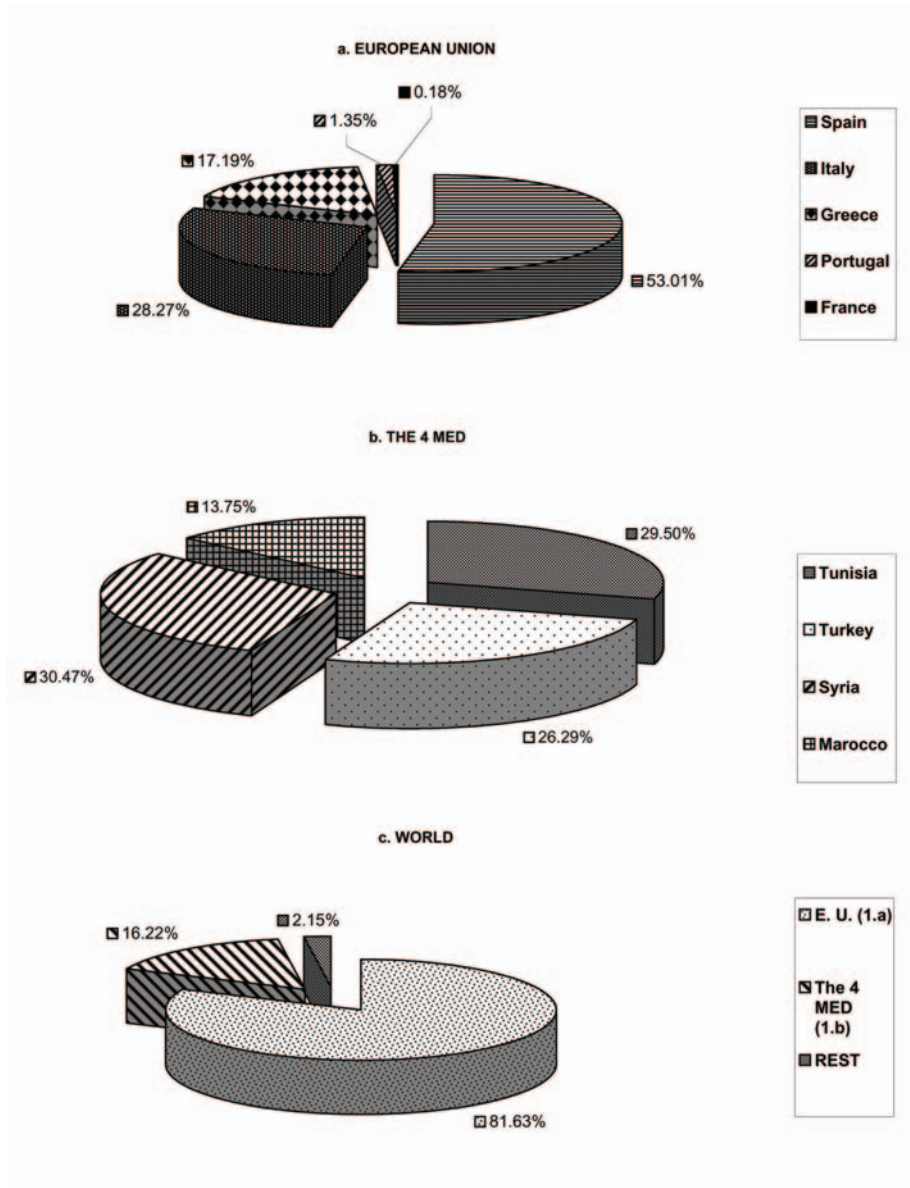


Fig. 3.1. % share of World Production in selected countries and groupings as at average 2000/01–2003/04

The most interesting group is that of the non-producing countries (France included) of the E.U. and the "Big New 5" (U.S.A., Australia, Canada, Japan, Brazil). With an annual rate of growth 10.06% and 8.42% respectively, their consumption has increased, in the last 20 years, by 417 thousand tons and has accumulatively reached 542 thousand tons (see Table 2) which already correspond to 20% of world consumption. This development is mainly due to the U.S. which has increased its consumption from 42 thousand in 1984 (kick off of the International Olive Oil Council promotional activities) to 248 thousand tons in 2004. In world consumption almost one million tons (+54%) were added to, during the 20-year period under consideration. This means an annual compound rate of growth of 2.6%.

The major olive oil producer countries are traditionally the world's leading consumers. Greece has the highest per capita consumption, 25 Kgr per capita, with Italy and Spain 12.3 and 12.6, respectively (European Commission, 2003). These three countries alone account for approximately 60% of world consumption and 83% of total European Community consumption. Their current high levels of consumption, which runs at an annual rate of 1.7%, limit their potential for future growth. Consumption trend in the 4 Mediterranean countries (Tunisia, Turkey, Syria and Morocco) is more or less similar, i.e. 1.9%. The key role for the increase of consumption lies, therefore, in the new, non-producer and non-traditionally consumer countries, which exhibit powerful trends.

Olive oil consumption has been for centuries restricted to the Mediterranean

TABLE 3.2
The World Olive Oil Consumption, in selected countries and groupings during 20 crop years 1985/86–2004/05 (Quantities in '000 tonnes)

Selected countries and groupings	Average	Average	Change		Annual Growth Rate (%)	World Share (%)
	1985/86 1988/89	2001/02 2004/05	tn	(%)		
(1) Italy + Spain+Greece +Portugal	1,283.10	1,708.05	424.95	33.12	1.67	62.38 %
(2) Tunisia+Turkey+Syria +Morocco	194.60	270.50	75.90	39.00	1.91	9.88 %
(3) Main producers (3)=(1)+(2)	1,477.70	1,978.55	500.85	33.89	1.70	72.26 %
(4) Rest of European Union (Northern)	38.68	229.05	190.37	492.24	10.06	9.81 %
(5) USA+Australia+Canada +Japan+Brazil	85.73	312.88	227.15	265.00	8.42	11.43 %
(6) New Consumers (6)=(4)+(5)	124.41	541.93	417.53	335.63	9.05	19.79 %
(7) Rest of the World	171.03	217.53	46.50	27.19	1.27	7.94 %
(8) World	1,773.13	2,738.00	964.88	54.42	2.59	100.00 %

Source: I.O.O.C., various documents

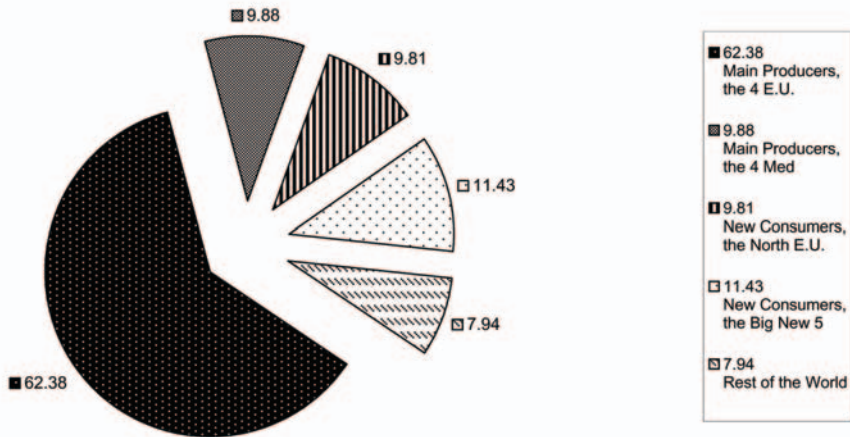


Fig. 3.2. % share of World Consumption in selected countries and groupings as at average 2000/01–2003/04

people. For the rest of the world olive oil was an unfamiliar oil, much more expensive than other vegetable (seed) oils, with the additional shortcoming of a strong flavor, not easily acceptable to non-trained consumers, as the taste for olive oil is usually acquired during childhood.

This consumer behavior had the following results: Firstly, a very limited total consumption outside the Mediterranean. Secondly, from all olive oil categories, the one best known was the category of “Olive Oil” (the term “Pure Olive Oil” has been abolished), a blend of refined olive oil and virgin olive oil. Obviously the absolute minimum of virgin olive oil is used to make the blend in order to tone down the strong flavor and bring the product as close as possible to the totally refined seed oils. Thirdly, the interest of olive oil producers was focused on the price ratio between olive oil and seed oils, which were considered as substitutes and directly competitive. In the beginning of the 80’s the European Community had seriously contemplated to impose a so-called “tax on oils and fats,” in order to safeguard that the price ratio would remain below 2:1. This was considered to be the limit that permitted olive oil to compete successfully with seed oils.

When examining the Consumption data (Table 2) it is evident that a drastic change took place in the 1990’s. This striking change in olive oil consumption was due to the very effective promotional campaigns organized by the International Olive Oil Council and financed by the European Union upgraded its image as a product of

high nutritional value (European Commission, 2003). Consumption started to climb rapidly and the “Olive Oil” category was gradually replaced by extra virgin olive oil. Obviously, all interest in fixing a price ratio was withdrawn. Olive oil began to break away from all other vegetable oils, as an altogether different product and could easily deal with competition in complete disregard to the price ratio.

Nevertheless, market surveys reveal that the demand function exhibits a different behavior depending on the country and the segment of the market under consideration but in all cases the cross elasticity coefficient has a positive sign, indicating substitutability between olive oil and seed oils (Zampounis, 1983).

If olive oil is approached as a segment of the overall vegetable edible oils market, then a particularly complicated and quite lengthy discussion of the issue is necessary. Comparisons between olive oil and other vegetable oils, bring out the following:

1. Quantitatively, olive oil consists of a very small portion, smaller than 3.5% of the world vegetable oils market.
2. Geographically, olive oil is restricted to the Mediterranean basin, while vegetable oils, tropical oils included (e.g. palm kernel oil) are met in all farmable lands of the world (e.g. soybean, sunflower, rapeseed, etc)
3. The cultivation of the olive is an enduring multiyear cultivation, labor intensive and met in relatively arid lands. The cultivation of vegetable oilseeds is annual, with high yields and is mechanized to a great extent. The production of olive oil, notwithstanding the table olive sector, is the economic reason for the cultivation of the olive tree. On the contrary, in many cases, vegetable oils are a by-product and the main economic reason of their cultivation is, following their crushing, the production of protein rich oilcakes (oil meals) for livestock feeding stuffs. The above account for the difference in price, that is the reason why olive oil merits a much higher price when compared to vegetable oils.
4. Among all vegetable oils, olive oil is the main commercially significant edible oil obtained by mechanical or physical processes (“virgin”).

Imports, Exports, and Balances

Tables 3 and 4 provide the basic data. In order to achieve a better understanding of world trade it is useful to separate countries into three separate groups.

The group of the “4Med” i.e. Tunisia, Turkey, Syria and Morocco, comprises countries that are clearly export orientated, with bulk olive oil transported to the Italian (and Spanish, to a lesser extent) packaging industry and with only some quantities exported in small packages directly to the U.S.A., North Europe, and other markets.

The second group consists of the clearly importing countries and can be divided into two sub-groups. The “Big New 5”(the U.S.A., Australia, Japan, Canada and Brazil) and “Northern Europe,” the E.U. non-olive oil producer countries, which, for analytical purposes, includes France also. These countries account for approximately 600 thousand tons of imports and consumption (Tables 2 and 3).

TABLE 3.3
The World Olive Oil Imports, in selected countries and groupings during 20 crop years 1985/86–2004/05 (Quantities in '000 tonnes)

Countries	The 4 crop year averages					change	
	1985/86	1989/90	1993/94	1997/98	2001/02		
	1988/89	1992/93	1996/97	2000/01	2004/05	tn	%
Spain	5.83	36.70	65.53	47.38	38.82	33.00	566.04
Italy	216.33	294.30	313.65	430.98	509.37	293.04	135.46
Greece	5.40	12.18	3.13	1.40	2.68	-2.72	-50.37
Northern Europe	87.8	92.15	142.52	225.07	280.54	19.74	219.52
European Union	315.33	435.33	524.83	704.83	831.41	516.09	163.67
USA	55.63	89.50	119.50	168.50	201.83	146.91	264.08
Australia	7.38	13.63	18.13	24.00	29.67	22.29	302.03
Canada	5.53	10.00	15.00	21.13	25.00	19.48	352.26
Japan	2.63	4.38	14.38	29.63	31.67	29.04	1104.18
Brazil	11.05	13.75	20.38	25.63	22.33	11.28	102.08
The Big New 5	82.20	131.25	187.38	268.88	310.50	228.30	277.79
Rest of the World	123.88	63.50	60.00	76.88	96.10	-27.78	-22.42
World	521.40	630.08	772.20	1,050.58	1,238.01	716.61	137.44

Source: I.O.O.C., various documents

TABLE 3.4
The World Olive Oil Exports, in selected countries and groupings during 20 crop years 1985/86–2004/05 (Quantities in '000 tonnes)

Countries	The 4 crop year averages					change	
	1985/86	1989/90	1993/94	1997/98	2001/02		
	1988/89	1992/93	1996/97	2000/01	2004/05	tn	%
Spain	179.60	238.98	250.05	387.83	604.65	425.05	236.66
Italy	82.58	122.25	160.00	229.57	303.81	221.24	267.91
Greece	80.55	99.53	126.84	148.88	105.68	25.13	31.20
European Union	396.48	488.9	569.11	809.77	"1,062.25"	665.78	167.92
Tunisia	50.23	104.5	105.88	124.75	90.17	39.94	79.51
Turkey	30.30	7.13	30.88	57.38	48.00	17.70	58.42
Syria	0.00	0.00	5.50	4.88	23.67	23.67	...
Morocco	0.03	8.38	12.88	5.88	9.50	9.48	...
The 4 Med	80.55	120.00	155.13	192.88	171.33	90.78	112.70
Rest of the World	7.45	15.38	17.63	15.75	18.00	10.55	141.61
World	484.48	624.40	741.86	"1,018.40"	"1,251.58"	767.11	158.34

Source: I.O.O.C., various documents

The U.S. market has become the biggest outside the Mediterranean. During the last 20 years imports and consumption have increased more than five times and they are now surpassing 200 thousand tons. Optimists claim that the 21st century belongs to olive oil because since 2000:

TABLE 3.5**The Balance of the 4 Med countries, during 1985/86–2004/05 (Quantities in '000 tonnes)**

	1985/86	1989/90	1993/94	1997/98	2001/02
(1) Production (1)	271	334	425	463	431
(2) Consumption (2)	195	206	232	278	271
(3) Availabilities (3) = (1) - (2)	76	128	193	185	160
(4) Exports (4)	81	120	155	193	171
(5) (4) : (3)	+ 6.6 %	- 6.3 %	-19.7% ¹	+ 4.3 %	+ 6.9 %

¹The 1996/97 crop year was exceptional at 705 thousand tonnes.

Source: [Tables 1, 2, 3](#) and [4](#)

- more than 30 % of households consume olive oil
- the gross value of retail sales surpassed 400 million \$ and olive oil enjoys the largest market share compared to all other vegetable oils
- according to market surveys the typical olive oil consumer is: well educated, prosperous, over 55 years old, lives at East Coast and belongs to a two member family. (NAOOA, 2001)

The third group consists of the “4 E.U.” producer countries, although each one of them presents a different profile.

Portugal consumes approximately 60-65 thousand tons, half of which are locally produced while the rest is imported, from the neighboring Spain.

Greece is the 3rd world producer, has the highest per capita consumption, negligible imports and is a net exporter at an average of 130 thousand tons which are directed: 87% to the Italian packaging industry in bulk, 2.5% similarly to the Spanish industry and the rest 10-11 % in small packages to the “Northern Europe” group of countries (4%) and outside Europe (7%).

The structural inadequacy of the Greek olive oil industry to export packaged product and hence to make the most of the added value of it, is reflected on internal prices ([Table 6](#) and [Figures 4a, 4b, 7](#)), which are lower than the equivalent Italian or the Spanish, despite the generally admitted high quality of Greek olive oil.

Spain, with a production that has already exceeded the one million tons, has become the unquestionable leader in world production (>40%) and in the E.U. (>50%). At the same time it puts forward a firm claim on the leadership of olive oil world trade. Spanish production is supplemented by a negligible 3% of bulk imports; 30 thousand tons from 3rd countries (the 4Med) and 10 thousand tons from Italy and Greece. In bulk exports to Italy remain still the principal export destination (250 thousand tons) and Portugal (45 thousand tons) a traditional market. However, Spanish promotional activities are focused on high value markets, where Spain, has been increasing its market share. Exports in small packaging have climbed to 180 thousand tons, which is almost equally divided, between 3rd countries (U.S.A., Australia, Japan etc) and the E.U. (France, U.K. etc).

The Spanish olive oil sector is characterized by a concentration of supply in the hands of the agricultural cooperatives with a subsequent strong negotiating power.

At the same time Spanish industry becomes stronger through local, European and international buyouts. Given the specific qualitative characteristics and despite the rapid increase of the volume of the Spanish production, producer prices follow a satisfactory trend when compared with prices of other countries (Table 6 and Figures 4a, 4b and 5)

Italy is the crossroads of world olive oil trade. A market open to all other countries either as an exporter, or an importer, or both simultaneously.

Italy is a net importer, since imports exceed exports by 214 thousand tons. Spain is the main supplier, with 249 thousand tons, Greece the second with 111 thousand

a. Spain			
Availabilities		Disappearance	
4.1 %	imports = 42		
1.1 %	other E.U. 11	Italy 249	45.9 %
3.0 %	4 Med 31	Greece 1	24.2 %
		Portugal 45	0.1 %
		North Europe 89	4.4 %
		Big 5 and world 89	8.6 %
98.7 %	Production 1.018		
		Consumption 558	54.1 %
	Stocks -29		

b. Italy			
Availabilities		Disappearance	
47.5 %	imports = 461		
25.7 %	Spain 249	Italy 245	25.3 %
11.4 %	Greece 111	Big 5 and world 163	16.8 %
10.4 %	4 Med 101	North Europe 82	8.5 %
59.5 %	Production 577	Consumption 725	74.7 %
	Stocks -68		

Source: Tables 1, 2, 3 and 4

Fig. 3.3. Balance of Spain and Italy, during 1996/97 - 2003/04 (Quantities in '000 tonnes). Source: Tables 1, 2, 3, and 4.

tons and 3rd countries (Tunisia, etc) with 101 thousand tons. These supplement the total available quantities, which amount to one million tons approximately.

Italy is also a traditional exporter, supplying the world market with attractive small packaging and well-known brands ever since the end of 19th century.

Making the most out of the considerable imported quantities and its high value added exports, the Italian industry is in the position to secure steadily for the Italian producer the highest producer prices (Table 6 and Figures 4a, 4b and 6) when compared with Greece and Spain.

Ending Stocks

During the ever continuing discussions on the reduction of the Common Agricultural Policy (CAP) budget, the press often used the phrase “the mountains of butter and the lakes of olive oil” in the ‘80s, in order to describe the surpluses resulting from the unrestricted quantities benefiting from production aid.

Although official statistics occasionally showed large stocks of olive oil, prices on the other hand, refused, as they logically ought to, fall.

Another point that should be taken into account is that, due to the yearly fluctuations in production and to changeable natural phenomena (drought, frost), large companies are obliged to have in stock a considerable amount of product in order to be able to satisfy demand and fulfill the agreements they have signed.

In the olive oil producing countries ending stocks seem to be moving around normal levels i.e. 5-10% of their total availabilities.

Prices

Olive oil, unlike seed oils (soya, colza, etc.), is not a commodity with one world reference price. Due to the heterogeneity of the physical product and its quality categorization, smaller, local markets are shaped, resulting in a variation of dissimilar prices, which do not bear easily to comparison or can be easily interpreted.

Table 6 presents a group of basic price series that have been extracted as averages of local markets. Out of these more broad-spectrum data some very useful conclusions could be drawn, taking always into account, the reservations expressed earlier about the difficulties attributable to production data.

The peaks at 1990/91 and mainly at 1995/96 illustrate the frost in Italy and Greece (1990/91) and the draught in Spain (1995/96).

During the last decade, price fluctuations tend to be much smoother because, apart from the ups and downs of the volume of production, they have been influenced by the following three factors:

- The operation of the single market in the European Union and the introduction of the Euro as of 2001, which have facilitated transactions.
- The preferential agreements and the inward processing agreements allow imports

TABLE 3.6**Producer Prices in Spain, Italy and Greece, during crop years 1989/90–2004/05 (prices in €/kg¹)**

a. SPAIN

Category	1989 /90	1990 /91	1991 /92	1992 /93	1993 /94	1994 /95	1995 /96	1996 /97	1997 /98	1998 /99	1999 /00	2000 /01	2001 /02	2002 /03	2003 /04	2004 /05 ²
Extra	1.63	1.69	1.71	1.95	2.32	2.76	3.64	2.46	1.83	2.33	1.91	1.71	1.85	2.03	2.34	2.57
Lampante	1.51	1.57	1.61	1.87	2.22	2.61	3.45	2.07	1.58	2.17	1.78	1.59	1.73	1.90	2.24	2.40

b. ITALY

Category	1989 /90	1990 /91	1991 /92	1992 /93	1993 /94	1994 /95	1995 /96	1996 /97	1997 /98	1998 /99	1999 /00	2000 /01	2001 /02	2002 /03	2003 /04	2004 /05 ²
Extra	2.70	4.30	2.48	2.45	2.78	3.27	4.22	3.64	2.57	2.62	2.26	2.25	2.44	2.58	2.60	2.68
Lampante	1.97	1.95	1.86	1.94	2.23	2.85	3.63	2.21	1.58	2.10	1.80	1.57	1.71	1.77	2.22	2.25

c. GREECE

Category	1989 /90	1990 /91	1991 /92	1992 /93	1993 /94	1994 /95	1995 /96	1996 /97	1997 /98	1998 /99	1999 /00	2000 /01	2001 /02	2002 /03	2003 /04	2004 /05 ²
Extra	1.53	2.44	1.71	1.72	1.99	2.25	3.28	2.53	1.95	2.14	1.81	1.75	2.11	1.98	2.51	2.48
Lampante	1.25	1.52	1.34	1.36	1.62	1.86	2.62	1.82	1.33	1.76	1.49	1.40	1.48	1.48	2.07	2.03

¹For the period 1989 to 2000 prices in national currencies were converted into euro according to the official parity of 1/1/2001:
1 € = 166.386 pta, 1,936.27 lire and 340.75 drachma

²Until May 2005

Source: Oils and Fats Management Committee of E.U., weekly editions.

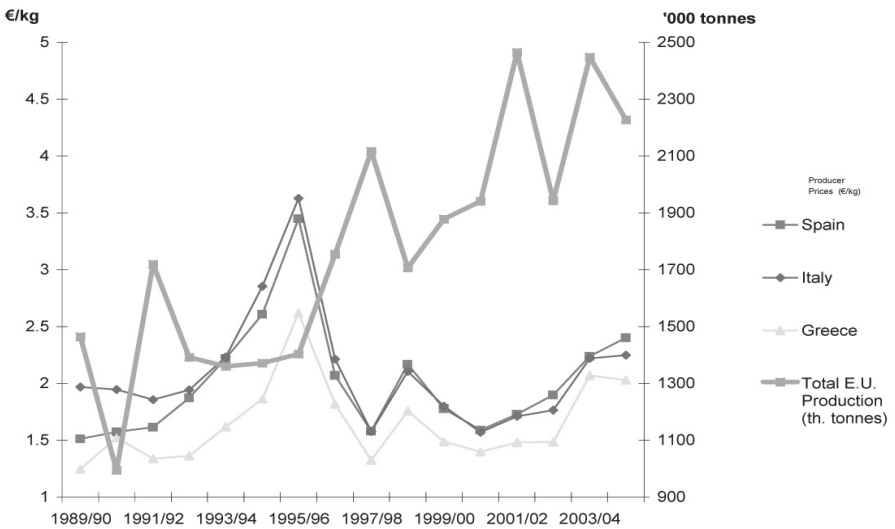
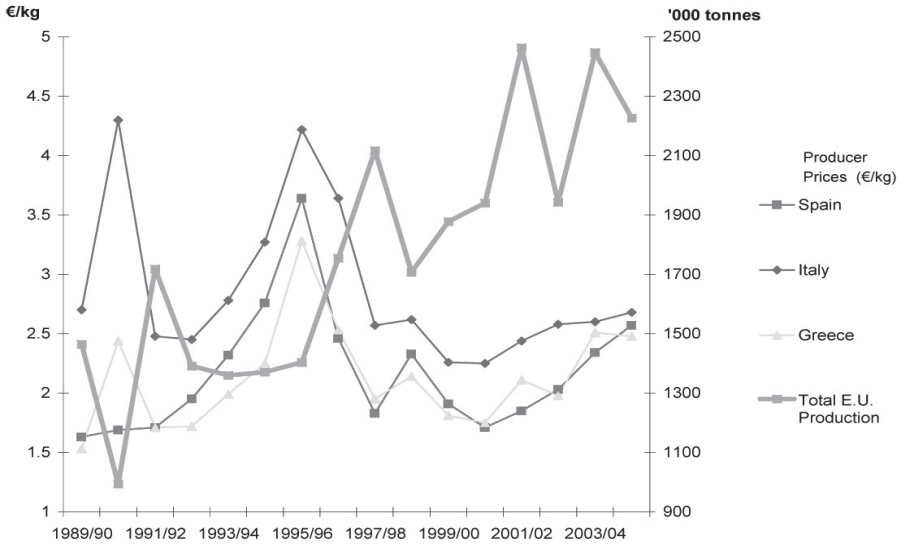


Fig. 3.4. (A) Producer Prices of Extra Virgin Olive Oil in Spain, Italy and Greece in relation to total E.U. production. (b) Producer Prices of Lampante Virgin Olive Oil in Spain, Italy and Greece in relation to total E.U. production

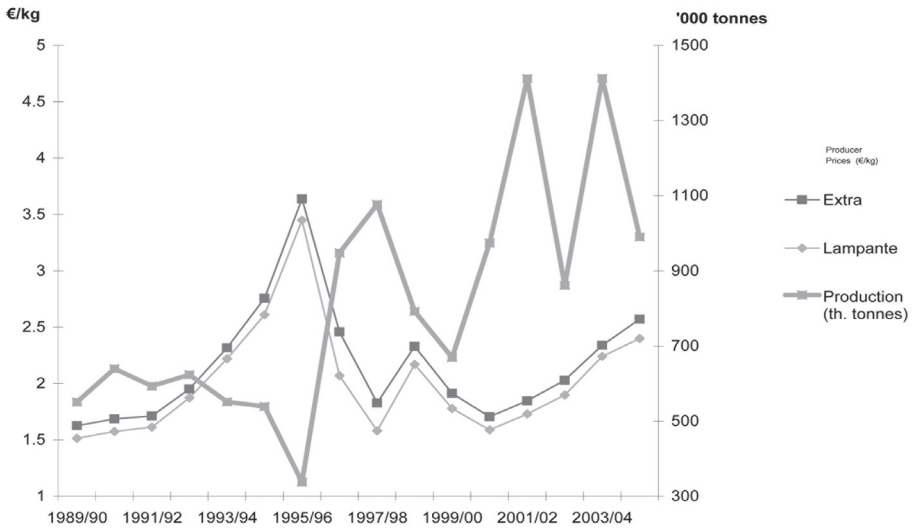


Fig. 3.5. Spain: Producer Prices of Virgin Olive Oil in relation to national production

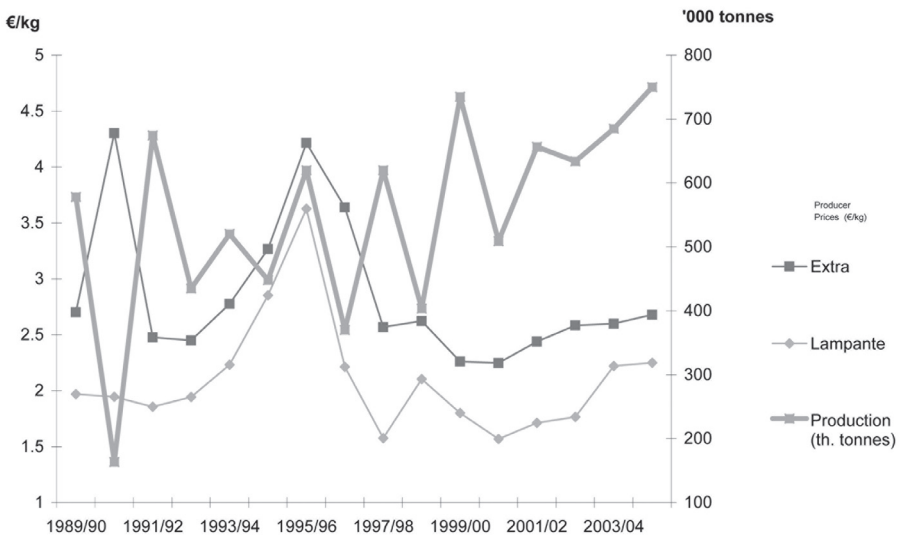


Fig. 3.6. Italy: Producer Prices of Virgin Olive Oil in relation to national production

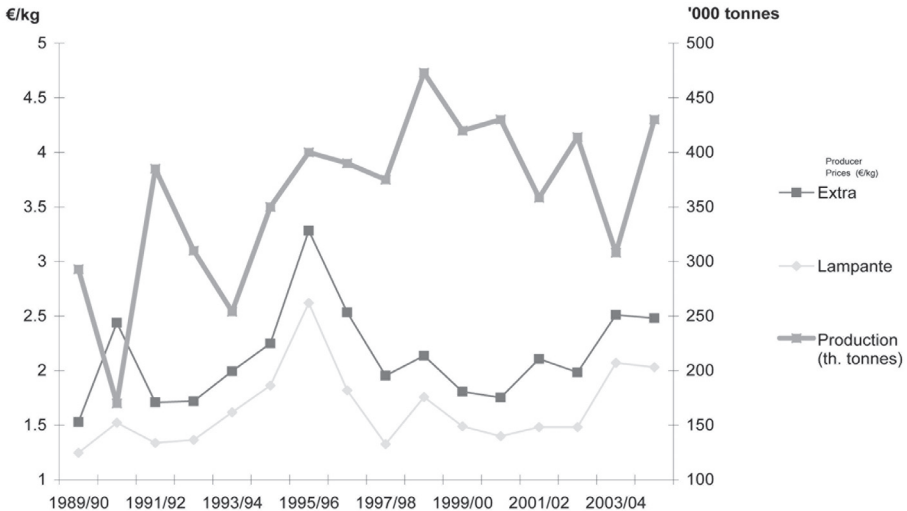


Fig. 3.7. Greece: Producer Prices of Virgin Olive Oil in relation to national production

into the E.U., from third countries (e.g. Tunisia), with insignificant or no import duties at all.

- The stocks held by the industry and the cooperatives following the years of rich crops.

European Agricultural Policies in the Olive Oil Sector

After the accession of Greece (1981) and especially that of Spain and Portugal (1986) to the E.U., no other policy can be said to have influenced the world olive oil market more than the olive oil Common Market Organisation (CMO), that makes part of the overall Common Agricultural Policy (CAP) of the E.U.

The olive oil CMO was established in 1966 with EU Regulation L 136/66 (OJEC 173, 30/09/1966). Even though the above regulation has been amended many times over the years, olive oil CMO has always comprised a network of cohesive policy measures aiming at specific targets:

1. To secure a fair income to the producer by granting a direct subsidy (Producer Aid), which is still in force and has always been absorbing the great part (more than 90 % after 1998) of the total available budget.

The other support mechanism, the Intervention, with minimum guaranteed prices and public stocks, was abolished in 1998.

TABLE 3.7
Allocation of the E.U. budget (EAGGF - Guarantee) for the olive oil sector during 1986 to 2000

Policy mechanism	% share	Member State	
Production Aid	69.3	Italy	43.90%
Consumption Aid	22.1	Spain	31.50%
Export Refunds	3.2	Greece	21.40%
Intervention	1.4		

Average budget

* 1986 to 2000 = 1,664 million ECU or 5.0 % of total EAGGF - Guarantee

* 2001 to 2003 = 2,398 million Euro or 5.5 % of total EAGGF - Guarantee

Source: Annual EAGGF budgets (Official Journals)

2. To encourage consumption by defending the competitiveness of olive oil in relation to other vegetable oils. This marketing subsidy was granted directly to the packing industries. It was abolished in 1998 while all efforts were concentrated on generic olive oil promotional activities.
3. To protect the Community olive oil from the abundant third country imports at low prices, various tools were established. Over the years these were adjusted to meet market developments and the WTO Agreement.

Export refunds were abolished in 1998.

A Common Customs Tariff and variable levy on imports are still in force, but significant preferential agreements with the other Mediterranean countries (especially with Tunisia) have been concluded, so that olive oils are imported at reduced, or even at zero custom duties. Under the inward processing system, olive oil is processed or packaged by the European industry and re-exported without any charge or refund.

4. To safeguard authenticity and quality of the product, at all stages till the final consumption.

Designations and definitions of all olive oil categories are obligatory for marketing within the Community and in trade with other countries. Methods of analysis are specific, detailed and regularly updated in order to take into account technical progress. Rules on labeling and packing in small containers are also obligatory at retail level.

The latest enlargement of E.U. to 25 Member States and the conclusion of Doha Round within WTO, have determined a new framework for the CAP, which has been put in force gradually according to the basic EU Regulation L 1782/03 (OJEC L 270, 21/10/2003). It brings a new philosophy, the “decoupling”, in other words the producer receives a fixed subsidy (production aid), that is fully disconnected from his/her actual production i.e. the volume of the olive oil produced.

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Chemistry, Properties, Health Effects

4

Olive Oil Composition

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Introduction

The composition of olive oil is primarily triacylglycerols (~99%) and secondarily free fatty acids, mono- and diacylglycerols, and an array of lipids such as hydrocarbons, sterols, aliphatic alcohols, tocopherols, and pigments. A plethora of phenolic and volatile compounds are also present. Some of these compounds contribute to the unique character of the oil.

Fatty Acids, Triacylglycerols, and Partial Glycerides

Fatty acids present in olive oil are palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3). Myristic (C14:0), heptadecanoic and eicosanoic acids are found in trace amounts. Scano and co-workers (1999), using ¹³C-Nuclear Magnetic Resonance Spectroscopy, detected traces of 11-*cis*-vaccenic and eicosenoic acids. Fatty acid compositional limits adopted in the most recent editions of Codex Alimentarius and International Olive Oil Council are given in Table 3.1.

IOOC also sets limits for *trans* fatty acids in each commercial category. For edible virgin olive oil categories the levels for C18:1*t* and for the sum of C18:2*t* and C18:3*t* isomers are extremely low (< 0.05% in each case).

Fatty acid composition may differ from sample to sample, depending on the zone of production, the latitude, the climate, the variety, and the stage of maturity of the fruit. Greek, Italian, and Spanish olive oils are low in linoleic and palmitic acids and they have a high percentage of oleic acid. Tunisian olive oils are high in linoleic and palmitic acids and lower in oleic acid. On the basis of the analysis of samples from various countries olive oils are classified in two types, one with a low linoleic-palmitic and high oleic acid content, and the other with a high linoleic-palmitic and low oleic acid content. Fatty acid composition of oil depends on the maturity stage. Ninni (1999) reported that oleic acid is formed first in the fruit and there is a strong

TABLE 4.1**Fatty Acid Composition as Determined by Gas Chromatography (% m/m methyl esters)**

Fatty Acid		Codex Alimentarius (2003)	IOOC*(2003)
lauric	C12:0	Not present in discernible amounts	Not specified
myristic	C14:0	< 0.1	< 0.05
palmitic	C16:0	7.5-20.0	7.5-20.0
palmitoleic	C16:1	0.3-3.5	0.3-3.5
heptadecanoic	C17:0	< 0.5	≤ 0.3
heptadecenoic	C17:1	< 0.6	≤ 0.3
stearic	C18:0	0.5-5.0	0.5-5.0
oleic	C18:1	55.0-83.0	55.0-83.0
linoleic	C18:2	3.5-21.0	3.5-21.0
linolenic	C18:3	**	≤ 1.0
arachidic	C20:0	0.8	≤ 0.6
eicosenoic	C20:1	Not specified	≤ 0.4
behenic	C22:0	< 0.3	≤ 0.2***
erucic	C22:1	Not present in discernible amounts	
lignoceric	C24:0	< 1.0	≤ 0.2

*The limits established include the precision values of the recommended method; **pending the results of IOOC survey and further consideration by the Committee on Fats and Oils, national limits may remain in place; ***Limit raised to <0.3 for olive-pomace oils.

antagonistic relationship between oleic and palmitic, palmitoleic and linoleic acids.

Characteristics and limits of physical and chemical indices and values, and of fatty acid composition for the various grades of virgin olive oils produced in each olive-growing area, determined at the onset and close to the olive oil production year are published yearly in national olive oil index files. Expansion of olive tree cultivation in countries of the Southern hemisphere (Australia, Argentina, New Zealand, and South Africa) as well as in California was the cause of a recent survey by the IOOC on the fatty acid composition of virgin olive oil produced around the world. Revision of Codex Alimentarius standards in order to be more representative of global production is therefore expected. Establishment of a maximum level for linolenic acid is of high priority as it can be used as a marker of adulteration. In the framework of this survey, 822 Australian olive oil samples were examined in the period 2002-03 at the Wagga Agricultural Institute (Mailer, 2005). Diversity in environment and cultivar characteristics had as a result wide ranges for the four major fatty acids (% methyl esters): (16:0), 7.8-18.8%; (18:1), 58.5-83.2%, (18:2), 2.8-21.1%; (18:3): 0.42-1.91%. The data deviated to a certain degree from those currently accepted by the Codex.

Slight deviations from the 1,3- random, 2-random distribution of fatty acids in the glycerol moiety of olive oil *triacylglycerols* have been found using advanced chromatographic and spectroscopic procedures (Santinelli et al., 1992; Vlahov, 2005). At the 2-position lower amounts of oleic and higher amounts of linoleic acid were evidenced in comparison to those obtained from calculations based on the theoretical

random pattern. Deviations depend on the total amount of oleic or linoleic acid in the oil. Like other vegetable oils, olive oil has a high concentration of oleic acid and a low concentration of palmitic ($\leq 2\%$) and stearic acids in position - 2 of the triacylglycerol molecules. The triacylglycerols found in significant proportions in olive oil are OOO (40-59%), POO (12-20%), OOL (12.5-20%), POL (5.5-7%) and SOO (3-7%) (Boskou, 1996). Smaller amounts of POP, POS, OLnL, LOL, OLnO, PLL, PLnO and LLL are also encountered (European Commission Regulation 282, 1998). Fully saturated moieties have not been reported and the same applies for the tri-unsaturated ones containing linolenic acid. Stearic and palmitic acids are absent from the 2-position of unsaturated species (tri- and tetraunsaturated) or from the molecule when there are more than five double bonds. Trilinolein or ECN 42 triacylglycerol content (as corrected recently), which is used as an authenticity marker by the EU, is the sum of the amounts of LLL, PoPoPo, SLnLn, PoPoL, PPOLn, OLLn, PLLn and PoOLn (positional isomers included). In the RP-HPLC chromatogram given in the same Regulation the triacylglycerol species assigned to the peaks according to elution order are LLL, OLnL, PLnL, LOL, OLnO, PLL, PLnO, LOL, PLO, OOO, SLO, POO, POL, SOO, SLS, and POS. Recent problems in the detection of hazelnut oil or almond oil in olive oil samples revitalized the interest in updating triacylglycerol compositional data (e.g. Parcerisa et al., 2000).

The presence of *partial glycerides* in olive oil is due either to incomplete triacylglycerol biosynthesis or hydrolytic reactions. In virgin olive oil, concentration of diacylglycerols (DG) range from 1 to 2.8% (Frega et al., 1993; Kiosseoglou and Kouzounas, 1993). In the diacylglycerol fraction C-34 and C-36 compounds prevail (Leone et al., 1988, Frega et al., 1993). Monoacylglycerols are present in much smaller quantities (less than 0.25%) whereas 1-species are considerably higher than the respective 2-monoglycerides. Their ratio depends on oil acidity (Paganuzzi, 1987; Paganuzzi, 1999). Storage conditions affect the distribution of fatty acids. 1,2-Diacylglycerols present in fresh oil tend to isomerize to the more stable 1,3-diacylglycerols. This rearrangement gives information about the age of the oil and storage conditions. The ratio of 1,3-/1,2-DG is considered as a useful criterion to monitor quality (Pérez-Camino et al., 2001; Spyros et al., 2004).

Hydrocarbons

Two hydrocarbons are present in considerable amounts in olive oil, squalene and β -carotene (the latter is discussed in the pigments section). Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is the last metabolite preceding sterol ring formation. Its presence is regarded as partially responsible for the beneficial health effects of olive oil and its chemopreventive action against certain cancers (Rao et al., 1998, Smith et al., 1998). It is the major constituent of the unsaponifiable matter (see [Glossary](#)) and makes up more than 90% of the hydrocarbon fraction (Perrin, 1992; Lanzón et al., 1994). It ranges from 200 to 7500 mg per kg oil (Perrin, 1992).

Even higher levels up to 12,000 mg/kg have been also reported (Lanzón et al., 1994). Squalene content depends on olive cultivar (De Leonardis et al., 1998; Manzi et al., 1998), oil extraction technology (Nergiz and Ünal, 1990), and it is dramatically reduced during the process of refining (Mariani et al., 1992; Lanzón et al., 1994). Variation in levels reported may be partially due to different analytical procedures used as commented by Nenadis and Tsimidou (2002).

Except for squalene, the hydrocarbon fraction of virgin olive oil is composed of diterpene and triterpene hydrocarbons, isoprenoidal polyolefins, and n-paraffins (Lanzón et al., 1994).

Tocopherols

Research on the occurrence and levels of tocopherols in virgin olive oils has shown that from the eight known "E-vitamins" the α -homologue comprises the 90% of the total tocopherol content. α -Tocopherol is found in the free form. The levels reported indicate a wide range of milligrams α -tocopherol per kg oil that depends on the cultivar potential and technological factors. Introduction of Good Manufacturing Practice and overall quality control programs supported by European Union for all edible types of olive oil had a positive impact on tocopherol levels in virgin olive oils of different origin. The levels currently found are much higher than the mean value of 100 mg/kg assigned to olive oil in the past (Gunstone et al., 1994; Belitz et al., 2004). This fact is important considering the *in vivo* antioxidant properties of the α -homologue (Kamal-Eldin and Appelqvist, 1996). Data for Italian and Spanish oils indicate a wide range for tocopherol levels: 55-264 mg/kg, $n=18$ (Conte et al., 1993); 97-315 mg/kg, $n=52$ (Fedeli and Cortesi, 1993); 100-320 mg/kg, $n=23$ (Esti et al., 1996); 147-187 mg/kg, $n=6$ (Ranalli and Angerosa, 1996); 103-283 mg/kg, $n=14$ (Cert et al., 1996); 160-253 mg/kg, $n=15$ (Manzi et al., 1998); 55-234 mg/kg, $n=65$ (Salvador et al., 1998). Compared to them, Greek oils have α -tocopherol levels that are among the highest reported (e.g. 127-370 mg/kg for 1994-95; 98-333 mg/kg for 1995-96 and 100-365 mg/kg for 1996-97 crop seasons) (Psomiadou et al., 2000). Low amounts of the homologues β -tocopherol (~ 10 mg/kg), δ -tocopherol (~ 10 mg/kg) and γ -tocopherol (~ 20 mg/kg) are usually reported. The levels of α -tocopherol may be related to the high levels of chlorophyll pigments and the concomitant requirement for singlet oxygen deactivation (Grams and Eskins, 1972). Tocopherol concentration seems to be reduced in the ripe fruits. Data on the influence of the extraction system vary (Psomiadou and Tsimidou, 1998; Beltran et al., 2005). Refining or hydrogenation causes loss of tocopherols (Andrikopoulos et al., 1989; Rabascall and Riera, 1987).

Pigments

Virgin olive oil color is the result of green and yellow hues due to the presence of chlorophylls and carotenoids. It is influenced by olive cultivar, maturation index,

production zone, extraction system, and storage conditions. Therefore it is considered as a quality index though no standardized method exists for its measurement.

Chlorophylls are encountered as pheophytins. Among the latter pheophytin α (Pheo α) is predominant (Mínguez-Mosquera et al., 1990; Mínguez-Mosquera et al., 1991; Rahmani and Csallany, 1991, Gandul-Rojas and Mínguez-Mosquera, 1996, Psomiadou and Tsimidou, 2001). The presence of Pheo α is related to processing conditions (Schwartz and Lorenzo, 1990), and enzymatic or enzymatic-like activity (Langmeier et al., 1993; Shioi et al., 1996). Handling and duration of storage cause further changes in pheophytin α content. The presence of pheophytin degradation products such as epimers, pyro-forms and allomers has been reported (e.g. Psomiadou and Tsimidou, 2001; Psomiadou and Tsimidou, 2002a). In a recent paper (Gallardo-Guerrero et al., 2005) the presence of pheophytin α degradation products was also evidenced for Spanish virgin olive oil samples stored under mild storage conditions for one year (15°C, dark, 3% headspace). These products, on the basis of previously reported findings, were identified as pyropheophytin α , 15¹-OH-lactone pheophytin α and 13²-OH-pheophytin α . Under light exposure green pigments degrade causing oil bleaching (Psomiadou and Tsimidou, 2002b). Pheophytin α levels depend heavily on analytical methodology. Spectrometric methods result in higher levels than those determined with NP- or RP-HPLC. Chlorophyll α can only be found in recently obtained oils. Chlorophyll and pheophytin b are also present though in minute amounts.

The main carotenoids present in olive oil are lutein and β -carotene (Mínguez-Mosquera et al., 1990; Ranalli, 1992; Rahmani and Csallany, 1991; Gandul-Rojas and Mínguez-Mosquera, 1996). Levels reported are related to analytical method used. The presence of carotenoids in olive oil is closely related to that of green pigments and is influenced by the same factors. The carotenoid fraction may also include several xanthophylls (violaxanthin, neoxanthin, luteoxanthin, antheraxanthin, mutatoxanthin, and β -cryptoxanthin). The ratio between the two major carotenoids seems to be cultivar dependent (Gandul-Rojas and Mínguez-Mosquera, 1996; Psomiadou and Tsimidou, 2001).

Aliphatic and Aromatic Alcohols

Aliphatic and aromatic alcohols present in olive oil are found in free and esterified form. The most important are fatty alcohols and diterpene alcohols. Alkanols and alkenols with less than ten carbon atoms in their molecule, which are present in free and esterified form, and some aromatic alcohols (benzyl alcohol and 2-phenylethanol) are constituents of the olive oil volatile fraction. Benzyl esters of hexacosanoic and octacosanoic acid have been also found in olive oil (Reiter and Lorbeer, 2001).

Fatty Alcohols. This class of minor constituents consists of linear saturated alcohols with more than 16 carbon atoms which are present in the free and esterified form.

The main fatty alcohols present in olive oil are docosanol, tetracosanol, hexacosanol, and octacosanol (Tiscornia et al., 1982; Boskou et al., 1983; Frega et al., 1992). Fatty alcohols with odd carbon atoms (tricosanol, pentacosanol, and heptacosanol) may be found in trace amounts (Tiscornia et al., 1982; Boskou et al., 1983).

Virgin olive oil total fatty alcohol levels are not usually higher than 250 mg/kg (Grob et al., 1990; Ranalli and Angerosa, 1996; Ranalli and Serraiocco, 1996; Cert et al., 1999; Aparicio and Luna, 2002). Tetracosanol and hexacosanol were found to be present at higher levels than the other fatty alcohols (Boskou et al., 1983; Ranalli and Angerosa, 1996; Ranalli and Serraiocco, 1996; Aparicio and Luna, 2002). Much higher levels were found in solvent extracted olive oils (Paganuzzi and Leoni, 1979; Tiscornia et al., 1982; Boskou et al., 1983; Grob et al., 1990). However, refined solvent extracted olive oils were found to contain fatty alcohols at concentrations similar to that of virgin olive oils (Grob et al., 1990). Fatty alcohol content is affected by cultivar, crop year, fruit ripeness, and processing (Camera and Angerosa, 1978; Ranalli and Angerosa, 1996; Ranalli and Serraiocco, 1996; Cert et al., 1999; Aparicio and Luna, 2002).

Esters of fatty alcohols with fatty acids (waxes) are important minor olive oil constituents because they can be used as a criterion to differentiate various olive oil types (EC Regulation 2568, 1991). The main waxes detected in olive oil are esters of oleic or palmitic acid with 36, 38, 40, 42, 44, and 46 carbon atoms (Reiter and Lorbeer, 2001). Virgin olive oils contain waxes at levels lower than 150 mg/kg, while raw and refined solvent extracted olive oils have wax content higher than 2000 mg/kg (Grob et al., 1990). This difference is officially used for the distinction between olive oil and solvent extracted olive oil. Wax content and composition are affected by cultivar, crop year, and processing (Ranalli and Angerosa, 1996; Ranalli and Serraiocco, 1996; Cert et al., 1999).

Diterpene Alcohols. Phytol and geranylgeraniol are two acyclic diterpenoids (Fig.4.1) present in the aliphatic alcohol fraction of olive oil in the free and esterified form (Camera and Angerosa, 1978; Paganuzzi and Leoni, 1979; Mariani et al., 1992; Cert et al., 1999; Reiter and Lorbeer, 2001). Phytol, which probably originates from chlorophyll, has been found in monovarietal virgin olive oils at levels ranging from 25 to 595 mg/kg (Aparicio and Luna, 2002). Geranylgeraniol is reported to be present in virgin olive oil from a new olive cultivar – I-77 at levels lower than 50 mg/kg (Ranalli et al., 2000). Its levels are used in the calculation of the alcoholic index, a useful parameter for detecting solvent extracted olive oil in virgin olive oil. Esters identified in the wax fraction of extra virgin olive oil are oleate, eicosanoate, eicosenoate, docosanoate, and tetracosanoate (Reiter and Lorbeer, 2001). They are mainly phytyl derivatives.

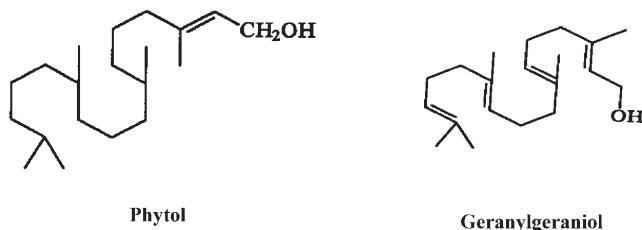


Fig. 4.1. Structural formulae of the olive oil diterpene alcohols.

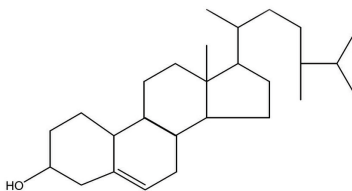
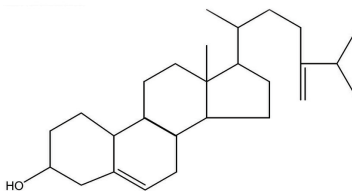
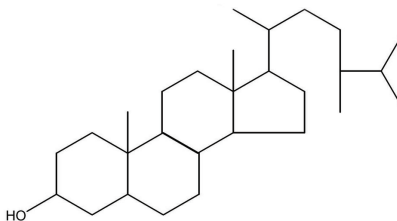
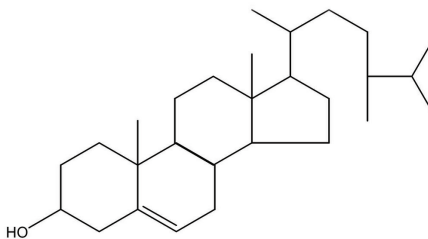
Sterols

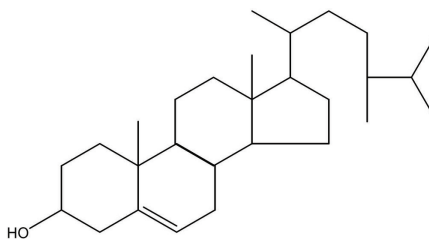
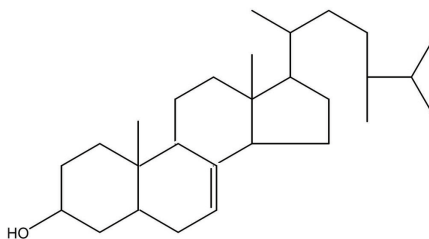
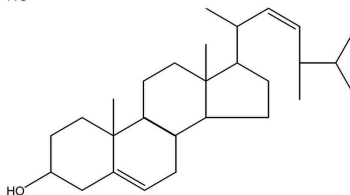
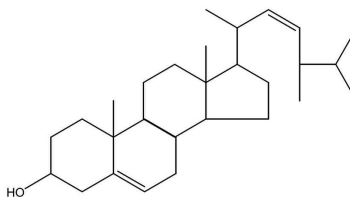
Sterols are important lipids related to the quality of the oil and broadly used for checking its genuineness. Four classes of sterols occur in olive oil: common sterols (4-desmethylsterols), 4α -methylsterols, triterpene alcohols (4, 4-dimethylsterols), and triterpene dialcohols.

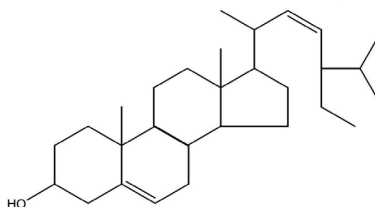
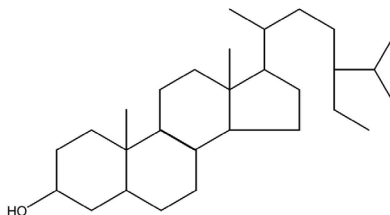
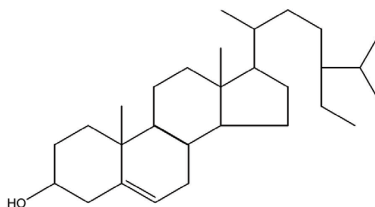
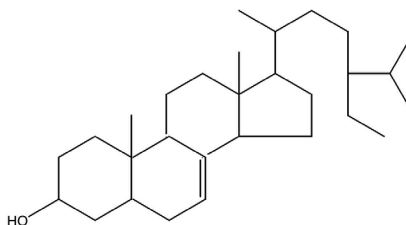
Common Sterols (4 α -desmethylsterols). Olive oil contains common sterols mainly in free and esterified form (Grob et al., 1990). However, these sterols have also been found as sterylglucosides and lipoproteins (Homberg and Bielefeld, 1985). The main components of this sterol fraction are β -sitosterol, Δ^5 -avenasterol, and campesterol (Itoh et al., 1973a; Boskou and Morton, 1975; Kornfeldt, 1981). Other sterols present in smaller quantities or in trace amounts are stigmasterol, cholesterol, brassicasterol, chlerosterol, ergosterol, sitostanol, campestanol, Δ^7 -avenasterol, Δ^7 -cholestenol, Δ^7 -campestenol, Δ^7 -stigmasterol, $\Delta^5,23$ -stigmastadienol, $\Delta^5,24$ -stigmastadienol, $\Delta^7,22$ -ergostadienol, $\Delta^7,24$ -ergostadienol, 24-methylene-cholesterol, and 22,23-dihydrobrassicasterol (Itoh et al., 1981; Calapaj et al., 1993; Mariani et al., 1995; Mariani, 1998). Structural formula of sterols reported to be present in olive oil are given in [Figure 4.2](#).

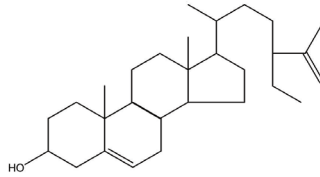
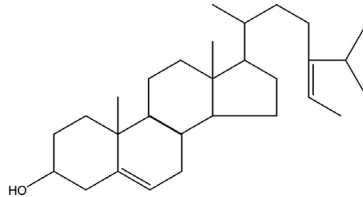
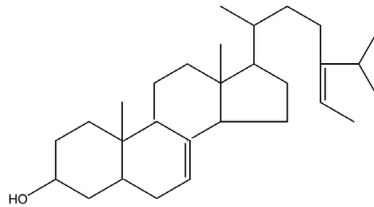
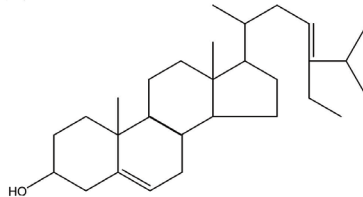
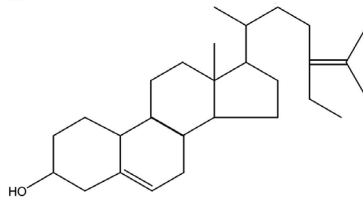
Total sterol content of virgin olive oils varies mainly between 1000 mg/kg, which is the lower limit set by the European Union Commission (EC Regulation 2568, 1991), and 2000 mg/kg (Morchio et al., 1987; Aparicio and Luna, 2002). Lampante olive oils contain higher amounts of total sterols (Morchio et al., 1987; Grob et al., 1990). Refined olive oils contain total sterols at lower levels because the refining process gives rise to significant losses of sterols, which may be as high as 25% (Morchio et al., 1987). Total sterol content of solvent extracted olive oils is up to three times higher than that of virgin olive oils (Morchio et al., 1987).

Studies on olive oil sterol composition show that β -sitosterol makes up 75 to 90% of the total sterol fraction, while Δ^5 -avenasterol usually ranges between 5% and 20% (Itoh et al., 1981; Calapaj et al., 1993). Percentages of Δ^5 -avenasterol, up to 36%, have been reported for Greek virgin olive oils (State Chemical Laboratory, 1994).

Cholesterol Δ^5 -Cholesten-3 β -ol**24-Methylene-cholesterol**24-Methylene- Δ^5 -cholesten-3 β -ol**Campestanol**(24R)-24-Methyl-cholestan-3 β -ol**Campesterol**(24R)-24-Methyl- Δ^5 -cholesten-3 β -ol**Fig. 4.2.** 4 α -Desmethylsterols present in olive oil.

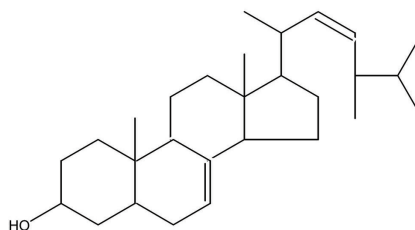
22,23-Dihydrobrassicasterol(24S)-24-Methyl- Δ^5 -cholesten-3 β -ol **Δ^7 -Campestenol**(24R)-24-Methyl- Δ^7 -cholesten-3 β -ol**Ergosterol**(24R)-24 Methyl- $\Delta^{5,22}$ -cholestadien-3 β -ol**Brassicasterol**(24S)-24 Methyl- $\Delta^{5,22}$ -cholestadien-3 β -ol**Fig. 4.2.** Continued

Stigmasterol(24R)-24-Ethyl- $\Delta^{5,22}$ -cholestadien-3 β -ol**Sitosterol**(24R)-24-Ethyl-cholestan-3 β -ol **β -Sitosterol**(24R)-24-Ethyl- Δ^5 -cholesten-3 β -ol **Δ^7 -Stigmasterol**(24R,S)-24-Ethyl- Δ^7 -cholesten-3 β -ol**Fig. 4.2.** Continued

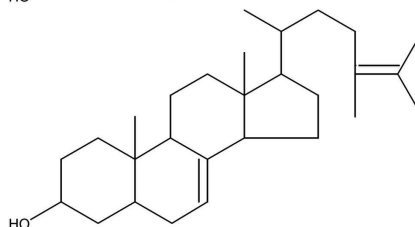
Cholesterol(24S)-24-Ethyl- $\Delta^{5,25}$ -cholestadien-3 β -ol **Δ^5 -Avenasterol**(24Z)-24-Ethylidene- Δ^5 -cholesten-3 β -ol **Δ^7 -Avenasterol**(24Z)-24-Ethylidene- Δ^7 -cholesten-3 β -ol **$\Delta^{5,23}$ -Stigmastadienol**(24R,S)-24-Ethyl- $\Delta^{5,23}$ -cholestadien-3 β -ol **$\Delta^{5,24}$ -Stigmastadienol**(24R,S)-24-Ethyl- $\Delta^{5,24}$ -cholestadien-3 β -ol**Fig. 4.2.** Continued

$\Delta^{7,22}$ -Ergostadienol(24R)-24 Methyl- $\Delta^{7,22}$ -cholestadien-3 β -

ol

 $\Delta^{7,24}$ -Ergostadienol(24R)-24 Methyl- $\Delta^{7,24}$ -cholestadien-3 β -

ol

**Fig. 4.2.** Continued

From the other sterols present in olive oil, campesterol and stigmasterol make up 4% and 2% of the total sterol fraction, respectively (Calapaj et al., 1993), but higher values have also been determined (Paganuzzi, 1985; Lanuzza et al., 1995; Rivera del Alamo et al., 2004). In all cases, the percentage of campesterol is higher than that of stigmasterol. The rest of the sterols occur in minute quantities. The levels of Δ^5 - and Δ^7 -avenasterol, Δ^7 -stigmasterol, stigmasterol, and chlosterol differentiate virgin, refined, and solvent extracted olive oils (De Blas and del Valle González, 1996).

Approximately 10 to 40% of total sterols are present as steryl esters (Boskou and Vlachopoulou, 1986; Grob et al., 1990). According to Mariani and his co-workers (1991), the composition of the two sterol fractions differs. Diunsaturated Δ^5 -sterols (Δ^5 -avenasterol, stigmasterol, and brassicasterol) are present at relatively higher levels in the free than in the esterified form. The opposite is the case of diunsaturated Δ^7 -sterols. Raw and refined lampante and solvent extracted olive oils have been found to contain sitosterol- C_{18} -esters at significantly higher levels than extra virgin olive oils (Grob et al., 1990). Therefore, the percentage of free β -sitosterol in total β -sitosterol can be used as a key parameter for assessing the quality and genuineness of a virgin olive oil. In the case of solvent extracted olive oils, losses during refining are more pronounced in the free sterol fraction (Grob et al., 1990).

Sterol composition and total sterol content are affected by cultivar, crop year, degree of fruit ripeness, storage time of fruits prior to oil extraction, processing, and also by geographic factors (Fedeli, 1993; Ranalli and Angerosa, 1996; Ranalli and

Serraiocco, 1996; Ranalli et al., 1997; Salvador et al., 1998; Cert et al., 1999; Gutiérrez et al., 1999; Koutsaftakis et al., 1999; Ranalli et al., 1999; Gutiérrez et al., 2000, Ranalli et al., 2000, Aparicio and Luna, 2002; Rivera del Alamo et al., 2004). Olive storage was found to be responsible for notable changes in the levels of individual sterols (Camera et al., 1978). Olive harvesting practices also affect individual sterol levels. Fedeli (1993) claims that stigmaterol rises to higher levels in olives left on the ground. Sterol composition is also affected by the refining process. Mariani and his coworkers (1992) studied the qualitative and quantitative variations occurring in both the free and esterified sterol fraction during bleaching with activated earths. They found that the esterified sterol fraction is subjected to more limited reductions than the free sterol fraction which is sometimes completely lost.

4-Methylsterols. Olive oil contains 4-monomethylsterols in small quantities (Fig. 4.3.), which are intermediates in sterol biosynthesis. They are present in free and esterified form (Kiosseoglou et al., 1987; Chryssafidis et al., 1992). The predominating components are obtusifoliol, gramisterol, cycloeucalenol, and citrostadienol (Itoh et al., 1973b; Boskou and Morton, 1975; Paganuzzi and Leoni, 1979; Itoh et al., 1981). They are Δ^7 - or Δ^8 -sterols except cycloeucalenol which has a 9,19-cyclopropane ring in the steroid skeleton.

The 4α -methylsterol fraction is extremely complex and gas chromatographic analysis reveals a considerable number of unknown minor components. Itoh and his co-investigators (1981) identified among these minor components 24-methyl-31-nor-9(11)-lanosterol, 24-methylene-31-nor-9(11)-lanosterol, 24-ethyllophenol, 24-methyl-(E)-23-dehydrolophenol, 24-ethyl-(E)-23-dehydrolophenol, 24-methyl-31-nor-(E)-23-dehydrocycloartanol, 28-isocitrostadienol, 24-ethyl-24(25)-dehydrolophenol, and 24-methyl-24(25)-dehydrolophenol.

The levels of total 4α -methylsterols are lower than that of common sterols and triterpene alcohols and vary between 50 and 360 mg/kg (Itoh et al., 1973b; Cert et al., 1999; Aparicio and Luna, 2002). In solvent extracted olive oils these levels are higher (Paganuzzi and Leoni, 1979; Kornfeldt, 1981). Citrostadienol has been found in oils obtained from fruits of a new olive cultivar - I-77 at levels up to 100 mg/kg (Ranalli et al., 2000). 4α -methylsterol content is affected by olive cultivar (Aparicio and Luna, 2002).

Triterpene Alcohols (4,4-dimethylsterols). The main components of the very complex 4,4-dimethylsterol fraction are β -amyrin, butyrospermol, 24-methylenecycloartanol, and cycloartanol (Fig. 4.4.) (Itoh et al., 1973b). Other triterpene alcohols identified in olive oil, but present in smaller quantities or in trace amounts, are cyclobranol, cyclosadol, dammaradienol, germanicol, 24-methylene-24-dihydroparkeol, taraxerol, α -amyrin, 7, 24-tirucalladienol, parkeol, and tirucallol (Itoh et al., 1981). Triterpene alcohols are present in the free and esterified form (Mariani et al., 1998). Significant

differences were observed between the distribution patterns of the total and esterified triterpene alcohol fraction in virgin olive oils, especially in the content of 24-methylenecycloartanol, butyrospermol, and cycloartenol (Chryssafidis et al., 1992). The triterpene alcohol fraction of olive oil and solvent extracted olive oil differ also in composition (Itoh et al., 1981; Grob et al., 1990).

Total triterpene alcohol levels have been found to vary between 350 and 1500 mg/kg (Kiosseoglou et al., 1987; Ranalli and Angerosa, 1996; Ranalli and Serraioco, 1996; Ranalli et al., 1997; Cert et al., 1999; Ranalli et al., 1999; Aparicio and Luna, 2002). Grob and his co-investigators (1990) determined the levels of free cycloartenol in virgin olive oils, lampante olive oils and solvent extracted olive oils. Italian virgin olive oils were found to contain cycloartenol at higher levels than Greek or Spanish virgin olive oils and Italian solvent extracted olive oils.

Triterpene alcohol composition and total triterpene alcohol content is affected by cultivar, crop year, and processing (Grob et al., 1990; Nergiz and Ünal K, 1990; Ranalli and Angerosa, 1996; Ranalli and Serraioco, 1996; Ranalli et al., 1997; Cert et al., 1999; Ranalli et al., 1999; Aparicio and Luna, 2002).

Significant structural modifications take place in the triterpene alcohol fraction during refining. Strocchi and Savino (1989) claim that when the oil is bleached with activated clays the 9,19-cyclopropane ring of 24-methylenecycloartanol opens giving rise to the formation of three derivatives (isomers which have a double bond at the carbon atom C₇, C₈, or C₉). A Δ^7 -isomer of 24-methylenecycloartanol (24-methyl-5 α -lanosta-7,24-dien-3 β -ol) has also been identified in refined olive oils (Lanzón et

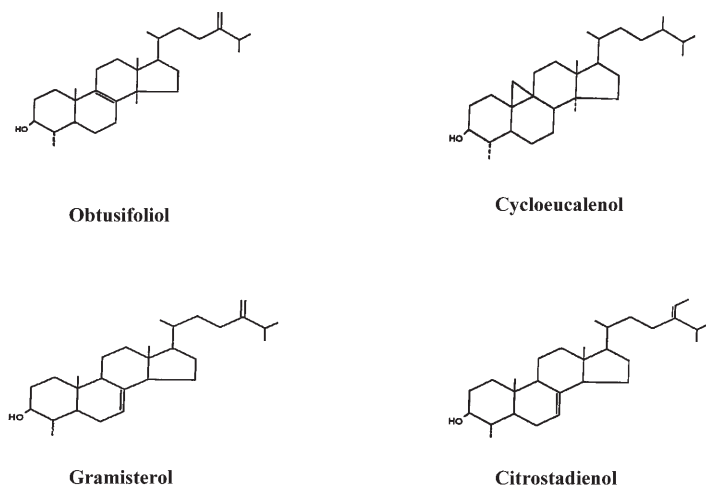


Fig. 4.3. Structural formulae of the main olive oil 4 α -methylsterols.

al., 1999). Qualitative and quantitative variations occurring in olive oil free and esterified triterpene alcohol fractions during bleaching with activated earths were evaluated by Mariani and his co-investigators (1992). They found a remarkable reduction of free triterpene alcohols and a more limited reduction of esterified triterpene alcohols.

Triterpene Dialcohols. Erythrodiol (homo-olestranol, 5α -olean-12-ene- 3β , 28-diol) and uvaol (Δ 12-ursen- 3β ,28-diol) are the main triterpene dialcohols identified in olive oil (Fig. 4.5.). Erythrodiol was found to be present in the free and esterified form (Mariani et al., 1998). Its content in olive oil is mainly affected by cultivar (Aparicio and Luna, 2002).

Virgin olive oils were found to contain total erythrodiol at levels ranging from 19 to 69 mg/kg (Aparicio and Luna, 2002) and free erythrodiol at levels usually lower than 50 mg/kg (Grob et al., 1990). Total and free erythrodiol levels of solvent extracted olive oils were found to be much higher than that of olive oils (Mariani et al., 1987; Grob et al., 1990).

The sum of erythrodiol and uvaol levels is usually given as a percentage of the whole sterol fraction because triterpene dialcohols are co-chromatographed with 4α -desmethylsterols. This sum, which must not exceed a limit set by the European Union Commission (Regulation 1989/2003), is used as a reliable indicator for distinguishing olive oil and solvent extracted olive oil (see Chapter 7, Analysis and Authentication).

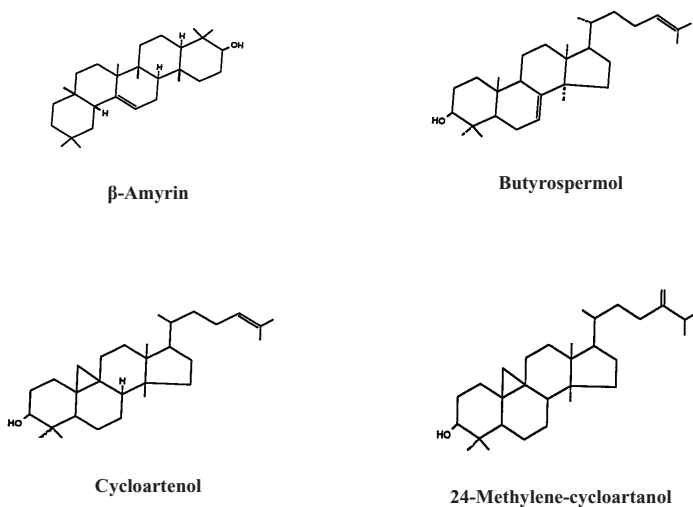


Fig. 4.4. Structural formulae of the main olive oil triterpene alcohols.

TABLE 4.2
Composition of Total and Esterified Triterpene Alcohol Fraction of Six Virgin Olive Oils

	RRT/Chol	Origin					
		Halkidiki	Kavala	Fthiotida	Keffalinia	Lesvos	Crete
		Total/ Esterified	Total/ Esterified	Total/ Esterified	Total/ Esterified	Total/ Esterified	Total/ Esterified
Cholesterol	1,00						
	1,13	Trace, 1	1, -	1, 5	-, -	-, -	4, -
β -Amyrin	1,16	2, 4	2, -	1, 6	1, -	2, 3	7, 7
Butyrospermol	1,17	7, 21	12, 17	7, 14	9, 27	4, 14	22, 37
	1,20	-, -	-, 4	-, -	-, -	-, -	-, -
Cycloartenol	1,21	1, 6	18, 51	12, 45	41, 50	21, 39	32, 46
	1,23	-, 2	-, -	-, 6	-, 3	-, -	-, -
24-Methylene cycloartanol	1,28	64, 31	63, 22	77, 22	47, 14	62, 44	28, 6
	1,29	-, -	1, -	-, 1	-, -	-, -	2, 2
	1,31	2, Trace	1, -	-, -	-, -	4, Trace	2, 1
	1,33	-, -	2, -	-, 1	-, -	7, -	3, 1
	1,35	-, 1	Trace, 1	2, -	2, -	-, -	-, Trace

Source: Chryssafidis et al. (1992). Reprinted with permission of J. Sci. Food Agric.

The relative triterpene dialcohol content in the whole sterol fraction is affected by cultivar and processing (Ranalli and Angerosa, 1996; Ranalli and Serraiocco, 1996; Ranalli et al., 1997; Cert et al., 1999; Koutsaftakis et al., 1999; Ranalli et al., 1999; Ranalli et al., 2000).

Triterpene Acids

Hydroxy pentacyclic triterpene acids are important olive fruit constituents (Fig. 4.6.). They are biologically active compounds and are present at trace amounts in olive oil. Oleanolic (3β -hydroxyolean-12-en-28-oic acid) and maslinic acid (2α , 3β -dihydroxyolean-12-en-28-oic acid) are the main triterpene acids present in virgin olive oil because they occur in the olive husk and a small quantity may be extracted during processing (Caputo et al., 1974). Both compounds and traces of ursolic acid (3β -hydroxyurs-12-en-28-oic acid) are also located in the reticular lipid layer of olive skin (Bianchi et al., 1994). Betulinic acid (3β -hydroxylup-20-(29)-en-28-oic acid) has also been identified in the skin of Coratina olive cultivar (Bianchi et al., 1992). Esterified derivatives of the triterpene acids were not found in olives and olive oils (Pérez-Camino and Cert, 1999).

Pérez-Camino and Cert (1999) claim that the main factor contributing to a higher level of hydroxy pentacyclic triterpene acids is oil acidity. Olive cultivar, olive ripeness, and oil extraction system have less influence on the levels of these acids. Total triterpene acid content of extra virgin olive oils obtained from fruits of different

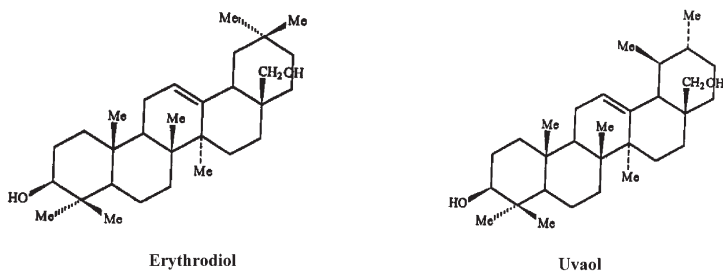


Fig. 4.5. Structural formulae of the olive oil triterpene dialcohols.

olive cultivars was found to range between 40 and 185 mg/kg. Virgin olive oils with free acidity higher than 1.0% and solvent extracted olive oils were found to contain these acids at levels higher than 300 mg/kg and 2400 mg/kg, respectively. The oleanoic and maslinic acid levels of virgin olive oils were found to be similar. Ursolic acid was present in traces. Oleanoic acid content of solvent extracted olive oils was much higher than that of maslinic acid. During chemical refining, total losses of oleanoic and maslinic acid were observed. Significant losses were also observed during physical refining (50-80% for oleanoic and 60-80% for maslinic acid).

Volatile and Aroma Compounds

Volatile compounds present in olive oil have been reported by Flath et al. (1973), Olías et al. (1977), Montedoro et al. (1978), Olías et al. (1980), Gutiérrez et al. (1981), Guth and Grosch (1991), Morales et al. (1994), Angerosa et al. (1998), Reiners and Grosch (1998), Bortolomeazzi et al. (2001), Cavalli et al. (2003), Servili et al. (2003), Vichi et al. (2003), Cavalli et al. (2004), and also Morales et al. (2005). Approximately two hundred and eighty compounds have been identified in the volatile fraction of virgin olive oils. They are hydrocarbons (more than 80 compounds), alcohols (45 compounds), aldehydes (44 compounds), ketones (26 compounds), acids (13 compounds), esters (55 compounds), ethers (5 compounds), furan derivatives (5 compounds), thiophene derivatives (5 compounds), pyranones (1 compound), thiols (1 compound), and pyrazines (1 compound). From this large number of compounds, only 67 were found to be present at levels higher than their odor threshold contribute to the aroma of virgin olive oil (see [table 4.3.](#)). About twenty of these compounds contribute to the flavor of virgin olive oils with sensory defects (Sanchez Saez et al., 1991; Angerosa et al., 1996; Morales et al., 2005).

The potent odorants of olive oil have been evaluated by applying aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry analysis of headspace samples (Guth and Grosch, 1991; Blekas et al., 1994; Blekas and Guth, 1995; Rein-

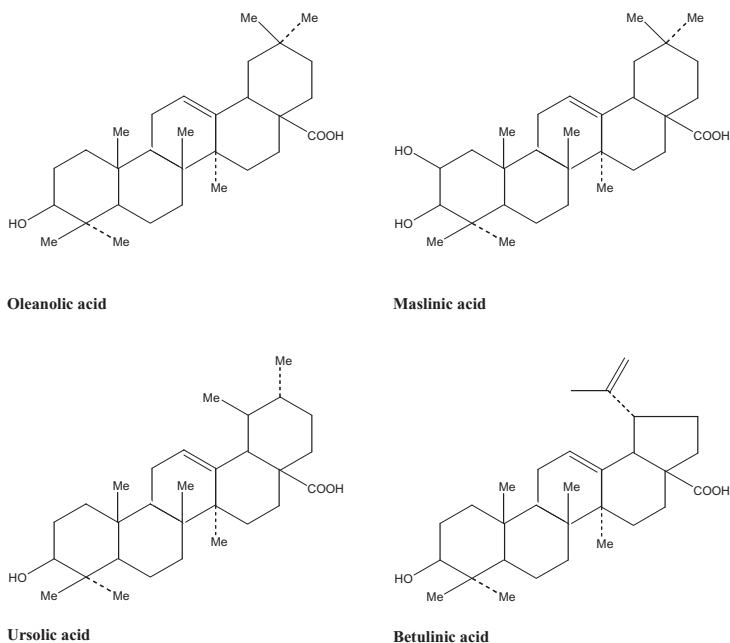


Fig. 4.6. Structural formulae of the olive oil hydroxy pentacyclic triterpene acids.

ers and Grosch, 1998). AEDA is a screening method which is applied to the volatiles isolated by a high vacuum codistillation of oil with diethyl ether. An aliquot of the concentrated by distillation and microdistillation sample is analyzed by HRGC while the effluent of the capillary column is sniffed. The aliquot is then diluted with diethyl ether in volume ratios 1 to 2^{n-1} ($n = 1, 2, 3$, etc) and the new samples are analyzed by gas chromatography-olfactometry until no odor is detected. In this way the flavor dilution factor (FD-factor) of each odorous compound can be estimated. The FD-factor is the highest dilution at which a volatile compound is still smelled during gas chromatography-olfactometry analysis and it is defined as the ratio of concentration of the odorant in the initial extract to its concentration in the most dilute extract in which odor is detected. FD-factor is proportional to the Odor Activity Value (OAV), which is the ratio of concentration to odor threshold of the compound in odorless oil (Grosch, 1994). The OAVs show the actual contribution of each odorant to the olive oil aroma (Guth and Grosch, 1993; Blekas et al., 1994; Blekas and Guth, 1995; Reiners and Grosch, 1998; Morales et al., 2005). The odor threshold values of olive oil odorants, which are determined retronasally (by tasting), are given in [Table 4.4](#).

[Table 4.5](#) presents odorants identified in Greek olive oils. Odor description des-

TABLE 4.3
Odorants Contributing to the Aroma of Olive Oil

Aldehydes	Esters	Alcohols
Ethanal	Ethyl acetate	Butan-2-ol
Propanal	Ethyl propanoate	2-Methylbutan-1-ol
2-Methylbutanal	Ethyl butanoate	3-Methylbutan-1-ol
3-Methylbutanal	Ethyl octanoate	(Z)-3-Hexen-1-ol
Pentanal	Ethyl cinnamate	Heptan-2-ol
Hexanal	Ethyl 2-methylpropanoate	1-Octen-3-ol
(E)-2-Hexenal	Ethyl 2-methylbutanoate	Nonan-1-ol
(Z)-3-Hexenal	Ethyl 3-methylbutanoate	2-Phenylethanol
Heptanal	Ethyl cyclohexylcarboxylate	
(E)-2-Heptenal	Butyl acetate	Ketones
Octanal	3-Methylbutyl acetate	1-Penten-3-one
(E)-2-Octenal	(Z)-3-Hexenyl acetate	Octan-2-one
Nonanal	2-Methylpropyl butanoate	1-Octen-3-one
(E)-2-Nonenal		2-Methyl-2-hepten-2-one
(Z)-2-Nonenal	Acids	(Z)-1,5-Octadien-3-one
(Z)-3-Nonenal	Acetic acid	(E)- β -Damascenone
(E,E)-2,4-Nonadienal	Propanoic acid	(Z)- β -Damascenone
(E,Z)-2,6-Nonadienal	Butanoic acid	
(E)-2-Decenal	Pentanoic acid	Others
(Z)-2-Decenal	2-Methylbutanoic acid	Guaiacol
(E,E)-2,4-Decadienal	3-Methylbutanoic acid	4-Ethylguaiacol
(E,Z)-2,4-Decadienal	Hexanoic acid	1-Octen-3-hydroperoxide
trans-4,5-Epoxy-(E)-2-decenal	Heptanoic acid	4-Methoxy-2-methyl-2-butanethiol
Phenylacetaldehyde		2-Isobutyl-3-methoxypyrazin
Vanillin		n-Octane

ignated during AEDA and FD-factors on an SE-54 capillary column are also given in the table (Blekas and Guth, 1995; Blekas and Guth, unpublished data).

By applying gas chromatography-olfactometry analysis of headspace samples to the screening for potent odorants, Blekas et al. (1994), and also Reinert and Grosch (1998), found also that ethanol also contributes to virgin olive oil aroma.

Levels of virgin olive oil odorants in commercially available samples with different aroma characteristics originated from Greece (a, b, c, d), Italy (e, f), and Spain (g, h) are given in Table 3.6. The odorants were quantified by stable isotope dilution analysis (Blekas and Guth, unpublished data).

It is clear that the qualitative composition of odorants present in virgin olive oils is similar. However, the quantitative composition differs strongly when the aroma characteristics are different. Olive oil obtained from healthy olives, harvested at the right ripening stage, and by proper extraction techniques contains mainly volatiles derived from linoleic and linolenic acid decomposition through the lipoxygenase pathway (Olias et al., 1993). The most abundant compounds are hexanal, (E)-2-hexenal, (Z)-3-hexenal, hexan-1-ol, (Z)-3-hexen-1-ol, hexyl acetate, and (Z)-3-hex-

enyl acetate. These volatiles are responsible for the green and fruity perception of the unique virgin olive oil aroma. C₅ aldehydes, ketones and alcohols, and pentene dimers also arise from a cleavage reaction of 13-hydroperoxide of linolenic acid (Salch et al., 1995), are usually present at levels lower than their odor threshold. The formation of these C₆ and C₅ compounds is affected by the cultivar, the degree of fruit ripeness, the storage time of fruits prior to oil extraction, and by the processing (Blekas et al., 1994; Aparicio and Morales, 1998; Angerosa et al., 1998a; Lercker et al., 1999; Morales et al., 1999; Salas and Sánchez, 1999; Angerosa et al., 1999; Morales and Aparicio, 1999; Angerosa et al., 1999a; Angerosa et al., 2000; Angerosa et al., 2001; Aparicio and Luna, 2002; Ridolfi et al., 2002; Servili et al., 2002; Angerosa and Basti, 2003; Benincasa et al., 2003; Luaces et al., 2003; Pérez et al., 2003; Servili et al., 2003; Tura

TABLE 4.4
Odor threshold of olive oil odorants determined retronasally

Compound	Odor threshold (µg/kg)	Compound	Odor threshold (µg/kg)
Ethanal	7,1 ^d	(E)-β-Damascenone	3,7 ^c
2-Methylbutanal	8,2 ^c	2-Methylbutan-1-ol	480 ^e
3-Methylbutanal	10,8 ^b	3-Methylbutan-1-ol	100 ^e
Pentanal	150 ^d	(Z)-3-Hexen-1-ol	360 ^c
Hexanal	75 ^d	2-Phenylethanol	120 ^c
(E)-2-Hexenal	250 ^d	Ethyl acetate	940 ^e
(Z)-3-Hexenal	1,2 ^d	Ethyl propanoate	100 ^e
Heptanal	50 ^d	Ethyl 2-methylpropanoate	0,75 ^a
(E)-2-Heptenal	400 ^d	Ethyl butanoate	3,5 ^c
Octanal	55 ^d	Ethyl 2-methylbutanoate	0,75 ^a
(E)-2-Octenal	125 ^d	Ethyl 3-methylbutanoate	0,50 ^c
Nonanal	260 ^d	Ethyl cyclohexylcarboxylate	0,06 ^c
(E)-2-Nonenal	65 ^d	Butyl acetate	300 ^e
(Z)-2-Nonenal	0,6 ^d	(Z)-3-Hexenyl acetate	750 ^a
(Z)-3-Nonenal	35 ^d	2-Methylpropyl butanoate	100 ^e
(E,E)-2,4-Nonadienal	460 ^d	Guaiacol	13 ^c
(E,Z)-2,6-Nonadienal	1,5 ^d	Acetic acid	380 ^c
(E)-2-Decenal	150 ^d	Propanoic acid	720 ^e
(E,E)-2,4-Decadienal	40 ^d	Butanoic acid	650 ^e
6-Methyl-5-hepten-2-one	1000 ^e	3-Methylbutanoic acid	25 ^c
1-Penten-3-one	3,2 ^d	Pentanoic acid	600 ^e
1-Octen-3-one	0,3 ^d	Hexanoic acid	700 ^e
(Z)-1,5-Octadien-3-one	0,03 ^d	Heptanoic acid	100 ^e
trans-4,5-Epoxy-(E)-2-decenal	3 ^d	4-Methoxy-2-methyl-2-butanethiol	0,025 ^c

^a Guth and Grosch, 1993

^b Preininger and Grosch, 1994

^c Reiners and Grosch, 1998

^d Belitz et al., 2004

^e Morales et al., 2005

TABLE 4.5
Odorants which contribute to the aroma of Greek virgin olive oils with different flavor profiles

Compound	RI on capillary		Odor description (assigned during AEDA)	FD-factor* on capillary SE-54 Samples			
	SE-54	OV-1701		A	B	C	D
3-Methylbutanal	650	731	Malty	2 ³	2 ³	2 ²	2 ³
1-Penten-3-one	680	775	Sharp, pungent	2	2	2 ⁴	2 ⁴
3-Methylbutan-1-ol Ethyl	735	848	Malty	2	1	<1	1
2-methylpropanoate	765	817	Fruity	2 ⁴	2 ⁵	2 ⁴	2 ⁶
(Z)-3-Hexenal	797	890	Green, apple-like	2 ⁵	2 ⁶	2 ¹¹	2 ⁹
Hexanal	800	883	Green, grassy	2 ⁸	2 ⁸	2 ⁶	2 ⁴
Ethyl							
2-methylbutanoate	849	906	Fruity	2 ⁶	2 ⁷	2 ⁶	2 ⁸
(E)-2-Hexenal	855	964	Green, bitter	2 ³	2 ³	2 ⁵	2 ⁶
(Z)-3-Hexen-1-ol	859	973	Green leaves	2 ³	2	2 ³	2 ²
Heptanal	907	989	Fatty	2 ⁵	2 ⁴	2 ³	2 ²
1-Octen-3-one	979	1070	Mushroom-like, earthy	2 ⁶	2 ⁷	2 ⁴	2 ⁴
Octanal	1003	1089	Soapy, citrus-like	2 ⁷	2 ⁶	2 ²	2 ²
(Z)-3-Hexenyl acetate	1008	1081	Fruity	2 ⁵	2 ⁴	2 ³	1
Phenylacetaldehyde	1051	1174	Sweet, honey-like	2	2 ²	1	2
(E)-2-Octenal	1060	1197	Fatty	2 ³	2 ³	1	<1
Guaiacol	1092	1232	Burnt	2 ³	2 ⁴	2 ²	2 ⁴
(Z)-3-Nonenal	1094	1184	Fatty	2 ²	2	1	<1
Nonanal	1105	1192	Soapy, citrus-like	2 ³	2 ⁴	1	<1
2-Phenylethanol	1117	1278	Sweet, honey-like	2 ³	2 ⁴	2 ³	2 ³
Ethyl cyclohexyl- carboxylate	1133	1215	Fruity	<1	2	1	<1
(Z)-2-Nonenal	1147	1257	Fatty	2 ⁵	2 ⁷	1	2
(E,Z)-2,6-Nonadienal	1158	1276	Cucumber-like	2 ⁴	2 ⁵	2 ³	2 ⁴
(E)-2-Nonenal	1161	1275	Fatty, tallowy	2 ⁴	2 ⁴	1	2
2,4-Nonadienal	1194	1324	Deep-fried	1	2	<1	<1
(E,E)-2,4-Nonadienal	1216	1343	Deep-fried	2	2	<1	2
2-Decenal	1251	1356	Fatty	2 ²	2 ³	<1	2
(E)-2-Decenal	1263	1381	Fatty	2 ³	2 ³	2	2
(E,Z)-2,4-Decadienal	1294	1429	Deep-fried	2 ³	2 ⁴	<1	<1
(E,E)-2,4-Decadienal	1317	1452	Deep-fried	2 ⁵	2 ⁸	2 ²	2 ²
trans-4,5-Epoxy-(E)-2-decenal	1378	1551	Metallic	2 ⁴	2 ⁵	2 ⁴	2 ⁴
Vanillin	1421	1631	Vanilla-like	2	2	2	2 ²

* All data are produced in the same laboratory

et al., 2004; Dhifi et al., 2005; Morales et al., 2005). In addition to these volatiles, linolenic, linoleic, and oleic autoxidation decomposition products, mainly aldehydes, and also aldehydes, alcohols, and esters deriving from biochemical transformations of amino acids such as isoleucine, leucin, phenylalanine, or valine (Schreier, 1984), have

TABLE 4.6
Levels of some odorants in virgin olive oil samples differing in flavor

Compound	Concentration ($\mu\text{g}/\text{kg}$)							
	a	b	c	d	e	f	g	h
Hexanal	620	460	95	370	260	180	460	1510
(E)-2-Hexenal	1150	7400	730	1550	650	330	1640	170
(Z)-3-Hexenal	31	165	10	57	31	25	37	8
Octanal	130	125	100	140	85	70	220	210
(Z)-2-Nonenal	9	10	7	11	16	15	14	15
(E)-2-Nonenal	21	22	11	25	33	19	44	46
(E,E)-2,4-Decadienal	77	75	39	73	880	1330	245	640
1-Octen-3-one	2.7	4.1	0.9	1.9	1.5	1.7	5.7	13
Ethyl 2-methylpropanoate	3.5	2.4	2.7	2.3	2.2	1.0	62	115
Ethyl 2-methylbutanoate	1.8	2.9	1.9	2.4	4.0	0.7	48	105
Ethyl cyclohexylcarboxylate	4.9	12.3	7.7	5.5	3.8	3.2	3.0	2.7
(Z)-3-Hexenylacetate	9130	7880	130	13440	300	20	130	75
(Z)-3-Hexen-1-ol	470	1310	600	1140	165	65	720	570
2-Phenylethanol	6170	6620	5770	7010	810	17	320	1470
Acetic acid	2880	5740	4610	3560	3180	2390	4970	14340

a low odor threshold and can contribute to the aroma of olive oil.

A greater number of volatile compounds have been found in virgin olive oils of poorer quality. Some of these volatiles give rise to sensory defects when they are present at high levels. They are produced by over-ripening of the fruit, significant attack of the fruits by molds and bacteria, when they are stored for a long period prior to oil extraction, and also by advanced autoxidation of the unsaturated fatty acids due to adverse storage conditions (Angerosa, 2002). Acids, esters, alcohols, aldehydes and ketones are mainly responsible for the most frequent off-flavors developed in virgin olive oil (Gutiérrez et al., 1981; Angerosa et al., 1996; Morales et al., 2000; Morales et al., 2005). According to Morales et al. (2005), the main contributors to the so called “fusty” off-flavor, which is characteristic for oils obtained from olive fruits being in an advanced stage of fermentation, are mainly ethyl butanoate, and also propanoic and butanoic acids. Responsible for the “mustiness-humidity” virgin olive oil off-flavor is 1-octen-3-ol, and to a lesser extent 1-octen-3-one. This off-flavor is characteristic for oils obtained from fruits piled under humid conditions for several days, giving rise to the development of various kinds of fungi. The main volatiles responsible for the “rancid” off-flavor are aldehydes which are decomposition products of linolenic, linoleic, and oleic acid hydroperoxides. Finally, the main odorants which contribute to the “winey-vinegary” off-flavor are acetic acid, 3-methylbutan-1-ol, and ethyl acetate.

The presence of terpene alcohols (Flath et al., 1973), monoterpene and sesquiterpene hydrocarbons in the virgin olive oil (Gutiérrez et al., 1981; Lanzón et al., 1994; Bortolomeazzi et al., 2001; Vichi et al., 2003), as well as aromatic hydrocarbons is

also interesting (Olias et al., 1980; Gutiérrez et al., 1981; Vichi et al., 2003). However, it is not clear whether all aromatic hydrocarbons are naturally present compounds or contaminants (Biedermann et al., 1995).

Other compounds identified in the volatile fraction are ethyl esters. Oils obtained from altered olive fruits or olive pomace have been found to contain high levels of ethyl palmitate, ethyl oleate and ethyl linoleate, while the levels of these esters in extra virgin olive oils are low (Pérez-Camino et al., 2002).

Other Minor Constituents

Some classes of minor constituents are present only in the crude oil. Filtration reduces the initial levels to a great extent whereas refining results in their removal.

Phospholipids. Experimental work for the identification of phospholipids in olive oil is rather limited. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine were reported to be the main phospholipids present in olive oil (Alter and Gutfinger, 1982). The fatty acid composition was found to be similar to that of triacylglycerols.

In a recent report Boukhchina et al. (2004) identified glycerophospholipids present in olive oil by liquid chromatography-mass spectrometry (GC-MS). The LC effluent was directly introduced into the mass spectrometer through an electrospray capillary while information about the fatty acid composition of each phospholipids class was given by the tandem mass spectra obtained for negatively charged lipids. Phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidic acid, and also phosphatidylglycerol were the phospholipids identified and quantified. Phosphatidylserine was not detected. However, the study was only based on one sample from the retail market.

The level of phospholipids may be important because these compounds have an antioxidant activity. According to Pokorny and Korczak (2001), these lipids may act as synergists (regeneration of antioxidants such as α -tocopherol or other phenols) or as metal scavengers. At high levels, however, phospholipids may cause foaming or darkening during frying. The possible contribution of phospholipids to the oxidative stability of olive oil has not been studied. Koidis and Boskou (unpublished data) determined phosphorous in cloudy olive oils, filtered oils and refined oils. Values obtained were in the range of 1-6 mg P/kg oil (n=26), corresponding to approximately 20-156 mg phospholipids/kg oil. The higher level of phospholipids in the unfiltered oils may be an additional antioxidant factor to phenols. These veiled oils were found to be more stable to oxidation and this was attributed to the higher levels of polar phenols (Tsimidou et al., 2004).

Proteins. Mainly in unfiltered oils minute quantities of proteins may be detected (see cloudy olive oil, [Chapter 11 Storage and Packing](#)).

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5

Polar Phenolic Compounds

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Introduction

Olive oil polar phenol fraction, known for many years as “polyphenols” (a term obsolete in recent publications) is in fact a complex mixture of compounds with different chemical structures obtained from the oil by extraction with methanol-water. Literature on these compounds has increased exponentially in the last 10 years for various reasons. Phenolic compounds are related to the stability of the oil but also to its biological properties. The latter have received much attention and today many phenolic compounds contained in the oil, mainly hydroxytyrosol and its derivatives, are thoroughly investigated with the aim of establishing a relationship between dietary intakes and the risk of cardiovascular disease or cancer. Ongoing and completed studies in this area associate these phenols with the beneficial role of olive oil in human health (for review see [Chapter 8](#)).

Virgin olive oil phenolic compounds belong to the following classes:

a. tyrosol, hydroxytyrosol, and their derivatives; b. derivatives of 4-hydroxybenzoic, 4-hydroxyphenylacetic, and 4-hydroxycinnamic acids; c. lignans, and d. flavonoids.

Chemistry, Analysis, and Levels

Individual phenolic compounds which often appear in lists of olive oil polar phenols are (in alphabetical order): 4-acetoxy-ethyl-1, 2-dihydroxybenzene, 1-acetoxy-pinoresinol, apigenin, caffeic acid, cinnamic acid (not a phenol), o- and p-coumaric acids, ferulic acid, gallic acid, homovanillic acid, p-hydroxybenzoic acid, hydroxytyrosol, luteolin, oleuropein, pinoresinol, protocatechuic acid, sinapic acid, syringic acid, tyrosol, vanillic acid, and vanillin. The presence of elenolic acid (not a phenol) in the same fraction has also been verified many times (Brenes et al., 2000, Morales and Tsimidou, 2000; Owen et al., 2000; García et al., 2001; Mateos et al., 2001; Boskou, 2002).

Tyrosol and hydroxytyrosol in their various forms are reported to be the major

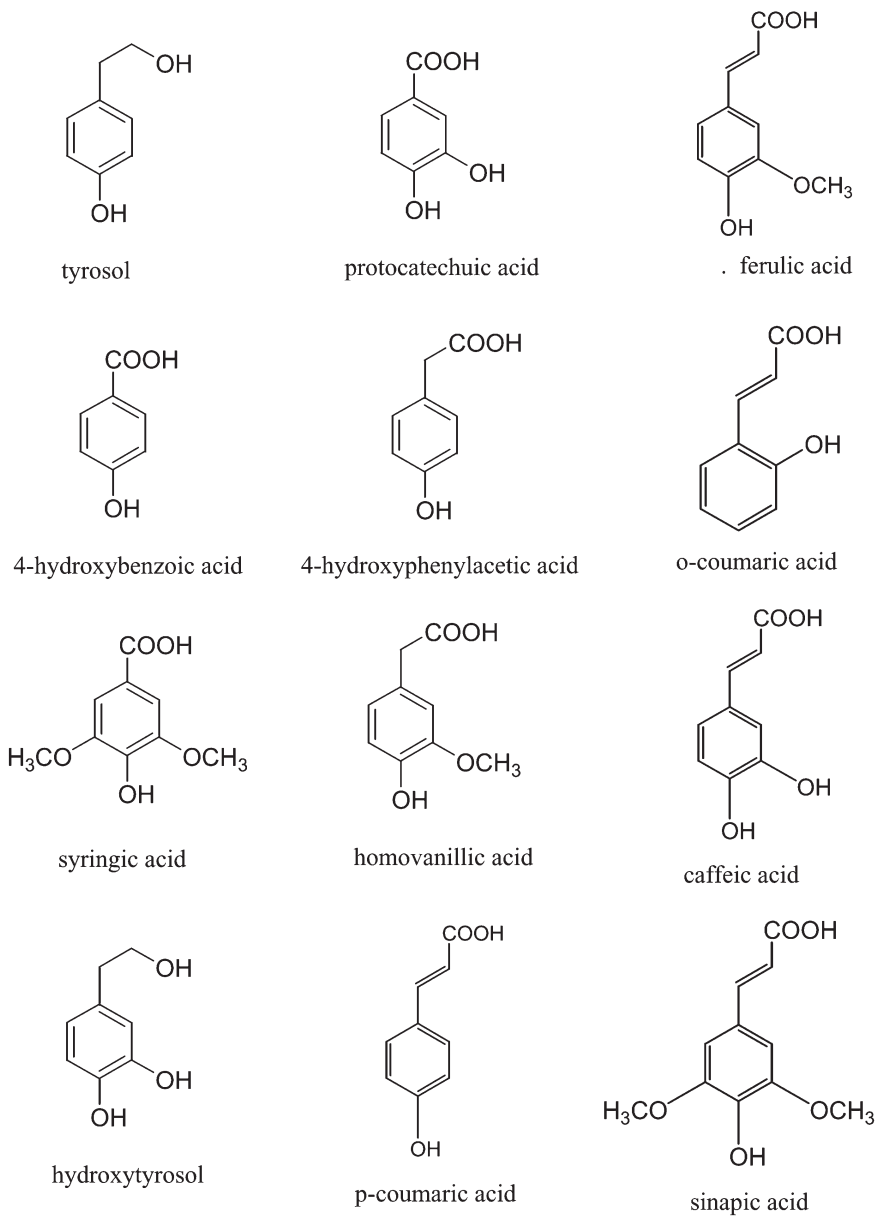


Fig. 5.1.

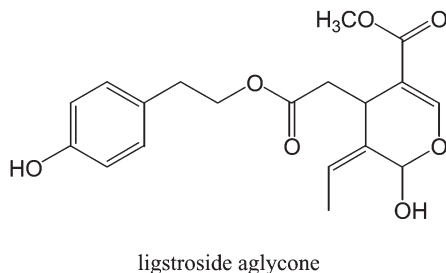
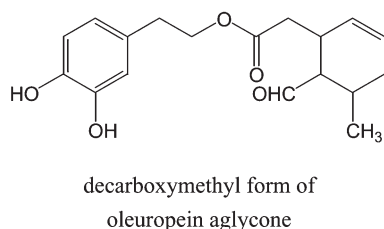
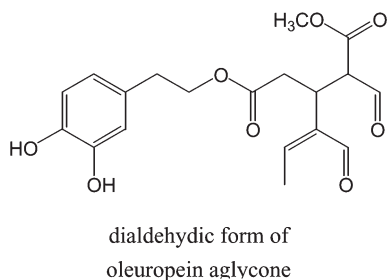
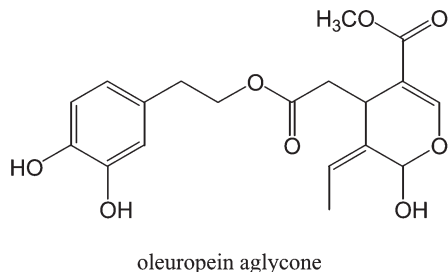
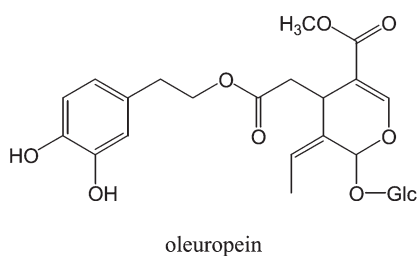
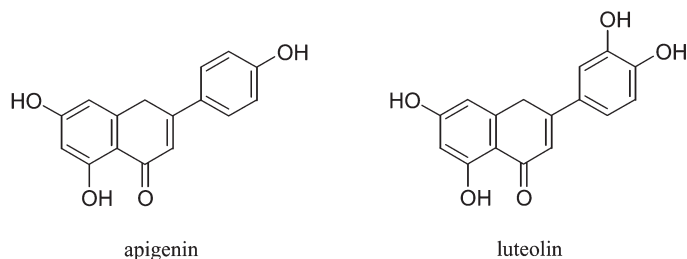
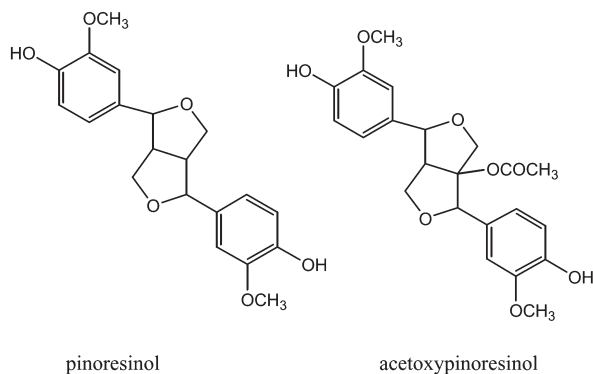


Fig. 5.2.

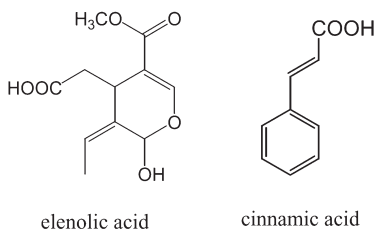
constituents. The more polar part of the methanol-water extract contains free phenols and phenolic acids (Fig. 5.1). The less polar part contains aglycones of oleuropein and ligstroside (the hydroxytyrosol and tyrosol esters of elenolic acid), deacetoxy and dialdehydic forms of these aglycones (Fig. 5.2), the flavones luteolin and apigenin (Fig. 5.3), the lignans 1-acetoxypinoresinol and pinoresinol (Fig. 5.4), and also elenolic acid and cinnamic acid (Fig. 5.5).

Litridou and coworkers (1997) reported the presence of an ester of tyrosol with a dicarboxylic acid. The same investigators demonstrated that total polar phenol (TPPC) and o-diphenol content was higher in the less polar part of the methanol-water extracts.

**Fig. 5.3.****Fig. 5.4.**

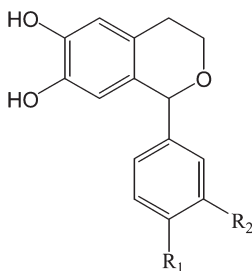
Glycosides were found to be present only in trace amounts. García and his coworkers (2001) determined the dialdehydic forms of elenolic acid linked to hydroxytyrosol and tyrosol, 1-acetoxy-ethyl-1,2-dihydroxybenzene (hydroxytyrosol acetate), 1-acetoxypinoresinol, pinoresinol, oleuropein aglycone, luteolin, and ligstroside aglycone as phenols with the higher concentration in Italian oils. More or less similar results were reported by Tovar et al. (2001) for Arbequina oils.

Bianco and his coworkers (2001) found a new class of phenols: hydroxyl-isochromans. The identity of two compounds of this class, 1-phenyl-6,7-dihydroxyisochroman and 1-(3"-methoxy-4"-hydroxy)phenyl-6,7-dihydroxyisochroman was confirmed by comparing the spectra of the biphenols, isolated from the oil by solid phase extraction, with the LC-MS spectra of compounds deriving from a reaction between hydroxytyrosol and aromatic aldehydes (benzaldehyde and vanillin). Such a reaction occurs also in a natural matrix and oleic acid acts as a catalyst. According

**Fig. 5.5.**

to the authors hydroxytyrosol present in olive fruits is in its glycosylated form but mainly linked as an ester to the aglycone moiety of oleuropein. During the malaxation step hydroxytyrosol is freed by glycosidases and esterases. This hydrolytic process, which also enhances the quantity of carbonyl compounds, favors the formation of isochroman derivatives. Hydroxy-isochromans are now investigated (Togna et al., 2003) for their antioxidant power and their ability to inhibit platelet aggregation. Another phenolic compound, 4-ethylphenol, not found in virgin olive oils, but characteristic of oils intended for refining, was detected and identified by Brenes et al. (2004). The level of this phenol is high in oils of “second centrifugation,” because its concentration increases with storage of olive paste.

More recently, Christoforidou et al. (2005) applied a very sophisticated technique, hyphenated liquid chromatography-solid phase extraction-nuclear magnetic resonance, to identify new phenols in the polar fraction of olive oil. The most interesting findings of this study were the verification of the presence of the lignan syringar-

 $R_1=H$ $R_2=H$ $R_1=OH$ $R_2=OCH_3$ **Fig. 5.6.** Hydroxy- isochromans

esinol (Fig. 5.7), the presence of two stereochemical isomers of the aldehydic form of oleuropein and the detection of homovanillyl alcohol.

Recent identification of oleocanthal (Beauchamp et al., 2005), of methyl acetal of the aglycone of ligstroside and of the β -hydroxytyrosol ester of methyl malate (Fig. 5.8) (Bianco et al., 2005) add to knowledge of more active forms of tyrosol and hydroxytyrosol derivatives present in olive oil. It remains to verify whether such compounds are found in specific oils (just extracted, from particular cultivars) or are inherent to any good quality virgin olive oil. For example, according to Beauchamp and coworkers, oleocanthal, a compound that has the same pharmacological activity as the anti-inflammatory drug ibuprofen, is found only in freshly pressed extra virgin olive oil and its presence is connected with the stinging sensation in the throat.

The colorimetric method universally applied for the determination of phenols in the water-methanol extract is based on the use of Folin-Ciocalteu reagent. Results are usually expressed as caffeic acid equivalents (mg caffeic acid/kg oil) though other phenols have been also used as standards (Tsimidou, 1999). The results may differ, depending on the standard used and the relative concentration of individual compounds, since the molar absorptivity per reactive group by each phenol is different (Singleton et al., 1999; Hrnčirik and Fritsche, 2004). However, the conventional colorimetric method is broadly applied for the quantitation of total polar phenols because it gives a good indication of the oil stability (Gutfinger, 1981; Aparicio et al., 1999; Blekas et al., 2002; Psomiadou et al., 2003).

Recently, very sophisticated gas chromatographic, but mainly liquid chromatographic methods, have been developed for the analysis of olive oil polar phenols. These methods are useful in elucidating the complex phenol structure of the so-called “polar fraction” of olive oil, but they cannot be easily applied for routine analysis. This is due to the high overall cost of the analysis taking into account the capital cost of chromatographic apparatus, the cost of consumables, the time needed for all the peaks to be eluted from the column, and the lack of commercially available

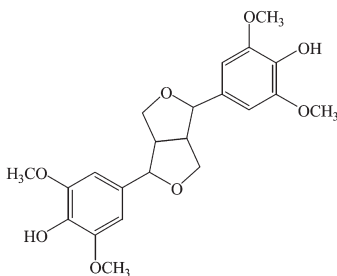


Fig. 5.7. Syringaresinol

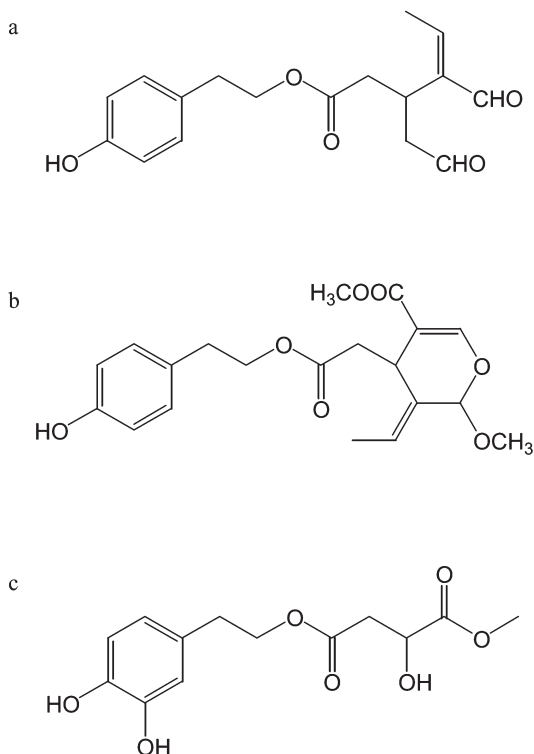


Fig. 5.8. Phenols recently identified in olive oil: a. oleocanthal, b. methyl acetal of the aglycone of ligstroside, c. β-hydroxytyrosol ester of methyl malate

standards. Another weak point of the procedures is the fact that the structure of all the constituents has not been fully elucidated. In a great number of review articles experimental details and critical appraisal of achievements has been repeatedly given (Tsimidou, 1998; 1999; Morales and Tsimidou, 2000; Gallina-Toschi et al., 2005; Carrasco-Pancorbo et al., 2005). Introduction of LC-MS and promising data from LC-SPE-NMR for the elucidation of the complex nature of olive oil phenolic compounds are not expected to improve routine quality control. Analytical sophistication is expected to answer research questions. The need of an easy to apply routine method for the determination of individual members is still existing. In this respect capillary electrophoretic separation of olive oil phenolic compounds is an interesting approach (Carrasco-Panorbo et al., 2004).

Olive oils differ in TPPC. Wide ranges have been reported (50-1000 mg/kg) but values are usually between 100 and 300 mg/kg (Tsimidou, 1998). The cultivar,

the system of extraction, and the conditions of processing, packing, distribution, and storage are critical factors that affect the final amount of TPPC in the oil. Aparicio and Luna (2002) gave useful figures for TPPC in ten monovarietal olive oils obtained using the Abencor system. The samples of olives were at the stage of normal maturity from the same olive orchard in Spain. In line with the characterization given by Montedoro et al. (1992), the Spanish drupes Blanqueta, Hojiblanca, Picudoand, and Pedondilla were found to be "medium" (200-500 mg/kg), Empeltre and Picual (Spain) "medium-high" (~500mg/kg), Koroneiki (Greece), Pajarero (Spain), Picholine (Morocco) "high" (500-1000 mg/kg) whereas Verdial de Velez (Spain) "low" (50-200 mg/kg) in TPPC. Commercial olive oils are expected to contain lower amounts of TPPC for obvious reasons. EEC Regulation no. 1019/2002 (EC, 2002), according to which the maximum capacity of packaging to consumers should be 5 L, may help to maintain high levels of phenols during domestic use (Grigoriadou et al., 2005).

Levels of individual phenols are difficult to establish due to natural variability and strong dependence on oil age and history after production. Free phenols are mainly found in stored oils whereas fresh oils contain more complex forms of secoiridoid aglycons. Servili and Montedoro (2002) gave average values for 116 oil samples obtained from industrial plants the size of which may be considered as typical of virgin olive oil irrespective of origin. Thus, median values for 3, 4-DHPEA, p-HPEA, vanillic acid, caffeic acid, 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA were 1.9, 2.6, 0.2, 0.4, 185.7, 22.7, 163.6, mg/kg, respectively.

Influence of Agronomic and Technological Factors

Effect of maturation is closely related to cultivar characteristics. Oleuropein and verbascoside relative levels are linked to drupe size (Amiot et al., 1986). During maturation oleuropein content is constantly reduced and is at a minimum in overripe drupes. At the same time, demethyl-oleuropein replaces oleuropein in about the same amounts. A biosynthetic relationship was also suggested for oleuropein, elenolic acid glucoside, and demethyloleuropein (Amiot et al., 1986). The fruit of *O. europea* appears to accumulate only glycosylated derivatives of oleuropein, which are probably less toxic than aglycones. Hydroxytyrosol is also related with ripe fruits. Probably oleuropein is converted by the action of glycosidases but the intermediate compounds are immediately re-metabolized in the fruit. Briante et al., (2002) examined the esterases activity not only during fruit maturation but also during processing, since activation may occur during crushing and malaxation. Oxidation of phenols by phenol oxidases and polymerization of free phenols are also expected to occur (Ryan et al., 2002). Delaying or anticipating the harvest time may, consequently, be crucial in maintaining oleuropein derivatives in olive oil (because of differences in their distribution between the oil and aqueous phases) and balancing bitter to pungent taste in the oil (Esti et al., 1998; Caponio et al., 2001, Skevin et al., 2003).

In recent years many papers have been published on the effect of maturation on

the fate of phenolics of various cultivars all over the world (see [Table 4.1](#)). The outcome of these studies is that it is difficult to generalize as to where the optimal stage of maturity is concerned.

The effect of storage of olives prior to milling is also important. Holding of olives results in considerable loss of antioxidants due to degradation of the cell structure and growth of lipolytic molds (Brenes et al., 1993; García et al., 1996; Agar et al., 1998).

There is plenty of research work relating the milling conditions to the level of both polar and non-polar phenols and oxidative stability. In a five year systematic comparative study, Salvador et al. (2003) examined samples from the three main extraction systems: pressure, dual-phase, and triple-phase. Total phenols and o-diphenols were found to be present at higher levels in the oil obtained by the two phase decanters. The oxidative stability and overall quality was superior in the oil obtained by these decanters. These properties were followed by a slightly higher index of bitterness. The lower phenol content of the oil extracted in three phase centrifuges is due to the addition of water, which reduces the concentration of the polar phenolic compounds. In the two-phase system this drawback is lessened because the waste water is recycled as soon as it is produced and used instead of the added water for the dilution of the paste. The above results are in accordance with data presented in other studies (Cert et al., 1996; Di Giovacchino et al., 2001).

In addition to the system of extraction, the crushing of fruits seems also to be of critical importance. To upgrade oil quality, olives richer in phenols can be crushed with a stone mill. In this way, the level of TPPC is reduced and bitterness and pungency are eliminated. On the contrary, to increase TPPC hammer crushers are recommended (Caponio et al., 1999). When hammer crushers are used, even the rotation rate may be critical, as indicated by Fogliano and his coworkers (1999). A change from 2200 rpm to 2900 rpm resulted in about 40% increase in the total antioxidant power of the polar fraction. This is due to a better fragmentation of olive tissues and a release of 3,4-DHPEA-EDA (the dialdehydic form of elenolic acid linked with hydroxytyrosol), caused by activation of hydrolytic enzymes during malaxation.

The conditions of kneading (temperature, time) are also important factors for the level of total phenols (García et al., 2001; Angerosa, 2001). According to García and his coworkers the malaxation stage may reduce the concentration of ortho-diphenols ca 50-70%. Other phenols seem to be more stable. Angerosa observed in the first 15 minutes of malaxation losses ranging from 40-55% due to change in the temperature from 25 to 35°C. When at laboratory scale the paste was malaxed under nitrogen, losses of phenols were avoided (García, 2001). This indicates the importance of chemical and enzymic reactions taking place because of the presence of oxidoreductases.

Glycosidases present in the olive fruit and consequently in the paste result in the formation of aglycone forms of secoiridoids. The latter are oxidized by the oxidizing enzymes. Servili and his co-investigators (2003) proposed that the time of exposure of

TABLE 5.1
Effect of maturation on the fate of phenolics of various cultivars all over the world.

Cultivars	References
Spanish	
Arbequina	Morello et al., 2004 a,b, Rovellini and Cortesi, 2003, Gimeno et al., 2002, Romero et al., 2002a, Zamora et al., 2001
Cornicabra	Vinha et al., 2005, Salvador et al., 2001
Farga	Morello et al., 2004b
Cacereña	Romero et al., 2002a
Gordal	Romero et al., 2002a
Hojiblanca	Beltrán et al., 2005, Romero et al., 2002a, Brenes et al., 1999
Lechín	Romero et al., 2002a
Manzanilla	Romero et al., 2002 a,b
Morrut	Morello et al., 2004
Picual	Vinha et al., 2005, García et al., 2002, Romero et al., 2002a, Zamora et al., 2001, Brenes et al., 1999
Italian	
Ascolana Tenera	Briante et al., 2002
Coratina	Caponio et al., 2001, Cortesi et al., 2000, Esti et al., 1998; Catalano and Caponio, 1996
Frantoio	Rovellini and Cortesi, 2003, Cortesi et al., 2000
Frantoio Seedling No 17 (FS17)	Briante et al., 2002
Gentile	Esti et al., 1998
Leccino	Škevin et al., 2003, Esti et al., 1998
Nostrana di Brisighella	Rotondi et al., 2004
Ogliarola Salentina	Caponio et al., 2001, Catalano and Caponio, 1996
Picudo	Brenes et al., 1999
Portuguese	
Bical, Bical de Castelo, Borrenta, Cobrançosa, Cordovil de Castelo, Galega, Madural, Verdeal Transmontana	Vinha et al., 2005
Greek	
Koroneiki	Rovellini and Cortesi, 2003, Koutsaftakis et al., 1999
Mastoidis	Rovellini and Cortesi, 2003
Tunisian	
Chemlali	Bouaziz et al., 2004
Croatian	
Bianchera	Škevin et al., 2003
Busa	Škevin et al., 2003

olive paste to air during the malaxation is considered a processing parameter that can be used to control endogenous oxidoreductases such as phenol oxidase, peroxidase

and lipoxygenase.

(For the level of phenols and processing conditions see more in [Chapter 9](#).)

Contribution to Oil Sensory Properties

The bitter taste of virgin olive oil, if not excessive, is a positive attribute and is related to the concentration of TPPs (Angerosa et al., 2001). Gutierrez-Rosales et al. (2003) have correlated bitter intensity of many virgin olive oil samples with the level of individual phenols. The samples were evaluated for bitterness by a panel on the basis of a 1-5 scale, imperceptible, slight, moderate, great, extreme. Solid phase extraction, preparative HPLC, analytical HPLC, and on-line electrospray ionization-collision induced dissociation-mass spectrometry were used to separate, identify and quantify individual phenols. The results indicated that mainly the dialdehydic and aldehydic forms of decarboxymethyl-oleuropein aglycone and the dialdehydic form of decarboxymethyl-ligstroside aglycone are responsible for the bitterness. In another study conducted by Andrewes et al. in 2003, relations were found between sensory properties and concentration of individual phenols. The dialdehydic forms of the deacetoxy-oleuropein and deacetoxy-ligstroside aglycones, derivatives and isomers of ligstroside, and oleuropein aglycones as well as other compounds with bitter or astringent taste were tentatively identified. The fraction containing deacetoxy-ligstroside aglycone produced a strong burning pungent sensation at the back of the throat. The similar aglycone of oleuropein was found to be slightly burning and the sensation was perceived mainly on the tongue. Tyrosol was not bitter but astringent. The final conclusion was that pungent virgin olive oil has a higher deacetoxy-ligstroside aglycone level.

The bitterness of virgin olive oil was also evaluated by Mateos et al. (2004) who quantified by HPLC secoiridoid derivatives. The aldehydic form of oleuropein aglycone was found responsible for the bitter attribute correlations between the level of secoiridoids and sensory bitterness of oils from different varieties. The authors proposed an equation involving the concentration of the aldehydic form of oleuropein.

Bitter Index

The bitter index was proposed by Gutierrez and his coworkers (1992) as an objective method to measure bitterness. The oil is dissolved in hexane, passes over a C18 column and after elution with hexane to remove fats, the retained compounds are recovered with methanol-water. The absorbance of the extract is measured at 225 nm. This conventional method has the drawback that non-bitter phenolic compounds absorb at 225 nm and, as indicated by Mateos et al. (2004), it cannot be correctly applied for comparison to samples from olive varieties with different phenolic profiles.

Antioxidant Properties with Technological Importance

Polar phenols are important for the stability of the oil. A high TPPC appears to be beneficial for the shelf life of the oil and there is a good correlation of stability and total or individual phenol content (Tsimidou et al., 1992; Monteleone et al., 1998; Gutierrez-Rosales and Arnaud (2001). Some oleuropein aglycones have been identified in olive oil but they are not thoroughly investigated experimentally for their activity because of their oxidative instability or difficulties in isolation. These aglycones are expected to be very potent radical scavengers as reported in a recent quantum-chemical study (Nenadis et al., 2005). A detailed presentation of the contribution of phenolic compounds to oil stability is given in Chapter 5.

Antioxidant Properties with Biological Importance

Olive oil phenols, especially oleuropein and hydroxytyrosol, have been studied with respect to their potential to scavenge synthetic radicals, peroxy radicals, superoxide radicals, and hypochlorous acid and to reduce damages induced by hydrogen peroxide and peroxynitrate ion (for review see [Visioli et al., 2004](#), [Boskou et al., 2005](#), and also [Chapter 8](#)).

Free radical scavenging activity of hydroxytyrosol and its derivatives using 1,7-diphenyl-2-picrylhydrazyl radical (DPPH) was measured by [Visioli et al. \(1998a\)](#), [Saija et al. \(1998\)](#), [Gordon et al. \(2001\)](#), [Tuck et al. \(2002\)](#), and [Lavelli, \(2002\)](#). [Tuck et al. \(2002\)](#) studied the scavenging activity not only of hydroxytyrosol but also of its metabolites in rats (homovanillic acid, homovanillic alcohol, glucuronide conjugate, and sulphate conjugate). The glucuronide was found to be a more potent antioxidant compared to hydroxytyrosol itself. Other metabolites of hydroxytyrosol that are expected to be effective radical scavengers are 3,4-dihydroxyphenylacetic acid and its corresponding aldehyde ([Nenadis et al., 2005](#)). [Saija et al. \(1998\)](#), in addition to DPPH test conducted measurements to obtain more information for the scavenging activity of hydroxytyrosol and oleuropein against peroxy radicals near the membrane surface and within the membranes, using a model system consisting of dipalmitoylphosphatidylcholine/linoleic acid unilamellar vesicles and a water-soluble azo compound as a free radical generator.

The radical scavenging capacity of the major phenols present in olive oil was also measured by [Briante et al. \(2003\)](#), who used the stable red radical cation DMPD⁺. The same authors attempted to differentiate phenols not only by their activity to scavenge radicals but also by their ability to chelate metal ions. A metal-chelate mechanism for antioxidant activity of olive oil phenols was also suggested by [Visioli and Galli \(1998\)](#). In order to evaluate olive oil as a source of antioxidants, many researchers attempted to measure ORAC (oxygen radical absorbance capacity) values, which indicate a capacity to protect against oxidation by peroxy radicals ([Ninfalli et al., 2001](#)).

Pellegrini et al. (2003) determined the TEAC (Trolox equivalent antioxidant capacity), FRAP (Ferric-reducing antioxidant capacity) and TRAP (total-radical-trapping antioxidant parameter) in plant food, beverages, and various edible oils including olive oil in an attempt to obtain additional information necessary to investigate the relation between antioxidant intake and oxidative stress related diseases. More recently, Gorinstein and coworkers (2003), measured antioxidant activity using four different techniques to evaluate Spanish olive oils; a. total radical-trapping antioxidative potential by ABAB (TRAP-ABAB), b. radical scavenging activity by DPPH (RSA-DPPH), c. antioxidant assay by beta-carotene–linoleate model system, d. total antioxidant status by ABTS (TAA-ABTS). The best correlation ($R=0.9958$) between total phenols and antioxidant capacity measured by the four methods was found for the beta–carotene /linoleate conjugated oxidation system.

Deiana and others (1999) found that hydroxytyrosol was very protective against peroxynitrite dependent nitration of tyrosine and DNA damage by peroxynitrite *in vitro*.

Scavenging activities of the major olive oil phenols against reactive nitrogen species (peroxynitrite), were studied by De la Puerta and his coworkers (2001). Caffeic acid, oleuropein, and hydroxytyrosol reduced the amount of nitric oxide formed by sodium nitroprusside and were also found to have the ability to reduce chemically generated peroxynitrite. However, as Visioli and his coworkers demonstrated (1998b) oleuropein seems to enhance NO production, from LPS-challenged mouse macrophages. De la Puerta and his co-investigators concluded that this glycoside has both the ability to scavenge nitric oxide but also to cause an increase in the inducible nitric oxide synthase (iNOS) expression in the cell.

Alternative Sources for Olive Oil Phenols

Recovery of Phenols from Milling Wastes

The increasing number of publications indicating a beneficial health role of phenols present in virgin olive oil has led to efforts to recover phenols from waste products and use them in lower quality oils and pharmaceutical products or to apply unusual manufacturing practices to increase phenol content in the oil. A methodology proposed by Visioli and his collaborators (1999) is based on defatting of the waste water, extraction with ethyl acetate (a solvent selective for low and medium m.w. phenols) and further fractionation on a Sephadex column. Skaltsounis and his collaborators (2004), in the framework of an international project (Life Eur/Gr 000611) for an integrated olive mill waste management and the recovery of natural antioxidants, proposed a filtration system followed by absorbance in a suitable resin. The resin outflow is treated in a nanofiltration/reverse osmosis system and recovery of the phenols is obtained by extraction with an organic solvent and chromatographic separation. Leaves were used as a raw material by Guinda et al. (2004). The ethanolic extract was concentrated and

partially purified for the removal of compounds without antioxidant properties. The extract was further fractionated using a countercurrent supercritical fluid extraction at a pilot scale plant.

An attempt to obtain a concentrate rich in hydroxytyrosol was also made by Fernandez-Bolanos et al. (2002) who treated the waste product from the two-phase decanter with steam to increase the solubility of the phenols in water. The estimated yield was 4-5 kg of hydroxytyrosol from 1000 kg of waste product with 70% humidity. When the recovered hydroxytyrosol was added to refined olive oil at a level of 100 mg/kg stability was increased by a ratio of 1:7. Fki et al. (2005) prepared ethyl acetate extracts with a continuous counter-current extraction unit from olive mill waste waters and suggested that the recovered phenols could be used for the stabilization of refined oils instead of synthetic antioxidants. Recently, Crea (2005) developed a process to obtain hydroxytyrosol concentrates. In the patented process the pits are removed from the olives prior to milling. Citric acid is added to the fruit water, which is rich in polar phenols. The latter protects against oxidation and hydrolyses oleuropein and other large molecules. The method is claimed to be completely solvent free.

Isolation from Olive Leaves and Other Related Sources

Other techniques aim at increasing the level of polyphenols in olive oil by using unusual processing techniques or by extracting olive leaves. Kachouri and Hamdi (2004) proposed fermentation by *Lactobacillus plantarum* added to olive mill waste water to depolymerize high molecular weight phenols and increase the transport of simple phenols from the vegetation water to olive oil. Gibriel and his coworkers (2004) suggest the addition of leaves during the crushing of olives to obtain oils with a higher TPPC. Paiva-Martins et al. (2004) proposed extraction of olive leaves and the addition of the extract to refined olive oil to restore the stability lost during refining.

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6

Olive Oil Quality

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Introduction

The questions “What is virgin olive oil quality?” or “How can quality be objectively determined?” as stated elsewhere (Psomiadou et al., 2003), seem to be rather rhetorical and their meaning may differ depending on the person who poses the question. Authenticity of an olive oil is the first priority because of the high price of the product. In the case of monovarietal oils or oils of protected denomination of origin, misbranding issues become important.

The list of methodologies and purity criteria developed over the years is long and continuously updated. When genuineness is guaranteed, commercial quality of the product is mainly characterized by sensorial features (Angerosa, 2000) and free acidity content. The maximum level of initial free acidity is rather low for edible types of the product (0–2%, expressed as oleic acid). Oxidative status that affects nutritional and sensorial value of olive oil is not considered in depth in the existing legislation. In our view the overall quality of the oil from production to consumption is strongly related to oxidative stability and its impact on the evolution of flavor, taste, color, and the content of endogenous antioxidants and other minor constituents beneficial to health. Therefore, this chapter emphasizes the factors and conditions affecting oxidative stability of virgin olive oil and the changes observed in the macro- and micro-constituents of the product from production to consumption. Changes due to enzymatic activity during storage (oxidative and hydrolytic) are also discussed. Effects of cultivar and extraction practices to the initial virgin olive oil quality characteristics are not detailed since they are covered in [Chapters 4, 5, 7, 10, and 11](#). Commercial quality criteria of the product as defined by international legislative bodies, given in Chapter 7, are discussed only when necessary.

Extended chapters on olive oil quality can be found in relevant publications edited by Boskou (1996), Kiritsakis (1998), and Harwood and Aparicio (2000). Recent reviews on oxidative stability and related subjects of virgin olive oil were published by Velasco and Dobarganes (2002), Servili and Montedoro (2002), and Kiritsakis et al.

(2002). Spanish, Italian, and Greek literature is rich in editions on the quality of oils from local cultivars.

Virgin Olive Oil Shelf Life

Although “olive oil is best when fresh and wine after aging,” according to the common belief, virgin olive oil is a stable oil and its shelf life is longer in comparison to that of other edible oils. The characteristic triacylglycerol composition and the presence of antioxidants (mainly polar phenols and α -tocopherol) are established key factors for the resistance of the oil to autoxidation. A series of other constituents, namely, free fatty acids, pigments, unsaturated hydrocarbons, enzymes, and trace metals are expected to affect positively or negatively, though to a lesser extent, oxidative stability. According to regulations product shelf life cannot exceed an 18-month period. However, as no specific instructions for storage conditions are given to consumers, maltreatment of the oil, even by those who traditionally use it, is common.

Virgin olive oil “keepability” can be defined as the length of time under ambient temperature within which no off-flavors are developed and quality parameters such as peroxide value and specific absorbance values are retained within accepted limits for a stated commercial category.

Prediction of food shelf life is a desirable goal in the food industry. To estimate olive oil resistance to oxidation, the Rancimat or AOM test results were found meaningful (Psomiadou et al., 2003; Hrnčirik and Fritsche, 2005). Efforts for prediction based on kinetic and/or mathematical approaches seem promising as far as they concern “the possibility of the packaged product not to reach the end of its shelf life under certain conditions” (Monteleone et al., 1998; Pagliarini et al., 2000; Goutierrez and Fernandez, 2002; Psomiadou et al., 2003; Coutelieris and Kanavouras, 2006; Kanavouras and Coutelieris, 2006). Oxidative Stability Index (OSI) values are reported to correlate with results produced by fast-ultrasound assisted method for the determination of the oxidative stability of virgin olive oil (Canizares-Macias et al., 2004). With this new technique the determination time is reduced many folds.

Olive oil shelf life depends on the material of the container used (De Leonardis and Macciola, 1998). Stainless steel tanks, tinsplate metal vessels, and glass containers protect the oil from oxygen and light. Polymers with special characteristics such as polyethyleneterephthalate coated with high barrier resin or with high barrier resin including “oxygen scavenger” has been recently suggested as a promising alternative to traditional glass, though mechanical compatibility remains to be tested (Cambacorta et al., 2004).

Storage of oils under nitrogen can extend shelf life as shown by Di Giovacchino and coworkers (2002). The importance of introduction of nitrogen to an olive oil production line has often been stressed (Garcia et al., 2001).

Storage in the Dark

Examination of the real keepability of virgin olive oil is now gaining popularity in contrast to studies based on accelerated oxidation that prevailed in the 1980's. Trials carried out at room temperature (25-30°C or lower) for samples stored in open or closed vials indicated a wide range of shelf life periods that are related to the combined effect of minor and major constituents.

Changes in the Lipid Substrate

Recent revision of EC legislation (EC, 2003) concerning olive oil characteristics reduced further initial values for free acidity of edible types of virgin olive oil. If an oil sample does not meet the limits set for free acidity, then, it is examined for its conformity to other quality parameters. Free acidity increases with time in both filtered and unfiltered oils (Brenes et al., 2001). However, acidity is not expected to contribute to an increase in oil stability as was indicated from the results of Mateos and coworkers (2003). The authors using purified olive oil as a model system, devoid of minor constituents, examined the effect of increased amounts of oleic acid (0-1%) in the absence and presence of polar phenols. The effect was not statistically significant in the presence of polar phenols.

Changes in the content of positional isomers of diacylglycerols (DGs) in storage have been proposed as a quantitative measure to assess olive oil history (Frega et al., 1993). In particular, the ratio of 1,3 -DGs/1,2-DGs or the ratio of 1,2-DGs to the total amount of DGs has been proposed for such an objective (Perez-Camino et al., 2001). Recently, Spyros et al. (2004), using ³¹P NMR showed that the isomerization depends strongly on the rate of the TG hydrolysis, the initial free acidity of the oil and storage conditions. The authors applied their model to several olive oil samples of known and unknown storage history and found a very good agreement for a storage period of up to 12 months. Longer periods of storage cannot be predicted from this index alone, since isomerization of DGs reaches equilibrium with time.

In almost every paper cited herein on olive oil stability, there are data regarding changes in the lipid substrate during autoxidation. These changes depend on an array of factors that are not always examined. Many of the trials involved storage of oil in open vials or in petri dishes at elevated temperatures. In this way autoxidation proceeds faster and changes in minor compounds evolve in a pace and manner possibly different from that occurring under reduced oxygen atmosphere. Generally, good quality virgin olive oil resists autoxidation longer than other oils rich in mono-unsaturated fatty acids (Koski et al., 2002). More recently, investigations focused on alterations of oil under conditions, mimicking storage in tanks, retail containers or domestic use. The main drawback of such trials is that monitoring periods cover only the initial stage of the oxidation process. Oxidative changes in the oil matrix are very slow when the container is filled with oil or nitrogen has been flushed through. Slow

rates of oxidative changes have also been observed in veiled virgin olive oils (Tsimidou et al., 2005). Virgin olive oils stored in sealed bottles for 12 to 24 months at ambient temperatures did not show any alterations in peroxide and K_{232} values (Brenes et al. 2001; Psomiadou and Tsimidou, 2002a) or in the percent composition of polyunsaturated fatty acids (Rastrelli et al., 2002; Morelló et al., 2004). Oils oxidized faster when the containers were opened periodically (Psomiadou and Tsimidou, 2002a) or were half empty (Rastrelli et al., 2002). The pattern of oxidation depends on the fatty acid composition of selected samples and the level of anti/pro-oxidants present. Therefore, observations and conclusions depend heavily on the experimental design. A random scheme in the selection of samples (Gomez-Alonso et al., 2005) also restricts generalizations.

Monitoring of changes in the substrate is based on two parameters: the peroxide value and absorbance at 232 nm. Changes in absorbance at 270 nm are much lower, especially in the first stage of autoxidation. Grigoriadou and Tsimidou (2006) examined whether K_{232} values and other UV absorbance characteristics could replace the determination of peroxide value (PV) in routine quality control of ready to be consumed or stored virgin olive oil. For this reason PV and extinction coefficients were determined for a large number of virgin olive oil samples ($n=40$). The samples were then stored at 45°C for several weeks. Changes in the lipid matrices were monitored by periodic measurements of the same quality indices. UV spectra and derivative spectra were obtained before and during storage. Regression data showed that the PV is correlated with the K_{232} value not only at time zero but also during storage. Evidence derived from the first derivative spectrum was found very useful for the evaluation of the oxidative status of the oil. These findings may be used to simplify the decision tree suggested for “the verification of consistency of a virgin olive oil sample with commercial category declared” in the EEC Regulation N° 1989 (2003), which takes into account acidity measurement, PV and absorbance values (K_{270} , ΔK , K_{232}).

UV absorbance seems very useful for the collection of information about the oxidation process during storage. Peroxide value determination can, therefore, be excluded from the routine control and the use of unwanted chemicals can be avoided.

Volatile Compounds Content and Development of Sensory Defects. The current olive oil legislation and trade standards refer to four groups of off-flavors: fusty, mustiness-humidity, winey-vinegary, and rancid. The three first groups are related to olive quality whereas the last one, rancid, develops in storage. Lampante oils are those characterized by intense defects. Such an oil is subjected to refining for further industrial use. Morales and coworkers (2005) identified the major components responsible for the negative characteristics of oils for three major European varieties. The highest sensory significance, evaluated by odor activity values, corresponded to 1-octen-3-ol for mustiness-humidity; ethyl butanoate, propanoic, and butanoic acids for fusty sensory defect; acetic acid, 3-methyl butanol, and ethyl acetate for winey-vinegary; and several

saturated and unsaturated aldehydes and acids for rancid defect. The presence of acids (acetic, butanoic, hexanoic, and heptanoic) indicates a high level of oxidative alteration in the oil. Rancid flavor comes from the contribution of 2-octenal, 2-heptanal, and 2-decenal with high odor thresholds. Saturated aldehydes—hexanal, nonanal, octanal, pentanal, and heptanal—influence the final aroma. Temperatures higher than the ambient favor increase in total volatile compounds content due to an increase in the content of hexanal and trans-2-hexenal in the presence of air. This increase leads to the development of rancid flavor (Di Giovacchino et al., 2002). Kanavouras and coworkers (2004) and Coutelieris and Kanavouras (2006) determined and used evolution of hexanal over time as the main indicator of the oxidative changes that take place in the oil matrix. Gómez-Alonso et al. (2004) observed that under accelerated oxidation of purified olive oil triacylglycerols at 40–60°C, the rancidity threshold coincided with the induction period for the kinetics of 2,4-decadienal formation.

Changes and Effect of Polar Phenols

The crucial role of polar phenolic compounds has been well established over the years (Tsimidou, 1998; Velasco and Dobarganes, 2002; Briante et al., 2003). High correlation coefficients have been repeatedly reported between total polar phenol content (or individual phenols) and peroxide values after storage for a given period or OSI values (Tsimidou et al., 1992; Pagliarini et al. 2000; Cinquanta et al., 2001; Blekas et al., 2002; Del Carlo et al., 2004). Ninfali et al. (2001) reported such a relationship with Orac Radical Absorbance Capacity values, i.e. the antioxidant potential of the oil. Gorinstein et al. (2003) and Pellegrini et al. (2003) extended this approach using more tests. Correlations for monovarietal oils vary with year of production. Similar relationships have been reported for *o*-diphenol content or for individual diphenols over the years. Despite variations in methodology, all the evidence supports the great significance of polar phenols for the oil stability (Montedoro et al., 1992; Litridou et al., 1997; Tsimidou, 1999; Hrnčirik and Fritsche, 2004; Gallina-Toschi et al., 2005).

Total polar phenols change during storage due to hydrolytic and oxidative processes. Total polar phenol content reduction was found to follow changes in the lipid substrate (Psomiadou and Tsimidou, 2002a). This reduction is limited (20–30%) under reduced oxygen availability. More recent is the interest in the contribution of individual phenols to stability. Information for the evolution of phenolic compounds in storage was very valuable (Cinquanta et al., 1997; Brenes et al., 2001; Lavelli, 2002; Tsimidou et al., 2005). Although sometimes conflicting results have been reported, it seems that tyrosol and hydroxytyrosol levels are continuously enriched due to hydrolysis of bound phenols. At the same time, hydroxytyrosol, an unstable compound, is oxidized rapidly in contrast to tyrosol. Loss in bound forms of hydroxytyrosol is greater than that of tyrosol derivatives (Gómez-Alonso et al., 2005). Lignan levels are less affected by storage conditions (Morelló et al., 2004). Type and levels of individual

phenolic compounds influence antioxidant capacity of virgin olive oil differently. The positive contribution of complex aglycone forms of hydroxytyrosol is stressed by many investigators. Changes in oil taste occur to a greater or a lesser extent since, for example, hydroxytyrosol aglycones are bitter and the free phenol, hydroxytyrosol, is not. Mateos et al. (2003) found that the concentration of *o*-diphenols influences the performance of α -tocopherol. This observation is not in accordance with previous reports on the behavior of α -tocopherol or *o*-diphenols (e.g. Yanishlieva and Marinova, 1992; Blekas et al., 1995). Studies on interactions of polar phenolic compounds with α -tocopherol in model systems are expected to be meaningful if the substrate used is purified olive oil (devoid of pro/antioxidants), the test compounds are added at realistic levels and experiments are carried out at ambient temperature. Such experiments are so lengthy that in most cases induction period is not reached; even after 3 month storage (45°C, open vials) purified triolein to which a low amount of caffeic acid (10 ppm) had been added did not reach induction period.

Changes and Effect of Tocopherols, Squalene, Pigments, and Other Minor Components

For years, the possible contribution of α -tocopherol, squalene (the major minor constituent of the oil) and pigments (chlorophyll derivatives and carotenoids) to the stability of the oil remained poorly understood. Many investigators (Papadopoulos et al., 1993; Blekas et al., 1995; Baldioli et al., 1996; Manzi et al., 1998; Aparicio et al., 1999; Psomiadou and Tsimidou, 2002a; Deiana et al., 2002) focused on the role of α -tocopherol in the storage period. The content in tocopherols depends on many factors, cultivar being an important one. The oxidation pattern seems to be greatly influenced by the initial level of tocopherols. Deiana and coworkers (2002) reported a high correlation between α -tocopherol level and conjugated dienes in storage. Handling may cause a continuous loss if oxygen supply is renewed. This was shown by Psomiadou et al. (2000) in a trial with samples stored for 24 months which were opened periodically and samples that remained in closed vials for 24 months.

In olive oil models devoid of pro/antioxidants the effect of α -tocopherols was found to be antioxidant in the range of 100-1000 mg/kg. The best effect was found for the lowest levels of addition (Blekas et al., 1995). Some authors argue that α -tocopherol participates in the autoxidation process after a sufficient amount of hydroperoxides is accumulated. Others (Morelló et al., 2004) infer that α -tocopherol is consumed from the beginning. In all cases, α -tocopherol, though not so strongly in comparison to polar phenols, correlates with oil stability and it contributes significantly to the oil resistance to oxidation. It has also been found to interact with carotenoids and squalene but the results of such interactions are strongly dependent on experimental conditions (e.g. Rastrelli et al., 2002). Both pro-oxidant and anti-oxidant activities have been assigned to carotenoids depending on the substrate, concentration, and presence/level of individual tocopherols. However, the low amount of

β -carotene and lutein present in virgin olive oils limits their importance in autoxidation process, which is expected to cause further reduction of their initial levels.

Psomiadou and Tsimidou (1999) assigned the weak antioxidant activity of squalene in olive oil to competitive oxidation phenomena. The authors reported on the confined role of squalene in the presence of primary antioxidants. Using HPLC methods, the same authors (2002a) monitored changes in α -tocopherols content (at 100 mg/kg oil level of addition) and squalene content (7000 mg/kg oil) and found that the decrease of the former is not in expense of the latter or *vice versa*. Therefore, it is not clear if tocopherols have a protective effect on squalene, as suggested by Manzi et al. (1998).

The importance of chlorophyll derivatives (mainly of pheophytin α) in the dark had really been overlooked because their role under light exposure attracted all the interest. Virgin olive oil is a natural lipid system that varies significantly in green pigment content (Mínguez-Mosquera et al., 1990; Gandul-Rojas and Mínguez-Mosquera, 1996; Psomiadou and Tsimidou, 2001). Psomiadou and Tsimidou (2002a), used the HPLC method they developed for the simultaneous elution of tocopherols and pigments (Psomiadou and Tsimidou, 1998), and found that the loss of pheophytin α in samples stored in sealed bottles for two years (ambient temperature) ranged from 50-90%. Changes in individual compounds were observed from the first stages of autoxidation. These were attributed to epimeric, pyro- and allomeric forms. Since then much discussion and work has been added as to whether the evolution of pheophytin derivatives (pyro and/or allomeric forms) can be used as indices of the oil history (production and storage conditions) (Gallardo-Guerrero et al. 2005; Anniva et al., 2006). Sterol contribution in the dark at low temperatures is negligible (Gutiérrez and Fernández, 2002) as expected.

In conclusion, it can be said that autoxidation of virgin olive oil during storage is an extremely slow process if technology delivers products with low initial peroxide/ K_{232} values and high levels of polar phenols/ α -tocopherol content. Exclusion of air in bottled virgin olive oil is a prerequisite.

Storage Under Light Exposure

Virgin olive oil should not be exposed to light, as it contains endogenous photosensitizers at significant levels (5-40 mg pheophytin α). Pheophytin α has a higher photosensitizing effect in comparison with the respective chlorophyll (Endo et al., 1984). This effect is concentration-dependent and further enhanced by oxygen availability (Psomiadou and Tsimidou, 2002b). Changes in the lipid substrate are mainly monitored by the peroxide value measurement due to an oxidation mechanism that restricts formation of conjugated dienes. In open vessels autoxidation and photosensitized oxidation progress in parallel so that increase in K_{232} values is also expected.

Investigators have always suggested protection from light with the use of suitable containers. In spite of the suggestions, companies still prefer transparent contain-

TABLE 6.1
Experimental Conditions in Olive Oil Storage Studies (2000-present) Under Light Exposure

Reference	Type of container/sample amount	Light exposure conditions	Storage period/temperature	Analytical characteristics determined
Pagliarini et al., 2000	Dark glass bottles, 500 ml screw capped	Supermarket shelves, uncontrolled light	14-17 months/uncontrolled temperature	PV, K ₂₃₂ , OSI, TChC [*] , total carotenoids, tyrosol, hydroxytyrosol, α -tocopherol
Psomiadou and Tsimidou, 2002b	transparent glass bottles, 10ml; 7% headspace	Fluorescence light 12100lx	Till Chl loss>90%/thermostated chamber, 25 \pm 1 $^{\circ}$ C	PV, K ₂₃₂ , OSI, individual chlorophylls and carotenoids TPPC ^{**} , α -tocopherol, squalene
Okogeri and Tasioula-Margari, 2002	Transparent glass bottles, 100 ml, 3% headspace	Diffused light	12 months/6-18 $^{\circ}$ C	PV, K ₂₃₂ , TPPC, phenolic fractions, α -tocopherol
Rastrelli et al., 2002	transparent glass bottles, 500ml; series a: half-empty, series b: 3% headspace	Diffused light	24 months/room temperature	PV, α -tocopherol, squalene, individual phenolic compounds, PUFA, sterols
Škevin et al., 2003	Open Petri dish/10g	UV Hanau lamp (365 nm)	8h/?	K ₂₃₂ , K ₂₇₀ , PV, TChC, TPPC, TdiPC ^{***}
Kanavouras et al., 2004	Glass, PET and PVC bottles (500 ml), sealed with standard polypropylene threaded caps	Fluorescence light bulbs (4x 40 W),	12 months/15, 30, 40, $^{\circ}$ C, 60%RH	PV, K ₂₃₂ , volatiles

*TChC: total chlorophyll content; **TPPC: total polar phenol content; ***TdiPC:total o-diphenol content

ers following general trends in marketing. The latter are expected to change soon as consumers' preference for virgin oil grows. Consumers are aware of the nutritional benefits of the oil and appreciate that these properties are preserved better in the dark under reduced oxygen availability. Studies on photo-oxidation of virgin olive oil (Gutierrez-Rosales et al., 1992; De Leonardis and Macciola, 1998; Rahmani and Csallany, 1998; Psomiadou and Tsimidou, 1998) or on olive oil models (Kiritsakis and Dugan, 1985; Fakourelis et al., 1987; Rahmani and Saad, 1989) were rather limited until 2000. Monitoring of oxidation was mainly based on changes of the lipid substrate. Since 2000, there have been studies on photosensitized oxidation and other factors which either included in the stability experiments light exposure or mimicked conditions at the consumer sale points. Experimental conditions used in such studies vary; and as shown in the table changes in the content of photosensitizers and quenchers are not always taken into consideration (Table 6.1).

Loss of α -tocopherol is greater than that of polar phenols in closed transparent bottles. Efficiency of α -tocopherol under light exposure has been attributed to quenching of singlet oxygen by a charge transfer mechanism. Pirisi et al. (1998) have reported on the photolysis kinetics of α -tocopherol under sunlight and artificial light ($\lambda > 290$ nm) in virgin olive oils and triolein. Psomiadou and Tsimidou (2002b) presented for the first time data on the protective role of squalene towards α -tocopherol. This was demonstrated by HPLC monitoring of the two compounds. Two possible explanations were given by the authors: 1) regeneration of tocopherols by squalene and 2) formation of stable cyclic hydroperoxides by squalene, which has been reported to trap two molecules of oxygen (Psomiadou and Tsimidou, 1999).

Carotenoids quench singlet oxygen and excited states of photosensitizers. The physical quenching mechanism is explained by their low singlet energy state and also by a light filtering effect due to extended conjugation. This activity explains why their loss was found to be negligible under storage in closed vessels compared to losses observed when oils were oxidized in open vessels (Psomiadou and Tsimidou, 2002b).

Virgin olive oil storage conditions should exclude light. Attention should be paid to outlet positions in the supermarkets and also in domestic use.

Alterations Due to Enzymatic Activity

The sequences of enzymatic actions that are expected to contribute to deterioration of olive oil have been outlined by Morales and Przybylski (2000). Lipase, lipoxigenase, and phenoloxidase and hydroperoxide lyase/isomerase or dehydrogenase activities have been observed during olive ripening, storage and the extraction process, mainly in the malaxation stage (Sciancalepore and Longone, 1984; Sciancalepore, 1985; Goupy et al., 1991; Angerosa et al., 1998; Morales et al., 1999; Salas and Sanchez, 1999). In this view undesirable enzymatic activity can be confined if GMP principles for virgin olive oil production are followed. Simultaneous grinding of stones and pulp has as a result the transfer of enzymes to *orujo* oil, which contribute to its deteriora-

tion in storage. (Boskou, 1996; Kiritsakis, 1998). Since desirable volatiles, responsible for the green odors are also accumulated in the same way (Olias et al., 1993; Morales et al., 1996; Aparicio and Morales, 1998; Angerosa et al., 2000; Ridolfi et al., 2002) enzymatic activity cannot be precluded in oil production line. Georgalaki and coworkers (1998) reported inconclusive information on the potential presence of proteins and oxidative enzyme (lipoxygenase and phenoloxidase) activities in virgin olive oils. The interest in olive lipoxygenase and other enzymatic activities and olive oil sensory quality is increasing (Williams et al., 2000; Briante et al., 2002).

Microbiological Quality

Microbiological studies on olive oil are limited and it is not clear whether or not microorganisms are involved in the improvement of the sensory attributes of the oil during the decanting process. Ciafardini and Zullo (2002a) carried out a trial during the sedimentation period, when the solid particles and microdrops of vegetation water present in the newly produced olive oil separate from the oily phase. They identified yeasts as the most prominent microbial population in the oil. The authors suggested filtration as a means to ensure top-quality extra virgin olive oil despite the serious reduction in polar phenols. The same authors (2002b) claim that microbiological glycosidases in stored olive oil could also be responsible for the loss in bitterness in addition to other hydrolytic enzymes. Hazard Analysis Critical Control Point preventive system adoption in the food business is expected to confer microbiological safety on olive oil production (Pardo et al., 2002).

Olive Oil Quality Indices

Many efforts are found in the literature for the establishment of indices of "authentication," "cultivar characterization or differentiation," "age," or "history of oil characterization," etc. All these efforts have tremendous limitations that should be considered before limits are proposed in legislation. Tunisian virgin olive oil exports suffered from limits set for the trilinolein content that did not consider natural variability. When such indices are designed to explain "history" of oils regarding storage conditions, the case becomes more complex. Data should be verified many times before adopted in official control. Quality control within the industry is free to apply any index that appears to protect its interests most.

In the early 1990s, the International Olive Oil Council proposed a quality index to numerically express the quality of virgin olive oil. The Global Index of Quality (I.G.Q.), a scale from 0-100, was given by a linear model based on sensory evaluation score, free acidity, specific absorbance value K_{270} , and peroxide value. The legislative limits that have been recently revised (EC, 1991; 2003) made this model invalid because of changes in the limits set for each analytical parameter. However, the coefficient of correlation for the respective quality criteria were 0.50, 0.25, 0.125 and

0.125. Tsimidou et al. (1997) criticized this approach and stressed that commercial classification of olive oils does not always coincide with the actual stability of the oil and that sensory quality does not guarantee resistance to oxidation. Based on a multidimensional statistical analysis the authors proposed that a more complex factor expressing stability should be inserted to the equation. Such a factor could be the outcome of the co-evaluation of all parameters related with stability. Alternatively, total quality estimation could be expressed by two different indices, one for the sensory and the other for the oxidative stability of the oils. In this view, Psomiadou et al. (2003) proposed that α -tocopherol content, total polar phenol content and total chlorophyll content, routinely determined, can also be considered for shelf life prediction and expiration dating.

Consumer Preference

As shown by Monteleone and coworkers (1997), the relative importance of the sensory characteristics as a driver of overall liking for Italian extra virgin olive oil was taste/flavor > odor > appearance. Differences were not driven by sex. Males were more responsive to odor liking as a driver of overall liking than were females. Older consumers (more than 37 years) were more attracted by appearance than younger consumers.

International commerce takes into account odor/taste preference as it is exemplified by the requirements of international competitions. The Mario Solinas Quality Award is an international competition for extra virgin olive oil that was first launched in the 2000-2001 crop year. The award is devoted to the memory of the Italian pioneer scientist on sensory evaluation of olive oil. The sponsor, IOOC, classifies the oils into three groups according to the fruity attribute given by the panel that issues the sensory analysis certificate (Table 6.2).

This classification reflects consumer perception and appreciation of the fruity attribute of virgin olive oil. This view is different from current requirements of legislation. Legislative provisions are (a) no defects for extra virgin olive oil and a score ≤ 2.5 for virgin olive grades, and (b) fruity attribute score just above zero for both commercial grades. From the outcome of the last 4 year competitions it seems that Italian oils prevail in medium fruitiness whereas Greek and Spanish oil had more intense flavor. More evidence on positive and negative sensory attributes of olive oil can be found

TABLE 6.2
Mario Solinas Award Requirements for Fruity Attribute

Group	Median of the fruity attribute
Intense fruitiness	≥ 5
Medium fruitiness	$2.5 \leq m < 5$
Slight fruitiness	< 2.5

in published papers (Morales et al., 2000; Morales, and Tsimidou, 2000). Although color is an important quality attribute at present it has not attracted the interest of legislative bodies. Traditional consumers' preference for greenish or yellowish hues of oils depends strongly on area of origin (Rahmani and Csallany, 1991; Ranalli, 1992). The scientific community has been working on the development of objective evaluation of olive oil color for many years (Minguez-Mosquera et al., 1991).

Instead of an Epilogue

Olive oil quality is a multifaceted issue that starts in the olive grove and is expected to end after digestion. Recent findings, including the discovery of an anti-inflammatory compound, oleocanthal, in newly pressed high quality extra virgin olive oils proved that olive oil quality has effects that remain even after digestion. Nature was generous with the olive tree and its products. Gods and people of the Mediterranean region adored it. Spanish, Italian, and Greek scientists struggled hard in the last century to establish it as an exceptional lipid source. The international scientific community became friendly during the last 15 years and this attitude added much to knowledge about its merits. Industry makes a lot of profit from scientific evidence and support. The ultimate consumer expects to receive the best quality product at a reasonable price throughout the year. The latter is a goal for the next few years.

Last but not least, the consensus report of eminent scientists on the healthy effect of virgin olive oil is quoted for those who are really interested in "olive oil quality." The signatories of the Jaen consensus report in 2004 were: Perez-Jimenez F, de Cienfuegos GA, Badimon L, Barja G, Battino M, Blanco A, Bonanome A, Colomer R, Corella-Piquer D, Covas I, Chamorro-Quiros J, Escrich E, Gaforio JJ, Luna PPG, Hidalgo L, Kafatos A, Kris-Etherton PM, Lairon D, Lamuela-Raventos R, Lopez-Miranda J, Lopez-Segura F, Martinez-Gonzalez MA, Mata P, Mataix J, Ordovas J, Osada J, Pacheco-Reyes R, Perucho M, Pineda-Priego M, Quiles JL, Ramirez-Tortosa MC, Ruiz-Gutierrez V, Sanchez-Rovira P, Solfrizzi V, Soriguer-Escofet F, de la Torre-Fornell R, Trichopoulos A, Villalba-Montoro JM, Villar-Ortiz JR (Perez-Jimenez et al., 2005).

1. Aging represents a great concern in developed countries because of the number of people involved and the pathologies related to it, like atherosclerosis, morbus Parkinson, Alzheimer's disease, vascular dementia, cognitive decline, diabetes, and cancer.
2. Epidemiological studies suggest that a Mediterranean diet (which is rich in virgin olive oil) decreases the risk of cardiovascular disease.
3. The Mediterranean diet, rich in virgin olive oil, improves the major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose metabolism, and antithrombotic profile. Endothelial function, inflammation, and oxidative stress are also positively modulated. Some of these effects are at-

- tributed to minor components of virgin olive oil. Therefore, the definition of the Mediterranean diet should include virgin olive oil.
4. Different observational studies conducted in humans show that the intake of monounsaturated fat may protect against age-related cognitive decline and Alzheimer's disease.
 5. Microconstituents from virgin olive oil are bioavailable in humans and show antioxidant properties and capacity to improve endothelial function. The anti-thrombotic properties of virgin olive oil can also help to modify hemostasis.
 6. In countries where the populations follow a typical Mediterranean diet, such as Spain, Greece, and Italy, where virgin olive oil is the principal source of fat, cancer incidence rates are lower than in northern European countries.
 7. The protective effect of virgin olive oil can be most important in the first decades of life. This suggests that the dietetic benefit of virgin olive oil intake should begin before puberty, and continue throughout life.
 8. The Mediterranean diet, based on virgin olive oil, is compatible with a healthier aging and increased longevity.
 9. However, despite the significant advances of the recent years, the final proof about the specific mechanisms and contributing role of the different components of virgin olive oil to its beneficial effects requires further investigation.

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7

Analysis and Authentication

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Introduction

Olive oil, differently from most vegetable oils, is obtained by means of some technological operations which have the purpose to liberate the oil droplets from the cells of olive flesh. Due to its mechanical extraction, it is a natural juice and preserves its unique composition and its delicate aroma, and therefore can be consumed without further treatments. However, a refining process is necessary for making edible lampante virgin olive oils. Lampante oils cannot be directly consumed because of the presence of organoleptic defects or because chemical-physical constants exceeding the limits established by International Organizations.

Consumers are becoming continuously more aware of potential health and therapeutic benefits of virgin olive oils and their choice is oils of high quality which preserve unchanged the aromatic compounds and the natural elements that give the typical taste and flavor.

Because of the steady increasing demand and its high cost of production virgin olive oil demands a higher price than other vegetable oils. Therefore, there is a great temptation to mix it with less expensive vegetable oils and olive residue oils. On the other hand even refined olive oils, due to high mono-unsaturated fatty acids content and other properties, often have prices higher than those of olive residue oil or seed oils. Thus, there are attempts to partially or totally substitute both virgin and refined olive oils with pomace oil, seed oils, or synthetic products prepared from olive oil fatty acids recovered as by-products in the refining process. The substitution or adulteration of food products with a cheap ingredient is not only an economic fraud, but may also have severe health implications to consumers. Such is the case of the Spanish toxic oil syndrome (TOS), resulting from the consumption of aniline-denaturated rapeseed oil that involved more than 20,000 people (World Health Organization, 1992; Wood et al., 1994; Gelpi et al., 2002).

Therefore, there is always a need to protect consumers through effective and clear regulations that assure uniformity of definitions, labelling rules, instrumental tech-

niques and methodologies, limits, and identity characteristics in all countries.

At the moment Codex Alimentarius, European Commission (EC), and International Olive Oil Council (IOOC) generally give the same limits for the olive oil identity characteristics. However, there are some differences between EC regulations and IOOC Trade Standards due to the fact that this last organization must take into account characteristics of all olive oils and pomace oils produced by all IOOC members. These characteristics can be different from those of European Union countries because of different cultivars and climate conditions.

In the last 20 years a great analytical effort was made from food chemists and many gas chromatographic, high pressure liquid chromatographic, and spectrometric methodologies were developed to evidence possible frauds. Several analytical approaches are currently included in regulations of the European Community, the Draft of Codex Alimentarius Standards, and the International Olive Oil Trade Standards.

The application of new reliable analytical approaches had, as a consequence, a reduction of adulteration, but there are still problems with sophisticated practices. These are the addition of: i) hazelnut oil; ii) olive oils subjected to forbidden deodorization in mild conditions; iii) olive oil obtained by second centrifugation of olive pastes (*remolido*).

The evaluation of quality and the checking genuineness of olive oils is made on the basis of analytical data of a number of parameters which must be within limit values established by the European Commission (EC Reg No 2568/1991 and its latest amendment EC Reg No 1989/2003), the Codex Alimentarius Norm (Codex Alimentarius Commission Draft, 2003) and the IOOC Trade Standards (International Olive Oil Council Trade Standards, 2003). The methods generally applied can be divided into two groups: i) methods adopted by national and international organizations such as IOOC, Codex Alimentarius, and the European Commission; ii) methods not evaluated by standardizing bodies, but proposed by researchers, which are either used to support nonconclusive results of official analyses, when sophisticated adulterations have to be evidenced, or to obtain a rapid and a more complete evaluation of olive oil quality.

Definitions

Olive oils can be distinguished in virgin olive oils mechanically or physically extracted from olive fruits, olive oils coming from further refining treatments and olive pomace oils, obtained by refining of the oil extracted from the olive pomace with a suitable solvent. All categories of olive oils are summarized in [Table 7.1](#). The European Regulations do not permit the trade of refined olive oil or refined pomace olive, but allow trading their blends with virgin olive oils. EC (EC Reg. No 2568/91) fixes the following categories: extra virgin, virgin and lampante, whereas IOOC and Codex also include, among edible olive oils, the ordinary grade. Codex Alimentarius does not consider oils not fit for human consumption.

TABLE 7.1
Definition of all categories of olive oils according to the different International Organizations

Category	Definition according to EC, IOOC and Codex Alimentarius
Extra virgin olive oil	Virgin olive oil having free acidity, as % of oleic acid, up to 0.8 and the other characteristics according to regulations in force
Virgin olive oil	Virgin olive oil having free acidity, as % of oleic acid, up to 2.0 and the other characteristics according to regulations in force
Ordinary virgin olive oil	Virgin olive oil having free acidity, as % of oleic acid, up to 3.3 and the other characteristics according to regulations in force. EC does not include this category
Virgin lampante olive oil	Virgin olive oil having free acidity, as % of oleic acid, greater than 3.3 and the other characteristics according to regulations in force
Refined olive oil	Olive oil obtained from virgin olive oil refining that preserves its natural glyceridic composition, having free acidity, as % of oleic acid, up to 0.3 and the other characteristics according to regulations in force
Olive oil	Oil obtained by blending refined olive oil and virgin olive oil having free acidity, as % of oleic acid, up to 1.0 and the other characteristics according to regulations in force
Crude pomace olive oil	Oil extracted from olive pomace by means of a solvent having the characteristics according to regulations in force
Refined olive residue oil	Olive oil obtained from crude olive oil refining that preserves its natural glyceridic composition, having free acidity, as % of oleic acid, up to 0.3 and the other characteristics according to regulations in force
Olive residue oil	Oil obtained by blending refined olive residue oil and virgin olive oil having free acidity, as % of oleic acid, up to 1.0 and the other characteristics according to regulations in force

Quality Parameters

Olive oils are classified by different International Organisms according to their quality which is established on the basis of certain parameters. These parameters verify hydrolytic and oxidative processes that take place in the fruits and during the technological procedures for extracting and refining, and also during the oil preservation. Parameters used by the different international organizations to check olive oil quality are reported in [Table 7.2](#).

Common to all international organizations are the determination of free fatty acids, peroxide value, spectrophotometric absorbances in the UV region, organoleptic characteristics, and halogenated solvents. In addition, the Codex Alimentarius and IOOC Standards include insoluble impurities, some metals and unsaponifiable mat-

TABLE 7.2
Quality parameters fixed by the different International Organizations.

Parameter	IOOC	Codex Alimentarius	EC
Sampling method	x	x	x
Free acidity	x	x	x
Peroxide value	x	x	x
Absorbance in UV region	x	x	x
Organoleptic assessment	x	x	x
Volatile halogenated solvents	x	x	x
α -tocopherol	x	x	—
Cu, Fe, Pb, As determination	x	x	—
Oil content in pomace residue	—	—	x
Insoluble impurities	x	x	—
Unsaponifiable matter content	x	x	—

ter determinations. These standards have common rules for sampling.

Methods for Olive Oil Quality Evaluation

Included in International Standards

Olive Oil Sampling and Laboratory Sample Preparation

[IOOC, Codex Alimentarius, EC according to EN ISO 61 and EN ISO 5555]

The different international organizations adopted the same rules for olive oil and pomace olive oil sampling. An exception to the norms is made for many of the olive oils and pomace oils formed by packages containing up to 100 liters. Detailed procedures are described in the Annex I bis of EC Regulation No 2568/91.

Free Fatty Acids (Free Acidity)

[IOOC: COI/T.15/NC n.3 (2003); Codex Alimentarius according to ISO 660 or AOCs Cd 3d-63(99), EC Reg. No 2568/91 Annex II]

Free acidity is the oldest parameter used for evaluating the olive oil quality since it represents the extent of hydrolytic activities. The determination is carried out by titration of free fatty acids of oils, diluted in a suitable mixture of solvents, with an aqueous or ethanolic potassium hydroxide solution. Maximum levels (Table 7.3) have been fixed by Regulations to establish the category, since it is tightly related to the quality of raw material. Oils obtained from healthy fruits, regardless of the cultivar, processed just after harvesting, show very low values of free acidity. But, if fruits are damaged by fly (*Bactrocera oleae*) attacks or are submitted to a prolonged preservation before processing, hydrolytic enzymes become active and the free acidity of the oil slightly increases. The possible invasion of olives from molds causes a notable increase of free acidity because of the presence of lipolytic enzymes in the mold.

TABLE 7.3

Limits of free fatty acidity, as oleic acid percent, fixed by the International Organizations for each olive oil category. nl = no limit

Category	IOOC	Codex Alimentarius	EC
Extra virgin olive oil	≤ 0.8	≤ 0.8	≤ 0.8
Virgin olive oil	≤ 2.0	≤ 2.0	≤ 2.0
Ordinary virgin olive oil	≤ 3.3	≤ 3.3	-
Lampante oil	> 3.3	-	> 2.0
Refined olive oil	≤ 0.3	≤ 0.3	≤ 0.3
Olive oil	≤ 1.0	≤ 1.0	≤ 1.0
Crude olive residue oil	nl	-	nl
Refined olive residue oil	≤ 0.3	≤ 0.3	≤ 0.3
Olive residue oil	≤ 1.0	≤ 1.0	≤ 1.0

Peroxide Value (PV)

[Codex Alimentarius and IOOC: according to ISO 3960 or AOCS Cd 8b-90; EC Reg. No 2568/91 Annex III]

The evaluation of the degree of olive oil oxidation is based on determinations of both the primary and the secondary products of oxidation. The primary stage of oxidation is the formation of hydroperoxides from polyunsaturated fatty acids through a radicalic mechanism.

The analysis is carried out by an iodometric procedure, which involves the dissolution of oil in a mixture of acetic acid-chloroform, and the addition of an excess of potassium iodide solution. Iodine formed is titrated with a standardized solution of sodium thiosulfate. The level of hydroperoxides (PV) is expressed as milliequivalents of active oxygen per kilogram of oil (meqO₂/kg). A limit value of 20 meqO₂/kg has been established for virgin olive oils, 5 for refined ones and 15 for blends of virgin olive oils with refined olive oils or refined olive pomace oils.

Peroxide value is a parameter which increases, and depends on the storage conditions (oxygen admittance, light, preservation temperature and time). After reaching a maximum, PV decreases because of the formation of secondary products, typical of rancidity.

Absorbances in Ultraviolet Region

[IOOC and Codex Alimentarius: according to COI/T.20/Doc. No. 19 or ISO 3656 or AOCS Ch 5-91(01), EC Reg. No 2568/91 Annex IX]

The evaluation of the degree of olive oil oxidation can be made also by means of the measurements of extinctions on oil sample diluted in an adequate solvent. Specific absorbances, conventionally indicated as K, are measured in the UV region at the wavelengths corresponding to the maximum absorption of the conjugated dienes and trienes, respectively at about 232 and 270 nm. The conjugated dienes and trienes

are formed in the autoxidation process from the hydroperoxides of unsaturated fatty acids and their fragmentation products. The absorption around 270 nm could also be caused by substances formed during earth treatment in the refining process. K_{232} evaluation is considered optional by IOOC Trade Standards. In addition to K_{232} and K_{270} , often, especially in trade negotiations, ΔK value is considered useful, calculated according to the following equation:

$$\Delta K = K_{\max} - [1/2(K_{\max+4} + K_{\max-4})] \quad [1]$$

where K_{\max} is the maximum absorbance near 270 nm.

Table 7.4 summarizes specific absorbances at 232 and 270 nm and ΔK value for each olive oil category.

Organoleptic Assessment of Virgin Olive Oil

[Codex Alimentarius: according to COI/T.20/Doc. No. 15; IOOC: COI/T.15/NC n.3 (2003); EC Reg. No 2568/91 Annex XII]

Current regulations also compel determination of organoleptic characteristics of virgin olive oils because they are considered as a very important criterion of quality evaluation. Although values of free acidity, peroxide index, and absorbances in the UV region are within limits fixed by regulations in force, virgin olive oils may have some organoleptic defects which obviously lower their quality. The methodology for evaluating organoleptic characteristics of virgin olive oils, known as Panel Test method, was developed in 1980's by IOOC, and later included into EC legislation.

The method involves as a measurement instrument, a group of 8 to 12 persons, suitably selected and trained to identify and evaluate the intensities of positive and negative sensory perceptions. The group uses a vocabulary specifically developed for

TABLE 7.4
Limits of the absorbances at 232 and 270 nm and ΔK value for each olive oil category fixed by the different International Organizations.

Category	IOOC			Codex Alimentarius			EC		
	K_{232}	K_{270}	ΔK	K_{270}	ΔK	K_{232}	K_{270}	ΔK	
Extra virgin olive oil	≤ 2.50	≤ 0.22	≤ 0.01	≤ 0.22	≤ 0.01	≤ 2.50	≤ 0.22	≤ 0.01	
Virgin olive oil	≤ 2.60	≤ 0.25	≤ 0.01	≤ 0.25	≤ 0.01	≤ 2.60	≤ 0.25	≤ 0.01	
Ordinary virgin olive oil	nl	≤ 0.30	≤ 0.01	≤ 0.30	≤ 0.01	—	—	—	
Lampante oil	nl	nl	nl	—	—	nl	nl	nl	
Refined olive oil	nl	≤ 1.10	≤ 0.16	≤ 1.10	≤ 0.16	nl	≤ 1.10	≤ 0.16	
Olive oil	nl	≤ 0.90	≤ 0.15	≤ 0.90	≤ 0.15	nl	≤ 0.90	≤ 0.15	
Crude olive residue oil	nl	nl	nl	—	—	nl	nl	nl	
Refined olive residue oil	nl	≤ 2.00	≤ 0.20	≤ 2.00	≤ 0.20	nl	≤ 2.00	≤ 0.20	
Olive residue oil	nl	≤ 1.70	≤ 0.18	≤ 1.70	≤ 0.18	nl	≤ 1.70	≤ 0.18	

the virgin olive oil assessment and taste virgin olive oils in pre-established conditions. Official methodology fixes a number of facilities concerning volume and temperature of oil sample, tasting room temperature and moisture, shape and size, and color of tasting glass. Samples are randomly presented and tasters are requested to mark the sensations they experienced during the tasting on a profile sheet and to evaluate their intensity on an unstructured scale 10 cm long, ranked from 0 to 10. Data provided by tasters are statistically processed to verify the reliability of the test.

The median value of the defect perceived with the higher intensity identifies the oil category. For extra virgin olive oil and virgin olive oil categories, the median of defects must be zero and the fruity value has to be greater than zero (Table 7.5).

Volatile Halogenated Solvents in Olive Oil

[IOOC and Codex Alimentarius: according to IOOC T20/DOC. No 8/Corr.1 (1990); EC Reg. No 2568/91 Annex XI]

Halogenated solvents such as chloroform, and tetrachloroethylene are contaminants that can be detected in trace amounts in virgin olive oils. Their determination is carried out in the volatile fraction (isolated by a headspace technique) by GC coupled to an Electron Capture Detector (ECD) or by direct injection of the oil into the gas chromatograph by using suitable precolumns. In the latter case after a few injections it is necessary to clean or to replace the precolumn with the disadvantage of discontinuous work. The limit for each halogenated compound is fixed at 0.1 ppm, whereas the sum of all of them must not exceed 0.2 ppm.

Metals

[Copper and Iron: IOOC and Codex Alimentarius according to ISO 8294 or AOCS Cd 3-25 (02)]

[Lead: IOOC according to ISO 12193 or AOCS Ca 18c-91(97) or AOAC 994.02 (02)]

TABLE 7.5
Median limits of defects (Md) and fruity attribute (Mf) of virgin olive oil categories fixed by the International Organizations.

Category	IOOC		Codex Alimentarius		EC	
	Md	Mf	Md	Mf	Md	Mf
Extra virgin olive oil	0	> 0	0	> 0	0	> 0
Virgin olive oil	≤ 2.5	> 0	≤ 2.5	> 0	≤ 2.5	> 0
Ordinary virgin olive oil	> 2.5 ≤ 6.0	0	> 2.5 ≤ 6.0	≥ 0	-	-
Lampante oil	≤ 2.5	0	≤ 2.5	0	≤ 2.5	0
Lampante oil	> 6.0	> 0	> 6.0	> 0	> 6.0	> 0

[Arsenic: IOOC according to AOAC 952.13 or 942.17 or 985.16]

Trace metals in vegetable oils may originate from endogenous factors connected with plant metabolism, or hexogenous factors such as the soil, fertilizers, and processing equipment.

There are few metals reported to be present in olive oils: copper (few tens of ng/g), iron, nickel (2 to 50 ng/g), manganese, cobalt, chromium (2 to 500 ng/g), tin (3 to 15 ng/g), lead (<40 ng/g), mercury (2 ng/g), and cadmium (<10 ng/g). Iron is certainly the element present at the highest concentration (70 to 3600 ng/g) (Garrido et al., 1994). In refined olive oils, the metal content is lower than in virgin oils, due to the refining process. Some transition metals (copper, iron) are related to the oxidative stability of olive oils because of their catalytic effect on the decomposition of hydroperoxides.

Both IOOC and Codex Alimentarius have fixed the same legal limits only for some metal concentration in olive oils. Iron content must not exceed 3 ppm, while a limit of 0.1 ppm was established for copper, lead, and arsenic. Most of the procedures reported in the literature for the determination of trace elements involve the use of atomic absorption spectrometry (AAS) equipped with a graphite furnace, with an ashing pretreatment of the sample before the analysis, or just a dilution of the olive oil sample in methyl isobutyl ketone (Garrido et al., 1994; Martin-Povlillo et al., 1994). Karadjova et al. (1998) made the electrothermal atomic absorption spectrometric determination of several metals in olive oil using universal modifiers for their thermal stabilization during the pretreatment step.

Other techniques such as Inductively Coupled Plasma Atomic Emission Spectrometry (ICP AES) (Murillo et al., 1999) and voltammetry (Galeano Diaz et al., 2003) are also used.

Very recently, derivative potentiometric stripping analysis (dPSA) was utilized to evaluate trace metals in olive oil (La Pera et al., 2002; Dugo et al., 2004). The dPSA provides a sensitive and convenient procedure for trace metal determination, representing an attractive alternative to spectroscopic and voltammetric techniques. The method has a slow dry ashing step with respect to sample pre-treatment but it requires a short time of analysis.

α -Tocopherol

[IOOC and Codex Alimentarius: according to ISO 9936]

The tocopherol content varies widely in relation to olive varieties and ripeness, to processing, and also to storage conditions of oils with values ranging from 100 to 300 ppm, for oils of good quality (Beltran et al., 2005; Psomiadou et al., 2000). During processing (e.g. deodorization), tocopherol concentration decreases drastically (Hernandez Rabascall and Riera Boatella, 1987). Because of its activity against oxidation (Blekas et al., 1995), α -tocopherol is added to refined olive oils to improve their stability.

Tocopherols can be determined after saponification by RP-HPLC with amperometric detector (Dionisi et al., 1995), or by the direct injection of the oil, dissolved in a suitable solvent, into a normal or a reversed phase HPLC apparatus with UV or spectrofluorimetric detector. Their individual content is calculated using calibration factors determined from standard solutions (International Standard ISO 9936). Besides the HPLC methods, tocopherols and tocotrienols can be determined also by gas chromatographic technique, after trimethylsilyl-derivatization (Rovellini et al., 1997).

Moisture and Volatile Matter Content

[IOOC and Codex Alimentarius: according to ISO 662]

The oil sample is heated at 105°C on a sand-bath, until moisture and volatile substances are completely removed. Table 7.6 reports limit values fixed by IOOC and Codex Alimentarius for each olive oil category.

Insoluble Impurities in Petroleum Ether

[IOOC and Codex Alimentarius: according to ISO 662]

Oil sample is treated with an excess of solvent, the solution is filtered, and the filtered dried and weighted at 105°C. Limits are shown in [Table 7.7](#).

Not Included in International Standards

Other analyses, in addition to official ones, are useful to complete the assessment of olive oil quality. They are measurements related to the level of antioxidants, the state of oxidation, the hydrolysis, the shelf life and the possible presence of contaminants and volatile compounds, especially those related to organoleptic defects arising from

TABLE 7.6.
Limits of moisture and volatile matter percentages fixed by IOOC and Codex Alimentarius for each olive oil category.

Category	IOOC	Codex A.
Extra virgin olive oil	≤ 0.2	≤ 0.2
Virgin olive oil	≤ 0.2	≤ 0.2
Ordinary virgin olive oil	≤ 0.2	≤ 0.2
Lampante virgin olive oil	≤ 0.3	-
Refined olive oil	≤ 0.1	≤ 0.1
Olive oil	< 0.1	≤ 0.1
Crude olive oil	≤ 1.5	-
Refined olive residue oil	≤ 0.1	≤ 0.1
Olive residue oil	≤ 0.1	≤ 0.1

TABLE 7.7
Limits of the percentage of insoluble impurities in petroleum ether for each olive oil category fixed by IOOC and Codex Alimentarius for each olive oil category.

Category	IOOC	Codex A.
Extra virgin olive oil	≤ 0.1	≤ 0.1
Virgin olive oil	≤ 0.1	≤ 0.1
Ordinary virgin olive oil	≤ 0.1	≤ 0.1
Lampante virgin olive oil	≤ 0.2	-
Refined olive oil	≤ 0.05	≤ 0.05
Olive oil	< 0.05	≤ 0.05
Crude olive oil	-	-
Refined olive residue oil	≤ 0.05	≤ 0.05
Olive residue oil	≤ 0.05	≤ 0.05

microbiological/fermentative or chemical oxidative processes.

Phenolic Compounds

Virgin olive oils contains phenolic substances responsible for their stability against oxidation (Baldioli et al., 1996; Servili and Montedoro, 2002; Del Carlo et al., 2004), beneficial properties in relation to human health (Visioli et al., 2002; Boskou et al., 2005) and for bitter, pungent, and astringent sensory notes (Gutierrez-Rosales et al., 2003; Angerosa and Di Giacinto, 1995; Andrewes et al., 2003; Mateos et al., 2004). Phenolic compounds are transferred into the oil during the olive processing, but their concentration is dramatically reduced during refining (Servili and Montedoro, 2002; Servili et al., 2004) and storage of oils (Cinquanta et al., 1997; Okogeri and Tasioula-Margari, 2002).

Phenolic fraction includes simple phenols, tyrosol, and hydroxytyrosol, derivatives of hydroxybenzoic and hydroxycinnamic acids, aglycons of some glucosides, namely oleuropein, demethyloleuropein, ligstroside, and verbascoside (Montedoro et al., 1993; Angerosa et al., 1995, 1996a; Cortesi et al., 1995; Bianchi, 2003). Free and esterified cinnamic and elenolic acids, some flavones, hydroxy-isochromans (Bianco et al., 2002), pinoresinol and 1-acetoxypinoresinol (Brenes et al., 2000; Owen et al., 2000) have also been identified (see also [Chapter 5](#)).

The analysis of phenolic substances involves their extraction from the oil, a clean-up step, the separation into single compounds, and finally their quantification. Several Authors (Morales and Tsimidou, 2000; Tsimidou, 1998; Pirisi et al., 2000; Hrcirik and Fritsche, 2004) have recently reviewed the different analytical approaches for the determination of phenolic compounds, in order to explain the controversial data reported in the literature. Extraction is usually performed by liquid-liquid partition with mixtures of methanol and water, in different ratios, or absolute methanol or tetrahydrofuran. Some researchers consider that the mixture methanol:water 80:20 v/v

allows the best recoveries (Montedoro et al., 1992), others obtained better or at least similar recoveries by adopting absolute methanol (Angerosa et al., 1995). The use of tetrahydrofuran seems to increase significantly recoveries in relation to those obtained with methanol:water 60:40 v/v (Cortesi et al., 1995). Solid phase extraction (SPE) with amino-modified C18 or polyvinylpyrrolidone packing material cartridges were also applied, but a selective retention of phenols was observed (Mannino et al., 1993; Favati et al., 1994; Cortesi et al., 1995). The use of C18 or end capped C18 cartridges provided unsatisfactory recoveries, probably due to the different interactions between the sorbing material and the analyte (Liberatore et al., 2001). The comparison of different methods (liquid-liquid extraction and C8, C18, and diol stationary phases) to isolate phenolic compounds gave evidence that the better results were obtained with liquid-liquid extraction in terms of recovery of the phenolic fraction (Bendini et al., 2003).

The methanolic extract of oil samples can be used to determine the total phenolic content colorimetrically. An aliquot of the phenolic extract is diluted with water, then 0.5 mL of Folin-Ciocalteu reagent, and a sodium carbonate solution are added, and finally the solution is spectrophotometrically examined at 725 nm, using a calibration curve obtained with caffeic acid standard solutions (Gutfinger, 1981). The response of single phenols to the Folin–Ciocalteu reagent is quite different. However, in spite of the drawbacks of the method, it remains a good practical, means to evaluate the stability of the virgin olive oil as suggested by Blekas et al. (2002).

The most common technique to separate the phenolic fraction into single compounds is RP-HPLC, by using acidic water-methanol or water-acetonitrile mixtures, and detection at 280 nm. Detection can be made also by means of amperometric technique or capillary zone electrophoresis (Mannino et al., 1993; Bendini et al., 2003). Cortesi et al. (1995) applied a MS detector operating in chemical negative ionization mode for characterizing phenolic compounds.

MS detection under EI at 70 eV or CI mode of single phenolic compounds, derivatized as trimethylsilyl ethers and separated by HRGC, have proved to be a useful tool to elucidate the structure of phenols present in virgin olive oils (Angerosa et al., 1995, 1996a; Liberatore et al., 2001).

^1H and ^{13}C NMR were also used to identify phenolic compounds (Montedoro et al., 1993; Sacchi et al., 1996) and products of degradation of oleuropein by β -glucosidase (Limiroli et al., 1995). More recently, separation and identification of phenolic compounds were achieved by means of the hyphenated LC-SPE-NMR technique (Christophoridou et al., 2005), thus enabling the identification of several new phenolic components, which had not been reported previously in the polar part of olive oil.

Volatile Compounds

Virgin olive oils can be affected by several sensory defects related to some volatile

compounds that can arise from microbiological deterioration or fermentations of olive fruits or by chemical oxidative processes (Angerosa, 2002; Morales and Aparicio, 2005). Some volatiles have been related to positive attributes by means of statistical techniques (Morales et al., 1995; Aparicio et al., 1996a; Angerosa et al., 2000a), others to the defects that more usually can be perceived in virgin olive oils, so that volatile determination can support results of sensory analysis. Fusty defect was related to the presence of 2-methyl butan-1-ol + 3-methyl butan-1-ol (Angerosa et al., 1996b), winy attributes to ethanol, ethyl acetate and acetic acid (Angerosa et al., 1996b; Morales et al., 2000), whereas musty perceptions to compounds with eight carbon number (Angerosa et al., 1999a). Some aldehydes were found to be related to rancidity, especially *trans*-2-heptenal, hexanal and *trans*-2-pentenal (Solinas et al., 1987). Also the ratio hexanal/nonanal represents an appropriate way to detect the beginning and the evolution of the autoxidation process (Morales et al., 1997).

The analytical approach to volatile determination requires an isolation method. Good results are obtained using techniques that involve an enrichment step such as dynamic headspace, supercritical fluid extraction (SFE) and solid phase microextraction (SPME) (Morales et al., 1994; Angerosa et al., 1997a; Vichi et al., 2003; Cavalli et al., 2003). Among the techniques with an enrichment step the most popular one is the isolation by means of dynamic headspace. Volatile compounds of an oil sample, submitted to a given temperature, are purged with an inert gas at a controlled flow, obliged to pass through a trap where they are retained. They are later desorbed thermally (Morales et al., 1994) or by solvent (Angerosa et al., 1997a) and injected into the gas chromatograph for their separation and quantification. The construction of calibration curves is needed to perform their quantification, otherwise volatile compounds are expressed as ppm of a suitable standard.

A special method which allows to quantify volatiles is the technique known as Stable Isotope Dilution Assay (SIDA) that uses deuterated forms of the volatiles to be quantified (Guth and Grosch, 1993). The method, although has a very good sensitivity, is poorly applied since it requires the preparation of a number of deuterated compounds not commercially available.

Partial Glycerides

Monoacylglycerols. Monoacylglycerols do not naturally occur in olive fruit, but are formed during olive processing. Their production is affected by the conditions of both the olive storage and the oil extraction.

Currently, the proposed methodology includes oil extraction with acetonitrile, TLC separation on silica gel plate, and recovery of monoacylglycerols with diethyl ether, GC analysis of silylated fraction (Leone et al., 1989). Paganuzzi (1987) suggested an impregnation of the silicagel layer with boric acid and controlled silylation conditions to avoid isomerization between 2- and 1-monoacylglycerols.

Diacylglycerols. Diacylglycerols may originate from both incomplete biosynthesis of

triacylglycerols and partial hydrolysis enzymatically mediated. The measurement of total diglycerides is helpful for evaluating the quality of olives used for the oil production.

The ratio of 1,2- to 1,3-diacylglycerols can be considered a good parameter of freshness of virgin olive oils, since only 1,2-diacylglycerols are practically present in fresh oils whereas 1,3-ones are formed during the oil preservation (Figure 7.1). A high value of this ratio is related to oils of very high quality level for which high prices can be justified (Pérez-Camino et al., 2001).

Diacylglycerols can be determined by HRGC of the oil previously silylated (Serani et al., 2001).

Pérez-Camino et al. (1996) have set up a simple analytical method for diacylglycerol determination, using a solid-phase extraction (SPE) of these compounds from the oil, followed by GC on a polar column. With the aid of this method, the isomerization of diacylglycerols during the isolation and GC analysis is negligible (Pérez-Camino et al., 1996; Conte et al., 1997).

Also NMR spectroscopy has been applied in the determination of mono- and di-

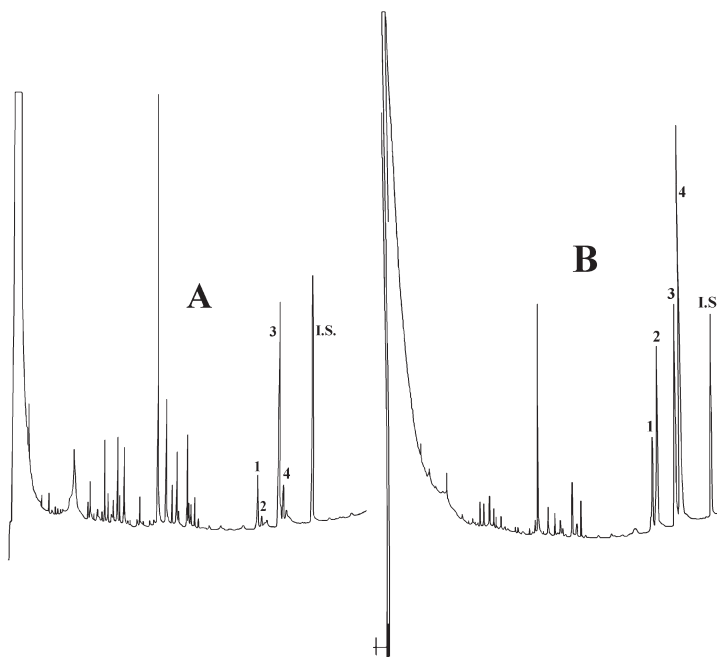


Fig. 7.1. HRGC diacylglycerol profile of (A) a newly extracted virgin olive oil; (B) the same sample after 6 months of preservation. 1: 1,2-C34-diglycerides; 2: 1,3-C34-diglycerides; 3: 1,2-C36-diglycerides; 4: 1,3-C36-diglycerides. Angerosa et al, unpublished data

glycerides. Sacchi et al. (1990, 1991) quantified the contents of partial glycerides using ^1H and ^{13}C NMR, whereas Spyros and Dais (2000) used ^{31}P NMR spectroscopy, after derivatization of free hydroxyls of partial glycerides with a specific phosphorous reagent.

Accelerated Oxidation Tests

Accelerated oxidation tests are useful to evaluate the effects of natural or chemical antioxidants on olive oil resistance toward oxidation, and to compare the storage stability of different oils.

Oven Test. Oil is put into an oven at a constant temperature and the oxidation is followed by chemical and sensory tests. The resistance of olive oil to oxidation is represented by the time expressed in days necessary for the beginning of oxidation.

Rancimat-OSI. It is based on the change of conductivity of the distillate collected from an oil subjected to an accelerated oxidation at a prefixed temperature. The change of conductivity is due to the production of formic and other carboxylic acids because of the oxidation of secondary products during the forced oxidation.

Pigments

The green-yellowish color is due to various pigments, i.e. chlorophylls, pheophytins and carotenoids. The levels of these compounds has been traditionally determined with spectrophotometrical methods measuring the total content in chlorophylls and carotenoids with value ranging, as the chlorophylls are concerned, from 1 to 10 ppm, and for the carotenoids, from a few up to 100 ppm.

According to the analytical procedure proposed by Minguéz-Mosquera and Gandul-Rojas (1992), both chlorophyllic and carotenoid pigments can be preliminary separated with solid-phase extraction (SPE) on octadecyl columns and liquid-liquid extraction. They can be well detected and identified by HPLC coupled with a diode array detector (DAD) (Minguéz-Mosquera and Gandul-Rojas, 1992; Gandul-Rojas et al., 1999; Mangos and Berger, 1997) or a fluorimetric detector (Endo et al., 1992.). Simultaneous detection of oil pigments such as pheophytins, carotenoids and tocopherols can be achieved by HPLC, using isopropanol-hexane mixtures as mobile phases, and a programmable UV/Vis spectrophotometer (Psomiadou and Tsimidou, 1998; Seppänen et al., 2003). Chlorophylls a and b, pheophytins a and b, and β -carotene can be measured at their appropriate absorption maxima (430, 452, 409, 433, and 452 nm respectively).

Contaminants

Pesticides. To protect olive groves from *Bactrocera oleae*, the key insect pest of olive fruit, and from other parasites, several pesticides are used, such as synthetic pyrethroids, organochlorine, and especially, organophosphorous insecticides (Lentza-Rizos and Avramides, 1995). In general, since most pesticides are nonpolar, residues tend to be distributed mostly in the olive oil. Due to an increasing awareness of the possible risks involved with the widespread use of pesticides, strict regulation of maximum residue limits (MRLs) for these contaminants have been fixed by the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO), and also by the European Community (Lacoste et al., 2004).

The analytical methodology, for determining pesticide residues in oils, uses different procedures, as reviewed by Lentza-Rizos and Avramides (1995). The main differences between existing methods lie in the sample clean-up step; the final determination is usually carried out by GC, in combination with one or more element specific detectors, such as ECD or NPD. The simplest and most rapid technique was that used by Morchio et al. (1992) who injected oil samples, previously diluted 1:1 with acetone, directly into a gas chromatograph. This method leads to a rapid decrease in column resolution, due to the lipidic material, so it can be used only for a few samples.

No clean-up is necessary in the method described by Dugo and coworkers (2005) because there were no interferences in the GC coupled with FPD detector.

Most of the clean-up methods for pesticide determination applied to olive oil are based on liquid-liquid partitioning with solvent of different polarity, such as hexane and acetonitrile (Cabras et al., 1997), followed by Florisil or size-exclusion chromatography (Barrek et al., 2003) or solid phase extraction (SPE) (Ramesh and Balasubramian, 1998). Lentza-Rizos et al. (2001) have also used a low-temperature lipid precipitation for the rapid analysis of pesticides, obtaining good recoveries. Alternative procedures which include supercritical fluid extraction (SFE) with CO₂ containing 3% acetonitrile have shown an equivalence with liquid extraction (Hopper, 1999).

To further improve the detectability of analytes, and reduce the working time, very recently a fully automated method has been developed. It employs an on-line combination of RP-HPLC and GC by using an oven transfer adsorption-desorption interface and does not require any sample pre-treatment step other than filtration (Sanchez et al., 2004).

Polycyclic Aromatic Hydrocarbons (PAHs). Polycyclic Aromatic Hydrocarbons (PAHs) are a well-known group of chemical contaminants widely distributed in the environment and considered to be carcinogens, as recently evidenced by the Scientific Committee on Food of the European Union (http://europa.eu.int/comm/food/fs/sc/scf/index_en.html). Due to their high lipophilic characteristics, PAHs can contaminate oils and fats. Olive pomace could be significantly contaminated by PAHs, during its

drying because this process is done in direct contact with combustion fumes. PAHs, however, can be removed from edible oils by treatment with activated charcoal. On February 2005 EC (Reg No 208/2005) established the maximum concentration (2 $\mu\text{g}/\text{kg}$) for benzo(a)pyrene considered as a marker of the presence and the effects of cancer producing PAHs in edible foods. EC did not adopt any method as official to quantify the amount of benzo(a)pyrene, but the methodologies to be used must satisfy some criteria such as a recovery range, limit values for precision, quantification, etc.

Identification and assessment of PAHs in olive oils have been carried out by several methods. PAHs have been extracted by solvent-solvent partition, with a previous saponification step (Stijve and Hischenhuber, 1987), while clean-up of the extract has been performed by column chromatography on silica gel, alumina, Sephadex, or Florisil (International Standard ISO 15302; Moret and Conte, 2000). To avoid long handling times and the use of large volumes of organic solvents, solid-phase extraction (SPE) has been successfully used instead of packed chromatography column, due to a wide range of available sorbents and extraction conditions (Cortesi et al., 2001; Barranco et al., 2003; Moret and Conte, 2002; Weisshaar, 2002; Moreda et al., 2004). Very recently also supercritical fluid extraction (SFE) has also been used to isolate PAHs from vegetable oils (Lage Yusty and Cortizo Davina, 2005). The separation and quantification of PAHs is usually performed on the extract by reverse phase HPLC with fluorescence detection (International Standard ISO 15302; Moreda et al., 2004), but also high-resolution gas chromatography coupled with a FID detector or mass spectrometry has been applied (Menichini et al., 1991; Guillen et al., 2004). Cortesi et al. (2001) performed the PAH analysis by coupled HPLC multiwavelength UV detection and programmed fluorimetry. Hyphenated techniques like liquid chromatography coupled with GC appear to be very promising (Bogusz et al., 2004). Using isotope dilution technique, Diletti et al. (2005) have quantified the PAH content at level below 1 ppb, with a GC-MS method.

Methods for Checking Olive Oil Genuineness

Included in International Standards

The Official methodologies include measurements of some chemical and physical constants, such as iodine value, refractive index determination, and specific color reactions, which may be useful in revealing adulteration with seed oils. Nowadays, these methods have been completely replaced by modern chromatographic and spectrometric determinations that provide more information and may lead to more conclusive results. The current methods to check olive oil genuineness are founded on two essential principles: 1) the different composition of olive oil; 2) the changes occurring in some constituents due to refining; 3) the differences in the composition between

virgin olive oil and pomace olive oil.

Chemical classes that are affected by botanical origin are fatty acid composition, triacylglycerols, and sterols whereas those influenced by the kind of extraction (mechanical or by solvent) are aliphatic alcohols, waxes, and the triterpene dialcohols.

Refining process modifies the natural olive oil composition and some new compounds are produced. These are some sterols, sterolic hydrocarbons, and *trans* isomers of unsaturated fatty acids (Lanzón et al., 1994; 1999; León-Camacho et al., 1999, 2001, 2004; Mariani et al., 1992; Paganuzzi, 1984; Strocchi and Savino, 1989).

The limits adopted by International bodies are reported in [Table 7.8](#).

Fatty Acid Composition

[IOOC: according to COI/T.20/Doc. No. 24 or AOCS Ch 2-91(02); Codex Alimentarius: according to COI/T.20/Doc. No. 24 and ISO 5508 or AOCS Ch 2-91(02) or AOCS Ce 1f-96(02); EC Reg. No 2568/91 Annex X A]

The determination is performed by gas chromatographic analysis of fatty acid methyl esters, after transesterification of olive oil triglycerides on very polar capillary columns. Preparation of fatty acid methyl esters (FAME) can be carried out in a methanolic medium with alkaline, acid, or alkaline and acid catalysis. Methylation with diazomethane represents an alternative procedure for free acids. Cert et al. (2000) have statistically assessed the precision and reproducibility in the preparation of FAME using (1) cold methylation with methanolic potassium hydroxide and (2) hot methylation with sodium methylate followed by acidification with sulfuric acid in methanol and heating. In oils with low acidities, the results obtained for both methylation methods were equivalent. However, the olive pomace oil sample (acidity 15.5%) showed significant differences between the fatty acid composition obtained with the two methylation methods. The methylation with the alkaline and acid catalysis did not yield an increase of the *trans*-isomers.

Fatty acids profiles are very different in edible oils ([Table 7.9](#)).

Wide ranges of percentages are seen for the more abundant fatty acids, whereas limit values are fixed for the minor ones (myristic acid, linolenic acid, arachidic acid, eicosenoic acid, behenic acid, and lignoceric acid). The oleic acid, the most representative fatty acid of olive oil, ranges from 55% to 83%. Hot climate modifies the fatty acid composition of olive oils. North African oils have a lower percentage of oleic acid and higher percentages of linoleic and palmitic acids than oils from the Mediterranean basin. Minor fatty acids are prominent in seed oils. For instance, linolenic acid, always $\leq 0.9\%$ in olive oil, can reach 8% in seed oils. The limit for linolenic acid has been fixed at 1% to include some genuine Moroccan olive oils, characterized by 1% of linolenic acid. The level of other minor fatty acids is useful to reveal seed oil adulteration. High amounts of eicosenoic and behenic acids are characteristic of soybean and rapeseed oils, erucic acid of rapeseed oils, and lignoceric acid of peanut oil. The limits fixed for fatty acids are not useful for detection of frauds if the addition of seed oil is

Table 7.8
Identity characteristics of olive oil categories fixed by IOOC. Limits adopted by EC and Codex Alimentarius are the same for the olive oil categories.

Categories	Waxes mg/ kg(1)	Saturated acids in 2- position of triacylglyc- erol %	Stigma- stadienes mg/kg	Δ ECN42	Trans oleic isomers %	Trans linoleic + trans linolenic isomers %	Chole- sterol %	Bras- sica- sterol %	Campe- sterol %
Extra virgin olive oil	≤250	≤1.5	≤0.15	≤0.2	≤0.05	≤0.05	≤0.5	≤0.1	≤4.0
Virgin olive oil	≤250	≤1.5	≤0.15	≤0.2	≤0.05	≤0.05	≤0.5	≤0.1	≤4.0
Ordinary virgin olive oil	≤250	≤1.5	≤0.15	≤0.2	≤0.05	≤0.05	≤0.5	≤0.1	≤4.0
Lampante virgin olive oil	≤300	≤1.5	≤0.50	≤0.3	≤0.1	≤0.1	≤0.5	≤0.1	≤4.0
Refined olive oil	≤350	≤1.8	-	≤0.3	≤0.2	≤0.3	≤0.5	≤0.1	≤4.0
Olive oil	≤350	≤1.8	-	≤0.3	≤0.2	≤0.3	≤0.5	≤0.1	≤4.0
Crude pomace olive oil	>350	≤2.2	-	≤0.6	≤0.2	≤0.1	≤0.5	≤0.2	≤4.0
Refined olive residue oil	>350	≤2.2	-	≤0.5	≤0.4	≤0.35	≤0.5	≤0.2	≤4.0
Olive residue oil	>350	≤2.2	-	≤0.5	≤0.4	≤0.35	≤0.5	≤0.2	≤4.0

(1) Oils with a wax content between 300 and 350 mg/kg are considered to be: lampante olive oil if the total aliphatic alcohol is less than or equal to 350 mg/kg or if the percentage of erythrodiol and uvaol is less than or equal to 3.5

crude olive pomace oil if total aliphatic alcohols are greater than 350 mg/kg and the percentage of erythrodiol and uvaol is greater than 3.5.

(2) β -sitosterol is the sum of $\Delta^{5,23}$ -stigmastadienol, chlerosterol, β -sitosterol, sitostanol, Δ^5 -avenasterol and $\Delta^{5,24}$ -stigmastadienol

(3) Percentages of other fatty acids: C16= 7.5-20.0; C16:1= 0.3-3.5; C17:0= ≤ 0.3; C17:1= ≤ 0.3; C18:0= 0.5-5.0; C18:1= 55.0-83.0; C18:2= 3.5-21.0

of about 5% (Christopoulou et al., 2004).

The detection becomes harder when the composition of oils in the mixture is very similar, such as in the case of hazelnut and sunflower oils, or oils obtained from seed plants biotechnologically modified. In these cases, other analyses are requested.

Trans Unsaturated Fatty Acids

[IOOC and Codex Alimentarius: according to COI/T.20/Doc. No. 17 and ISO 15304 or AOCS Ce 1f-96(02); EC Reg. No 2568/91 Annex X A]

Virgin olive oils contain only *cis* isomers of unsaturated fatty acids. In the refining process there is a partial isomerization of unsaturated fatty acids the extent of which is related to the conditions of the process. The official method of *trans* unsaturated fatty acids determination involves the quantitative conversion of triacylglycerols into methyl esters followed by High Resolution Gas Chromatography (HRGC) quantification of *trans* fatty acid methyl esters, by using capillary columns covered by a

Table 7.8 continued.

Stigma-sterol %	β -sito-sterol (2)	Δ^7 -stigma-sterol %	Total sterols mg/kg	Erithrodiol + Uvaol %(3)	C14:0%	C18:3 %	C20:0 %	C20:1 %	C22:0 %	C24:0 %
< camp	≥ 93.0	≤ 0.5	≥ 1000.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 1000.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 1000.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 1000.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 1000.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 1000.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.2	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 2500.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.3	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 1800.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.3	≤ 0.2
< camp	≥ 93.0	≤ 0.5	≥ 1600.0	≤ 4.5	≤ 0.05	≤ 1.0	≤ 0.6	≤ 0.4	≤ 0.3	≤ 0.2

Notes:

- The results of the tests must be expressed to the same number of significant digits as that specified for each characteristic. The last significant digit must be rounded up to the next digit if the non-significant digit that follows is greater than 4.
- An oil has to be placed in a different category or declared not in conformity in terms of purity if any one of the characteristics exceeds the limit.
- The limits for the characteristics (1) and (3) do not have to be respected simultaneously for all olive pomace oils.

cyanopropylsilicone stationary phase. To avoid artificial increases of isomers, a cold methylation with methanolic potassium hydroxide or diazomethane, an analysis temperature no higher than 225°C and a cleanliness control of the injector are recommended (León-Camacho, 2001). Peaks formed by ethyl or other esters, produced when the column has an insufficient polarity, could overlap with the *trans*-linolenic acid methyl ester one, and give wrong results.

The presence of *trans* isomers of olive oil unsaturated fatty acids is not a specific kind of adulteration.

Fatty Acid in the 2-Position of Triacylglycerol

[IOOC and Codex Alimentarius: according to ISO 6800:199 or AOCS Ch 3-91(97), EC Reg. No 2568/91 Annex VII]

It is well known that unsaturated fatty acids are oriented during the biosynthesis of triacylglycerols to 2-position and only a very low amount of saturated ones esteri-

TABLE 7.9
Fatty acid composition of the main seed oils according to Codex Alimentarius.

Fatty acid	Olive oil	Rapeseed oil	Rapeseed oil (low erucic acid)	Safflower-seed oil	Safflower-seed oil (high oleic acid)	Soyabean oil	Sunflower-seed oil	Sunflower-seed oil (high oleic acid)	Peanut oil	Maize oil	Grapeseed oil
C12:0	ND	ND	ND	ND	ND-0.2	ND-0.1	ND-0.1	ND	ND-0.1	ND-0.3	ND
C14:0	0.0-0.05	ND-0.2	ND-0.2	ND-0.2	ND-0.2	ND-0.2	ND-0.2	ND-0.1	ND-0.1	ND-0.3	ND-0.3
C16:0	7.5-20.0	1.5-6.0	2.5-7.0	5.3-8.0	3.6-6.0	8.0-13.5	5.0-7.6	2.6-5.0	8.0-14.0	8.6-16.5	5.5-11.0
C16:1	0.3-3.5	ND-3.0	ND-0.6	ND-0.2	ND-0.2	ND-0.2	ND-0.3	ND-0.1	ND-0.2	ND-0.5	ND-1.2
C17:0	0.0-0.3	ND-0.1	ND-0.3	ND-0.1	ND-0.1	ND-0.1	ND-0.2	ND-0.1	ND-0.1	ND-0.1	ND-0.2
C17:1	0.0-0.3	ND-0.1	ND-0.3	ND-0.1	ND-0.1	ND-0.1	ND-0.1	ND-0.1	ND-0.1	ND-0.1	ND-0.1
C18:0	0.5-5.0	0.5-3.1	0.8-3.0	1.9-2.9	1.5-2.4	2.0-5.4	2.7-6.5	2.9-6.2	1.0-4.5	ND-3.3	3.0-6.5
C18:1	55.0-83.0	8.0-60.0	51.0-70.0	8.4-21.3	70.0-83.7	17.0-30.0	14.0-39.4	75.0-90.7	35.0-69.0	20.0-42.2	12.0-28.0
C18:2	3.5-21.0	11.0-23.0	15.0-30.0	67.8-83.2	9.0-19.9	48.0-59.0	48.3-74.0	2.1-17.0	12.0-43.0	34.0-65.6	8.0-78.0
C18:3	0.0-1.0	5.0-13.0	5.0-14.0	ND-0.1	ND-1.2	4.5-11.0	ND-0.3	ND-0.3	ND-0.3	ND-2.0	ND-1.0
C20:0	0.0-0.6	ND-3.0	0.2-1.2	0.2-0.4	0.3-0.6	0.1-0.6	0.1-0.5	0.2-0.5	1.0-2.0	0.3-1.0	ND-1.0
C20:1	0.0-0.4	3.0-15.0	0.1-4.3	0.1-0.3	0.1-0.5	ND-0.5	ND-0.3	0.1-0.5	0.7-1.7	0.2-0.6	ND-0.3
C20:2	ND	ND-1.0	ND-0.1	ND	ND	ND-0.1	ND	ND	ND	ND-0.1	ND
C22:0	0.0-0.2	ND-2.0	ND-0.6	ND-1.0	ND-0.4	ND-0.7	0.3-1.5	0.5-1.6	1.5-4.5	ND-0.5	ND-0.5
C22:1	ND	>2.0-60.0	ND-2.0	ND-1.8	ND-0.3	ND-0.3	ND-0.3	ND-0.3	ND-0.3	ND-0.3	ND-0.3
C22:2	ND	ND-2.0	ND-0.1	ND	ND	ND	ND-0.3	ND	ND	ND	ND
C24:0	0.0-0.2	ND-2.0	ND-0.3	ND-0.2	ND-0.3	ND-0.5	ND-0.5	ND-0.5	0.5-2.5	ND-0.5	ND-0.4
C24:1	ND	ND-3.0	ND-0.4	ND-0.2	ND-0.3	ND	ND	ND	ND-0.3	ND	ND

ND = not detectable

fies this position of glycerol. According to specific distribution rules there is a prominent concentration of saturated fatty acids in 1- and 3- positions.

Oils with a fatty acid composition identical to that of genuine olive oils can be chemically prepared by esterifying by-products of olive oil refining process. In these products the 1,3 random, 2 random distribution cannot be reproduced. Therefore the amount of saturated fatty acids is notably higher in the 2-position than in genuine oils.

The determination of fatty acids in the 2-position of glycerol includes 1) neutralization, if the free acidity exceeds 3%, 2) chromatographic separation on a column of alumina, 3) partial hydrolysis of triacylglycerols mediated by porcine pancreatic lipase for a defined time, 4) isolation of monoacylglycerols in the 2-position by TLC, 5) methanolic transesterification and 6) HRGC analysis of methyl esters.

EC regulation fixed limits for the sum of palmitic plus stearic acid percentages, for the different olive oil categories (1.5% for virgin olive oils, 1.8% for refined olive oils, and 2.2% for pomace oils); percentages higher than limits evidence the addition of esterified oil.

ΔECN42 Values

[IOOC and Codex Alimentarius: according to COI/T.20/Doc. No. 20 or AOCS Ce 5b-89(97); EC Reg. No 2568/91 Annex XVIII]

The availability in the market of deesterolized oils with fatty acid composition very similar to that of olive oils lead to the quest of new methods to reveal possible adulterations. From a practical point of view, it is very useful to cluster triglycerides with the same chromatographic behavior by Equivalent Chain Number (ECN). ECN is the actual carbon number minus twice the number of double bonds per molecule. For an example glycerol trilinoleate has an ECN equal $42 (3 \times 18 = 54, 2 \times (3 \times 2) = 12, 54 - 12 = 42)$.

Olive oil, differently from the most seed oils, has mainly triglycerides with ECNs 44, 46, 48, and 50; triglycerides with ECN40 and ECN42 are absent or found at trace amounts, respectively. Therefore, the evaluation of ECN42, which varies according to content of glycerol trilinoleate, is an effective tool to detect more unsaturated oils. More effective information can be drawn from Δ ECN42, the difference between theoretical ECN42 (calculated by a special computer programme based on the GC determination of fatty acid composition and 1,3-random, 2-random distribution theory) and the experimental ECN42 (determined by HPLC technique). The current HPLC method for determining triacylglycerols is based on the resolution into single glycerides, according to both molecular weight and total number of double bonds. The separation is made in isocratic conditions, using a mixture of acetonitrile and acetone as mobile phase. The detection is performed by means of an RI detector. RI detector has the disadvantage that it is greatly affected by both temperature and composition of the mobile phase. Therefore, any increase of temperature should be avoided

to reduce inevitable deflection of baseline. This is obtained with suitable thermostated cells. Under these experimental conditions, the resolution into single glycerides is not complete and it can be only partially improved with the use of a 4 mm i.d. (internal diameter) RP-18 column with 4 μm particle diameter. Moreda and coworkers (2003) successfully overcame the poor reproducibility of the mobile phase composition by replacing the mixture of acetonitrile and acetone with propionitrile.

$\Delta\text{ECN}42$ must not exceed 0.2 for extra and virgin olive oils, 0.3 for lampante and refined olive oils, 0.5 for refined pomace oil and olive pomace oil, and can reach 0.6 for crude pomace oil. $\Delta\text{ECN}42$ is a very useful and effective tool in detecting the presence of most of the vegetable oils (El-Hamdy and El-Fizga, 1995). However, the fixed limits for $\Delta\text{ECN}42$ are not sufficient to detect percentages lower than or equal to 5% of hazelnut, peanut, and mustard oils in mixtures with olive oils according to Christopoulou et al. (2004).

Sterol Composition

[IOOC and Codex Alimentarius: according to COI/T.20/Doc. No. 10 or ISO 12228 or AOCS Ch 6-91(97); EC Reg. No 2568/91 Annex V]

Sterol content, and especially sterol profile, are quite characteristic of each botanical species (Table 7.10).

Therefore the determination of sterol composition is widely applied as an effective and reliable means to detect the adulteration with foreign oils. The content of some sterols, such as campesterol, stigmasterol, and β -sitosterol, decreases during the refining process since they suffer a dehydration. The resulting oils with a low sterol content (desterolized) can be used to adulterate olive oil. In this case sterol composition does not give conclusive information but the suspicion can be supported by the determination of both total amount of sterols, which must be $\geq 1,000$ ppm, and dehydration by-products of sterols. Also some stigmastadienols, such as $\Delta^{5,23}$ - and $\Delta^{5,24}$ -stigmastadienols, not naturally occurring in virgin olive oils, but formed during the refining process may be present (Amelotti et al., 1985).

Olive oil must contain not more than 0.5% of cholesterol. Higher percentages of this sterol evidence the presence of animal fats, palm oil, or its fractions. A limit of 0.1% of brassicasterol has been fixed. Higher values indicate the adulteration with oils from the *Brassicaceae* family. Percentages higher than 0.5% of Δ^7 -stigmasterol indicate the adulteration with sunflower oil (Figure 7.2).

A maximum value of 4.0% has been fixed for campesterol, present at high levels in soybean, rapeseed, and sunflower oils. In addition as campesterol percentage is greater than that of stigmasterol, this relation is useful to evidence mixtures with soybean oil. Some genuine virgin olive oils showing a campesterol content exceeding the upper limit established by EU regulations (Rivera del Alamo et al., 2004). The apparent β -sitosterol (the sum of contents of $\Delta^{5,23}$ - and $\Delta^{5,24}$ -stigmastadienols, chlosterol, β -sitosterol, sitostanol, and Δ^5 -avenasterol) must cover 93%.

TABLE 7.10
Sterolic composition of main seed oils according to Codex Alimentarius.

	Rapeseed	Safflowerseed		Soyabean oil	Sunflowerseed		Peanut oil	Maize oil	Grapeseed oil
	oil (low erucic acid)	Safflower- seed oil	oil (high oleic acid)		Sunflower- seed oil	oil (high oleic acid)			
Cholesterol	ND-1.3	ND-0.7	ND-0.5	0.2-1.4	ND-0.7	ND-0.5	ND-3.8	0.2-0.6	ND-0.5
Brassicasterol	5.0-13.0	ND-0.4	ND-2.2	ND-0.3	ND-0.2	ND-0.3	ND-0.2	ND-0.2	ND-0.2
Campesterol	24.7-38.6	9.2-13.3	8.9-19.9	15.8-24.2	6.5-13.0	5.0-13.0	12.0-19.8	16.0-24.1	7.5-14.0
Stigmasterol	0.2-1.0	4.5-9.6	2.9-8.9	14.9-19.1	6.0-13.0	4.5-13.0	5.4-13.2	4.3-8.0	7.5-12.0
beta-sitosterol	45.1-57.9	40.2-50.6	40.1-66.9	47.0-60.0	50.0-70.0	42.0-70.0	47.4-69.0	54.8-66.6	64.0-70.0
delta-5-avenasterol	2.5-6.6	0.8-4.8	0.2-8.9	1.5-3.7	ND-6.9	1.5-6.9	5.0-18.8	1.5-8.2	1.0-3.5
delta-7-stigmasterol	ND-1.3	13.7-24.6	3.4-16.4	1.4-5.2	6.5-24.0	6.5-24.0	ND-5.1	0.2-4.2	0.5-3.5
delta-7-avenasterol	ND-0.8	2.2-6.3	ND-8.3	1.0-4.6	3.0-7.5	ND-9.0	ND-5.5	0.3-2.7	0.5-1.5
Others	ND-4.2	0.5-6.4	4.4-11.9	ND-1.8	ND-5.3	3.5-9.5	ND-1.4	ND-2.4	ND-5.1
Total sterols (mg/kg)	4500- 11300	2100- 4600	2000- 4100	1800- 4500	2400- 5000	1700- 5200	900- 2900	7000- 22100	2000- 7000

ND not detectable

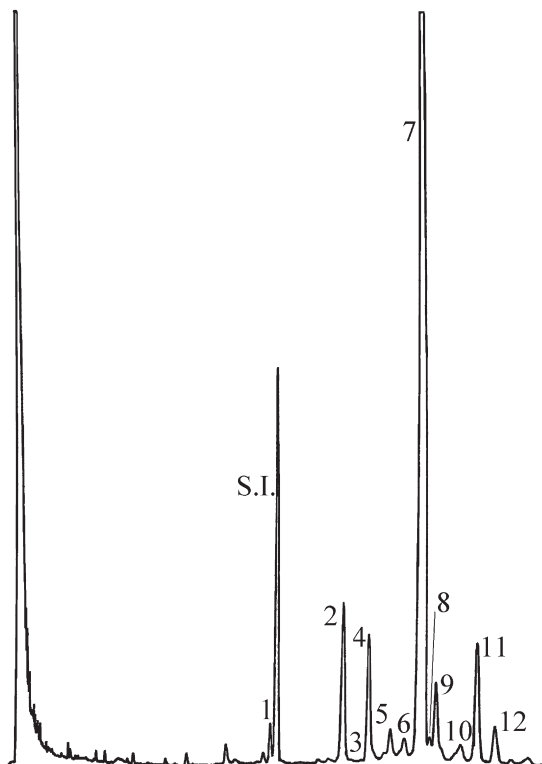


Fig. 7.2. HRGC of sterolic fraction of a mixture of olive oil with 20% of sunflower seed oil. 1: cholesterol; 2: 24-methylencholesterol; 3: campesterol; 4: campestanol; 5: stigmasterol; 6: Δ^7 -campesterol; 7: $\Delta^{5,23}$ -stigmastadienol; 8: clerosterol; 9: β -sitosterol; 10: sitostanol; 11: Δ^5 -avenasterol; 12: $\Delta^{5,24}$ -stigmastadienol. IS: internal standard (α -cholestanol). Angerosa et al, unpublished data

Sterol determination involves: a. the saponification of the oil sample, after the addition of a suitable internal standard (e.g. α -cholestanol) with an ethanolic potassium hydroxide solution, b. the extraction of unsaponifiable matter with diethyl ether, c. the isolation of sterolic fraction by means of TLC on a plate impregnated with potassium hydroxide and d. the quantification of single sterols, previously silylated, by HRGC. The analysis may show some problems because of an ineffective separation of sterolic fraction from the unsaponifiable matter thin layer chromatography. It is possible that small amounts of cycloartenol and 24-methylene-cycloartenol are scraped off with the sterol band, thus overlapping with Δ^7 -stigmastanol and β -sitosterol respectively, in the usual condition of analysis (Morales and León-Camacho, 2000).

Some researchers performed capillary GC analysis of silyl-derivatives of sterols previously separated by means of isocratic HPLC (Cert et al., 1997). When compared with the official TLC sterol determination method, the HPLC technique shows no difference except of a higher Δ^7 -sterol recovery (Cert et al., 1997).

The official method is time consuming, therefore several researchers tried to simplify it by removing the TLC step. Bello (1992) achieved the separation of the sterolic fraction from the unsaponifiable matter using a commercial (Sep-Pak) silica cartridge and petroleum ether-diethyl ether elution. Results obtained with this method were in good agreement with those deriving from the time-consuming TLC step. A similar approach was also followed by Lechner et al. (1999) who, prior to capillary gas chromatography, successfully applied SPE to separate sterols from the triacylglycerol matrix. Another interesting approach is the extraction with a semicontinuous counter-current supercritical carbon dioxide extraction (Ibanez et al., 2002).

Erythrodiol and Uvaol

[IOOC and Codex Alimentarius: according to IUPAC Method 2.431; EC Reg. No 2568/91 Annex VI]

A very high content of erythrodiol, uvaol, waxes, and aliphatic alcohols is accumulated in the flesh and skin of olive fruits so that oils obtained by solvent from solid residue after the mechanical extraction of olive pastes is particularly rich in these compounds. Percentages of erythrodiol and uvaol in relation to that of sterols can provide a good means of differentiation between mechanically obtained oils and solvent extracted. Since triterpenic dialcohols essentially occur as free or mono- and di-esters of fatty acids, the determination of these ester classes can be useful for a better identification of different kinds of olive oil (Mariani et al., 1998).

Triterpenic dialcohols are separated and analyzed with sterols, using the same methodology. The sum of erythrodiol and uvaol, in the total sterol fraction, does not exceed 4.5% in virgin and olive oils. In pomace oils it can be as high as 30%. Percentages higher than 4.5% indicate blending with olive pomace oil (Figure 7.3).

Such results have to be confirmed by wax level, since genuine virgin oils produced in certain regions contain erythrodiol and uvaol in percentages higher than the fixed limits (Albi et al., 1990).

Alternatively to the GC official methodology, the separation of unsaponifiables can be performed, by preparative HPLC with refractive index detection, preparation of silyl-derivatives, and analysis by capillary GC. The HPLC determination of sterols/dialcohols gave higher Δ^7 -sterol amount. All the other sterols and erythrodiol and uvaol recoveries were similar to those of the official TLC sterol determination method (Cert et al., 1997).

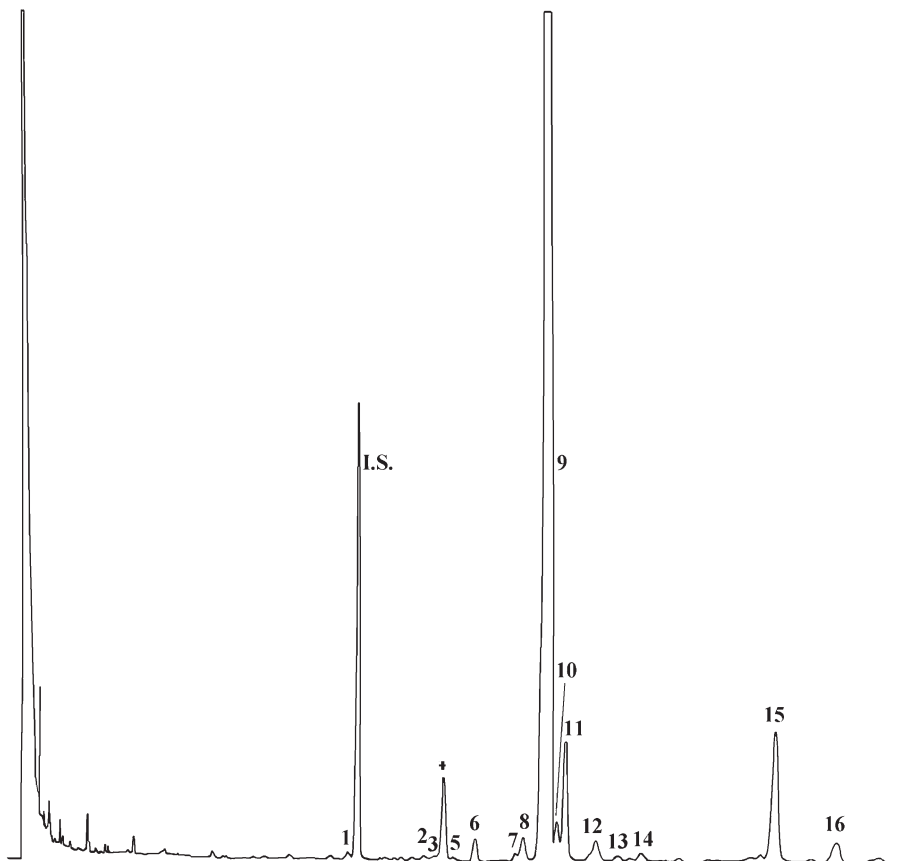


Fig. 7.3. HRGC of both sterolic fraction and triterpenic dialcohols of a mixture of olive oil with 15% pomace olive oil. 1: cholesterol; 2: 24-methylencholesterol; 3: campesterol; 4: campestanol; 5: stigmasterol; 6: Δ^7 -campesterol; 7: $\Delta^{5,23}$ -stigmastadienol; 8: chlosterol; 9: β -sitosterol; 10: sitostanol; 11: Δ^5 -avenasterol; 12: $\Delta^{5,24}$ -stigmastadienol; 13: Δ^7 -stigmastanol; 14: Δ^7 -avenasterol; 15: erythrodiol; 16: uvaol. IS: internal standard (α -cholestanol). Angerosa et al, unpublished data

Wax Content

[IOOC and Codex Alimentarius: according to COI/T.20/Doc. No. 18/Rev. 2 or AOCS Ch 8-02(02), EC Reg. No 2568/91 Annex IV]

Waxes, esters of fatty acids with fatty alcohols, found in olive oil are esters C36, C38, C40, C42, C44, and C46. As they accumulate in the skin of olives, higher amounts of them can be detected in olive pomace oils rather than in olive oils (Bi-

anchi et al., 1994). Since the waxy fraction C40-46 esters are the least affected by the dewaxing process (Amelio et al., 1993), the determination of the sum of C40-46 aliphatic waxes can be considered a reliable parameter to detect olive-residue oil in olive oil (Grob et al., 1990).

For the determination of waxes separation by silica gel chromatography, after the addition of a suitable internal standard (e.g. lauryl arachidate) is necessary. The waxy fraction eluted with hexane:diethyl ether 99:1 is analyzed by GC, using a capillary column and on-column injection. Limits are fixed by EC to guarantee the purity and to classify the various grades of olive oil; these are: 250 mg/kg for virgin olive oils, 300 mg/kg for lampante olive oils, 350 mg/kg for olive, and refined olive oils. Contents higher than 350 mg/kg are present in solvent-extracted oils. However wax quantification should be supported by erythrodiol and uvaol determination, since several studies proved that wax content increases during the oil preservation (Mariani and Venturini, 1996; Paganuzzi et al., 1997), because of a natural esterification of fatty alcohols and free fatty acids. In oils with high free acidity the extent of esterification is relevant.

The esterification of several different fatty acids and fatty alcohols can lead to waxes with the same carbon atom number and therefore the content of a given wax will be given by the sum of more peaks. León-Camacho and Cert (1994) suggested to shorten the gas chromatographic column or to increase the carrier gas flow, to avoid the splitting of wax peaks.

An attempt to make automatic wax content determination was made by Amelio et al. (1993) who replaced column chromatography by a separation in HPLC and automatic collection of wax fraction that later is analyzed by HRGC.

Recently Pérez-Camino and coworkers (2003) proposed a simplification of the official method. They isolated wax fraction from the oil using solid-phase extraction on silica-gel cartridges. The fraction was later analyzed by capillary GC using on-column injection. The determination of aliphatic waxes had the same precision as the EC official method.

Aliphatic Alcohol Content

[IOOC: COI/T.20/Doc. No. 26; Codex Alimentarius: NGD C 76-1989; EC Reg. No 2568/91 Annex XIX]

In olive oils saturated linear fatty alcohols form a homologue series, mainly with an even chain of carbon atoms which range from 20 to 32. Some seed oils have linear fatty acids with an odd chain.

Aliphatic alcohols accumulate in the flesh and skin of olive fruits and, as a consequence, they are contained in solvent extracted oils in higher amounts than in mechanically extracted oils (Christopoulou et al., 1996; Tacchino and Borgoni, 1983).

An aliphatic alcohol content higher than values usually found in genuine olive oils may be indicative of a fraudulent addition of olive pomace oil, but it cannot be

considered conclusive since some genuine oils also show levels exceeding the proposed limits. Erroneous evaluations could be made, due to an increase of the free alkanols level after solvent crystallization in the dewaxing process (Amelio et al., 1993). In these cases, other supporting analyses are necessary to confirm adulteration.

The alcoholic fraction is isolated from the unsaponifiable matter by TLC after the addition of a suitable internal standard (e.g. 1-eicosanol); quantification is carried out on the silyl derivatives, using GC on a capillary column. The separation of linear from triterpenic alcohols and methylsterols by TLC before GC analysis is advisable (Morales and León-Camacho, 2000). Depending on the mobile phase a band with a R_f slightly higher than that of linear alcohols can be observed; this band is due to a tertiary polyisoprenoid alcohol which is readily decomposed during GC to form a hydrocarbon artifact, with a lower molecular weight (Lanzón et al., 1992).

Stigmastadienes

[IOOC and Codex Alimentarius: according to COI/T.20/Doc. No. 11 or ISO 15778-1 or AOCS Cd 26-96(02); EC Reg. No 2568/91 Annex XVII]

Several unsaturated hydrocarbons with a steroidal structure, known as sterenes, are formed by dehydration of sterols, during olive oil refining. Among them, stigmasta-3,5-diene originates from the dehydration of β -sitosterol (Cert et al., 1994), and it is considered as an effective marker of oils subjected to a bleaching process or to a thermal treatment (Lanzón et al., 1994). Limits set by International bodies are stigmastadienes not more than 0.15 ppm in virgin olive oil, and 0.5 ppm for lampante.

Stigmastadiene determination is especially useful to evidence the addition of des-terolized oils since the high temperatures needed for the removal of sterols during refining process promote the formation of sterenes. Dehydration products from the other sterols are also good tracers of olive oil adulteration with seed oils (Grob et al., 1994a, 1994b; Mariani et al., 1995).

The official method involves the extraction of unsaponifiable matter, the fractionation of steroidal hydrocarbons with silica gel column chromatography and GC analysis. A typical chromatogram is showed in [Figure 7.4](#). Sterenes could also be determined by RP-HPLC coupled with an UV detector since they have characteristic absorptions due to the presence of a conjugated double bond system (Schulte, 1994; Amelio et al., 1998). More effective is the sterene determination with on-line coupled LC-GC-MS techniques (Grob et al., 1994b).

Spectrophotometric Analysis in the Ultraviolet Region

[IOOC: COI/T.15/NC n.3 (2003); Codex Alimentarius: according to COI/T.20/Doc. No. 19 or ISO 3656 or AOCS Ch 5-91 (01), EC Reg. No 2568/91 Annex IX].

The detection of adulteration of virgin olive oils with refined olive oil and olive

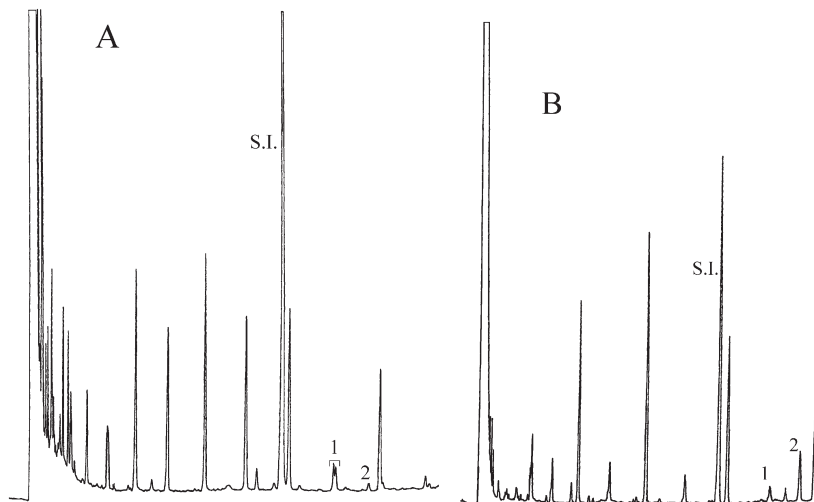


Figure 7.4. HRGC steradienes profile of (A) a virgin olive oil; (B) a refined olive oil. 1: campestadienes; 2: stigmastadienes. Angerosa et al, unpublished data

residue oils can be carried out by measuring specific absorbances in the UV region (Chiricosta et al., 1996) at the wavelengths typical of conjugated polyenes. Measurements are made on an oil sample diluted in an adequate solvent. A number of products due to autoxidation of the oil interfere, since they adsorb in the same region. A passage of the sample through an alumina (Di Sipio and Trulli, 2001, 2002) or a silica gel column (Morchio et al., 2000) is necessary before the spectrophotometric analysis. Some years ago the use of a modern refining process resulted in oils with negligible UV absorption values. An admixture of such oils with virgin olive oil cannot be revealed by UV absorbance measurements. Other analyses, e.g. *trans* isomer fatty acid determination, are suggested to evidence this adulteration (Morchio et al., 1989).

Not Included in International Standards

Other methodologies to check olive oil genuineness, although not included in official methods, can usefully support attempts to reveal adulteration. These methods are based on the analysis of both triacylglycerols and non-triacylglycerols components.

Triacylglycerols

The current method for determining triacylglycerols is based on the resolution into individual compounds using HPLC with a refractive index (RI) detector (Figure 7.5).

However, in the usual HPLC determination of triacylglycerols (Cortesi et al., 1990) RI detection does not allow adoption solvent gradients which are essential for the better separation of triacylglycerols.

Some researchers used light scattering detection with solvent gradients, thus obtaining efficient separations of triglycerides (Palmer and Palmer, 1989; Caboni et al., 1992). More recently, the detection of triacylglycerols from vegetable oils has been made with evaporative light scattering detectors (ELSD) which allow a solvent gradient to be used as mobile phase, improving their separation. ELSD show a sensitivity 200-400 times greater than the refractive index (RI) (Mancini et al., 1997).

Some ratios of major triacylglycerols were used to differentiate genuine olive oils from mixtures with reesterified oils or to evidence the presence of hazelnut oil (Casadei, 1987). Triacylglycerol profiles were also processed by chemometrical techniques,

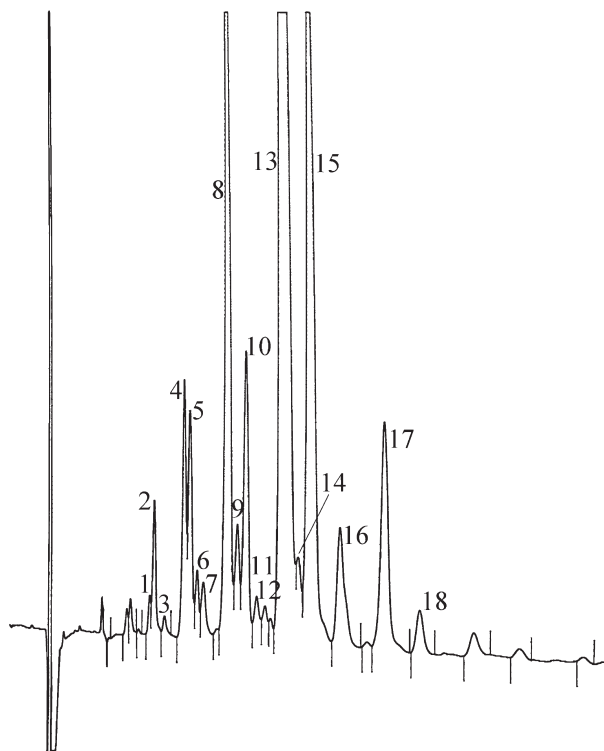


Figure 7.5. HPLC triacylglycerol profile of a mixture of olive oil with 20% of rapeseed oil. 1: LLL; 2: OLLn+PoLL; 3: PLLn; 4: OLL; 5: OOLn+PoOL; 6: PLL+PoPoO; 7: POLn+PPoPo+PPoL; 8: OOL+LnPP; 9: PoOO; 10: SLL+PLO; 11: PoOP+SpoL+SOLn+SpoPo; 12: PLP; 13: OOO+PoPP; 14: SOL; 15: POO; 16: POP; 17: SOO; 18: POS+SLS. Angerosa et al, unpublished data

to reveal olive oil falsification (Tsimidou et al., 1987a).

Trilinolein (LLL) content (Christopoulou et al., 2004) can give useful information about possible adulteration with rich linoleic seed oils; however, the low LLL content in some seed oils or the addition of canola oil up to 7.5% w/w cannot allow the detection of adulteration (Salivaras and McCurdy, 1992).

The gas chromatographic approach is not widespread because of poor volatility of triacylglycerols and high temperatures (350°C) required for the analysis. Several years ago, GC methods were not able to determine triacylglycerol composition since stationary phases could not resist the high temperatures needed to volatilize the sample. Nowadays the use of phenyl-methyl-silicone phases, able to endure temperatures greater than 350°C for a long time, allows the separation of triglycerides according to carbon atom number and unsaturation (Antoniosi Filho et al., 1993; Geeraert and Sandra, 1987). To avoid losses or thermal decomposition in the split injection, an on-column injection is generally used due to different volatility of triglycerides. The gas chromatographic method, although able to resolve triglycerides, has the disadvantage of column deterioration.

Alcoholic Fraction

Refining leads to isomerization of the triterpenic fraction because of the opening of a 3-carbon atom ring and the translocation of a double bond in the side chain from the 24-28 to the 24-25 position (Paganuzzi, 1984; Strocchi and Savino, 1989; Lanzón et al., 1999). Thus the detection of triterpenic isomers can serve as a means of revealing illegal additions of refined oils to virgin olive oil.

The isolation of triterpenic alcoholic fractions is achieved by TLC fractionation of the unsaponifiable matter, while the quantification is carried out by HRGC. Frega and coworkers (1993) achieved the separation of the different compounds through HRGC analysis of the silylated unsaponifiable matter. Some researchers (Mariani et al., 1993) suggested to avoid the time-consuming isolation of unsaponifiable; they carried out the separation of the oil previously silylated by silica gel column followed by HRGC analysis.

The determination of alcoholic index (I.A.) can be a useful means to detect the addition of olive pomace oil to olive oil, since it is significantly higher in olive residue oils than in virgin olive oils (Camera, 1978/1980). Alcoholic index is a numerical factor calculated from a ratio of areas of some peaks of the alcoholic fraction of the unsaponifiable matter. It is given by the following equation

$$(I.A.) = \frac{C_{22}}{C_x} \times \frac{(C_{22} + C_{24} + C_{26} + C_{28})}{(CA + 24MeCA)} \quad [2]$$

where C_{22} - C_{28} are the correspondent aliphatic alcohol areas, C_x is the area of geranylgeraniol and CA and 24MeCA are the areas of cycloartenol and 24-methylen-cyclo-

artanol respectively.

Other Absorptions in the Visible and UV Spectra

An interesting region of UV spectra is between 310 and 320 nm, where conjugated tetraenes absorb. The display of derivative indices of dienes, triene, and tetraene bands by three-dimensional graphs allows a discrimination of virgin olive oils, refined olive oils, and seed oils even in the presence of autoxidation products according to Chiricosta et al. (1994). The derivative spectrophotometry is very rapid, has a low cost, and provides evidence for the presence of seed oils at low percentages (Calapaj et al., 1993).

Spectrophotometry in the visible region is a good means to detect the presence of virgin olive oil in olive oil category. Olive oils are obtained by blending virgin olive oils with refined olive oils. Virgin olive oils, differently from refined olive oils, show emission at 673 nm due to the presence of chlorophyll. Thus the presence of virgin olive oil, even in a small quantity, can be evidenced measuring spectrofluorimetrically the emission at 673 nm. However, due to the variability of the chlorophyll content in virgin olive oil related to agronomic and technological factors, it is not possible to quantify the amount of virgin olive oil.

Hydrocarbons

Refining causes, in addition to a loss of volatile compounds (especially sesquiterpenes), the appearance of hydrocarbons not naturally occurring in virgin olive oils such as alkadienes (mainly *n*-hexacosadiene), stigmasta-3,5-diene, isomerization products of squalene, isoprenoidal olefins from hydroxy derivatives of squalene, and steroidal hydrocarbons deriving from 24-methylene cycloartanol (Lanzon et al., 1994).

The procedure to determine squalene isomers, according to Mariani and coworkers (1993), involves a chromatographic separation on a silica gel column (2% H₂O) of oil sample previously silylated, the isolation of squalene isomer fraction and its analysis by HRGC on SE-52 columns with flame-ionization detection.

The carbon number profile of *n*-alkanes could be a good means to determine adulteration of extra virgin olive oil with very low percentages of both crude rapeseed and sunflower seed oils (Webster et al., 2000). Analysis of the *n*-alkane pattern by Principal Component Analysis has been suggested as a possible means to identify these adulterants at levels of about 0.5% (Webster et al., 2000).

Authentication

Food authenticity is an important issue that includes adulterations, varietal and geographical characterization, and verification of some properties of olive oils with Denomination of Protected Origin (DOP) (EEC Reg No 2081/92), through analytical

methodologies.

Adulteration

Extra virgin olive oil has a much higher price compared to olive oils of other categories or olive pomace oils and seed oils. Because of this, its adulteration with cheaper products can be an attractive practice.

International bodies such as the European Commission and IOOC defined strict identity characteristics of the different olive oil categories (Table 7.8) and adopted modern instrumental techniques replacing classical purity tests.

Current methodologies adopted in official methods resulted in a significant improvement of the control of olive oils, but some questions have been raised by researchers who indicated that such methods are not able to reveal all sophisticated adulterations (Paganuzzi, 1997) and, in addition, can classify some genuine oils outside their natural category (Proto, 1992).

Fatty acid composition can only give some but not conclusive information about the possible presence in a mixture of linoleic rich vegetable oils. The adulteration is detected by the Δ ECN42 determination, which has proved to be very effective, and by the analysis of sterols, which is especially useful for detecting the botanical origin of the added seed oil.

Monoacylglycerol content could differentiate between genuine virgin olive oils and oils fraudulently deacidified (Leone et al., 1989), whereas total diglycerides content is helpful for detecting a possible fraudulent raising of the category of a given product (Leone et al., 1988).

Problems related to the detection of the addition of one of different kinds of desterolized oils to olive oil have been overcome since processes needed for removing sterols involve the production of *trans* unsaturated C18 fatty acids, of stigmasta-3,5-diene and n-alkadienes (e.g. n-hexacosadiene) and isomerization products of squalene. Isomerizations that convert Δ^7 sterols into $\Delta^{8(14)}$ and Δ^{14} sterols (Biedermann et al., 1995) are of practical interest in such cases because they reveal the addition of small amounts of desterolized sunflower oils.

Addition of refined pomace olive oil to refined olive oil is generally detected by the determination of waxes, aliphatic alcohols, and erythrodiol+uvaol.

The adulteration of virgin olive oil with low proportion of refined olive oil, in addition to the official determinations has already been discussed. The spectrofluorimetric detection at 673 nm (Marini et al., 1990), typical of chlorophyll pigment, can evidence the presence of virgin olive oil in other grades, although it is not possible to measure levels because of the wide variability of chlorophyll content. Atomic absorption is considered only a preliminary screening tool, to detect the presence of synthetic chlorophyll. In the latter, the central magnesium ion of the chlorophyll molecule is replaced by a transition element, such as a copper ion. Serani and Piacenti (2001b) have developed an analytical approach to detect copper pheophytin (E141),

a coloring agent illegal in olive oil. More recently Del Giovine and Fabietti (2005) have proposed a capillary zone electrophoresis (CZE) method, with a laser induced fluorescence (LIF) detector, to determine copper chlorophyll from natural pigments. Results obtained confirm a good repeatability, reproducibility, and accuracy of this method, compared to other methods such as HPLC.

In the last years authenticity and adulteration of olive oil have been extensively monitored using spectroscopic techniques which show advantages in terms of speed and expense per test.

Mid-infrared (MIR; 4000–400 cm^{-1}) and near-infrared (NIR; 15000–4000 cm^{-1}) spectroscopy have been successfully used for the detection of oil adulterants, providing direct molecular specific information without extensive sample preparation (Sato, 1994; Guillen and Cabo, 1999). The MIR region is where the fundamental groups appear. For vegetable oils, MIR spectra are dominated by the vibrations of polymethylene chains of triglycerides. Two distinct regions are present in a MIR spectrum: the first (3100–1700 cm^{-1}) is formed by well-resolved peaks. In this part of the spectrum there is absorption due to the C-H stretching vibration of *cis* fatty acid ($-\text{CH}=\text{CH}-$) that appears near 3005 cm^{-1} in triolein, shifts towards higher frequencies as the degree of unsaturation increases. The corresponding *trans* form absorbs near 3025 cm^{-1} . The second part of a MIR spectrum (1500–700 cm^{-1}) is called the fingerprint region and shows overlapping peaks. The fingerprint region is closely related to the degree and type of unsaturation, and also to the content of *cis* and *trans* isomers. The intensity of the band near 1400 cm^{-1} depends on the percentage of monounsaturated acyl groups; that of the band near 1160 cm^{-1} on the content of saturated acyl groups. The presence or absence of bands near 915 cm^{-1} , very weak in olive oil, can be useful in detecting the existence of blends with high linoleic oils (Guillen and Cabo, 1999).

NIR spectra generally contain a number of broad and overlapping bands, arising from the overtones (first and second) and combinations of functional groups present in oil samples. The most intense bands in the oil spectra can be found at 4260 and 4370 cm^{-1} , and are characteristic of the combinations of C-H stretching vibrations of $-\text{CH}_3$ and $-\text{CH}_2$ with other vibrations. The two bands at 5700 and 5750 cm^{-1} correspond to the first overtone of the C-H stretching vibration of $-\text{CH}_3$, $-\text{CH}_2$ and $-\text{HC}=\text{CH}-$. The absorption band near 6010 cm^{-1} is due to C-H vibration of *cis*-unsaturation. Fatty acids having *cis* double bond exhibit strong absorption bands in the region around 6010 cm^{-1} , and the intensity of these bands increases with increasing unsaturation. In the region between 7700 and 9100 cm^{-1} , the second overtone of the C-H stretching vibration of $-\text{CH}_3$, $-\text{CH}_2$ and $-\text{HC}=\text{CH}-$ can be found.

Data handling of MIR and NIR spectra is very difficult, and useful information can be drawn only in combination with chemometrics. Lai et al. (1995) demonstrated the potential of MIR spectroscopy for the quantitative determination of the level of refined olive and walnut oils in extra virgin olive oil. Downey et al. (2002) applied discriminant analysis and PLS to NIR data for the quantification of sunflower adul-

teration in extra virgin olive oils. The presence of adulterants, such as corn oil, sunflower oil, soya oil, walnut oil, and hazelnut oil in pure olive oil, could be predicted on the basis of NIR data with very low error limit ranging from ± 0.57 to ± 1.32 % w/w, as reported by Christy and coworkers (2004).

NMR spectroscopy has also played an ever-increasing role in the study of properties of vegetable oils, as a tool for authentication and quality assessment of virgin olive oil. Recently, Vlahov (1999) and Sacchi and coworkers (1997) have reviewed the usefulness of NMR to the study of olive oils.

^1H NMR spectrum of any edible oil shows about 10 signals, due to protons of the main components, triglycerides. The proportion of various acyl groups in oils of different botanical origin provides a great deal of information which permits good discrimination between oils of different composition (Guillen and Ruiz, 2003a, 2003b). Fauhl et al. (2000) have applied discriminant statistical analysis to some concrete signals of the ^1H NMR spectra, to show the effectiveness in discriminating between olive, hazelnut and sunflower oils. The high resolving power of ^{13}C allows the characterization of triglyceride mixtures, the fatty acid compositions, without distinguishing, however, the homologous chains, i.e. C16:0 and C18:0, that appear as a single resonance.

The analysis of ^{13}C NMR spectra discriminates among virgin olive oils, oils with a high content of oleic acid, and oils with a high content of linoleic acid by using step-wise discriminant analysis. Zamora et al. (2001) obtained a 97.1% correct validated classification for different oils, suggesting that ^{13}C NMR may be used satisfactorily for discriminating some specific groups of oil. To obtain 100% correct classifications for the different oils and mixtures, more information is needed than that obtained from the direct analysis of the oils. More recently ^{31}P NMR spectroscopy has also been applied for the detection of extra virgin olive oil adulteration (Fragaki et al., 2005).

Other techniques, such as carbon stable isotope ratio (Angerosa et al., 1997b; Spangenberg et al., 1998), Curie-point Pyrolysis mass spectrometry (Py-MS) (Goodacre et al., 1993), FT-Raman spectroscopy (Baeten et al., 1996) and electrospray ionization-mass spectrometry (ESI-MS) (Goodacre et al., 2002) have been applied for assessing the adulterations.

Current Problems

A series of olive oil adulteration problems is related to the addition of high oleic acid oils, such as hazelnut oil. Other problems are a: the illegal addition to virgin olive oils of olive oil subjected to forbidden deodorization under mild conditions that do not cause the formation of hydrocarbons not naturally occurring in virgin olive oils, *trans* isomers of fatty acids, or isomerization products of squalene, which are all useful tracers of refined oils; b: the addition of oils obtained by a second centrifugation of olive pastes (*remolido*).

Addition of Hazelnut Oil

Adulteration of olive oil with hazelnut oil is one of the most difficult to detect, due to similar triacylglycerol composition, total sterol content, and fatty acid profile (Benitez-Sanchez et al., 2003).

Detection of hazelnut oil in mixtures with olive oil is especially difficult at adulteration levels below 20%. A method of detecting the adulteration with pressed hazelnut oil is based on the determination of filbertone, (*E*)-5-methylhept-2-en-4-one, a characteristic volatile compound of hazelnut oil with a great flavor impact (Blanch et al., 1998, 2000). Blanch et al. (1998) have tested different techniques, such as simultaneous distillation-extraction (SDE) and supercritical fluid extraction (SFE) to determine their suitability for the detection of filbertone. RPLC-GC was the most satisfactory for detecting compositional differences between olive and hazelnut oils. The off-line coupling of HPLC and $^1\text{H-NMR}$ for detecting filbertone shows good sensitivity and selectivity (Ruiz del Castillo et al., 2001). Peña et al. (2005) developed a new methodology to detect low percentages of hazelnut oil very recently, combining direct analysis of oil samples by headspace-mass spectrometry and various multivariate statistical techniques. Low levels of pressed hazelnut oil adulteration can be evidenced by RP-HPLC analysis of the polar component (Gordon et al., 2001; Zabaras and Gordon, 2004), using a marker present in the polar fraction of hazelnut oils, but not in olive oils (Gordon et al., 2001).

Ollivier et al. (1999) proposed to search for α -amyryn and lupeol that are present in great proportion in hazelnut and almond oils and absent in virgin olive oils to detect possible adulteration of olive oil at levels of addition $>5\%$.

Several ketosteroids (e.g. sitostan-3-one), recently identified (Mariani et al., 2001) may also be used as markers to identify the addition of hazelnut oil to olive oil.

Due to the natural variability of tocopherol pattern in different oils and their degradation during refining, detection of adulterations presents serious limitation. Recently (Mariani et al., 1999a; Morchio et al., 1999) investigated the content of tocopherols to detect the adulteration of olive oils with hazelnut oils. Olive oils contain a higher percentage of β -tocopherol than γ -tocopherol compared with hazelnut oils. Conversely, olive oils have traces of δ -tocopherol, whereas hazelnut oils contain higher amounts. The authors have suggested that genuine oils should have a ratio γ/β -tocopherol <5 . However, tocopherol determination is not actually of great interest because of the large variability of the tocopherol composition and the tocopherol degradation during refining.

Some researchers have considered the possibility of using more than one parameter to solve the problem. Mariani et al. (1999b) have recently set up a chromatographic method for the determination of esterified sterols which allows the detection of admixtures with hazelnut oil by calculating the ratio in the esterified sterol fraction. This ratio is always ≤ 1 for non-adulterated olive oils. The method permits the detection of levels 5-10% of hazelnut oil, but it has to be combined with $\Delta\text{ECN}42$,

to avoid falsely positive results (Vichi et al., 2001). Cert and Moreda (2000) used the comparison of several triglyceride algorithms with a reference database, to detect low percentages of hazelnut oil. They evaluated the ratio $R = rECN_{42}/rECN_{44}$ where $rECN_x$ is the ratio between the value experimentally determined by means of HPLC and theoretical content calculated from the HRGC composition of C16 and C18 fatty acids, assuming a 1,3-random,2-random distribution of fatty acids in the triacylglycerol with restrictions for saturated fatty acids in the 2-position. R value in relation to the ratio oleic acid/linoleic acid allows one to assess the olive oil genuineness. To overcome problems related to the possibility of some falsely positive results, IOOC experts (International Olive Oil Council T.20/Doc no. 25 February 2004) proposed to adopt, in addition to R, a decision tree, founded on the agreement between results from several mathematical algorithms (calculated from theoretical and experimental triacylglycerol composition) and those from a database built from genuine oils. Algorithms take into account the following parameters: LLL_{theor} , ΔOOL (where ΔOOL has the same meaning $\Delta ECN42$ that is the difference between theoretical and experimental OOL), ΔLLL , $\Delta ECN44$, and percent of linolenic acid.

Other potential discriminant factors, such as $(LLL/ECN42) \times 100$, $ECN46/LLL$ and $(ECN44+ECN46)/LLL$, useful for blends with seed oils, could not reveal adulteration at low percentages of hazelnut oil (Christopoulou et al., 2004).

More recently, spectroscopic techniques have been widely applied to assess adulterations. FT-Raman spectroscopy, together with Partial Least Squares and Genetic Programming, were employed to verify the level of hazelnut oil added to virgin olive oil. Results seemed to successfully predict the addition of hazelnut oil in the range 0–20% (López-Díez et al., 2003). Classification of hazelnut oil, olive oil, and other types of oils was successfully achieved with FT-IR spectroscopy (Christy et al., 2004; Ozen and Mauer, 2002). Depending on the adulterant oil, detection limits for olive oil adulteration were as low as 2%, adulteration of virgin olive oil with hazelnut oil could be detected only at levels 25% and higher (Ozen and Mauer, 2002). 1H -NMR (Mannina et al., 1999) and ^{13}C -NMR (Zamora et al., 2001) have been investigated as alternative approaches to detect hazelnut adulteration, also in combination with artificial neural networks (Garcia-Gonzalez et al., 2004) giving a detection limit of the model around 8% of hazelnut oil.

Olive Oils Subjected to Forbidden Deodorization in Mild Conditions

The “deodorized oils” are olive oils that have been subjected to a deodorization under low temperature and in vacuum to remove undesirable volatile compounds or to the bleaching and deodorization under conditions that avoid significant modifications of their composition.

Serani and coworkers (Serani et al., 2001; Serani and Piacenti, 2001a, 2001b,) studied both the effects of thermal treatments on the transformation of pheohytins and the effects of chemical-physical treatments on diglyceride composition. They ob-

tained a mathematical function that allows determination if a virgin olive oil has been subjected to a thermal treatment, and named this function Cold index. The function is always > 0.10 in deodorized oils, whereas it is near zero or negative in the majority of extra virgin olive oils (Serani and Piacenti, 2001a, 2001b). In addition the same authors studied oils subjected to chemical and physical treatments and related the absolute content of diglycerides with the free acidity of the oil and the isomerization time of 1,2-diglycerides. The latter is calculated by kinetics of isomerization and mathematically expressed as a function of the ratio between 1,2- and 1,3-diglyceride isomers and free fatty acids (Serani and Piacenti 2001a; Serani et al., 2001). Treated oils show isomerization times notably longer than genuine virgin olive oils. Limit values were suggested to discriminate genuine virgin olive oils.

Mixtures of Virgin Olive Oil With Olive Oil Obtained by Second Centrifugation of Olive Pastes (Remolido)

The processing of olive pastes obtained from the first centrifugation can be performed immediately or after storing. Oils from the second centrifugation of fresh pastes show characteristics very similar to those from the first centrifugation, but they are closer to those of olive pomace oils, if pastes are processed after several days, because of a greater amount of erythrodiol, waxes and free aliphatic alcohols.

There are not consolidated methodologies for detecting addition of oils from the second centrifugation. IOOC experts (International Olive Oil Council, T.20/Doc. N. 39-1 1998 and T.20/Doc. N. 38-4 1998) proposed to determine both total aliphatic alcohol content and alcoholic index (I.A.) to reveal fraudulent admixtures with oils from the second centrifugation. Alcoholic index is significantly higher in oils from the second centrifugation than in oils from the first one, in extra and virgin categories, and in lampante grade. Alcoholic index has already been described in the Alcoholic fraction section.

Varietal Characterization.

There is a huge number of *Olea europaea* cultivars and some of them were recently planted in new areas different from regions where they were autochthonous.

A great research work has been made in an effort to understand the modifications of the qualitative and quantitative composition of most oil fractions, according to variety. Several investigations indicated that some parameters can be used to differentiate oils from various cultivars (Aparicio and Luna, 2002; Stefanoudaki et al., 2000). Esti and coworkers (1996a) found that the total content of alcohols could be a useful tool for varietal characterization. On the other hand, Gandul-Rojas and Minguéz-Mosquera (1996) reported differences in the contents of chlorophylls and carotenoids useful for discriminating some Spanish varieties. Fatty acids and unsaturated and aliphatic hydrocarbons were used to distinguish Croatian cultivars (Koprivnjak

and Conte, 1996). Koprivnjak et al. (2005) obtained to differentiate three different Croatian varieties by applying a linear discriminant analysis (LDA) to n-alkanes of oils obtained during four consecutive years.

Relationships between cultivars and sensory quality were investigated by several researchers using sensory evaluations and volatile composition analysis (Aparicio and Luna, 2002; Stefanoudaki et al., 2000; Cavalli et al., 2004; Tura et al., 2004).

The characterization of monovarietal virgin olive oils is very difficult since their composition is affected by a number of variables such as pedoclimatic conditions (Aparicio et al., 1994a; Morello et al., 2003), ripening degree of fruits, and extraction systems. Moreover, there is also the difficulty related to the variability of the contents of single compounds over the years.

The most important changes have been observed during the ripening process, which in turn is affected by climate, agronomic practices, and irrigation (Romero et al., 2002). Nitrogen fertilization would slaken fruit ripeness, a greater availability of water as in the case of irrigation (Goldhamer et al., 1994) promotes the maturation, thus causing a reduction of phenols, weakening of bitterness and a modification of volatile composition and sensory profile (Salas et al., 1997).

Climate, and in particular temperatures, modify the metabolic activities of fruit and affect unsaturated fatty acid content (Esti et al., 1996a; Beltran et al., 2004) and phenolic content (Beltran et al., 2005).

Statistically significant changes were observed in triterpenic and sterolic fractions (Esti et al., 1996a; Christopoulou et al., 1996) and in the diacylglycerol ratio (Vlahov, 1996). The volatile composition shows a different evolution pattern in relation to fruit maturity and the extension of fruit pigmentation (Morales et al., 1996; Angerosa and Basti, 2001) with notable changes in sensory odor note intensities. Phenolic compounds show a dramatic reduction that can reach about 60% during the last 4 months of fruit ripening (Škevin et al., 2003; Mousa et al., 1996; Esti et al., 1996b). This decrease of phenolic compounds is responsible for a weakening of the bitter sensory note.

All the mentioned variations in composition are greater in "cold" areas. Oil from mountainous regions generally shows a higher content of linoleic acid, lower oxidative stability, and lower concentrations of sterols, tocopherols, phenols, and chlorophylls than oil from areas at low altitude (Aparicio et al., 1994a; Mousa et al., 1996).

Technological conditions during the oil extraction also modify the composition. The concentration of volatile compounds and polyphenols in olive oils depends on the type of grinding machines, malaxation conditions, and extraction system. A greater recovery of phenolic compounds is observed by using metallic crushers. Conversely, the amount of volatile compounds is significantly higher in oils obtained with a mill stone (Angerosa and Di Giacinto, 1995). Malaxation time and especially temperature negatively affect the composition of metabolites arising from the lipoxygenase pathway, reduce volatile compounds displaying pleasant odors and increasing those

giving less attractive perceptions (Morales and Aparicio, 1999; Morales et al., 1999; Angerosa et al., 2001). In addition, due to oxidative mechanisms mediated by endogenous peroxidases and polyphenoloxidases and interactions with polysaccharides, phenolic compounds are reduced significantly (Servili et al., 2003) and this causes a significant loss of bitterness.

Oils extracted by pressure are significantly more stable and have more intense grass notes and bitter taste in relation to oils extracted by the three phase decanters. This is attributed to a higher concentration of phenols and volatile compounds (Aparicio et al., 1994b; Di Giovacchino et al., 1994; Angerosa et al., 2000b). Oils from two-phase systems are characterized by a reduced loss of *o*-diphenols, tocopherols (Jiménez-Márquez et al., 1995; Angerosa and Di Giovacchino, 1996), and volatile compounds (Ranalli and Angerosa, 1996) as well as low levels of aliphatic and triterpenic alcohols and waxes (Ranalli and Angerosa, 1996).

Because of the different influences of the processing conditions, the characterization of oils from different cultivars can only be achieved through the information from various glyceridic and nonglyceridic fractions. To obtain a reliable differentiation of monovarietal oils, it is necessary to have a large set of oil samples representative of all pedoclimatic, technological and agronomic variables, a large number of chemical compounds and/or sensory attributes, and to apply to them statistical techniques or artificial intelligence algorithms. Bucci et al. (2002) claimed that good results can be obtained by applying supervised chemometric procedures to official quality parameters, such as linear discriminant analysis (LDA) and artificial neural networks (ANNs).

Giansante and coworkers (2003) used fatty acids, fatty alcohols, polycyclic triterpenes, and squalene to discriminate oils from four cultivars. Experimental data were processed by unsupervised and supervised chemometrics. PCA and SIMCA statistical procedures were applied to triglycerides and sterols to distinguish oils from different cultivars (Galeano Diaz et al., 2005).

Excellent results were obtained by Aparicio and his group by applying multivariate statistical procedures to several oil fractions as well as to volatile and sensory descriptors (Aparicio et al., 1997). Volatile compounds are strongly related to sensory descriptors. Sensory notes deriving by the construction of a statistical sensory wheel (Aparicio and Morales, 1995; Aparicio et al., 1996a) were successfully used for the characterization of cultivars (Aparicio et al., 1996b) by means of fuzzy logic profiles (Calvente and Aparicio, 1995).

A completely different approach to the monovarietal oil characterization is based on the evaluation of the percent distribution of volatile metabolites arising from oxidation of linolenic acid (LnA) mediated by lipoxygenase. Metabolite content is strictly connected with the cultivar variable because of the enzyme differences genetically determined and not significantly influenced by the environmental conditions of olive growing areas (Angerosa et al., 1999b). Therefore, cultivars are grouped and

differentiated according to activity of hydroperoxide lyases (% *trans*-2-hexenal), acyl-hydrolases (% of both *trans*-2-hexen-1-ol and *cis*-3-hexen-1-ol) and alcohol acyltransferase (% *cis*-3-hexenyl acetate), and the amount of *trans*-2-hexenal. (Angerosa et al., 2004). Moreover, the percent distribution is the same in oils from the beginning of purple coloring of the fruit. This means that the main metabolites from LnA are independent of the degree of fruit ripening (Angerosa and Basti, 2001). The independence of volatile compositions from the growing area and ripening stage, and the consistency over years, suggest that the cultivar is the dominant factor in the formation of the aroma. Therefore, the determination of metabolites from LnA, together with the concentration of *trans*-2-hexenal, could be considered an effective tool to differentiate monovarietal oils (Table 7.11) (Angerosa et al., 2004).

One of the most innovative approaches to identify variety is the characterization of virgin olive oils by DNA (Cresti et al., 1997). This is especially important for olive oils with a DOP (Denomination of Protect Origin) designation. Their certification implies that the oil composition related to cultivars grown in a given growing area, is in accordance with the registration of the denomination. Labels generally report the country of origin, but do not provide any detail about cultivars. Assay of DNA, present in olive oil and even in refined oil (Hellebrand et al., 1998), can provide reliable information about varieties used for its production (Angiolillo et al., 1999). It is

TABLE 7.11
trans-2-hexenal (ppm) and percent distribution of C₆ metabolites from enzymatic oxidation of linolenic acid. Source: Angerosa et al, 2004.

Cultivar	trans-2-hexenal ppm	% trans-2-hexenal	% trans-2-hexen-1-ol	% cis-3-hexen-1-ol	% cis-3-hexenyl acetate
Mastoidis	17.1	99.4	0.1	0.5	0.0
Coratina	43.5	97.8	1.5	0.7	0.0
Frantoio	53.4	96.6	1.2	0.7	1.5
Taggiasca	17.2	94.9	1.6	1.6	1.9
Canino	30.3	94.8	2.8	2.2	0.2
Picual	23.2	92.6	1.2	5.0	1.2
Leccino	47.3	89.0	10.1	0.9	0.0
Dritta	11.4	84.5	10.9	1.5	3.1
Bosana	12.1	82.7	10.1	2.0	5.2
Carolea	7.4	83.4	2.2	14.4	0.0
Provenzale	5.7	79.4	1.4	9.6	9.6
Nocellara del Belice	6.8	78.4	1.1	15.8	5.0
Gentile di Chieti	6.5	75.1	2.3	18.1	4.5
Maurino	6.3	74.4	2.3	20.9	2.4
Koroneiki	4.6	58.7	3.8	16.3	21.3
Pisciottana	11.0	52.6	4.7	32.9	9.9
Moraiolo	1.8	45.6	5.0	42.4	7.0

possible to construct a DNA database of varieties used for oil production by analyzing DNA of leaves, since the profile of purified DNA from a monovarietal oil correspond to the profile of DNA isolated from the leaves of the same cultivar (Busconi et al., 2003). The DNA assay requires an amplification with suitable techniques (Testolin and Lain, 2005) because of the low level in olive oils. The technique is useful in the verification of the cultivar used for the production of monovarietal oils. However, often DOP oils are produced by processing olives from two or more cultivars. In these cases at the moment, even if the composition of different cultivars is known, DNA analysis can only lead to the identification of the main variety used if this has a proportion above 80%. Therefore, the application of DNA assays for the identification of production cultivars does not currently give conclusive results (Breton et al., 2004).

Characterization of Virgin Olive Oils by Geographical Origin

Verifying the declared origin or determining the origin of unidentified olive oil is not yet an easy task. Standard limits, introduced by International bodies, are able to reveal most of the adulterations, but they are not useful in differentiating oils according to olive growing areas. Oil composition is an expression of biosynthetic genetically controlled pathways, modulated through the action of specific enzymes whose activity is affected by climate, cultivar, soil kind, and the extraction process. This means that the identification of geographical origin can be achieved only when very strict relationships between compositional and sensory data, and the agronomic and climatic characteristics of a given growing area are understood. On the assumption that the composition of virgin olive oils is related to the geographical area where they are produced, oils with the Denomination of Protected Origin (DOP) designation and Indication Geographical Protect (IGP) are marketed within countries of the European Union. In fact the control of a DOP or IGP products is obtained by administrative measures of oil production. Many efforts are made by researchers to control these commodities by objective analytical methods.

Researchers trying to elucidate relationships between composition and geographical origin use HRGC and HPLC methods to determine major and minor components of olive oils. Experimental data are generally processed by multivariate statistical procedures or expert systems for the classification of the olive oils. Interesting results were obtained by applying many multivariate procedures, but the more encouraging differentiations were made by means of expert systems that use a very large database. For the creation of a database, all the possible information about climate, cultivars, growing area, altitude, longitude, latitude, etc, must be taken into account. In addition, sampling should include oils produced in many olive crops to obviate to the variability induced by olive producing year.

The major components of olive oil give useful information which may be used to differentiate the oils. Statistical procedures have been applied to fatty acids (Tsimidou and Karakostas, 1993; Stefanoudaki et al., 1999). The effect of latitude, which dif-

ferentiates oils of the North regions from those of the South areas, is clearly delineated by fatty acids and triacylglycerols (Tsimidou et al., 1987b; Tsimidou and Karakostas, 1993; Alonso and Aparicio, 1993). However, more information can be drawn from minor constituents. For instance, the longitude may be indicated by the triterpenic alcohol content which decreases from coastal to inland regions (Aparicio et al., 1994a).

Chemometric methods have been applied to sterolic composition and triglycerides (Galeano Diaz et al., 2005), fatty acids, fatty alcohols, and triterpenes (Giansante et al., 2003; Bianchi et al., 2001), triglycerides (Brescia et al., 2003), volatile compounds (Vichi et al., 2003), whereas unsaturated and aliphatic hydrocarbons were used to differentiate Croatian oils (Koprivnjak and Conte, 1996; Koprivnjak et al., 2005). An expert system, labelled SEXIA, has been successfully applied to data of unsaponifiable components, also sometimes including volatile compounds and sensory descriptors (Aparicio and Alonso, 1994; Aparicio et al., 1994c; Morales et al., 1995; Aparicio et al., 1996b).

Recently, emergent techniques were also investigated for their ability to differentiate geographical origin of virgin olive oils. Angerosa and coworkers (1999c) applied stable isotope ratio to gain information about the geographical origin of oil samples. ^{13}C NMR spectroscopy was able to discriminate monovarietal oils from different Italian production areas (Shaw et al., 1997; Vlahov et al., 2001, 2003; Vlahov 2005). This result was explained by the differences in fatty acid composition. Satisfactory results were obtained by Sacchi (Sacchi et al., 1998) and Sacco et al., (2000), who applied Principal Component Analysis or Hierarchical Clustering to high-field ^1H NMR spectroscopic data of minor components. They obtained a very good classification of oil from traditional cultivars with respect to the region of origin. However samples from new cultivars were not correctly classified. This indicates a strong contribution of olive variety on chemical composition of virgin olive oils.

FT-IR and NIR, in combination with different multivariate procedures were also tested as a means to differentiate oils from different producing countries (Downey et al., 2003; Tapp et al., 2003).

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8

Healthful Properties of Olive Oil Minor Components

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Introduction

Adherence to a Mediterranean diet is likely to lower the risk for cardiovascular disease (Trichopoulou et al., 2003; Trichopoulou et al., 2005) and certain cancers (Trichopoulou et al., 2000; Trichopoulou et al., 2003). Even though cardiovascular risk and coronary heart disease (CHD) have always been associated with classic risk factors such as high serum cholesterol and blood pressure, evidence shows that the prevalence of such factors does not differ significantly between the populations of the Mediterranean area—where the incidence of CHD and certain cancers, e.g. breast and colon cancers, is lowest—and those of other North-European and Western countries (Parfitt et al., 1994). Moreover, there are several observations that do not completely link CHD incidence, fat intake, and absorption (Mancini and Rubba, 2000). Taken together, these data suggest that other, as yet unexplored, risk factors may be favorably affected by a healthful diet (Mancini and Rubba, 2000). Indeed, several studies demonstrate that oxidative processes in the endothelium play a role—the extent of which is yet to be fully understood—in the onset of atherosclerosis (Steinberg et al., 1989; Stocker and Keaney, 2004). These processes exacerbate inflammation and greatly increase the risk for atherosclerosis and CHD (Ross, 1999). Such experimental data led to the formulation of an oxidant/atherosclerosis hypothesis, which has been receiving increasing experimental support. The precise nature of the phenomena that trigger the development of atheroma and the extent of their contribution to CHD are yet to be fully elucidated. Based on this evidence, experimental and epidemiological studies are being carried out on the possible role of antioxidants in the relative protection from CHD observed in the Mediterranean area.

In the past, coupled with the low consumption of meat, major emphasis was put on the low saturated fat content (and the concomitant high proportion of monounsaturated fat) of the Mediterranean diet. More recently, research has underlined the importance of plant foods (including carbohydrates and non-digestible fiber) and of the regular use of olive oil. The latter has been traditionally endorsed with healthful

and even medicinal properties. As far as the cardiovascular system is concerned, the protective properties of olive oil have been, until recently, exclusively attributed to its high monounsaturated fatty acid (MFA) content, mostly in the form of oleic acid (18:1n-9). Indeed, monounsaturate supplementation leads to enhanced resistance of LDL to oxidation (Bonanome et al., 1992), hence lowering one of the risk factors for CHD (Witztum and Steinberg, 2001). Appropriately, the US Food and Drug Administration recently allowed a qualified health claim for monounsaturates from olive oil and reduced risk of CHD (FDA, P04-100, 2004). However, several observations argue against the hypothesis of oleic acid as the exclusive responsible factor for the lower rates of CHD of the Mediterranean area. For example, the effects of MFA on circulating lipids and lipoprotein have not been fully clarified. While the major effects of high monounsaturated fatty acid intakes on serum cholesterol are generally thought to be indirect and have been attributed to the associated replacement of saturated fatty acids (Belkner et al., 1993; Hegsted et al., 1993; Gardner and Kraemer, 1995), some studies (reviewed by (Mensink et al., 2003), attributed a direct, although modest, cholesterol-lowering effect to MFA alone, when they equicalorically replace carbohydrates. Also, MFA increases the levels of the protective high-density lipoprotein (HDL) more than polyunsaturates (PUFAs) when these two classes of fatty acids replace carbohydrates in the diet (Mensink et al., 2003); however, there are reports of a neutral effect of MFA on plasma lipids or even a total- and LDL-cholesterol lowering activity. In turn, while oleic acid might exert some beneficial effects on the serum lipid profile, its actions are of moderate magnitude at best. Most important, oleic acid is one of the predominant fatty acids in largely-consumed animal foods such as poultry and pork. Thus, contrary to the common belief, the percentage of oleic acid in the Mediterranean diet as a whole is only slightly higher than that of other kinds of Western diets, *e.g.* the North American one (Dougherty et al., 1987; Katan, 1995). It is therefore unlikely that oleic acid is exclusively accountable for the healthful properties of olive oil. Finally, it is also noteworthy that several seed oils obtained through genetic selection, such as sunflower, soybean, and rapeseed oils are nowadays rich in monounsaturated, albeit devoid of phenolics (Owen et al., 2000), and are commercially available. Consumption of such oils, namely rapeseed, is widespread in several areas of the world (Gunstone, 2004): if oleic acid were endowed with strong cardioprotective effects, similar low incidence of CHD and high longevity would be observed outside the Mediterranean basin.

This chapter reviews the evidence that indicates how the phenolic components of extra virgin olive oil may play a role in the protection from CHD and cancer observed in the Mediterranean area.

Olive Oil Minor Constituents—Role in Human Health?

The epidemiological evidence of a lower incidence of CHD and certain cancers in the Mediterranean area (Keys, 1995) stimulated research on the potentially protective

activities of olive oil minor constituents, some of which have recently become commercially available.

It must be accentuated, as mentioned in other parts of this book, that only olive oil marketed as “extra virgin,” *i.e.* the one with a degree of acidity lower than 0.8% according to the current regulations, contains substantial amounts of phenolic compounds. Other kinds of olive oil, including the one simply marketed as “olive oil” are poor in or devoid of phenolics, due to the chemical procedures employed to reduce the acidity. This has important consequences in terms of agronomic and marketing policies. If olive oil phenols do have an impact on human health (see below), then the various procedures that influence olive oil quality are to be implemented and the general public should be informed to choose high quality olive oil. In particular, the phenolic constituents confer a bitter and pungent taste to the oil, as a result of complex interactions between such “minor constituents” and the taste buds, including inactivation of ptyalin. Slightly bitter and pungent tastes are positive attributes. This is known to members of panel tests, who evaluate the organoleptic quality of the oil and often appreciate high-phenol olive oils. Choice of an extra virgin olive oil, in addition to providing enjoyable meals, might thus positively influence human health, as described below.

Evaluation of the Role of Olive Oil Phenols in Human Health – Methodological Caveats

The major limitation of the qualitative/quantitative evaluation of olive oil phenolic compounds lies in the current lack of simple and easy to apply methodologies, as in the case of other foods (Hammerstone *et al.*, 2000; Manach *et al.*, 2004; Manach *et al.*, 2005; Williamson and Manach, 2005). This negative aspect prevents the correct assessment of a relationship between olive oil phenol consumption and incidence of disease. Currently, the most widely employed methods for evaluating the total polyphenolic content of olive oil are the Folin-Ciocalteu colorimetric assay (Visioli *et al.*, 1995b) and the HPLC (Montedoro *et al.*, 1992). The former is simple to perform and does not require expensive equipment, but is limited by the low specificity of the reagent toward phenolic compounds; further, it does not provide qualitative information of the composition of the phenolic fraction. Conversely, HPLC is very sensitive and specific, but it is time-consuming (samples run for about one hour) and does not provide information on phenolic molecules for which reference standards are unavailable. An enzymatic assay for the quantitative determination of olive oil phenolics has been proposed by Mosca *et al.* (Mosca *et al.*, 2000). This method is rapid and easy to perform and is more sensitive and specific for phenolic compounds than the Folin-Ciocalteu method. Alas, it also provides quantitative information only and does not detect other “minor constituents” such as cinnamic and vanillic acids. Finally, a rapid and sensitive method to evaluate the phenolic components of olive oil by Atmospher-

ic Pressure Chemical Ionization-Mass Spectrometry (APCI-MS) has been described by Caruso et al. (Caruso et al., 2000). This method allows for quick analyses of crude methanolic extracts of olive oil, does not need extensive analytical workup, and makes it possible to quantify oleuropein aglycone. The apparatus is, however, very expensive and requires trained personnel to operate.

Olive Oil Phenolics and Human Health

In Vitro Studies

1. Antioxidant Activities

The first experiments of our group on the biological activities of olive oil phenolics started over a decade ago thanks to the availability of oleuropein, obtained from Extrasynthese (France), and hydroxytyrosol, isolated in pure form from olive oil and provided to our lab by Professor Montedoro. The experimental model employed chemically-induced oxidation of LDL, which, back then, was considered prototypic to what was supposed to take place *in vivo*.

Both hydroxytyrosol (HT) and oleuropein (OE) potently and dose-dependently inhibit copper sulfate-induced and metal-independent oxidation of LDL, at concentrations of 10^{-6} to 10^{-4} M (Visioli and Galli, 1994; Visioli et al., 1995a). The protective effects of HT and OE were demonstrated through the assessment of various markers of LDL oxidation, such as a) a reduced formation of short-chain aldehydes (evaluated as thiobarbituric acid-reacting substances, TBARS) and of lipid peroxides, by b) a higher vitamin E content in the residual LDL (indicating sparing of endogenous antioxidants), by c) an extension of the lag phase to form conjugated dienes and by d) a reduced formation of malondialdehyde-lysine and 4-hydroxynonenal-lysine adducts, indicating protection of the apoprotein layer (Visioli et al., 1995a).

The antioxidant activities of hydroxytyrosol and oleuropein were further investigated and confirmed by the use of metal-independent oxidative systems, which indicated that these compounds are potent free radical scavengers (Visioli et al., 1998). In particular, both HT and OE effectively scavenge superoxide anion generated by either human polymorphonuclear cells or by the xanthine/xanthine oxidase system (Visioli et al., 1998); it is noteworthy that, in these experimental setups, both vitamin E and butylated hydroxytoluene were found to be inactive. The free radical-scavenging properties of olive oil phenolics have been confirmed over the past few years by several groups in various experimental models (see [Table 8.1](#)).

Relevant to the development of atherosclerosis, a scavenging effect of hydroxytyrosol and oleuropein was also demonstrated with respect to hypochlorous acid (Visioli et al., 1998), which is a potent oxidant species produced *in vivo* by activated neutrophils at the site of inflammation (Aruoma and Halliwell, 1987): evidence is rapidly

TABLE 8.1
Biological activities of olive oil phenolics

Model	Activity	References
In vitro	Antioxidant activity	(Grignaffini et al., 1994; Salami et al., 1995; Visioli et al., 1995a; Visioli et al., 1995b; Saenz et al., 1998; Saija et al., 1998; Speroni et al., 1998; Visioli et al., 1998; Deiana et al., 1999; Manna et al., 1999; Fito et al., 2000; Pellegrini et al., 2001; Deiana et al., 2002; Lavelli, 2002; Leenen et al., 2002; Manna et al., 2002; Stupans et al., 2002; Carluccio et al., 2003; Gorinstein et al., 2003; Moreno, 2003; Pellegrini et al., 2003; Hashimoto et al., 2004; Masella et al., 2004; Valavanidis et al., 2004)
	Increase NO production	(Visioli et al., 2001)
	Anti-inflammatory activity	(Petroni et al., 1995; Kohyama et al., 1997; de la Puerta et al., 1999; Miles et al., 2005)
	Antiatherogenic activity	(Carluccio et al., 2003; Turner et al., 2005)
	Metabolism	(Edgecombe et al., 2000; Manna et al., 2000)
	Cytostatic activity	(Tranter et al., 1993; Saenz et al., 1998)
	Antimicrobial activity	(Tranter et al., 1993; Bisignano et al., 1999)
Animal	Chemoprevention	(Budiyanto et al., 2000 Perino et al., 1988)
	Antioxidant activity	(Grignaffini et al., 1994; Wiseman et al., 1996; Coni et al., 2000; Alarcon de la Lastra et al., 2002; Wiseman et al., 2002)
	Anti inflammatory effect	(Martinez-Dominguez et al., 2001)
	Inhibition of progression of aortic lesions	(Aguilera et al., 2002)
	Antithrombotic effect	(Brzosko et al., 2002)
	Absorption and metabolism	(Bai et al., 1998; Ruiz-Gutierrez et al., 2000; Tan et al., 2003; Visioli et al., 2003)
	Human	Blood lipid modulation and eicosanoid production
Oxidative stress		(Ramirez-Tortosa et al., 1999a; Ramirez-Tortosa et al., 1999b; Masella et al., 2001; Vissers et al., 2001a; Vissers et al., 2001b; Nagyova et al., 2003; Haban et al., 2004)
Postprandial lipemia		(Nicolaiiew et al., 1998; Bonanome et al., 2000; Piers et al., 2002; Soares et al., 2004; Weinbrenner et al., 2004)
Absorption and metabolism		(Bonanome et al., 2000; Visioli et al., 2000a; D'Angelo et al., 2001; Miro-Casas et al., 2001; Visioli et al., 2001; Miro-Casas et al., 2003a; Visioli et al., 2003)

accumulating that the formation of chloramines via the myeloperoxidase-catalized formation of HOCl and subsequent chlorination of apoB-100 is responsible for LDL modification and peroxidation (Carr et al., 2000).

Most studies of olive oil phenolics focus on their cardioprotective potential. However, there is epidemiological evidence of lower incidence of certain cancers (breast, colon) in the Mediterranean area (Trichopoulou et al., 2000). As DNA mutation plays a key role in carcinogenesis, it is important to investigate the chemopreventive properties of olive oil minor constituents in ad hoc models (Owen et al., 2004). Deiana, Aruoma, and collaborators first investigated the activities of hydroxytyrosol toward chemically-induced DNA and aminoacid modification (Deiana et al., 1999). Low concentrations of hydroxytyrosol, i.e. 50 μM , are able to scavenge peroxynitrite and therefore to prevent ONOO⁻-dependent DNA damage and tyrosine nitration. The pro-oxidant activities of hydroxytyrosol (which are due to its copper-reducing properties and might potentially and paradoxically exacerbate DNA damage), were investigated in a model of copper-induced DNA damage. It was found that pro-oxidant actions were 40-fold weaker than those of ascorbate and occurred at very high, non-physiological concentrations (>500 μM) (Deiana et al., 1999). In synthesis, hydroxytyrosol is endowed with chemopreventive potential, which is being confirmed by human trials (see below). The hypothesis that both oleuropein and hydroxytyrosol, similarly to other phenolic compounds (Gehm et al., 1997) possess estrogenic or androgenic activities, was also tested. However, both compounds were found to be inactive (Visioli, Galli, and Poletti, unpublished data).

2. Activities on Enzymes

In addition to their antioxidant actions, the activities of olive oil phenolics on enzymes have been tested in a variety of cellular models, (i.e. platelets, leukocytes, and macrophages) relevant to human pathology. Most olive oil phenolics are amphiphilic and possess the ability to modulate enzymes such as cyclo- and lipoxygenases, NAD(P)H oxidase, and nitric oxide synthase, that are involved in key functions of those cells.

Hydroxytyrosol was found to inhibit a) chemically-induced *in vitro* platelet aggregation, b) the accumulation of the pro-aggregant agent thromboxane in human serum, c) the production of the pro-inflammatory molecules leukotrienes by activated human leukocytes, and d) to inhibit arachidonate lipoxygenase activity (Petroni et al., 1995; Kohyama et al., 1997; de la Puerta et al., 1999; Turner et al., 2005). IC₅₀s are in the 10⁻⁵ M range, similar to those of other NSAIDs and aspirin, indicating that the effects of olive oil phenolics on human health extend beyond mere antioxidant properties. Relevant to the onset and development of atherosclerosis as localized at the arterial wall level, a report by Carluccio et al. (Carluccio et al., 2003) demonstrated that olive oil phenolics inhibit endothelial activation and the related expression of adhesion molecules, hence lessening the consequences of inflammation, namely the recruitment of circulating cells (monocytes/macrophages). The effects of olive oil phe-

nolics were comparable or even superior to those of red wine components such as resveratrol, providing yet another piece of evidence for the cardioprotective effects of the Mediterranean diet.

Modulation of macrophagic response to bacterial challenge is a multi-faceted phenomenon that is involved both in host immune response and in atherogenesis via intimal inflammation (Ross, 1999). In fact, during acute sepsis and inflammation, macrophages react to the endotoxin challenge by increasing the production of reactive species such as nitric oxide, which inhibits platelet aggregation and adherence, and maintains a proper end-organ perfusion rate through increased vasorelaxation. Accordingly, inhibition of nitric oxide synthesis during sepsis increases cellular damage and animal mortality. Moreover, macrophagic nitric oxide exerts a protective role in preventing oxidative LDL modification that may occur at the site of inflammation as a consequence of enhanced reactive oxygen species production (Jessup et al., 1999; Bloodsworth et al., 2000). Yet, macrophage-mediated production of inflammatory mediators in the arterial wall, namely in the intima, exacerbates the onset and development of atherosclerosis and is being recognized as a key contributor to atherogenesis and CHD. We reported that, when added to murine macrophages together with bacterial lipopolysaccharide (LPS), oleuropein increases the functional response of these immune-competent cells, as evaluated by a significant increase ($+ 58.7 \pm 4.6\%$, mean \pm SD) in the production of nitric oxide (Visioli et al., 1998). This increase is due to a direct tonic effect of oleuropein on both the activity and the expression of the inducible form of the enzyme nitric oxide synthase (iNOS), as demonstrated by Western blot analyses of cell homogenates and by the coincubation of LPS-challenged cells with the iNOS inhibitor L-nitromethylarginine methylester (Visioli et al., 1998). These findings were not confirmed by a recent report (Turner et al., 2005) and await further investigation.

In Vivo Studies

The first step toward demonstrating *in vivo* effects of olive oil phenolics was to assess their bioavailability. In fact, experimental evidence that flavonoids and phenolic compounds are absorbed from the diet is accumulating (Williamson and Manach, 2005). Earlier suggestions of *in vivo* activities came from laboratory animals, *e.g.* rats or rabbits, which demonstrated a higher resistance to oxidation of LDL obtained from animals fed virgin olive oil, as compared to LDL separated from animals that were only administered an equivalent amount of oleic acid as either triolein (Scaccini et al., 1992) or olive oil devoid of phenols (Wiseman et al., 1996). In the year 2000, we demonstrated that olive oil phenolics are dose-dependently absorbed by humans and that they are excreted in the urine as glucuronide conjugates; another interesting finding of that study was that increasing amounts of phenolics administered with olive oil stimulated the rate of conjugation with glucuronide (Visioli et al., 2000b). Further studies elucidated the metabolic pathways of hydroxytyrosol and oleuropein,

which form elevated quantities of homovanillyl alcohol and homovanillic acid (Caruso et al., 2001; Miro-Casas et al., 2003b). The most complete study in this area is from Miro-Casas and collaborators, who developed a method to quantify hydroxytyrosol and its metabolites in plasma (Miro-Casas et al., 2003a). In brief, absorption of hydroxytyrosol is nearly complete and its plasma half-life is 2.43 h.

It is noteworthy that hydroxytyrosol (previously also known as DOPET) is a derivative of dopamine metabolism, formed via monoamino oxidase-catalyzed deamination and subsequent reduction (Lamensdorf et al., 2000), and is found in the brain and other tissues. Accordingly, the formation of homovanillyl alcohol in CACO-2 cells has been reported following incubation with hydroxytyrosol (Manna et al., 2000). Likely, hydroxytyrosol is recognized and metabolized by the catecholamine enzymatic systems, such as the catechol-*O*-methyltransferase.

Mechanistically, studies carried out in CACO-2 intestinal cells demonstrated that hydroxytyrosol is absorbed from the gut by passive diffusion (Manna et al., 2000). Finally, Bonanome and coworkers (Bonanome et al., 2000) demonstrated the postprandial absorption of olive oil phenolics and their incorporation into human lipoproteins.

In addition to the elucidation of metabolic pathways that follow absorption, research is concentrating on the possible *in vivo* activities of olive oil phenolics. While some evidence has been built by animal experiments, human studies are more scant. The first, albeit limited, evidence of *in vivo* antioxidant activity was published in 2000 (Visioli et al., 2000a), when a moderate, but significant, decrease in F₂-isoprostane excretion by healthy volunteers who ingested phenol-rich olive oils was reported. To date, approximately a dozen randomized, crossover, and controlled studies have been published yielding non-univocal results on the activities of olive oil phenolics. This is possibly due to limited sample sizes, different choices of biomarkers, confounding by other dietary components, etc. (Covas et al., in press).

To investigate the effects of olive oil phenols on postprandial events (Sies et al., 2005), we recently ran a study to evaluate the effects of moderate, real life doses of two olive oils, differing only in their phenolic content, on some *in vivo* indexes of oxidative stress (plasma antioxidant capacity and urinary hydrogen peroxide levels) in a postprandial setting. Moreover, we assessed whether phenolic compounds influence a few arachidonic acid metabolites involved in the atherosclerotic processes, such as leukotriene B₄ (LTB₄) and thromboxane B₂ (TXB₂). Six subjects in each group received one of the two oils (30 mL/day of olive oil, OO, or extra virgin olive oil, EVOO, distributed among meals) over seven consecutive days (intervention period). On the morning of days 2 and 7 of the intervention and after an overnight fast, 50 mL of the same oil was administered with 25 g of bread to the volunteers to evaluate postprandial changes. On days 1 and 8, subjects received 75 mL of glucose in 150 mL of water to assess their glycemic response. Comparison of OO and EVOO treatments showed no significant differences between groups for all parameters (Figures 1 and

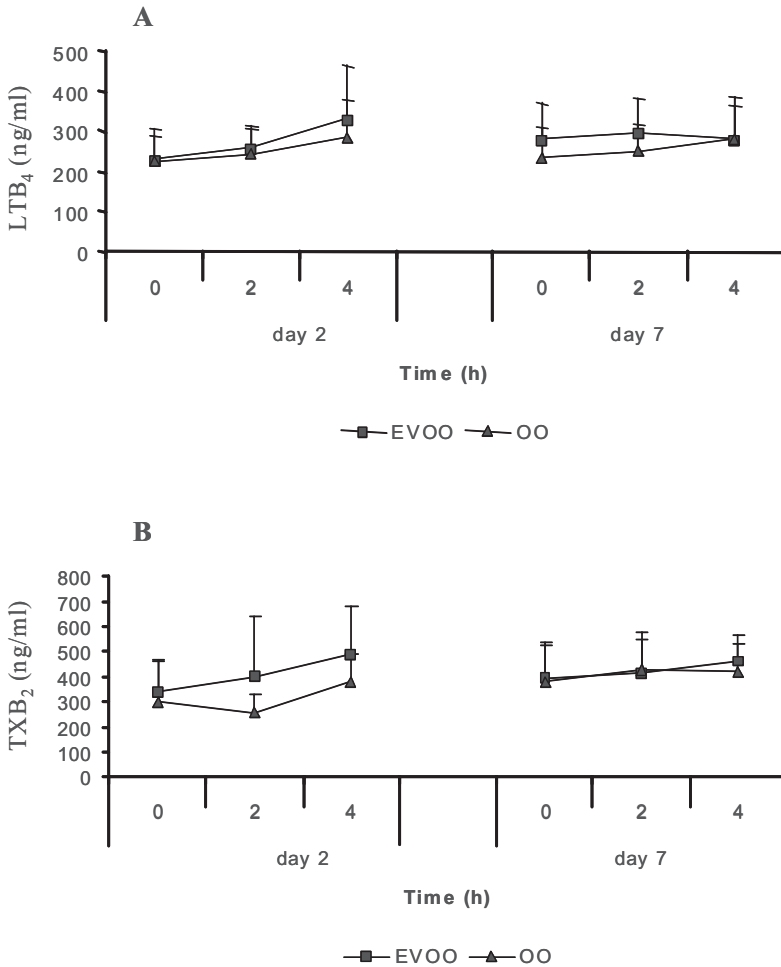


Fig. 8.1. Mean (\pm S.D.) plasma postprandial concentrations of LTB₄ (A) and TXB₂ (B). The volunteers received 30 mL of EVOO (n = 6) or OO (n = 6), distributed among meals, for one week. In the morning of days 2 and 7, 50 mL of the same oil was administered with 25 g of bread to evaluate post prandial changes. Blood aliquots at baseline (0 h), 2, and 4 h after meal were incubated for 1 h at 37 ° C, to stimulate TXB₂ production. Other aliquots were immediately added with the calcium-ionophore A37129 50 μ M for 30 min at 37 ° C, to stimulate LTB₄ production. The productions of LTB₄ and TXB₂ were evaluated by immunoassay (Cayman Chemical, Ann Arbor, MI). All data were subjected to repeated Measures Analysis of Variance (softwares: Stata version 8.0 and SAS version 8.2). EVOO: extra virgin olive oil; OO: olive oil.

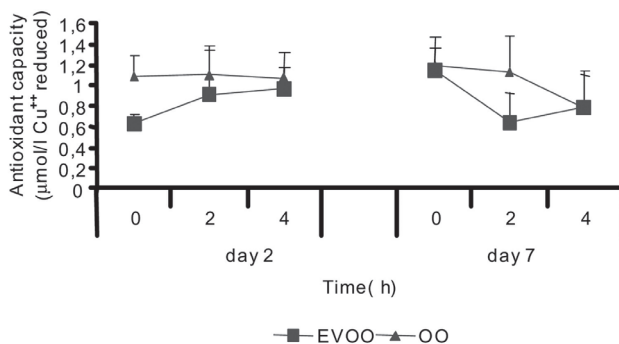


Fig. 8.2. Postprandial plasma antioxidant capacity (means \pm SEM). The volunteers received 30 mL of EVOO ($n = 6$) or OO ($n = 6$), distributed among meals, for one week. In the morning of days 2 and 7.50 mL of the same oil was administered with 25 g of bread to evaluate postprandial changes. The total antioxidant capacity of plasma was measured by a validated method based on the reduction of Cu^{++} to Cu^+ , with uric acid as the reference compound (Pellegrini et al., 2001; Visioli et al., 2003). The results are expressed as $\mu\text{mol/l Cu}^{++}$ reduced. All data were subjected to repeated Measures Analysis of Variance (softwares: Stata version 8.0 and SAS version 8.2). EVOO: extra virgin olive oil; OO: olive oil.

2).

It can be argued that one limitation of this study is the short-term period of sustained specific nutrient consumption. The short-term design, however, permitted volunteers to be restricted to a controlled low-antioxidant diet, thus avoiding consumption of other antioxidants as well as other possible confounding variables, such as fast changes in lifestyle factors which often mask and blur the results of this kind of study. In synthesis, even though human evidence is accumulating and many data are promising, the information available to date does not allow researchers to make any conclusive inference on the specific role played by olive oil phenolics in human health.

As far as toxicology is concerned, there are very few published data that address this issue. In all of the studies a toxic activity of hydroxytyrosol was excluded, even at high doses (D'Angelo et al., 2001; Babich and Visioli, 2003; Christian et al., 2004). However, in view of the potential future formulation of nutraceuticals, consolidation of these data is mandatory.

The recovery of olive phenolics from waste waters (for nutraceutical purposes) is another interesting and emerging field. It is noteworthy that the olive paste is continuously hosed with lukewarm water during the milling, a process that is called malaxation. The resulting "waste water" is produced in extremely large quantities ($\sim 800,000$ tons/year in Italy) and, despite the fact that it contains a considerable

amount of phenols (more than 1% w/v), is currently disposed of. A decade ago, we demonstrated that waste water extracts have powerful (low ppm range) *in vitro* antioxidant activity (Visioli et al., 1995b; Visioli et al., 1999); thus, olive mill waste water could be recovered and employed as a cheap source of natural antioxidants. Indeed, animal experiments (Visioli et al., 2000c; Visioli et al., 2001) and a couple of human studies (Visioli et al., 2003; Leger et al., 2005) confirmed that waste waters are a source of bioactive phenols and dietary supplements derived from olive mill waste water are already available in the market.

Conclusions

The observation that in the Mediterranean area there is a lower incidence of CHD (Keys, 1995; Willett et al., 1995; Trichopoulou et al., 2003; Trichopoulou et al., 2005) and certain types of cancers (Trichopoulou, 1995; Lipworth et al., 1997) demonstrates that the Mediterranean diet, rich in grain, legumes, fresh fruits and vegetables, wine in moderate amounts, and olive oil has beneficial effects on human health, as further confirmed by a cross-cultural trial of Mediterranean diet (Singh et al., 2002). While the beneficial effects of the Mediterranean diet on the cardiovascular system have so far been mostly attributed to its lipid profile (i.e. high oleic acid and low saturates), evidence of the contribution of natural antioxidants and other components of the diet, such as fiber, to this effect is accumulating and should also be taken into account. The evidence reviewed in this chapter suggests that choosing a phenols-rich, extra virgin olive oil would contribute to the dietary intake of biologically-active compounds in quantities that have been correlated with a reduced risk of developing CHD (Hertog et al., 1993; Hertog et al., 1995). Moreover, a phenols-rich, tasty olive oil exerts “indirect” effects, such as the need to be used only in small amounts to dress foods. This reduces the overall calorie intake and is associated with the consumption of fresh vegetables.

In conclusion, even though solid human evidence is yet to be gathered, the biologically-relevant properties of olive oil phenolics described in this chapter and summarized in [Table 1](#) provide substantial evidence to support the hypothesis that virgin olive oil consumption may contribute to lower CHD mortality.

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Processing and Application

9

Olive Oil Extraction

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Introduction

Olive oil is the oily juice of the olive, separated from the other components of the fruit. Properly extracted from fresh, mature fruit of good quality, the oil has a characteristic sensory profile. Its fatty acid composition is characterized by a good balance between saturated, monounsaturated, and polyunsaturated acids. It is also unique among common vegetable oils in that it can be consumed in the crude form, thus conserving vitamin content and phenolic compounds of nutritional importance.

According to the Codex Alimentarius, IOOC, and EC regulations: 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 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 having a chemical or biochemical action.

The ideal objective of any extraction method is to extract the largest possible amount of oil without altering its original quality. However, if quality is not to be modified, it is essential to use only mechanical or physical methods for extracting the oil, avoiding chemical and enzymatic reactions that might change its natural composition.

When treating the olive as prime material, one must consider two groups of phases: the solid elements of the skin, pulp, and kernel, and the liquid phases made up of the oil and the vegetable water. The preparation of olive oil is an industrial process, the purpose of which is to separate one of the liquid phases—the oil—from the other constituents of the fruit. Thus, beginning with healthy, whole, clean fruit, harvested at the moment of optimum maturity, it is necessary to make a paste preparation by means of breaking the vegetal structure; to liberate the oil from the cells and finally achieve the formation of solid and liquid phases. By means of pressure, percolation, or centrifugation, the solid and liquid phases are then separated. Finally, the liquid phases are separated into oil and vegetable water by decantation and/or vertical cen-

trifugation.

The separation between the solid and the liquid phases is not complete: the mass of solids with varying percentages of humidity and oil content form the sub-product called olive pomace and the liquids with varying percentages of fine solid material constitute the oily must.

Extraction methods became more effective with the use of hydraulic presses and transmission mechanisms. Over the years they became more and more mechanized, driven by the need to spare labor expenses in order to lower costs, but the whole process was discontinuous.

The first tests conducted on continuous-flow facilities date back to the second half of the 1960s by Alpha Laval. Improvements enabled the oil to be extracted through the centrifugal effect produced by devices rotating at high speed; the use of stainless steel instead of ordinary steel raised the quality and hygiene standards of the oils produced. These facilities exploit the effect of centrifugal force, which operates by drawing off the liquids. When they came into use after years of testing, they helped to lower labor costs and raise processing capacity.

The extraction of olive oil commences from the olive tree and ends with the storage of the product. There are limitations in a series of factors prior to the extraction process which influence the quantity and quality of the oils. The main factors (which are beyond the scope of this chapter) are: the varieties of the olives, the microclimatic conditions, the variability of soils, the systems of cultivation, which regulate the absorption capacity of terrains and retain rain or irrigation water (Montedoro et al., 1989, 1992; Inglese et al., 1996; Reiners et al., 1998; Gutierrez et al., 1999; Tovar et al., 2001; Romero et al., 2002; Morello et al., 2003; El Antari et al., 2003; Servili et al., 2004; Royo et al., 2005); and pest monitoring and control (Zunin et al., 1993).

Olive Ripening

A very important factor is the maturity stage of the olives for harvesting. Recent analysis data show the great variability in the content and type of phenols present and of volatile substances, which influence the aroma of the oil, during maturation (Esti et al., 1998; Koutsaftakis et al., 2000; Aparicio et al., 1998; Ryan et al., 2002; Schiratti et al., 1999; Rovellini et al., 2003; Caponio et al., 2001; Skevin et al., 2003; Bouaziz et al., 2004; Morello et al., 2004; Angerosa et al., 2004).

The most specific index to test the ripening is the oil accumulation in the olives. It is interesting to note that while the percentage of oil in fresh olive fruit continuously increases as the olive ripens, the percentage of the oil in dry substances reaches a maximum value and remains constant. This occurs because the triglyceride biosynthesis proceeds to a certain ripening stage after which it stops (Garcia and Mancha, 1992).

Recent studies, by Beltran et al (2004), on three of the most important Spanish and Italian cultivars (Picual, Hojiblanca, and Frantoio) indicated that during ripen-

ing the oil content on a dry weight basis increased in the fruit, but oil biosynthesis in flesh ceased from November. Each olive cultivar showed a different ripening pattern, “Hojiblanca” being the last one to mature. Oil content, when expressed on a fresh weight basis, increased in all cultivars, although there are variations due to climatic conditions. Olive fruits presented lower oil and higher dry matter contents in the year of lowest rainfall. Therefore, fruit harvesting should be carried out from the middle of November in order to obtain the highest oil yield and avoid natural fruit drop.

Traditionally, olives are harvested at the green-yellow or black-purple stage. Since all of the fruit does not mature simultaneously even on the same tree, harvesting should take place when the majority of the fruit are at optimum maturity. This is not always possible because other factors may also affect harvest time such as weather conditions, availability of farm labor, availability of olive oil mills, etc.

Harvesting and Transport

The optimal harvesting time is when oil levels are high in the olive fruit. Harvest should begin before natural fruit drop. In normal-ripening varieties the time to start harvesting can be judged by the color of the fruit skin. When there are no green olives left on the tree, perhaps only some fruits at color-change, oil biosynthesis has ceased and harvesting can begin (Tombesi et al., 1996).

Methods used to harvest olives depend on cultural techniques, tree size and shape, and orchard terrain. Most olives are harvested by hand and/or with shakers. Newly-planted orchards are more likely to be mechanically harvested. The high trees of some varieties are harvested with the aid of nets after the natural drop of the fruit. Precautions should be taken to avoid fruit breakage through mechanical damage and fruit contamination by soil material.

Olive transportation and storage should be considered as critical phases for controlling both mechanical damage and temperature. Improper handling during these phases can result in undesirable enzymatic reactions and the growth of yeasts and molds.

The best way to transport the olives is in open-mesh plastic crates that allow air to circulate and prevent the harmful heating caused by the catabolic activity of the fruit (Kiritsakis, 1998). When stored before processing, the olives must be spread in shallow layers and kept in well-ventilated, cool, dry areas. Storing of the olives in jute sacks has to be avoided.

To ensure that the olives retain the quality characteristics they possessed at the time of harvesting they must be delivered immediately to the extraction plant for processing.

Olive Oil Extraction

The flow sheet of the recently used extraction plants comprises four main operations:

- Fruit cleaning (defoliation, olive washing)
- Preparation of the paste (crushing, malaxation)
- Separation of the solid (pomace) and liquid phases (oily must and wastewater)
- Separation of the liquid phases (oil/wastewater)

Fruit cleaning

Fruit cleaning entails two operations: leaf removal and washing. Defoliators suck the leaves, twigs and dirt through a powerful airflow generated by an exhaust fan. After that, the olives are washed in a current of water. This water is recycled after decanting and clean water is constantly mixed in pre-set proportions. To improve washer efficiency, the washing vat is equipped with a shaker that shakes any impurities through screens as well as with an air injection system to create turbulence in the mass.

Crushing

This operation is designed to tear the fruit cells to release the droplets of oil from the inner cavity (vacuole). Not all the oil can be released because it is virtually impossible to lacerate all the cells. Moreover, the droplets are surrounded by an amphoteric pseudo-membrane that tends to keep the oil in a state of emulsion, the stability of which depends on the size of the droplets: the smaller they are, the more stable they are. Also, a small amount of oil remains caught in the colloidal system formed by the pectins in the paste.

The latest version of stone mills consists of a metal basin of a suitable width (with a side shutter to allow the paste to be discharged) on which upright granite millstones (2 till 4) gyrate at 12-15 rpm. Millstones (grindstones) are cylindrically-shaped, have a 120-140 cm diameter and are 30-40 cm wide on their traveling edges.

Stone mills do an optimal job because the combined pressing-and-pushing action of the millstones performs two functions: it crushes and partially mixes the paste. The drawbacks of stone mills are that they are expensive, crushing is slow and not continuous and they have to be carefully operated by skilled staff.

When continuous-extraction facilities came into use, metal crushers—hammer or toothed-disc—were used to grind the olives. They consist of a metal part that throws the olives against a fixed or slowly gyrating metal screen by rotating at high speed. The screen size is 5 mm, 6 mm, or 7 mm and they must be chosen in connection to the extraction system and the maturation stage of the olives to obtain the most suitable stone particle size.

Hammer (or toothed-disc) crushers have come into widespread use because they have a high handling capacity, they operate continuously and they are coupled with malaxing machines. However, there are still doubts as to whether they do the proper job. This is the reason why processors ask for traditional stone mills to be incorporated into continuous-extraction facilities.

Hammer mills are the type of crusher in greatest use, but they have some drawbacks.

- They facilitate the creation of emulsions because of the high speed rotation of the hammers, which is necessary to prolong malaxation.
- They raise the temperature of the olive paste and produce oils with a pronounced bitterness.

Recent research by Amirante et al (2002) has helped to arrive at a better understanding of the effects of the crushing mechanism on the size of the stone fragments (Fig. 9.1).

Figure 9.1 plots the size distribution of the solid particulates obtained by three different crushing methods: stone mill and finishing toothed-disc crusher; toothed-disc crusher and hammer crusher regulated at two different settings.

It can be seen from this figure that, in general, at the normal settings the hammer crusher produces a finer fragment size (curve A) although crushing is still quite rough due to the combined dynamic action of the hammers and the subsequent extrusion through the grating.

This effect can be attenuated by positioning the hammers farther away from the grating, and by using a grating with a larger aperture size (curve B).

When the stone mill and finishing crusher are used (curve C), the results obtained are in-between and characterized by a more uniform stone fragment size whereas the toothed-disc crusher on its own produces larger-sized particles (curve D).

Consequently, it is not easy to say which is the best method for crushing olive fruits: the quality and cultivar of the olives should be the deciding factor. Generally, it is better to use stone mills to crush olive fruits that tend to give “bitter-pungent” oils while it is wiser to use hammer crushers for fruits that tend to give rather “sweet” oils.

On the other hand, hammer crushers produce a smaller stone fragment size than disc crushers, leading to differing increases in paste temperature. When the paste is ground

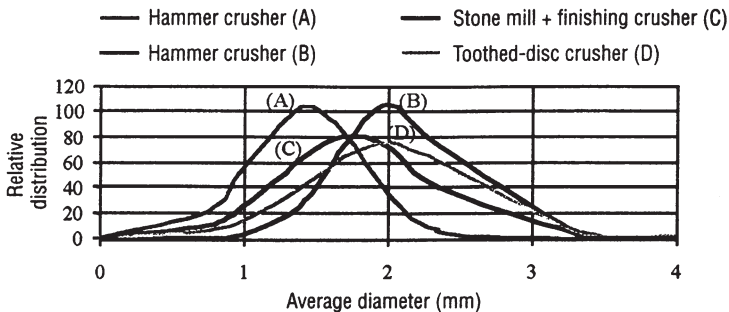


Fig. 9.1. Stone fragment size using different crushers to prepare the olive paste. Amirante et al., 2002).

using disc crushers, the keeping properties of the oils are better than those of hammer-crushed oils. The oils obtained from de-stoned olive paste have a slightly higher total phenol content than oils obtained from mash that has been pounded by hammer crushers, although both types of oils give comparable stability results. When compared with crushing, de-stoning results in higher total phenol contents and higher induction times. Data obtained permit suitable criteria to be suggested for choosing the right machinery to produce top-quality virgin oils that keep well. Improvements in machinery manufacturing will make it possible to achieve greater dispersion of the thermal energy released in stone fragmentation and a large reduction in the absolute amount of energy produced by removing the whole stone.

Malaxation

The oil in olives (about 20-25 %) is found in the mesocarp cells, for the most part, in the vacuoles and scattered to a lesser extent through the cytoplasm in the form of small lipid inclusions.

The oil to be extracted by mechanical means has to be released from the tissues in such a way that the droplets can merge into larger drops until they form what are known as “pockets.”

Malaxation (also mentioned as beating or kneading) is fundamental for increasing extraction yields. It is designed to enhance the effect of crushing and to make the paste uniform. The prime aim is to break up the oil/water emulsion, so that the droplets of oil join together to form larger drops.

The percentages of differently-sized oil drops found in olive paste after crushing and beating have been discussed by Di-Giovacchino (1989, 1996). After crushing, only 45 % of the drops have a diameter of more than 30 microns, which is the minimum size for continuous-process separation of the oil, while this percentage rises to 80% after beating, with an accompanying large increase in the number of drops with a bigger diameter (Table 9.1).

Not all the oil in the olives can be released: some remains enclosed in the unshattered cells, some is spread through the colloidal system (micro gels) of the olive paste, and some is bound in an emulsion with the vegetable water.

The main difficulty in recovering this “bound” oil is that the droplets of dispersed or

TABLE 9.1
Percentages of differently-sized oil drops in the paste after crushing and mixing.
Source: Di Giovacchino,1996

	oil drop diameter μm					
	<15	15-30	30-45	45-75	75-150	>150
After Crushing	6	49	21	14	4	6
After Malaxation	2	18	18	18	19	25

emulsified oil are surrounded by a lipoprotein membrane (phospholipids and proteins) which stabilizes the oil's emulsification or dispersion. The smaller the size of the droplets the greater their degree of stabilization, which means that they are prevented from fusing to form larger drops.

When millstones are employed to crush the olives, the oil emulsion is optimally broken up after 10-15 minutes of mixing at room temperature. In mills where continuous centrifugation is employed, which are normally equipped with metal crushers, malaxation, either in 2 or 3 stages, takes 60 to 90 min. Raising the temperature makes the olive paste less viscous and it is easier to separate the liquid phases by centrifugation. It is well known that an increase of the duration and temperature of the malaxing followed by direct centrifugation of olive pastes, results in higher extraction yields, especially in the case of "difficult" olives.

Malaxing vats are made of stainless steel inside, and are semi-cylindrical or semi-spherical. They have upright or horizontal rotors and a heating system using hot water (45-50°C) running through an outer chamber. The rotating arms are fitted with specially designed stainless steel blades of varying shapes and sizes, which mix the paste by slowly spinning at 15-20 rpm. To protect against any oxidation of the olive paste during the malaxing process, machines are also designed to work with an inert gas (nitrogen) under light pressure, if required. Sometimes, malaxing can make the paste emulsify more and may have a negative effect on oil yields. This happens when the movement of the blades is too fast and the temperature and times are not properly adjusted to the rheological characteristics of the paste being processed.

Separation of the Solid and Liquid Phases (Pomace/Oily Must and Wastewater)

Pressing

Pressing is based on the principle that when a combined solid/liquid mass, like olive paste, is subjected to pressure, the volume of mass decreases because the liquid phase—the oily must—is forced out with the help of the drainage effect of the mats and the stone fragments and is separated from the solid phase. It is an operation that can be compared to filtration and, in fact, it shares the same kinetic properties; but it is more complex.

In a normal filtering process, the volume of the filter bed is fixed, while the volume of the drainage channels gradually decreases as the solid matter is deposited there until it completely obstructs the channels, thus preventing the liquid from running through. When the paste is pressed, the filter bed is variable and the volume of the drainage channels decreases in exact proportion to the amount of liquid that is discharged and is only reduced to zero when all the liquid has been forced out.

Automatic paste distributors are used to apply the paste on the mats in collaboration with a kneader batcher, which accepts the paste from the stone mills. Inox disc diaphragms

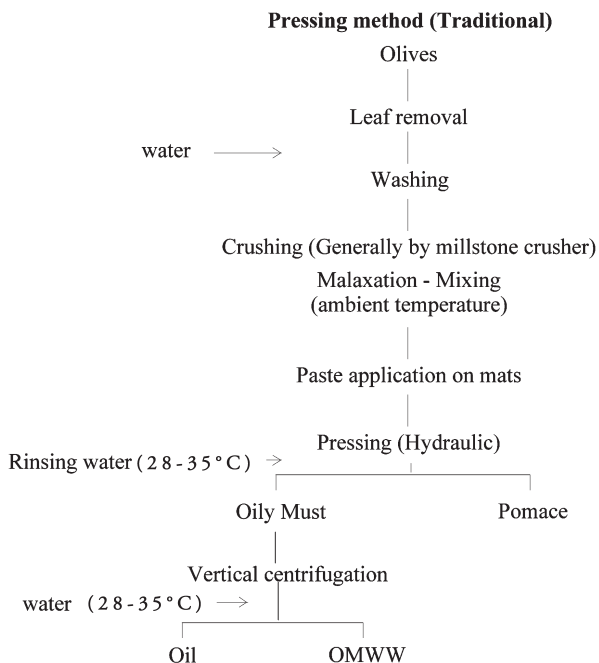


Fig. 9.2. Extraction by the traditional pressing method

are placed among every five mats so that the charge is uniform for pressing. Pressure is applied to a large stack of mats spread with olive paste that is placed on a trolley with a central spike. The perforations in the central spike have been a decisive factor for improving the separation of the oily must (olive oil plus vegetation water) from the pomace because they allow the liquid phases to flow out from the middle of the stack. The large stack of the mats and discs is placed under the press formed by an open monoblock scaffolding and a piston (35-40 cm in diameter) that pushes the pile from the bottom.

Today, pressure extraction is normally carried out in hydraulic super-presses with a service pressure up to 400 atm (which refers to the area of the piston). Super-presses work in single press mode with gradual increase of the pressure up to the maximum value within 45-60 min, remaining at that high pressure for an additional 10-20 minutes. After pressing, a little quantity of water is used to rinse the stuck material off the mats and transfer the oily must for clarification. In practice, a processing yield of 86-90% is obtained and the humidity of the pomace is about 28 %. This method therefore guarantees a top quality oil because of the short beating time and the low temperatures throughout the entire operation, provided that the quality of olives and the state of the mats are also good. The old drawback of vegetable fiber mats has now been altogether overcome through the use of inert polypropylene fiber mats that are more easily cleaned.

Restraints on the practical suitability of pressing are, above all, the cost of the labor it requires, the fact that it is not a continuous operation, and that filter materials have to be used in optimum conditions. Pressure is the oldest method of extraction. It is still in use, though not widespread.

Centrifugation

The appearance of continuous-operating plants contributed to the reduction of costs and to the increase of processing capacity. These plants work on the basis of centrifugal force that operates by sucking out the liquid from the paste.

This separation method is based on the principle that any combination of immiscible liquids with differing densities tends to split up spontaneously into its individual constituents. The reason is that the natural force of gravity affects liquids differently, depending on the density.

If only gravitational force is applied, the speed of separation can be extremely slow but if the mixture is subjected to an artificial gravitational force the speed of separation can be increased. This is done with rotary machines whose speed and separation efficiency are directly proportional to the angular speed and rotation radius, as well as to the difference in the density of the liquids that have to be separated. The machines in use are horizontal centrifuges that operate at an angular speed producing up to 3000 times greater acceleration than natural gravitational acceleration. When

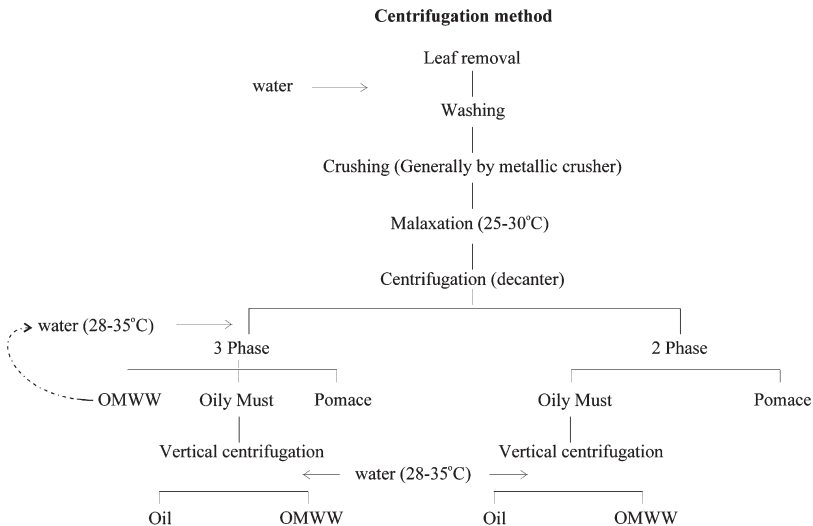


Fig. 9.3. Extraction by the centrifugation method

subjected to such forces, the denser solid particles are pressed outwards against the rotating bowl wall, while the less dense liquid phase forms a concentric inner layer. Different dam plates are used to vary the depth of the liquid—the so-called pond—as required. The sediment formed by the solid particles is continuously removed by the screw conveyor, which rotates at a different speed than the bowl. As a result, the solids are gradually “ploughed” out of the pond and up the conical “beach.”

Very important sections of any decanter centrifuge are:

- Inlet zone

The inlet zone accelerates the feed slurry up to the speed of the bowl. A properly designed inlet zone keeps any degradation of the feed solids to a minimum so that disturbance of the sediment in the bowl is avoided.

- Screw conveyor

The key to good decanter performance lies in the efficient and effective scrolling of the sedimented solids. The design of the screw conveyor is therefore crucial.

- Solid discharge section

Depending on the application, the consistency of the separated solids can vary from a dry powder to a paste. The configuration of the discharge zone is therefore chosen to enable such “cakes” to exit as effectively as possible.

- Liquid discharge section

In a two-phase decanter, the liquid level is regulated by dam plates. When operating in a three-phase mode, each phase discharges over a set of dam plates into separate baffled compartments in the casing. In certain applications, an adjustable pairing tube or a centripetal pump is used to discharge the oil.

Obviously, by increasing the separation speed the mixture stays for a shorter time in the machine and so the amount of mixture that is separated per unit of time increases, i.e. processing capacity is raised.

The churning effect that rotation produces on the water-diluted olive paste leads to the formation of an emulsion in the interface of the two oil/water phases as a result of which a small proportion of oil tends to be lost in the vegetable water. With the advent of improved centrifuges, this drawback has been considerably lessened. However, although very close to those of presses, centrifuge extraction yields are still slightly lower. The increased processing capacity of these types of plants has substantially cut the length of time that olives lie in the mill lofts waiting to be processed, thereby lowering the average acidity of the resultant oils and improving the quality from this aspect.

As far as the other aspects are concerned, it has been acknowledged that the higher temperatures and longer beating times involved, coupled with the use of hot water to dilute the olive paste, partly remove the minor compounds that give the oil its stability and flavor.

The continuous centrifugation involves the steps of: leaf removal and washing, crushing of the olives, malaxing the olive paste, and centrifuging with or without water addition according to the “three-phase” or “two-phase” mode, respectively.

Three-phase Centrifugation. For many years, olive pastes undergoing centrifugal extraction had to be quite fluid to facilitate separation of the fractions with different specific weights; this was done by adding lukewarm water, equivalent to approximately 40-60% of the weight of the olive fruits. The water-thinned paste is centrifuged in the decanter. Three phases are obtained: an oily must, vegetable water mixed with the added water (OMWW), and olive pomace (stones and pulp residue). Disadvantages of this process include increased amounts of wastewater that is produced due to increased water utilization (1.25 to 1.75 times more water than press extraction), loss of valuable components (e.g. natural antioxidants) in the water phase, and problems of disposal of the Oil Mill Waste Water.

To reduce this problem the water phase can be recycled as soon as it comes out of the decanter, to thin the olive paste by injection into the pump that delivers the paste into the decanter. This technique has made it possible to reduce the volume of wastewater by approximately 35% and to improve the total polyphenol content of the oil by approximately 30% (Khlif et al., 2003). However, the practice negatively affects the quality of the produced oil and it is hardly used anymore.

Two-phase Centrifugation. The failure to develop a suitable end-of-pipe wastewater treatment technology gave the opportunity to technology manufacturers to develop the two-phase process, which uses no water process, delivers oil as the liquid phase, and a very wet olive pomace (humidity 60 ± 5 %) as the solid phase using a more effective centrifugation technology. This technology has attracted special interest where water supply is restricted and/or aqueous effluent must be reduced. When fresh olives are used, the paste is produced without addition of water, whereas, when dried olives are used, a small amount of water is added. The disrupted paste is centrifuged in the decanter from which two phases are obtained: oily must and a solid/water mixture (pomace).

Decanters based on the two-phase process were developed by several companies. The performance of the two-phase decanters was evaluated in comparison to the traditional three-phase extraction process and was found to produce olive oil in similar yields to the three-phase process, but of a superior quality in terms of polyphenols and o-diphenols content and keepability. In addition, the two-phase process did not produce wastewater during oil extraction. The two-phase decanting reduces the water requirements. However, it creates a high humidity pomace, named in Spain "Alperujo," which is difficult to handle. The application of a second three-phase centrifugation after malaxing and appropriate dilution of the paste obtained from the first two-phase centrifugation pomace decreases the humidity of the final pomace, but only a small percentage of oil is recovered. This oil is green and has a higher aliphatic alcohols, waxes, and triterpene-alcohol content.

Percolation (or Selective Filtration) in Combination with Centrifugation

Olive oil extraction from olives by the percolation method is based on the difference of the surface tension between olive oil and vegetable water. Because of this difference, when a steel blade is plunged into olive paste, it is preferably coated with oil. When the blade is withdrawn, olive oil drips off and parts from the other phases, thus creating a flow of oily must. This is due to the fact that, in the presence of the solids of olive paste, olive oil has an interfacial tension less than vegetable water in relation to the steel blade.

The first percolation extractor version (1911) was called “Acapulco,” the second version (1930) was called “Acapulco-Quintanilla,” and the third version (1951), which was manufactured in Spain was called “Alfin.”

Continuous improvements have been accomplished in the equipment of percolation. In 1972 a processing system based on both percolation (Sinolea System) and centrifugation was introduced. Sinolea consists of a stainless steel semicylindrical

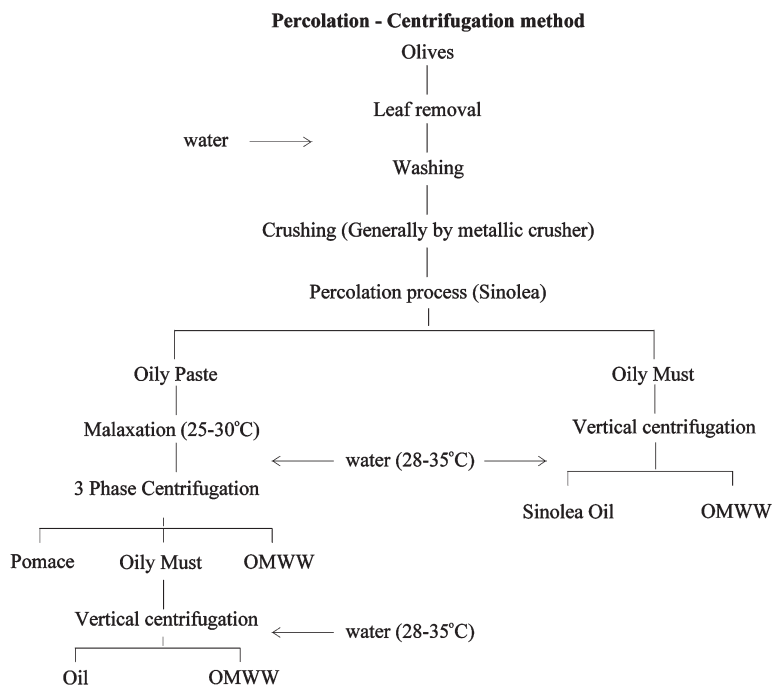


Fig. 9.4. Extraction by percolation-centrifugation method.

grating and many small blades moving through the slits in the gravity. The movement of the blades is slow; therefore, when they plunge into the olive paste as it is continuously renewed, they are coated with oil. The oil drips off the blades when the blades are withdrawn.

The following modified equation, for practical reasons, is used to calculate the oily flow leaving the extractor:

$$\log \frac{Q_r}{Q_0} = H - h \log \frac{t}{10},$$

where, Q_r = the residual oil in the olive paste at a specific time t ; Q_0 = the oil contained in the initial paste and H and h are kinetic constants of process (Di Giovancchino, 1991_. In actual fact, operating over a suitable length of time (30 min until maximum 60 min) with Sinolea extractor, 50-70% of the oil is extracted from the paste. The yield depends on the variety of the olives, the duration of extraction and the rheological properties of the paste.

The remaining oily paste, after an additional malaxation for 20-40 minutes, is thinned with water and then is farther centrifuged with a 3 phase decanter, in order to recover the main part of the remaining oil. The Sinolea oil is a high quality virgin oil (high polyphenol content and perfect organoleptic characteristics), because percolation takes place at ambient temperature, without the addition of water and without employing mats; thus any possibility of contamination is avoided. The combined process produces a quality oil in similar yields to those obtained by pressing, but it has the advantage that it is continuous, which reduces cost and increases capacity.

Separation of the Liquid Phases

The oily must obtained in the various extraction systems has to undergo one last operation for the separation of the oil from suspended solids and the vegetable water. With a clarifier or settling tank, particles and liquid phases will fall to the bottom, but the lack of control and the length of time required makes natural settling unsuitable for modern industrial processes.

Today, disc stack centrifuges with a self-cleaning bowl do this job and they are known as clarifiers. Mainly a three-phase separation clarifier is used to separate two immiscible liquids including separating solids simultaneously. Rotating this unit rapidly means that effect of gravity is replaced by a controllable centrifugal force: the effect of which can be more than 10,000 times greater than gravity on solids suspended in liquids. When subject to such forces, the denser solid particles are pressed outwards against the rotating bowl wall, while the less dense liquid phases form concentric inner layers.

The oily must obtained from many extraction systems is fed, with the addition of a small amount of water (25% - 35% of the oily must) to the disc stack centrifuge in which pure oil and separated water are obtained. Solids are discharged from the disc centrifuge

periodically.

Main Manufactures of Olive Oil Extraction Plants

Currently, main manufactures of olive oil extraction plants are (in alphabetical order):

1. Alpha Laval <http://www.alfalaval.com>
2. Amenduni <http://www.amenduni.com>
3. Flottweg GmbH <http://www.flottweg.com>

TABLE 9.2

Quality characteristics of oils obtained by pressing, percolation and three-phase centrifugation. Source: Di Giovacchino, 1996.

Determinations	System	Average	Minimum	Maximum
Acidity, %	Pressing	0.23 a	0.18	0.28
	Percolation	0.23 a	0.20	0.27
	Centrifugation	0.22 a	0.16	0.28
Peroxide value, meq.O ₂ /kg	Pressing	4.0 a	2.8	5.5
	Percolation	4.6 a	3.9	5.3
	Centrifugation	4.9 a	4.0	6.3
Total polyphenols, gallic acid mg/l	Pressing	158 a	111	197
	Percolation	157 a	103	185
	Centrifugation	121 b	87	158
o-diphenols, caffeic acid mg/l	Pressing	100 a	66	154
	Percolation	99 a	62	149
	Centrifugation	61 b	32	92
Induction time, hr	Pressing	11.7 a	8.7	16.6
	Percolation	11.2 a	8.9	15.0
	Centrifugation	8.9 b	7.4	10.9
Chlorophyll pigments, ppm	Pressing	5.0 a	3.2	8.1
	Percolation	8.9 b	6.1	18.5
	Centrifugation	9.1 b	6.5	13.7
K ₂₃₂	Pressing	1.93 a	1.82	2.11
	Percolation	2.03 a	1.89	2.27
	Centrifugation	2.01 a	1.90	2.16
K ₂₇₀	Pressing	0.120 a	0.110	0.132
	Percolation	0.124 a	0.110	0.132
	Centrifugation	0.127 a	0.090	0.153
Organoleptic rating	Pressing	6.9 a	6.2	7.4
	Percolation	7.0 a	6.7	7.4
	Centrifugation	7.0 a	6.7	7.2

Different letters indicate significant differences at P < 0.05

4. Gea Westfalia Separator <http://www.westfalia-separator.com>
5. Hiller GmbH <http://www.hillerzentri.de>
6. Pieralisi Group <http://www.pieralisi.com>
7. Rapanelli Fioravante spa <http://www.rapanelli.com>, <http://www.sinolea.net>

Quality Characteristics of Oils Obtained by Different Extraction Systems

The value of extra virgin olive oil, like every other product of agro-food processing, depends on the characteristics of the raw material. It is impossible to obtain an excellent product by starting with poor raw material, even if the most efficient extraction procedures are used. The cultivars and the harvest time must be selected carefully to correspond to the optimal level of fruit maturity (Amiot et al., 1986; Montedoro et al., 1989; Esti et al., 1998; Cortesi et al., 2000; Caponio et al., 2001).

The effect of the extraction process on olive oil quality is well documented (Montedoro et al., 1992; Di Giovacchino et al., 1996; Ranalli et al., 1996; Koutsaftakis et al., 1999; Cert et al., 1999; Cortesi et al., 2000; Servili et al., 2004). According to Tsimidou (1998), Good Manufacturing Practices as well as decisions about the cultivation of certain olive varieties should take into consideration the factor of “polyphenols.” Enrichment of an olive oil with phenolic components has its limitations because very high concentrations of polar phenols affect the sensory quality of the oil (see also chapter on phenols). More bitter olive oils may not be acceptable by the consumers even if they contain nutritional components or have longer shelf lives.

Unfortunately, there are different analytical methods for the estimation of the total polyphenol content varying in extraction and separation procedures as well as in the expression of results, which makes the comparison of the quantitative results obtained difficult; (Hrncirik et al, 2004). Blekas et al (2002) proposed that the colorimetric determination of total polyphenols with the Folin-Ciocalteu reagent is a good practical means to evaluate virgin olive oil stability. Sensory evaluation of virgin olive oil is directly correlated with the variety, ripeness (Aparicio et al., 1997), and extraction conditions (Morales et al., 1999). Sensory properties are largely affected by phenolic composition. In particular, these compounds were associated to the bitter and pungent sensory profile of the virgin olive oil. However, the relationships between individual hydrophilic phenols and sensory characteristics were not clearly defined (Servili et al., 2004). Recent research work by Andrewes et al (2003) and Mateos et al (2004) correlates virgin olive oil pungency and bitterness with individual phenols (see also chapter on phenols). Volatile compounds and their relationship with quality have been discussed by Aparicio and Morales (1998), Morales et al (1999) and Angerosa et al (2004).

Another aspect which has not been extensively studied is the reliability of trials. Recently, it was indicated (Stefanouadaki et al., 1999; Angerosa et al., 2000) that oils

from laboratory mills were clearly different from samples extracted industrially. This implies that results derived from investigations carried out using oils obtained from laboratory mills cannot be immediately transferred to oils from industrial plants. A comparison of the three processes for good quality olives easy in malaxing is given by Di Giovancchino et al (1996) (Table 9.2).

After the introduction of the two-phase centrifugation the only published data of commercial olive mills by two-phase, three-phase centrifugation, and pressing process are given by Salvador et al (2003) for Cornicabra virgin olive oils obtained in five crop seasons (Table 9.3).

Generally, centrifugation extraction in comparison to traditional pressing gives oils with higher chlorophyll content. The older technique to crush a small portion of olive leaves together with the olives is not recommended, because the organoleptic characteristics are altered, beside the increase of the chlorophyll pigments. In the presence of light, chlorophylls and their derivatives are the most active promoters of photosensitized oxidation (Fakourelis, et al., 1987).

TABLE 9.3

Quality indices of Cornicabra virgin olive oils from crop seasons 1994/1995 to 1998/1999 obtained by different extraction systems (n =140) Salvador et al., 2003

Quality indices	Extraction system			
	ANOVA F-ratio	C2	C3	P
Number of samples ^a		68	63	9
Free fatty acid (% oleic)	1.8	0.58 a	0.58 a	0.86 a
Peroxide value (meq/kg)	0.7	10.2 a	9.4 a	11.1 a
K ₂₃₂	0.1	1.619 a	1.616 a	1.653 a
K ₂₇₀	1.0	0.139 a	0.132 a	0.140 a
Oxidative stability (h)	3.6*	65.8 b	57.2 a	46.3 a
Total phenols (mg/kg)	4.8**	160 b	142 b	100 a
ortho-diphenols (mg/kg)	3.1	9.2 a	6.9 a	-
α-Tocopherol (mg/kg)	8.3***	178 c	160 b	134 a
Chlorophylls (mg/kg)	5.7	11.4 b	8.6 a	11.4 a, b
Carotenoids (mg/kg)	4.1	7.6 b	6.5 a	6.8 a, b
Intensity of bitterness	6.5**	2.0 b	1.6 a	-
Overall quality index	1.7	6.4 a	6.5 a	6.1 a

Dual phase (C2), triple-phase (C3) decanter centrifugation and pressure system (P).

^a By crop season. C2: 12 samples from crop 1994/1995; 9, 1995/1996; 9, 1996/1997; 17, 1997/1998; 21, 1998/1999. C3: 11, 1994/1995; 6, 1995/1996; 9, 1996/1997; 19, 1997/1998; 18, 1998/1999. P: 4, 1994/1995; 2, 1995/1996; 3, 1996/1997.

* P ≤ 0.05 (95%).

** P ≤ 0.01 (99%).

*** P ≤ 0.001 (99.9%).

Values with different letters are statistically different (P < 0.05).

Other results obtained from more recent research work indicate that: Virgin olive oils from percolation (first extraction) compared with oils from centrifugation (second extraction) (Ranalli et al., 1999) are characterized by (i) higher contents of total phenols, o-diphenols, hydroxytyrosol, tyrosol-aglycons, tocopherols, trans-2-hexenal, total volatiles, and waxes; (ii) higher resistance to autoxidation and turbidity; (iii) higher sensory scores; (iv) higher ratios of campesterol/stigmasterol, trans-2-hexenal/hexenal, and trans-2-hexenal/total volatiles; (v) lower contents of chlorophylls, pheophytins, sterols, and aliphatic and triterpene alcohols; (vi) lower alcoholic index and color indices; (vii) similar values of acidity, peroxide index, and UV (ultraviolet) spectrophotometric indices; (viii) similar percentages of saturated and unsaturated fatty acids, triglycerides, and diglycerides; and (ix) similar values of glyceridic indices. Stigmastadienes, trans-oleic, trans-linoleic, and trans-linolenic acid isomers were not detected in the two genuine oil kinds. Based on these parameters Ranalli concluded that the first extraction method (percolation) provides oil superior in quality.

Lercker et al (1999) found that after crushing Italian olives the volatile fraction contained approximately 20% trans-2-hexenal and after 70 min of kneading, the percentage was increased to 50%. Hexenal content also increased, but its level remained significantly lower than that of *trans* 2-hexenal. When kneading was over, a different tendency was observed—an increase in hexenal and a decrease in *trans* 2-hexenal. The authors concluded that strong enzyme activity and extended kneading periods generate desirable aroma compounds at the expense of stability through loss of antioxidants.

Morales et al (1999) studied the conditions of extraction and showed that a temperature of 25°C and a malaxing time of 30 – 45 min produce volatiles contributing to the best sensory quality. Higher temperatures (>35°C) with minimum malaxing time (<30 min) produce oils with pleasant green notes.

In another study, Ranalli et al (2000) compared oils extracted from olive pastes by the direct centrifugation mode with the oils produced by the indirect centrifugation (after percolation) mode. The former were characterized by (i) higher contents of total phenols, o-diphenols, hydroxytyrosol, tyrosol-aglycons, total volatiles, trans-2-hexenal and other pleasant volatiles, total tocopherols, total sterols, and waxes; (ii) lower contents of triterpene dialcohols, aliphatic and triterpene alcohols, chlorophylls, and pheophytins; (iii) lower values of color index; (iv) higher values of turbidity, campesterols/stigmasterol ratio, 1,2-diglycerides/1,3-diglycerides ratio, oxidative stability, and overall quality indices; and (v) higher sensory score. Stigmastadienes and trans-isomers of C₁₈ fatty acids were not always detected. The average oil outputs of the two centrifugation extraction procedures were comparable, as confirmed by similar overall oil amounts found in the by-products.

Garcia et al (2001) evaluated simple and complex olive oil phenols in the streams generated in the two-phase extraction system using *Arbequina* and *Picual* cultivars. The malaxation stage reduced the concentration of ortho-diphenols

in the oil ca. 50-70%, while the concentration of monortho-diphenols remained constant, particularly the lignans: 1-acetoxypino-resinol and pinoresinol. Malaxation of the paste under nitrogen atmosphere resulted in reduced oxidation. Phenolic compounds in the wash water from the vertical centrifuge were also identified. 3,4-DHPEA (hydroxytyrosol), p-HPEA (tyrosol) and 3,4-DHPEA-EDA were the most representative phenols in these waters.

Angerosa et al (2001) came to the conclusion that malaxing temperature was mainly responsible for (i) the sensory flattening of oils, (ii) very considerable losses of secoiridoid compounds, (iii) the marked decrease of concentration of C₆ esters, very important contributors of delicate green perceptions, and of cis-3-hexen-1-ol which gives pleasant green sensations, (iv) the increase of hexan-1-ol and trans-2-hexen-1-ol, elicitors of less attractive perceptions.

Amirante et al (2001) concluded that the time and the temperature of malaxing, as well as olive paste dilution, greatly affect both oil quality and extraction yields. In particular, under some of the most extreme conditions involving, for instance, 19% and/or 41% water additions to the olive paste, the extraction yields decreased, on average, by about 2 kg/100kg of processed olives, as compared to the yields obtainable with 32% dilutions. Moreover, by malaxing at 35°C, the phenolic compound content and resistance to oxidation decreased by about 30%, on average, as compared with malaxing performed at 27°C.

Ranalli et al (2001) compared oils extracted by two-phase centrifugation, after stone mill crushing, with the traditional stone mill-press line. With olives easy to process, the two-phase centrifugation oils showed higher contents of pleasant volatiles and tocopherols, lower contents of unpleasant volatiles, and comparable contents of phenols and lipochromes.

Gimeno et al (2002) studied the effects of harvesting and two processing systems (two-phase centrifugation and three-phase centrifugation) on olive oil quality. Oils extracted from high quality olives do not differ in free acidity, peroxide value, and ultraviolet light absorption. Nor was the fatty acid composition affected. However, the antioxidant content of the oil was higher from green olives than from ripe olives. Neither method affected α -tocopherol and β -carotene levels but the phenolic content was higher in the two-phase method. This is due to the addition of lukewarm water used to dilute the olive paste.

Koutsaftakis et al (2002) compared oils extracted by two-phase centrifugation, after crushing the olives with a hammer crusher equipped with an inverter for the adjustment of its rotating speed and with a toothed disc crusher. Comparative trials were carried out using 5 mm and 6 mm hole gratings. Oils obtained with hammer crusher operating at 3000 and 2500 rpm showed significantly higher values of total phenols and stability measured by the induction period. Concentration of total phenols and resistance to oxidation, were found to be proportional to the velocity of the hammer crusher. Additionally, the oils obtained using a 6 mm screen size presented

higher values of total phenols. No marked differences were monitored in the rest qualitative parameters caused either by the velocity of the hammer crusher or the sieve size. These data confirm the results presented by Cortesi et al (2000).

Servili et al. (2002) studied the effect of a blade crusher in comparison with the traditional hammer crusher on the quality of virgin olive oil from two different Italian cultivars. Results obtained showed that quality parameters i.e. free fatty acids, peroxide value, UV absorption, and total phenol content as well as the phenolic composition of oils were not significantly affected by the two different crushers used. On the contrary, the use of the blade crusher influenced the composition of the volatile compounds.

Phenols present in olive paste are soluble in water and oil, depending on their partition coefficients (K_p) and temperature. Addition of water to the paste alters the partition equilibrium between aqueous and oil phases and causes a reduction of phenolic concentration through dilution of the aqueous phase. A coincident lower concentration of these substances occurs in the oil phase. In fact, a large amount of the antioxidants is lost with the wastewater during processing or remain in the pomace (Mulinacci et al., 2001; Lesage-Meessen et al., 2001).

According to Rodis et al (2002), the partition coefficient (K_p) was calculated by dividing the equilibrium concentration of the phenolic antioxidant compounds in the oil and water phases. This coefficient was estimated to be from as low as 0.0006 for oleuropein to a maximum of 1.5 for 3,4-DHPEA-EA (3,4-dihydroxyphenylethanol-elenolic acid). Because the K_p values were very low, some changes in the process were introduced in order to achieve a higher concentration of antioxidants in the oil. A temperature increase could lead to increasing the partition coefficient. Limiting the quantity of water during oil extraction was the basis for designing alternative processes for increasing the antioxidant concentration in the olive oil.

Luaces et al (2005) investigated the effect of heat treatment of olives on olive oil pigment composition and bitterness. Results obtained indicate that a decrease of bitterness intensity of virgin olive oil obtained after water-heat treatment of olive fruit (60-68°C) for 3 minutes prior to oil extraction was most effective, but there is an increase of chlorophyll compounds as well as lutein and β -carotene.

According to EC regulation 1019/2002: The indication “first cold pressing” may appear only for virgin or extra virgin olive oils obtained at a temperature below 27°C from a first mechanical pressing of the olive paste by a traditional extraction system using hydraulic presses. The indication “cold extraction” may appear only for virgin or extra virgin olive oils obtained at a temperature below 27°C by percolation or centrifugation of the olive paste.

The main advantages and disadvantages of the recent in use olive oil extraction plants appear in [Table 9.4](#).

Coadjuvants Used for Olive Oil Extraction

Irrespective of the extraction method involved, the yields obtained from “difficult” olives are lower (less than 80%) than those normally obtained from “easy” olives (more than 85%).

In the difficult olive pastes the oil is trapped in the colloidal tissues of cytoplasm or emulsified with vegetation water, the so-called “not free” olive oil. The first coadjuvants were lyophilized enzymes having a pectolytic and cellulolytic action. At present, liquid enzymatic products having more specific pectolytic activity are preferred. However, the use of coadjuvants is not in accordance with the legal definition of virgin olive oil and the oil must be obtained from the fruits solely by mechanical or other physical means.

An enzymatic complex produced by a fungus, *Aspergillus aculeatus*, primarily breaks down pectins, celluloses, and hemicelluloses. It is added to the olive paste in the crushing or malaxing step (20 mL per 100kg of olives). It degrades membranes of non-crushed oily cells, permitting the recovery of the oil even when the vegetal colloidal structures are degraded. These structures bind the oil drops, which are thus liberated. A mass of free oil is then formed, which is easily extractable or recoverable by mechanical equipment. The complex brakes oil-water emulsions, and induces positive effects on the rheological characteristics of the pastes. These latter are better centrifuged with a more efficient separation of the phases (oil, water, and husk).

Ranalli and Serraiocco (1996) showed that enzymatic complexes increased the extraction yields more by using the percolation-centrifugation system and less by using pressing. Qualitative characteristics of the oils obtained with the use of enzymes were found to be only slightly superior.

Vierhuis et al (2001) used another enzyme complex (pectolytic enzyme from *Aspergillus niger*) to study the effect of the use of cell-wall-degrading-enzyme prepara-

TABLE 9.4
Comparison between extraction systems in use

	Hydraulic Presses Extraction	Centrifugation 3 Phase	Centrifugation 2 Phase	Percolation Centrifugation 3 Phase
Process	Discontinuous	Continuous	Continuous	Continuous
Capacity	Small	Medium High	Medium High	Medium High
Labor Cost	High	Low	Low	Low
Energy Consumption	Low	High	High	High
Water Consumption	Low	High	Low	High
Processing Yield (%)	86-90	85-89	85-89	85-89
Pomace Moisture (%)	28 ± 4	48 ± 4	60 ± 5	48 ± 4
OMWW Quantity	Low	High	Low	High
Polyphenol Content	High	Low	High	High (Sinolea oil)
Contamination Risk	High	Low	Low	Low

tions during extraction of virgin olive oil. The use of nitrogen flush during processing was investigated. The content of secoiridoid derivatives (3,4-DHPEA-EDA) and (3,4-DHPEA-EA) increased significantly in the olive oil obtained in this manner. Ranalli et al (1999) tested a complex formulation containing pectinase, cellulolytic, and hemicellulolytic enzymes, using the percolation-centrifugation system. This enzyme aid produced better quality oils and improved yield. Di Giovacchino et al. (1990) used a plant fiber extraction aid derived from the trunk of the alder (*Alnus rubra*). The fiber is very hydrophilic (ability to absorb water: 1100 – 1300 %), thus improving percolation and pressing extraction rates when “difficult” olives are being worked with.

Specific research has also been conducted into other types of aids such as microtalc, which has lipophilic properties and its positive action is due to the reduction of the oil / water emulsion and to the consequent increase of free oil (Di Giovacchino et al., 1991; Cert et al., 1996). Due to its lipophilic properties, the talc does not raise percolation extraction yields to any substantial extent, but it has a positive effect in reducing the emulsion in the paste, thus increasing the amount of oil released. An additional feature of talc, approximately 95% of which is dragged along with the paste, is that it raises the oil content of the pomace and reduces that of the vegetable water. The use of microtalc does not affect the sensory quality and chemical composition of the saponifiable and unsaponifiable fractions of virgin olive oil.

Improvements and Innovations

The new technological concept in olive oil production is to raise the quality of oil that is extracted by reducing triglyceride deterioration and by preserving the level of minor components to improve its oxidative stability and nutritional properties of the oil.

Recent research has focused on the rheological characteristics of olive pastes and most recently extraction from de-stoned olives, on controlled malaxing in an inert-gas atmosphere and on the new, short-coned, water-saving decanter centrifuges.

Destoning

In the last twenty years a number of authors have examined the possibility of the large-scale production of virgin olive oil from de-stoned olives. Some of the findings of this research show that de-stoning lowers oil yield by no more than 1.5%. Lately, the olive machinery manufacturing industry has brought out destoners in an attempt to eliminate the temperature-raising effect of hammer crushing. The substitution of a hammer or disc crusher by a destoner coupled with a finisher should, in addition, allow the plant working capacity to be increased and the energy consumption to be decreased. According to Angerosa et al (1999) the concentrations of volatile compounds and in particular of hexanal increased in oils from de-stoned fruits obtained from a laboratory mill. Hexanal, reminiscent of green apple or green fruit odor notes (Aparicio et al., 1998), makes a great contribution to the olive oil flavor because of its

low odor threshold.

When the stones are removed without crushing, the olive paste contains less solid particulates. The liquid phase accounts for 75-78% of this paste versus 70% of conventional mash. Data provided by Amirante et al (2002) indicate that the paste is less viscous (Table 9.5), although, when handled more gently, the solid and liquid phases in the flesh are more closely bound.

Conventional olive pastes containing stone fragments have higher viscosity than de-stoned pastes (Table 9.6) which is also lowered considerably by malaxing and thinning of the paste by adding warm water and to improve centrifugal separation efficiency.

According to Amirante et al (2002), the oils extracted from de-stoned olive paste have a higher total phenol content than those produced from hammer-crushed paste, although the oxidative stability was more or less identical. This phenomenon has been confirmed in subsequent tests and might be explained by the fact that the oils obtained from de-stoned paste emulsify to a greater extent.

Patumi et al (2003) having studied the effect of fruit stoning on olive oil quality concluded that the main organoleptic and physicochemical parameters, as well as oxidative stability showed no obvious influence of stoning on olive oil quality. Chemometric analysis of the data showed that the oils can be better grouped according to cultivar than to technology. Lipoxygenase activity in the paste from whole and stoned olives showed no effect that could be attributed to the technology. Furthermore, the stone did not contribute significantly to increasing the lipoxygenase activity in the olive paste. It is concluded that it is harder to separate the liquid phases from the solid

TABLE 9.5
Variation, under different conditions, in the apparent viscosity (centipoise) of de-stoned olive paste. Source: Amirante et al, 2002,

Conditions	27 °C	27 °C	35 °C	35 °C
	no dilution	30% dilution	no dilution	30% dilution
After crushing	18,050	7,400	16,350	6,750
After malaxation	16,550	6,200	14,350	5,200

TABLE 9.6
Variation, under different conditions, in the apparent viscosity (centipoise) of conventional olive paste containing stone fragments. Source: Amirante et al, 2002

Conditions	27 °C	27 °C	35 °C	35 °C
	no dilution	30% dilution	no dilution	30% dilution
After crushing	51,500	36,050	45,350	15,500
After malaxation	40,650	22,950	23,400	11,800

phase due to the poor drainage caused by the absence of stone fragments.

Lavelli and Bondesan (2005) studied the secoiridoids, tocopherols, and antioxidant activity of monovarietal extra virgin olive oils from destoned fruits. The results showed that the oils obtained from both stoned and destoned olives had a very low degradation level, which was not affected by destoning. Destoning slightly lowered the α -tocopherol content, but increased the total secoiridoid content and the antioxidant activity of the oils. However, these effects were mainly variety dependent.

Particle Size and Decanters

For many years, olive pastes undergoing centrifugal extraction had to be quite fluid to facilitate separation of the fractions. This was done by adding warm water equivalent to approximately 40-60% of the weight of the olive fruits.

It is well known that oils produced in a conventional three-phase continuous-flow facility have the lowest content of polyphenols due the dilution of the paste, while those produced in two-phase decanters have the highest. In addition, no particular crushing method can be claimed to be superior to the others since optimal results are obtained when crushing is combined properly with the right malaxing times and temperatures. Nevertheless, more vigorous crushing is recommended for varieties with a low content of minor components.

Merely crushing the olives is not enough for subsequent centrifugal separation because unwanted emulsions may form or it may not be possible to separate the three phases: oil, vegetable water, and solid particulates.

After recent theoretical studies by Amirante et al., (1995) and Catalano et al. (2003), it was observed that decanters could operate optimally when centrifuging low-dilution pastes (10-20%), if the differential scroll/bowl speed was adjusted. The theoretical and test results showed that the particle size of the olive paste produced by present-day crushers was such that, when centrifuged with no or very little water, approximately 75% of the solid particles were pushed close to the decanter discharge outlet. This prompted some major thinking on how to adjust the decanter to improve its quantitative and qualitative performance. As a result of the data obtained from the research, barriers (weirs) were positioned close to the decanter discharge outlet, variable-speed scrolls were used and improvements were made to the drainage section in order to enhance the separation of the liquid phases from the solids.

Enzymes

Another objective of recent research was to further study ways to inhibit polyphenoloxidase and peroxidase activity during malaxation of the pastes by flushing with nitrogen. The time of exposure of olive pastes to air was studied by Servili et al (2004) as a processing parameter to regulate the averaged concentration of oxygen in the paste and consequently the phenolic amount in the oil.

Future Trends

The next stage of research will entail malaxing destoned paste in an inert-gas atmosphere in order to examine the possibility of extracting better minor components, even with longer mixing times, but without causing oil oxidation.

Results obtained so far confirm that the oils obtained from destoned olive mash in an inert-gas atmosphere are superior in quality to those obtained from conventional mash although the oil yields are slightly lower. It is clear from the above that all innovations proposed are based on fundamental physical and chemical principles. As Professor E. Fedeli said in 1992 at the Olive Oil Symposium in Toronto (Fedeli 1993): “what technology tries to do is to preserve nature’s work.”

Good Manufacturing Practice (GMP) Guidelines for Virgin Olive Oil Production

Quality and origin certificates should be based on quality assurance systems. These entail process control. An effective process control is based on standardized operating conditions and procedures.

The GMP represents a set of minimum standard conditions that should be followed throughout the whole production chain, from cultural operations in the olive grove to the shelves of the supermarket (Petrakis, 1994, Aparicio et al., 1994). In addition, Hazard Analysis Critical Point Control (HACCP) is necessary for the consumer’s protection.

Agricultural Aspects

Pest monitoring and control is a critical phase of olive production for both olive quality and safety. For extra virgin olive oil, less than 3% infestation rate of olive fruits is obligatory. The olives should be harvesting at their optimal ripeness grade. New analyzers working on the principle of NIR or NMR for the quick estimation of the oil content in the olives, instead of the time consuming old system of SOXHLET extraction, facilitate this work (Garcia Sanchez et al., 2005). Harvesting should be carried out manually or by mechanical means.

Precautions should be taken to avoid fruit breakage through mechanical damage

and fruit contamination by soil material. Spontaneous fruit drop, resulting from over-ripeness, damage by parasites, or atmospheric agents, should be avoided as much as possible.

Olives should be harvested on nets lifted up from the ground or on other similar materials, so that olives avoid contact with the soil. Such direct contact of olives with the soil should be avoided and the thickness of the layers should not exceed 50 cm.

Olive Transportation and Storage

Olive transportation and storage should be considered as critical phases for controlling both mechanical damage and temperature. Improper handling during these phases can result in undesirable enzymatic reactions and the growth of yeasts and molds. Olives should be processed as soon as possible after harvesting. The best way to transport the olives is in open-mesh, plastic crates that allow air to circulate and prevent harmful heating caused by the catabolic activity of the fruit. Olives should be kept in well ventilated places, at temperatures below 25°C and relative humidities below 75%, until processing.

Olive Oil Extraction

The critical factors of the extraction process for oil quality are time-temperature relationship and contamination of the oil by accidental contaminants and processing aid.

Defoliation and Olive Washing. Leaves and small branches that are normally collected with olives during harvesting should be removed (Di Giovacchino et al, 2002). The weight of these materials should not exceed 1%. The water used for washing must be of potable quality. This water is recycled after decanting and clean water must be constantly added.

Milling, Malaxing, Extraction, and Separation. It is advisable to optimize the crushing systems to avoid rough pastes and to choose low temperatures in the malaxing to avoid oxidation processes (Ranalli et al., 2001). More vigorous crushing is recommended for varieties with a lower content of phenolic compounds. The temperature of heating water in the jacket of malaxing machines should never exceed 45°C.

The temperature of olive paste and oil should never exceed 27°C for first cold pressing or cold extraction extra virgin or virgin olive oil. The process water must be of potable quality. Chlorination of the water must be avoided, because the trihalomethanes formed are liposoluble and will be trapped by the oil (Mariani *et al*, 1990). The temperature of water for the dilution of the paste or in the clarifier must be 28-35°C. We should never dilute the paste with a high quantity of water or use a high quantity of water in the clarification of the must. Recycled vegetable water, freshly separated

from good quality olives by centrifugation, is permitted as a process water. The malaxing time depends on olive variety and maturity. Normally a 60 minute malaxing time is enough and in any case it should never exceed 90 min.

Olive Oil Storage

During this phase the following two factors are to be controlled: temperature and contact of the oil with traces of water, solid residues, or colloidal impurities, in order to avoid degradative (lipolytic or oxidative) reactions. During storage, oil should be kept in the dark, at temperatures lower than 25°C, in completely filled inox tanks. Within three months of production, oil should be separated from solid impurities and water by decantation or filtration and transferred into clean, dry containers. For prolonged storage, it is recommended to flash the empty space of the tank with nitrogen. Loading and unloading of the tanks must be from the bottom in order to minimize the contact of the oil with the air.

Materials and Buildings

All the materials that may come into contact with olive paste or oil should be food grade. Special care should be taken for the cleaning of the mats used in the traditional hydraulic pressing system. The following areas in the olive oil extraction factory should be separated: olive reception, extraction plant, oil storage tanks, bathrooms, dressing rooms, and other rest rooms for workers. By-product storage, boiler, and laboratories should be clearly separated from the extraction plant. In all processing and storage rooms, walls should be painted with washable, antimold, non-porous coatings. Floors should be made with washable, non-slippery, non-porous, and inert materials. Special care should be taken in the cleaning and hygiene of draining pads. Special care should be taken to separate the oil factory from any sources of aerosol and smoke contamination. Halon fire extinguishers should be avoided.

Contamination by volatile solvents used in laboratories should be avoided.

Attention should be paid to avoid oil contamination by lubricants used in equipment transmissions and gears as well as by water condensate drips.

Extraction of Crude Olive Pomace Oil and Refining

Olive pomace is a valuable byproduct from olive oil extraction contrary to olive mill wastewater, which is an unwanted industrial effluent (high organic load, with COD up to 220 g/L) (Fiorentino et al., 2003). Olive pomace obtained from traditional pressing and three phase decanter centrifugation is a source of income for the oil mills because they sell it to industrial factories where the residual oil (crude olive pomace oil) is extracted by hexane.

Olive pomace should be delivered immediately to the extraction factory for dry-

ing in order to stop fermentation and preserve the oil from hydrolysis and enzymatic deterioration. Pomace must be dried to 7-8% moisture content to obtain optimum extraction yield. The driers consist of slowly rotating (3-5 rpm) large cylinders with specific blades inside for mixing. An inclination of about 3% is enough for the exit of dry pomace. A large mass of hot air ($450\pm 50^{\circ}\text{C}$) blows by the aid of a ventilator. The hot air blows through the drier from a furnace which burns exhausted olive pomace. At the exit of the ventilator the temperature of the humid air is $95\pm 5^{\circ}\text{C}$. Rotary dryers operate in parallel streams, i.e. the pomace and the hot air advance in the same direction. The aim of the parallel steam circulation is to prevent the low moisture pomace from lying in a high temperature zone of the dryer.

These dryers are hardly ever built for capacities of more than 200 tons per 24 hours.

When the amount of pomace to be dried exceeds this tonnage, two to four rotary driers are installed. For the evaporation of 1000 kg of water with these rotary driers, 300 kg of exhausted olive pomace is consumed. This explains why factories are not interested very much in the two-phase decanter pomace.

The dried pomace is then ground and finally extracted with the aid of hexane.

The batch extractors are arranged in a battery form (5-8 according to the capacity of the extraction plant). The hexane passes from one extractor to the next. The solvent/oil mixture, named miscella, is continuously collected. Once the oil in one extractor has been recovered, steam is injected to eliminate the hexane residue.

Continuous high capacity percolation extractors are also used. Hexane or miscella is pumped over a bed of dry pomace and percolates down through the bed. The miscella obtained is distilled to recover the hexane and finally the crude pomace oil remains.

Normally, 8% crude pomace oil is extracted from the dried pomace and in the exhausted olive pomace the remaining oil is less than 1% oil. By mechanical sieving, the exhausted olive pomace may be further separated into pulp and the rest. Pulp is used as an animal feed and the rest is used as a fuel. The crude pomace oil obtained is a high acidity (more than 3% as oleic acid), dark green oil (high chlorophyll content), with a high waxes content and with unpleasant odor and taste. Therefore, it needs to undergo a refining procedure to become edible. The crude pomace oil refining steps are: degumming, alkali neutralization, bleaching, deodorization, and winterization.

Degumming is achieved by hydrating the gums using 0.1% concentrated phosphoric acid (at $60\text{--}80^{\circ}\text{C}$) to make them insoluble in oil. They can be separated from the oil phase by decantation in the batch systems or continuous centrifugation. Alkali neutralization is carried out with NaOH solutions (20-22 Degrees Baumé) and removes nearly all free-fatty acid by converting them into oil-insoluble soaps. Furthermore, pigments and metals contributing to lipid oxidation reactions are partially removed. The oil-insoluble soaps are separated by decantation in the batch neutralizers. In the continuous plants, degumming and alkali neutralization go in series and the

separation is taking place by the aid of centrifugation.

The principal goal of the bleaching step is to remove the unpleasant colors of the alkali neutralized pomace oil. The method is based on the heat/oxidation/adsorption of the pigments to colorless or slightly colored materials, thus providing a product of pleasant color. The bleaching step is carried out with 2-3% acid-activated earth. The oil is stirred with the bleaching earth for 30 min under a vacuum (30-40 mmHg) after heating of the oil at 90-100°C. It is necessary to use in conjunction 0.5-1% activated charcoal to ameliorate the color and to trap PAHs (Cert et al., 2000). The bleached oil is removed from the activated earth and charcoal by filtration.

Deodorization is essentially a steam distillation at reduced pressures (4-8 mmHg) and elevated temperatures (180-200°C). Steam is sparged into the oil to remove traces of volatile components, including flavor and odor compounds (mostly aldehydes and ketones), pesticides, free fatty acids, etc. Usually, the deodorization time in batch systems ranges from a minimum of 5-8 hours, whereas in the continuous plants this time is kept within the range of 2-3 hours, depending on the amount of the stripping steam used. Citric acid at about 0.01% should be added during the cooling stage to chelate metals and increase stability.

Winterization is applied to pomace oil particularly rich in waxes in order to avoid precipitation or cloudiness during storage. It is achieved by slowly cooling the oil and holding for a specific period of time in the crystallizers. Solids formed are separated by filtration. In the batch systems the winterization takes place after the deodorization. In the continuous systems it takes place after the alkali neutralization and the separation of the waxes by the aid of centrifugal clarifiers.

Physical refining may also be carried out by deodorizing at higher temperature (240°C) and under reduced pressures (< 2 mmHg), but it is not recommended for pomace olive oil because interesterification reactions are promoted. Attempts are now being made to deodorize olive pomace oil using nitrogen as stripping gas. The two phase decanter pomace has to be managed properly to minimize its environmental impact due to its high humidity content. Nowadays, composting methods are used (Cegarra et al., 2004) for applications in agriculture (organic fertilizers, soil conditioners).

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10

Treatments and Modifications

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Introduction

Like most vegetable oils, non-edible forms of olive oil are neutralized, bleached, and deodorized to obtain a bland fatty material which is usually blended with natural oil. The industrial process of refining should be considered as a means to restore a defective but still valuable product. Lampante oils usually have market prices higher than those of seed oils.

Factors such as acidity, peroxide value, and flavor score determine whether an oil is suitable for consumption or has to be refined. Each processing step has specific functions for removing certain major or minor constituents. Alkali refining removes free fatty acid, phospholipids, and pigments. In the presence of water, mucilage and resinous substances become insoluble and separable. Thus, the two treatments, neutralization and removal of mucilaginous substances, can take place at the same time. The elimination of mucilage is important because such substances reduce the capacity of activated earths and carbon used for bleaching. Bleaching reduces chlorophylls, carotenoids, and residual fatty acid salts. Deodorization removes volatiles, oxidation products, carotenoids, free fatty acids, pesticide residues and part of sterols, tocopherols, and hydrocarbons. Refining also destroys peroxides and thus the stability of the oil is increased. If the oil is winterized, waxes are removed. This additional step is necessary for olive oil-residue oil.

An ideal refining process aims to keep unchanged the structure of triacylglycerols and minimize configuration changes of fatty acids as well as losses of valuable constituents such as tocopherols. However, such losses are inevitable; therefore, the addition of alpha-tocopherol at a maximum level of 200 mg/kg to refined olive oil and olive-pomace oil is permitted by the International Olive Oil Council (COI,2003) and the Codex Alimentarius to restore natural tocopherols lost in the refining process.

The first step of refining is neutralization of free fatty acids. Low acidity oils are easily treated by sodium hydroxide solution. Neutralization of high acidity oils, especially husk oil, is more difficult. Oils neutralized by alkali are subjected to bleaching

by earths and, if necessary, by activated carbon. Synthetic silicas can be used in combination with bleaching earths.

If free acids are removed by physical refining, the oil needs a prerefining process. It is first degummed and bleached and then deacidified by deodorization. Free fatty acids do not disturb the activity of decolorizing earths. Physical refining takes place in a stainless steel deodorizer where there is a vacuum of 0.1 mm of residual pressure, the temperature is approximately 230°C and there is a flow of direct steam. When the process is over, the oil has practically zero acidity and peroxide value.

Refining with Supercritical Carbon Dioxide.

This technique was tested by Bondioli and his co-workers (1992). It is an alternative for the preparation of refined oil without the negative characteristics of the conventional methods. The production cost of the method is rather high and its practical feasibility questionable.

Winterization

Neutralized olive residue oils are usually winterized to remove waxes and high melting point triacylglycerols. This treatment significantly improves the resistance to clouding and sedimentation. The oil is kept at crystallization tanks at a low temperature for 24-36 hours. Then the oil is filtered. The residual solid fraction contains waxes and should be used only for industrial purposes. Olive oils winterized in solvent usually have lower percentages of unsaponifiable matter compared to the starting material.

Mild Purification

Various attempts have been made to obtain olive oil without resorting to the complete refining process to remove undesirable acidity and flavoring compounds and to spare the valuable minor components. The practical results of such attempts are not well known, because they are either recent laboratory scale experimental approaches or patents. It must also be stressed that the products of such methods should not be used to adulterate virgin olive oil, which is protected by strict standards and regulations (set by the International Olive Oil Council, The European Commission and other bodies. See [Chapter 7](#) Analysis and Authentication).

In a recent report Hafidi et al. (2005) proposed a soft purification of lampante oils based on a new deacidification method, a combination of sodium hydroxide neutralization and membrane microfiltration. Van Buuren et al. (2005) patented a method for the manufacture of a spread with a high content of olive oil polyphenols and no adverse flavor. The deodorized oil used is obtained by a “mild” refining process which removes only a small portion of the minor constituents. The whole procedure is based on sparging with an inert gas, which removes fatty acids and offensive odor-

ous substances.

Modifications

Hardening

Olive oil is too valuable to be hydrogenated since even lampante oils are usually more expensive than seed oils. It appears that only small quantities of non-edible olive oils after refining were hydrogenated in areas where there is a surplus of raw material.

In order to obtain a product suitable for the preparation of cooking fats or margarines, olive oil has to be hydrogenated under conditions which favor isomerization. The finished products have a low percentage of dienoic fatty acids and a relatively high level of geometrical and positional isomers (Boskou and Karapostolakis, 1983, Boskou and Chryssafidis, 1986).

Interesterification and Glycerolysis

Olive oil has been co-randomized with highly hydrogenated seed oils on a pilot-plant scale. From the interesterification product, a cocoa butter-like fat was obtained by fractional crystallization (Landmann et al., 1961).

Intesterification of refined olive oil-tristearin blends would give zero *trans* plastic fats with a higher percentage of polyunsaturated fatty acids than hydrogenated olive oil. Gavriilidou and Boskou (1989, 1991) interesterified, in a laboratory scale, refined olive oil-glycerol blends using sodium methoxide as a catalyst. The rearranged fats were found to have properties very close to those of soft tube packed margarines. The interesterification induced changes in olive oil and partially hydrogenated palm oil blends were described by Alpalsan and Karaali (1998). A 30:70 olive oil-hydrogenated palm oil mixture after enzymic interesterification had properties similar to those of package margarines with the additional advantage of higher amounts of monounsaturated fatty acids.

Vural et al. (2004) prepared interesterified olive oil and used it as a beef fat substitute in frankfurters. The objective was to obtain a better ratio of unsaturated to saturated fatty acids.

Ferreira-Dias and collaborators (2001) used two commercially available immobilized lipases as biocatalysts for the glycerolysis of olive residue oil in hexane to produce mono- and diacylglycerols. The value added products from the residue oil (emulsifiers) could contribute to the valorization of the olive oil industry. Fomuso et al. (2001) prepared a mayonnaise and a salad dressing based on an enzymatically synthesized structured lipid from caprylic acid and olive oil. The enzyme was a *Rhizomucor miehei* lipase. Caprylic acid was used for the synthesis of a triacylglycerol mixture containing an octanoic acid at the 1,3-position and long chain fatty acids in the 2-position, which is more rapidly hydrolyzed and absorbed than typical long chain triacylglycer-

ols. The product of enzymatic transesterification was found to have similar viscoelastic properties with conventional olive oil based mayonnaise. Structured triacylglycerols containing behenic and oleic acids (low calorie structured lipids) were prepared by Tynek and Ledochowska (2005) who used enzymic interesterification of olive oil and hydrogenated rape seed oil and acidolysis of olive oil with behenic acid. Behenic acid was incorporated mainly in the sn-1,3 position of the triacylglycerol molecules, while the distribution of oleic acid at the sn-2 position varied. The structured lipids had solid fat content suitable for bakery and confectionery products.

Changes in Olive Oil Composition due to Processing

Modern analytical techniques have been extensively applied to olive oil to study quantitative changes due to alkali refining, physical refining, bleaching with activated earths, and deodorization. The formation of trace amounts of new compounds due to these treatments has been broadly used as a means to detect purity and authenticity of olive oil.

Conjugation

A usual structural modification accompanying bleaching with activated earths is the conjugation of part of the double bonds of the di- and triunsaturated acids, and the formation of dienes and trienes which absorb at 232 nm and around 270 nm.

Formation of Geometrical Isomers.

Geometrical isomers of natural 18:1, 18:2, and 18:3 fatty acids are not found in natural olive oils except in minute quantities. Decolorization causes some modification in the structure of fatty acids and may generate *trans* isomers in virgin olive oil. Higher percentages are found in esterified or refined oils, especially when the latter are deodorized at high temperatures (Mariani et al., 1991). Illicit industrial processes that

TABLE 10.1

	Sum of <i>trans</i> monoenes	Sum of <i>trans</i> dienes and trienes
Extra virgin olive oil	≤ 0.05	≤ 0.05
Virgin olive oil	≤ 0.05	≤ 0.05
Lampante	≤ 0.10	≤ 0.10
Refined olive oil	≤ 0.20	≤ 0.30
Blended olive oil (a mixture of refined and virgin)	≤ 0.20	≤ 0.30
Crude olive pomace oil	≤ 0.20	≤ 0.10
Refined olive-pomace oil	≤ 0.40	≤ 0.35
Olive pomace oil	≤ 0.40	≤ 0.35

tend to mask a seed oil (e.g. desterolization) cause some modifications in the structure of fatty acids and may generate *trans* isomers. The European Commission Regulation EC 1989/2003 sets the limits in [Table 10.1](#) as a guarantee of authenticity and good manufacturing practice.

Hydrocarbons

Squalene is the major hydrocarbon in virgin olive oil and may represent as much as 50% of the unsaponifiable matter. Processing of crude olive oil causes significant reduction of squalene due to bleaching (Mariani et al., 1992), but mainly due to deodorization (Bondioli, 1993). Squalene is recovered from the sludge of deodorization (European Union, Shared cost project FAIR-CT 95-1075, 1996-1999).

Losses of Sterols and Formation of Steroid Hydrocarbons

Sterols are lost during processing. Neutralization causes a 15% loss of total sterols, according to Morchio et al. (1987). Smaller losses accompany decolorization and deodorization: that is a total loss of approximately 15-25% for all steps of refining. Free sterols concentration is reduced to a greater extent compared to that of sterol esters (Mariani et al., 1992, Phillips, 2002). Pasqualone and Catalano (2000) studied the free x 100 / total sterols ratio in many natural and neutralized oils. They concluded that when this ratio exceeds 70%, the presence of neutralized oils in extra virgin olive oil should be excluded.

The composition of the sterol dehydration products in refined olive oil was studied by Grob and his workers, who in a series of publications (1990, 1994, 1995), attempted to solve analytical problems related to stigmastadiene, stigmastatriene, campestadiene, and campestatriene determinations and proposed methods to evaluate quality and authenticity of virgin olive oil. The presence of steroidal hydrocar-

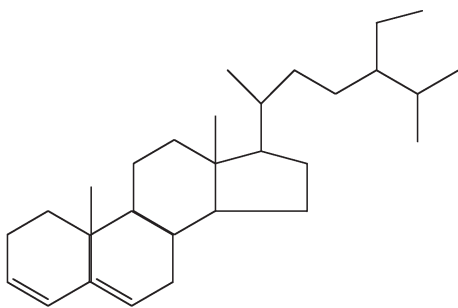


Fig. 10.1. Stigmastadiene

bons in refined oils was also studied by Bartolomeazzi et al. (2000), who proposed a mechanism for the formation of trienes from the decomposition of 5a-, 7a-, and 7b-hydroxy derivatives of phytosterols. Conditions for the analysis of steradienes by gas chromatography were studied by Dobarganes et al. (1999) who prepared the IUPAC method for the analysis of stigmastadienes. Recently, Verleyen et al. (2002) compared the methods for the quantitation of 3,5-stigmastadiene, formed from the dehydration of beta-sitosterol, by gas chromatography and high pressure liquid chromatography.

Present limits for the stigmastadiene are (IOOC Standard, COI 15/NC /Rev 1, 2003, Commission Regulation EC 1989/2003):

Virgin olive oil 0.15 mg/kg

Lampante 0.50 mg/kg

Triterpene Alcohols

In refined olive oils, either refined by alkali or by physical processes, a triterpene alcohol, 24-methyl-5a-lanosta-24-dien-3 β -ol, a 24-methylene cycloartanol isomer was determined by Lanzon et al. (1999). This triterpene alcohol is formed during refining by the opening of the 9,19-cyclo ring, the creation of a double bond in the delta-7 position and the translocation of the double bond from the 24-28 to the 24-25 position. According to the authors, it can be used for the detection of refined olive oil in virgin olive oil.

Tocopherols

Alpha-tocopherol concentration is eliminated when olive oil is processed. Greater losses are observed mainly after deodorization (Rabascal, 1987). According to IOOC standards it is permitted to restore natural tocopherols lost in the refining process by adding alpha-tocopherol to refined olive oil, refined olive-pomace oil and olive-pomace oil at a maximum level of 200 mg/kg.

Alcohols and Waxes

Alkanols, waxes, and other esters are relatively stable and they are subjected to more limited reductions during bleaching (Mariani, 1992). In contrast to other minor components, fatty alcohols concentration increases several-fold during the neutralization step. This is due to the liberation of alcohols by hydrolysis of waxes (Grob, 1990).

Triacylglycerols

Prolonged deodorization at high temperatures may cause rearrangement of fatty acids in the 1,2,3-positions of the glycerol molecules and an increase of saturated fatty acids in position 2. Such modifications should be avoided in genuine olive oil because the percentage of saturated fatty acids in position 2 is used as an index for the detection

of esterified oils (see also [Chapter 7](#), Analysis and Authentication).

Other Constituents

Various other minor constituents are drastically reduced or disappear completely in the various stages of refining. These are pigments, phospholipids, polar phenols, aroma compounds, and contaminants such as aromatic hydrocarbons and insecticide residues.

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11

Storage and Packing

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Olive oil may have to be stored for many months. If specific precautions against deterioration are not taken, this will cause an increase of acidity due to the action of lipases and the development of rancidity.

Tanks or drums for storage should be constructed of material which is impermeable to oil. The interior should be inert so that clearing can be done easily and absorption of odors or other substances (e.g. trace metals), which accelerate oxidation, is prevented. The oil should be protected from air, light, and fluctuation of temperature above 15°C. Normally the oil should be kept indoors. If, however, the tanks are stored outdoors, they should be coated with an external lining to prevent extreme changes in temperature.

Stainless steel containers are considered ideal for storing. They have a cone shaped bottom to purge sediments periodically. Nitrogen may be added to the air space. Metallic drums can have a significant negative effect on flavor quality and promote deterioration if not lined with epoxy resins. The storage of olive oil in an iron tank and a polyester-glass fiber (PGF) tank was studied by Perez-Cerezal et al. (1977). Measurements of peroxide values, spectrophotometric constants, and organoleptic evaluation after 10 months showed a significantly more rapid deterioration in the quality of oil stored in the iron container.

Packaging

Packing can be designed with the objective to obtain better oxidation stability and to ensure adequate shelf life. Three factors are important for choosing packing materials: impermeability to fat, impermeability to gases, and protection from light. Materials used for bottling and packing of olive oil are plastic, glass, (especially tinted glass), tin plates, ceramics, and plastic-coated cardboard. Tin plates are not transparent and they have excellent impermeability properties. These containers are also resistant to damages from handling and suitable for lithographic labeling. Glass is an inert material and glass bottles are resistant to gas permeation, but their protective effect against

light may vary. Consumers usually prefer transparent glass because the oil is visible, but this is not scientifically advisable since photo-oxidation takes place easily in transparent glass. Green bottles protect oil from light rays in the range 300-500nm. Big glass containers (demijohns) should be covered outside.

Polivinyllchloride (PVC) is impermeable to fats and gases, but its ability to protect from light is moderate. Other polymeric materials such as polypropylene (PP) and polyethylene (PE) have average characteristics. Polyethylene terephthalate (PET) is considered to be a better plastic material than PVC, PP, and PE because of its good barrier properties and also its mechanical qualities. The properties of various types of containers used in olive oil were studied by Gutierrez Gonzales-Quijano and Olias Jimenez (1970). They compared samples stored in tin plates, glass, PVC, and polyethylene bottles in darkness and light at 28-30°C. Spoilage times, as indicated by an increase of peroxide value above acceptable limits, were: polyethylene in light 9-20 days, in dark 120-190 days, all in other packs 225 days. Kiritsakis and Hernandez (1998) have discussed the drawbacks of plastics in relation to migration of oxygen, migration of constituents of the packaging material into the oil, as well as the absorption of the different constituents of the oil by the plastic packaging material (scalping). Mendez and Falque (2002) studied the influence of the container on the quality of commercial mixtures of refined olive mark (orujo) oils with virgin olive oil. They compared plastic containers, glass, tin plate, and carton. The evolution of peroxide values was found to be more rapid in plastic and glass containers and slower in opaque plastic, tin plate, and carton containers. In a recent report, Del Nobile and his collaborators (2003) studied the properties of traditional plastic containers and two innovative materials containing an oxygen scavenger. Their measurements showed that a slower rate of quality decline can be obtained by using an oxygen scavenger or by reducing the concentration of oxygen dissolved in the oil prior to bottling.

The shelf life of extra virgin olive oil stored for 12 months in packages with different oxygen barrier properties was studied by Gambacorta et al. (2004). Five different materials were tested: polyethyleneterephthalate (PET), PET containing 1% oxygen barrier, PET containing 3% oxygen barrier, PET coated with high barrier resin containing an oxygen scavenger, and glass (used as a control). Containers having high oxygen barrier properties, (PETC, PET) maintained the initial quality parameters practically unchanged, both at room temperature and at 37°C. High values of the (E)-2-hexenal to hexanal ratio and organoleptic examination indicated only minimum changes and absence of off-flavors.

Psomiadou and Tsimidou (2002) studied the photooxidation of virgin olive oil and the changes in pheophytin, alpha-tocopherol, squalene, and total polar phenols content. They concluded that to preserve the precious characteristics of the oil, it is necessary to change practices of bottling and use dark glass bottles or paper bags as much as possible. If transparent glass bottles are used, these should be protected from light in carton boxes. Factors influencing the shelf life of packaged olive oil were also

studied by Coutelieris and Kanavouras (2005), who used the activation energy concept to estimate reduction of quality of packaged olive oil.

If properly stored in a dark place and a temperature below 15°C, the shelf life of olive oil can be extended to almost 2 years, especially when the container remains unopened. Even 20°C may work well, provided there are no big fluctuations. The ideal spot would be a cabinet far from the stove, like a wine cellar, where the temperature is low and it is dark. Storing in a refrigerator may extend the life of certain grades without any serious harm in the quality. The oil becomes cloudy but when warmed at room temperature it easily returns in its original form. Of course, such practices should be avoided in the case of expensive extra virgin olive oils intended for gourmet palates.

Defects Due to Bad Storing

Rancidity (heat, time, light)

Metallic-plastic (depending on the container)

Dirty (no proper removal of sediment)

Putrid (long period of storage)

International Olive Oil Council Standards

According to International Olive Oil Council trade standards (IOOC, COI / T 15/ NC no 3/1,2003) olive oil and olive pomace oil intended for trade shall be packed in containers which comply with basic principles of food hygiene recommended by the Codex Alimentarius Commission (CAC/RP 1-1969, Rev.3 -1997). These containers may be:

Tanks, containers, vats, which permit transportation in bulk of olive oils and olive-pomace oils.

Metal drums, in good condition, hermetically-sealed, which should be internally covered with a suitable varnish.

Metal tins and cans, lithographed, new, hermetically-sealed, which should be internally covered with a suitable varnish.

Demi-johns, glass bottles or bottles made of suitable macromolecular material.

Container Filling Tolerance

The volume occupied by the contents shall under no circumstances be less than 90% of the capacity of the container, except in the case of tin containers with a capacity of, or less than, 1 L in which the volume occupied shall under no circumstances be less than 80% of the capacity of the container; this capacity is equal to the volume of distilled water at 20°C which the container can hold when full.

Container Labeling

In addition to sections 2, 3, 7, and 8 of the Codex General Standard for the Labeling of Prepacked Foods (CODEX STAN 1-1985, Rev. 1-1991) and the guidelines applying to food not intended for direct sale to consumers, the specific provisions providing the filling information shall be applied:

1. On Containers Intended for Direct Sale to Consumers

1.1 Name of the product

The labeling on each container shall indicate the specific designation of the product contained, complying in every way with the relevant provision of this standard.

1.1.1 Designation of olive oils:

Extra virgin olive oil

Virgin olive oil

Ordinary virgin olive oil¹

Refined olive oil¹

Olive oil²

1.1.2 Designation of olive-pomace

Refined olive-pomace oil¹

Olive-pomace oil²

1 This product may only be sold direct to the consumer if permitted in the country of retail sale.

2 The country of retail sale may require a more specific designation.

1.2 Net contents

The net contents shall be declared by volume in the metric system ("System International" units)

1.3 Name and address

The name and address of the manufacturer, packer, distributor, importer, exporter, or seller shall be declared.

1.4 Country of origin

The name of the country of origin shall be declared. When the product undergoes substantial processing in a second country, the country in which such processing is carried out shall be considered as the country of origin for labeling purposes.

1.5 Indication of source and appellation of origin.

1.5.1 Indication of source

The labels of virgin olive oil may indicate their source (country, region, or locality) when they have been empowered to do so by their country of origin and when such virgin olive oils have been produced, packed, and originate exclusively in the country, region, or locality mentioned.

1.5.2 Appellations of origin

The labels of extra virgin olive oil may indicate their appellation of origin (country, region, or locality) when they have been awarded such an appellation in accordance with the terms provided under the regulation of their country of origin and when such extra virgin olive oil has been produced, packed, and originates exclusively in the country, region, or locality mentioned.

1.6 Lot identification

Each container shall be embossed or otherwise permanently marked in code or in clear to identify the producing factory and the lot.

1.7 Date marking and storage conditions

1.7.1 Date of minimum durability

In the case of pre-packaged products intended for the end consumer, the date of minimum durability (preceded by the words “best before end”) shall be declared by the month and year in uncoded numerical sequence. The month may be indicated by letters in those countries where such use will not confuse the consumer; if the shelf life of the product is valid to December, the expression “end (stated year)” may be used as an alternative.

1.7.2 Storage instructions

Any special conditions for storage shall be declared on the label if the validity of the date of minimum durability depends thereon.

2. On Forwarding Packs of Oils Intended for Human Consumption

In addition to the details noted under section 1., the following inscription shall appear:

-number and type of containers held in pack.

3. On Containers Allowing the Transportation in Bulk of Olive Oils and Olive-pomace Oils

The labeling on each container shall include:

3.1 Name of the product.

The name shall indicate the specific designation of the product contained, complying in every way with the provisions of this standard.

3.2 Net contents

The net contents shall be declared by weight or volume in the metric system (System International units).

3.3 Name and address

The name and address of the manufacturer, distributor or exporter shall be declared.

3.4 Country of origin

The name of the exporting country shall be declared.

Nutrition Facts		
Serving Size 1 Tbsp (15mL)		
Servings Per Container		
Amount Per Serving		
Calories 120	Fat Cal. 120	
% Daily value*		
Total Fat 14 g		21%
Saturated Fat 2 g		9%
Polyunsaturated Fat		1.5
Monounsaturated Fat		10 g
Cholesterol 0 mg		0%
Sodium 0 mg		0%
Total Carbohydrate	0 g	0%
Protein	0 g	
Not a significant source of dietary fiber, sugars, vitamin A, vitamin C, calcium and iron.		
*Percent Daily Values are based on a 2,000 calorie diet.		

Figure 11.1

Nutrition Labeling

A model for nutritional labeling of packed oils based on existing Food and Drug Administration nutritional labeling rules is given in [Figure 11.1](#).

Cloudy and Unfiltered Olive Oil

Virgin olive oil is produced in a form of emulsion-dispersion, which can persist for several months before full deposition of a residue. Small quantities of cloudy (veiled) oil (the fresh olive juice) are sold to consumers who consider this type more “green,” not over-processed, and richer in flavor. Many chefs also prefer this natural slight cloudiness in salads or in gourmet dishes. The oil is usually sold in bulk to the consumers directly from the mills, but it is also available in bottles. Unfiltered oils are virgin olive oils not filtered through filter paper or diatomaceous earth, but bottled only after settling. There are many firms specializing in the trade of packed unfiltered raw olive oil with varying flavoring characteristics (e.g. sweet and fruity, peppery etc.), but the usual practice is with oils of early harvest from green olives. Very often the oils are advertised as “stone made” or “cold pressed” to emphasize that a traditional process has been used. The term “cold pressed” means that the temperature during the production is kept at 35°C. The product is considered ideal for use in tavernas, restaurants, and other “fine-eating” establishments.

Freshly pressed extra virgin oil has been recently found to contain oleocanthal, a tyrosol derivative (see chapter “[Polar Phenolic Compounds](#)”). This compound has the same pharmacological properties as the drug Ibuprofen, a nonsteroidal anti-inflammatory compound and this is attributed to some structural similarities. The presence of oleocanthal is also related to the stinging sensation in the back of the throat (“a throaty bite”). This finding adds to the healthful effects of the Mediterranean diet and olive oil, especially of the freshly prepared virgin olive oil.

Veiled oils were found to have longer induction periods compared to filtered ones (Lercker et al., 1994). It appears that the material in suspension-dispersion that “veils” the oil plays a significant role against oxidation, although there is little evidence concerning the chemical nature of the compounds participating in the stable dispersion system. In a recent report Tsimidou et al. (2005) found a higher total phenolic compounds content in veiled oils in relation to the filtered and this may partly explain the higher stability.

One other possible explanation might be the presence of emulsifiers. There are compounds in the oil which may act as tensioactive solutes, e.g. traces of phosphatides or partial glycerides (Kiosseoglou and Kouzounas, 1993). Bianco and his co-workers (1998) identified two galactosyl glycosides in freshly produced oils, the α -1,6 digalactosyl derivative of the 1,2 diglyceride of linolenic acid and the α -1,6 digalactosyl derivative of the glycerol linolenate-oleate diester. The physicochemical characteristics of such compounds and the stable emulsions formed may allow an increase in the

transfer of hydrophilic compounds, such as phenols, which are strong antioxidants. It has also been suggested that small quantities of proteins may contribute to the higher oxidative stability of unfiltered olive oil (Zamora et al., 2001). There is a discrepancy in the literature concerning the level of proteins and values varying from 0.1 to 400 mg / Kg have been reported (Georgalaki, 1998, Hidalgo, 2002). In a recent report (Koidis and Boskou 2005) it was demonstrated that the level of proteins in cloudy olive oil is very low, not exceeding 2.5 mg/ kg oil. This indicates that a significant antioxidant activity cannot be expected from proteins in the presence of strong antioxidants (α -tocopherol, o-diophenols) at much higher concentrations.

A lipoxygenase activity has been detected in freshly prepared olive oil (Georgalaki et al., 1998). In spite of the presence of a small quantity of water in the non-filtered oils (a favorable condition for an enzymic activity), these oils have higher oxidative stabilities. It can be postulated that the polar phenolic compounds present not only act as primary antioxidants, but also as inhibitors of oxidizing enzymes.

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12

Culinary Applications

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Domestic and Other Uses

Olive oil, a food staple in the warmer regions around the Mediterranean Sea, is now becoming popular throughout Europe and in the United States, Canada, and other countries. This is due to its highly characteristic flavor but also to the promotion of the health benefits of Mediterranean dietary patterns.

Olive oil has a remarkable stability and can be stored for 18 months or more. The resistance to development of rancidity is combined with a vast array of flavor and color hues and distinct features depending on the cultivar of olives from which the oil is extracted. These virtues offer opportunities for a variety of culinary applications with very little or no processing. Anyone coming from the Mediterranean region of the world could tell about the flavor of a good dose of olive oil on salads, fish, vegetables, and almost everything else.

Olive oil contributes complex flavors that are reflected throughout the whole dish and adds body and depth to food. A good quality olive oil blends perfectly with the greens. Traditional vegetable dishes are prepared with seasonal vegetables, pulses, and grains. Although very old, these recipes contain wisely balanced ingredients and meet health criteria as defined by modern science.

In vegetarian dishes olive oils with herbal hues are usually preferred. For salad, a pronounced hint of apple is suitable, while for grilled meats a peppery flavor is desirable. Other dishes such as pies, mayonnaise, fried eggs, etc require different hues for those who can go deep into sensorial characteristics like mouthful, bouquet, taste, aftertaste, etc., and have developed their own personal preferences. “Freshly cut grass flavor,” “flowery aroma,” “pepperness,” and other such comments are very likely to be heard not only in oil-tasting parties but even in common discussions of consumers with a sophisticated palate. One has to stress also that differences in soil, climate, cultivar, year, maturity of the fruit, and processing conditions hardly allow for two identical olive oils. The chefs have already understood that, as with wine, each extra virgin olive oil has its very own identity.

The taste of olive oil is very often complemented by the sharp taste of vinegar, lemon, or tomato. Olive oil serves as a buffer against high acidity from fruit juices such as lemon, vinegar, and tomatoes. A simple traditional salad dressing is an instantly beaten mixture of olive oil and lemon juice, a source of both lipid-soluble and water soluble vitamins (α -tocopherol, carotenoids, ascorbic acid) and biophenols (hydroxytyrosol derivatives and other o-diphenols). In a recent report Paraskevopoulou et al. (2005) demonstrated how a stable olive oil-lemon juice salad dressing can be developed with the use of xanthan gum as a stabilizer and arabic gum or propylene glycol alginate as emulsifier. Samples of the prepared dressing were found to have a remarkable stability against oil droplet coalescence.

In salads or in cooking, olive oil is often mixed with herbs and spices, which are also an important element of the Mediterranean diet. Herbs like oregano, rosemary, thyme, or other herbs from the plants of the *Lamiaceae* family are rich sources of phenolic compounds with strong antioxidant activity (Nakatami, 1994, Tsimidou, and Boskou, 1997, Exarchou et al., 2001, Exarchou et al., 2002). These herbs maintain the nutritional value of the food and enhance the shelf life of the product.

Antoun and Tsimidou (1997) prepared gourmet olive oils which contained dry oregano and rosemary. Such oils, containing small amounts of the herbs, not only satisfied sensory requirements, even among non olive oil consumers, but also had improved resistance to auto-oxidation. This is obviously due to the contribution of antioxidants present in the herbs, like rosmarinic acid, caffeic acid, carnosol, carnosic acid, carvacrol, thymol, and others (Nakatami, 1994, Tsimidou and Boskou, 1994, Exarchou et al. 2001, Exarchou et al., 2002). In retail outlets it is now possible to purchase olive oil flavored with a variety of herbs and spices. These specialty oils, containing the additional antioxidants from the herbs, should be stored carefully in dark places because of the presence of increased levels of chlorophylls transferred from the plant material. It is known that chlorophylls promote photosensitized oxidation. Thus, total chlorophyll content may be a critical factor for the shelf life of these preparations. Damechki et al. (2001) proposed a suitable labelling for oregano and rosemary gourmet olive oils suggesting careful avoidance of light for a safe domestic use.

In addition to salads and cooking, olive oil is also used in marinades, pasta sauces, for preserving fish, cheese, sausage, and vegetables, for the preparation of breakfast toasts (tostada con aceite), as a dip for bread and in sweets, savory dishes, and home bread.

Due to the biological importance of olive oil, new attempts are continuously made to extend its uses in a variety of fatty products (e.g. margarines, cholesterol lowering spreads, reduced fat mayonnaise, butter creams, chocolate products or pastes made of almonds, hazelnuts and other nuts). The replacement of other fats by olive oil in such products is not well known since most of these applications are patented. Recently, Ansorena and Astiazaran (2004) suggested a new application for olive oil.

They conducted a study to obtain better oxidative stability in dry fermented sausages. Pork backfat was partially substituted with olive oil and antioxidants (BHA, BHT) were added to the product. The substitution resulted in lower rates of lipid oxidation during storage and better monounsaturated plus polyunsaturated to saturated fatty acid ratios. Kayaardi and Gok (2004) also suggest replacement of animal fat in meat products to reduce cholesterol levels.

Positive and Negative Attributes

The International Olive Oil Council in its Trade Standards (COI/T.15/NC no 3-25,2003) defined 3 positive attributes: bitter, fruity, and pungent and 11 negative attributes: fusty, musty, muddy, sediment winey-vinegary, rancid, heated or burnt, hay or woody, greasy, vegetable water, brine, and earthy (for the definitions see [glossary](#) at the end of the book).

Some of these properties are processing defects, (e.g. fusty, burnt, excessively bitter), others are due to storage and packaging. Ethyl acetate and acetic acid produced by acetic acid bacteria, which grow during the storage of olives, are responsible for the vinegary defect (Garcia-Gonzalez and Aparicio, 2002).

In addition to the three positive attributes defined by the IOOC, there are many other characterizations encountered when the flavor of olive oil is judged by experts. These may be: apple, artichoke, astringent, sweet, banana, buttery, fresh, grass, green, melon, peppery, flat, rough, impersonal, harmonious, and others.

Some of the positive attributes can be declared in the label of the packaged olive oil according to regulation EC 1019/02. This, however, has to wait till July 2006, when the IOOC finalizes the sensory evaluation methods (Regulation EC 1750/2004, OJEU, L312/7, 9.10.2004).

Olive Oil in Frying

Olive oil has a remarkable stability during domestic deep-frying or in other uses that require frying temperatures (Boskou, 1999). In comparison to sunflower, cottonseed, corn, and soybean oil, olive oil has a significantly lower rate of alteration. This increased stability to thermal oxidation explains why the oil can be used for repeated frying. The resistance of olive oil to rapid deterioration at elevated temperatures is attributed to its fatty acid composition and the presence of natural antioxidants such as squalene, alpha-tocopherol, and Delta-5-avenasterol (Boskou, 1999, Blekas and Boskou, 1999). The above properties are well known to people who traditionally use olive oil in cooking and prefer olive oil as a means of shallow frying.

According to Varela (1992), deep frying in olive oil offers a means to improve the profile of lipid intake. During the frying process, changes occur in the fat composition since the oil penetrates into the fried food. Western diets using vegetable oils and animal fats are very often rich in saturated fatty acids and also n-6 fatty acids. When

meat is cooked in olive oil there is a favorable change in saturated to polyunsaturated fatty acids ratio. A better combination is to fry fish. In sardine, for example, the nutritional benefits of the oil are combined with those of the n-3 fatty acids from the fish (Cuesta et al.1998).

Heating and Phenolic Compounds

In the last decade, researchers have focused on the level of phenolic compounds such as hydroxytyrosol in heated olive oil, since these compounds contribute to the stability of the oil against auto-oxidation but they are also considered components with an important biological role. Most of the published reports indicate that phenolics in virgin olive oil deteriorate relatively rapidly. Andrikopoulos et al. (2002) determined total phenols during successive pan-fryings and deep-fryings of virgin olive oil under conditions applied in domestic cooking. The loss of polar phenols and tocopherols was significant. Brenes et al. (2002) investigated the changes occurring in virgin olive oil subjected to simulated domestic frying, microwave heating, and boiling with water in a pressure cooker. Heating at 180°C caused a significant loss of tocopherols and hydroxytyrosol derivatives, but lignans (pinoselinol and 1-acetoxypinoselinol) were relatively stable. Microwave heating caused lower losses of phenolic compounds. Boiling in the pressure cooker caused rapid hydrolysis of the secoiridoid aglycons. The hydrolysis products were diffused in the water phase.

Pellegrini and coworkers (2001) used the ABTS decolorization assay of antioxidant activity to study the effect of heating on the total antioxidant activity (TAA) of extra virgin olive oil and alpha-tocopherol content, in the presence of 14 polar phenolic compounds occurring in the oil. Their results indicate that heating causes a significant loss of olive polyphenols, which act as stabilizers of alpha-tocopherol during olive oil heating. Similar results for a stabilizing effect of polar phenols on alpha-tocopherol were also reported by Valavanidis et al. (2004). Gomez-Alonso et al. (2003) found that the antioxidant activity of the phenolic extract, measured with the DPPH radical test, diminishes during the first six frying processes (each frying process: 10 min at 180°C). A rapid loss was observed mainly in the concentration of hydroxytyrosol and its secoiridoid derivatives (aldehydic forms).

A loss of the antioxidant capacity of olive oil and other vegetable oils due to heating at frying temperatures was also reported by Quiles et al. (2002) who used electron spin resonance and also by Carlos-Espin (2000) and his coworkers who studied the kinetics for the disappearance of total free radical scavenging capacity (RSC) using the DPPH test. Kalantzakis and others (2003) examined the loss of antioxidant capacity (evaluated by the DPPH test) and the polar transformation products formed from various vegetable oils heated at 180°C for 10 hours. It was observed that olive oil lost its radical scavenging capacity at a shorter time of heating in relation to soybean, sunflower, cottonseed oil, and a commercial frying oil. However, olive oil reached the level of 25% Total Polar Compounds (rejection point) after prolonged heating, while

all the other oils reached this upper limit in shorter periods (10 hours of heating). It can be concluded that olive oil as a frying medium has a remarkable stability and a resistance to oxidative polymerization due to frying. When, however, health effects are expected from the presence of natural antioxidants, the number of heating operations should be restricted to a minimum.

Another important aspect of frying in olive oil was examined by Persson and his coworkers (2003) who fried beefburgers in various oils. The burgers were analyzed for the levels of 12 different heterocyclic amines(HA), such as 2-amino-3,8-dimethylimidazol(4,5-*f*) quinoxaline, and 2-amino-1-methyl-6-phenyl-imidazol(4,5-*b*)pyridine. The intake HA amines has been associated to the development of cancer in some epidemiological studies. During cooking of animal tissue these amines are formed at low levels via the Maillard reactions and a free radical mechanism. Frying in virgin olive oil reduced the formation of heterocyclic amines and this was related to the presence of secoiridoid phenols. Loss of these phenols by storage or heating caused a decrease in the HA-reducing capacity of the oil.

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Glossary

Alpechin The waste extraction water of oil mills (IOOC).

Alperujo The solid by-product of the two-phase centrifugation method for olive oil extraction.

Appellation of Origin Trade term which may be awarded to virgin olive oils in accordance with the regulation existing in each country for the production of such an oil in a specific region.

Astringent (positive attribute) A puckering sensation in the mouth created by tannins.

Beta – Residue Oil Pomace oil.

Bitter (positive attribute) A preferred characteristic, when it is not excessive. Usually obtained from green olives or olives turning color. Perceived on the back of the tongue.

Bland Processing Defect due to excessive water added.

Cake The residue remaining after the mechanical extraction of the oil from the olives.

Centrifugation Rotary Operation for separating the constituents of the paste or oily must through the differences in their density (IOOC).

Centrifugal System Processing System based on the use of a decanter for the liquid phase from the pomace.

Clarification Operation An operation to remove water from the oily must by decantation or centrifugation (IOOC).

Classic System The traditional batch method of olive processing using hydraulic plate presses.

Cold-pressing Process of extracting virgin olive oil by applying mechanical pressure to olive paste at a temperature of less than 25°C (IOOC).

Continuous System Processing of olives within a system which uses a horizontal centrifugal decanter.

Crude Olive-pomace Oil Oil obtained by treating olive pomace with a solvent.

Dacus oleae The olive fly causing damage to the fruit.

Decanted Oil Oil purified by decantation after storing (not filtered).

Desterolization A fraudulent action. The removal of sterols from cheaper oils in order to render them undetectable in olive oil.

Dirty Oil which has unpleasant odors. Processing defect due to poor oil cleaning.

Earthy Negative attribute due to olives collected with earth or mud and not washed.

Flat An oil which has lost its characteristic aroma.

Fresh (positive attribute) Good aroma, fruity, not oxidized.

Fruity (positive attribute) Set of the olfactory sensations characteristic of the oil, which depends on the variety and comes from sound, fresh olives, either ripe or unripe. It is perceived directly or through the back of the nose (retro-nasal).

Fusty (negative attribute) Characteristic flavor of oil obtained from olives stored in piles, which have undergone an advanced stage of anaerobic fermentation. Associated with n-octane, produced from the decomposition of 10-hydroxyperoxide of oleic acid and isoamyl alcohol formed from fermentation.

Grass The taste of grass, seen in green olives or those crushed with leaves.

Greasy (negative attribute) Flavor of oil reminiscent of that of diesel oil, grease or

mineral oil.

Green (positive attribute) A young oil, usually with a spicy-bitter taste.

Harmonious (positive attribute) All the qualities of the oil blend and work well with each other.

Hay or Woody (negative attribute) Characteristic flavor of certain oils produced from olives that have dried out or were frozen.

Heated or Burned (negative attribute) Characteristic flavor of oils caused by excessive and/or prolonged heating during processing.

Husk Residue solids after pressing of the pulp.

Lampante Virgin Olive Oil Virgin olive oil not suitable for consumption with an acidity more than 2% (expressed as oleic acid).

Malaxation The phase of mixing after crushing the olives in the centrifugation process, which promotes the coalescing of small oil drops.

Milling Processing of olives for the production of olive oil.

Muddy (negative attribute) Sediment in tanks and vats.

Musty (negative attribute) Characteristic moldy flavor of oils obtained from fruit in which large numbers of fungi and yeast have developed as a result of storing in humid conditions.

Natural Olive Oil Virgin olive oil.

Oleaster The botanical progenitor of the olive (*Olea sylvestris*).

Olive Kernel Oil The oil obtained from olive pomace.

Olive Paste Paste produced by grinding the olives.

Olive Pomace A by-product of olive processing containing fragments of skin, pulp and kernel.

Olive Pomace Oil Blend of refined olive pomace oil and virgin olive oil for consump-

tion

Olive Residue Oil Olive pomace oil.

Orujo Oil Spanish term, equivalent to sulphur olive oil.

Panel Test Scoring of olive oil by a group of specially trained assessors under specified conditions.

Peppery A peppery bite in the back of the throat which can force a cough.

Press Machine that squeezes the oily must from the oily paste.

Pungent (positive attribute) “Picante” or biting tactile sensation characteristic of certain olive varieties or oil produced from unripe olives. Perceived in the throat (peppery).

Rancid (negative attribute) Flavor of oils, which have undergone a process of oxidation and fragmentation of hydroperoxides into compounds with characteristic disagreeable odors such as aldehydes, ketones, alcohols, lactones, furans, esters, and others.

Reesterification (or Esterification) Illegal procedure to restore acidity by chemically esterifying glycerol with fatty acids.

Reextracted Olive Pomace Oil Oil obtained by recrushing olive pomace.

Refined Olive Oil Oil obtained from virgin olive oils by refining methods that do not change its initial triacylglycerol composition.

Remolido Repaso Second centrifugation oil.

Riviera Type Olive Oil The oil produced by mixing 5-20% virgin olive oil to refined olive oil.

Sensory Wheel A circular diagram; a condensed set of sensory attributes for describing virgin olive oil.

Stoned Olive Paste Paste obtained by grinding stoned olives.

Sulphur Olive Oil The oil obtained by extraction with solvents of the cakes derived

from the pressing of the olive paste.

Super Press Press that uses several pistons.

Traditional Mill Classic system of extraction with hydraulic presses.

Unsaponifiable Matter The whole of the products present in the substance analyzed which, after saponification thereof with an alkaline hydroxide and extraction by a specified solvent, remains non-volatile under the defined conditions of test.

Vegetable Water (negative attribute) Flavor acquired by the oil as a result of prolonged contact with the liquid, non-oil fraction of the olive,-also called fruit water.

Veiled Virgin Olive Oil Cloudy virgin olive oil.

Winey-vinegary (negative attribute) Characteristic flavor of certain oils reminiscent of wine or vinegar. This flavor is mainly due to aerobic fermentation in the olives leading to the formation of acetic acid, ethyl acetate, and ethanol.