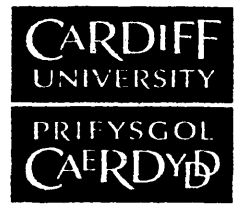

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Factors Affecting Olive Oil Quality

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A thesis presented for the degree of Doctor of Philosophy
at Cardiff University

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Dedicated to the memory of my parents

Abbreviations list

3,4-DHPEA	3,4-Dihydroxy-Phenyl-Ethanol or Hydroxytyrosol
3,4-DHPEA-EA	Isomer of Oleuropein Aglycon
3,4-DHPEA-EDA	Dialdehidic Form of Elenolic Acid linked to 3,4-DHPEA
AaOO	C17:0- C18:1- C18:1
AAT,	Alcohol Acyltransferase
ACCase,	Acetyl-CoA Carboxylase
ACP	Acyl Carrier Protein
ADHs	Alcohol Dehydrogenases
ADP	Adenosine Diphosphate
AOO	C20:0- C18:1- C18:1
ATP	Adenosine Triphosphate
BCCP	Biotin Carboxyl Carrier Protein.
C16:0	Palmitic acid
C16:1	Palmioleic acid
C17:0	Margaric
C17:1	Margaroleic
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	α-Linolenic acid
C20:0	Arachidic acid
C20:1	Eicosenoic acid
C22:0	Behenic acid
C24:0	Lignoceric acid
CHD	Coronary Heart Disease
CoA	Coenzyme A
DAG	Diacylglycerol
DAGAT	Diacylglycerol Acyltransferase
DHS	Dynamic Headspace
DPPH	2,2-Diphenyl-1- Picrylhydrazyl radical
dS/m	deciSiemens/m

EC	European Commission
EC	Electrical Conductivity
ECe	Electrical Conductivity of The Saturated Soil Extract
ECiw	Electrical Conductivity of Irrigation Water
EeOO	C17:1- C 18:1- C 18:1
EFA	Essential Fatty acid
FAO	Food and Agriculture Organization of the United Nations
FAS	Fatty acid Synthase
G3-P	Glycerol 3-phosphate
G3PAT	Glycerol 3-phosphate acyltransferase
GaOO	C20:1- C18:1- C18:1
HOSO	High Oleic Sunflower Oil
HPL	Hydroperoxide Lyase
IOOC	International Olive Oil Council (IOOC)
KAS	β-ketoacyl-ACP synthase
KAS III	β-ketoacyl-ACP synthase
LA	Linoleic acid
LDL	Low Density Lipoprotein
LLL	C18:2-C18:2-C18:2
LOX	Lipoxygenase
LPAAT	Lysophosphatidate (1-acylglycerol 3-phosphate) acyltransferase
MUFAs	Monounsaturated Fatty acids
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
ND	Not detected
NEFA	Non-esterified fatty acids
OLL	C18:1-C18:2-C18:2
OLLn	C18:1-C18:2-C18:3
OOL	C18:1- C18:1- C18:2
OOLn	C18:1- C18:1- C18:3
OOO	C18:1- C18:1- C18:1
PAP	Phosphatidate Phosphohydrolase

p-HPEA	p-Hydroxy-Phenyl-Ethanol or Tyrosol
p-HPEA	p- Hydroxyphenylethanol, or Tyrosol
p-HPEA- EDA	Dialdehidic form of elenolic acid linked to p-HPEA
PLL	C16:0- C18:2-C18:2
PLLn	C16:0-C18:2-C18:3
POD	Peroxidase
POL	C16:0- C18:1- C18:2
POO+SOL	C16:0- C18:1- C18:1 + C18:0- C18:1- C18:2
PoOL	C16:1- C18:1-C18:2
POP	C16:0-C18:1-C16:0
POS	C16:0- C18:1- C18:0
PPL	C16:0- C16:0 C18:2
PPO	Polyphenoloxidase
PV	Peroxide Value
RRT	Relative Retention Times
SCL	Sandy Clay Loam
SFA	Saturated Fatty acid
SOO	C18:0- C18:1- C18:1
SOS	C18:0- C18:1- C18:0
SPE	Solid Phase Extraction
TAG	Triacylglycerol
USDA	United States Department of Agriculture
VLDL	Very Low Density Lipoprotein

Summary

Olive (*olea europaea, L.*), an evergreen tree has been cultivated for thousands years, in many semi-arid and arid mountainous areas surrounding the Mediterranean Sea. Olive oil is extracted from the olive fruit by mechanical means, without any chemical treatment thus preserving all of its natural constituents, which are responsible for the unique flavor, as appreciated by consumers.

This thesis concentrates on the important factors that might affect the quality of olive oil. These comprise: cultivar / agricultural methods / weather /processing /storage.

Of the quantitative and qualitative results obtained, the following are of particular interest.

- The olive variety influenced the chemical composition and sensory quality of the olive oil. Particularly affected were the composition of fatty acids, sterols, aliphatic and triterpene alcohols, phenolic compounds and sensory attributes.
- Osmotic stress, due to no irrigation or to saline irrigation, influenced the size and oil content of the fruit and the composition of triacylglycerol molecular species, fatty acids, total phenols, secoiridoid derivatives as well as volatile compounds and sensory attributes of olive oil
- The processing methods affected the sensory quality and the phenolic compounds of olive oil. The most significant variations occured mainly due to the quantity of water added to the system of extraction and the duration and temperature of malaxation. The alteration of these during the extraction process was reflected in oxidative processes.
- The rate of olive oil oxidation was a function of both the time and the various conditions of storage. Virgin olive oil keeps its qualitative characteristics under the category of extra virgin olive oil for 15 months if it is stored in tin containers indoors at room temperature and with nitrogen in the headspace.

The present studies provide information for the olive oil industry in order to improve the overall olive oil quality by optimising each step of the production chain.

Chapter 1. General Introduction

1.1. Edible oils

Fat and oils are water insoluble organic substances found in cells, which are extractable by non-polar solvents such as chloroform, ether and benzene. Fats and oils are complex mixtures containing a wide range of lipid classes. Information on the various component classes is crucial for understanding the behavior of the fat or oil during processing, to monitor or assess its quality or, for example, to detect adulteration. The most important group of compounds in edible fats and oils are the triacylglycerols (TAGs), a set of compounds consisting of three fatty acids linked to a glycerol backbone. Because of the large number of possible fatty acids, an enormous number of TAGs are possible (Boskou, 1996).

Storage fats are mainly triacylglycerols and are accumulated in specific depots in the tissues of plants and animals. These fats are an important energy source in the diet and provide essential nutrients. The composition of the fatty acids in these triacylglycerols is an important dietary consideration. Dietary fat requirements are usually recommended at about 30% of total calories although Western diets frequently contain more.

Fats and lipids are essential to life and they have five important functions:
(FAO/WHO 1977)

a) as a source of energy,
b) as essential membrane components
c) for surface coverings of many organisms
d) as biological effector molecules or sources of such components
e) other specific functions e.g. vitamins.

Neutral oils have a high caloric value (9 Kcal/g) and can be stored in a nearly anhydrous form as intracellular fat droplets. The fatty acid components of lipids usually have an even number of carbon atoms and are mainly from 12 to 22 carbon atoms long. Fatty acids may be saturated or unsaturated. The double bonds of unsaturated fatty acids have the *cis* geometric configuration. The *cis* forms are less stable than the *trans* forms and they may be converted into the latter by heating with

certain catalysts. In this way, oleic acid can be readily converted to its *trans* isomer elaidic acid, which has a higher melting point.

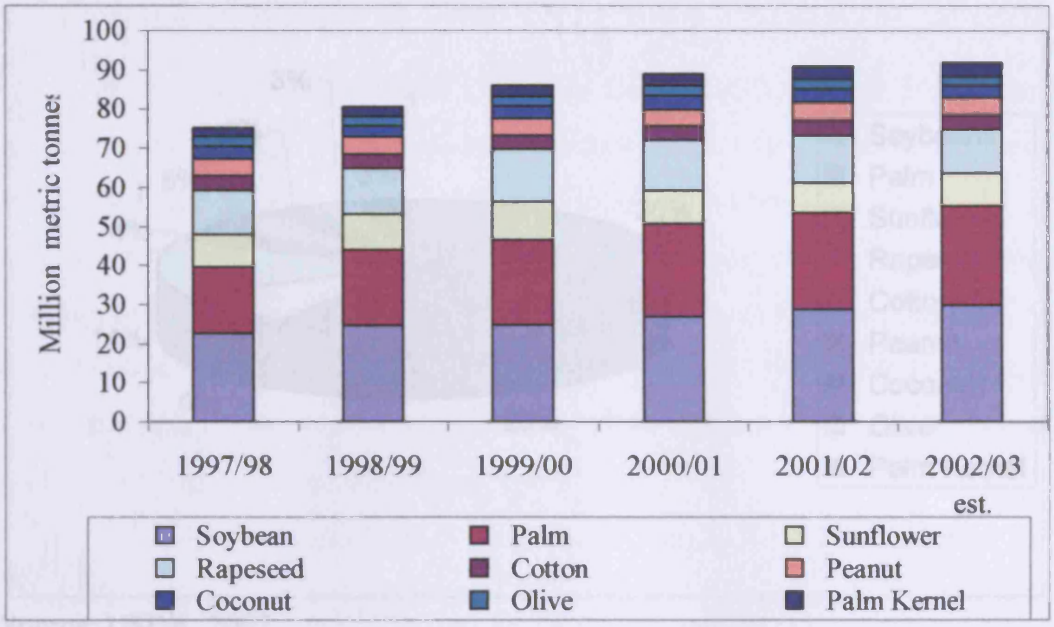
In fatty acids with multiple double bonds, their *cis* configuration causes the hydrocarbon chain to become kinked and shortened. These structural features of unsaturated fatty acid chains are biologically significant, particular in membranes.

Linoleic (18:2, n-6) and α-linolenic (18:3, n-3) acids are required for the normal growth and function of animal tissues. They have double bonds positioned six carbons (n-6) and three carbons (n-3) from the methyl end of the molecule, respectively. Moreover, linoleic and α-linolenic acids are essential fatty acids because they cannot be synthesised by mammals and must be obtained from the diet. Essential fatty acids play various roles as constituents of cell membrane phospholipids. They are needed for eicosanoid synthesis (e.g. prostaglandins, prostacyclins, thromboxanes and leukotrienes) influencing many diverse properties such as platelet aggregation, blood pressure and heart functionality (FAO 1977; Berra et al 1997; Harwood & Yaqoob, 2002). The n-3 and n-6 series of essential fatty acids have many other health-giving properties apart from acting as precursors of eicosanoids. Many of these benefits may be due to their selective effects on gene transcription (see Harwood and Yaqoob, 2002).

World production of vegetable oil showed 20 per cent increase between 1997-98 and 2001-02. However, the annual rate of increase between years has gradually decreased over this period (Figure 1.1). Similarly, consumption of vegetable oils has shown an increase by 23.55 per cent over this period, again showing a gradual decline in terms of the annual increase. Among the vegetable oils, palm oil and soybean oil constitute the largest components of world trade.

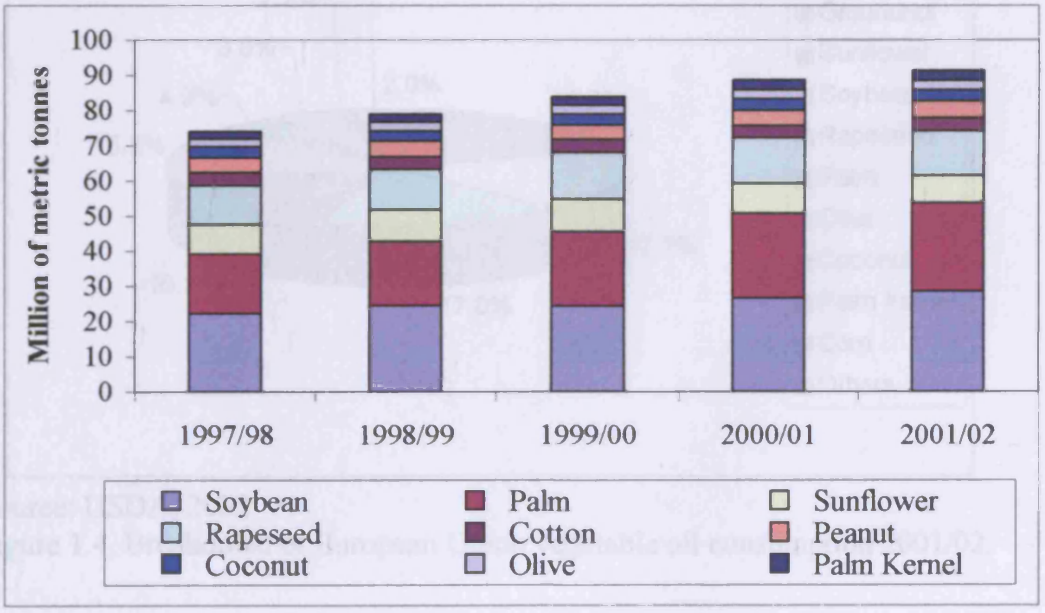
The % share of soybean oil in the world consumption in 2002 was the highest (30 %) followed by the palm oil (28 %) and rapeseed oil (14%) (Figure 1.3).

Olive oil accounts for only 3 %. However, taking into account the total amount of vegetable and seed oils consumed in the European Union, olive oil competes favourably with other popular oil types. It represents 15.2 % of total oil consumption, compared with 18.2 % for sunflower, 17.7 % for Soya, 17.3 % for rape and 16.0 % for palm oils.



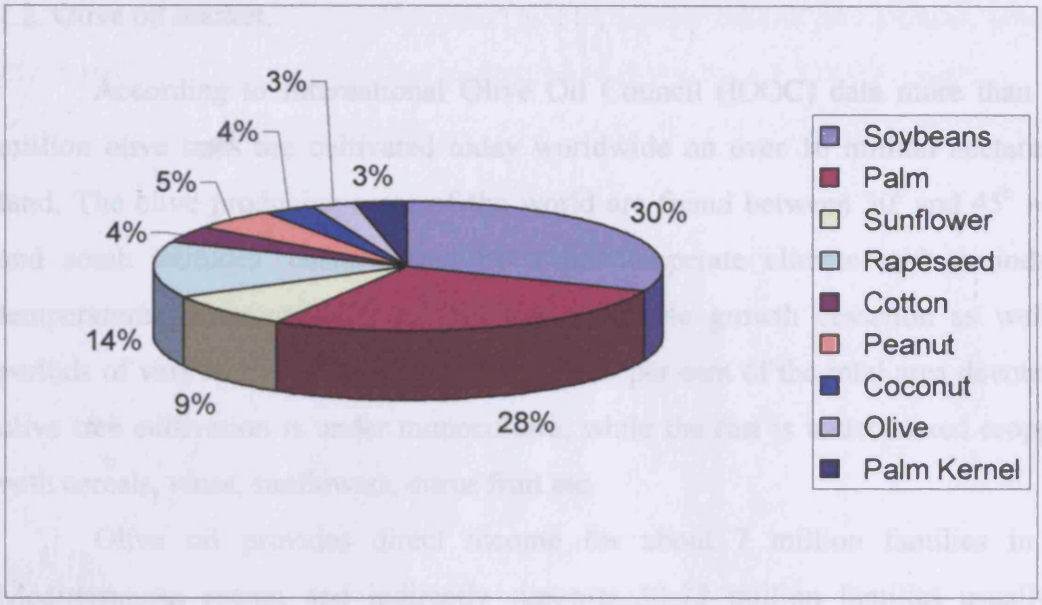
Source: USDA, 2002

Figure 1.1. Major vegetable oil world production.



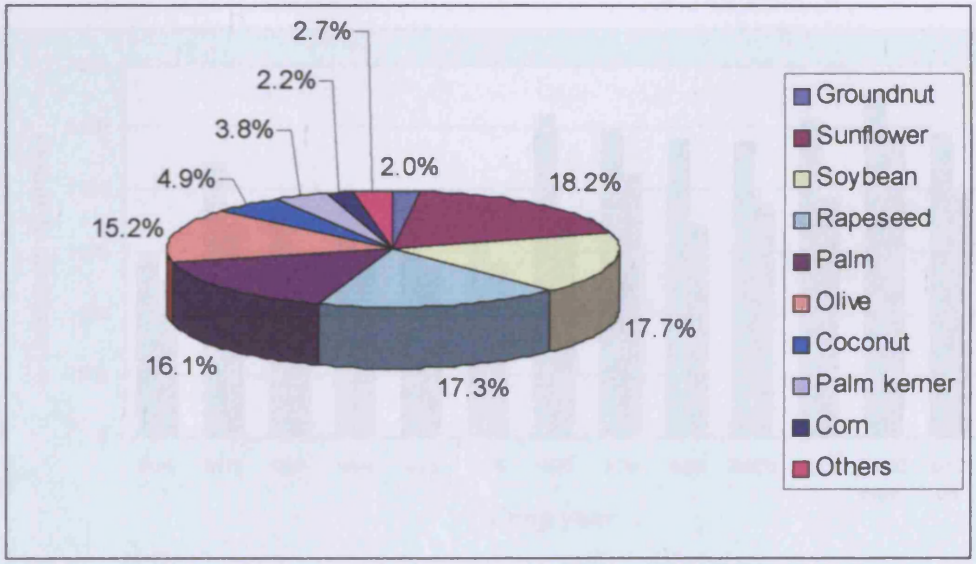
Source: USDA, 2002

Figure 1.2. Major vegetable oil world consumption.



Source: USDA, 2002

Figure 1.3. Breakdown of world vegetable oil consumption 2001/02.



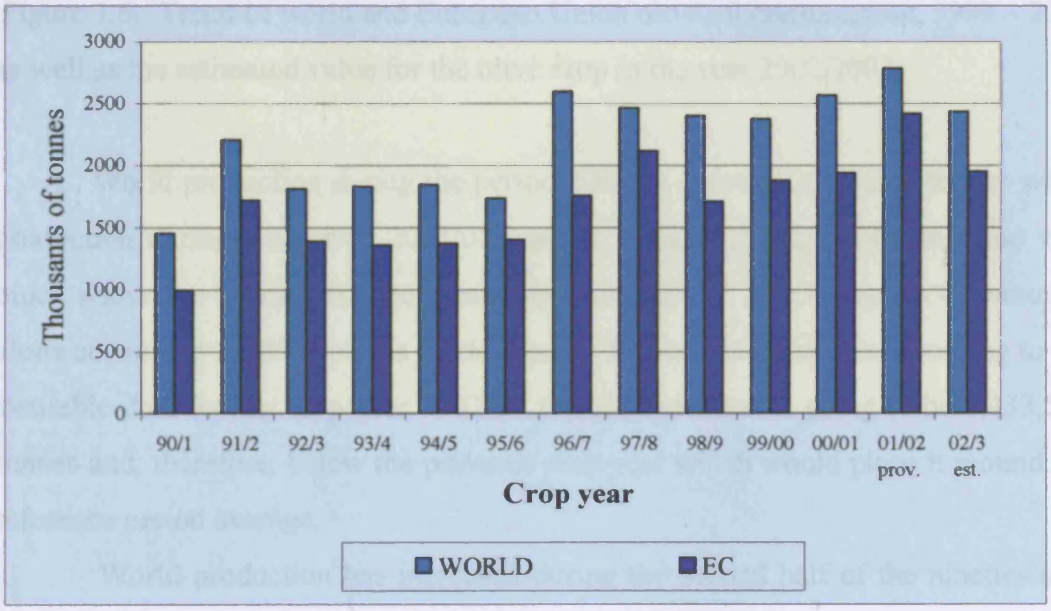
Source: USDA, 2002

Figure 1.4. Breakdown of European Union vegetable oil consumption 2001/02.

1.2. Olive oil market.

According to International Olive Oil Council (IOOC) data more than 830 million olive trees are cultivated today worldwide on over 10 million hectares of land. The olive producing areas of the world are found between 30° and 45° north and south latitudes, characterized by a hot–temperate climate with periods of temperatures of around 0°C to facilitate vegetable growth cessation as well as periods of very hot temperatures. More than 60 per cent of the total area devoted to olive tree cultivation is under monoculture, while the rest is under mixed cropping with cereals, vines, sunflowers, citrus fruit etc.

Olive oil provides direct income for about 7 million families in the Mediterranean region and indirectly supports 30-35 million families usually in economically deprived areas.

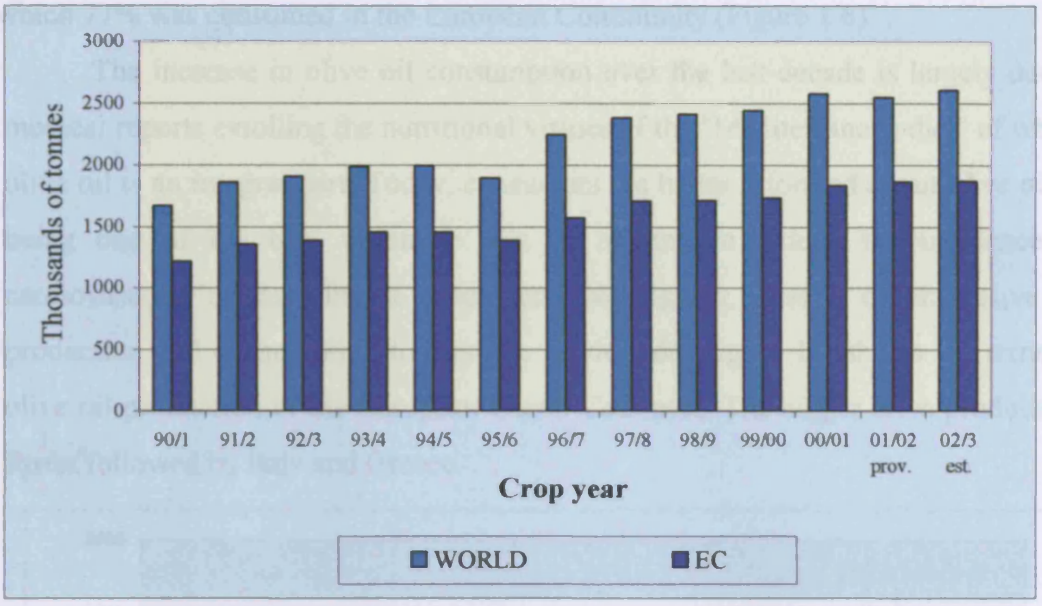


Source: IOOC, 2002

Figure 1.5. Trend of world and European Union olive oil production, 1990 – 2002 as well as the estimated value for the olive crop in the year 2002/2003.

The trend of olive oil production and consumption in the world and in European Union are shown on Figures 1.5 and 1.6. The average olive oil production between 1990 and 2000 crop years was 2,071,200 tonnes (IOOC, 2002). However,

production growth was much greater in the second half of the decade, when it averaged 2,314,200 compared to the first, when it averaged 1,828,000 tonnes.



Source :IOOC, 2002

Figure 1.6. Trend of world and European Union olive oil consumption, 1990 – 2002 as well as the estimated value for the olive crop in the year 2002/2003.

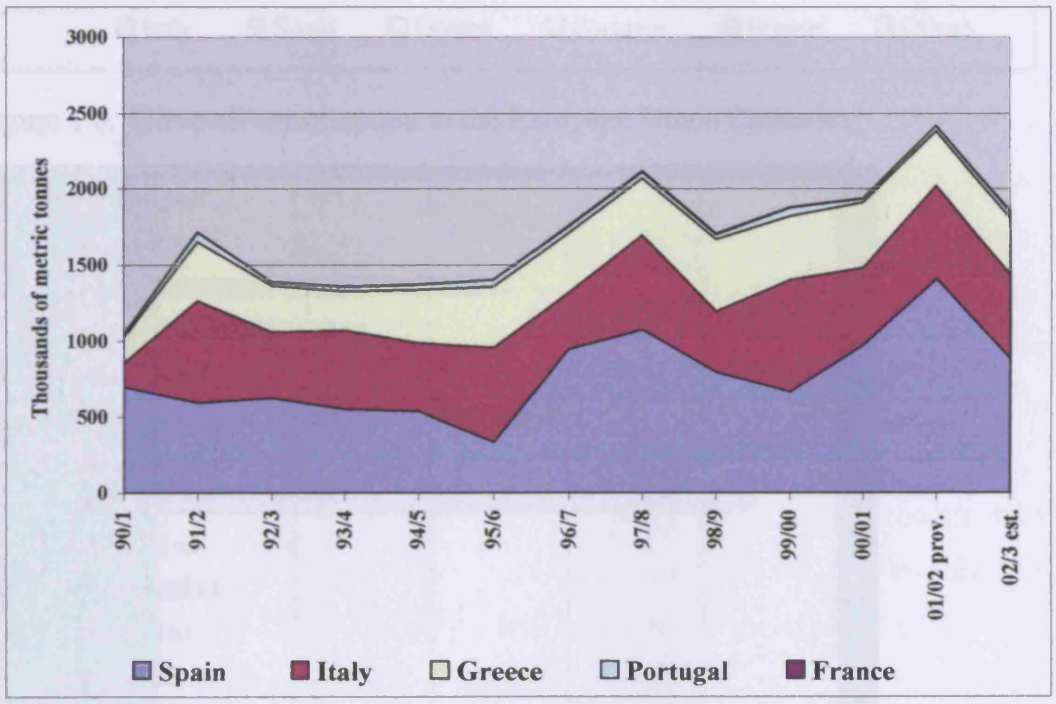
World production during the period 2000/01 came to 2,565,500 tonnes while production during the period 2001/02 was the highest (2,782,500 tonnes) and very much above the average for the preceding four seasons. The European Community alone accounted for 87% of this world figure. The estimated values according to the available data for the crop year 2002/03 world production is going to be 2,433,500 tonnes and, therefore, below the previous crop year which would place it around the reference period average.

World production has increased during the second half of the nineties as a result of the entry into production of important plantation areas in a number of producing countries, particularly in European Community and non-Mediterranean countries, such as Argentina and Australia. Simultaneously, consumption of olive oil has kept up with the production trends following promotion efforts carried out by the International Olive Oil Council.

However, the favourable climatic conditions, the start of production in new orchards, improved inputs and technology mean that the world market might be

entering a new phase where production exceeds consumption. At present, world consumption continues upwards and has reached a figure of 2,548,500 tonnes, of which 77% was consumed in the European Community (Figure 1.6).

The increase in olive oil consumption over the last decade is largely due to medical reports extolling the nutritional virtues of the "Mediterranean diet" of which olive oil is an integral part. Today, consumers are better informed about olive oil as being one of the best vegetable oils in helping to reduce the incidence of cardiovascular disease, breast cancer and cell ageing. This is causing olive oil production and consumption to increase worldwide. Figure 1.7 shows the trend of olive oil production in the European Union Countries. The bigger olive producer is Spain followed by Italy and Greece.



Source: IOOC, 2002.

Figure 1.7. Trend of olive oil production in European Union countries.

The European Union also accounts for the bulk of world consumption (about 71%). The highest percentage (about 92%) of consumption in the EC corresponds to the three leading countries, Italy, Spain, and Greece. Figure 1.8 shows the trend of olive oil consumption during the year's 1990- 2003 in the EC. It is striking to see the small consumption share of Portugal, France and the other European countries. When expressed per head, it is obvious that the same producer countries are also the

leading olive oil consumers (Figure 1.9). It is remarkable, that the annual Greek consumption is almost twice that of the average Spanish and Italian consumption

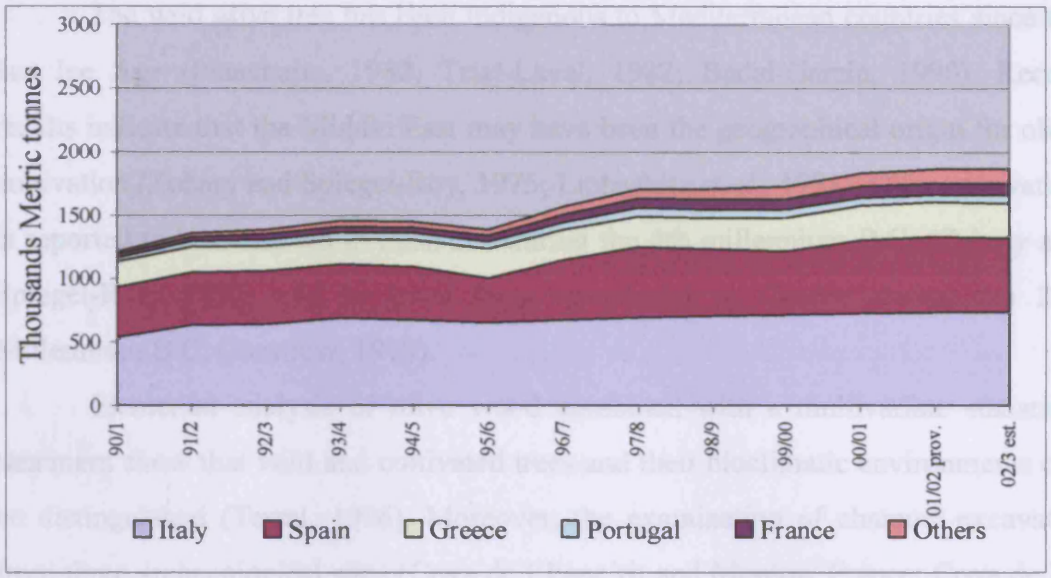
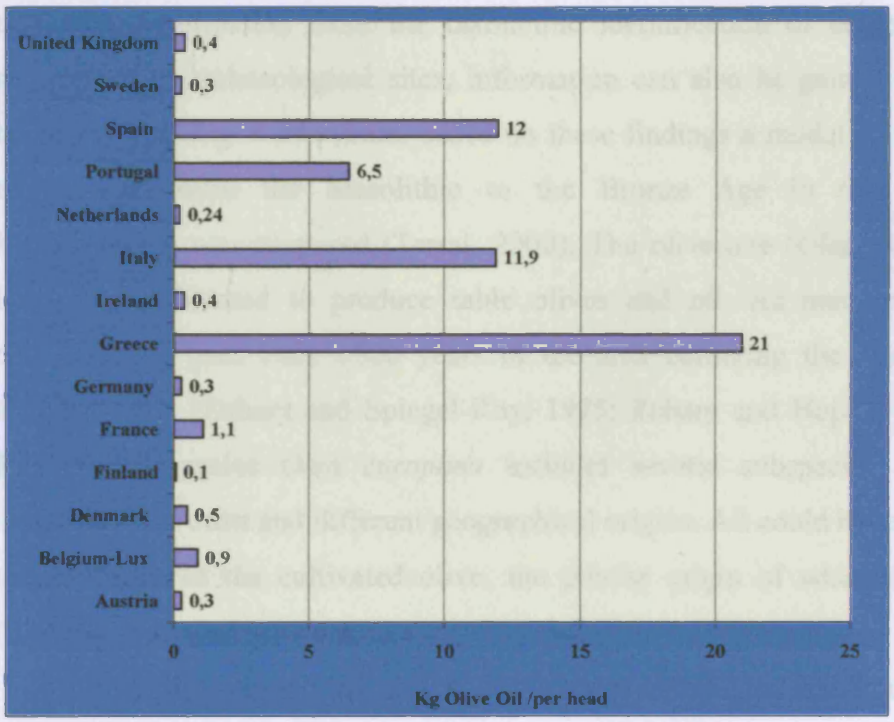


Figure 1.8. Olive oil consumption in the European Union Countries.



Source: Luchetti, 2002

Figure 1.9. Average olive oil consumption per head in the EU countries: Olive oil crop year 1999-2000.

1.3. Historical Review

The wild olive tree has been indigenous to Mediterranean countries since the last Ice Age (Planchairs, 1982; Triat-Laval, 1982; Badal-Garcia, 1990). Recent results indicate that the Middle East may have been the geographical origin for olive cultivation (Zohary and Spiegel-Roy, 1975; Liphschitz *et al.*, 1991). Olive cultivation is reported to have started in Palestine during the 4th millennium B.C. (Zohary and Spiegel-Roy, 1975) and to have been introduced to Greece during the 2nd Millennium B.C. (Renfrew, 1973)

Elemental analysis of olive wood combined with a multivariate statistical treatment show that wild and cultivated trees and their bioclimatic environments can be distinguished (Terral, 1996). Moreover, the examination of charcoal excavated from three archaeological sites (Cova de l'Espe´rit and Montou, France; Cova de les Cendres, Spain) showed the deliberate collection of this wood by man. In addition to ecological information from the taxonomic identification of ecological artefacts sampled from archaeological sites, information can also be gained on their ethno botanical meaning. Furthermore based on these findings a model of exploitation of olive wood from the Mesolithic to the Bronze Age in the North-western Mediterranean was proposed (Terral, 2000). The olive tree (*Olea europaea L)* has long been cultivated to produce table olives and oil. As mentioned above, its domestication goes back 6000 years to the area bordering the east coast of the Mediterranean (Zohary and Spiegel-Roy, 1975; Zohary and Hopf, 1993; Civantos, 1995). The species *Olea europaea* includes several subspecies with different morphological traits and different geographical origins. All could have contributed to the evolution of the cultivated olive, the precise origin of which is still unclear (Besnard *et al.*, 2001a; Besnard *et al.*, 2001b).

Several words remind us today of this ancient heritage. For example, from the Greek word elaia derived the Latin words olea (oil) and olivum (olive); and from the Hebrew zait derived the Arabian terms of az-zait (aceite in modern Spanish) and zaitum (aceituna in modern Spanish).

The benefits of olive trees are clear: they grow in rather arid areas, protect the soil against desertisation and erosion and help to regulate the water cycle. The olive tree (*Olea europea, L*) is a plant native to the Mediterranean basin. As a sacred tree,

the olive tree played a significant role in both Hellenic and Roman Mythology and olive oil played a significant role in the religious, socio-cultural, medical and nutritional history of man since its cultivation 6000 years ago. Olive oil in ancient Mediterranean countries was used as a basic food source, principal lighting fuel (Kimpe *et al.*, 2001) and main ingredient in body-care products. It is not clear yet if olive fruit was utilised first for oil-extraction or as a food after some type of processing. The lipid fraction of residues in ancient oil lamps found at the archaeological site of Sagalassos (south-west Turkey) were analysed by gas chromatography (GC) coupled to mass spectrometry (MS). Results indicate that olive oil was not exclusively used as a fuel. Possibly olive oil was mixed with leftovers of other used oils. In some samples even saturated TAGs were present, probably stemming from traces of animal fat. However, as no cholesterol was detected by GC–MS, it is believed that the additions of animal fat were minor (Kimpe *et al.*, 2001)

The leaf of the tree is used to honour victory, wisdom and peace. An olive branch was returned to Noah on the ark by a dove, signalling the end of the great flood and olive oil still holds a sacred place in the solemn rites of various religions, from baptisms, to oil lamps, as well as in traditional monuments found throughout the Mediterranean region.

The olive tree was widespread, and particularly in ancient Greece as confirmed by the Greek mythology and the numerous histories and literary testimonies, especially in the poems of Homer. It is reported in mythology that Athena, the goddess of wisdom and the arts, was in competition with Poseidon, the sea god. Each was charged with presenting humankind with the most valued gift. Poseidon donated the horse. Athena caused an olive tree to grow at the gates of the Acropolis. It was Athena's gift the people deemed most valuable. In return for her favours, Athens, the most powerful city in Greece, was named in her honour and Greek gods were believed to be born under the branches of the olive tree. Aristotle pondered the olive tree at great length and eventually elevated its cultivation to a science. Solon established the first laws to protect it. Homer deemed olive oil the "liquid gold." Hippocrates prescribed it as the "great therapeutic."

Major archaeological evidence of olive oil in the economy of Crete in 2500 BC can be seen today in the storerooms of the palace of King Minos in Knossos. The olive fruit was not only important in the diet of ancient Crete as it is today 4500 years later,

but may also have been a major source of export revenue. Olive oil was also chiefly responsible for establishing the commercial progress of Greece at a time when the entire Mediterranean region was in depression.

1.4. Unique properties of olive oil

The beneficial health properties of olive oil have been known empirically for centuries, particularly around Mediterranean basin. In recent years these same benefits have been confirmed by medical and epidemiological research (Jacotot, 1994; Trichopoulou *et al,* 1995b; Keys, 1995; Trichopoulou and Lagiou, 1997; Roche *et al.,* 2000; Wartburg *et al.,* 2002). Today, there is evidence that the amount and type of dietary fat have profound effects on plasma lipids and lipoproteins and on the risk factors associated with several major diseases such as incidence of cardiovascular diseases, and cancer which are the main causes of death among populations in develop countries (Trichopoulou *et al,* 1995a; Lipworth, 1997; Weisburger, 1997; Rose and Connolly, 1999; Gurr, 2000; Gurr *et al.,* 2002).

"The Seven Countries study", carried out by Angel Keys, presented the first scientific evidence for relationship between a diet low in animal products as well as saturated fat and low mean population levels of serum cholesterol with low CHD incidence and mortality. This study started in 1960, finished in 1986, and was carried out with about 13000 men, aged 40 to 59 years old, enrolled in 15 population samples located in seven different countries (Italy, Greece, former Yugoslavia, Netherlands, Finland, United States, and Japan). The study also demonstrated that the type of fat rather than level of fat was related to the coronary heart disease. Mediterranean countries with relative high intakes of total fats had lower rates of CHD and this was strongly correlated with low intake of saturated fatty acid. Furthermore, a strong inverse relationship between the intake of monounsaturated fats and the MUFA to SFA ratio and CHD incidence, cancer and all-cause mortality was also confirmed. Among all groups from southern Europe, Crete showed the lowest mortality from CHD. More than other Mediterranean Diets, the Cretan diet was, at least in the 1960s, rich in legumes, fruit, and edible fats that were mostly olive oil. The Cretan diet contained little meat, moderate amounts of fish and alcohol, mostly in form of red wine (Keys, 1980; Keys *et al.,* 1986; Keys, 1995; Willet *et al.,* 1995; Trichopoulou *et al.,* 2000).

Recent research work suggests that the components of olive oil have a greater role in disease prevention than previously thought and the benefits of the "Mediterranean diet" to health have enhanced the popularity of olive oil. Nowadays, worldwide health organisations recommend olive oil, due its fatty acid composition and the presence of certain minor constituents, as a healthy food. As a result, its daily consumption is increasing throughout Europe and in the United States.

The beneficial effects of a diet rich in extra virgin olive oil are attributed to its composition, which is the major determinant of its metabolic effects. Several factors must be taken into consideration. The fatty acid profile, with the content of monounsaturated is high in olive oil compared with seed oils. Oleic acid (C18: 1, n-9) is the main component of olive oil and its content ranges from 65 to 83 % (mean 75%) of fatty acids Monounsaturated fatty acids are proven to have beneficial effects on serum lipids and lipoproteins (Mensink and Katan, 1992), on composition of human VLDL triacylglycerols (Ruiz-Gutierrez *et al.,* 1997) on membrane lipid composition and morphology in rat erythrocytes (Escudero *et al.,* 1998). Olive oil is low in saturated fatty acids (mean 11.5 %) and polyunsaturated fatty acids with linoleic (C18:2, n-6) and α-linolenic (C18:3, n-3) acids having mean values of 9.5 % and 1.0 %, respectively (Kiritsakis, 1990; Boskou, 1996; Stefanoudaki *et al.,* 1999)

Essential fatty acids are also commonly referred to as omega-6 and omega-3 FAs respectively. Omega-3 and omega-6 fatty acids are important in the normal functioning of all tissues of the body. Deficiencies are responsible for a host of symptoms and disorders including abnormalities in the liver and kidney, changes in the blood, reduced growth rates, decreased immune function, and skin changes including dryness (Table1.1) (Gurr *et al.,* 2002). Adequate intake of the essential fatty acids results in numerous health benefits. Recommendations for dietary intake of EFAs are not easy to propose since optimal requirements are not fully known. However, in order to ensure the best biological functions, an intake of about 3–6% of total fat is generally recommended. Within the n-6 family, LA is the major dietary EFA and, based on animal studies, about 1–2% of total caloric intake is required to relieve symptoms induced by its deficiency. (Approximately 8-10% linoleic acid is in agreement with this requirement for essential fatty acids when olive oil is used as sole source of fat).

Table1.1. Major effects of n-6 essential fatty acid deficiency in rats.

Skin	Dermatosis, water permeability increased Sebum secretion decreased Epithelial hyperplasia
Body weight	Decreased
Circulation	Heart enlargement Capillary resistance decreased
Kidney	Enlargement; intertubular haemorrhage
Lung	Cholesterol accumulation
Endocrine glands	Adrenals; weight decreased in females, increased in males Thyroids; weight increased
Reproduction	Females: irregular oestrus; impaired lactation, reproduction Males: degeneration of seminiferous tubulus
Metabolism	Changes in tissue fatty acid composition. Reduced cholesterol concentration in plasma. Increased cholesterol concentration in liver, adrenals and skin. Mitochondrial swelling and uncoupled oxidative phosphorilation. Increased triacylglycerol output by the liver

Adapted from Gurr *et al.* (2002).

Moreover, the biological properties of olive oil are also related to its antioxidant composition, namely, vitamin E (alpha-tocopherol), carotenoids and phenolic compounds (simple phenols such as hydroxytyrosol and complex phenols such as secoiridoids). Phenolic compounds are potent antioxidants whose activity has been demonstrated in *vitro* with inhibited LDL oxidation and preventing the peroxidative chain reaction (Visioli *et al.*, 1995; Owen *et al.*, 2000a; Owen *et al.*, 2000b). The in *vivo* oxidation of LDL is strongly linked to the formation of atherosclerotic plaques which in turn contibute to the development of coronary heart disease (Visioli and Galli, 1998; Saija *et al.*, 1998). Nowadays phenolic compounds are receiving increased attention. Many studies have been reported on the health properties of plenolic compounds of olive oil such as 3,4-DHPEA, 3,4-DHPEA-EDA and 3,4-DHPEA-EA and on the possibility that phenolic compounds might act as

antioxidants, thus avoiding lipid oxidation inside the arteries and therefore accumulation of oxidised LDL in the arterial walls, a primary cause of coronary diseases (Soler-Rivas *et al.,* 2000). Phenolic compounds have obvious antiatherogenic effects by protecting the LDL against oxidative modifications (Visioli & Galli, 1998). The biological activities of olive oil phenolics are not limited to their antioxidant potency but extend to their interaction with relevant enzymatic systems. In particular, 3,4-DHPEA inhibits platelet aggregation (Petroni *et al,* 1995) and reduces the formation of pro-inflammatory molecules, thromboxane B2 and leukotriene B, by activated human leukocytes during immune responses (Visioli *et al.,* 1998; Visioli and Galli, 1998).

The process of olive oil extraction involves mild mechanical procedures obtained without the use of chemicals, high temperatures and organic solvents. As a result, the integrity of olive oil minor constituents, which include antioxidants polyphenols and vitamins such as α-tocopherols (around 200 ppm) and b-carotene (which, together with chlorophylls is responsible for the oil color), phytosterols, pigments, terpenic acids, flavonoids such as luteolin and quercetin, squalene is preserved.

The physiological role of vitamin E centres on its ability to react with and quench free radicals in cell membranes and other lipid environments, thus preventing (PUFA) damage by lipid oxidation (Bramley *et al.,* 2000). However, there is a disagreement between scientists on the optimum daily dietary intake of vitamin E connected with health benefits.

On the other hand, many plant oils need further elaboration such as hydrogenation in order to be useful. The use of hydrogenated oils by the food industry is decreasing in order to reduce *trans* fatty acids. *Trans* fatty acids are formed from *cis*-unsaturated fatty acids during hydrogenation. In nature, hydrogenation occurs in the rumen of cattle, and consequently, milk and beef have been sources of *trans* fatty acids. The health implications of trans fatty acids in our diet are a matter of some controversy, but it has been demonstrated that their metabolic behaviour is often similar to saturated fats.

In recent years, the interest in *trans* fatty acids was renewed due to publications reporting that high intakes of trans fatty acids increase serum LDL and lower HDL cholesterol concentrations in volunteers. *Trans* fatty acids also increase

LDL cholesterol and epidemiological studies also indicate that high intakes of trans fatty acids increase coronary heart disease (CHD) risk ((Mensink and Katan, 1990; Zock and Katan, 1992; Valenzuela *et al.*, 1995; Gurr, 1996a).

Mediterranean populations are characterised by low prevalence of coronary heart disease (CHD) despite the consumption of diets with a relative high proportion of fat (40% of total energy). It has been postulated that the apparent protection from CHD may be due to the high proportion of MUFA, relative to SFA, consumed by these populations (Keys *et al.*, 1986)

High oleic sunflower oil (HOSO) is a new vegetable oil which contains a proportion of oleic acid similar to that of olive oil, but the oils differ in their composition of minor fatty acids, in triacylglycerol species composition and sn-2 positional distribution of other minor fatty acids.

Olive oil and high oleic sunflower oil (HOSO) were used as the major fats in the diet of healthy volunteers (about 23 years old) in order to study the effects of these MUFA enriched diets on very low-density lipoprotein (VLDL) triacylglycerol composition, on fatty acid composition and sn-2 positional distribution Ruiz-Gutierrez *et al.* (1998). Results showed that both the monounsaturated enriched oils, decreased significantly the content of sn-glycerol-palmitate-linoleate-oleate, sn-glycerol-palmitoleate-dioleate-and sn-glycerol-palmitate-dilinoleate in VLDL whereas they increased the content of sn-glycerol-trioleate. In addition olive oil but not HOSO further increased VLDL triacylglycerols that contained α-linolenic and docosahexaenoic acids acylated at sn-2 -position. These results suggest that differences in the composition of VLDL triacylglycerols may be of major importance in explaining the beneficial role of dietary olive oil in reducing the atherogenic risk profile. The beneficial effect of an olive oil diet was proven also, in a study done in 1997 by the same authors who found a decrease in free and esterified cholesterol in erythrocyte membrane only after olive oil diet and not after a HOSO diet. Furthermore, the effect of a diet rich in mounsaturated fatty acid rich-oils, olive oil and HOSO on platelet aggregation on postmenopausal women (about 63 years old) with high fat diets was studied by Sanchez-Muniz *et al.* (1998). Their data suggest that other compounds present in the oils besides the fatty acid composition may play an important role in modulating platelet aggregation in postmenopausal women.

1.5. The olive fruit

The fruit of olive tree is a drupe, which is green usually becoming blackish-purple when fully ripe. A few varieties are green when ripe and some turn a shade of copper brown. The shapes range from almost round to oval or elongated with pointed ends. There is no difference, between the olive fruit and other drupes, in regard to their anatomy, since the component parts in all cases are the same:

a) the epicarp or epidermis or skin

b) the mesocarp or flesh,

c) endocarp or stone

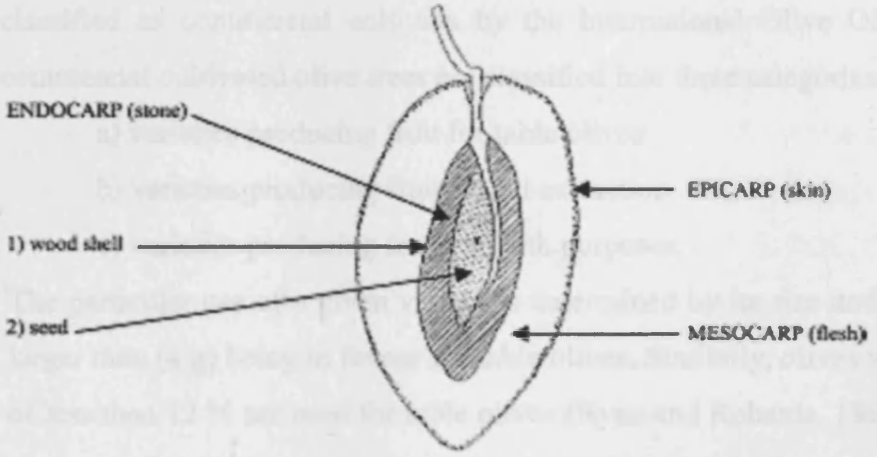


Figure 1.10. Cross section of olive fruit (adapted from Bianchi, 2003).

The endocarp or stone consists of the woody shell, which encloses the seed. The greater quantity of the oil on a wet weight basis (15-30%) is contained in the mesocarp of the olive fruit while in the endocarp the oil content is 1-3 percent. Fruit and seed oils have also differences because they have different functions in the plant. Seeds play important role in the propagation and development of plant species consequently storage substances such as lipids, carbohydrates and proteins are accumulated in order to be used by the embryo during the germination period. On the contrary, fruits play roles in the dissemination of the seeds by accumulated storage substances, which will attract animals, which then facilitate spreading the seeds (Sanchez and Harwood, 2002). Accordingly, the different parts of olive drupe, mesocarp and endocarp, produce two different kinds of oils (Cortesi *et al.*, 1997;

Cortesi *et al.*, 1999; El Antari *et al.*, 2003). Furthermore, the distribution of phenolic compounds in the various parts of the olive fruit (peel, pulp and seed) was different (Servili *et al.*, 1999; Servili *et al.*,1999). Oleuropeine glucoside, demethyloleuropeine, and verbascoside were present in all parts of the fruit, whereas luteolin-7-glucoside and rutin were present in the peel of all cultivars during all of the ripening steps studied. Nuzhenide was the most concentrated phenolic compound in the seed and was detected exclusively in this part of the fruit. In addition Ranalli *et al.* (2002) reported differences in triacylglycerol, diacylglycerols molecular species and fatty acid composition of flesh and seed of the olive fruits of seven major Italian varieties.

There are approximately 1000 recorded varieties of olives, 250 of which are classified as commercial cultivars by the International Olive Oil Council. These commercial cultivated olive trees are classified into three categories

a) varieties producing fruit for table olives

b) varieties producing fruit for oil extraction

c) varieties producing fruit for both purposes, .

The particular use of a given variety is determined by its size and oil content. Fruit larger than (4 g) being in favour for table olives. Similarly, olives with an oil content of less than 12 % are used for table olives (Ryan and Robards, 1998). However, the anatomy of the fruit and its chemical composition in all olive varieties are basically the same.

The olive fruit contains about 50% water, between 20 and 25% oil, 25% carbohydrates, cellulose, proteins and minerals. The level of these constituents varies depending on the cultivar, the growth conditions and the fruit ripeness (Solinas *et al.*, 1987b; Boskou, 1996; Nergiz and Engez, 2000; Vierhuis, 2002). In addition the oil content, fruit size and yield of olive cultivars varies between cultivars, environmental and cultural conditions. Yield and fruit characteristics vary with location and between years in the same orchard (Lavee and Wodner, 1991; Lavee and Wodner, 2004). In addition to oil, the pulp and kernel contain small amounts of various polar lipids characteristic of membranes, such as phosphatidylcholine, phosphatidylethanol alamine, phosphatidylglycerol and phosphatidylinositol as well as isoprenoid lipids including squalene, sterols and steryl esters and the pigments, chlorophylls and beta-carotene. In addition, volatile hydrocarbons, alcohols, aldehydes, ketones, and acids

are found. Olives also contain phenolic compounds and vitamins, especially vitamins E, B_1, B_2, and B_6. Significant mineral components are sodium, potassium, magnesium, calcium, manganese and iron. The amount of these constituents varies with cultivars and environmental conditions.

The flowering of the olive tree marks the beginning of fruit development and in the following months the olive fruit achieves its maximum fruit weight. Actually, after fruit set, olives grow rapidly for 30 or 40 days, after which time the seed is formed and the pit hardens. From then on, pit weight hardly changes. Pulp weight increases slowly during the summer and increases more rapidly from mid-September. Fruit growth continues to increase after the epicarp turns reddish, reaching a maximum when the highest percentages of fruits are changing colour (Gutierrez *et al.*, 1999; Loupassaki *et al.*, 1993). Dry matter acquisition is continuous and constant during fruit growth, while seed, stone, pulp and oil show specific intense periods of growth, when they can be influenced by changes in source-sink ratios. The texture changes and the activities of cellulolytic and pectinolytic enzymes can be evaluated with respect to future fruit utilization. (Tombesi, 1994).

Ripening of the olive is characterised by an increase in fruit size and changes in the skin colour, which turns from green to yellow then, usually, a reddish-violet colour and, finally, a deep violet. Olive fruit ripening is caused by physiological and biochemical pathways that can be influenced by climatic and cultural conditions even if most of the events are under strict genetic control (Tombesi, 1994).

A lot of publications are available on the changes in chemical composition of olive fruit as well as olive oil during ripening (Solinas *et al.*, 1987b; Amiot *et al.*, 1986; Amiot *et al.*, 1989; Garcia *et al.*, 1996; Aparicio and Morales, 1998; Monteleone *et al.*, 1998; Gutierrez *et al.*, 1999; Salvador *et al.*, 2001; Briante *et al.*, 2002). Generally fruit growth is a result of cell division, cell enlargement and air space formation. In the case of olive fruit we can add oil formation. The double sigmoid growth curve typical for many drupes has frequently been regarded as applicable to olive (Loupassaki *et al.*, 1993; Zarrouk *et al.*, 1996).

1.6. Biosynthesis of lipids

1.6.1. *De novo* production of fatty acids

During the last decade, interest in the biosynthesis of triacylglycerol (TAG) of plant oils has increased greatly and is summarised in a number of review articles (Harwood, 1996; Salas *et al.*, 2000; Harwood and Sanchez, 2000). The most important precursor for the *de novo* synthesis of fatty acids is acetyl-CoA. Previous studies have proposed two main mechanisms for the formation of acetyl-CoA (Harwood 1988; Harwood and Sanchez, 2000)

- The first involves the degradation of six-carbon sugars via glycolysis in the plastid, which can yield acetyl-CoA by the action of plastid pyruvate dehydroganase. Labelling experiments show that most plastids contain all the necessary enzymes to convert sucrose to acetyl-CoA and consequently synthesize fatty acids. This is the preferred route in photosynthetic tissues.
- The second route consists of acetyl-CoA production via mitochondrial pyruvate dehydrogenase, followed by cleavage of this precursor to acetic acid. The latter is then transported to the plastid, where it is re-activated to acetyl-CoA.

The contribution of both pathways to fatty acid biosynthesis has not been extensively studied so far in oil fruits. However, labelling experiments using either acetate or pyruvate suggest that both pathways take place in olive pulp (Harwood & Sanchez, 2000). *De novo* fatty acid synthesis is a process that takes place in the plastids of plants through the combined activity of acetyl-CoA carboxylase and fatty acid synthase.

The first committed step in the *de novo* synthesis is the ATP dependent formation of malonyl-CoA from bicarbonate and acetyl-CoA. Malonyl-CoA is the key intermediate in fatty acid biosynthesis (Figure 1.11)

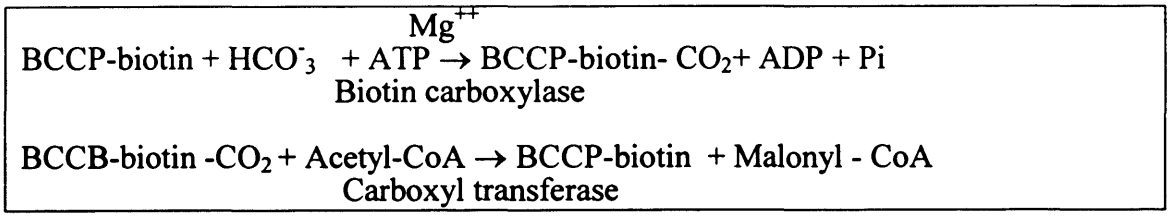


Figure 1.11. Acetyl-CoA carboxylase reaction. The two partial reactions (biotin carboxylation and carboxyl transfer) Note: BCCP, biotin carboxyl carrier protein.

The reaction takes place in two main steps, where intermediate products ADP and Pi are released before acetyl-CoA binds. In this model, the carboxylated biotin of BCCP formed at one site by the hydrolysis of ATP is able to swing to the carboxyltransferase active side (Harwood, 1988; Harwood, 1996). Malonyl-CoA produced by acetyl-CoA carboxylase can be transformed to malonyl-acyl carrier protein (ACP) by a malonyl-CoA: ACP transacylase before being used by fatty acid synthase (FAS).

In grasses there are two isoforms of acetyl-CoA carboxylases, both of which are multifunctional proteins of about 220 kDa. These proteins contain at least three domains: biotin carboxyl carrier protein, biotin carboxylase, and carboxyl transferase. The major isoform (about 85% of total leaf activity) is localised in the plastid whereas the minor form is in the cytosol of epithelial cells (Harwood & Sanchez, 2000).

In other types of plants, however, there are two different molecular forms of acetyl-CoA carboxylase. In plastids exists a multienzyme complex of four proteins. There are separate biotin carboxylase and biotin carboxylcarrier proteins. The carboxyltransferase enzyme consists of two dissimilar proteins. In contrast to the plastid, the cytosolic acetyl-CoA carboxylase in a large molecular mass (200-240 kDa) multifunctional protein.

There are two types of fatty acid synthases (FAS Type I and Type II). Research work done in different laboratories showed that FAS in plants is a multi-polypeptide Type II synthase. Three β-ketoacyl-ACP synthases (named I, II, III) are found in the fatty acid synthase of plants. The reactions of plant fatty acid synthesis are shown in Figure 1.12 β-ketoacyl-ACP synthase III (KAS III) catalyses the first condensation reaction and may determine the overall rate of fatty acid synthesis. KAS III is different from the other enzymes because it uses acetyl-CoA, rather than two acyl-ACP substrates and is not inhibited by cerulenin (Harwood, 1996). Thus, fatty acid biosynthesis starts with the condensation of malonyl-ACP with acetyl-CoA by the action of KAS III to produce acetoacetyl-ACP.

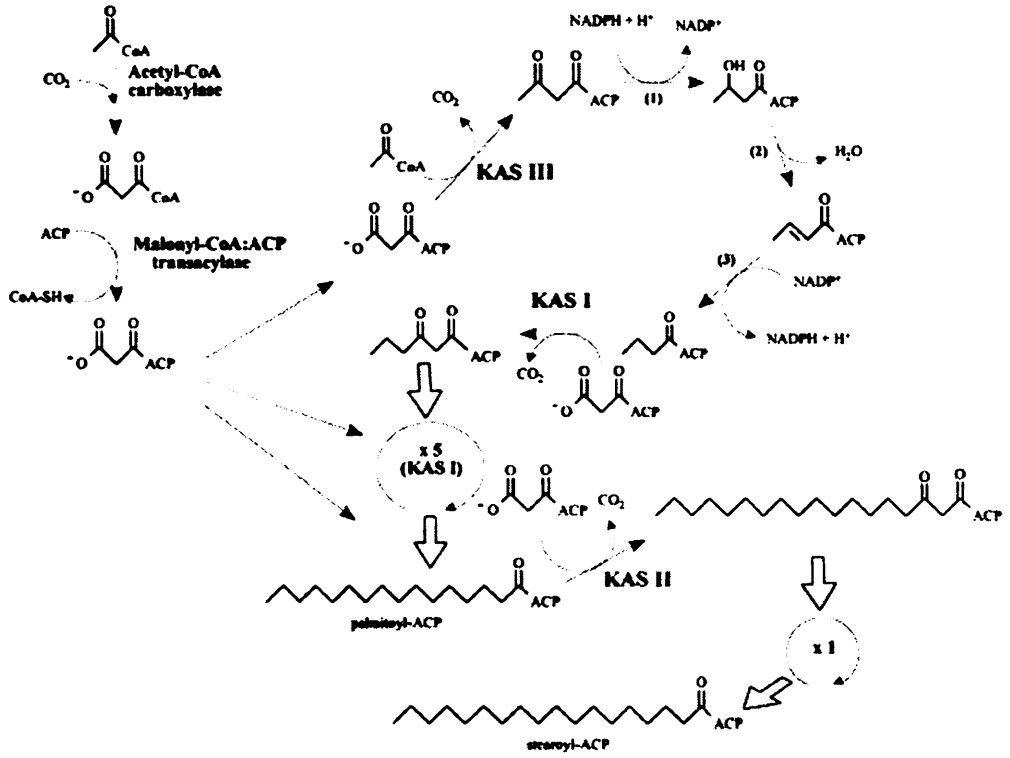


Figure 1.12. Fatty acid synthesis in plants. Adapted from Harwood and Sanchez,2002.

This compound is subsequently reduced by β -keto-ACP reductase (an enzyme dependent on NADPH) to yield the β-hydroxyacyl derivative. β-hydroxyacyl derivative is dehydrated by β-hydroxyacyl-ACP dehydrase, and the unsaturated acyl-ACP is reduced by enoyl reductase. These results in a four carbon (butyryl) acyl derivative, which can be submitted to a further elongation cycle. The next isoforms of β-ketoacyl-ACP synthase (KAS I) uses acyl-ACPs as the primer substrates instead of acetyl-CoA. This enzyme is responsible for condensations necessary to produce the acyl-chains of 6-16C, so the final product will be palmitoyl-ACP.

β-ketoacyl-ACP synthase II (KAS II) is then responsible for the formation of stearoyl-ACP from palmitoyl-ACP. It is only moderately sensitive to cerulenin but is strongly inhibited by arsenite. This step is very important because it determines the ratio of 16C to 18C products.

Therefore, the cycle of elongations involves four different reactions: condensation, reduction, dehydration and a second reduction. The enzymes catalysing these partial reactions have been purified and studied from several plants, among them the

oleaginous fruits avocado and oil palm. Furthermore the overall fatty acid synthase activity has been studied in soluble fractions of olive pulp by using radiolabeled malonyl-CoA as precursor (Sanchez & Harwood, 1992) This activity was stimulated, as expected, by ACP and inhibited by cerulenin, and also showed a strong dependence on the addition of exogenous reduced pyridine nucleotides (NADH or NADPH) and thiol reagents (2-mercaptoethanol).

Possible mechanisms for the termination of acyl chain formation can be through the activity of plastid acyltransferase or thioesterase (Harwood, 1996). Acyl-ACP thioesterases are though to play a very important role in terminating chain elongation. These enzymes contribute in the release of fatty acids from ACP so that they can be exported out of the plastid into the cytoplasm where they can be incorporated into triacylglycerols. Most species, including oil fruits, have thioesterases showing a high substrate specificity for 18C oleoyl-acyl-ACP. Fatty acyl-ACPs can also be used directly in the plastid for the production of plastid glycerolipids (*prokaryotic pathway*) as an alternative to hydrolysis, conversion to acyl-CoAs and export to the cytoplasm. The cytoplasmic acyl-CoAs are used to produce other glycerolipids via endoplasmic reticulum processing (*eukaryotic pathway*) (Browse and Somerville, 1991).

1.6.2. Modification of fatty acids

The long chain saturated fatty acids produced by d*e novo* synthesis can be desaturated either by chloroplast (plastid) desaturases or by those located on the endoplasmic reticulum. In addition, because of substrate specificities of plastid acyltransferases as opposed to the endoplasmic reticulum acyltransferases, glycerolipids synthesized in the chloroplast have an sn-1, sn-2 distribution of acids. Showing 18C at the 1-position and 16C at the 2-position. On the other hand, glycerolipids synthesized from glycerol 3-phosphate outside the chloroplast have 16C or 18C combinations at sn-1 position but only 18C acids at the sn-2 position (Harwood, 1996).

In plants there are two types of desaturases, soluble and membrane-bond. The Δ^9-desaturase is soluble and is found in the chloroplast stroma, by contrast, the Δ^{12} and Δ^{15} desaturases are membrane-bound. The desaturases introduce double bonds into fatty acid chains at specific positions (e.g. at Δ^9, Δ^{12}, Δ^{15}) to produce mono- and

polyunsaturated fatty acids, which are important determinants for oil quality (Los and Murata, 1998). Some acyl lipid desaturases use complex liquids and recognize specific polar head groups, as well as the sn- position on the glycerol backbone to which the fatty acid is esterified. Desaturation reactions take place in both plastid and endoplasmic reticulum membranes with acyl lipid substrates. The soluble Δ^9 desaturate uses an acyl (stearoyl)-ACP substrate (Harwood, 1996; Haralambidis *et al.*, 1998). All these enzymes control the insertion of cis double bonds into acyl chains. As a generalization, chemical nomenclature references the carboxyl end of fatty acid as carbon one and double bonds are introduced from the carboxyl end of the acyl chain, starting in the Δ^9- position such that oleate has its double bond in the Δ^9- position. However, desaturases could also introduce the double bond counting from the methyl end and, in the case of polyunsaturated fatty acids, from an existing double bond. However, because the exact specificity is not known for most desaturases, convention uses the Δ-numbering system.

As mentioned above, stearoyl-ACP Δ^9-desaturase is a soluble enzyme, located in the plastid stroma. This enzyme is very active in relation to β-ketoacyl-ACP synthase II and this explains why stearate does not accumulate in plants (Harwood, 1996). The significance of stearoyl-ACP Δ^9 desaturase in olive and avocado fruits can be inferred from the very high concentration of oleate in their oils. Further desaturation of oleate to produce polyunsaturated fatty acids can take place in the plastid or in the endoplasmic reticulum, via the so-called prokaryotic or eukaryotic pathways, respectively.

In many plants including olive, palm and avocado the desaturation of oleic acid involves its transportation out of the plastid to the acyl-CoA pool of the cytosol. The acyl-CoA intermediates are incorporated into phosphatidylcholine in the endoplasmic reticulum via the Kennedy pathway (see below) and the oleate acyl chains can be further desaturated to linoleate and then to a-linolenate (while attached to phosphatidylcholine) by Δ^{12} and Δ^{15}-desaturases (Salas *et al.*, 2000).

1.6.3. The Kennedy pathway

Triacylglycerols are the major storage lipids of plants and are formed via the classic Kennedy pathway (Kennedy, 1961; Harwood and Page, 1994). This involves the attachment of acyl chains to the glycerol backbone to produce glycerolipids

throughout a series of reactions that occurs in the endoplasmic reticulum (Figure 1.13.).

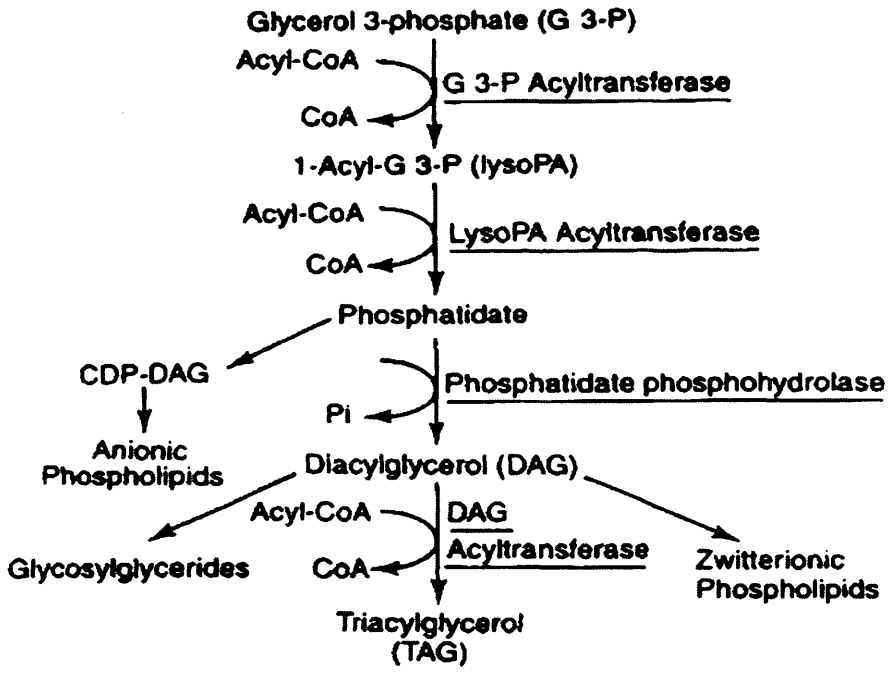


Figure 1.13. The Kennedy pathway for acyl lipid assembly.
Note: G3-P, glycerol 3- phosphate; PA, phosphatidic acid (phosphatidate); DAG, diacylglycerol; TAG, triacylglycerol.

The following enzymes catalyse the four reactions used to produce TAG. The first enzyme, glycerol 3-phosphate: acyl-CoA acyltransferase (G3PAT) catalyzes the transfer of an acyl group from an acyl donor to the *sn*-1 position of glycerol 3-phosphate. The plant cell contains three types of G3PAT, which are located in the chloroplasts, mitochondria and cytoplasm, respectively (Murata and Tasaka, 1997). The enzyme yields 1-monoacylglycerol 3- phosphate (lysophosphatidate). The second enzyme, which is 1-monoacylglycerol 3-phosphate: acyl-CoA acyltransferase (LPAAT), produces phosphatidate. Phosphatidate is a branch point for anionic phosphoglyceride synthesis whereas diacylglycerol is used for zwitterionic phosphoglyceride and glycosylglyceride formation (Harwood & Sanchez, 2000). LPAAT has been shown to be selective for unsaturated fatty acids. In safflower, linoleate was preferred to oleate with almost total exclusion of saturated fatty acids.

The first two acyltransferases of the pathway are responsible for the non-random distribution of fatty acids at the sn-1 and sn-2 positions of the glycerol backbone.

The third step in the pathway is the conversion of phosphatidate to diacylglycerol through the action of phosphatidate phosphohydrolase (PAP). This enzyme has been studied in a number of plant systems. PAP has been reported to be present in both microsomal fractions and chloroplast envelopes, and to be subject to control by translocation from the cytosol to the endoplasmic reticulum, where it is much more active (Salas *et al.*, 2000).

The final step in the Kennedy pathway is the acylation of diacylglycerol at the sn-3 position catalyzed by 1,2-diacylglycerol: acyl-CoA acyltransferase (DAGAT). The enzyme usually has a broad substrate specificity but with some degree of selectivity (Harwood and Page, 1994). Various radiolabeling experiments showed that DAGAT and, possibly, phosphatidate phosphohydrolase might limit triacylglycerol formation when lipid accumulation rates are high (Perry & Harwood, 1994). The substrate selectivity of all three Kennedy pathway acyltransferases for their acyl-CoA substrates plays a strong influence on the quality of the triacylglycerols accumulated.

In olives, the pathway of TAG synthesis has been studied in microsomal fractions both from the pulp tissue of developing olives by using radiolabelled acyl-CoAs as substrates (Sanchez *et al.*, 1992) and from tissue cultures by using radiolabelled glycerol-3-phosphate (Rutter *et al.*, 1997).

1.6.4. Storage of triacylglycerols

In olives there are two sites for oil accumulation. The mesocarp (or pericarp) tissue of the fruit is the major site of oil accumulation. In addition, TAG is also stored within the seed in oil bodies of definite size and structure through a well-known process in which oleosin protein participates (Harwood and Sanchez, 2000). Ultrastructural studies have shown that, in olive mesocarp, TAGs tend to fuse to produce oil droplets that develop with fruit maturation to a size of about 30 μm. This process probably takes place because oleosins are absent in olive pulp, so there is no limitation on oil coalescence, and explains also why olive oil can be easily extracted from olive pulp by mild mechanical methods.

1.7. Agricultural production of olives and olive oil.

Olive production involves important environmental, social and economic considerations. In recent years, olive culture has changed dramatically, due to improved farming practices and the achievement of new technologies. However, the increased requirements of productivity, quality and reduced costs has led to the necessity for more research in special areas such as cultivation techniques

The quality of olive oil is affected by several factors, such as environmental (Montedoro *et al.*, 1989; Cimato 1990; Ferreiro and Aparicio, 1992; Mousa *et al.*,1996; Tous *et al.*, 1997; Stefanoudaki *et al.*, 1999) genetic (Montedoro *et al.*, 1995; Angeroasa *et al.*, 1999; Campeol *et al.*, 2001) and agricultural (cultivation techniques, harvesting, fruit transportation and storage and processing technology). All cultural practices are aimed at raising product yields and quality.

Numerous experimental studies have been focused on defining the factors affecting quality of the oils throughout the production cycle. Montedoro (1989) reported the % contribution of each agricultural factor affecting the quality characteristics of olive oil (Table 1.2).

Table 1.2. Factors affecting the qualitative characteristics of an olive oil.

Factors	Percent contribution
Variety	20
Ripeness	30
Methods of harvesting	5
Means of transport	5
Storage methods and times	10
Extraction system	20
Olive oil storage	10

Adapted from Montedoro *et al.*, 1989.

In addition, Fontanazza *et al.* (1993) reports that environmental and genetic (cultivars) factors are among the most important. Moreover, cultural practices such as soil management, fertilization, irrigation, pruning and harvesting are the factors,

which not only affect quality of olive oil but also have an impact on pest and diseases of olive (Civantos, 1996; Campos and Civantos, 2000).

1.7.1. Growing Area

The area, in which the olive is grown, is concentrated in the Mediterranean countries. The olive tree is part of the natural Mediterranean forest together with carob, pistachio and oak. Its early domestication led to large extensive mono cultural hilly and mountainous terrains. The olive developed high adaptability to the harsh climatic conditions and soil conditions of the Mediterranean mountainous environment. Resistance to water stress, high temperature, shallow and uneven soils are among the factors affecting olive tree evolution (Lavee, 2003).

The average density (tree per ha) is very different among producing countries, being highly dependent on the availability and physical structure of the land. 87 per cent of olive oil cultivation is concentrated in the Mediterranean region, which underlines its social, ecological and economic importance for this area. With the discovery of America in 1492 the olive tree was carried to the New World; by 1560 olives were grown in Mexico, then Peru, California, Chile and Argentina. More recently the olive has continued to spread and it is cultivated in South Africa, Australia, Japan and China, and more recently, in the south west of the USA and Australia (Luchetti, 2002). As mentioned before, the olive-producing areas are found between 30° and 45° north and south latitudes, and in some areas up to about 1000 meters above sea level As a result, olive growing is characterised by considerable environmental variability in climatic, soil, agronomic, and socio-economic terms (Cirio, 1997).

Conditions are optimal for growing when the minimum temperature does not fall below -5°C. However, olive trees can withstand low temperature of -8 or -10 °C and even lower as long as they are not exposed for prolonged periods. When the winter temperature drops to –10°C the aerial part of the tree can be damaged and reconstitution of the damaged olive groves is costly for farmers. Therefore, strong limiting factors in olive growing are the minimum temperatures in winter and early spring. Current knowledge of frost resistance of olive trees is based on field observations after frost damage. Resistance to cold is influenced by the variety.

Bartolozzi and Fontanazza (1999) tested the survival to freezing stress of twelve olive cultivars/selection from different parts of Italy and one French cultivar for their resistance to frost. The plants were three-years-old, planted in pots and were subjected to four different freezing temperatures (-8°, -10°, -12° and -14°C). At the same time, laboratory analyses were used as a test to verify field observations from previous years. `Borsciona' was the most sensitive to freezing. However, visual observations of the damage confirmed `Bouteillan' to be an olive genotype with frost hardiness traits. The results presented in that work should be considered as preliminary observations, which need further confirmation. In fact, frost hardiness in three-year-old plants is not comparable with that in old plants.

Furthermore, D'Angeli *et al.,* (2003) studied and measured free cytosolic calcium signalling in the olive tree during cold acclimation and assessed the possibility of using Ca^{2+} as an early genotype-selection marker of cold susceptibility in the olive tree during cold acclimation. Their results suggested that Ca^{2+} signalling could be used as an early biochemical marker of such genotype selection. Since frost resistance is a highly complex and not well-known subject in olive, further studies on the compounds involved in this mechanism are needed. However, there are olive tree requirements for cold estimated to be 150-300 hours at temperatures less than 9° C between November and February. The action of low temperature in fact promotes differentiation in latent vegetative buds. These buds will, under the effect of hormones induced by the cold, develop into flower buds.

In addition, the effect of freezing damage to olive fruits during the harvest period and on virgin olive oil composition and quality indices, as well as the effect on the oil sensory attributes was studied by Morello *et al.* (2003). No differences were found in the quality indices of oils affected by freeze injuries, because olives were harvested and processed in a short-time period. The most important changes were observed in the minor components of olive oil, i.e. pigments and the phenolic fraction with an important decrease in the concentration of secoiridoid, which play an important role in oil stability and the sensory attributes. After frost, oils showed lower stability and important changes in their sensory attributes, leading to an absence of bitter taste and a less pungent taste.

Recently, olive growing has expanded in areas ecologically different from areas where commercial plantations now exist. Therefore a high probability of failure

exists due to different climatic conditions and especially changes in environmental temperature. The best olive production areas have mild winters and long, warm, dry summers (Noggle and Fritz, 1983; Fitter and Hay, 1987; Sibbett and Osgood, 1994). Extreme temperatures also affect the reproductive cycle of the olive (Lavee, 1996a)

While the olive tree is very tolerant on difficult environments, in order to have high yields, optimal conditions are needed (Scaramuzzi and Roselli, 1986).

1.7.2. Irrigation

The positive effect of irrigation on vegetative activity and production of olive fruits has been reported by many authors. However, the olive tree is adapted to a climate with little annual rain. Not only does the olive tree exhibit significant resistance against drought it can survive in a climate with only 200 mm rainfall and in moderately saline or calcareous soils (Ryan *et al.*, 1998). The olive tree can survive in periods of drought stress because it develops defence mechanisms (Xyloyianis *et al.*, 1999; Chartzoulakis *et al.*, 1999). However, because the distribution of rain is not uniform during the year and, in a lot of cases there is extended drought in the summer months, irrigation (particularly during the summer) has positive effects on yield, increased vegetative growth, better budding and flowering, higher percentage of fruit set and greater size of fruits (Lavee *et al.*, 1990; Michelakis, 1990; Dettori and Russo, 1993; Goldhamer *et al.*, 1994; Chartzoulakis *et al.*, 1992; Michelakis, 1995; Michelakis *et al.*, 1995).

On the other hand, in order to have high production and good yields, rainfalls of 600-800 mm are essential. The best time for complementary irrigation is during the critical stages of growth to favour new shoots and inflorescence production and good setting of fruit (Orgaz *et al.*, 1996; Tombesi *et al.*, 1996). Table 1.3 details the effects that water deficit at different periods and phenological stages can have on the olive tree.

The effect of water quantity and its time of application on the yield and water utilisation efficiency of cultivar Koroneiki in a moderately deep clay soil was studied by Chartzoulakis *et al.* (1992). Results showed that irrigation increased fruit production by increasing both the size as well as the number of the fruits. However, the oil content (% fresh weight) was higher for non-irrigated trees. It was reported by Inglese *et al.* (1996), that oil accumulation on a dry basis showed similar basic

patterns for irrigated and non irrigated trees except for the reduction of oil accumulation which was apparent under non- irrigated conditions consistently during phase C of the fruit, which occurred from 90 to 120 days after flowering. In fact irrigation during the third stage of fruit development, delayed ripening time and increased fruit size and oil content.

Table 1.3. Effect of water deficit on various stages in olive growth.

Period	Growth events	Effect of soil water deficit
February	Flower bud differentiation	Less inflorescence
to April	Flower bud development.	Higher production of incomplete
	Vegetable bud burst	flowers
	Start of shoot growth	Pestil abortion
		Decrease shoot growth
May to June	Flowering	Reduced fruit set
	Fruit set	Reduced fruit growth
	Fruit growth (cell division)	Reduced shoot growth and increased
	Shoot growth	alternate bearing
July to August	Fruit growth	Reduced fruit growth
	(Cell enlargement)	Fruit drop
	Flower induction	Permanent decrease in fruit size
September	Fruit growth	Small fruit sized
to harvest	Oil formation	Low flesh/stone ratio
	Shoot growth (trees on' off	Reduced oil yield
	' years)	Reduced shoot growth
	Build- up of reserves	Poorer-quality flowers next season.

Adapted from Tombesi *et al.*, 1996.

On the other hand, prolonged water deficit reduced fruit growth and oil accumulation rate. However, harvest time affected oil characteristics more than water availability. Fatty acid composition was not affected by the water regime but polyphenols content increased and alcohol content decreased in the oil produced by irrigated trees (Inglese *et al.*, 1996)

1.8. Extraction Technology

For the production of high quality oil with optimum organoleptic properties it is necessary for special care to be taken at each successive stage in the production process. Olive processing, therefore, is a chain with important tasks that starts at the tree and ends when the bottle reaches the consumers table. If one link of the chain is broken, the final objectives may not be reached.

1.8.1. Olive harvesting and transportation

As mentioned before, maturation of the olive fruit is a slow and long process which lasts several months and which varies according to the latitude of the growing area, the olive variety and water availability. During the ripening process, the percentage of oil on dry basis increases as the colour changes until the whole surface of the fruit becomes black. The colour change is due to formation of anthocyanins. Harvesting overripe olives beyond the point at which the entire fruit surface has turned colour results in lower oil content, more dry matter and, therefore, more olive pomace. In addition extraction yields are lower due to formation of emulsions when the oily paste is centrifuged and the quality of olive oil is inferior (Montedoro, 1989). Consequently, optimum ripeness stage is very important for harvesting in order to obtain better quality oil since fruit maturity stage may directly or indirectly affect the oil composition and quality (Frega *et. al.*, 1991; Mariani *et. al.*, 1991; Frega *et. al.*, 1992; Loupassaki *et al.*, 1993; Garcia *et. al.*, 1996). Various methods have been proposed for determining the state of olive maturity. The method for determining the ripeness stages of fruit was proposed by the National Institute of Agronomical Research of Spain, San Jaen Station and is today generally accepted as an index of maturity. The index is based on the colour and texture changes of olive fruit (see 2.1.1). According to this approach, the best harvesting period for excellent quality oil is when the maturity values are between 3.5 and 4.5.

The different ways of harvesting depend on cultural techniques, tree size and shape and orchard terrain. Olives are harvested by hand or by shakers onto nets

spread out underneath the trees. Precautions must be taken to avoid breakage during mechanical harvesting and contamination of the fruits by soil in cases where nets are used. Fruits must be transported with great care and, like other sensitive fruits, should be in perforated boxes. In order to obtain high quality oil, olives should be processed within 24 hours of harvesting. In cases where oil extraction is impossible, olives should be stored in well-ventilated places at temperature below 25°C and a relative humidity below 75% in order to avoid fermentation or mould growth. However, olive fruit storage, even if carried out in ideal conditions can have harmful effects on oil quality and this can be measured both by analytical and organoleptic indices (Perez-Camino et al., 1992; Angerosa *et. al.,* 1989; Angerosa *et. al.,* 1990; Garcia *et. al.,* 1996; Kiritsakis *et al.,* 1998; Pereira *et al.,* 2002).

1.8.2. Olive Processing

The extraction process for virgin olive oil uses one of three distinct systems for oil extraction namely, centrifugation, pressure and percolation. Pressure and centrifugation are self-sufficient because they are exhaustive processes. On the other hand, selective filtration is connected with either pressure methods or centrifugation, as it is insufficient alone for efficient processing. These systems differ not only in the physical forces applied to the paste in order to separate the oil, but also in the amount of water added during extraction. However, no matter what system is used for olive oil extraction, some processing steps are the same for all systems in accordance with the diagrams shown in Figures 1.15 and 1.16.

Olives must be crushed to release the oil. To accomplish this, a wide range of equipment has been used since antiquity; pestle and mortar or various types of rotary mills with stones and millstones (Amirante, 1993; Angerosa and Di Giacinto, 1995; Di Giovacchino, 2000; Servili *et al.,* 2002; Caponio *et al.,* 2003). Today two basic types of crushers are used:

a) Roller or stone crusher (Figure 1.14)

b) Metal crusher

Di Giovacchino, 2000; Servili *et al.*, 2002; Caponio *et al.*, 2003). Today two basic types of crushers are used:

a) Roller or stone crusher (Figure 1.14)

b) Metal crusher

Figure 1.14. Classical mill stone.

Roller or stone crushers are used in classical systems with stone mills being generally used for preparing the paste. These stones are granite and have various shapes ranging from cylindrical to truncated conical shapes. The number of stones can be from one to four according to their size and the efficiency of the operation. Roller mills can handle 300-500kg of olive, require 5-8 KW of power and operate with a speed of 10-14 rpm. The cells are well broken without the formation of emulsions. Moreover the paste doesn't warm up and is not contaminated by metals. However, the mills have disadvantages. They are too large and very expensive and crushing is slow discontinuous and time consuming. Furthermore, stones are difficult to be cleaned.

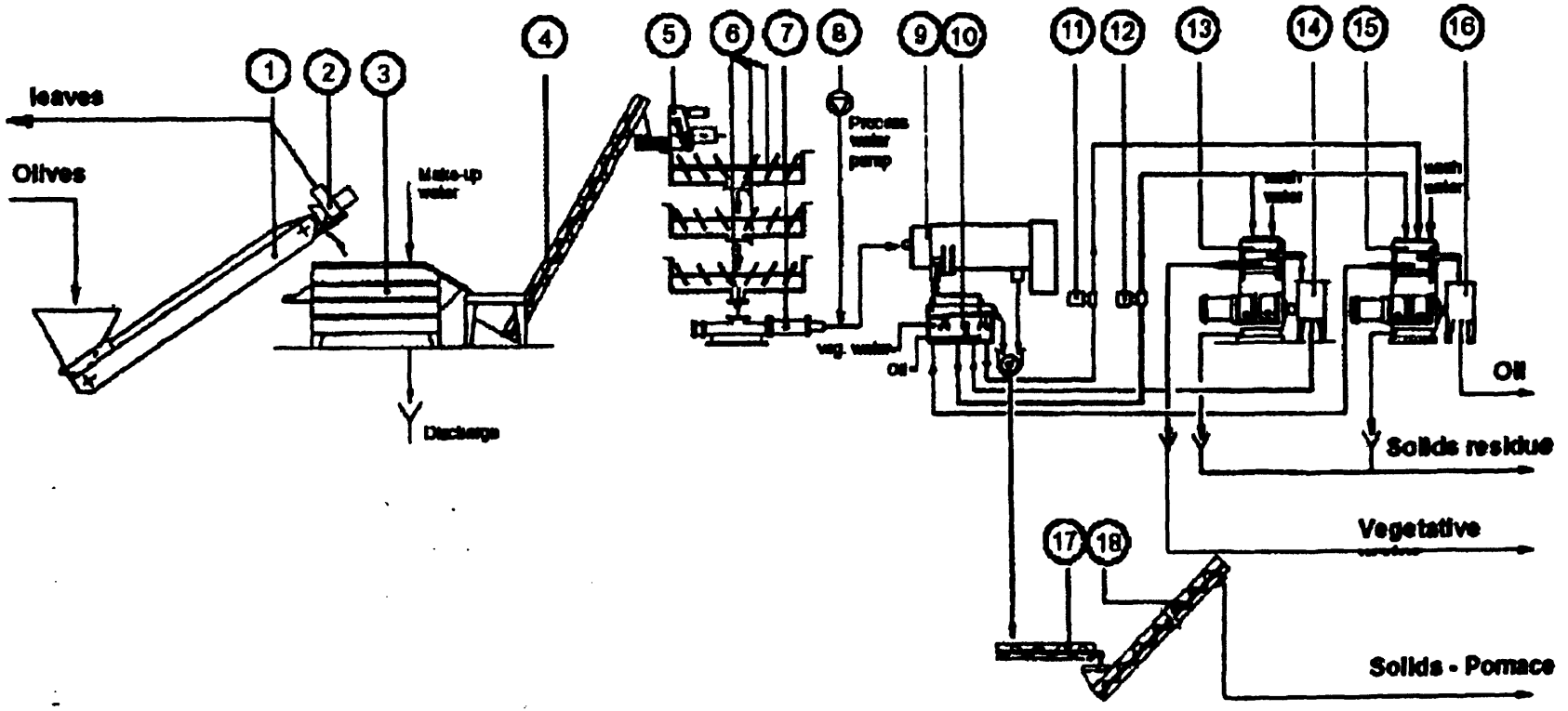


Figure 1.15. Continuous cycle olive extraction plant- Flow diagram for 3-phase decanter. Adapted from Pieralisi

Note: 1, belt elevator; 2, leaf remover; 3, washing machine; 4, screw elevator; 5, crusher unit; 6, kneader unit; 7, monopump for paste; 8, process water; 9, horizontal centrifugal decanter; 10, vibrofiltration tank; 11, oil phase feed pump; 12, water phase feed pump; 13, vertical separator – water; 14, oil recycle tank; 15, vertical separator- oil phase; 16, oil collector tank; 17, pomace screw conveyor; 18, pomace screw elevator. When operating in 2-phase extraction mode, the water phase vertical separator is not in the decanter.

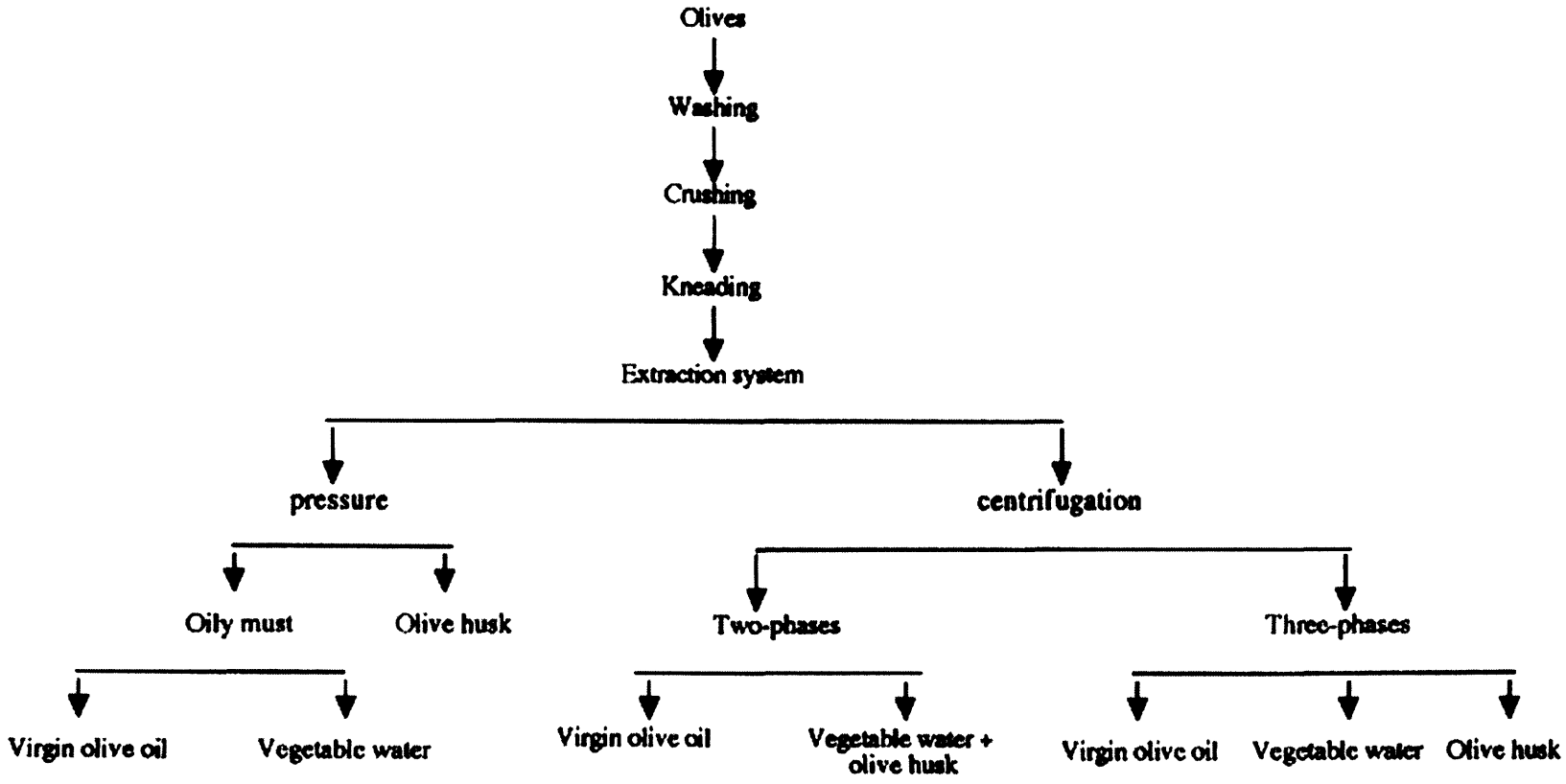


Figure 1.16. Flux diagram of the processing procedures using pressure and centrifugation systems.

In continuous centrifugation systems the olive crushing is usually carried out with a metallic crusher, such as mobile or fixed-hammer, toothed discs cones or rollers. However, most of the mills today use hammer crushers. They consist of a metal part of different shapes, which, by rotating at high speed, throws the olives against a fixed metal grating. The advantages of metal crushers are their ability to operate continuously and automatically, their high capacity, they are not expensive, tolerate debris such stones and grit and extract more phenols. The disadvantages of these crushers are the risk of emulsion formation, that the paste is not always correctly prepared and crushing is very quick and can affect organoleptic characteristics - oils can have bitter, stronger, pungent attributes due to more phenols (Angerosa and Di Giacinto, 1995; Catalano *et. al.*, 1995). In addition, the olive paste temperature is increased (Bianchi and Catalano, 1996). Crushing plays a very important role in the extraction process of olive oil. The degree of milling should be adjusted according to the olive variety and its stage of maturity. The type of crusher used and the degree of milling will also have a direct influence on later operations (Mendoza, 1975; Cert *et al.*, 1999; Koutsaftakis *et al.*, 2000).

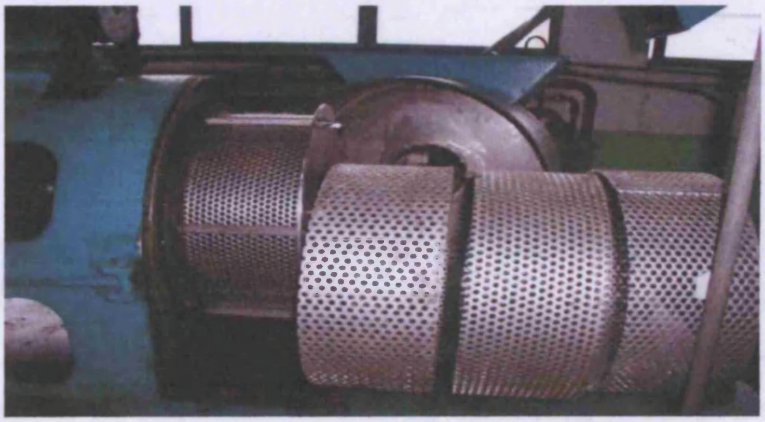


Figure 1.17. Hammer crusher with different sieves size.

It has been noted that, during traditional crushing (roller crushing) the paste temperature increases about 4 °C above the environmental temperature. In contrast, following hammer crushing the olive paste comes out at a temperature about 12 °C above the environmental temperature (Bianchi and Catalano, 1996). Furthermore, these authors studied power consumption in the hammer crushing in relation to olive delivery (Kg/h) and olive oil quality.

Several studies have shown that sensory (fruitiness) and nutritional characteristics of the oil depend on agronomic and, especially, on technological factors such as crushing and malaxation. Many important flavour compounds are not present in the vacuole oil but appear during crushing. Thus, metal crushing system have been shown to influence oil quality and, particularly, bitter or fruity tastes and the chlorophyll and phenolic contents (Angerosa and Di Giacinto, 1995; Caponio *et al.,* 1999; Caponio *et al.,* 2003). In contrast, the degree of stone-grinding does not influence oil quality very much. (Ranalli, 1998). The influence of a new crusher *i.e.* blade crusher on the quality of virgin olive oil from two different Italian cultivars (Coratina and Oliarola) was determined by Servili *et al.* (2002). Results showed that quality parameters 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 and consequently improved sensory quality for the oils obtained due to an increase of the cut grass and floral sensory notes.

1.8.3. Mixing

Paste malaxing (mixing), is a basic step (following crushing) of the mechanical olive oil extraction process and has been studied by several authors (Montedoro,1989; Di Giovachino, 1991; Servili *et al.,*1994; Servili *et al.,*1998; Angerosa *et al.,* 2001; Ranalli *et al.,* 2001; Amirante *et al.,* 2001). No matter which method is used to create the olive paste it has to be mixed. The mixing entails stirring the paste slowly and continuously in order to increase the percentage of freed small oil droplets formed during the milling to merge into large drops that can be easily separated through mechanical systems and to breaking oil/water emulsions. After crushing only 55% of the drops have diameter of more than 30 microns while after mixing the percentage is rises to 80 % (Martinez Moreno *et al.,* 1957). This phenomenon is due to the mechanical action of the equipment, which breaks the oil/water emulsions and to the activity of enzymes naturally present in the olives (Di Giovacchino, 1991). Table 1.4 shows the percentages of different size drops in olive paste after crushing and mixing.

Table 1.4. Percentage of oil drops, per diameter size, after crushing and mixing

	Diameter of oil drops (micron)					
Stage in processing	< 15	15-30	30-45	45-75	75-150	>150
After crushing	6	49	21	14	4	6
After mixing	2	15	18	18	19	25

(Di Giovacchino, 1991).

Figure 1.18. presents a mixing unit, which consists of semi cylindrical, large-capacity vats fitted with a horizontal shaft and an outer chamber for circulation of warm water. In the continuous process with 3-phase or 2-phase decanters two or three mixing units are used, depending on the industrial yield of the mill.

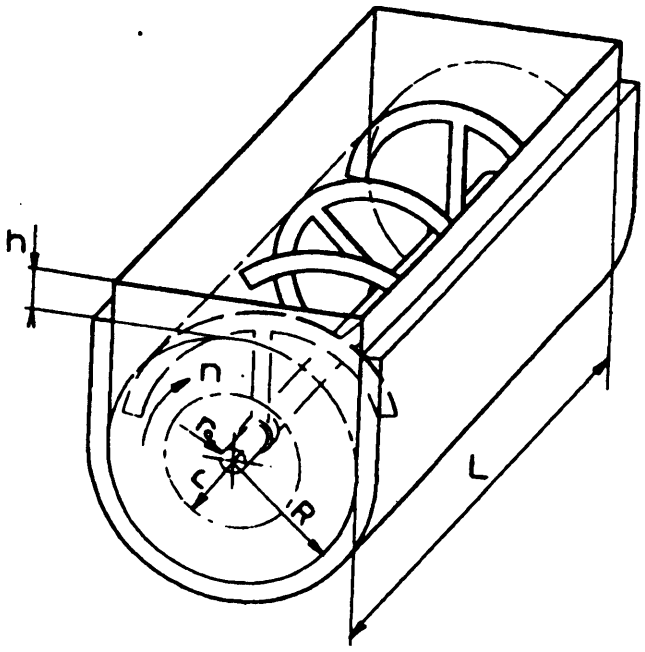


Figure 1.18. Typical mixer of olive paste fitted with a horizontal shaft that is used when the centrifugation method is employed.

1.8.4. Traditional system with hydraulic press

The classic olive oil extraction process using hydraulic pressure is the oldest method for extracting olive oil. The olive paste is squeezed between pulp mats. The structure of the system for the best extraction of the paste has to be arranged as follows: one iron disc with two or three layers of paste on a corresponding filter disc, according to the rheological characteristics of the paste. The oily must (olive+ vegetation water) separates from the solid phase as it runs through the needle onto a trolley table. Martinez-Moreno (1975) has described pressing as a filtering process, the kinetics of which are based on Carman's equation for the extraction of oily must from pastes.

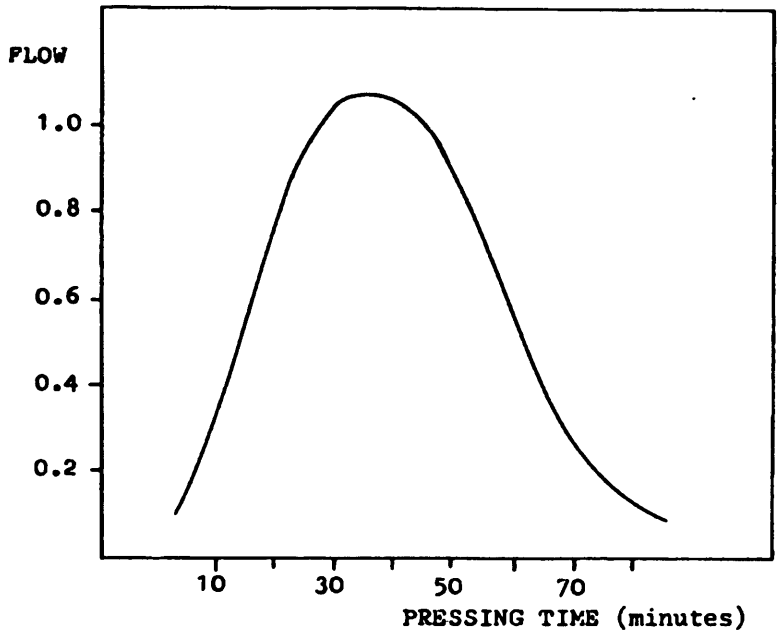


Figure 1.19. Variation of the flow (L/100 kg olives / min.) of the oily must (Vegetation water + olive oil) during olive paste processing. Adapted from Boskou, 1996

The separation of the solid phase from the liquid is by pushing the stacked load under the head of the press frame. By applying pressure the liquid phases are able to run through the olive cake and the mesh of the mat, producing two flows of oily must, one centrifugal, and the other centripetal.

The speed at which the oily must runs out of the press varies over time (Figure 1.19) The shape of the curve is affected by the characteristics of the olive fruits and the operating conditions of crushing and mixing (Di Giovacchino, 1991). The flow of the oily must is higher when the moisture of the olive paste is low. Furthermore, the non-compressible solid matter such as stones positively affects the flow rate.

The advantages of the pressure process are the following: limited investment is needed, the machine is simple and reliable, energy consumption is low and the pomace has a low moisture content so that only a small amount of vegetation water is produced. Disadvantages are: mats become contaminated, it is labour intensive and the process is discontinuous.

1.8.5. Centrifugal system.

In the beginning of the 1970's the centrifugal system was introduced to the market. This system is called three-phase because the centrifugal decanter allows the separation of three flows of matter, olive oil, pomace and vegetable wastewater.

In this method the centrifugal force is applied to the water-diluted paste in a high-speed rotor (operating at 3000 to 4000 r.p.m.). Each individual decanter has a maximum hourly capacity, (depending on its manufactured and dynamic characteristics) which must be complied with if satisfactory extraction yields are to be obtained.

The optimum amount of water needed to dilute the paste is determined by the characteristics of the olives and the centrifuge. The amount of water added can affect extraction yields. It is, therefore, necessary to achieve the right ratio between olive paste and water and optimum paste /water ratios may vary from 1:0.7 to 1:1.2.

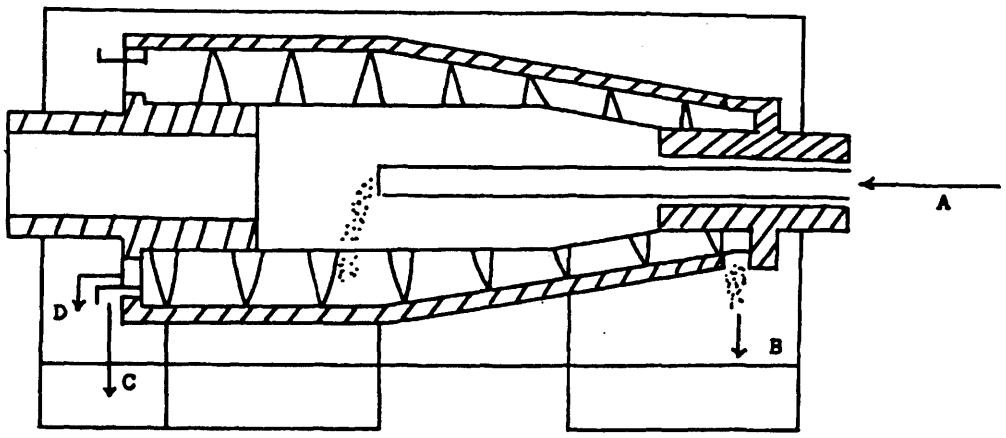


Figure 1.20. Diagram of a horizontal centrifuge: A: Supply of olive paste and water; B: pomace outlet; C: vegetation water outlet; D: oil outlet.

The 3-phase extraction method was developed in order to reduce labour costs and increase processing capacity and yield. The classical methods can process around 8-10 tonnes of olives per day whereas the 3-phase continuous systems can process 30-32 tonnes per day with a fraction of the labour requirement

On the other hand, this technology uses around 50 percent more water than the simple pressing method (around 80-100 liters of water per 100 kg. of fruit processed) and generates nearly twice as much wastewater per unit mass of fruit processed (1.7 kg wastewater per kg. olives processed compared to 0.9 in the classical method). However, the dilution of the paste causes reduction of natural antioxidants in the oil as well as increased water content of pomace.

1.8.6. Continuous two-phase centrifugal systems

Since 1992 some olive oil plant manufacturers brought to the market the two phase 'Decanters' which are able to centrifuge the oily phase without diluting it with water. However, in some cases the addition of a small quantity of water (0–20 l/100 kg of olives) to dilute the olive paste may be needed. The new method of decanting, extensively adopted in Spain and now expanded to Italy and Greece, is a modification of the horizontal three phases centrifuge. The 2-phase decanters have two products: oil and alperujo (two phase pomace – mixture of solid residue and vegetation water in the form of a semi fluid paste). However, this very wet pomace (55 and 60 %

moisture) has several characteristics that have caused new problems for the subsequent treatments and particularly in drying. This is due to higher moisture content and a lot of polyphenols (Ranalli and Martinelli, 1995) and polysaccharides. These compounds are water-soluble and are common in vegetation water, but their presence in pomace causes problems in the driers (Arjona *et al.,* 1999). The most typical effects produced by the sugars are the caramelisation and agglomeration of particles during drying. Compared to three-phase decanting, this process presents the following advantages: lower energy consumption, more antioxidants in oil and very small quantities of vegetation water.

1.8.7. Percolation (Selective Filtration)

The principle of this separation is that oil and vegetation water have different surface tensions with respect to steel plates. The system consists of about 1500 stainless steel metal sheets of 12x 2 mm. These metal sheets are attached to a very slow moving base. As the system is moving the blades get into the paste and, when they are withdrawn, they remove oil droplets. About (70-80%) of the oil contained in the olive fruit is separated by selective filtration. The yield, of course, depends on the rheological characteristics of the paste. Better results are obtained with pastes, which have lower moisture and higher solid matter contents (Di Giovacchino *et al.,* 2000). The pomace from selective filtration has a high moisture content (about 50-60 %) and oil content and must be subsequently extracted by pressing or centrifugation.

1.8. 8. Separation of olive oil from vegetation water

The final step in the olive oil extraction process (regardless of the system used) is the separation of oil and the vegetation water mixture. These two immiscible liquids have different densities and can be separated either by natural settling or by centrifugation in vertical centrifuge. Depending on the degree of separation, the olive oil can be from perfectly clear to opalescent or turbid, as a function of the customer's preference.

At present the separation of oil and vegetation water mixture is done mainly with vertical centrifuges operating with speeds of 6500-7000 r.p.m. (Di Giovacchino *et al* 2002). The vertical centrifuge is self-cleaning. This means that it automatically

discharges the fragments of pulp as the liquid is spun and separates the water from the oil.

Virgin olive oil, after production in the olive mill should be stored properly until the time of package. Storage should be in stainless steel tanks at an even temperature and purging the stored product under nitrogen is recommended. Upright tanks have a smaller surface area for the oil to be exposed and are recommended over horizontal tanks. A conical base for tanks allows any settling during storage to be monitored and oil can be drawn off above any sediment, which may have appeared.

1.9. Quality measurements of olive oil

Olive oil is one of the few vegetable oils that can be consumed in its natural form, and thus preserving all its natural constituents. Moreover, olive oil receives wide recognition throughout the world for its superior quality in comparison with other vegetable oils and for its impact on health. Quality and authenticity characteristics of the various types of olive oils are described in detail in the International Olive Oil Council trade standards (the *Codex Alimentarius*) and in the European Commission Regulation 2568/91 and its amendments. The quality of virgin olive oil is monitored via the measurement of analytical parameters for which certain limit values are set. In Tables 1.5 and 1.6 are the different parameters and limits for olive oil classifications reported in the European Commission Regulation 2568/91 and its amendment 796/2002. According to these characteristics and the limits, virgin olive oil is classified in four categories (extra virgin, virgin, ordinary and Lampante). Principal characteristics that determine the quality of olive oil are acidity, peroxide value, absorbance in the ultraviolet region and organoleptic score. Free acidity indicates the free acid content of the oil (expressed as oleic acid because of the high levels of oleic acid in olive oil) and is an important parameter in trade classification of the oil. Peroxide values and ultraviolet absorptions are measures of the extent of oxidation. Peroxides are primary products of lipid oxidation. Spectrophotometric examination in the ultraviolet can provide information also on the quality of the oil, its state of preservation and changes caused during extraction processes. Fatty acids with conjugated diene or triene systems absorb strongly in the area 230-270. Sensory analysis plays a significant role in the overall quality of virgin olive oil and is a measure of the preference of consumers.

Table 1.5. Quality characteristics of olive oil and olive pomace oil.

Category	Acidity (%) (*)	Peroxide value mEq 02/kg (*)	Halogenated solvents mg/kg (*) (1)	Waxes mg/kg (**)	Saturated fatty acids in position 2 triglyceride (%)	Stigmasta-dienes mg/kg (2)	Difference between HPLC and theoretical calculation of ECN42	K_{232} (*)	K_{270} (*)	K_{270} after alumina (3)	Delta-K (*)	Organoleptic assessment Median of defects (Md) (*)	Organoleptic assessment Median for "fruity" (Mf) (*)
1. Extra virgin olive oil	≤ 1,0	≤ 20	≤ 0,20	≤ 250	≤ 1,3	≤ 0,15	≤ 0,2	≤ 2,50	≤ 0,20	≤ 0,10	≤ 0,01	Md = 0	Mf > 0
2. Virgin olive oil	≤ 2,0	≤ 20	≤ 0,20	≤ 250	≤ 1,3	≤ 0,15	≤ 0,2	≤ 2,60	≤ 0,25	≤ 0,10	≤ 0,01	Md ≤ 2,5	Mf > 0
3. Ordinary virgin olive oil	≤ 3,3	≤ 20	≤ 0,20	≤ 250	≤ 1,3	≤ 0,15	≤ 0,2	≤ 2,60	≤ 0,25	≤ 0,10	≤ 0,01	Md ≤ 6,0 (4)	—
4. Virgin lampante olive oil	> 3,3	> 20	> 0,20	≤ 300 (5)	≤ 1,3	≤ 0,50	≤ 0,3	≤ 3,70	> 0,25	≤ 0,11	—	Md > 6	—
5. Refined olive oil	≤ 0,5	≤ 5	≤ 0,20	≤ 350	≤ 1,5	—	≤ 0,3	≤ 3,40	≤ 1,20	—	≤ 0,16	—	—
6. Olive oil	≤ 1,5	≤ 15	≤ 0,20	≤ 350	≤ 1,5	—	≤ 0,3	≤ 3,30	≤ 1,00	—	≤ 0,13	—	—
7. Crude olive-pomace oil	> 0,5 (**)	—	—	>350 (6)	≤ 1,8	—	≤ 0,6	—	—	—	—	—	—
8. Refined olive-pomace oil	≤ 0,5	≤ 5	≤ 0,20	> 350	≤ 2,0	—	≤ 0,5	≤ 5,50	≤ 2,50	—	≤ 0,25	—	—
9. Olive-pomace oil	≤ 1,5	≤ 15	≤ 0,20	> 350	≤ 2,0	—	≤ 0,5	≤ 5,30	≤ 2,00	—	≤ 0,20	—	—

1) Overal upper limit for compounds detected by electron capture detector.
For compounds detected individually the upper limit is 0,10 mg/kg.
(2) Sum of isomers that could (or could not) be separated by capillary column.
(3) For the purpose of determining the presence of refined oil, where the K270 exceeds the limit for the category concerned, it must be determined again after passage over alumina.
(4) Where the median for "fruity" is 0, the median of the defects must be not more than 2,5.
(5) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be lampante olive oil if 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.
(6) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be crude olive-pomace oil if total aliphatic alcohol is greater than 350 mg/kg and the percentage of erythrodiol and uvaol is greater than 3,5.

Table 1.6. Quality Characteristics of olive oil and olive pomace oil.

Category	Acid content						Sum of the translino-leic isomers (%)	Sum of the translino-leic and translino-lenic isomers (%)	Cholesterol (%)	Brassica-sterol (%)	Campe-sterol (%)	Stigma-sterol (%)	Betasito-sterol (%) (1)	Delta-7-Stigma-stenol (%)	Total sterols (mg/kg)	Erythrodiol and uvaol (%) (**)
	Myristic (%)	Linolenic (%)	Arachidic (%)	Eicosenoic (%)	Behenic (%)	Lignoceric (%)										
1. Extra virgin olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
2. Virgin olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
3. Ordinary virgin olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
4. Virgin lampante olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,10	≤ 0,10	≤ 0,5	≤ 0,1	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5 (2)
5. Refined olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,20	≤ 0,30	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
6. Olive oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,20	≤ 0,30	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
7. Crude olive-pomace oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,20	≤ 0,10	≤ 0,5	≤ 0,2	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 2 500	> 4,5 (3)
8. Refined olive-pomace oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,40	≤ 0,35	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 800	> 4,5
9. Olive-pomace oil	≤ 0,05	≤ 0,9	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,40	≤ 0,35	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 600	> 4,5

(1) Delta-5,23-Stigmastadienol + Chlerosterol + Beta-Sitosterol + Sitostanol + Delta-5-Avenasterol + Delta-5,24-Stigmastadienol.

(2) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be lampante olive oil if 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.

(3) Oils with a wax content between 300 mg/kg and 350 mg/kg are considered to be crude olive-pomace oil if total aliphatic alcohol is greater than 350 mg/kg and the percentage of erythrodiol and uvaol is greater than 3,5.

Notes:

(a) The results of the tests must be expressed to the same number of significant digits as that specified for each characteristic.

(b) An oil is to be placed in a different category or declared not in conformity in terms of purity if any one of the characteristics lies outside the limit laid down.

(c) The characteristics marked with an asterisk (*), relating to the quality of the oil, mean that:— for virgin lampante olive oil, these limits (with the exception of K232) do not have to be respected simultaneously,— in the case of other virgin olive oils, non-compliance with at least one of these limits shall involve a change in category, while remaining classed in one of the categories foe virgin olive oils.

(d) The characteristics marked with two asterisk (**) mean that, for all olive-pomace oils concerned, these limits do not have to be respected simultaneously.'

1.9.1. Olive oil flavour and aroma

The unique aroma of olive oil is attributed to a complex mixture of volatile and some non-volatile components. Around one hundred and eighty compounds belonging to several chemical classes have been detected from the volatile fractions of different quality virgin olive oils (Angerosa, 2002). These compounds are able to stimulate the olfactory gustatory epithelium and give rise to the perception of olfactory attributes while non volatiles, such as phenols compounds can modify taste perception.

Many analytical extraction and chromatographic techniques have been developed to carry out the analysis of volatiles and phenolic compounds. Gas chromatography coupled with mass spectrometry (GC-MS) has been used mostly to isolate as well to identify volatile compounds present in the headspace of olive oil (Montedoro *et al.,* 1978; Angerosa *et al.,* 1990; Morales *et al.,* 1994; Servili *et al.,* 1995). Today the dynamic headspace sampling in conjunction with (GC-MS) seems to be one of the most popular and suitable methods for the separation and identification of these complex combinations with evidence of linear relationships between the detected compounds and both attributes and defects of a sensory nature. The volatile compounds responsible for olive oil flavour come from the olive fruit. Some are present in the intact fruit tissue, others are secondary products related to the cell destruction during crushing of the fruits (Montedoro *et al.,* 1993; Morales and Aparicio, 1999).

The primary precursors of the volatiles compounds are 18C polyunsaturated fatty acids (particularly linoleic and linolenic acids) and amino acids (leucine, isoleucine, and valine) (Morales and Tsimidou, 2000).

Disruption of cell structure results in the release of enzymes (e.g LOXs) contained endogenously in the olive fruit, which immediately become active and promote the formation of 6C compounds through a cascade of biochemical reactions known as the lipoxygenase (LOX) pathway (Olias *et al.,* 1993; Hatanaka, 1993). The 6C compounds include saturated and unsaturated aldehydes, alcohols, ketones, hydrocarbons and esters of alcohols, which make up to 80% of total volatiles of Spanish and Italian oils analysed so far (Sanchez and Harwood, 2002). Among volatile compounds present in olive oil aromas, 6C compounds and, in particular, 6C

aldehydes show the highest concentrations. Aliphatic 6C compounds contribute to the so-called green notes characteristic of the green leaves odour in of olive oil (Flath *et al.,* 1973; Montedoro *et al.,* 1978; Olias *et al.,* 1993; Morales *et al.,* 1994). The concentration of volatile compounds present in oils changes in relation to the ripening degree and storage conditions of the fruits as well as the operation conditions during the oil extraction process (Montedoro and Garofolo, 1984; Montedoro *et al.,* 1978; Angerosa & Di Giacinto, 1995; Angerosa *et al.,* 1998; Morales & Aparicio 1999; Angerosa *et al.,* 2001). The differences in the content of volatiles produced from intact olive and cut olive fruit cv. Picual was shown in experimental work done by Morales *et al.* (1999) in order to detect if the LOX pathway becomes active when olive fruits are damaged. In intact olives only hexane was a significant volatile while the volatile profile of cut olives included many 5 and 6 carbon aldehydes and alcohols confirming that volatile compounds are produced mainly by the LOX pathway immediately after olive crushing (Figure 1.21).

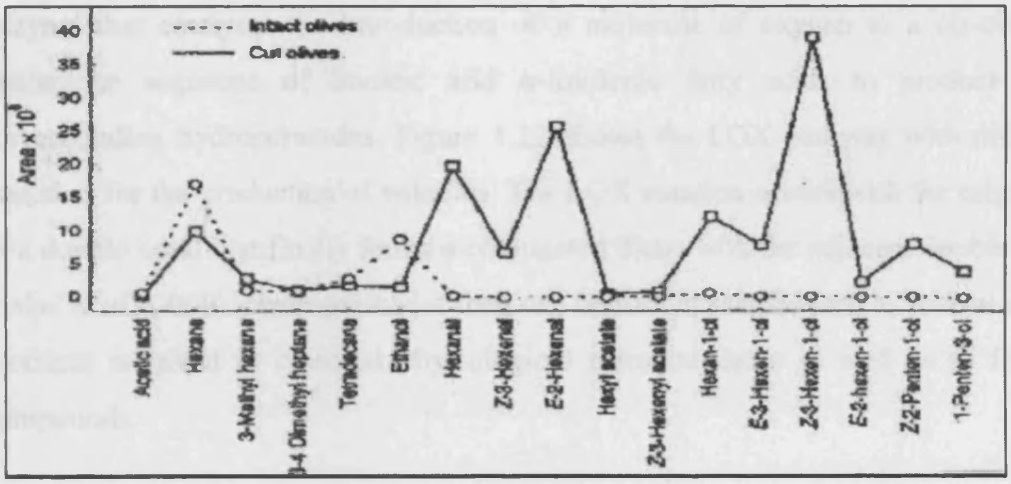


Figure 1.21. Production of some volatile compounds in cut and intact olives. Adapted from Morales and Aparicio (1999).

In addition, a strong effect of cultivar on quantitative differences in the volatile constituents of three Italian cultivars grown under identical conditions of environment and cultivation was observed by Montedoro *et al.* (1978). Furthermore, Angerosa *et al.* (1999) studied the effect of cultivar on the 6C volatile composition of the oils of seven cultivars, chosen to be among the most representative of the Mediterranean basin oil production. Oils produced from olive fruits harvested at the

same ripening degree from Picual and Koroneiki varieties, respectively, when cultivated in Spain and Greece showed similar content of volatiles to those obtained in the oils of the same cultivar in Italy. However the volatiles were different between Picual and Koroneiki showing the importance of the olive variety.

1.9.2. Lipoxygenase pathway

It is well established from experimental work that many volatile compounds responsible for the flavor of olive oil are enzymatically produced from polyunsaturated fatty acids (particular linoleic and α-linolenic) through the cascade of reactions described in the literature as "lipoxygenase pathway" (Vick & Zimmerman, 1987; Hatanaka, 1993; Olias *et al.*, 1993). Many enzymes are involved in this pathway and some of them were recently isolated and characterised in olive pulp (Salas and & Sanchez, 1998 a, b, c). The qualitative and quantitative concentration of 6C products is highly dependent on the level of enzymes involved in the LOX pathway and on their activity. Lipoxygenase (LOX) is a non-heme iron-containing enzyme that catalyses the introduction of a molecule of oxygen to a cis-cis 1,4-pentadiene sequence of linoleic and α-linolenic fatty acids to produce their corresponding hydroperoxides. Figure 1.22 shows the LOX pathway with different branches for the production of volatiles. The LOX reaction occurs with the migration of a double bond that finally forms a conjugated diene with the adjacent double bond (Salas *et al.*, 2000). Hydroperoxides then can be further metabolised to several active products involved in essential physiological roles in plants as well as to flavour compounds.

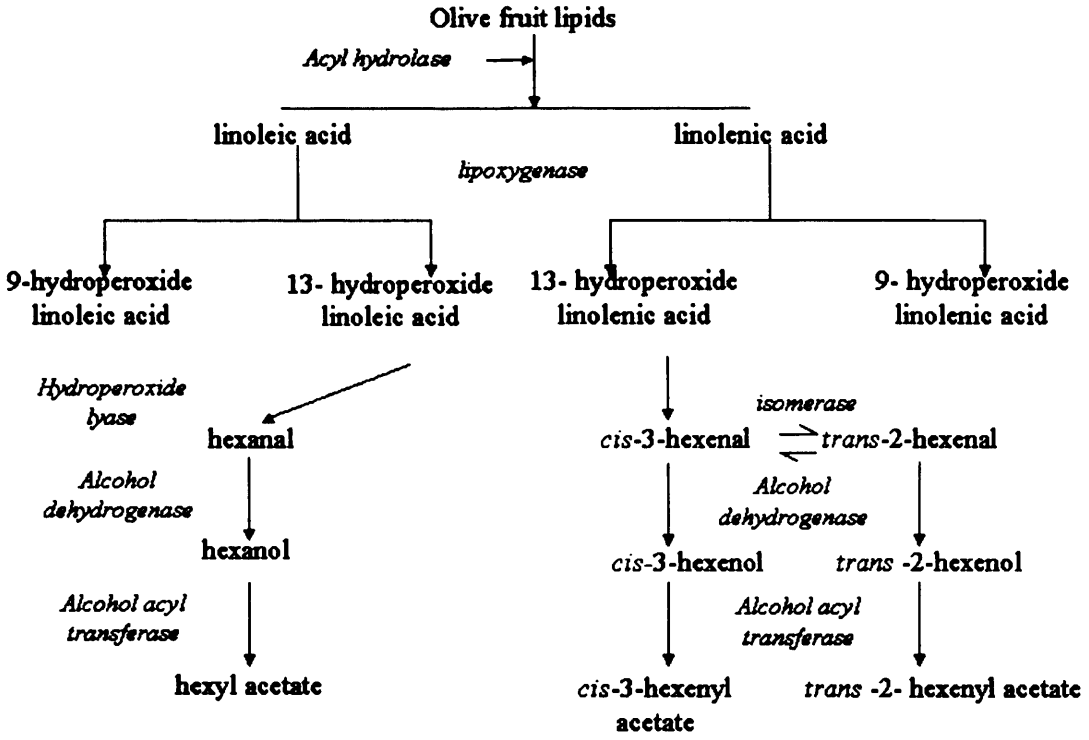


Figure 1.22. Lipoxygenase pathway for volatile production.

Through the action of hydroperoxide dehydratase, allene oxides are formed, which can be metabolised further to yield jasmonic acid. Jasmonic acid (JA) is a plant growth regulator, and plays a major role in the plant's response to many stress conditions. Alternatively peroxygenase produce fatty acid hydroperoxides that are involved in the biosynthesis of cutin monomers (Blee and Schuber, 1993)
Lipoxygenase also catalyses the anaerobic cleavage of n-6 (S)- hydroperoxy linolenic acid to produce a 13C-oxoacid (13-oxo-12, 9-tridecadienoic acid) and a 5C alcohol (*cis*-2- pentenol or 1-penten-3-ol) (Figure 1.23) (Salas *et al.*, 1999). The formation of the *cis*--2- pentenol present in olive oil is due to this reaction (Morales *et al.*, 1995; Angerosa *et al.*, 1998). In addition, lipoxygenase can catalyse the formation of other secondary products such as ketodienes, fatty acid dimmers and hydrocarbons under anaerobic conditions.

A key reaction for the generation of sensory compounds is that of hydroperoxide lyase. Hydroperoxide lyase cleaves the fatty acid hydroperoxide molecule of the C-C bond located between the hydroperoxide function and the

double bond with *trans* configuration in the 13(S) or 9(S) hydroperoxide of linoleic and linolenic acid. The products of this reaction are a volatile aldehyde and a non–volatile oxoacid. The Oxoacid may be a precursor of traumatic acid, a wound hormone.

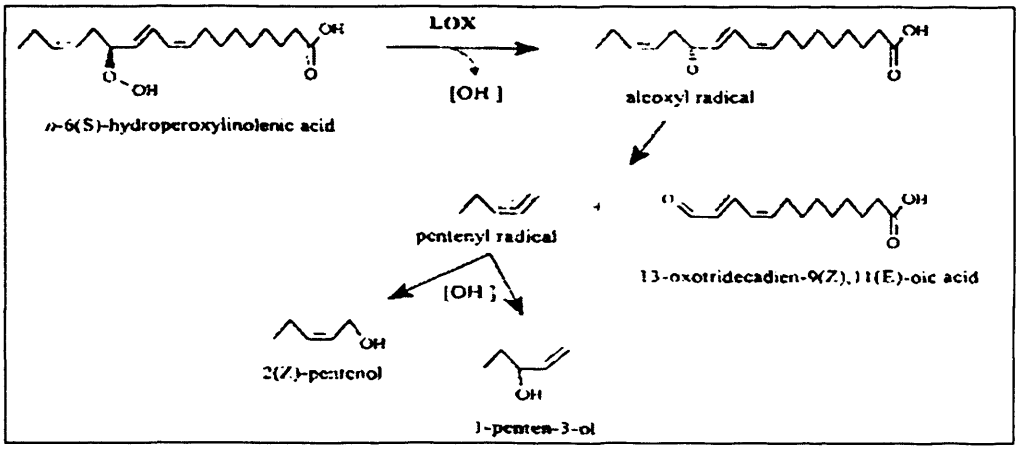


Figure1.23. β-Scission of the n-6 (S)- hydroperoxylinolenic acid induced by lipoxygenase. Adapted from Sanchez & Salas (2000).

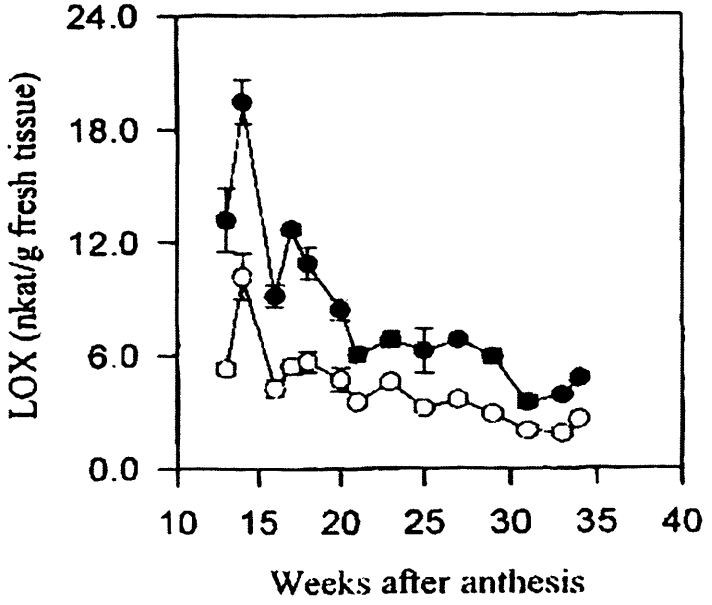


Figure 1.24. LOX changes during the development of the olive fruits. The activity was measured using both linoleic (o) and linolenic(•) acids as the substrates. Adapted from Salas *et al.* (1999).

In research done by Salas et al. (1999) lipoxygenase activity in olive fruits was measured and characterized in order to determined its significance for the

biosynthesis of virgin olive oil aromas. In their experiments, LOX activity was measured at different stages of fruit ripeness from 13 to 34 weeks after anthesis. Maximum activity was observed about 15 weeks after anthesis, with a steady decrease at later stages of fruit maturation (Figure 1.24). In addition, under standard conditions, LOX activity measured in particular fractions of olive pulp ranged between 0,5 and 5 nkat/g fresh tissue, depending on the development stage of the fruit.

Changes noticed in the concentration of LOX during the ripening of olive fruits were in good agreement with the volatile profile of oils produced at different stages of maturation and, especially, in overripe fruits which showed a decrease in hexenal and hexenols (Aparicio and Morales, 1998). Regarding the substrate specificity of this enzyme, results showed that olive LOX oxidizes free unsaturated fatty acids at a much higher rate than esterified ones (between 50- and 100-fold higher), which, of course, means that its preferred substrates must be released by acyl hydrolase activity. Furthermore, experiments carried out with different fatty acids to investigate olive lipoxygenase substrate specificity have shown that it has a preference for α-linolenic acid (Table 1.7) (Sanchez & Salas, 2000).

Table 1.7. Relative substrate activity of lipoxyganase from the microsomal fraction from olive pulp.

Substrates	Lipoxygenase relative activity (%)
Linoleic acid	61
α-Linolenic acid	100

Adapted from Sanchez & Salas, (2000).

Olive LOX regiospecificity was investigated by analysing hydroperoxide products resulting from incubation of linoleic or α-linolenic acid with olive membrane fractions (Salas *et al.*, 1999). Studies indicated that the olive pulp lipoxygenase showed a high regiospecificity for position Δ-13 of the linoleic and α-linolenic fatty acids yielding preferentially the Δ-13 –ZE(Z) isomers (Table 1.8). This isomer is the precursor of six-carbon aldehydes via the hydroperoxide lyase reaction.

Table 1.8. Regiospecificity of the olive fruit lipoxygenasea

	Hydroperoxide isomers (%)			
Precursor	13-ZE(Z)	13-EE(Z)	9-EZ(Z)	9-EE(Z)
Linoleic acid	67	11	17	7
Linolenic acid	73	15	12	N.D.

The 15K membrane fraction isolated from fruits harvested 28 wk after anthesis was used as the source of enzyme. The concentration of fatty acid substrates was 1 mM. N.D, not detected. Adapted from Salas *et al.*, (1999).

The activity of lipoxygenase from olive fruit pulp in different membrane fractions showed optimal pH at the range 5-5.5 (Figure 1.25), which is very close to that of the olive paste during malaxation. Therefore, during the extraction procedure of olive oil there are optimum conditions for the enzymatic formation of hydroperoxides

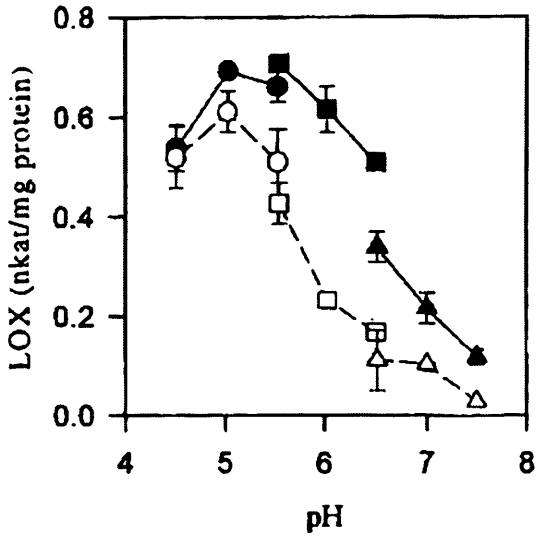


Figure 1.25. Effect of pH on lipoxygenase (LOX) activity of particulate fractions from olive fruit pulp; 3K (—) and 150 K (----) fractions from olives harvested 28 wk after anthesis. Results are means of three determinations + SD, carried out using linoleic acid as substrate. Sodium acetate (●○), MES(□, ■), and potassium phosphate (▲,Δ) were used as the assay buffers, Abbreviations: K,1000x g ; nkat, nmoles of

substrate transformed per second under reaction conditions; MES, 2 –(N-morpholino) ethanesulfonic acid. Adapted from Salas *et al.*, (1999).

1.9.3. The cleavage reaction: hydroperoxide lyase (HPL)

Fatty acid hydroperoxide lyases (HPLs) catalyse the cleavage of polyunsaturated fatty acid hydroperoxides at the bond situated between the carbon atom carrying the hydroperoxide group and the adjacent (E) double bond (Salas *et al.*, 1999). The products of this reaction are volatile aldehydes of six or nine carbon atoms (depending on the positional isomer cleaved) and non- volatile oxoacids of nine or twelve carbon atoms. Hydroperoxide lyase is the first enzyme of the LOX pathway producing volatile compounds. Olive HPL is a membrane-bound enzyme located mainly in the chloroplast thylakoid fractions. Two isoforms of HPL with similar characteristics have been purified from olive pericarp. Both showed optimum pH values at 6.0 and identical substrate specificities. Only the n-6-hydroperoxides of linoleic or a-linolenic acid were substrates of these enzymes (Table 1.9). The specificity of these enzymes gives an explanation for the total absence of 9C aldehydes in olive oil (Salas et al., 1998). Moreover, 6C unsaturated aldehydes produced from n-6-hydroperoxylinolenic acid (which is the preferred substrate of olive HPLs) are the most abundant in olive oil aroma. Furthermore, olive HPL is 2.5-fold more active with n-6 –hydroperoxides of linolenic acid than the analogous isomer of linoleic acid (Salas and Sanchez, 1999). This explains why 6C unsaturated volatiles compounds are more abundant than saturated ones in the aroma of virgin olive oil

Table 1.9. Substrate specificity of hydroperoxide lyases partially purified by ion – exchange and hydroxylapatite chromatography from olive pulp.

Substrate	HPL-I Relative Activity (%)	HPL-II Relative Activity (%)
n-6-hydroperoxylinolenic acid	100	100
n-10-hydroperoxylinolenic acid	0	0
n-6-hydroperoxylinoleic acid	42	38

n-10-hydroperoxylinoleic acid	0	0

Adapted from Sanchez & Salas (2000).

The highest activity of HPL was observed at the beginning of fruit growth and, after that time, it decreased slightly although it maintained a high level during the whole maturation period. Therefore, the decrease in the content of 6C volatile compounds during olive ripening cannot be attributed to a lower HPL activity of pulp. Moreover, HPL is more easily inactivated at higher temperatures than LOX, so that extraction conditions during processing may be important for volatile production (Figure1.26).

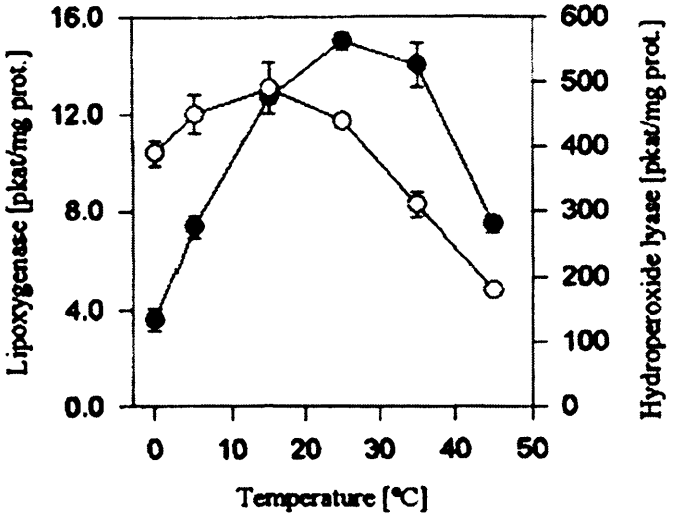


Figure 1.26. Higher temperatures inactivate lipoxygenase pathway enzymes at different rates. LOX (filled circles) and HPL (empty circles) activities were determined by using end-point colourimetric methods after 30 min incubation
Adapted from Salas & Sanchez (1999).

1.9.4. Alcohol dehydrogenase

Alcohol dehydrogenases (ADHs), alcohol: NAD oxidoreductase and alcohol: NADP oxidoreductase) catalyze the reversible reduction of volatile aliphatic aldehydes to their corresponding alcohols in a reaction dependent on pyridine nucleotides. ADHs are found in different plant species and are also involved in the synthesis of cinnamic acid and resistance to anoxia. Volatile alcohols present in the aroma of olive oil, which have been identified as responsible for the characteristic «green note», are formed from the corresponding aldehydes by the action of NADP-

dependent ADHs present in the pulp tissues of developing olives (Salas and Sanchez, 1998). Total activity in the crude extract was found to be 20-fold higher with NADPH than with NADH. However, both NAD and NADP-dependent ADH activities have been detected. Three ADH isoforms were purified, one dependent on NAD (NAD-ADH) and two dependent on NADP (NADP-ADH I and NADP-ADH II) Among them, only the enzyme NADP-ADH II displayed high affinity for the 6C aldehydes produced through the LOX pathway. The highest activity of NADP-ADH II levels was found 25 weeks after anthesis, when more than half of the final oil content had accumulated in the green fruits. The enzyme showed an optimum pH value of 8.5, which explains the low ratio of alcohols to aldehydes in the aroma composition of oil, since the olive paste has an acidic pH. Thus one would expect that the activity level of this enzyme would be low during olive oil manufacturing (Salas & Sanchez.1998).

1.9.5. Alcohol acyltransferase

The volatile alcohols produced by the ADHs can be esterified with acyl-CoA moieties to produce volatile esters by the action of alcohol acyltransferase (AAT). The esters formed give rise to sweet, green and fruity notes in olive oil. Olias *et al.* (1993) reported that tissues from olive fruits are able to form hexyl acetate upon incubation with hexyl alcohol and acetic acid. Recently, alcohol acyltransferase activity was measured in enzymes extracts prepared from developing olives by using hexanol and acetyl-CoA substrates (Salas and Sanchez, 2000). The activity of the latter enzyme in olives was low with an optimal pH value of 7.5 (Salas, and Sanchez, 1999). The low level of enzyme activity during the extraction process (pH of olive paste is 4.5 to 5) explains the low concentration of volatile esters in olive oil compared to aldehydes or alcohols.

1.10. Analytical Methods (including detection of adulteration)

Olive oil quality control is necessary not only because of its connection with good health but also due to the high price commanded for extra virgin and virgin olive oil especially in years of low production. The high price constitutes the main driving force behind frequent commercial fraud. Adulteration of olive oil includes addition of seed oils, refined olive oil, olive residue oils, synthetic products prepared from olive oil, fatty acids recovered as by-products in the refining process, and high oleic acid oils (hazelnut oil, sunflower oil, palm olein) which are desterolized by strong reffination (Firestone et al., 1988; Salivaras and McCurdy, 1992; Boskou, 1996; Aparicio and Aparicio-Ruiz, 2000; Christopoulou et al., 2004). Proof of authenticity is an ultimate but often unattainable goal of all adulteration studies. It must be recognised that, while the existence of adulteration can be demonstrated, the absence of adulteration can never be proved.

Recently significant progress has been made in analytical methods and chemical techniques that can be used in order to ascertain the purity and quality characteristics of olive oil. The development of instrumentation and the special methods for the detection of minor components have contributed a lot in the fight against adulteration.

Traditional tests such as iodine number, refractive index, saponification value and unsaponifiable matter have very limited value because today it is easy to prepare fraudulent mixtures that have characteristics which fall within the accepted limits for olive oils. This is especially a problem with olive oil because being a natural product, there is variation in the content of individual components in the oil due to the effect of variety, soil, climate, fruit ripeness, agricultural practices, pest attack, harvest and storage conditions and the system of extraction. (Montedoro and Garofolo, 1984; Cimato, 1990; Kiritsakis, 1990). As mentioned before, the quality and authenticity characteristics of the various types of olive oils are described in International Olive Oil Council trade standards (the *Codex Alimentarius*) and the European Commission Regulations .In Tables 1.5 and 1.6 the limits established for each criterion are presented.

The determination of UV absorption at 270 nm provides a measure of the state of oxidation of the oil. Further examination of the UV absorption spectrum can

be used as criteria for authenticity (Kapoulas and Andrikopoulos, 1987; Passaloglou-Emmanouilidou, 1990). Refining causes formation of conjugated dienes and trienes and increased values of 232 and 270 nm may indicate the presence of refined oils. However, interpretation of results based on UV absorption is not always easy (Boskou, 1996) although the method is useful when adulteration exceed 10%. Fatty acid composition by GLC can be used as a routine analysis for the purity of various edible oils and fats (Ulberth and Buchgraber, 2000). Christopoulou *et al.* (2004) could detect adulteration of olive oil with vegetable oils (soyabean, walnut, canola, rapeseed, peanut and mustard) above a level of 5% by using fatty acid and triacylglycerol determinations. Addition of seed oils (canola, sunflower oil, soybean oil) to olive oil can be detected by an increase in both trilinoleate and dilinoleyl-linolenate, which have limits (< 0.5%) in olive oil.

Analysis of minor components in the unsaponifiable matter can be used as a useful tool for the identification of oils, and thus may be helpful for the detection of foreign oils in olive oil. The presence or absence of long-chain aliphatic and triterpenic alcohols, methylsterols, sterols and triterpenic dialcohols are important for the characterization of vegetable oils. As it is well known, the concentration of sterols is very important for the detection of adulteration of olive oil with seed oils. The sterol composition of extra virgin olive oil is very characteristic and, thus, has become a helpful tool to detect adulterations with other vegetable oils.

The limits of each sterol concentration in olive oil and olive pomace oil have been established by the IOOC and the European Community and are useful for the detection adulteration in olive oil. However, significant differences and, in some cases, deviations from the above established limits have been found in certain samples of olive oil this can be due to olive variety and is reflected in both the olive oil composition and the pomace oil. (Christopoulou *et al.*, 1996).

According to Gomez, (1992) oligopolymeric compounds can verify the level of oxidative and thermal degradation of olive oil. Data obtained from oxidative degradation and from thermal and catalytic activity at certain stages during refining showed that oligopolymers were absent in extra virgin olive oil but were present in refined olive and pomace oils. The mean level of oligopolymers in refined oils was 0.7 % and was more than twice as high in refined olive pomace oils. Gomez (1992)

concluded that the absence of oligopolymers in virgin olive oils could be used as a quality standard for detection its possible adulteration with refined oils.

Beaten *et al.* (1996) have found that the combination of Fourier transform- Raman (FT-Raman) spectroscopy with multivariate procedures can be used for predicting the level of adulteration in a set of virgin olive oil samples that were adulterated with soybean, corn and raw olive residue (olive pomace) at 1.5 or 10% respectively. Results showed 100 % correct discrimination between the genuine and adultered samples and 91.3 % correct classification at different adulteration levels. They also concluded that FT-Raman) spectroscopy could be useful in detecting adulteration beyond the limits achieved by techniques suggested by the EC standard methods (1995)

In addition Beaten *et al.* (1998) analysed one hundred and thirty-eight samples from 21 different sources (vegetable and animal sources) The results showed that FT-Raman spectra not only provide information of the degree of unsaturation but also the balance among the amounts of saturated, monounsaturated and polyunsaturated fatty acids. The scattering intensities near different Raman shifts (3013, 1663 and 1264 cm-1) showed a high correlation with the fatty acid profile determined by gas chromatography. Therefore, if instrumentation is available, the FT-Raman spectroscopy is useful for analysis as well as being important for the detection of adulteration.

1.11. Deterioration of olive oil.

It is well known that most fats and oils are susceptible to deterioration. This deterioration may be caused by contact with unsuitable materials, by prolonged contact with aqueous impurities and by oxidation. The main processes involved in olive oil deterioration are hydrolytic rancidity or hydrolysis and oxidative rancidity. The former is caused by lipolysis while oxidation may be autoxidation or photooxidation (Kiritsakis, 1990).

1.11.1. Hydrolytic rancidity (lipolysis).

In lipolysis triacylglycerols are split into glycerol and free fatty acids. Lipolysis is the only source of generation of free fatty acids. The determination of free non-esterified fatty acids (reported as mass of free oleic acid as a % of total oil) is the simplest method of monitoring hydrolytic acidity.

For olive oil, lipolysis usually starts in the fruit due mainly to enzymes in the fruit or exogenous lipase from microbial infection. It may be accelerated by damage during harvesting and storage of olive fruits before processing (Kiritsakis, 1990; Kiritsakis and Tsipeli, 1992). Limited lipolysis will also occur during processing. Factors affecting hydrolytic rancidity are enzyme content, fruit contamination, temperature and conditions during processing.

1.11 2. Autoxidation

Olive oil becomes rancid as a result of oxidation, which begins after the oil is obtained from the fruit and during storage. Rancidity is the unpleasant flavour that results from the accumulation of decomposition products of the oxidation reaction. The initial volatiles responsible for the pleasant olive oil sensory characteristics are produced mainly through the lipoxygenase pathway. These can disappear during oxidation and be replaced by other volatiles (Solinas *et al.*, 1987a; Morales *et al.*, 1997) with a consequent decrease in nutritional quality and safety caused by the formation of secondary compounds (Moure *et al.*, 2001). It is an extremely complex process involving numerous reactions, which proceed via a free radical chain mechanism involving three stages. The initial products of autoxidation are primary unstable hydroperoxides, which readily decompose to a range of products (Figure

1.27), particularly mixtures of volatile aldehyde compounds (Morales and Przybylski, 2000).

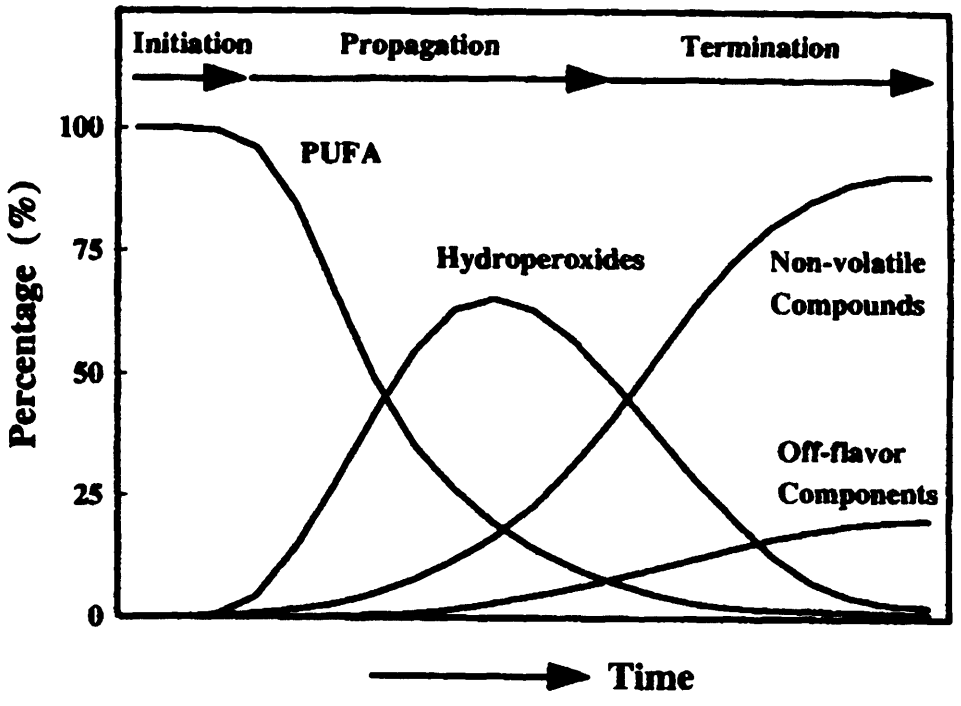


Figure 1.27. Oxidation of polyunsaturated fatty acids (PUFA) and formation of primary (hydroperoxides) and secondary (non-volatile and off- flavor components) products. Adapted from Morales and Tsimidou (2000).

The structure of fatty acids, i.e. their unsaturation degree and the position of the double bonds are main factors determining the rate and the mechanism of autoxidation. In contrast to saturated fatty acids, polyunsaturated fatty acids are highly susceptible to oxidation. The rate of oxidation increases geometrically with the number of double bonds. In addition, it has been established that when the unsaturated fatty acids are in the form of esters of glycerol (natural glycerides) the autoxidation rate is much lower due to an increase in steric hindrance and a decrease in the mobility in the molecule (Yanishieva-Maslarova, 1985). Therefore, release of fatty acids will affect the rate of autoxidation. In addition, the process is controlled by oxygen, temperature, light and metal content (especially Cu and Fe).

Mechanism of autoxidation

The autoxidation of unsaturated fatty acids can be divided into three phases: initiation, propagation and termination.

Initiation

$$RH + Initiator \rightarrow R^{\bullet} + H^{\bullet}$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

Propagation:

$$ROO^{\bullet} + R_1H \rightarrow ROOH + R^{\bullet}$$

Termination:

$$R^{\bullet} + R^{\bullet} \rightarrow RR$$

$$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$$

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$$

$$2RO^{\bullet} + 2ROO^{\bullet} \rightarrow 2ROOR + O_2$$

RH : refers to any unsaturated fatty acid and the H is adjacent to a double bond

$R^{\bullet}$ and $ROO^{\bullet}$: refer to free radical.

ROOH: hydroperoxides

RR and ROOR: refer to inactive end products.

Free radicals react with oxygen to produce peroxide radicals ($ROO^{\bullet}$). Peroxide radicals have the ability to attack another fatty acid RH: the result is peroxide and a new free radical. The quantity of these highly reactive compounds rises constantly until they begin to interact.

Regardless of the mechanism of formation, hydroperoxides will decompose further into a high variety of compounds of different polarity, stability, and molecular weight. Depending on the mode of this reaction the secondary oxidation products may be aldehydes, ketones, alcohols, hydrocarbons or polymeric compounds.

Autoxidation is an irreversible process, which it is not possible to stop completely, but may be prevented or delayed in different ways:

1 Initiation energy -light and high temperature can be excluded by storage of oil in a cool, dark place

2 Heavy metals: traces (even in very small concentrations of 0.1to 1 ppm) of these catalysts will decrease olive oil stability.

3 Oxygen has to be substantially excluded.

4 Radicals can be inactivated, by combining them with agents known as radical scavengers. These compounds interrupt the chain reaction either by donating one hydrogen atom to a free radical or by combining directly with radicals to form inert products. Oxygen and radical scavengers are often called antioxidants (Valenzuela, and Nieto 1996)

1.11.3. Photooxidation

Photoxidation requires the presence of substances known as photosensitizers or chromophores, which can capture the energy of light (Carlsson *et al.,* 1976). This energy can be transmitted to oxygen molecule which is present in the triplet state ($^3O_2$), converting it to a more active singlet state that reacts directly with the double bond of fatty acids by a symmetrical addition known as ''ene '' reaction. Oxygen in the singlet state is about 1500 times more reactive with linoleic acid than oxygen in the triplet ground state at ambient temperature

Olive oil is very sensitive to radiation between 320-700 nm because of their chlorophyll content. The prooxidant activities of chlorophyll and pheophytin on olive oil, seed oils and fatty acid esters has been demonstrated (Carlsson *et al.,* 1976; Kiritsakis and Dugan 1985; Fakourelis, 1985; Fakourelis *et al.,* 1987.) They observed that virgin olive oil oxidised faster in the light than in the dark. Light deterioration is due to the presence of photosensitisers or chromophores and to photodecomposition of hydroperoxides.

Mechanism of photoxidation:

^{1}Sensitiser + hv $\rightarrow$ (^{1}Sensitiser)* $\rightarrow$ (^{3}Sensitiser)*

(^{3}Sensitiser)* + $^3O_2$* $\rightarrow$ $^1O_2$* + ^{1}Sensitiser

$^1O_2$* +RH $\rightarrow$ RCOOH $\rightarrow$ free radical

Where:

(^{1}Sensitiser)*: activated single state sensitiser

(^{3}Sensitiser)*: activated triple state sensitiser

$^1O_2$*: activated single state oxygen

$^3O_2$*: activated triple state oxygen

It is characteristic of photosensitised oxidation that no significant difference of reactivity was observed among 18:1, 18:2, and 18:3. On the other hand, there are great differences in the oxidation rate among the three fatty acids in free radical oxidation. It is noteworthy to say that photooxidation cannot be preventing or delayed by the addition of antioxidants, but only by single state oxygen quenchers such as nickel chelates, β-carotene and α-tocopherols (Carlsson *et al.*, 1976: Kiritsakis and Dugan, 1985; Boskou, 1996).

According to Frankel, (1991) and Rovelini *et al.*, (1997) the volatile components produced during oxidation depend on the type mechanism (either autoxidation or photooxidation) as well as the nature of fatty acid.

Table 1.10 shows the hydroperoxides formed from autoxidised and photooxidized oleic, linoleic and linolenic Acids. Often the same compounds are produced during the two process but, in addition, there are further products and the percetage mixtures are distinct.

Table. 1.10. Hydroperoxide products caused by unsaturated fatty acid oxidation.

Acid	Process	Hydroperoxide isomers
C18:1	autoxidation	8-OOH 9-OOH 10-OOH 11-OOH
	photoxidation	9-OOH 10-OOH
C18:2	autoxidation	9-OOH 13-OOH
	photoxidation	12-OOH 9-OOH 10-OOH 13-OOH
C18:3	autoxidation	9-OOH 13-OOH
	photoxidation	12-OOH 9-OOH 10-OOH 13-OOH 15-OOH 16-OOH

Adapted from Rovelini *et al.*, 1997.

1.11.4. Antioxidants

Antioxidants are organic molecules, which can prevent or delay the development or the progress of oxidation. Some of these substances are naturally found in the plant kingdom and others are synthesized by man. In the last two decades a lot of research has been done on synthetic antioxidants due to concerns about some deleterious and dangerous effects that have been observed for some of them.

Virgin olive oils, being mechanically extracted from olive fruits (*Olea europaea L.*), preserve all the constituents that are present in the fruit such as antioxidants and volatile compounds, which are mainly responsible for delicate flavour which makes them highly appreciated by consumers.

In contrast, most vegetable oils, which are extracted from seeds by solvents, lose these important substances during the various refining stages (Montedoro and Cantarelli, 1969; Angerosa, 2002).

Among the antioxidants naturally occurring in olive oil are tocopherols (around 200 ppm), and β-carotene (which, together with chlorophylls, is responsible for the oil color), phytosterols, pigments, terpenic acids, flavonoids such as luteolin and quercetin, squalene, and phenolic compounds (Visioli *et al.,* 2002). All these antioxidants contribute to the stability of olive oil. The most important fat-soluble antioxidants in vegetable oils are the tocopherols.

Chlorophyll pigments are known to act as photosensitises of edible oils, accompanied with the production of singlet oxygen. In contrast, β-carotene acts as a singlet oxygen quencher (Kiritsakis and Dugan, 1985; Boskou, 1996).

The predominant phenolic compounds present in olive oil are the phenol alcohols 3,4-DHPEA and p-HPEA, vanillic, caffeic, protocatechuic, sinapic and ferrulic acids. However, the prevalent phenolic compounds in the oil are secoiridoid derivatives, such as the dialdehydic form of elenolic acid linked to 3,4-DHPEA or p-HPEA (3,4-DHPEA-EDA or p- HPEA-EDA, respectively) and an isomer of oleuropein aglycon (3,4-DHPEA-EA) Their content of these compounds is affected by the variety, environment, maturity degree and oil extraction procedure. (Solinas *et al.,* 1987c ; Montedoro and Garofolo,1984 ; Amiot *et al.,* 1986 ; Montedoro *et al.,* 1993, Angerosa, *et al.,* 1995 ; Cortesi *et al.,* 1995).

The antioxidant activity of the above phenolic compounds has been studied by many researchers (e.g. Chimi *et al.*, 1991; Baldioli *et al.*, 1996; Litridou *et al.*, 1997). According to Chimi *et al.* (1991) phenolic compounds inhibit the autoxidation of oils RH by trapping intermediate peroxide radical in two ways

$RCOO^{\bullet} + ArOH \rightarrow RCOOH + ArO^{\bullet}$ (I)

$RCOO^{\bullet} + ArO^{\bullet} \rightarrow RCOO\text{-}ArO$ (II)

First, the peroxyl radical abstracts a proton from the phenolic antioxidant to yield hydroperoxide and aroxyl radical. (Eq.1)

Second, the aroxyl free radical undergoes radical -radical coupling to produce a peroxide product. (Eq.2). The rate of oxidation of a lipid inhibited by a phenolic antioxidant requires consideration of other reaction as:

$ArO^{\bullet} + RCOOH \rightarrow RCOO^{\bullet} + ArOH$ (III)

$2ArO^{\bullet} \rightarrow$ Non- radical product (IV)

$ArO^{\bullet} + RH \rightarrow ArOH + R^{\bullet}$ (V)

For sterically hindered phenols (hydroxytyrosol and oleuropeine, caffeic acid) the rates of reactions (II) and (IV) greatly exceed the rates of reactions (I) and (V) and as a result, phenolic free radical and peroxide radical are withdrawn from the chain reaction that consequently breaks the autoxidation process. Thus, phenols are effective antioxidants.

Further work on the antioxidant activity of naturally phenolics by Chimi *et al.* (1991) concluded that the antioxidant activity of the phenolic compounds is correlated with the number of hydroxy groups substituted at the aromatic ring and with the nature of the substituent at p-position. Diphenols such as caffeic acid, oleuropeine and hydroxy- tyrosol had higher $OH^{\bullet}$ radical scavenging capacity than tyrosol.

Tocopherols are also very important constituents of olive oil because of their contribution to the oxidative stability and nutritional quality value of olive oil. According to Valenzuela and Nieto (1996) tocopherols work as antioxidants by donating the hydrogen of the hydroxyl group to a fatty acid free radical. The resulting tocopherol radical is very stable and does not continue the chain reaction of producing new fatty acid radicals. The antioxidant activity of tocopherols range in the order of delta isomer>gamma isomer> beta isomer >alpha isomer (the least active).

The relative effectiveness of tocopherols isomers as antioxidants is show on Table 1.11.

Table 1.11. Antioxidant activity of tocopherols compared to biological activity.

	Antioxidant activity	Biological activity
Alpha Tocopherol	100	1·49
Gamma Tocopherol	200	0·15
Delta Tocopherol	400	0·05

•International units /mg

α-Tocopherol has a synergetic effect with o- diphenols and contribute significantly to the retardation of peroxide formation (Valenzuela and Nieto 1996; Blekas et al., 1995; Blekas and Boskou, 1998).

1.11.5. Pigments

Virgin olive oil has a color ranging from yellow green to gold due to the presence of natural pigments such as chlorophyll and pheophytin and carotenoids.
The chlorophylls content of olive oil ranges from 1 to 20 ppm. The content of chlorophylls and carotenoids decrease with the maturity of the fruit. Moreover, the violet or purple color in ripe fruit is due to the formation of anthocyanins, which appear at the end of maturation stage (Minguez-Mosquera and Garrido-Fernaandez 1989).
Pheophytin a is the main pigment found in virgin olive oil. In oils from green olives, pheophytin a can represent up to 42% of the total pigment composition. By contrast, in oils from overripe black olives, lutein is the predominant component accounting for up to 61 % of the total pigments (Minguez-Mosquera *et al.*, 1991).

1.12 Aims of the Research

As discussed in the Introduction, olive oil is an extremely important crop in the Mediterranean region and, its quality can be affected by a host of different factors. In order to maximize the production of the best quality oils, it is clearly vital to understand the contribution of the different factors so that bad effects can be minimized and good effects maximized.

In the work reported in this thesis, the cultivars Koroneiki, Mastoidis and Coratina were studied. Most of the experiments were carried out on cv. Koroneiki, which is the main cultivar grown in Crete. The effects of processing conditions, of water or saline irrigation and storage were considered. The analysis of quality has used all the standard IOOC methods but, in addition, a more comprehensive analysis for certain components was used.

By these means, the intention was to establish when clear effects on olive oil composition are produced by the various conditions. This should then be of use to the agricultural industry in maximizing the production of high quality oils.

Chapter 2. Materials and Methods

2.1. Materials

2.1.1. Olive fruit

Olive fruits (*Olea europaea L.*) of two Greek cultivars Koroneiki, Mastoidis and one Italian variety Coratina were used in this project. These cultivars are the main oil producing cultivars grown in Greece and Italy. The olive fruits used for laboratory experiments were handpicked while, for industrial experiments, olive fruits were harvested using mechanical methods.

The maturity index (MI) was calculated according to the method proposed by the National Institute of Agronomical Research of Spain, San Jean Station (Hermoso *et al.*, 1991). The procedure is based on dividing a randomly taken sample of 100 olives, according to the colour. The index is given by the following formula:

$$\text{Maturation index.} = (0 \times n_0)\ (1 \times n_1) + (2 \times n_2) + \ldots (7 \times n_7)/100$$

Where: n_0, n_1, n_2,..., n_7 is the number of olives belonging to each of the following eight categories.

Group 0: Intense green skin.

Group 1: Yellowish green skin.

Group 2: Green skin with red spots in less than half the skin of the fruits.

Group 3: Reddish or purple areas in more than half the skin of the fruits.

Group 4: Black skin and white pulp.

Group 5: Black skin and some purple pulp.

Group 6: Black skin more than half of the pulp purple.

Group 7: Black skin and totally dark pulp.

2.1.2. Irrigation experiments

2.1.2. Irrigation experiments

Experiments were conducted at a commercial olive orchard at Kissamos, Crete, Greece during two successive year crops 1998 and 1999. The soil was sandy-loam (SL) with pH 8·2 and electrical conductivity (EC) of the saturated extract 0·35 dS/m.

The climate at Kissamos is typical Mediterranean with an average annual precipitation of 560 mm concentrated in the autumn and winter months. Fifty-year-old olive trees (*Olea europaea L cv* Koroneiki), planted at 5 x 6 m spacing in the orchard, were used. All trees were cared for in the same way (i.e. fertilisation, pruning, weed control and pest management) during the experiment. During the years of the experiment trees were subjected to two irrigation treatments: (a) irrigation with 0·4 Epan coefficient and (b) dry farming (no irrigation). Irrigation with in-line drippers, one line per plant row, started in mid-May and continued until the end of September each year. The seasonal application of irrigation water was 275 mm and 247 mm for 1998 and 1999 respectively.

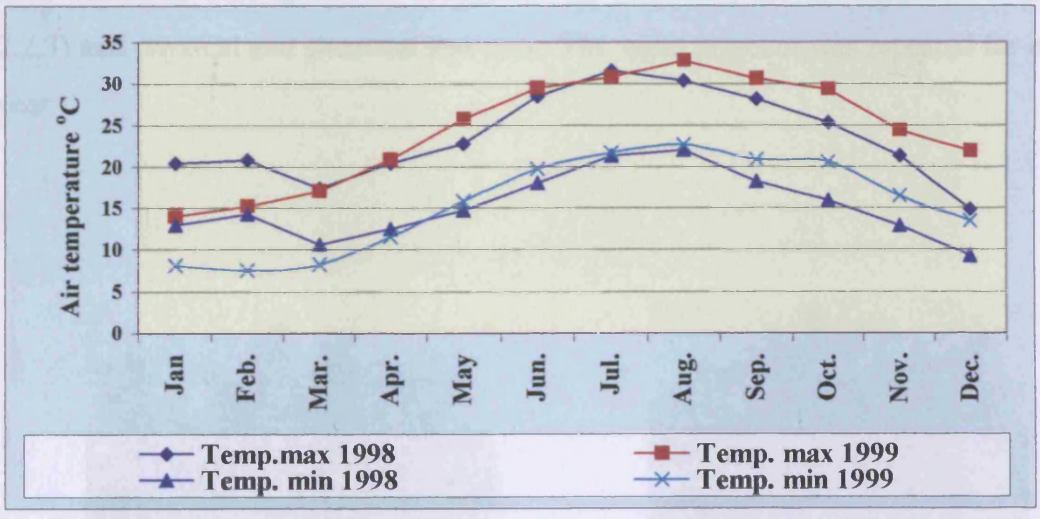


Figure 2.1. Minimum and maximum air temperatures recorded during the years 1998 and 1999 in the olive trees orchard.

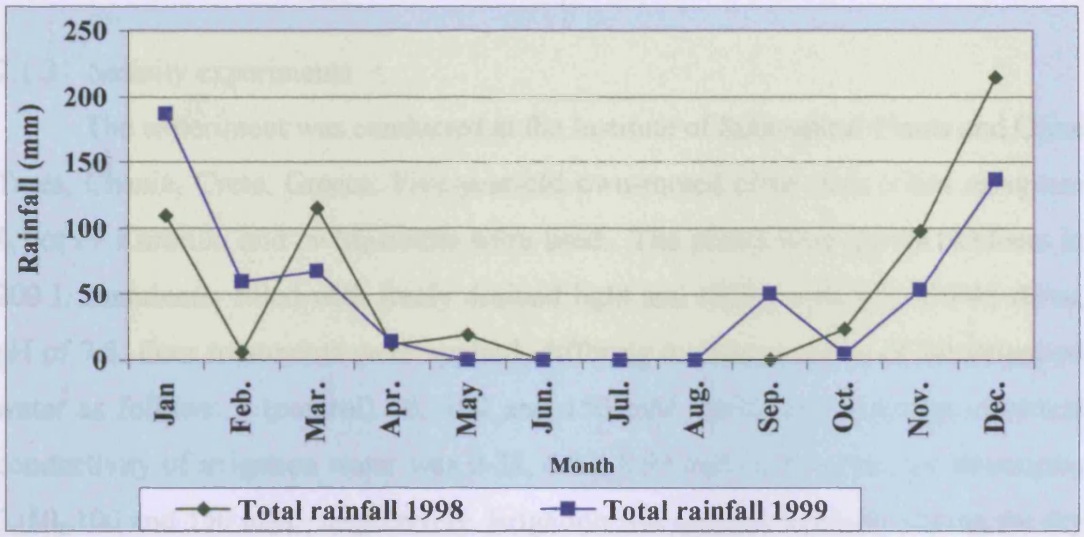


Figure 2.2. Total rainfall, as mm, recorded during the years 1998 and 1999 in the olive trees orchard.

Olive fruits from irrigated and non-irrigated olive trees were harvested randomly at two stages during ripening, first at an immature stage (green olives) in the middle of October and, secondly, at the normal harvesting stage (semi-black olives) in the last ten days of November. Three representative olive samples from each treatment were taken and brought to the laboratory for oil extraction (section 2.2.3) and physical and chemical analysis. The same protocol was repeated for each year.

A B

Figure 2.3. Irrigated (A) and non-irrigated (B) orchard at Kissamos, Crete.

2.1.3. Salinity experiments

The experiment was conducted at the Institute of Subtropical Plants and Olive Trees, Chania, Crete, Greece. Five-year-old own-rooted olive trees (*Olea europaea L.*) of *cv* Koroniki and *cv* Mastoidis were used. The plants were grown outdoors in 200 L containers, filled with freely drained light soil (SCL) with EC of 0·48 dS/m, pH of 7·5. Four treatments were applied, differing in concentration of the irrigation water as follows: 0 (control) 50, 100 and 150 mM NaCl. The resulting electrical conductivity of irrigation water was 0·32, 4·82, 8·94 and 12·50 dS/m. for treatments 0, 50, 100 and 150 mM respectively. Irrigation was applied regularly, during the dry season (April to October) while during the wet season they received the natural rainfall. Plants in all treatments were irrigated when soil water potential reached at 30 cm reached –40 kPa.

Figure 2.4. Olive trees irrigated at 50, 100, and 150 mM salinity levels.

Leaching was not applied throughout the irrigation period. Four trees from each cultivar were used as replication for each irrigation treatment in a completely randomised experimental plot design. Olive fruits were harvested on November. Olive oil was obtained as described (section 2.2.3)

2.1.4. Stability experiments

Extra virgin olive oil of Koroneiki cultivar extracted by the 2-phase decanter method was used for this experiment. The oil was divided into two parts. One part was used without filtration (not filtered) and had moisture content of 0·19% and the other part was filtered through cotton (filtered) with moisture content of 0·07 %.

Tin containers with a capacity of 500 ml were used. These containers were filled with 460 ml of oil thus leaving a headspace of about 40ml. After adding the oils to the containers, the unfiltered oil and one part of the filtered oil were tightly capped with air in the headspace. The other part of the filtered oil was purged for 4 minutes with nitrogen gas.

One set of samples was stored indoors in the dark at ambient temperature (23°C± 5°C) and the other was stored outside (in the roof of the building) where the temperature varied from 0°C to 20°C in the winter and from 20°C to 40 °C in the summer. In addition a number of containers had loose fitting covers and were stored for two months outside in the beginning of the storage period. As a result, we obtained the following combinations of samples:

- Filtered, unfiltered, filtered plus N_2 and unfiltered in open containers (outside)
- Filtered, unfiltered, filtered plus N_2 (indoors)

Enough tin containers for each oil sample were stored under the various conditions so that no container, once withdrawn from storage and analysed, had to be re-used.

The extent of oxidation was monitored by periodic measurements of peroxide value and conjugated dienes, of the rate of degradation of total phenols, the phenolic fraction, α-tocopherol and fatty acids, and resistance to oxidation.

2.1.5. Reagents and standards

Standards: gallic acid was purchased from Sigma Co. (Poole,U.K.), p-HPEA (98%) from Aldrich Co. (Milwaukee,U.S.A.), oleuropein from Extrasynthese Co.(Genay, France). 3,4-DHPEA and the dialdehydic form elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA) and the isomer of oleuropeine aglycon (3,4-DHPEA-EA) were kind gifts from Prof. Montedoro (University of Perugia, Italy). Tocopherol standards (alpha, beta, gamma, delta) were purchased from Merck (Darmstadt, Germany). Sterol standards were purchased from Sigma Co. (Poole, U.K.). The trimethylsilyl ethers (TMSEs) of sterols were prepared by the addition of silylation reagent which consisted of a 9:3:1 (v/v/v) mixture of pyridine /hexamethyl disilazane/trimethyl chlorosilane. Fatty acid methyl ester standards of high purity were purchased from Polyscience (Niles, Illinois)

Reagents: glacial acetic acid, (analytical reagent quality), potassium iodide, (saturated aqueous solution, recently prepared, free from iodine and iodates), sodium thiosulphate, starch, chloroform, methanol, acetic acid, hexane, methanol (HPLC gradient-grade) and Folin -Ciocaltea reagent were purchased from Merck (Darmstadt, Germany). High-purity water was produced with a Millipore system. Isooctane (2,2,4-trimethylpentane spectrophotometrically pure), heptane, (chromatographic quality) were obtained from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Determination of moisture content of olive fruits

Olives were crushed in a laboratory hammer crusher using sieves with 5 mm holes. 50 g of crushed paste was weighed in a tared mash beaker, which had been previously dried and cooled in a desiccator. The sample was kept in an oven at 105 °C until constant weight.

Moisture in the sample % = loss in weight x 100/weight of the sample.

2.2.2. Determination of fruit oil content

Oil content was determined following the official methods of AOAC (Official Methods of analysis, 1985). From a homogeneous representative sample of crushed olive paste, 50 g were taken and dried in an oven at 105 °C to constant weight. The dried paste was extracted using a Soxhlet extraction apparatus with hexane as solvent. After 12 h of extraction, the solvent was evaporated and the oil content was determined. The use of hexane or light petroleum as extraction solvents is a standard method, which permits a quantitative determination. However, the method is time-consuming and can be awkward with the sample drying out. Some other methods which had been proposed were also tried out but excluded because of their drawbacks.

2.2.3. Extraction using a laboratory scale oil mill.

Two experiments were carried out using laboratory mills.

a) Comparison of the two cultivars Koroneiki and Coratina.

The experiment was carried out with fruits (*Olea europaea* L) of two different cultivars Coratina and Koroneiki. The fruits were harvested by hand at the same stage of maturation, when 50% of the olives displayed partial or total purple color. A careful selection of healthy fruits was done and olives infected by insects were discarded. Olive oil was extracted on the same day. Oil from cv. Koroneiki was extracted using a laboratory scale olive mill (Callis Company). This mill consisted of a hammer crusher, eight one-liter capacity mixers and a basket centrifuge. While, Coratina fruits were processed in a laboratory oil-mill (Valpesana Company, S. Casciano Val di Pesa, Italy) using a hammer crusher, a single mixer and a basket centrifuge. The two cultivars were processed further using the following conditions:

Crushing of olives was carried out using a hammer mill operating at 3000 r.p.m. Sieves with 5 mm holes were used at the exit.

Malaxation. The paste obtained from the hammer mill was mixed at 50 r.p.m. for 20 min at 28 ±2 °C. Subsequently 200 ml of distilled water at 28 ±2 °C was added and malaxation continued for ten minutes more .

Centrifugation. After malaxation, the paste was centrifuged at 1400 x g for 1 min to remove the solid remains. The liquid phase (oil plus water) was put into a

Centrifugation. After malaxation, the paste was centrifuged at 1400 x g for 1 min to remove the solid remains. The liquid phase (oil plus water) was put into a cylinder and allowed to decant for 10 min. The oil was filtered through a cotton layer. Oil samples were kept at -20 °C until analysis.

b) Malaxation of olive paste

The experiments were carried out with cv. Koroneiki. A homogeneous batch of semi-black olive fruits was divided into six lots. The olives were submitted to the extraction process, with operating conditions changed only with respect to the malaxing time of the oily paste, which was performed without addition of water and for 0 min, 30 min and 60min respectively. The separation of oil was carried out using a laboratory hydraulic press (maximum press 220 bar).

Figure 2.5. Laboratory mill with six-chamber and an olive crusher.

2.2.4. Industrial extraction procedures

Olive fruits of varieties Koroneiki or Coratina at a maturity stage 3.7 (section 2.1.1.) were processed by means of a two or a three- phase decanter (Figure 2.6). Extraction of olive oil from the cultivar Coratina was carried out at the Institute Sperimentale per la Elaiotecnica in Pescara, Italy and olive oil from the cultivar Koroneiki was carried out at the Subtropical Plants and Olive Tree Institute in Chania. For each cultivar, fruits were divided into six lots of 400 kg.

The oil extraction of cv. Koroneiki was carried out with a three-phase decanter Alfa-Laval -Type 314 (Monza, Italy) and 2 phase decanter Pieralisi Major (Jesi Italy) On the other hand, for cv. Coratina the *Novoil EDJ/1* equipment (*Rapanelli Company*, Foligno, Italy) system was used to separate the oily must with a centrifugal decanter operating with 3 phase or 2- phase.

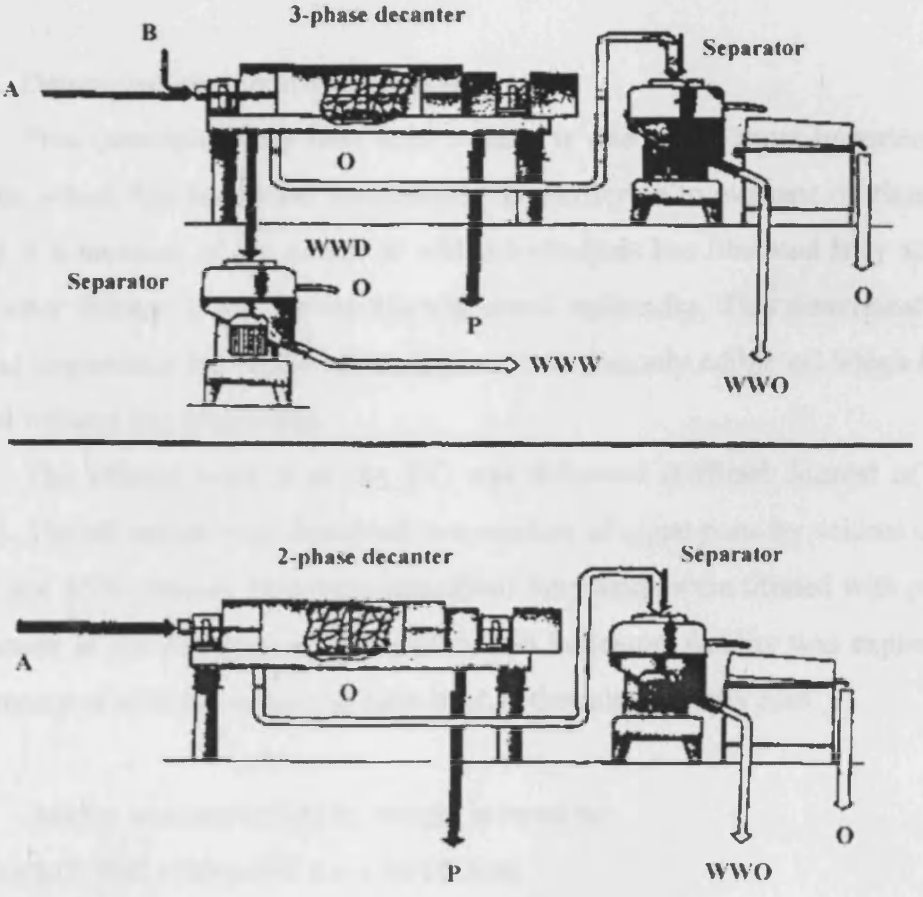


Figure 2.6. Diagram showing 2-phase and 3-phase decanters connected to oil separators: A: supply of olive paste; B: supply of water; O: oil; P: olive pomace; WWW: wastewater from wastewater separator; WWO: waste water from olive oil separator.

The industrial process included the following steps and within each step the same operating conditions were maintained for both cultivars.

- Elimination of leaves from olive samples.
- Milling of drupes by a hammer crusher operating at 2800 r.p.m. with a sieve of 6 mm holes operating at 100 r.p.m in the same direction as the crusher.
- The paste obtained was malaxed at 23 r.p.m for 20, 30 or 45 min for cv. Koroneiki and 30, 45 or 60 for *cv.* Coratina. The malaxation temperature was kept at 28 ±2 °C.
- In the case of three-phase horizontal decanter fluidification of the paste with tap water, 50 l of water was added to 100 kg of olive paste.

  Separation of the oily must into oil and vegetation water was carried out by an upright automatic discharge centrifugal oil separator operating at 1700 r.p.m

2.2.5. Determination of non-esterified fatty acids

Free (non-esterified) fatty acid content is one of the most important quality indices, which has been used traditionally as a criterion to evaluate or classify olive oil. It is a measure of the extent to which hydrolysis has liberated fatty acids from their ester linkage in the parent triacylglycerol molecules. This determination is of special importance for virgin olive oil since it is the only edible oil which has to be traded without any processing.

The official method of the EC was followed (Official Journal of the EC, 1991). The oil sample was dissolved in a mixture of equal parts by volume of diethyl ether and 95% ethanol. Free (non-esterified) fatty acids were titrated with potassium hydroxide in the presence of phenolphthalein indicator. Acidity was expressed as a percentage of total oil, assuming oleic acid as the released fatty acid.

Acidity as a percentage by weight is equal to:

V x c x M/1 000 x100/m =V x c x M/10 X m

where:

V= the volume of titrated potassium hydroxide solution used, (in millilitres)

c = the exact concentration in moles per litre of the titrated solution of potassium hydroxide used

M= the molar mass in grams per mole of the acid used as the standard (i.e. oleic acid= 282)

m= the weight in grams of the sample.

The amount of the sample used was adjusted depending on the expected acid value. The procedure is time-consuming, since one can analyse only 7–8 samples per hour and, in addition, the method consumes large quantities of organic solvents.

2.2.6. Determination of peroxide value.

Peroxide value (PV, meq O_2 /kg oil) is a measure of oxidative rancidity of olive oil. Peroxides or hydroperoxides are called primary oxidation products of unsaturated fatty acids, which subsequently degrade to secondary oxidation products (aldehydes, ketones, alcohols, acids, etc). Peroxides may be used as an indicator of the current oxidation status of oil but they cannot give information on the relative potential of the sample to be oxidized.

The official European Community analytical method is an iodometric titration method, which measures the iodine liberated from potassium iodide by peroxides present in the oil at room temperature in an acetic acid/ chloroform medium.

The oil sample (5g) was dissolved in a solvent mixture of acetic acid: chloroform (3:2,v/v). 1 ml of a saturated aqueous solution of potassium iodide was added and the mixture put into the dark for exactly five minutes. The liberated iodine was titrated with a standardised sodium thiosulphate solution in the presence of 1% aqueous starch solution as indicator. The peroxide value (PV), expressed in meq of active oxygen per kilogram of oil, is given by the formula:

PV= V x N x 1000/m, where:

V= the number of ml of the standard sodium thiosulphate solution used for the test, corrected to take into account the blank value

N= the exact normality of the sodium thiosulphate solution used; m=the weight in g of the test sample

The amount of the sample used for the analysis was adjusted depending on the expected peroxide value.

Errors in the iodimetric method have been attributed to the addition of iodine to double bonds of unsaturated fatty acids as well as to the liberation of iodine by

oxidation of iodide in air. This method is highly empirical and accuracy depends on a variety of experimental parameters such as accurate timing as well as protection of the reaction mixture from light and atmospheric oxygen. The procedure is also time-consuming and requires large quantities of organic solvents.

2.2.7 Ultraviolet (UV) spectroscopy

Spectrophotometric examination in the ultraviolet region can provide information on the quality of oil, its state of preservation and changes brought about by technological processes. The absorption at wavelengths of 232 and 270 nm is due to the presence of conjugated diene and triene systems. These absorptions are expressed as specific extinctions E (1%) cm, (the extinction of a 1 % solution of the fat in the specified solvent, in a standard cell) conventionally indicated by K. The detection of adulteration of virgin olive oil with refined olive oil or olive pomace oil (olive residue oil) can be based on measurements at UV region. In fact the extinction coefficient at 270nm, corresponding to the absorption of the conjugated trienes induced by certain types of bleaching earths during the bleaching process is a very important measurement. The official method of the European Community was followed (Official Journal of the EC, 1991).

Oil samples were dissolved in pure iso-octane (2,2,4-trimethylpentane) and the extinction of the solution was determined at wavelengths of 232 and 270 nm with reference to pure solvent. Calibration of the spectrophotometer was checked before the analysis for both wavelength response and the accuracy of the response.

2.2.8. Sensory evaluation of the oil sample

The sensory evaluation of olive oils was performed according to Annex XII of EC regulation no. 2568/91 EC (1991).

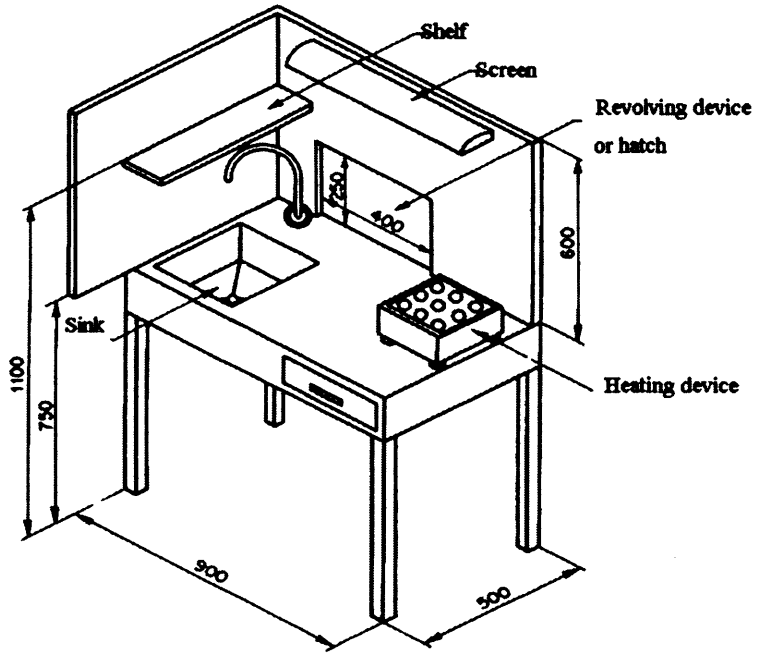


Figure 2.7. Arrangement of the booth for olive oil sensory analysis.

The sensory profile is made up of 16 descriptors (eight positive and eight defective) of which three were generic (Table 2.1). Moreover, the number of descriptors of the official panel was increased in order to allow a more careful description of "green" perceptions (Table 2.2). For this purpose, ten tasters were trained with oil flavours coming from unripe fruits of different cultivars as well as the changes occurring during olive paste malaxation. After training, tasters were asked to evaluate the sensory characteristics of virgin olive oils to verify whether the new profile sheet was able to differentiate oils in relation to cultivars, as well as the environment and technological steps of the oil extraction process.

Evaluation of the samples was performed under the conditions described in the COI regulation (COI, 1986) regarding sensory booths, time of day, oil temperature (28 ±2 °C), amount of oil tasted (15 ml) and the glass appropriate for this.

Sample presentation was fully randomised and each sample was evaluated in triplicate.

Table 2.1. Profile sheet and grading table used in the official European Community methodology.

Profile sheet
Olfactory-gustatory-tactile notes (2)

	0	1	2	3	4	5
Olive fruity (ripe and green) (1)						
Apple						
Other ripe fruit						
Green (leaves, grass)						
Bitter						
Pungent						
Sweet						
Other allowable attribute(s)						
(Specify						
.....)						
Sour/winey/vinegary/acid (1)						
Rough						
Metalic						
Mustiness/humidity (1)						
Muddy sediment						
Fusty ('Atrojado')						
Rancid						
Other unallowable attribute(s)						
(Specify						
.....)						

Grading table

Defects	Characteristics	Overall mark: points
None	Olive fruity	9
	Olive fruity and fruitiness of other fresh fruit	8
		7
Slight and barely perceptible	Weak fruitiness of any type	6
Perceptible	Rather imperfect fruitiness, anomalous odours and tastes	5
Considerable, on the border of acceptability	Clearly imperfect, unpleasant odours and tastes	4
Great and/or serious, clearly perceptible	Totally inadmissible odours and tastes for consumption	3
		2
		1

Remarks: .....

.....

Name of taster: .....

.....

Legend of sample: .....

Date: .....

(1) Delete where not applicable
0: not detectable
1: barely perceptible,
2: slight
3: average,
4:great,
5:extreme

Table 2.2. Profile sheet of sensory attributes which contribute to "green" perceptions of virgin olive oils.

Descriptors	Intensities					
	0	1	2	3	4	5
Cut green lawn						
Green leaf or twig						
Green olives						
Green banana or its skin						
Green tomato						
Almond						
Green fruit (which?)						
Artichoke						
Apple (explain which one)						
Walnut husk						
Green hay						
Bitter						
Pungent						

0: not detectable
1: barely perceptible,
2: slight
3: average,
4:great,
5:extreme

2.2.9. Sterol analysis

The presence of other seed oils in olive or olive oil pomace can be detected by the sterol pattern. The official method of the EC was followed (Official Journal of the EC, 1991). Oil samples (with the addition of 500 μl of 0.2% alpha-cholestanol solution in chloroform as internal standard) were saponified with 2N ethanolic potassium hydroxide and the unsaponifiable matter was extracted with diethyl ether. The extract was fractionated by TLC on silica gel plates yielding four bands containing: aliphatic alcohols (together with triterpenic alcohols), methylsterols (together with oleanolic aldehyde), sterols and triterpenic dialcohols and hydrocarbons (Figure 2.8). The solvent used was a 65:35 (by volume) mixture of hexane and diethyl ether. The sterol fraction was removed from the other unsaponificable compounds on the silica -gel plate by scraping the corresponding band and extracted with diethyl ether.

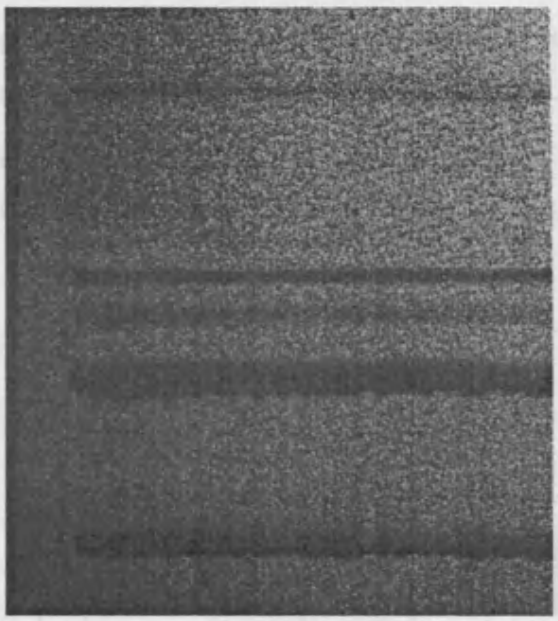


Figure 2.8. Separation of the unsaponifiable fraction from olive oil by thin layer chromatography. Adapted from Morales and Leon-Camacho, 2000.

After removing diethyl ether the sterols were converted to trimethylsilyl ethers and separated on a HP-5 5% phenyl methyl siloxane capillary column (30.0m x 320μm internal diameter x 0.25 μm thickness) at 285 °C. Identification of individual peaks was done on the basis of retention times and by comparison with standard mixtures of sterol TMSEs analysed under the same conditions. Quantitative analysis was performed with (3β-hydroxy-5α- cholestane) as the internal standard. Results were expressed as mg/100g of oil and as the sum of the total sterols using HP ChemStation software.

This method is laborious and time-consuming because it entails extraction and purification prior to GLC determination in order to remove the very high proportion of triacylglycerols. In fact, the major problem encountered in GLC determination of sterols is due to the high proportion of triacylglycerols in olive oil which may deposit on the column and affect resolution by giving rise to broadened peaks or absorbing polar solutes (Ballesteros *et al.,* 1995). Capillary columns can give greater resolution but problems arise in detecting compounds at low concentration. This may be due to

the incomplete separation of the sterol fraction during TLC on silica gel. According to Morales and Leon-Camacho (2000) problems can be attributed to the amount of the unsaponifiable compounds deposited on the plate, inadequate development of the plate and defective scraping of the sterol band. In cases where sterols were purified by means of HPLC, higher values for Δ^7- sterols have been observed. This might point to their loss following TLC because of inaccurate scraping of the sterol band or inefficient elution.

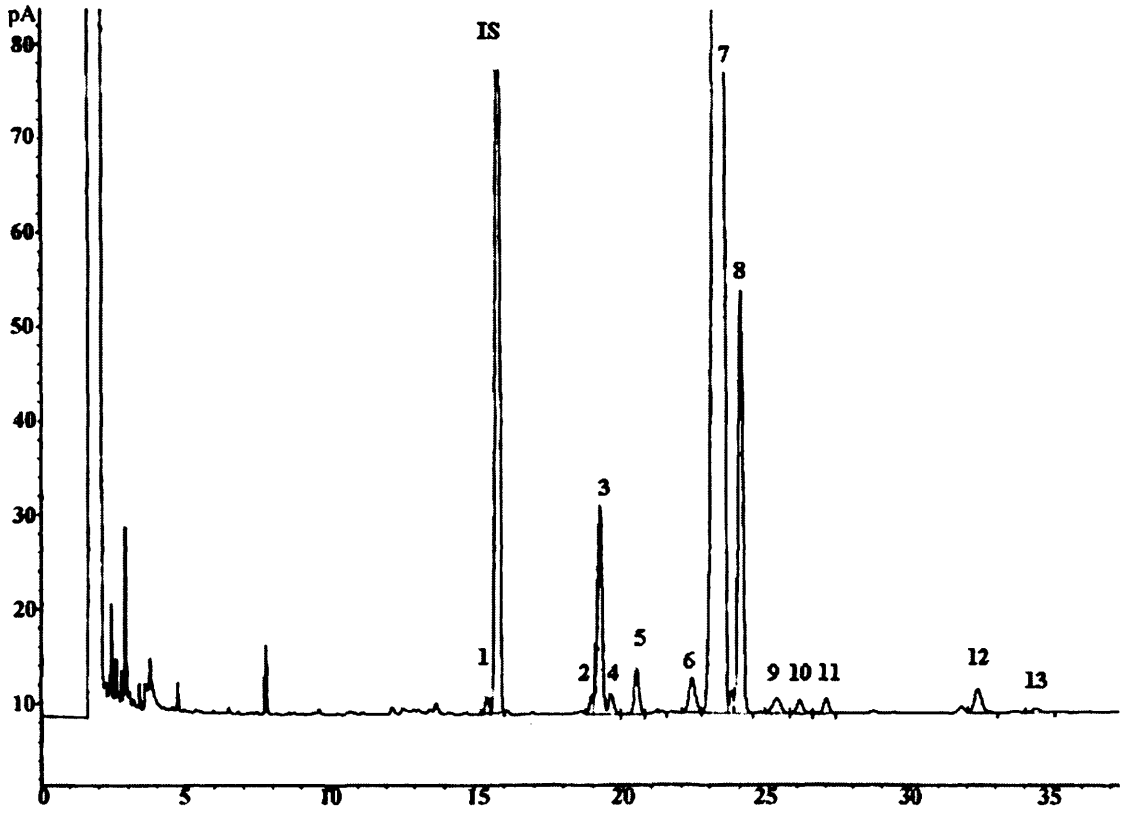


Figure 2.9. Representative chromatogram of sterols and triterpenic dialcohols from olive oil of *cv* Koroneiki . *Note:* 1, cholesterol; 2, 24-methyl cholesterol; 3, campesterol; 4, campestanol; 5, stigmasterol; 6, 5,23-stigmasta dienol; 7, β-sitosterol; 8, Δ^5-avenasterol; 9, 5,24-stigmastadienol; 10, Δ^7 -stigmastenol; 11, ^{7}avenasterol; 12, erythrodiol; 13, uvaol.; IS, internal standard (3β- hydroxy- 5α -cholestane).

2.2.10. Determination of aliphatic alcohols.

Olive oil samples (with 1-eicosanol added as internal standard) were saponified with methanolic potassium hydroxide and the unsaponifiable matter

extracted with ethyl ether. The total alcoholic fraction was separated from the unsaponifiable matter by chromatography on a layer of potassium hydroxide impregnated silica gel (Figure 2.7). The alcohols recovered from the silica gel were transformed into trimethylsilyl ethers. The trimethylsilyl ethers were separated by a Hewlett Packard 6890 gas chromatograph with a split injector and a FID detector on a HP-5 5% phenyl methyl siloxane capillary column (30.0m x 320μm internal diameter x 0.25 μm) at 285 °C. Identification of individual peaks was done by comparison of retention times with standard mixtures analysed under the same conditions.

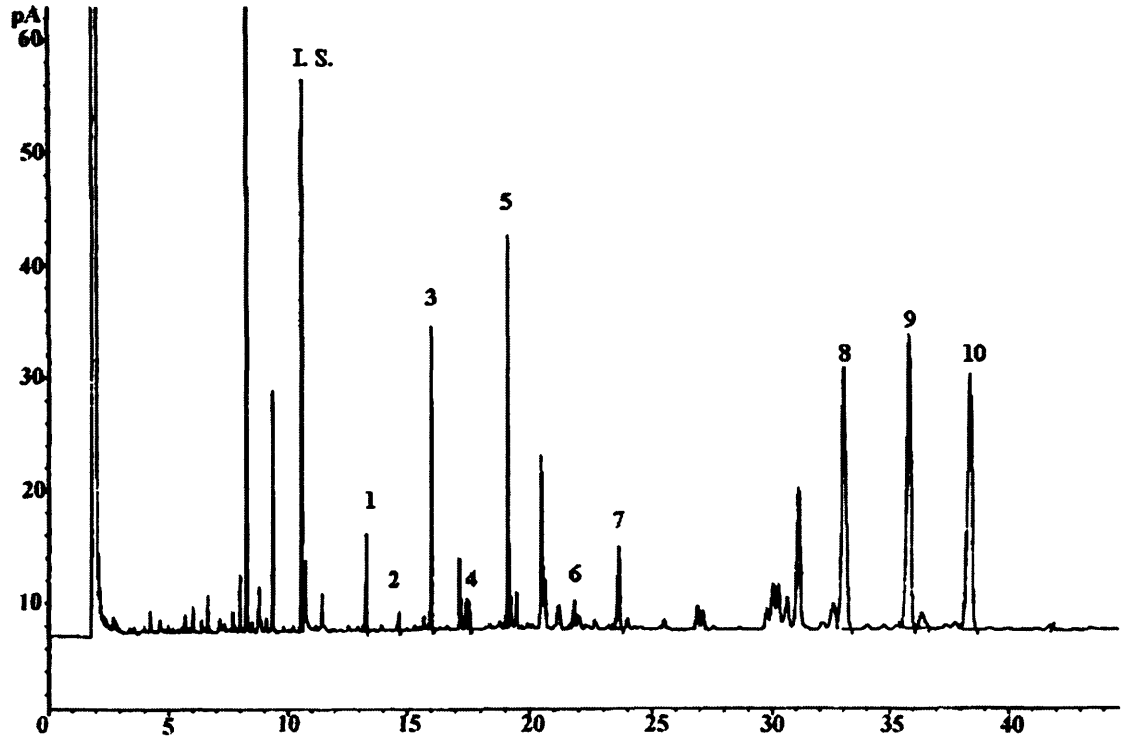


Figure 2.10. Representative chromatogram of olive oil aliphatic and triterpenic alcohols of olive oil cv Koroneiki. Note: 1, docosanol; 2, tricosanol; 3, tetracosanol; 5, hexacosanol; 6, heptacosanol; 7, octacosanol; 8, cicloartenol; 9, 24-methylene cycloartenol; 10, citrostadienol; I.S. internal standard (arachidyl alchohol).

Quantitative analysis was carried out with arachidyl alcohol as internal standard. Results were expressed as total aliphatic alcohols (mg/100g of oil) and as % distribution of individual aliphatic alcohols by using HP ChemStation software. Like sterol analysis, this procedure is time-consuming and needs extra attention with

regard to inadequate development of plates, defective scraping of the alcohol band etc.

2.2.11. Triacylglycerol analysis

The analytical separation of triacylglycerols was performed according to the EC method (Official Journal of the EC, 1991). The apparatus consisted of a Jasco (Tokio, Japan) liquid chromatograph (Model PU 980) coupled with a refractive index (RI) detector (Jasco 830-RI) and a software interface for processing of the acquired data. Injection was by means of a rheodyne injection valve (Model 7125) with 10 µl fixed loop (Rheodyne, California, USA). The chromatographic separation was achieved on a Kromasil 100 C18, column (25m x 4 mm i.d.), obtained from MZ Analysentechnik (Wohlerstr, Mainz, Germany) at 40°C. Isocratic elution was carried out at a flow rate of 0.7ml min^{-1} with a mixture of acetone: acetonitrile (60:40, v/v) as the mobile phase.

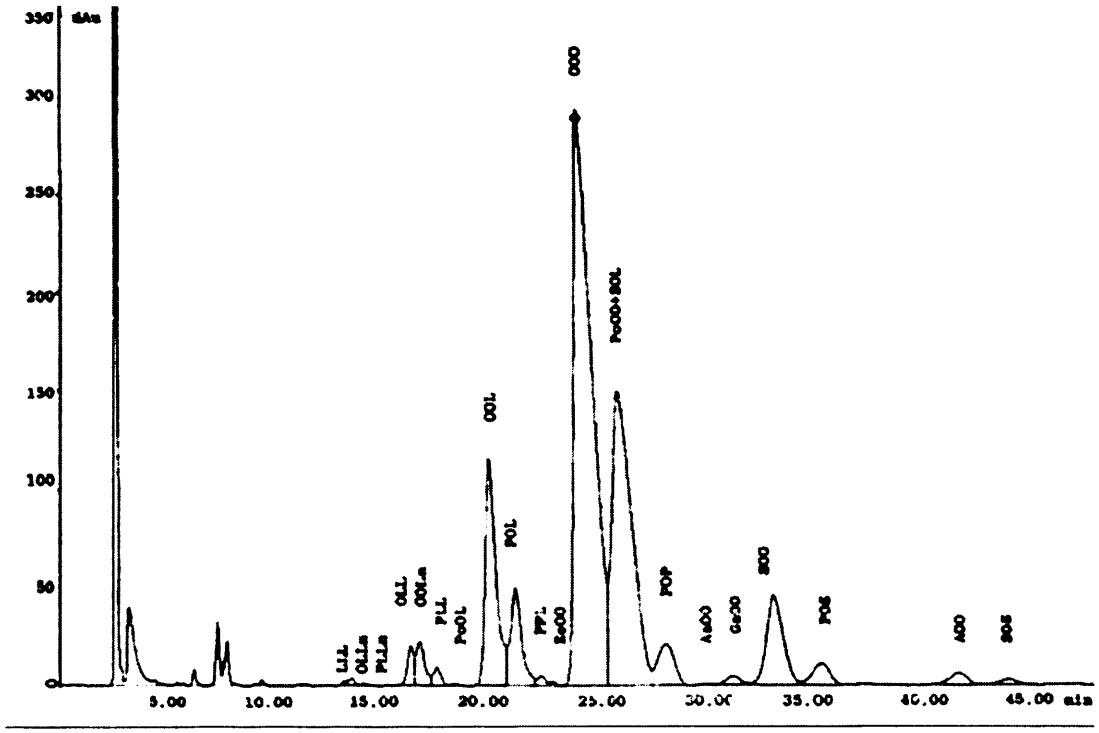


Figure 2.11. High performance liquid chromatogram of triacylglycerols of olive oil of *cv* Koroneiki. *Note*: L, linoleic acid; Ln, linolenic acid O, oleic acid; P, palmitic; Po, palmitoleic; S, stearic; A, arachidic acid; Ee, eicosanoic acid.
See: List of Abbreviations for nomenclature of triacylglycerols.

Triacylglycerols were separated according to number of carbon atoms and their unsaturation. Peak identification was done on the basis of retention times by comparison with mixtures of triacylglycerols analysed under the same conditions. Results were expressed as a % composition of the total triacylglycerols using the HP ChemStation software.

The main disadvantage of refractive index detection is the limited sensitivity to isocratic operating conditions. The isocratic elution system was used because the variation of the composition of the mobile phase affects the resulting signal. Bubble elimination from solution (degassing) must be carried out before analysis. The RI detector is also affected by temperature variations and, for this reason, it is necessary to work with thermostatic cells. The detector also requires pressure variation to be minimal during operation (Leon-Camacho, 1993; Morales and Leon-Camacho, 2000).

2.2.12. Fatty acid methyl ester (FAME) preparation and analysis by GC

The composition of fatty acids has been customarily used in as an indicator of olive oil purity. However, the variation of fatty acid patterns in olive oils due to geographic origins, olive variety and maturation stage make the analysis of the data with regard to adulteration very difficult. However, the presence of trans isomers of oleic, linoleic and linolenic acids above the limits established by the EC regulations can indicate adulteration with hydrogenated seeds oils.

In order to analyse fatty acids by gas liquid chromatography (GLC) it is necessary to derivatise fatty acids by forming methyl esters. Methyl esters are more volatile and this makes them easier to elute from chromatographic column. Fatty acid methyl esters were prepared using a cold saponification method. According to EC regulations their rapid method is recommended for transesterification when the free fatty acid content of the olive oil sample is less than 3·3 %. 0.25g of olive oil was transferred to a 5 ml screw-top test tube. 5ml hexane and 0.5ml of methanolic potassium hydroxide solution (2N) were added. The cap was tighten, and the tube shaken vigorously for 30 seconds. The mixture was centrifuged at 3,500 r.p.m. for 2min after which 1µl of the upper layer was injected into the GC.

The fatty acid profile was analysed using a Hewlett Packard gas chromatograph (HP 6890 series) with an auto injector connected to a flame ionisation

detector and fitted with a capillary column BPX70 (SGE scientific Pty Ltd. Australia), (50m length, 0.22 mm i.d. and 0.25μm film thickness). Helium was used as carrier gas and nitrogen as the make up gas. The oven temperature was held at 165°C for 5min and increased to 220°C with a rate of 2°C/min and then held for 15min. The inlet temperature was 250°C and the detector temperature was 280°C. The flow rate was 0.8ml/min and the split ratio was 1/80. Peak identification was made using retention times with respect to those of reference standard mixtures analysed under the same operational conditions. The results were expressed as a percentage of total fatty acids using HP ChemStation software.

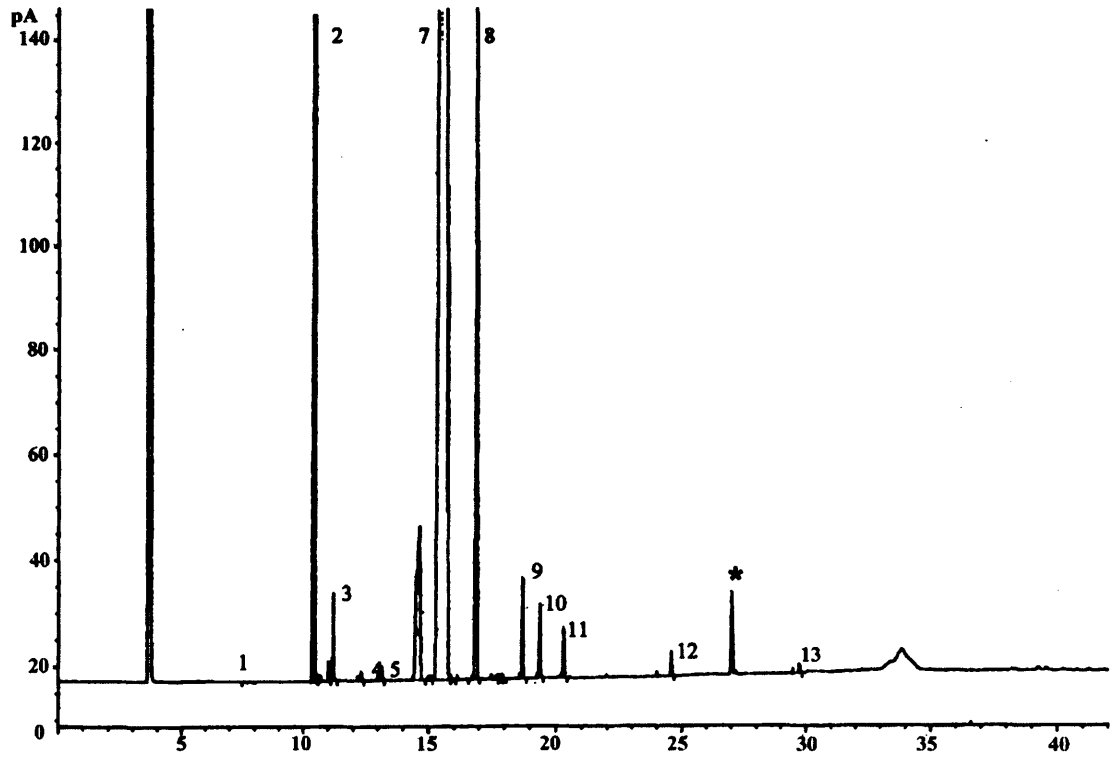


Figure 2.12. Chromatogram showing the fatty acid methyl esters of olive oil. *Note*: 1, Myristic; 2, Palmitic; 3, Palmitoleic; 4, Margaric; 5, Margaroleic; 6, Stearic; 7, Oleic; 8, Linoleic; 9 Arachidic; 10, Linolenic; 11, Eicosenoic; 12, Behenic; 13, Lignoceric acid. * Probably Squalene

2.2.13. Extraction of the phenolic compounds of olive oil

The extraction of phenolic compounds was carried out according to Montedoro et al. (1992). Phenolic constituents were extracted with a methanol /water (80:20,v/v) mixture. 30 ml of the solvent were added to 30 g of olive oil and mixed using an Ultra Turrax homogenizer T25 at 15000 r.p.m. for 1 min and centrifuged at 5000 r.p.m. for 10 min. The extraction was repeated two times. The combined methanolic extracts were concentrated in a vacuum rotary evaporator at 35°C until syrup was obtained. Purification of phenolic extract was done as follows. 10 ml of acetonitrile was added to the syrup and then it was subjected to two consecutive washings with 20 ml of hexane. The acetonitrile layer was brought to dryness using a rotary evaporator. Finally, the sample was dissolved in HPLC grade methanol and was quantified calorimetrically for total phenols (section 2.2.14) and separated by HPLC for the fractionation of phenolic compounds (section 2.2.15).

The isolation of the phenolic fraction from olive oils is an important aspect accounting for differences in the results described in the literature. These extraction procedures are very laborious, and alteration of the phenolic compounds can occur during the technique. Consequently, attempts to isolate the phenolic fraction of olive oil by solid phase extraction (SPE) using C_{18} cartridges have been made. Unfortunately, this method was inefficient for olive oil extraction although SPE gave good results for olive fruit and vegetation water.

According to the work done by Montedoro *et al.* (1992) the optimal extraction solvent ratio is methanol /water, (80:20, v/v) and purification of polar extract with acetonitrile –hexane (1:4, v/v) gave the best recovery. Pure methanol is not suitable because some polar lipids are also dissolved. The above ratios of solvents were the ones used in the present work.

2.2.14 Colorimetric determination of total phenols.

The concentration of total (poly) phenols in a methanolic extract was estimated with Folin -Ciocalteau reagent. The procedure consisted of dilution of a suitable aliquot of the phenolic extract with water in a 25 ml volumetric flask and addition of 0.25 ml Folin -Ciocalteau reagent. After 3 min, 1 ml of saturated (ca. 35

%) Na_2CO_3 solution was added and the content was mixed and diluted to 25ml with water. The absorbance was measured at 725 nm against a reagent blank. A calibration curve was calculated using gallic acid concentrations ranging from 25-180 µl /25 ml with a straight-line relationship (regression coefficient of 0.9995).

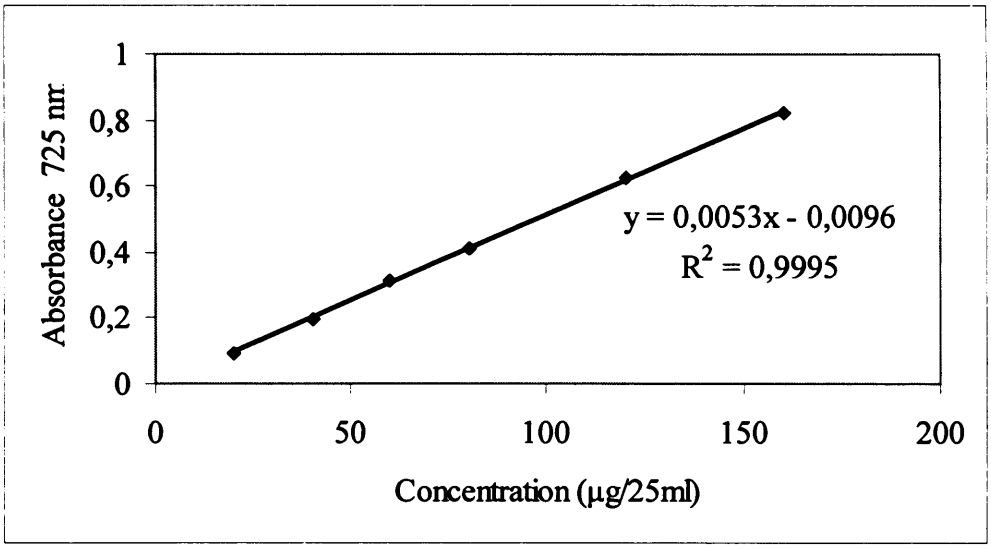


Figure 2.13. Calibration curve of gallic acid.

The method is based on the oxidation of phenols using phosphotungsten ($H_3PW_{12}O_{40}$) phosphomolybdic ($H_3PMo_{12}O_{40}$) acid reagent in an alkaline environment (pH 11.5) (AOAC 1960). The intensity of the developed bluish colour that is due to the formation of tungsten and molybdenum oxides (W_8O_{23}, Mo_8O_{23}) is used as an estimate of the level of total phenols. Two reagents, Folin-Ciocalteau and Folin-Denis, are used for the colorimetric determination of phenols in olive oil polar fraction. Folin-Ciocalteau reagent is now commercially available. This reagent is more suitable due to its composition and preparation method (Morales & Tsimidou, 2000). It contains a higher percentage of molybdate, which increases the reaction rate and, in addition, the presence of sulphate ions improves the solubility of salts and yields less turbid solutions. Reducing compounds, as well as ferric or zinc ions interfere with the determination. Choice of the standard, amount of the reagent, length of the reaction period and other experimental conditions influence quantification.

2.2.15. HPLC separation of phenolic compounds

The separation of phenolic compounds was performed according to Montedoro et al. (1992). The HPLC system consisted of a Hewlett Packard liquid chromatograph (series 1100) equipped with a Lichrospher 100 RP-18 5μm (250 mm x 4mm i.d.) and a 5cm pre- column. The column was coupled to a UV detector (Jasco UV 970). The eluates were monitored at 278nm. Samples were dissolved in methanol. Sample injection was carried out with a Rheodyne injector (model 7125) with 10-μl loop (Rheodyne, CA, USA). Solvent flow rate was 1ml/min.

The mobile phase used was 0.2% acetic acid (pH 3·1) in water (A) and in methanol (B) for a total running time of 60 min with the gradient changed as follows: 95% A/5%B for 2 min, 80% A /20% B in 8 min, 70% A /30% B for 10 min, 60% A /40% B for 10 min, 40% A /60% B for 10 min, 0% A /100% B for 10 min and then return to the initial conditions.

Free phenolic compounds were identified by comparing their relative retention times (RRT) with standards. Hydrolysable phenolic compounds were identified by comparing their RRT with standards which were kindly provided by Prof. Montedoro, University of Perugia, Italy (section 2.1.4). The results were expressed as ppm calculated using the HP ChemStation software.

The phenolic fraction from olive oil represents a complex mixture that is difficult to determine without the use of a complex gradient. Because phenolic compounds show several absorption maxima, the use of simultaneous multiple UV (photodiode array) detection is recommended for identification purposes. Comparisons of chromatograms at different detection wavelengths support the use of 280 nm as a compromise for the detection of phenolics. In fact, detection at 280 nm shows considerably more detail than detection at other wavelengths in the range 250 to 360 nm (Ryan et al., 1999). In addition, the presence of acetic acid in the mobile phase suppressed the dissociation of the phenolic compounds and enhanced the selectivity of the elution system (Tsimidou *et al.*, 1992).

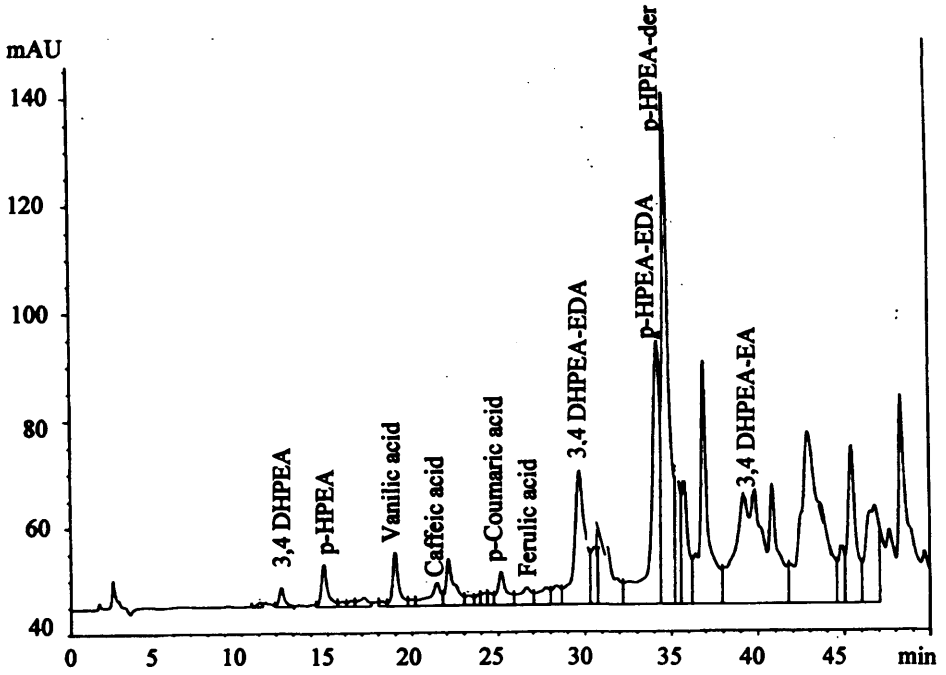


Figure 2.14. High performance liquid chromatogram of phenolic compounds of virgin olive oil sample of *cv* Koroneiki.

2.2.16. Resistance to oxidation

The induction time of oil oxidation was determined using the Rancimat 679 apparatus (Fig. 2.15) (Methrohm, Switzerland) at 120°C with airflow of 20 l/h (Laubli & Bruttel, 1986).

This test is based on the decomposition of hydroperoxides and the formation of short chain fatty acids that change the conductivity of water. The acids produced when oils are heated at 100 °C or higher, are mainly formic acid and acetic acid. The conductivity cells continuously monitor the conductivity of deionised water. The conductivity of deionised water is increased because volatile oxidation products (produced when the oil is heated under a stream of air) are trapped. A steep rise in conductivity showed that the reaction had gone to completion (Figure 2.16). Normally one observes an induction period followed by a rapidly rising response as oxidation of the oil is accelerated. This induction period corresponds to the stability

of the oil and is defined as the time, (expressed in hours), that is needed to reach the maximum change of conductivity. The Rancimat apparatus permits the analysis of 6 oils at a time. The method is time-consuming and suffers from problems of large errors caused by variations in the airflow. Moreover, significant losses of formic acid from the collector may occur if collection temperature exceeds 20°C.

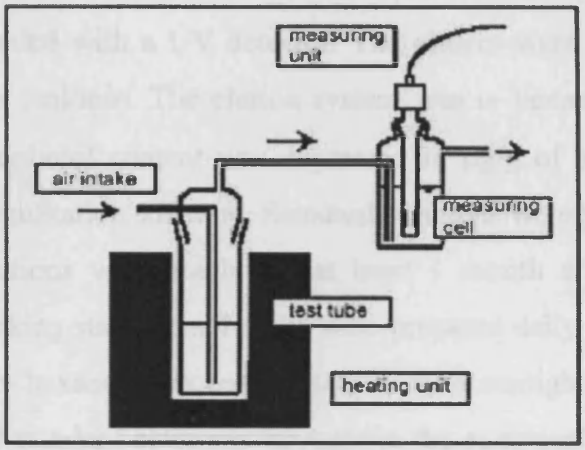


Figure 2.15.Diagram showing the reaction vessel and conductivity-measuring cell of Rancimat apparatus.

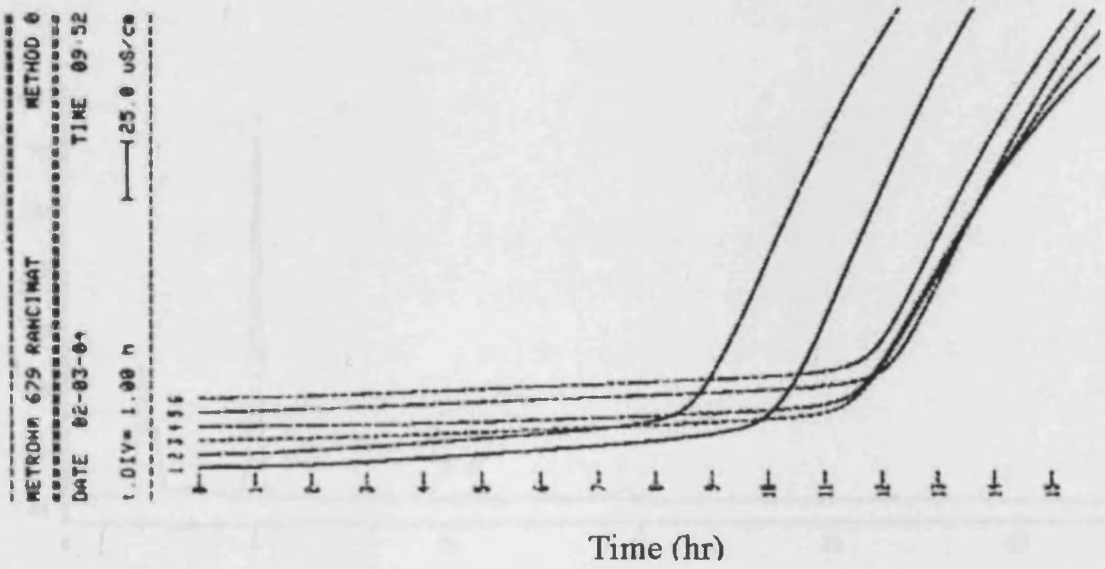


Figure 2.16. A typical plot of rancimat apparatus for the determination of induction time versus conductivity of olive oil sample of *cv* Koroneiki.

2.2.17. Tocopherol analysis

Tocopherol analysis was carried out following the IUPAC 2.432 method. The chromatographic separation of tocopherols was achieved with HPLC (Hewlett Packard series 1100) with direct injection of the oil dissolved in n- hexane using a 250x4mm Lichrosorb Si 60, 5μm column (analyzentechnik, Merck, Germany) coupled with a UV detector. The eluates were monitored at 292nm. The flow rate was 1ml/min. The elution system was n- hexane/ 2-propanol (99.5: 0.5,v/v). The tocopherol content was expressed in μg/g of oil and data was analysed using HP ChemStation software. Standard solutions were prepared in n-hexane. These standard solutions were stable for at least 1 month at 4-8°C in argon atmosphere. Fresh working standard solutions were prepared daily by appropriate dilutions of standards in n- hexane. Care was taken to exclude sunlight and diffuse light exposure of sample and standard solutions throughout the analytical procedure by wrapping the samples and standards with aluminium foil. Injections in duplicate were made at each concentration for both standards and samples. Calibration curves were tested daily and injections of standard solutions were made after every two samples.

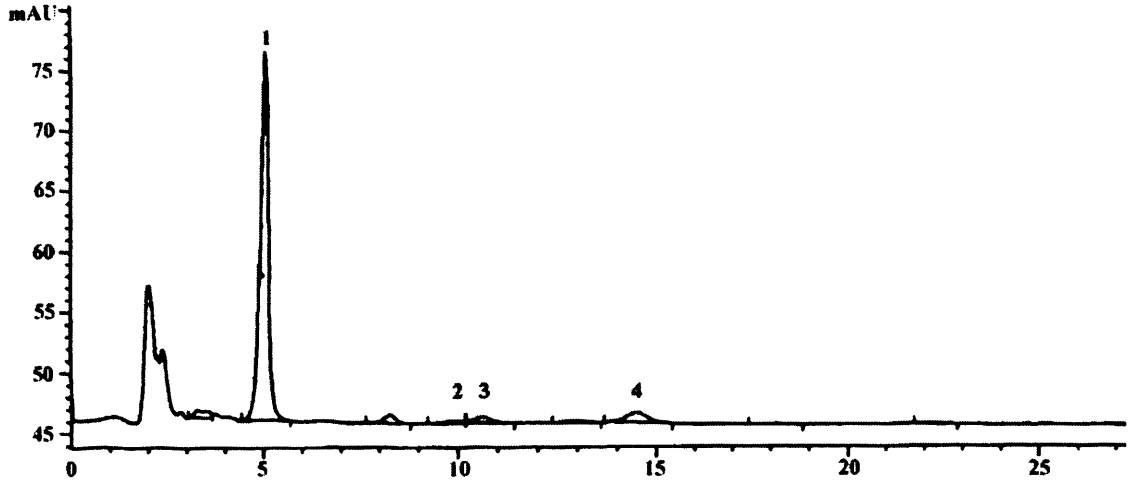


Figure 2.17. Chromatogram of tocopherols in olive oil samples of cv Koroneiki.
Note: 1 α-tocopherol; 2, β- tocopherol; 3, γ- tocopherol; 4, δ- tocopherol;

2.2.18. Chlorophyll analysis

The content of chlorophylls and their derivatives was determined according to the American Oil Chemist official method (AOCS official method Cc13d-55). The concentration of chlorophyll in the oil is expressed in p.p.m. from the spectrophotometric absorpion measurements at 630, 670 and 710nm according to the equation.

$$\text{Chlorophyll (ppm)} = [A_{670} - (A_{630} + A_{710}) \times 0{\cdot}5] / 0{\cdot}101\ L$$

Where A= absorbance, L= thickness of cuvette (1cm).

The absorptions were measured in crude oil against a blank of carbon tetrachloride.

The chlorophyll fraction of olive oil is made up of chlorophylls a and b and their magnesium- free derivatives, pheophytins a and b. The content of chlorophylls and their derivatives depends on the stage of ripeness and decreases continuously from green to black olive fruits (Minguez- Mosquera *et al.*, 1991; Stefanoudaki *et al.*, 1992). In oils that have undergone refining, the content of these pigments decreases remarkably because of their absorption at the decolorization stage and because of degradation during the whole process (Morales and Camacho, 2000).

2.2.19. Determination of volatiles

The content of volatiles was determined by dynamic headspace gas chromatography according Morales *et al.* (1994). 0·5 gr of olive oil with 3·33 ppm (w/w) of isobutyl acetate added as internal standard was placed in a vessel, stirred and heated at 40 °C to facilitate the removal of the volatiles. A stream of nitrogen gas was flushed over the surface of the oil at a rate of 200 ml min^{-1}. The carrier gas was passed through a condenser in order to remove any water vapour, which might have hindered the chromatographic analysis.

The volatiles were then trapped in a sample tube packed with Tenax TA (Perkin Elmer). The sampling time was 30 min. Thermal desorption of the trapped volatiles was achieved using an Automatic Thermal Desorption System (Perkin Elmer ATD 400 model) set at 250. The volatiles were then flushed from the sample

tube again using nitrogen carrier gas (18 –22 psi) and concentrated on a second trap containing Tenax Tabut at temperature of -30°C for 10 min. The cold trap was flushed heated to 250°C and the volatiles were automatically injected onto a GC capillary column. A Perkin Elmer (PE) Autosystem GC was fitted with a fused-silica Supelcowax 10 capillary column (60m x 0.32 mm ID, 0.5 μm film thickness) using helium as carrier gas and nitrogen as a make- up gas and the oven temperature was set initially at 40 °C for 4 min and was programmed to rise at 4 °C min^{-1} until 240°C was reached. The injection temperature was 175°C and the detector temperature was 275°C. Both the Autosystem GC and the ATD 400 were controlled automatically by a PE Nelson Model Integrator Programmer. Tentative identification was made by comparing retention times of the GC peaks with authentic standards. Peaks were identified by mass spectra by using a Fisons MD800 Mass Selective Detector. The software used was MassLab VI3. Sample components were verified by comparison of the mass spectral data with those of authentic reference standards. When standards were not available, the components were identified by mass spectra obtained from the mass spectral library collection.

Dynamic headspace (DHS) sampling methods prior to capillary column gas chromatography are especially suitable for the determination of volatile compounds at a wide range of concentrations. The great advantage of DHS is concentrating the sample so that it is possible to detect compounds at very low concentrations (Morales et al., 1994). The method consisted of purging the volatile compounds from the sample, which was subjected to a determined temperature, with an inert gas at a controlled flow and passing them through a trap where they retained. They are later desorbed and injected into the chromatograph for their separation. The amount of volatile compounds depend on temperature, sample size, absolute quantity of the gas used for the stripping, the type of compounds to be analysed and the material employed for the trapping as well as diameter and length of the traps (Morales and Tsimidou, 2000).

2.2.20. Statistical analysis

The Independent-Samples t-test procedure was used for equality of means of olive oils between varieties, irrigated and non-irrigated olive trees, stage of maturation as well as 2-phase and 3-phase extraction methods. Differences were considered to be significant at $p \leq 0.05$.

Furthermore, General Linear Model (GLM) multivariate analysis was applied to the data set in order to study the effect of temperature and time of olive paste malaxation and salinity levels on olive oil quality parameters. Duncan's multiple range test was performed to show differences at $p \leq 0.05$ level of significance.

All statistical analyses were conducted using SPSS of the Windows Statistical package version 8.0 (SPSS 1986).

Chapter 3. Olive oil processing

3.1. Introduction

According to EC regulations, virgin olive oil is the only product from olive tree fruits (*Olea europea L.)* obtained under mechanical conditions, and at temperatures, which do not lead to alterations in the oil. Moreover, the latter should not undergo any treatment other than washing and filtration. Because of these stipulations about the extraction of oil from olive fruits, virgin olive oil has a characteristic aroma, taste and colour that distinguish it from other vegetable oils. Olive oil can be used directly for human consumption and does not need complex processing steps such as bleaching, deodorizing and refining.

Olive fruits can contain 15-30 % of oil on a wet weight basis. Most of the oil is located in the flesh of mesocarp (Kiritsakis *et al.,* 1990). There is some oil in the skin (epicarp) and about 1-3 % in the endocarp. The oil inside the mesocarp cells is found as ill-defined oil droplets, which are, essentially, part of the cytoplasm. They differ from oil droplets in seeds (including olive seeds) where the storage triacylglycerol is contained in lipid bodies of a defined size and which a half-unit membrane bound. Associated with seed oil bodies are oleosin proteins that may play a role in storage lipid breakdown during germination (Murphy, 1993)

During extraction, as a result of crushing and the rheological properties of the fruits, a substantial part of the storage oil is dispersed. The resultant droplets form a micro gel that is mainly distributed in pockets and channels in the olive mash. If this colloidal dispersion is not treated correctly, a major fraction of the emulsion oil is removed and lost with the by-products. Indeed, the oil that is emulsified with the tissue water cannot be recovered by most current centrifugal methods. However, the slow malaxation of paste makes the production of large drops of oil easier and leads to a continuous oil phase separated from the paste solids and vegetable water (Di Giovacchino, 1991; Angerosa *et al.,* 2001). Thus, olive oil processing involves a number of basic operations, each of which must be carried out correctly if good extraction and high quality oil is to be achieved.

During the last 30 years remarkable progress has been made in the field of olive oil processing. The classical mill, which has been used over centuries for oil

extraction, has been almost completely replaced by centrifugal separation. This centrifugal system was introduced in an attempt to improve the efficiency of the olive oil extraction process and to improve the quality of oil, as well as to reduce labour. In this system, centrifugal force is applied to the water-diluted paste in a high-speed rotated machine (3000 to 4000 r.p.m.), which is called a horizontal centrifuge or decanter. The optimum amount of water needed to dilute the paste is determined by the characteristics of the olives and the operating parameters of the centrifuge. Recent experiments on the influence of oil paste dilution on extraction yield and quality showed that best results in terms of extraction yield, were obtained with 32% water added to olive paste which itself came from olives with a moisture content of 43 to 45% (Amirante *et al.*, 2001).

In 1992 new oil processors were introduced in the form of the 2-phase decanter, which is able to centrifuge the oily phase without diluting it with water. These decanters produce a minimum amount of liquid waste effluent, which is about 8 to 9 times less than that for three-phase decanters (Koutsaftakis and Stefanoudaki, 1995; Ranalli and Martinelli, 1995; Ranalli and Angerosa, 1996). Because the endogenous olive fruit water (vegetation water) is not separated from the husk the moisture of pomace is up to 60 %. The high content of pomace moisture is a disadvantage because the methods for transportation and its treatment at the factories have to be modified. On the other hand, the reduction in wastewater is a considerable environmental advantage.

The aim of the experimental work described in this chapter was to ascertain the effect of 2- phase, and 3-phase decanters on the qualitative and organoleptic characteristics of olive oils from two cultivars, Koroneiki and Coratina. The two cultivars were chosen because they represent major cultivars used for Greek and Italian olive oils, respectively, and because it is known that they have distinct organoleptic characteristics (see section 3.3.1). Furthermore, laboratory scale as well as industrial level extractions were carried out in order to gather further information of the effect of temperature and malaxation time, on qualitative characteristics of olive oil and its yield. Such detailed information should help industry to apply the most appropriate conditions during oil extraction in order to optimize the activities of endogenous enzymes that give rise to positive attributes in olive oil and to minimize the factors, which contribute to quality deterioration.

3.2. Effect of different extraction systems on the qualitative characteristics of olive oil

3.2.1. Differences between cultivars

Virgin olive oil composition is influenced by several factors, including the olive variety (Montedoro, 1989; Aparicio and Luna, 2002), agronomic techniques (Montedoro and Garofalo, 1984; Montedoro and Servili, 1992), environmental conditions and ripening stage (Frega *et al.*, 1991; Mariani *et al.*, 1991; Frega *et al.*, 1992; Garcia *et al.*, 1996; Morales *et al.*, 1996; Ranalli *et al.*, 1999) and extraction technology (Angerosa *et al.*, 2000b; Koutsaftakis *et al.*, 1999; Cert *et al.*, 1999).

In this research work both sensory and chemical characteristics of virgin olive oil obtained from Coratina and Koroneiki varieties were studied. The olives were picked when 50% of the olives displayed partial purple color. The mean values and standard deviations for major quality parameters and intensities of sensory attributes as detected by tasters are presented in Tables 3.1. to 3.4. The values of the analytical parameters responsible for quality and authenticity of oils samples studied fell within the ranges established by the European Commission (1991) for the higher quality category "extra virgin olive oil". α-tocopherol content was significantly higher in oils from Koroneiki cultivar. α-Tocopherol comprises about 98 % of the total tocopherol fraction and exerts both vitamin potency and antioxidant action. (Psomiadou and Tsimidou, 1998; Ranalli and Angerosa, 1996). Oils of Koroneiki cultivar showed higher contents of campesterol, Δ^5avenosterol and aliphatic alcohols but lower β-sitosterol than Coratina (Table 3.2). The fatty acid compositions of the two types of oils are reported in Table 3.3. In Coratina cultivars the concentrations of C16:0 and C16: 1 are lower and that of C18: 1 is significantly higher than Koroneiki cultivar. The percentage of certain individual fatty acids (which are markers of genuineness), such as myristic, linolenic, arachidonic, behenic and lignoceric acid were within the limits set by E.C. Regulation no. 2568 (1991). The results of sensory profile showed that oils of Koroneiki cultivar are characterized by high intensities of green leaf or twig and green olives attributes (Table 3.4). The latter was significantly higher than for Coratina. In contrast, oils of Coratina cultivars are characterized by high intensity of the bitter almond and pungent attributes (Table 3.4). The higher

intensities of pungent attributes may be due to the higher content of total phenolic compounds as well as to secoiridoid phenols analyzed by HPLC (Table 3.1).

Table 3.1. Comparison of general qualitative characteristics of virgin olive oils obtained by laboratory extraction mill from Koroneiki or Coratina cultivars.

Analytical oil parameters	Koroneiki	Coratina	Sig. (2-tailed)
Acidity (% total oil)	0·31± 0·09	0·22± 0·04	0·02
Peroxide (meqO_2/kg oil)	5·47± 1·43	3·25± 1·20	<0·005
K232 (OD 232 nm)	0·14± 0·03	0·14± 0·02	0·65
K270 (OD 270 nm)	1·54± 0·03	1·63± 0·16	0·15
Total Phenols (ppm gallic acid)	303.76± 76.96	347·11± 45·47	0.20
Oxidative stability (h)	20·60± 3·00	21·88± 1·29	0.29
α-tocopherol (mg/kg)	145·2± 7·40	116·25± 5·23	<0·005
Chlorophyll (ppm)	7·52± 0·86	6·44± 0·59	<0·005
3,4-DHPEA (ppm)	2·01± 1·06	2·54± 3·00	0·65
p-HPEA(ppm)	3·12± 0·53	2·97± 0·23	0·21
3,4-DHPEA- EDA (ppm)	540·10± 104·21	630·71± 97·76	0·45
p-HPEA-EDA (ppm)	322·18± 136·87	476·49± 101·44	0·02
p-HPEA-Derivative (ppm)	123·03± 89·79	161·00± 1·89	0·06
3,4-DHPEA- EA (ppm)	199·98± 66·86	230·63± 85·14	0·55

Data are expressed as means ± standard deviation (n=8), p values are shown for significance by 2 tailed t-test for equality of means between the two cultivars. Qualitative characteristics were quantified as described in Sections 2.2.5, 2.2.6, 2.2.7, 2.2.8, 2.14, 2.2.15, 2.2.16, 2.2.18. Phenolic abbreviations are in the abbreviation list.

In olive oil, the main phenols are secoiridoid compounds. It is generally thought that compounds mainly responsible for the bitter-pungent characteristics of oils are 3,4-DHPEA and p-HPEA and the aglycones that contain them, which

originated by degradation of the glucosides oleuropein and ligstroside through β-glucosidase enzyme activity (Servili and Montedoro 2002; Angerosa *et al.*, 2000a).

On the other hand, a good relationship was found between oxidative stability of olive oil and the content of phenolic compounds (r= 0·74 and 0·91) respectively for the Coratina and Koroneiki cultivars. These results are in agreement with those obtained by Baldioli *et al.* (1996).

Table 3.2. Comparison of the percentage composition of sterols in virgin olive oils obtained from Koroneiki or Coratina cultivars by laboratory mill.

	Koroneiki	Coratina	Sig. (2-tailed)
Cholesterol (%)	0·18± 0·06	0·20± 0·01	0·30
Campesterol (%)	3·81± 0·06	3·40± 0·21	<0·005
β-Sitosterol (%)	77·93± 1·70	85·19± 1·01	<0·005
Δ^5Avenosterol (%)	12·89± 2·20	5·46± 0·76	<0·005
Δ^7 Stigmastenol (%)	0·23± 0·06	0·28± 0·04	0·03
Δ^7 Avenasterol (%)	0·33± 0·07	0·35± 0·13	0·67
Erythrodiol+Uvaol (%)	2·86± 0·43	1·95± 0·24	<0·005

Data are expressed as means ± standard deviation (n=8), p values are shown for significance by 2 tailed t-test for equality of means between the two cultivars. Sterols were measured as described in section 2.2.9.

Table 3.3. Comparison of analytical parameters of virgin olive oils obtained from Koroneiki or Coratina cultivars by laboratory mill.

Analytical oil parameters	Koroneiki	Coratina	Sig. (2-tailed)
C16:0 (% total fatty acids)	11·31± 1·00	8·50± 0·95	<0·005
C16: 1 (% total fatty acids)	0·77± 0·17	0·34± 0·08	<0·005
C18:0 (% total fatty acids)	2·67± 0·21	2·41± 0·63	0·29
C18: 1 (% total fatty acids)	77·29 0·29	80·79± 0·99	<0·005
C18: 2 (% total fatty acids)	5·43± 0·32	5·80± 0·33	0·04
C18: 3 (% total fatty acids)	0·70± 0·05	0·74± 0·05	0·08
C20:0 (% total fatty acids)	0·49± 0·04	0·42± 0·09	0·06
LLL (% TAGs)	0·15± 0·07	0·14± 0·06	0·86
OOO (% TAGs)	51·27± 3·27	56·91± 2·02	<0·005
Total triterpenic alcohols (mg/100g)	40·94± 4·47	64·19 08·70	<0·005
Total aliphatic alcohols mg/100g)	15·42± 3·90	7·21± 1·05	<0·005

Data are expressed as means ± standard deviation (n=8), p values are shown for significance by 2 tailed t-test for equality of means between the two cultivars. Qualitative characteristics were measured as described in chapter 2. Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the numbers afterwards giving the number of double bonds. LLL= trilinolein. 000= triolein.

Table 3.4. Comparison of sensory attributes perceived in virgin olive oils obtained from Koroneiki or Coratina cultivars by laboratory mill.

.	Koroneiki	Coratina	Sig. (2-tailed)
Green Olives	3·06± 0·41	2·71± 0·45	<0·005
Green leaf or twig	1·28± 0·41	1·06± 0·38	0·80
Cut green lawn	0·70± 0·44	1·42± 0·28	0.03
Almond	0·30± 0·22	0·39± 0·24	0·26
Bitter almond	0·70± 0·17	1·30± 0·33	<0·005
Walnut husk	0·45± 0·17	0·43± 0·35	0·40
Artichoke	0·45± 0·38	0·56± 0·36	0·38
Apple	0·56± 0·33	0·21± 0·31	<0·005
Green banana	0·26± 0·12	0·49± 0·19	0·65
Bitter	2·36± 0·28	2·60± 0·71	0·12
Pungent	1·79± 0·47	2·25± 0·43	<0·005
Sweet	0·26± 0·13	0·19± 0·05	0·16

Data are expressed as means ± standard deviation (n=6), p values are shown for significance by 2 tailed t-test for equality of means between the two cultivars. Sensory attributes were measured as described in section 2.2.8. A 0-5 scale was used to score the sensory attributes.

3.2.2 Acidity, peroxide index, UV spectrophotometric indices with different extractions

These parameters are generally related to the quality and soundness of the olives and are often dependent on possible enzymatic alterations of the olive fruit constituents. Free (non-esterified) fatty acids show a prooxidant effect due to the presence of carbonyl group in their structure (Frega *et al.,* 1999). In addition, for example, free PUFAs are substrates for lipoxygenases. The probable mechanism for general oxidation is attributed to the catalytic action of the carbonyl group on the decomposition of a small amount of hydroperoxides formed in the initial stages of autooxidation (Morales and Prybylski, 2000). Measurement of peroxide values is an

indicator of the initial oxidation because it measures the hydroperoxide content. The specific extinction at 232 nm (K_{232}) is related to the primary oxidation products. During formation of peroxy radical and hydroperoxides, a shift in double bond configuration occurs, thus transferring the normal methelene-interrupting configuration into conjugated forms. K_{270} and ΔK (triene peak) are related to the secondary oxidation products. UV spectrophotometric indices are also useful to detect fraudulent addition of refined olive oil to virgin olive oil. (For the purpose of detecting refined oil, if the K_{270} exceeds the limit for the category concerned, it must be determined again after passage of the oil over Al_2O_3 column (E.C. regulations 2568/91).

Table 3.5. Comparison of the qualitative characteristics of virgin olive oils obtained from olive fruits of *cv* Koroneiki by the two and three- phase decanter methods

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
Acidity (% total oil)	0·56 ± 0·01	0·61 ± 0·02	0·174
Peroxide ($meqO_2$/kg oil)	7·71 ± 0·25	8·45 ± 0·57	0·015
K232 (OD 232nm)	1·35 ± 0·21	1·48 ± 0·05	0·173
K270 (OD 232nm	0·16 ± 0·01	0·17 ± 0·02	0·325
Total phenolics (ppm gallic acid)	135·17 ± 19·50	111·66 ± 13.49	<0·005
Resistance to oxidation (hr)	10·51 ± 0·26	8·63 ± 0·26	<0·005
Chlorophyll (p.p.m)	7·10 ± 1·04	7·93 ± 0·35	0·091
α- Tocopherol (mg/kg)	157·10 ± 10·63	163·65 ± 10·54	0·150

Data are expressed as means ± standard deviation (n=6). t-test for equality of means for the two extraction methods shown with P values given. Qualitative characteristics were quantified as described in Sections 2.2.5, 2.2.6, 2.2.7, 2.2.14, 2.2.16, 2.2.18.

Table 3.6. Comparison of the qualitative characteristics of virgin olive oils obtained from olive fruits of *cv* Coratina by two and three phase decanter methods.

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
Acidity (% total oil)	0·29 ± 0·02	0·30 ± 0·02	0·739
Peroxide ($meqO_2$/kg oil)	6·84 ± 0·87	6·83 ± 1·76	0·994
K232 (OD 232nm)	1·52 ± 0·01	1·39 ± 0·10	0·010
K270 (OD 232nm	0·13 ± 0·01	0·12 ± 0·01	0·040
Total phenolics (ppm gallic acid)	181·08 ± 10·67	105·13 ± 13·49	<0·005
Resistance to oxidation (hr)	14·18 ± 0·86	9·82 ± 1·15	<0·005
Chlorophyll (p.p.m)	6·58 ± 0·83	6·30 ± 0·14	0·444

Data are expressed as means ± standard deviation (n=6) t-test for equality of means for the two extraction methods shown with P values given Qualitative characteristics were quantified as described in Sections 2.2.5, 2.2.6, 2.2.7, 2.2.14, 2.2.16, 2.2.18.

There were no significant differences between acidity, peroxide index and ultra-violet absorption coefficients K_{232} and K_{270} between 2- and 3-phase decanter extraction methods (Table 3.5, 3.6) cultivars studied. These results confirm previous reports for the Coratina cultivar (Ranalli and Angerosa, 1996; Di Giovacchino, 2000).

3.2.3. Phenolic compounds

Phenolic compounds are considered to be the most important antioxidants in olive oils. The level of these compounds affects many of the qualitative parameters of virgin olive oils and is related to the oil's resistance to oxidation (Baldioli *et al.*, 1996). The concentration of phenolics also contributes to the organoleptic characteristics by preventing the degradation of volatile compounds and, furthermore, is responsible for the typical pungent and bitter taste of olive oil (Gutierrez, 1992; Olias *et al.*, 1993; Angerosa and Di Giacinto, 1995). Phenolics can contribute to nutritional properties by reducing the levels of conjugated dienes and trienes caused by oxidation of polyunsaturated fatty acids. In fact, the nutritional value of olive oil is directly related to the concentration of natural antioxidants such as 3,4-DHPEA, p-

HPEA, 3,4-DHPEA-EDA, 3,4- DHPEA-EA (Petroni *et al.,* 1995; Visioli *et al.,* 1995). The oils obtained by the two- phase decanters displayed significantly higher values in total phenols and in resistance to oxidation for both the cultivars studied (Tables 3.5 and 3.6). In addition, most secoiridoid compounds showed higher values in the 2-phase system. However, significant differences were only noted in 3,4-DHPEA-EDA, 3,4- DHPEA-EA, p-HPEA-EDA and p-HPEA derivative for oils of cultivar Coratina (Table 3.7and 3.8). The differences in total polyphenols are probably because, in the three- phase system, dilution of the paste with water causes the phenolic compounds in the oily phase to be partly transferred into the aqueous phase because of partitioning between the two immiscible liquids (oil and vegetation water). Each phenolic compound has its own specific distribution coefficient (K) between the oil and vegetation water (Ranalli and Angerosa, 1996; Rodis *et al.,* 2002). Thus, during the extraction process of olive oil, a significant amount of phenols is discarded with the wastewater. Direct consequences of the increased phenolics with two- phase extraction are the increase in oxidative stability and improved flavor (Vaquez-Roncero *et al.,* 1976; Chimi *et al.,* 1991; Papadopoulos and Boskou, 1991, Baldioli *et al.,* 1996).

The chromatographic profile of the phenolic substances present in olive oils of 2-phase and 3-phase extraction systems is shown in Figure 3.1. The two chromatograms show similar phenol profiles with differences relating only to the peak area (or quantity).

Table 3.7. Comparison of the contents of phenolic compounds in virgin olive oils obtained from cv. Koroneiki by two- and three-phase decanter methods.

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
3,4-DHPEA	0·57 ± 0·06	0·47 ± 0·06	0·10
p-HPEA	1·78 ± 0·03	1·10 ± 0·53	0·09
3,4-DHPEA-EDA	106·77 ± 17·53	51·02 ± 14·82	0·50
p-HPEA-EDA	76·84 ± 12·14	79·41 ± 9·03	0·55
p-HPEA der.	9·65 ± 1·95	8·54 ± 1·19	0·44
3,4-DHPEA-EA	294·35 ±76·35	230·3± 21·56	0·35

Data are expressed as means ± standard deviation (n=6). p values are shown for significance by 2 tailed t-test for equality of means between the two extraction methods. Phenolic compounds were measured as described in section 2.2.15 Phenolics are abbreviated as shown in the list of abbreviations.

Table 3.8. Comparison of the contents of phenolic compounds in virgin olive oils obtained from cv.Coratina by two- and three-phase decanter methods.

	2-phase decanter	3- phase decanter	Sig. (2-tailed)
3,4DHPEA	1·30 ± 0·17	1·13 ± 0·35	0·50
p-HPEA	2·97 ± 0·23	2·10 ± 0·17	0·01
3,4 DHPEA-EDA	253·92 ± 19·98	93·12 ± 4·10	<0·005
p-HPEA-EDA	153·44 ± 27·58	89·68 ± 5·99	<0·005
p-HPEA der.	11·00 ± 1·89	5·92 ± 0·52	<0·005
DHPEA-EA	96·63 ± 3·34	54·96 ± 3·09	<0·005

Data are expressed as means ± standard deviation (n=6) p values are shown for significance by 2-tailed t-test for equality of means between the two extraction methods. Phenolic compounds were measured as described in section 2.2.15.

Phenolics are abbreviated as shown in the list of abbreviations.

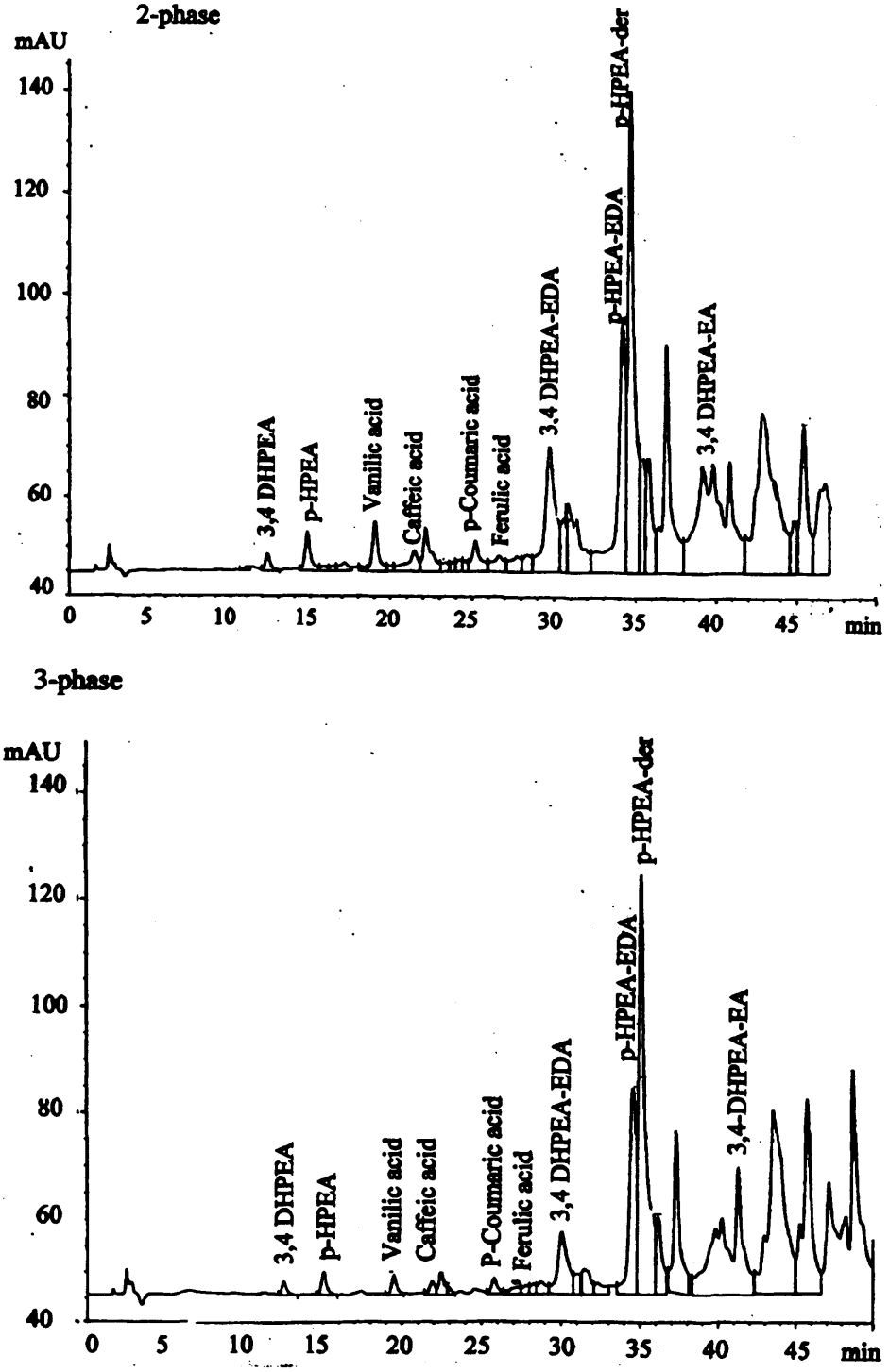


Fig. 3.1. Reverse- phase high performance liquid chromatography (at 278 nm) of phenolic extracts from virgin olive oil samples extracted by 2-phase and 3-phase extraction systems. (For method see section 2.15.).

3.2.4. Oxidative stability

Longer induction times for oxidation were observed for oils from the 2-phase decanter system (Table 3.5, 3.6). They were particularly increased for the Coratina cultivar, which in any case, showed better resistance to oxidation (Table 3.6). The higher stability values in the 2-phase decanter system can be attributed to the higher phenolic concentration in these oils. Moreover, it has been reported in previous research (Gutierrez *et al.*, 1977; Tsimidou and Boskou, 1997; Baldioli, *et al.*, 1996; Gutierrez and Fernandez, 2002; Vellasco and Dobarganes, 2002) that there is positive correlation between polyphenol content and stability of the oil. In the present work, the data confirmed this relationship in that longer inductions times were noted in the oils from the Coratina were the concentration of phenols were also higher (Table 3.1). Furthermore, there was a better increase in resistance to oxidation for Coratina where there was also a larger increase in phenolic content for the 2-phase extraction system (Tables 3.5 and 3.6). As discussed above, the higher phenolic content achieved by 2-phase extraction method should be beneficial, as it is most likely to lead to an improvement generally in both oxidative stability and in flavour.

3.2.5. Chlorophyll content

Although pigment analysis is not demanded by EC regulations, colour is a basic attribute in determining oil characteristics and is connected to quality in the minds of consumers. Although carotenoids make a contribution to olive oil color, chlorophylls are the main determinants and it is this green pigmentation that consumers look for. Chlorophyll content was quantified by spectrophotometric methods using wavelengths between 630 to 710 nm. The phaeophytins derived from chlorophyll a and chlorophyll b can also be significant and have their own absorbance characteristics. They can be differentiated from chlorophylls by appropriate absorption measurements. In our experiments, chlorophyll content was not affected by the extraction system for either cultivar (Tables 3.2 and 3.3). However, chlorophyll content was influenced significantly by the cultivar with values for Koroneiki being somewhat higher (Table 3.1). This is in keeping with the known differences between cultivars (Gandul-Rojas and Minguez- Mosquera, 1996).

3.2.6. Composition of sterols and triterpene dialcohols.

The Koroneiki cultivar is characterised by much higher content of delta 5-avenasterol than Coratina (Table 3.2). Otherwise the two olive cultivars were rather similar in sterol patterns with β-sitosterol as the major component (78% in Koroneiki and 85% in Coratina). The extraction method did not affect the concentration of individual sterols (Tables 3.9, 3.10) with the possible exception of β-sitosterol and Δ^5-avenasterol in the Koroneiki cultivar (Table 3.9). Because sterols are practically insoluble in aqueous systems, then it would not have been expected that the extraction method would have had much affect on their content in the oil. Therefore, the apparent increase in β-sitosterol and decrease in Δ^5-avenasterol for Konoreiki (Table 3.9) was surprising. However, there was considerable variation between samples so that the differences were only significant at the 10% level.

Table 3.9. Comparison of the sterol and triterpenic dialcohol percent composition of virgin olive oils obtained from cv. Koroneiki by two and three phase decanter methods.

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
Cholesterol (%)	0·20 ± 0·05	0·16 ± 0·08	0·329
Campesterol(%)	3·83 ± 0·07	3·80 ± 0·05	0·446
β-Sitosterol (%)	79·08 ± 1·46	76·78 ± 1·01	0·010
Δ^5-Avenasterol (%)	11·36 ± 1·72	14·43 ± 1·41	0·007
Δ^7- Stigmasterol (%)	0·23 ± 0·04	0·24 ± 0·08	0·808
Δ^7 Avenasterol (%)	0·34 ± 0·09	0·33 ± 0·04	0·682
Erythrodiol+ Uvaol(%	3·02 ± 0·38	2·69 ± 0·45	0·199

Data are expressed as means ± standard deviation (n=6). T-test for equality of means for the two extraction methods with P values shown. Sterols and triterpenic dialcohol were quantified as described in section 2.2.9.

Table 3.10. Comparison of the sterol and the triterpenic dialcohol percentage composition of virgin olive oils from Coratina extracted by two- and three- phase decanter methods.

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
Cholesterol (%)	0·21 ± 0·01	0·20 ± 0·01	0·270
Campesterol(%)	3·33 ± 0·18	3·48 ± 0·22	0·252
β-Sitosterol (%)	85·01 ± 0·84	85·38 ± 1·21	0·545
Δ^5-Avenasterol (%)	5·55 ± 0·76	5·38 ± 0·81	0·712
Δ^7- Stigmasterol (%)	0·30 ± 0·03	0·27 ± 0·04	0·092
Δ^7 Avenasterol (%)	0·37 ± 0·15	0·34 ± 0·12	0·711
Erythrodiol+ Uvaol(%)	1·93 ± 0·25	1·98 ± 0·26	0·748

Data are expressed as means ± standard deviation (n=6). T-test for equality of means for the two extraction methods with P values shown. Sterols and triterpenic dialcohol were quantified as described in section 2.2.9.

3.2.7. Long chain aliphatic and triterpene alcohol fraction

The aliphatic alcohol fraction includes saturated long chain alcohols (docosanol, tetracosanol, hexacosanol ,octacosanol) as well as treterpeneic alcohols (cycloartenol, 24-methylene cycloartenol). These compounds are useful to assess some authentic aspects of virgin olive oil (Paganuzzi, 1980; Tiscornia, 1992). No significant differences were observed in the content of aliphatic and triterpene alcohols in oils produced by the two-phase or the three–phase decanter. However, the total aliphatic alcohol content appeared to be higher (though not significantly) following the three-phase decanter (Table 3. 11 and Table 3.12).

Table 3.11. Comparison of the aliphatic and triterpene alcohol composition of virgin olive oils from Coratina extracted by two- and three- phase decanter methods.

	2-phase decanter	3- phase decanter	Sig. (2-tailed)
Docosanol (mg per 100g)	0·73 ± 0·42	0·50 ± 0·07	0·73
Tetracosanol (mg per 100g)	0·64 ± 0·48	0·99 ± 0·05	0·64
Hexacosanol (mg per 100g)	2·48 ± 1·68	3·57 ± 0·07	2·48
Octacosanol (mg per 100g)	1·31 ± 0·71	1·85 ± 0·05	1·31
Total aliphatic alcohols (mg per 100g)	6·58 ± 1·24	7·85 ± 0·19	0·15
Cycloartenol (mg per 100g)	40·98 ± 3·53	38·09 ± 2·95	0·34
24-Methylene cicloartenol (mg per 100g)	16·06 ± 1·27	15·06 ± 0·78	0·31
Citrostadienol (mg per 100g)	10·96 ± 5·96	7·23 ± 0·29	0·34

Data are expressed as means ± standard deviation (n=6). T-test for equality of means for the two extraction methods with P values shown. Aliphatic and triterpene alcohols were quantified as described in section 2.2.10.

Table 3.12. Comparison of the aliphatic and triterpene alcohol composition of virgin olive oils from Koroneiki extracted by two- and three- phase decanter methods.

	2-phase decanter	3- phase decanter	Sig. (2-tailed)
Docosanol (mg per 100g)	1·57 ±0·05	2·71 ±0·31	<0·005
Tetracosanol (mg per 100g)	2·92 ±0·46	4·11 ±0·53	0·04
Hexacosanol (mg per 100g)	5·23 ±0·96	7·45 ±2·50	0·22
Octacosanol (mg per 100g)	1·84 ±0·32	2·62 ±0·92	0·24
Total aliphatic alcohols (mg per 100g)	12·87± 1·90	17·97 ± 3·86	0·11
Cycloartenol (mg per 100g)	5·89± 1·91	5·31 ± 0·83	0·65
24-Methylene cicloartenol (mg per 100g)	32·82±2·41	33·41 ± 1·79	0·75
Citrostadienol (mg per 100g)	2·53± 1·58	1·93 ± 0·89	0·60

Data are expressed as means ± standard deviation (n=6). T-test for equality of means for the two extraction methods with P values shown. Aliphatic and triterpene alcohols were quantified as described in section 2.2.10.

3.2.8. Fatty acid composition

Although the fatty acid composition of oils was affected by the olive variety (Table 3.3) there were no significant changes (apart from in the minor component, palmitoleate in Koroneiki) caused by the two extraction methods (Tables 3.13 and 3.14). The ratio of C18: 2/C18: 3 determines the green aroma perception which can be additionally qualified with the tasting descriptors sweet or bitter. Empirical results indicate that the lower the ratio, the higher is the bitterness of olive oil (Aparicio, 2000).

Table 3.13. Comparison of the percent composition of fatty acids in virgin olive oils from cv. Koroneiki extracted by two- and three- phase decanter methods.

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
C16:0 (% total fatty acids)	10·83 ± 0·12	10·47 ± 0·06	0·01
C16:1 (% total fatty acids)	1·10 ± 0·17	1·50 ± 0·10	0·03
C18:0 (% total fatty acids)	2·53 ± 0·40	2·17 ± 0·06	0·19
C18:1 (% total fatty acids)	78·40 ± 0·20	78·07 ± 0·85	0·54
C18:2 (% total fatty acids)	5·43 ± 0·06	5·77 ± 0·31	0·14
C18:3 (% total fatty acids)	0·73 ± 0·06	0·70 ± 0·05	0·37
C18: 2/C18: 3 (% total fatty acids)	7·43 ± 0·47	8·24 ± 0·43	0·10

Data are expressed as means ± standard deviation (n=6). t-test for equality of means for the two extraction methods with P values shown. Fatty acids were quantified as described in section 2.2.12 Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the number afterwards giving the number of doubled bonds.

Table 3.14. Comparison of the percent composition of fatty acids in virgin olive oils from cv. Coratina extracted by two- and three- phase decanter methods.

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
C16:0 (% total fatty acids)	9·70 ± 0·17	9·90± 0·2	0·12
C16: 1 (% total fatty acids)	0·54 ± 0·10	0·56± 0·08	0·45
C18:0 (% total fatty acids)	2·33 ± 0·15	2·20± 0·20	0·32
C18: 1 (% total fatty acids)	79·77 ± 0·15	79·67± 0·21	0·54
C18: 2 (% total fatty acids)	5·67 ± 0·06	5·70± 0·09	0·37
C18: 3 (% total fatty acids)	0·82 ± 0·12	0·78± 0·09	0·19
C18: 2/C18: 3 (% total fatty acids)	6·98 ± 0·09	7·20± 0·12	0·37

Data are expressed as means ± standard deviation (n=6). t-test for equality of means for the two extraction methods with P values shown. Fatty acids were quantified as described in section 2.2.12 Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the number afterwards giving the number of doubled bonds.

3.2.9 Organoleptic Evaluation

Tables 3.15 and 3.16 show the sensory findings evaluated by the panel of 12 experts at the Subtropical Plants and Olive Tree Research Institute in Chania. The panelists were trained according to the olive oil quality standards of the European Community (E.C., 1991). These panelists work at the Institute and are habitual consumers of olive oil. The parameters assessed were biased toward the so-called "green" attributes. The extraction of olive oil was carried out from fruits at a particular stage of ripening (50% turning black under standardized conditions) (section 2.2.8). Thus, oils were extracted under the best conditions using the same process and the same operating conditions for both varieties. The only variation was that the paste in the two- phase decanter system was not diluted with water.

The oils obtained by the two- phase decanter system always exhibited higher values in the attributes bitter almond, bitter and pungent in both cultivars studied (Table 3.15, 3.16). However, statistically significant differences in these attributes were only noted in oils from the Coratina cultivar (Table 3.16). The Coratina cultivar also showed significant decreases in the values for green olives, sweet and apple attributes for the two-phase decanter system (Table 3.16).

Table 3.15. Comparison of the sensory intensities of virgin olive oils obtained from *cv* Koroneiki by two- and three- phase decanter methods.

	2-phase decanter	3-phase decanter	Sig. (2-tailed)
Green olives	3·18 ± 0·24	2·94 ± 0·51	0·224
Cut green lawn	1·09 ± 0·43	0·96 ± 0·41	0·500
Green hay	0·18 ± 0·12	0·34 ± 0·19	0·051
Green tomato	0·59 ± 0·32	0·45 ± 0·23	0·289
Bitter almond	0·34 ± 0·27	0·27 ± 0·28	0·569
Walnut husk	0·19 ± 0·17	0·10 ± 0·16	0·279
Artichoke	0·61 ± 0·42	0·30 ± 0·29	0·088
Apple	0·49 ± 0·30	0·62 ± 0·37	0·443
Green banana	0·15 ± 0·23	0·18 ± 0·22	0·779
Green leaf or twig	0·59 ± 0·29	0·71 ± 0·57	0·567
Bitter	0·50 ± 0·25	0·28 ± 0·26	0·089
Pungent	1·20 ± 0·39	0·89 ± 0·52	0·176
Sweet	2·29 ± 0·52	2·21 ± 0·92	0·821

Data are expressed as means ± standard deviation (n=9). T-test for equality of means for the two extraction methods with P values shown. Sensory attributes were quantified as described in section 2.2.8. Perception: 1. Barely perceptible, 2.Slightly, 3. Average, 4.Great, 5. Extreme.

Table 3.16. Comparison of the sensory intensities of virgin olive oils obtained from olive fruits of *cv.* Coratina by two- and three- phase decanter methods.

	Malaxation Time		
	2-phase decanter	3-phase decanter	Sig. (2-tailed)
Green olives	2·44 ± 0·50	2·95 ± 0·24	0·016
Cut green lawn	0·96 ± 0·28	1·15 ± 0·45	0·339
Green Hay	0·24 ± 0·24	0·29 ± 0·23	0·693
Green tomato	0·42 ± 0·24	0·53 ± 0·17	0·261
Bitter almond	1·27 ± 0·67	0·58 ± 0·39	0·019
Walnut husk	0·47 ± 0·47	0·40 ± 0·22	0·719
Artichoke	0·47 ± 0·36	0·64 ± 0·35	0·339
Apple	0·06 ± 0·16	0·36 ± 0·35	0·042
Green banana	0·17 ± 0·17	0·22 ± 0·21	0·624
Green leaf or twig	0·60 ± 0·32	0·47 ± 0·26	0·359
Bitter	1·77 ± 0·47	0·60 ± 0·29	<0·005
Pungent	2·04 ± 0·33	1·46 ± 0·31	<0·005
Sweet	0·84 ± 0·37	2·04 ± 0·49	<0·005

Data are expressed as means ± standard deviation (n=9) t-test for equality of means for the two extraction methods with P values shown. Sensory attributes were quantified as described in section 2.2.8 Perception: 1. Barely perceptible, 2.Slightly, 3. Average, 4.Great, 5. Extreme.

3.2.10. Conclusions

Despite differences in the oils derived from the two extraction systems, all values for the different components were within the limits for extra virgin olive oil as defined by the quality standards of European Community. However, 2-phase extraction seemed to enhance the phenolic content, in particular for oils from the Coratina cultivar (phenolics were increased 43% for Coratina and 18% for

Koroneiki). Furthermore, the differences in secoiridoid compounds between the 2-phase and 3-phase extraction systems were significantly higher but only in Coratina cultivar. Increased phenolic contents are most likely due to the addition of water to the olive paste in 3-phase decanter, which modifies the distribution of the hydrophilic phenol compounds between oily phase and aqueous phase. The low partition coefficient of most of olive oil phenolic compounds results in large losses in vegetation water. Thus, 2-phase decanter techniques can be considered as an alternative process for enriching olive oil with antioxidants. However, α-tocopherol content (because of its higher liposolubility) was not affected by the extraction system used. Oxidative stability was higher in oils prepared by the two-phase systems and this can be explained by the higher content of phenolic compounds. It is known that phenolic compounds play an important role in the antioxidant activity of olive oil (Vazquez Roncero 1978; Chimi *et al.*, 1991; Tsimidou *et al.*, 1992).

As regards the quality parameters of the oils, no clear trend was observed for oil acidity and the K_{270} and K_{232} values. The sensory evaluation of the samples from the two systems showed that higher bitter and pungent tasting attributes were noted in oils from the 2-phase system and there are usually regarded as desirable for consumers. Thus, 2-phase decanter represents an improved extraction method for olive oil quality, and at the same time seems to resolve olive-milling problems by significantly reducing the quantity of wastewater produced. This makes it the better choice from the environmental point of view when compared with 3-phase centrifugation. Furthermore, 2-phase decanter methodology decreased the costs of olive processing because it uses the minimum amount of hot water to dilute the pastes to be centrifuged. The vegetation water produced is very low and this, therefore, reduces the cost of its purification. The electrical energy consumption to warm up processing water is also reduced. However, the technique has the drawback that the pomace with the vegetation water (alpeorujo) has 60 –65 percent moisture content. Alpeorujo is a waste product of the olive oil mill industry that still has significant oil content. Before extracting the remaining oil with hexane, the moisture content of alpeorujo has to be reduced from approximately 65% to about 8% and this demands much more energy. In addition the sugars present in it make it sticky and difficult to dry (Ranalli and Martinelli, 1995; Arjona *et al.*, 1999).

3.3. Effect of malaxation on olive oil characteristics

Olive paste malaxing (mixing) is a basic step (following crushing) in the mechanical process of olive oil extraction and it involves stirring the paste slowly and continuously. This operation is important for the quantitative recovery of the oil from the paste, first by helping the droplets to merge into large drops (diameter >30μm) that can be separated by mechanical means (Martinez -Moreno *et al.,* 1957) and, second, by breaking the oil/water emulsions (Di Giovacchino, 1991; Servili *et al.,* 1998; Angerosa *et al.,* 2001; Ranalli *et al.,* 2001). Malaxation not only increases the 'free oil'' quantity but also contributes to the breaking of oily cells that remain uncrushed during the first step (grinding) and so permits the recovery of additional oil and, therefore, increases the final yield.

In addition, during malaxation, several chemical and enzymatic changes, which modify the oil's analytical characteristics, take place. Thus, during olive crushing and malaxation, a number of endogenous enzymes of olive fruit, such as polyphenoloxidase (PPO), peroxidase (POD), and lipoxygenase (LOX), are activated due to disruption of cellular tissues and these affect sensory and nutritional aspects of virgin olive oil quality (Servili *et al.*, 1998; Angerosa *et al.*, 1998). In particular, the enzymes involved in the LOX pathway which are activated during olive crushing and malaxation result in the formation of six and nine carbon atom volatile compounds that cause the 'green flavour' of virgin olive oil (Olias *et al.,* 1993; Angerosa *et al.*, 1998; Morales *et al.,* 1999) On the contrary, PPO and POD may promote oxidation of phenol compounds and the activity of these enzymes is related to the loss of phenolic compounds during malaxation (Servili *et al.,* 1998; Vierhuis *et al.*,2001).

In addition, changes also occur due to partitioning of compounds between oil and water. Thus, for example, the phenolic compounds are distributed during processing between the water and oil phases depending on their solubilities in the two phases (Di Giovacchino, 1991; Rodis *et al.*, 2002).

3.3.1. Influence of malaxation time

Analytical data, which relate oil basic quality characteristics to malaxation time, are presented in Tables 3.17, 3.20 to 3.23. The acidity, peroxide value and UV

spectrophotometric indices were in all cases below the legal upper limit for extra-virgin olive oils. In the laboratory scale experiments, these parameters and indices were not influenced significantly by malaxation time. However, in industrial based experiments (Table 3.20 to 3.23.) these parameters showed some changes due to kneading time, although consistent trends were not observed. Chlorophyll content increased significantly in all cases as the malaxation time increased. The oxidative stability of olive oil decreased from 0min to 60 min of malaxation time in laboratory scale experiments as well as from 20 min to 45min in industrial processes. These values showed the same trend as the total phenols. No differences were observed in the fatty acid composition (data not shown).

Table 3.17. Changes in qualitative characteristics of olive oils from semi-black olives of *cv* Koroneiki extracted by the laboratory mill process at 30 °C and at three malaxation times.

	Malaxation Time		
	0min	30min	60min
Acidity (% total oil)	0·21± 0·02 [a]	0·22±0·01 [a]	0·22±0·01 [a]
Peroxide (meqO_2/kg oil)	5·9± 0·73 [a]	6·40±0·77 [a]	6·38±0·05 [a]
K232 (OD 232nm)	1·73± 0·03 [a]	1·69±0·04 [a]	1·76±0·10 [a]
K270 (OD 270nm)	0·15± 0·02 [a]	0·11±0·01 [a]	0·13±0·02 [a]
Chlorophyll content (ppm)	1·43± 0·16 [a]	5·46±0·72 [a]	6·72±0·87 [a]
Total phenols (ppm gallic acid)	294·82±9·50 [a]	249·85±15·62 [b]	209·19±15·01 [c]
Oxidative stability (h)	19·67±4·31 [a]	17·43±2·33 [b]	13·77±2·45 [c]
α-Tocopherol (mg/kg)	167·55±4·99 [a]	172·57±6·06 [a]	176·21±5·70 [a]

Data are expressed as means ± standard deviation (n= 9). Means values in the same row with different superscripts are significantly different ($p<0·05$) Qualitative characteristics were quantified as described in 2.2.5, 2.2.6, 2.2.7, 2.2.14, 2.2.16, 2.2.17, 2.2.18.

Total phenol content of olive oil samples, measured calorimetrically, decreased during processing. In laboratory scale experiments the decrease was almost the same from 15% and 16% respectively from 0 min time to 30 min and from 30 min to 60 min (Table 3.17). In 3-phase decanter in both cultivars total phenols

increased from 20 min to 30 min and decreased from 30 min to 45 min for Koroneiki and to 60 min for Coratina (Table 3.21 and 3.23). It is important to note that the decrease never exceed 20% except for one case in 3-phase extraction system from 30 to 45 min in Koroneiki cultivar. However, none of these changes was statistically significant. Nevertheless, the apparent trends are in agreement with those obtained by other investigators ((Di Giovacchino *et al.*, 2000; Servili *et al.*, 1994).

On the other hand, α-tocopherol content did not change during malaxation (Table 3.17) and it should be noted that α-tocopherol comprises about 90% of the total tocopherols of olive oil. Previous research (Blekas and Boskou, 1998) studied the contribution of α-tocopherol on the oxidative stability of olive oil using a model system of purified olive oil triacylglycerols. It was found that α-tocopherol has a synergistic effect with the ortho- diphenols and can contribute significantly to the retardation of peroxide formation.

Table 3.18. Changes in phenolic compounds (mg/kg) of olive oils obtained from semi-black olives of *cv* Koroneiki extracted by laboratory mill processed at 30 °C and at 3 malaxation times.

	Malaxation Time		
	0min	30min	60min
3,4 –DHPEA	0·82± 0·09 [a]	1·18± 0·47 [a]	1·11± 0·46 [a]
p-HPEA	1·54± 0·24 [b]	2·56± 0·31 [a]	2·13± 0·43 [ab]
3,4 -DHPEA- EDA	181·17± 7·77 [b]	223·92± 43·00 [a]	86·97± 10·98 [c]
p-HPEA- EDA	212·0± 2·90 [a]	139·9± 7·68 [b]	100·6± 3·32 [c]
p-HPEA derivative	222·1± 1·65 [a]	119·1± 10·05 [b]	70·1± 7·40 [c]
3,4 –DHPEA- EA	393·6± 4·12 [a]	247·9± 23·23 [b]	147·7± 13·30 [c]

Data are expressed as means ± standard deviation (n= 9). Means values in the same row with different superscripts are significantly different ($p<0·05$) Phenolic compounds were measured as described in section 2.2.15

Phenolics are abbreviated as shown in the list of abbreviations.

The HPLC analysis of the polar olive oil (Table 3.18) showed that 3,4 – DHPEA, p-HPEA and 3,4 DHPEA-EDA increased (although the former was not statistically significant) from 0 min time to 30min during malaxation. The latter two compounds then decreased in amounts between 30 and 60 min. malaxation. p-HPEA-EDA, p-HPEA-ester and 3,4-DHPEA-EA decreased throughout malaxation. Secoiridoidal aglycons such 3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA are formed during crushing, by the hydrolysis of oleuropein, demethyloleuropein and ligstroside in reactions catalysed by endogenous β-glucosidases. In addition, since hydrophilic phenols in virgin olive oil depend on endogenous enzymes from the olive fruit, their concentration in the oil should depend on different extraction conditions. In addition, the distribution of phenolic compounds between water and oil (as pointed out before) is related to their solubility in the two media and will be dependent on any water added. In addition, reduction of phenolic compounds during malaxation can occur due to oxidative reactions catalyzed by endogenous enzymes such as PPO and POD.

The mixing process in industrial extractions takes place in semicylindrical or semispherical mixers of stainless steel where a large quantity of olive paste must be used according to good manufacturing practice (GMP). In such circumstances, only a small part of olive paste is in contact with atmospheric oxygen at the surface and the rest is protected. Recent experiments (Servili *et al.,* 1998) showed that reduction in the activity of PPO and POD, obtained by reducing oxygen levels in the paste during malaxation, strongly improved the concentration of hydrophilic phenols both in olive paste and virgin olive oil.

Changes in the content of aliphatic and triterpenic alcohols during malaxation are shown in Table 3.19. As can be seen from the data, a 113 % increase in total aliphatic alcohols was observed during 60 min of malaxation. The percent increase in concentration was higher from 0 to 30 min of malaxation where it was about 70 %.

Table 3.19. Change in content of aliphatic and triterpene alcohols of olive oils from semi-black olives of cv Koroneiki extracted by laboratory mill processed at 3 malaxation times and at 30 °C.

	Malaxation Time		
	0 min	30 min	60 min
Docosanol (mg/100g)	0·7	1·0	1·3
Tricosanol (mg/100g)	0·2	0·2	0·3
Tetracosanol (mg/100g)	1·5	2·7	3·2
Pentacosanol (mg/100g)	0·3	0·4	0·5
Hexacosanol (mg/100g)	2·9	4·8	6·3
Heptacosanol (mg/100g)	0·2	0·7	0·9
Octacosanol (mg/100g)	1·19	1·8	2·3
Total aliphatic alcohols (mg/100g)	6·9	11·7	14·7
Cycloartenol (mg/100g)	13·5	12·4	12·8
24-Methylene cycloartenol (mg/100g)	12·9	14·6	16·9
Citrostadienol (mg/100g)	14·1	12·8	10·2

Data of single determination

Table 3.20. Changes in qualitative characteristics of olive oils from semi-black olives of *cv* Koroneiki extracted by the 2 phase decanter process at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	30 min	45 min
Acidity (%total oil)	0·48±0·01 [a]	0·45± 0·01 [a]	0·46± 0·00 [a]
Peroxide (meqO_2/kg oil)	7·96±0·23 [a]	7·64± 0·19 [a]	7·53± 0·14 [b]
K232 (OD 232nm)	1·50±0·01 [a]	1·08± 0·01 [c]	1·46± 0·01 [b]
K270 (OD 270nm)	0·17±0·00 [a]	0·15± 0·00 [a]	0·16± 0·00 [a]
Total phenols (ppm gallic acid)	153·57±11·30 [a]	133·95± 23·97 [a]	108·98± 15·38 [a]
Oxidative stability (h)	10·20±0·85 [a]	11·20± 0·28 [a]	10·15± 0·07 [a]
Chlorophyll (ppm)	5·81±0·01 [c]	7·44± 0·05 [b]	8·05± 0·07 [a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different ($p<0·05$) Qualitative characteristics were quantified as described in Sections 2.2.5, 2.2.6, 2.2.7,2.2.14, 2.2.16, 2.2.18.

Table 3.21. Changes in qualitative characteristics of olive oils from semi-black olives of *cv* Koroneiki extracted by the 3- phase decanter process at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	30 min	45 min
Acidity (% total oil)	0·64± 0·01 [a]	0·59± 0·00 [a]	0·61± 0·00 [a]
Peroxide (meqO_2/kg oil)	8·61± 0·17 [a]	7·87± 0·45 [a]	8·87± 0·57 [a]
K232 (OD 232nm)	1·52± 0·01 [a]	1·50± 0·01 [a]	1·42± 0·01 [a]
K270 (OD 270nm)	0·19± 0·00 [a]	0·17± 0·01 [b]	0·14± 0·00 [c]
Total phenols (ppm gallic acid)	97·40± 4·67 [a]	124·05± 17·89 [a]	94·20± 25·46 [a]
Oxidative stability (h)	6·10± 0·71 [b]	10·50± 0·57 [a]	9·30± 0·28 [a]
Chlorophyll (ppm)	7·80± 0·01 [b]	7·63± 0·04 [b]	8·37± 0·03 [a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different ($p<0·05$) Qualitative characteristics were quantified as described in Sections 2.2.5, 2.2.6, 2.2.7, 2.2.14, 2.2.16, 2.2.18.

Table 3.22. Changes in qualitative characteristics of olive oils from semi-black olives of cv Coratina extracted by the 2- phase decanter process at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	45 min	60 min
Acidity (% total oil)	0·29± 0·01 [a]	0·29±0·03 [a]	0·30± 0·02 [a]
Peroxide (meqO_2/kg oil)	6·24± 0·01 [b]	6·33±0·20	7·96± 0·01 [a]
K232 (OD 232nm)	1·52± 0·01 [a]	1·51±0·01 [a]	1·54± 0·00 [a]
K270 (OD 270nm)	0·13± 0·01 [a]	0·13±0·00 [a]	0·13± 0·00 [a]
Total phenols (ppm gallic acid)	187·85± 0·21 [a]	182·00±14·14 [a]	173·40± 12·59 [a]
Oxidative stability (h)	15·20± 0·28 [a]	14·00±0·14 [b]	13·35± 0·21 [b]
Chlorophyll (ppm)	5·86± 0·05 [c]	6·25±0·02 [b]	7·63± 0·01 [a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different (p<0·05) Qualitative characteristics were quantified as described in Sections 2.2.5, 2.2.6, 2.2.7,2.2.14, 2.2.16, 2.2.18.

Table 3.23. Changes in qualitative characteristics of olive oils from semi-black olives of cv Coratina extracted by the 3- phase decanter process at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	45 min	60 min
Acidity (% total oil)	0·30± 0·02 [a]	0·31± 0·01 [a]	0·29± 0·02 [a]
Peroxide (meqO_2/kg oil)	5·46± 0·00 [c]	5·95± 0·01 [b]	9·09± 0·18 [a]
K232 (OD 232nm)	1·27± 0·01 [b]	1·41± 0·01 [a]	1·49± 0·01 [a]
K270 (OD 270nm)	0·12± 0·01 [a]	0·11± 0·01 [a]	0·11± 0·01 [a]
Total phenols (ppm gallic acid)	98·85± 8·56 [a]	112·50± 24·04 [a]	104·05± 14·21 [a]
Oxidative stability (h)	11·15± 0·07 [a]	9·70± 0·14 [b]	8·60± 0·14 [c]
Chlorophyll (p.p.m)	6·22± 0·04 [a]	6·45± 0·14 [a]	6·24± 0·09 [a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different (p<0·05) Qualitative characteristics were quantified as described in Sections 2.2.5, 2.2.6, 2.2.7,2.2.14, 2.2.16, 2.2.18.

3.3.2. Effect of malaxation time on sensory qualities

The main sensory adjectives responsible for the unique aroma of virgin olive oil and which consumers recognize are fruity, green, bitter and pungent. The fruity sensation is reminiscent of healthy olive fruits harvested at the optimum time of fruit ripening. The perceptions called "green notes" are reminiscent of green leaves or twigs, cut grass, almond, bitter almond, artichoke and walnut husk. Recent research has revealed that the sensory green profile is due to the different concentrations of the three C_6 and C_5 fractions of volatile compounds in the aroma of olive oil (aldehydes, alcohols and esters) (Morales *et al.*, 1994; Aparicio *et al.*, 1996b). Furthermore, the relationship between volatile compounds and sensory perceptions has been studied in detail (Solinas *et al.*, 1987b; Olias *et al.*, 1993; Morales *et al.*, 1994; Aparicio *et al.*, 1996; Angerosa *et al.*, 2001).

The data (Tables 3.25-3.28) show the intensities of attributes perceived by tasters for oils from cv. Koroneiki and cv. Coratina at malaxation times frequently used in the extraction process for each cultivar. The results showed that mixing time affects (but not significantly) the sensory attributes of olive oil. The perception of cut green lawn increases in all cases during malaxation whereas fruity sensation (green olives) shows an increasing tendency in oil samples from cv. Coratina but the opposite was observed in the oil samples from cv. Koroneiki. The almond sensation was perceived with stronger intensity in most cases with increased malaxation time.

In recent work (Angerosa *et al.*, 2001) the changes in concentration of volatile compounds at different malaxation times and temperature were studied. Their results showed a steady increase of volatile compounds in oils extracted from two Italian cultivars (Coratina and Frantoio) (with the exception of *cis*-3-hexenyl acetate) at increasing malaxation time. By comparing changes in the concentration in volatiles compounds reported in previous research with the alteration in sensory attributes detailed in Tables 3.24-3.27 a good agreement with the attributes detected is found.

Table 3.24. Change in sensory attributes of olive oils from semi-black olives of *cv* Koroneiki extracted by the 2 phase decanter process at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	30 min	45 min
Green olives	3·35 ± 0·08[a]	3·28 ± 0·23[a]	2·95 ± 0·23[a]
Cut green lawn	1·15 ± 0·21[b]	1·27 ± 0·09[a]	1·62 ± 0·08[a]
Almond	0·25 ± 0·06[a]	0·44 ± 0·00[a]	0·67 ± 0·47[a]
Bitter almond	0·22 ± 0·11[a]	0·39 ± 0·08[a]	0·44 ± 0·62[a]
Walnut husk	0·25 ± 0·35[a]	0·17 ± 0·23[a]	0·11 ± 0·00[a]
Artichoke	1·02 ± 0·17[a]	0·66 ± 0·47[a]	0·56 ± 0·47[a]
Green leaf or twing	0·77 ± 0·47[a]	0·67 ± 0·16[a]	0·61 ± 0·07[a]
Bitter	0·56 ± 0·17[a]	0·44 ± 0·16[a]	0·36 ± 0·17[a]
Pungent	1·13 ± 0·17[b]	1·50 ± 0·08[a]	1·07 ± 0·31[a]
Sweet	2·47 ± 0·47[a]	1·84 ± 0·08[a]	2·39 ± 0·40[a]
Overall grade (1-9)	7·57 ± 0·61[a]	7·39 ± 0·40[a]	7·22 ± 0·16[a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different ($p<0·05$). Sensory attributes were quantified as described in section 2.2.8. Perception: 1. Barely perceptible, 2.Slightly, 3. Average, 4.Great, 5. Extreme.

Table 3.25. Change in sensory attributes of olive oils from semi-black olives of *cv* Koroneiki extracted by the 3- phase decanter processed at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	30 min	45 min
Green olives	3·51± 0·29[a]	3·11± 0·00[a]	2·35± 0·49[a]
Cut green lawn	0·75± 0·16[a]	0·78± 0·00[a]	1·00± 0·00[a]
Almond	0·14± 0·19[a]	0·39± 0·08[a]	0·28± 0·04[a]
Bitter almond	0·32± 0·45[a]	0·54± 0·19[a]	0·34± 0·06[a]
Walnut husk	0·00± 0·00[a]	0·22± 0·31[a]	0·13± 0·18[a]
Artichoke	0·09± 0·13[a]	0·78± 0·00[a]	0·21± 0·13[a]
Green leaf or twig	0·15± 0·21[b]	0·95± 0·08[a]	0·72± 0·17[a]
Bitter	0·28± 0·01[a]	0·42± 0·12[a]	0·52± 0·45[a]
Pungent	0·84± 0·03[a]	1·28± 0·08[a]	1·19± 0·27[a]
Sweet	2·54± 0·15[a]	2·55± 0·49[a]	2·29± 0·58[a]
Overall grade (1-9)	7·21± 0·09[a]	7·28± 0·08[a]	7·03± 0·39[a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different ($p<0·05$). Sensory attributes were quantified as described in section 2.2.8. Perception: 1. Barely perceptible, 2.Slightly, 3. Average, 4.Great, 5. Extreme.

Table 3.26. Change in sensory descriptors of olive oils from semi-black olives of *cv* Coratina extracted by the 3- phase decanter process at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	45 min	60 min
Green olives	2·62± 0·08[a]	3·10± 0·29[a]	3·13± 0·18[a]
Cut green lawn	1·00± 0·00[a]	1·37± 0·59[a]	1·45± 0·17[a]
Almond	0·57± 0·10[a]	0·50± 0·08[a]	0·68± 0·25[a]
Bitter almond	0·51± 0·08[a]	0·43± 0·18[a]	0·38± 0·06[a]
Walnut husk	1·00± 0·00[a]	0·86± 0·36[a]	0·41± 0·22[a]
Artichoke	0·74± 0·15[a]	0·37± 0·10[a]	0·38± 0·06[a]
Green banana	0·23± 0·07[a]	0·61± 0·39[a]	0·27± 0·02[a]
Green leaf or twig	0·37± 0·00[b]	0·84± 0·08[a]	0·57± 0·01[b]
Bitter	0·37± 0·12[a]	0·84± 0·08[a]	0·57± 0·01[a]
Pungent	1·39± 0·04[a]	1·53± 0·11[a]	1·66± 0·13[a]
Sweet	2·51± 0·52[a]	2·06± 0·08[a]	2·15± 0·21[a]
Overall grade (1-9)	6·51± 0·52[a]	6·06± 0·08[a]	6·15± 0·21[a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different ($p<0·05$). Sensory attributes were quantified as described in section 2.2.8. Perception: 1. Barely perceptible, 2.Slightly, 3. Average, 4.Great, 5. Extreme.

Table 3.27. Change in sensory attributes of olive oils from semi-black olives of cv Coratina extracted by the 2- phase decanter process at 30 °C and at three malaxation times.

	Malaxation Time		
	20 min	45 min	60 min
Green olives	1·98± 0·59[a]	2·81± 0·58[a]	2·53± 0·47[a]
Cut green lawn	0·82± 0·54[a]	1·06± 0·23[a]	1·25± 0·07[a]
Almond	0·61± 0·55[a]	0·32± 0·02[a]	0·50± 0·14[a]
Bitter almond	0·88± 0·00[a]	1·20± 1·13[a]	1·70± 0·42[a]
Walnut husk	0·41± 0·27[a]	0·45± 0·63[a]	1·02± 0·18[a]
Artichoke	0·83± 0·24[a]	0·16± 0·08[a]	0·74± 0·09[a]
Green leaf or twig	0·70± 0·42[a]	0·74± 0·09[a]	0·90± 0·14[a]
Bitter	1·88± 0·45[a]	2·23± 0·47[a]	1·95± 0·07[a]
Pungent	2·41± 0·27[a]	2·10± 0·00[a]	2·09± 0·27[a]
Sweet	0·52± 0·11[a]	0·59± 0·12[a]	1·18± 0·54[a]
Overall grade (1-9)	6·38± 0·25[a]	6·30± 0·28[a]	6·35± 0·49[a]

Data are expressed as means ± standard deviation (n= 2). Means values in the same row with different superscripts are significantly different ($p<0·05$). Sensory attributes were quantified as described in section 2.2.8. Perception: 1. Barely perceptible, 2.Slightly, 3. Average, 4.Great, 5. Extreme.

3.3.3. Influence of olive paste temperature during malaxation.

Table 3.28 shows the changes in qualitative characteristic of olive oil extracted at different malaxation temperatures using laboratory scale experimentation. After malaxation no water was added in the paste and the laboratory press allowed separation of oil and solids.

The specific absorption coefficients (specific extinction) in the ultraviolet region, K_{232} and K_{270} did not change significantly. The peroxide value, on the other hand, fluctuated from 15°C to 37°C and increased significantly at 42°C. Chlorophyll and phenol content increased significantly with increasing paste temperature.

Oxidative stability as measured by Rancimat apparatus, followed the same trend as total phenols.

The content of individual phenols measured by H.P.L.C. is presented in Table 3.29. 3,4-DHPEA and ferrulic acid increased, vanillic acid decreased whereas p-HPEA, caffeic acid and p-coumaric acid increased from 15°C to 28°C and then decreased. Increasing the temperature of olive paste up to 42°C during the malaxation step increased significantly the content of secoiridoids such as 3,4 DHPEA-EDA , p-HPEA-EDA p-HPEA der and 3,4 DHPEA-EA. Furthermore, industrial scale experiments carried out with a 2- phase decanter and at malaxation temperatures of 30°C or 40°C and malaxation time of 10 min, 30 min and 60 min also showed an increased content of total phenols at 40°C paste temperature following 30 min malaxation (Fig. 3.2). However, contradictory results are reported in the literature concerning the above subject. In some research papers, a reduction in phenol content was reported (Servili *et al.,* 1994; Cert et *al.,* 1999; Angerosa *et al.,* 2001). In other studies, just the opposite was found (Di Giovacchino, 1991; Ranalli *et al.,* 2001).

Figure 3.3 shows the intensities of the attributes perceived by tasters for oils from Koroneiki cultivar following an increase of the paste temperature during malaxation from 15°C to 42°C. Results showed that the intensities of attributes were decreased by increasing malaxation temperature. Tasters generally perceived a weakening of flavour at 30 min of malaxation and by raising malaxation temperature from 15°C to 42°C. Our results can be explained by the results obtained by Salas and Sanchez (1999) on LOX and HPL, the first two enzymes of the lipoxygenase pathway. They showed that at temperatures higher than 35°C, LOX activity decreased and, furthermore, HPL showed maximum activity at 15°C and a clear decrease at 35°C. This means that above 25°C the production of C6 volatiles responsible for the green aroma of olive oil would be limited.

Figure 3.4 relates the overall score award by the taster to olive oil samples extracted at different olive paste malaxation temperature. Higher scores were achieved at lower malaxation temperatures.

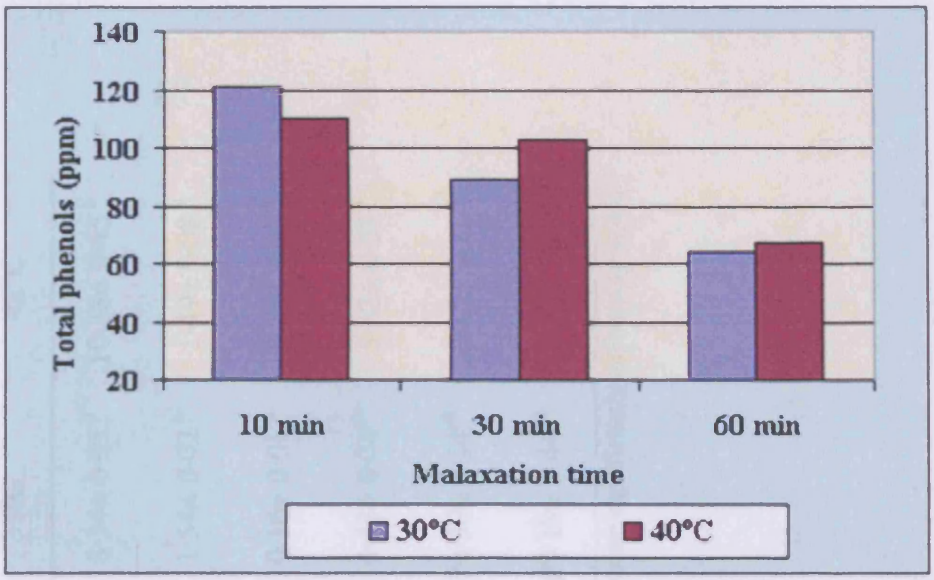


Figure 3.2. Total phenol content of olive oil of cv Koroneiki extracted by the 2-phase decanter process at three malaxation times and at 30°C and 40°C. Data of single determination.

Table 3.28. Effect of olive paste malaxation temperature on qualitative characteristics of olive oil extracted from olive fruits of cv Koroneiki in laboratory scale experiments.

	15°C	22°C	28°C	37°C	42°C
Chlorophyll (ppm)	3·23± 0·04 ^{c}	4·06± 0·01 ^{c}	4·33± 0·04 ^{c}	8·34± 0·05 ^{b}	10·38± 0·45 ^{a}
K232 (OD 232nm)	1·39± 0·03 ^{a}	1·43± 0·01 ^{a}	1·42± 0·01 ^{a}	1·54± 0·02 ^{a}	1·49± 0·08 ^{a}
K270 (OD 270nm)	0·16± 0·02 ^{a}	0·14± 0·00 ^{a}	0·14± 0·01 ^{a}	0·16± 0·01 ^{a}	0·15± 0·02 ^{a}
Peroxide (meqO_2/kg oil)	4·55± 0·07^{ab}	3·70± 0·43 ^{b}	4·07± 0·09 ^{b}	4·43± 0·04^{ab}	5·62± 0·03 ^{a}
Oxidative stability (h)	18·45± 1·06^{d}	23·05± 0·07^{bc}	21·30± 0·14^{cd}	26·30± 0·14^{ab}	27·15± 0·21 ^{a}
Total phenols (ppm gallic acid)	383·91± 36·04 ^{c}	540·89± 21·62 ^{b}	513·36± 66·31 ^{b}	609·19± 1·44^{ab}	711·12± 72·08 ^{a}

Data are expressed as means ± standard deviation (n= 4). Means values in the same row with different roman superscripts are significantly different ($p<0,05$)
Qualitative characteristics were quantified as described in Sections 2.2.6, 2.2.7, 2.2.14, 2.2.16, 2.2.18.

Table 3.29. Effect of olive paste malaxation temperature on phenolic composition of olive oil (mg/kg) extracted from olive fruits of cv Koroneiki in laboratory scale experiments.

	15°C	22°C	28°C	37°C	42°C
3,4- DHPEA	0·90± 0·13 ^{b}	0·73± 0·01 ^{b}	0·97± 0·06 ^{ab}	1·32± 0·42 ^{a}	1·47± 0·03 ^{a}
p-HPEA	1·43± 0·18 ^{a}	1·51± 0·09 ^{a}	1·72± 0·06 ^{a}	1·56± 0·40 ^{a}	1·48± 0·08 ^{a}
Vanillic acid	2·51± 0·06 ^{a}	2·23± 0·29 ^{b}	2·00± 0·11 ^{b}	1·59± 0·28 ^{b}	1·07± 0·09 ^{c}
Caffeic acid	0·24± 0·00 ^{b}	0·44± 0·05 ^{a}	0·80± 0·30 ^{ab}	0·63± 0·10 ^{ab}	0·44± 0·14 ^{ab}
p- Coumaric acid	0·48± 0·05 ^{a}	0·74± 0·06 ^{a}	0·73± 0·04 ^{a}	0·63± 0·09 ^{a}	0·41± 0·00 ^{a}
Ferrulic acid	0·37± 0·03 ^{c}	0·33± 0·11 ^{c}	0·32± 0·01 ^{c}	0·66± 0·01 ^{b}	0·85± 0·01 ^{a}
3,4- DHPEA-EDA	129·27± 4·19 ^{d}	216·67± 11·62 ^{c}	287·28± 6·65 ^{b}	508·00± 16·97 ^{a}	519·63± 0·53 ^{a}
p-HPEA-EDA	130·74± 0·08 ^{c}	167·75± 13·47 ^{b}	199·55± 4·17 ^{ab}	216·24± 22·97 ^{a}	218·06± 13·22 ^{a}
p-HPEA der	79·93± 3·74 ^{b}	129·88± 9·47 ^{a}	136·01± 7·94 ^{a}	122·83± 17·04 ^{a}	116·12± 8·69 ^{a}
3,4 DHPEA-EA	223·47± 21·10 ^{c}	425·73± 14·86 ^{b}	516·61± 9·68 ^{a}	586·50± 70·00 ^{a}	638·92± 15·68 ^{a}

Data are expressed as means ± standard deviation (n= 4). Means values in the same row with different roman superscripts are significantly different (P<0,05) Phenolic compounds were quantified as described in Section 2..15 Phenolics are abbreviated as shown in the list of abbreviations.

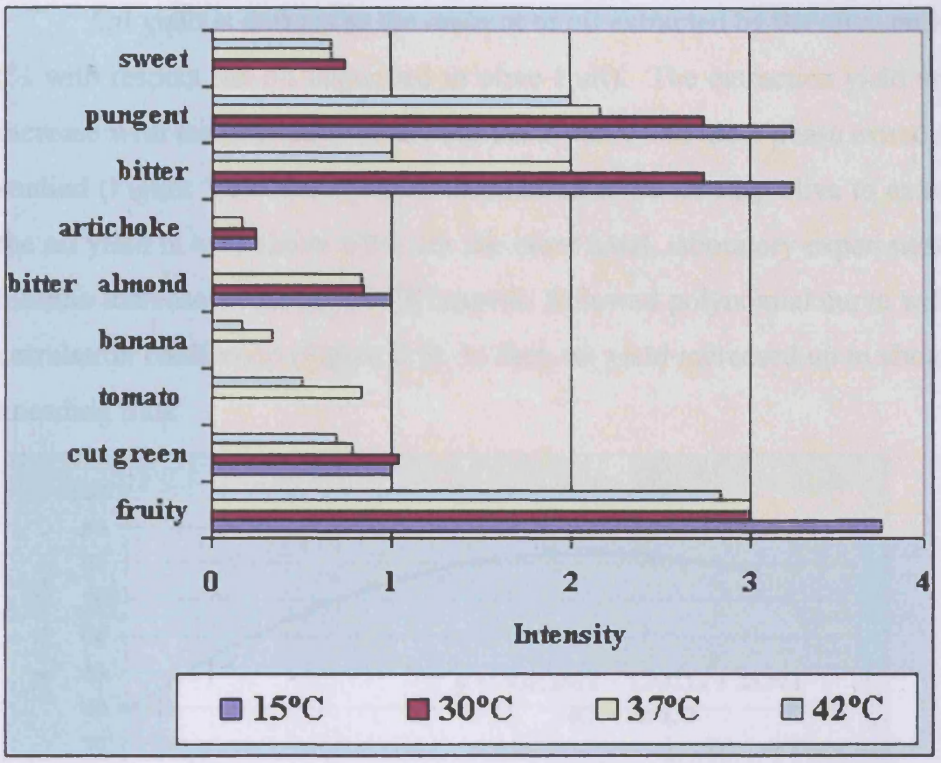


Figure 3.3. Intensities of attributes perceived by assessors for oils from *cv.* Koroneiki extracted at 15°C, 30°C, 37°C and 42°C after 30 min of malaxation.

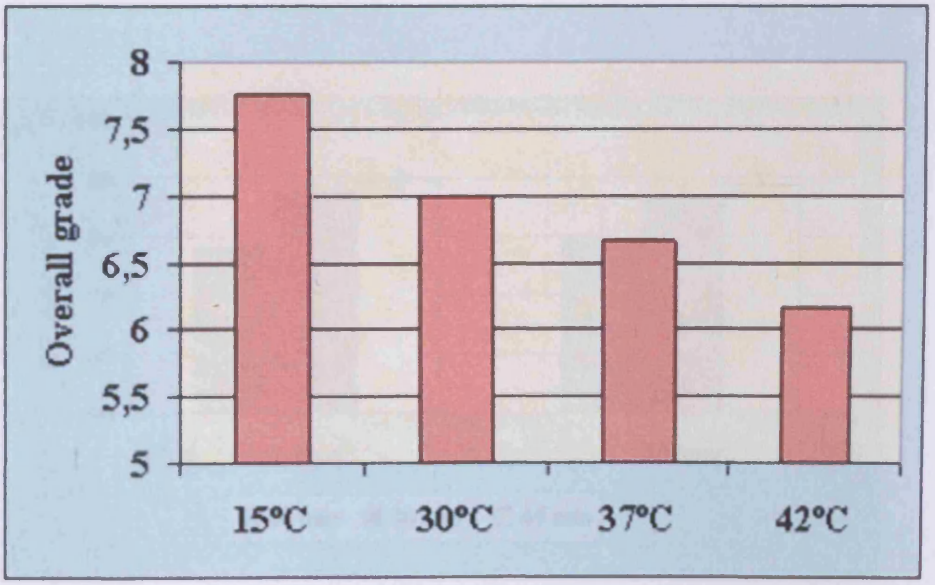


Figure 3.4. Overall grade of olive oils from *cv.* Koroneiki extracted at 15°C, 30°C, 37°C and 42°C after 30 min of malaxation.

3.3.4. Malaxation factors affecting oil yield.

Oil yield is defined as the amount of oil extracted by the olive milling process (% with respect the oil contained in olive fruit). The extraction yield was found to increase with malaxation time in both the 2-phase and the 3-phase extraction systems studied (Figure 3.6). Koroneiki is considered to be an easy olive to extract because the oil yield is well above 80%. On the other hand, laboratory experiments indicated that the increase in oil yield of Koroneiki followed polynomial curve with very high correlation coefficient (Figure 3.5). In fact, oil yield increased up to about 45 min of kneading time.

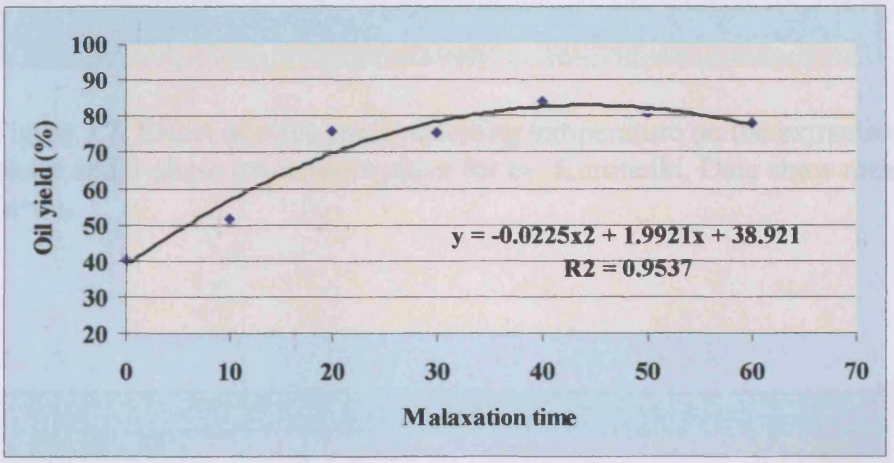


Figure 3.5. Effect of olive paste malaxation time on the oil extraction yield, in laboratory mill experiments for cv. Koroneiki.

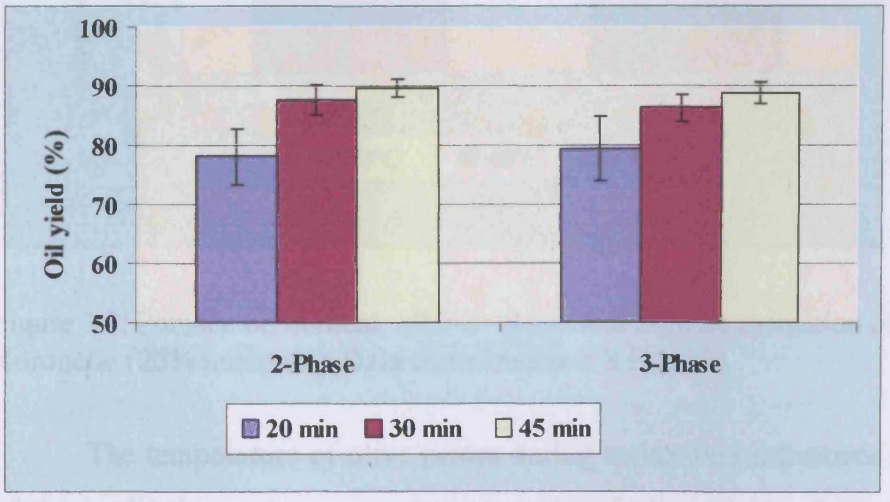


Figure 3.6. Effect of malaxation time of olive paste on the oil extraction yield obtained by 2-phase and 3 phase extraction systems for cv. Koroneiki. Data show means ± S.D (n=3).

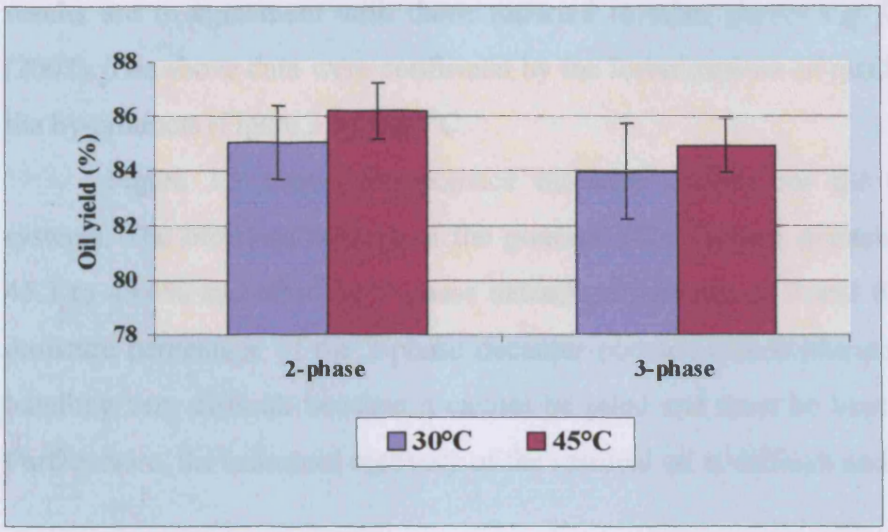


Figure 3.7. Effect of olive paste malaxing temperature on the extraction yield of 2-phase and 3-phase extraction system for cv. Koroneiki. Data show means ± S.D (n=3).

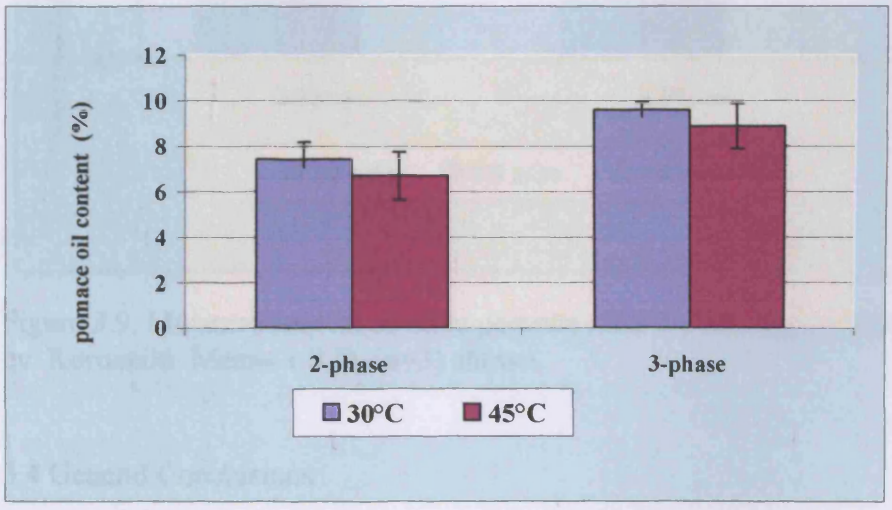


Figure 3.8. Pomace oil content, after 2- phase and 3-phase extraction of cv. Koroneiki (25% moisture). Data show means ± S.D (n=3).

The temperature of olive pastes during malaxation influences not only some of the qualitative and sensory characteristics of olive (Tables 3.28, 3.29) but may also affect the oil yield. Figure 3.7 shows that an increase in mixing temperature from 30°C to 45°C caused a small increase (but not statistically significant) in oil

extraction yields for both the 2- phase and the 3-phase extraction system. These results are in agreement with those reported in other papers e.g. Di Giovacchino (2002). The above data were confirmed by the lower amount of residual oil found in the by-products (Figure 3.8) at 45°C.

Figure 3.9 shows the pomace moisture content for the two processing systems. The moisture content of the pomace after 3-phase extraction varied from 45.3 to 48.6% and after the 2-phase extraction between 61.9 and 63.8%. The high moisture percentage of the 2-phase decanter pomace makes transport, storage, and handling very difficult because it cannot be piled and must be kept in large ponds. Furthermore, the industrial recovery of the residual oil is difficult and expensive.

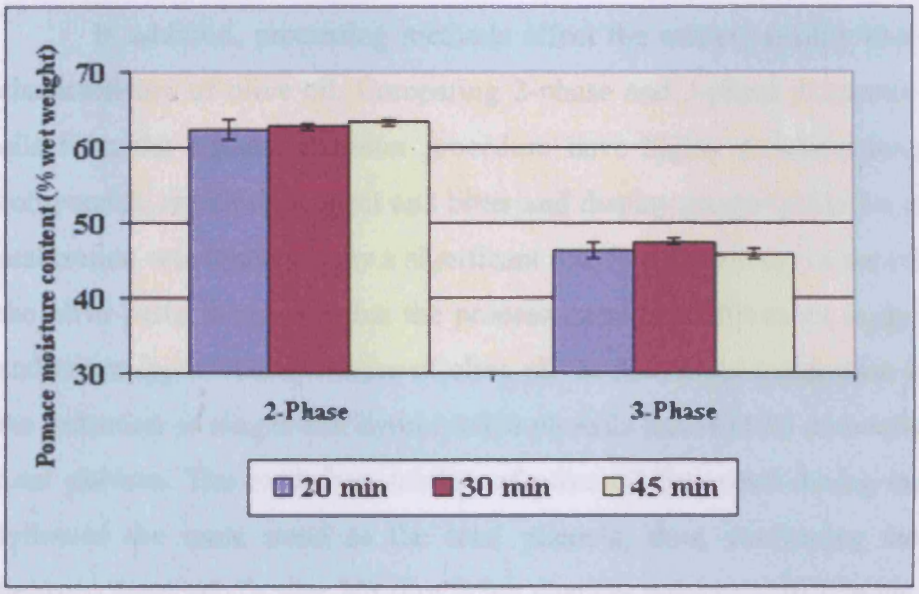


Figure 3.9. Moisture content of olive pomace after 2-phase and 3- phase extraction of cv. Koroneiki. Means ± S.D. (n=3) shown.

3.4 General Conclusions

The nature of the olive variety affected the chemical compositional data of the olive oil extracted, particularly the relative composition of fatty acids, sterols, aliphatic and triterpene alcohols and phenolic compounds. For example, the Coratina cultivar showed significantly higher concentrations of oleate, triterpenic alcohols and triolein. This variety is also rich in phenolic compounds, which are positively correlated to the bitter and pungent attributes. Koroneiki, on the other hand, is basically characterized by higher concentrations of campesterol and Δ^5-avenasterol,

total aliphatic alcohols, palmitate and palmitoleate. Significant correlations were found to exist between total phenols and induction times in both the oils obtained from Coratina and Koroneiki cultivars. However, it should be noted that the phenolic compounds analyzed by HPLC in all the chromatograms for each cultivar were similar, with differences relating only to their relative percentages. Moreover, differences between cultivars can be explained due to the fact that phenolic compounds dissolved in oils are mainly secoiridoid derivatives of tyrosol and hydroxytyrosol. These compounds are products of enzymatic breakdown of oleuropein and ligstroside in olives as a result of which their concentration in the paste depends on the extent of enzymatic process that occur during malaxation (Cert *et al.,* 1999).

In addition, processing methods affect the sensory quality and the chemical characteristics of olive oil. Comparing 2-phase and 3-phase extraction systems, the oils from the 2-phase decanter procedure have higher concentrations of phenolic compounds, are more pungent and bitter and display greater oxidative stability. Paste malaxation was found to play a significant role in the recovery of the oil contained in the olive paste. It appears that the process causes modifications in most qualitative and organoleptic characteristics of olive oil. In fact, paste malaxation time results in the reduction of simple and hydrolysable phenols (secoiridoid derivatives) as well as total phenols. The oxidative stability of olive oil decreased during malaxation and followed the same trend as the total phenols, thus, confirming the relationship between these two factors. No significant changes were perceived in the intensities of sensory attributes during malaxation time despite alteration in some oil components such as chlorophyll. However, the higher the temperature of the olive paste during malaxation the lower the intensities of green attributes and the lower the flavor scores for the olive oil. On the other hand, increasing the olive paste temperature and time of malaxation increased oil yield.

Consequently, the analytical quality, aroma and oxidative stability of the oils depend on cultivar, the extraction method used and the time and temperature of malaxation. In some cases, the processing techniques may have to carefully balance quantitative yields against the quality of the final oil.

3.5. Summary

From the work reported in this chapter, the factors affecting the mechanical extraction process and quality of olive oil in order of priority are:

- Variety (comparison between Koroneiki and Coratina variety)
- Temperature of malaxation

The increase in the temperature of the olive paste during malaxation reduces the intensities of the green attributes and the flavor scores for the olive oil.

- Time of malaxation

a) Malaxation time must be varied according to the olive variety.

b) An increase in the paste malaxation time results in the reduction of phenolic compounds as well as in the oxidative stability of olive oil.

- Oil extraction methodology (differences between 2-phase and 3-phase decanter)

According to the results, the highest quality and the highest yield of olive oil is a result of a compromise of the above factors.

Chapter 4. Effect of orchard irrigation with saline water on olive oil qualitative parameters

4.1. Introduction

Over the last two decades the demand for good quality fresh water for agricultural and industrial purposes has been increasing all over the world. Many countries are facing water shortages at present or it seems likely that they may have to cope with further demands on scarce water resources in the future. The problem is more intense in arid and semi-arid areas such as in the Mediterranean region, which is characterised by a water imbalance, particularly in the summer months. This is due to an uneven distribution and low precipitation of rain, high temperatures and, at the same time, increasing demands for irrigation and domestic water use by the tourist industry.

The availability of water resources of lower quality such as drainage water, saline ground water and treated wastewater has become an important consideration for the agricultural industry. In particular, the use of saline water is a potential source for irrigation.

The potential use of such low- quality water for irrigation will depend on four factors, which are interrelated:

a) electrical conductivity (EC)

b) the salinity tolerance of the crop concerned.

c) proper irrigation management including leaching requirements

d) potential damage to soil properties and the possibilities to minimise such damage.

The salt tolerance of crops is usually presented as a function of the electrical conductivity of the saturated soil extract (ECe) (Maas and Hoffman, 1977). However, it seems that the situation is more complex and other soil factors, including nutrients can influence crop salt tolerance. According to Maas and Hoffman (1977), crop yield is not reduced until a threshold of salinity is exceeded. Beyond the threshold level the crop yield decreases linearly with increasing salinity. Different crops and different cultivars of the same crop vary in their tolerance to salinity. These inter-and intragenetic variations in the salt tolerance of plants can be exploited to select crops

or cultivars which grow and produce reasonable yields under a given root-zone salinity. Different crops have different water uptake patterns but all take water from wherever it is most readily available within the root zone. Each irrigation leaches the upper root zone and maintains low salinity. On the other hand, salinity increases with depth and thus is greater in the lower part of the root zone. In 1990, a committee of consultants recommended guidelines for utilising poor- quality water and its wider applicability in different agro-ecological zones in India (Table 4.1).

Table 4.1. Guidelines for salinity classification of irrigation water. Electrical conductivity (EC) values indicate the limits between good quality and saline water.

Soil Texture (% Clay)	Crop tolerance	ECiw (dSm^{-1}) limit for rainfall regions		
		<350 mm	350-550 mm	>550 mm
Fine >30	Sensitive	1·0	1·0	1·5
	Semi-tolerant	1·5	2·0	3·0
	Tolerant	2·0	3·0	4·5
Moderately fine (20-30)	Sensitive	1·5	2·0	2·5
	Semi-tolerant	2·0	3·0	4·5
	Tolerant	4·0	6·0	8·0
Moderately coarse (10-20)	Sensitive	2·0	2·5	3·0
	Semi-tolerant	4·0	6·0	8·0
	Tolerant	6·0	8·0	10·0
coarse (<10)	Sensitive	n.m.	3·0	3·0
	Semi-tolerant	6·0	7·5	9·0
	Tolerant	8·0	10·0	12·5

Source: Minchas and Gupta (1992).[a] Rainfall region (mm). Electrical conductivity values are in deciSiemens/m (dS/m). The limits are based on field experiences in different agro-climatic zones. It is expected that with waters of salinity equal to or lower than these limits, there would be no yield reduction on long-term basis and no adverse effect on soil quality n.m., not measured.

The classical explanation of water stress for plants growing in a saline environment is that there is reduced water availability for the plant due to decrease in osmotic potential of soil water (West *et al.*, 1986; Yeo *et al.*, 1985; Katerji *et al.*, 2003). A reduction in root growth may provide a supplementary explanation. Thus, it is also possible that the reduction in root development and, thus, the consequential

poor penetration to greater depths causes less water and fewer nutrients to be taken up and become available to plants.

During their initial exposure to salinity, plants experience water stress, which reduces leaf expansion. During long-term exposure to salinity, plants also experience ionic stress, due to the continuous accumulation of salts, which can lead to premature senescence of adult leaves and, thus, a reduction of the photosynthetic area available to support continued growth (Sultana *et al.,* 1999).

The management tactics for optimal crop production with saline water include the prevention of salt build-up to levels which limit the future productivity of the soil and the control of salt balances in the soil - water system, as well as minimising the damaging effects of salinity on crop growth.

4.1.1 Salinity effects on olive growth and production.

The olive (*Olea europaea*, L.) is one of the best-adapted species to the semi-arid and arid, Mediterranean environment. It has been considered as a moderately salt tolerant tree crop (Maas and Hoffman, 1977; Rugini and Fedeli, 1990) with a very high drought resistance (Xyloyiannis *et al.,* 1999). It was suggested by Tattini *et al.* (1994), Benlloch *et al.* (1991) that the mechanism of salt tolerance in *Olea europaea* L. is located within the root system, which prevents salt accumulation in the growing organs with varying degrees of effectiveness. On the other hand, Bongi and Loreto (1989) reported the salt tolerance mechanism may be related to the capacity of olive to accumulate salt in leaf vacuoles. In comparison with other important Mediterranean tree crops, the olive tree is more tolerant than citrus but less tolerant than date palm (Table 4.2).

Benstein (1964) found a 10% growth reduction in olives when the electrical conductivity of the soil solution was 4-6 dS/m and the latter value can easily be as high as 6-8 dS/m in soils with high calcium status. Therios and Misopolinos (1988), using 3 year-old olive plants, observed a negative effect of NaCl on dry weight yields at salt concentrations > 4·67 g/l during a 90 days culture period. Furthermore, NaCl concentrations higher than 8g/l in the soil waters strongly reduces olive crop performance (Rugini and Fedeli, 1990). Different olive cultivars have been found to vary in the degree of their tolerance to salinity (Therios and Misopolinos, 1988; Benlloch *et al.,* 1991;Tattini, 1992; Chartzoulakis *et al.,* 2002).

Table 4.2. Yield reduction to be expected due to salinity of irrigation water (dS/m).

Crop	Reduction									
	0%		10%		25%		50%		100%	
	ECe	ECw	ECe	ECw	ECe	ECw	ECe	ECw	ECe	Ecw
Olives	<2·7	<1·8	<3·8	<2·6	<5·5	<3·7	<8·4	<5·6	>14·0	n.m
Citrus	<1·7	<1·1	<2·3	<1·6	<3·2	<2·2	<4·8	<3·2	>8·0	n.m
Date Palm	<4·0	<2·7	<6·8	<4·5	<10·9	<7·3	<17·9	<12·0	>32·0	n.m

Source: Maas and Hoffman, 1977) n.m.= not measured. Electrical conductivity values are in deciSiemens/m (dS/m). ECe, Conductivity of saturated soil extract; ECw, Conductivity of water.

Tattini *et al.* (1992) showed that olive genotypes differed in their ability to withstand high NaCl concentrations and concluded that an inverse relationship existed between salt tolerance and the vigour of the genotype. The response of 26 olive cultivars to an excess of NaCl in the growing medium was studied by Marin *et al.* (1995). The classification of olive cultivars reported in their study may be useful for selecting genotypes tolerant to high saline conditions, since wide differences in tolerances were obtained. Thus, Klein *et al.* (1993) have observed that high soil salinity (6.5 dS/m) did not affect fruit size and, moreover, caused a consistent increase in oil content in cv. Manzanillo and cv. Uovo di Piccione .

Most of the experiments carried out on olive plants under saline conditions have studied vegetative parameters, leaf gas exchange and salt accumulation in different organs. In contrast, very little research work has been published on the effect of saline irrigation water on productivity and oil quality. Because of this and since shortages of good quality water are likely to increase in the Mediterranean region in the future (partly because of climate change), we decided to study the effect of salinity on oil yield and quality in two important Greek cultivars.

4.2. Effect of saline irrigation on olive oil characteristics

4.2.1. Effect of NaCl on fruit characteristics

The mean values and standard deviations of olive fruit characteristics (oil content, moisture content and average weight of 100 olive fruits) from olive trees of the two cultivars studied (Koroneiki and Mastoidis) irrigated at four salinity levels are presented in Tables 4.3 & 4.4. In general, irrigation with saline water significantly changed these characteristics at all levels for cv. Koroneiki but only at the highest NaCl concentration (150 mM) for cv. Mastoidis (section 2.1.3. for salt treatments and respective electrical conductivity).

The reduction with respect to oil content for 150 mM irrigation was 46% and 13 % for Koroneiki and Mastoidis cultivars, respectively. However, whereas the oil content of Koroneiki fruits was reduced by all treatments, only treatment with 150 mM NaCl reduced that for Mastoidis. Moisture content of olive fruits was increased with increased salinity levels of irrigation water and, again, Koroneiki was more affected. The increase in water content is probably due to the fact that, following irrigation with saline water, the sodium chloride content increased in olive fruits encouraging water entry (Chartzoulakis K., personal communications). Furthermore, salt treatment decreased fruit weight only in the Koroneiki cultivar. This finding was in agreement with the results of Klein *et al.* (1994) that irrigation with saline water might occasionally reduce fruit size.

Table 4.3. Effect of NaCl treatments on fruit weight, oil and moisture content of olive fruits from Koroneiki cultivar.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
Weight of 100 fruits (g)	94·3± 5·4 ^{a}	62·6± 9·8 ^{b}	73·3± 8·0 ^{b}	74·9± 12·6 ^{b}
Moisture (% wet weight)	48·4± 3·7 ^{c}	51·3± 5·4 ^{c}	62·4± 2·9 ^{b}	69·0± 11·9 ^{a}
Oil content (%) on dry weight basis	51·2± 1·3 ^{a}	45·1± 0·7 ^{b}	36·4± 1·1 ^{c}	27·4± 11·5 ^{d}

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0{\cdot}05$). Quantitative characteristics were measured as described in sections 2.2.1, 2.2.2. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

Table 4.4. Effect of NaCl treatment on fruit weight, oil and moisture content of olive fruits from Mastoidis cultivar.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
Weight of 100 fruits (g)	201·3±16·6[a]	209·4±47·4[a]	207·8±24·7[a]	179·6±24·5[a]
Moisture (% wet weight)	41·4±1·0 [b]	42·5±0·1 [b]	44·9± 0·9 [b]	52·8± 1·6 [a]
Oil content (%) on dry weight basis	58·1±2·9 [a]	57·5±2·3 [a]	55·3± 0·7 [a]	50·7± 1·5 [b]

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0{\cdot}05$). Quantitative characteristics were measured as described in sections 2.2.1, 2.2.2. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

4.2.2. Effect of NaCl on U.V. spectrophotometric indices.

Tables 4.5 & 4.6 show that with increasing NaCl levels no significant variation was observed in spectrophotometric absorption in the UV region for oils extracted from either cultivar. Furthermore, it is of note that, in both cultivars, the values of these parameters are within the limits established by the European Community for extra virgin olive oil. These data show that oxidation of polyunsaturated fatty acids was not sufficient to increase the formation of conjugated double bonds.

Table 4.5. Effect of NaCl on the qualitative characteristics of oil from olive fruits of cv. Koroneiki.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
K270(OD 270)	0·19±0·01 [a]	0·16±0·03 [a]	0·18± 0·01 [a]	0·17± 0·02 [a]
K232(OD 232)	1·51±0·15 [a]	1·63±0·03 [a]	1·70± 0·07 [a]	1·73± 0·09 [a]
Total phenols (p.p.m.gallic acid)	319·43±32,50[a]	339·1±23·50[a]	314·63± 30·81 [a]	386·6± 50·2 [a]

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0{\cdot}05$). Quantitative characteristics were measured as described in sections 2.2.7, 2.2.14. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

Table 4.6. Effect of NaCl on qualitative characteristics of oils from olive fruits of cv. Mastoidis.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
K270 (OD 270)	0·30± 0·09 [a]	0·30± 0·08 [a]	0·22± 0·03 [a]	0·29± 0·03 [a]
K232 (OD 232)	1·97± 0·19 [a]	1·97± 0·21 [a]	1·80± 0·11 [a]	1·87± 0·04 [a]
Total phenols (p.p.m·gallic acid)	320·35± 44·45[a]	320·35± 40·03[a]	274·93± 44·11[a]	333·87± 36·61[a]

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0{\cdot}05$). Quantitative characteristics were measured as described in sections 2.2.7, 2.2.14. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

4.2.3. Effect of NaCl on phenolic compounds

According to previous data (Amiot *et al.*, 1989; Cinquanta *et al.*, 1997; Garcia *et al.*, 1996), the absolute concentration of phenolic compounds present in olive oil is the result of complex interaction between several factors including cultivar and genetics, the climate, degree of fruit maturation and its position on the tree, the rootstock used and agricultural practices. For the Koroneiki variety there may have been an increase in total phenolics at 150 mM NaCl but, because of variation between samples, this was not sufficient to be statistically significant (Table 4.5). For Mastoidis, there were no changes in total phenolic compounds (Table 4.6).

H.P.L.C. analysis of the oil extract allowed the separation of phenolic compounds (section 2.2.15). Simple phenols in both cultivars did not vary significantly with increasing salinity levels. The components most substantially represented in this fraction were 3,4 DHPEA and p-HPEA with the other simple phenols only giving small values of about 1mg/kg in the oils of either cultivar (Table 4.7. and Table 4.8.).

The other major phenolic fraction, the secoiridoid derivatives,were increased significantly in the Mastoidis cultivar with 150 mM NaCl treatment. These derivatives are major components of the phenolic fraction and their increase is probably because the olive trees were more stressed under high salinity levels. In cv. Koroneiki, only 3,4 DHPEA-EDA was increased by salt water irrigation (Table 4.7.).

Table 4.7. Composition of phenolic compounds in olive oils (ppm) from fruits of cv. Koroneiki irrigated at three salinity levels.

	Salt Treatment		
	Control	50 mM	100 mM
3,4 DHPEA	1·10± 0·06 [a]	0·80± 0·04 [a]	0·90± 0·03 [a]
p-HPEA	1·72± 0·06 [a]	1·33± 0·24 [b]	1·20± 0·11 [b]
3,4 DHPEA-EDA	237·50± 5·40 [b]	311·8± 19·44 [a]	308·00± 28·84 [a]
p-HPEA - EDA	302·08± 49·15 [a]	347·33 ± 33·49 [a]	314·33± 27·30 [a]
p-HPEA derivative	269·30± 49·48 [a]	262·50 ± 51·77 [a]	230·70± 22·25 [a]
3,4 DHPEA EA	340·30± 38·98 [a]	357·00 ± 36·59 [a]	345·83± 6·55 [a]

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0{\cdot}05$). Phenolic compounds were measured as described in section 2.2.15. Phenolics are abbreviated as shown in the list of abbreviations. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

Table 4.8. Composition of phenolic compounds in olive oils from fruits of cv. Mastoidis irrigated at four salinity levels.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
3,4 DHPEA	0·64±0·05 [a]	0·68 ±0·19 [a]	0·90 ± 0·18 [a]	1·00 ± 0·26 [a]
p-HPEA	2·17±0·31 [a]	1·80 ±0·17 [a]	2·73 ± 0·29 [a]	1·73 ± 0·35 [a]
3,4 DHPEA-EDA	65·64±12·52 [b]	154·87±53·42 [b]	160·50±52·92 [b]	451·19±50·65 [a]
p-HPEA - EDA	178·78±25·15 [c]	391·97±31·5 [b]	381·00±28·45 [b]	518·07±35·80 [a]
p-HPEA derivative	126·49±26·05 [b]	200·13±30·06 [a]	236·57±35·05 [a]	263·96±25·85 [a]
3,4 DHPEA EA	248·86±15·05 [b]	262·43±18·03 [b]	270·10± 20·8 [b]	411·89±23·20 [a]

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0{\cdot}05$). Phenolic compounds were measured as described in section 2.2.15. Phenolics are abbreviated as shown in the list of abbreviations. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

4.2.4 Effect of NaCl on fatty acid composition

The fatty acid composition of olive oils following control and saline treatments is tabulated in Tables 4.9. & 4.10. The fatty acid composition of olive oils was significantly affected by salinity. Palmitic acid (the major saturated fatty acid) as well as the total saturated fatty acids was increased by increasing the concentrations of salt. However, statistically significant differences for palmitic acid were observed only in cv. Koroneiki. As expected when saturated acids were increased, total unsaturated fatty acids decreased significantly (Tables 4.9. & 4.10).

Table 4. 9. Fatty acids of olive oils from fruits of cv. Koroneiki irrigated at four salinity levels.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
C16: 0	13·28± 0·22^{b}	13·96± 0·29^{b}	15·79± 0·94^{a}	15·49± 0·51^{a}
C16:1	1·06± 0·04^{ab}	0·86± 0·04^{b}	1·16± 0·15^{a}	1·06± 0·19^{ab}
C18:0	2·59± 0·16^{a}	3·27± 0·02^{a}	2·85± 0·03^{a}	2·88± 0·29^{a}
C18: 1	75·65± 1·04^{a}	74·19± 0·93^{ab}	72·21± 2·21^{b}	70·09± 0·60^{c}
C18:2	5·66± 0·75^{b}	6·49± 0·48^{ab}	6·65± 0·79^{ab}	7·72± 1·16^{a}
C18:3	0·73± 0·06^{c}	0·84± 0·03^{b}	1·28± 0·21^{ab}	1·36± 0·29^{a}
Saturated	16·53± 0·24^{c}	18·03± 0·29^{b}	19·63± 1·00^{a}	19·36± 0·24^{a}
Unsaturated	85·73± 0·16^{a}	85·90± 0·62^{a}	82·63± 1·39^{b}	82·91± 0·95^{b}
Unsat./sat	6·22± 0·10^{a}	5·90± 0·16^{a}	5·03 ± 0·38^{b}	5·12± 0·20^{b}
Oleic/Linoleic	13·56± 2·12^{a}	11·49± 0·94^{ab}	9·76 ± 1·35^{b}	9·21± 1·34^{b}

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0·05$). Fatty acids were quantified as described in section 2.2.12. Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the number afterwards giving the number of doubled bonds. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

However, within the unsaturated fatty acids, some interesting changes were noted. Thus, the percentage of oleic acid was reduced in both cultivars while both linoleic and α-linolenic acids were increased significantly. These results confirm previous data for a Tunisian olive variety (Zarrouk *et al.*, 1996). These changes in unsaturated acids are reflected in the ratio of oleic to linoleic acid, which was reduced by salt-water treatment. In addition, the ratio of unsaturated /saturated fatty acids was higher in the control and decreased significantly with irrigation at 100 mM and 150 mM of NaCl. These results are in agreement with those reported previously for cv. Frantoio (Cresti *et al.*, 1994) and cv. Barney (Wiesman *et al.*, 2004).

Table 4.10. Fatty acids of olive oils from fruits of cv. Mastoidis irrigated at four salinity levels.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
C16: 0	12·22± 0·03^{a}	12·35± 0·58^{a}	12·71± 0·60^{a}	13·01± 0·09^{a}
C16: 1	1·08± 0·09^{a}	0·94± 0·17^{a}	0·99± 0·15^{a}	0·95± 0·06^{a}
C18:0	2·65± 0·07^{a}	2·71± 0·17^{a}	2·78± 0·07^{a}	2·83± 0·11^{a}
C18: 1	76·42± 0·63^{a}	74·40± 1·56^{b}	73·42± 1·20^{b}	72·42± 0·24^{b}
C18:2	5·40± 0·44^{b}	7·22± 1·12^{a}	8·40± 1·26^{a}	8·23± 1·46^{a}
C18:3	0·51± 0·02^{c}	0·56± 0·03^{bc}	0·61± 0·04^{b}	0·68± 0·02^{a}
Saturated	15·81± 0·13^{b}	16·02± 0·54^{b}	16·55± 0·49$^{a\,b}$	16·94± 0·19^{a}
Unsaturated	86·07± 0·51^{a}	85·83± 0·41^{a}	86·22± 0·60^{a}	84·89± 0·58^{b}
Unsat/sat	6·81± 0·13^{a}	6·62± 0·32^{a}	6·46± 0·27^{ab}	6·18± 0·07^{b}
Oleic/Linoleic	14·22± 1·32^{a}	10·51± 1·81^{b}	8·90± 1·58^{a}	8·79 1·15^{b}

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0{\cdot}05$). Fatty acids were quantified as described in section 2.2.12. Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the number afterwards giving the number of doubled bonds. Results shown are % in total fatty acids. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

4.2.5. Effect of NaCl on triacylglycerol molecular species

The triacylglycerol fraction was separated into molecular species (as described in section 2.2.11) by HPLC. The results obtained are shown in Tables 4.11 & 4.12. Triolein (OOO), the major TAG of olive oil, showed significantly lower values as the salinity concentration of irrigation water increased in both varieties. These results differ from those obtained by El-Agaimy (1994) but agree with the fatty acid data in section 4.2.4, which in turn was in agreement with other workers (Zarrouk *et al.,* 1996; Cresti *et al.,* 1994). As expected also from the fatty acid results (Tables 4.9 & 4.10), those molecular species containing linoleic acid and α-linolenic acids or enriched with saturated acyl chains were increased (and usually significantly) in both varieties. Even so the value for the LLL containing fraction was still less than the maximum limit of 0·5 % permitted by EC regulations. As expected from the fatty acid data, the changes in TAG molecular species were greater for the Koroneiki cultivar. Thus, the reduction in the OOO concentration in olive oil of cv. Koroneiki following 150 mM NaCl treatment of irrigation water was about 25% in comparison to the control while in Mastoidis the reduction in this species was about 15 %. This can be explained because olive cultivars differ in their ability to withstand high NaCl concentrations (Tattini *et al.*, 1992; Marin L *et al.*, 1995; Charzoulakis *et al.*, 2002). In fact, the growth of cv. Mastoidis was affected only at high concentration of NaCl whereas cv. Koroneiki showed significantly lower concentration of OOO after low NaCl treatments (Charzoulakis *et al.*, 2002).

Table 4.2.11. Triacylglycerol molecular species composition of olive oil from fruits of cv. Koroneiki irrigated at four salinity levels.

	Salt Treatment			
	Control	50 mM	100 mM	150 mM
LLL+OLLn	0·23± 0·01^{b}	0·23 ± 0·03^{b}	0·38± 0·10^{a}	0·46± 0·04^{a}
OLL	0·97± 0·70^{b}	1·01 ± 0·02^{b}	1·17± 0·15^{b}	2·01± 0·37^{a}
OOLN	1·39± 0·02^{c}	1·38 ± 0·05^{c}	1·73± 0·07^{a}	2·06± 0·42^{a}
PLL+PoOL	0·60± 0·01^{a}	0·69 ± 0·11^{a}	1·04± 0·11^{a}	1·21± 0·75^{a}
OOL	9·82± 0·89^{a}	10·24 ± 0·65^{a}	9·54± 0·08^{ab}	11·79± 2·45^{a}
POL	5·45± 0·57^{c}	5·78 ± 0·46^{bc}	6·29± 0·23^{b}	8·45± 0·26^{a}
PLL	0·50± 0·05^{b}	0·60 ± 0·04^{b}	0·75± 0·11^{a}	0·84± 0·06^{a}
OOO	42·09± 1·01^{a}	38·73 ± 1·85^{b}	36·80± 0·98^{b}	31·93± 1·13^{c}
POO+SOL	27·91± 0·55^{a}	28·06 ± 1·79^{a}	29·18± 0·84^{a}	28·18± 1·84^{a}
POP	3·40± 0·18^{a}	3·36 ± 0·26^{a}	4·08± 0·19^{ab}	4·44± 0·96^{a}
GAOO	0·41± 0·10^{a}	0·37 ± 0·12^{a}	0·47± 0·08^{a}	0·27± 0·10^{a}
SOO	4·71± 0·29^{b}	5·93 ± 0·47^{a}	5·49± 0·65^{ab}	5·06± 0·67^{ab}
POS	1·19± 0·05^{b}	1·53 ± 0·07^{a}	1·52± 0·12^{a}	1·53± 0·16^{a}
AOO	0·81± 0·04^{a}	0·98 ± 0·02^{a}	0·97± 0·07^{a}	0·64± 0·51^{b}
SOS	0·30± 0·02^{b}	0·41 ± 0·03^{a}	0·35± 0·04^{ab}	0·36± 0·04^{a}

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0·05$). Triacylglycerols were quantified as described in section 2.2.11. Abbreviations: P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; Po, Palmitoleic acid; S, stearic acid; A, arachidic acid; Ga, gadoleic acid. Result shown are % in total TAGs. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

Table 4.2.12. Triacylglycerol molecular species composition of olive oil from fruits of cv. Mastoidis irrigated at four salinity levels.

	Salt Treatment ($dSm^{-1}EC$)			
	Control	50 mM	100 mM	150 mM
LLL+OLLn	0·18± 0·02^{b}	0·23± 0·09^{ab}	0·34±0·08^{a}	0·27± 0·06^{a}
OLL	0·93± 0·16^{b}	1·51± 0·30^{a}	2·00±0·49^{a}	1·83± 0·19^{a}
OOLN	1·08± 0·06^{b}	1·09± 0·22^{b}	1·35±0·22^{ab}	1·47± 0·21^{a}
PLL+PoOL	0·51± 0·06^{a}	0·60± 0·22^{a}	0·62±0·08^{a}	0·67± 0·06^{a}
OOL	10·09± 0·46^{b}	12·08± 1·33^{a}	13·10±1·17^{a}	12·69± 0·56^{a}
POL	5·09± 0·34^{b}	6·01± 1·07^{ab}	6·99±1·01^{a}	6·95± 0·28^{a}
PLL	0·92± 0·11^{b}	1·03± 0·10^{ab}	1·11±0·12^{ab}	1·23± 0·10^{a}
OOO	43·66± 1·01^{a}	41·10± 3·03^{ab}	37·91±2·91^{b}	37·29± 0·18^{b}
POO+SOL	26·24± 0·48^{a}	24·77± 0·28^{b}	24·80±0·32^{b}	25·33± 0·32^{b}
POP	3·02± 0·16^{a}	3·09± 0·37^{a}	3·18±0·25^{a}	3·45± 0·09^{a}
GAOO	0·86± 0·05^{b}	0·92± 0·02^{ab}	0·92±0·05^{ab}	1·04± 0·15^{a}
SOO	5·18± 0·11^{a}	5·11± 0·45^{a}	5·10±0·34^{a}	5·18± 0·15
POS	1·18± 0·04^{b}	1·22± 0·04^{b}	1·26±0·02^{b}	1·39± 0·07^{a}
AOO	0·65± 0·05^{a}	0·71± 0·02^{a}	0·76±0·04^{a}	0·72± 0·13^{a}
SOS	0·27± 0·07^{a}	0·27± 0·04^{a}	0·31±0·02^{a}	0·29± 0·06^{a}

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test ($p \leq 0·05$). Triacylglycerols were quantified as described in section 2.2.11. Abbreviations: P, palmitic acid ; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid Po, Palmitoleic acid; S, stearic acid; A, arachidic acid; Ga, gadoleic acid. . Result shown are % in total TAGs. Section 2.1.3 for salt treatments and respective electrical conductivity (dS/m).

4 .3. Discussion

Although olive trees are traditionally dry farmed in Mediterranean regions where water is scarce, research studies have shown (Fontanazza, 1994; Lavee *et al.,* 1990; Goldhamer *et al.,* 1994) that irrigation can have significant effects in ensuring that optimum water is available for tree growth and fruit production. Most recently, due to the necessity of using brackish water for agricultural requirements, considerable progress has been made in understanding the different mechanisms of olive tree resistance to drought stress and to salt tolerance. Salt stress changes the water relations of higher plants and salt tolerance is often related to drought tolerance (Greenway and Munns, 1980; Flowers and Yeo, 1989). These mechanisms comprise anatomical alterations in the roots, which prevent Na^+ and Cl^- translocation to the shoot. On the other hand, additional effects occur in leaf development and physiological processes. These include reduction of leaf size, closure of leaf stomata due to changes in the leaves water status, leaf rolling etc. (Tattini *et al.,* 1995; Loupasaki *et al.,* 2002; Charzoulakis *et al.,* 2002; Bosabadilis and Kofidis, 2003).

In the experiments, the two cultivars Koroneiki and Mastoidis, which are the main cultivars grown extensively on the island of Crete, were used. Koroneiki accounts for 85% of all olive trees planted and Mastoidis, 14%. Koroneiki in general is cultivated on the plains, lower hillsides and coastal areas of Crete where the climate is relatively warm. On the other hand, Mastoidis is grown in higher altitudes up to 800 m. Irrigation with saline water resulted in the reduction of fruit weight and oil content in both cultivars although Koroneiki was more strongly affected. Changes also occurred in the composition of fatty acids due to increasing salinity levels. In particular, oleate decreased while linoleate and linolenate increased. Furthermore, the content of total phenols and secoiridoids were higher when more concentrated saline solutions were used for irrigation. However, the degree of changes was different for each variety with more intense alterations found in Koroneiki. These results could be explained by comparison with the results found by Charzoulakis *et al.* (2004) on the same trees. They found that the decrease in photosynthesis was less in Mastoidis and the concentrations of $Na^+$and Cl^- (% dry weight) were higher in the fruits and leaves of Koroneiki. This suggested that Mastoidis excluded salts at the root level. Higher contents of sodium were determined in the roots of Mastoidis in comparison to Koroneiki (Charzoulakis *et al.,* 2002). Furthermore, it is known that increases in Na^+

and Cl^- concentrations in leaves cause reduced CO_2 assimilation rates (Charzoulakis *et al.,* 2002). Thus, it may be that cv. Mastoidis is less affected by saline irrigation because it possesses an adaptive mechanism for excluding some of the salts (and their harmful effects) from the photosynthetic tissues. Finally, it is worth mentioning that, although the different treatments alter various qualitative parameters, all of these remain within the limits established by the European Community for extra virgin olive oil.

4.4. Summary

In this chapter, the effect of irrigation with saline water on oil yield and quality was studied, in two important Greek cultivars, Koroneiki and Mastoidis.

Although results showed that Mastoidis was less affected in saline water, the following conclusions can be derived for both cultivars.

Increase in NaCl levels in irrigation water results in:

- A decrease in oil content in the fruits.
- An increase in total phenols and secoiridoid derivatives and oxidative stability of olive oil.
- Modification in the fatty acid and triacylglycerol composition of olive oil.

Chapter 5. Effect of irrigation on olive oil quality characteristics

5.1. Introduction

The olive tree (*Olea europaea L*) has been considered one of the most drought resistant crops. Many authors have reported that it has developed defence mechanisms in response to drought stress (Xyloyianis *et al.*, 1999; Chartzoulakis *et al.*, 1999). This means that it is well adapted to the semi-arid Mediterranean environment, where it traditionally grows under drought conditions (Rugini and Fedeli, 1990). Olive fruit growth is rapid during its first maturation stage (when water is more available), slower in the second (August, September) and rapid again in late autumn during the third stage, when water is usually available again. Pansiot and Rebour (1961) have reported that the olive is most sensitive to drought during the swelling stage when the stone hardens which occurs during the second stage in August and September.

Although olive trees can grow and produce adequate crops under low annual water supply, irrigation during drought periods is essential in order to give high yields (Agabbio, 1983; Scaramuzzi and Roselli, 1986). Irrigation increases fruit production by increasing both the size and the number of the fruits as well as their oil content (Lavee et al., 1990; Chartzoulakis *et al.*, 1992; Michelakis *et al.*, 1995; Michelakis, 1995; Inglese *et al.*, 1996; Lavee and Wonder, 2004).

Although a lot of research has been carried out on the protective mechanisms of the olive tree to prolonged periods of drought stress and the beneficial effect of proper irrigation on growth and yield, little information exists on the effect of drought on olive oil quality. In fact almost nothing is known about cv. Koroneiki the main subject of this theses. In fact, previous studies of irrigation effects on qualitative characteristics are often contradictory. Some authors have reported a beneficial effect of irrigation on triacylglycerol accumulation in the olive (Milella and Dettori, 1987) while the opposite or no effect has been reported by other authors (Gatto, 1989; Lavee and Wodner, 1991). Higher acidity, peroxide value, total phenols and oxidative stability have been reported in oils from irrigated olive trees compared to water-stressed trees (Dettori and Russo, 1993; Ismail *et al.*, 1999; Motilva *et al.*, 2000). On the other hand, higher contents of phenols, stability and bitterness have

been reported in oils from water- stressed olives by Salas *et al.* (1997), Patumi *et al.* (1999), and Patumi *et al.* (2002).

In order to clarify some of these discrepancies, as well as to compare drought stress with salinity effects (Chapter 4) in cv. Koroneiki, the qualitative characteristics of oils produced in two different years were examined. Moreover, because drought had been reported to have more effect at certain stages of ripening, samples were taken at two points during maturation.

5.2. Results of the effect of irrigation on olive oil quality

5.2.1. Effect of irrigation on olive fruit characteristics

The mean values for olive fruit characteristics including oil content, moisture content and the average weight of 100 fruits are presented for the two successive years of the experiment in Tables 5.1a and 5.1b. The weight of 100 fruits was significantly higher ($p< 0.05$) in irrigated trees for both years. Even for mature fruits the yield was increased around 25%. The percentage oil content in the first year of the experiment was significantly higher ($p< 0.05$) in irrigated fruits at both stages of ripening. These results are in agreement with previous studies (Michelakis *et al.*, 1995; Inglese *et al.*, 1996; Ismail *et al.*, 1999). However, in the second year of the experiment, irrigation did not increase oil content of olive fruits at either stage in ripening. This was probably because there was 50 mm rainfall in September 1999 in contrast to only 0·5 mm in September 1998 and, therefore, there was water available in the soil, which could be used by the trees.

There are other general effects when the olive trees are irrigated. For example, fruit growth is continuous until ripening and oil accumulation is linear throughout this period (Lavee and Wodner, 1991). In contrast, lower soil water availability results in higher stomatal resistance and, thus, lower photosynthetic activity, lower fruit water and weight, greater fruit drop and faster fruit color development (Panelli *et al.*, 1994). Furthermore, the higher maximum temperatures characteristic for 1999, in the months from August to November, should have positive effects on plant metabolism and fruit ripening (Jacoboni *et al.*, 1999).

Table 5.1a. Olive fruit characteristics for irrigated and non-irrigated olive crops at two ripening stages for year 1 (1998).

	Sample	Irrigated	Non- Irrigated	Sig. (2-tailed)
Weight of 100 fruits (g)	S1	82·64 ± 1·20	41·07 ± 0·88	<0·005
	S2	92·97 ± 4·01	65·08 ± 1·36	<0·005
Moisture (%)	S1	48·42 ± 0·51	36·61 ± 0·71	<0·005
	S2	49·21 ± 0·49	54·06 ± 0·73	<0·005
Oil content (% dry weight)	S1	44·94 ± 0·83	31·63 ± 1·31	<0·005
	S2	51·30 ± 2·09	41·72 ± 0·74	<0·005

Crop year 1998-99. S1= Green, S2= Semi-black. Data are expressed as means ± standard deviation (n=6). Olive fruit characteristics were quantified as described in sections 2.2.1, 2.2.2.

Table 5.1b. Olive fruit characteristics for irrigated and non-irrigated olive crops at two ripening stages for year 2 (1999).

	Sample	Irrigated	Non- Irrigated	Sig. (2-tailed)
Weight of 100 fruits (g)	S1	72·77 ± 1·40	42·08 ± 1·20	<0·005
	S2	77·09 ± 2·50	59·91 ± 2·45	<0·005
Moisture %	S1	46·40 ± 0·10	28·76 ± 0·17	<0·005
	S2	48·71 ± 0·66	46·54 ± 0·77	0·020
Oil content (% dry weight)	S1	36·45 ± 0·38	34·57 ± 0·63	0·012
	S2	45·51 ± 1·95	43·05 ± 1·49	0·160

Crop year 1999-2000. S1= Green, S2= Semi-black. Data are expressed as means ± standard deviation (n=6). Olive fruit characteristics were quantified as described in sections 2.2.1, 2.2.2.

5.2.2. Effect of irrigation on quality characteristics of virgin olive oil of cv. Koroneiki.

Qualitative characteristics of the oil such as acidity, peroxide value and spectrophotometric absorption in the UV region over the two years of the experiment were found to vary (Table 5.2a & 5.2b). Although there were often significant differences between irrigated and non-irrigated samples, these were often contradictory between the two collection years. The acidity of the oils from irrigated trees was increased significantly in both years for samples of semi-ripe (S2) olives. On the other hand, peroxide values were increased for irrigated samples in 1998 but decreased in 1999. The absorbancies in the UV region showed only small changes. Notably all the values obtained were well within the limits established by the European Economic Community legislation (1992) for extra virgin olive oil.

The oxidative stability of the oils, as measured by Rancimat apparatus, decreased during the ripening of fruits from both irrigated and non-irrigated olive trees. The decrease was greater in oils from the fruits of irrigated trees (28.6% and 27.8%) compare well non-irrigated trees 6.87% and 6.0% in the two years of the experiment. The decrease in stability can be explained by the loss of natural antioxidants (phenols, o-diphenols and tocopherols) during ripening. Although the content of total phenols was usually higher in the oil from fruits of non-irrigated trees the oxidative stability was often significantly higher in oils extracted from fruits of irrigated trees (Tables 5.2a and 5.2b). These results may be due to the higher percentage of polyunsaturated fatty acids in the oil of non-irrigated trees. A higher percent concentration in polyunsaturated fatty acids (linoleic and α-linolenic acids) would, of course, give rise to oxidation which in turn, could shorten shelf life.

The content of chlorophyll pigments of immature olives was higher in the samples from non-irrigated trees for both years. However, the mature (semi-black) olives yielded oils with less chlorophyll for the non- irrigated trees. In fact, the values obtained for semi-black olives in the 1999-2000 season were not much different between the irrigated and non-irrigated trees.

Table 5.2a. Effect of irrigation and ripening on qualitative characteristics of virgin olive oils obtained from fruits of *cv.* Koroneiki in 1998

		Irrigated	Non-Irrigated	Sig.(2-tailed)
Acidity (% total oil)	S1	0·41 ± 0·02	0·38 ± 0·07	0·35
	S2	0·34 ± 0·01	0·22 ± 0·00	<0·005
Peroxide (meq O_2/kg oil)	S1	5·88 ± 0·84	5·98 ± 0·82	0·84
	S2	6·72 ± 0·79	3·54 ± 0·66	<0·005
K232 (OD 232nm)	S1	1·70 ± 0·05	1·74 ± 0·03	0·052
	S2	1·44 ± 0·04	1·55 ± 0·04	<0·005
K270 (OD 270nm)	S1	0·18 ± 0·01	0·19 ± 0·01	0·002
	S2	0·14 ± 0·02	0·16 ± 0·02	0·152
Total phenol (ppm gallic acid)	S1	366·14 ± 14·54	342·36 ± 8·09	0·006
	S2	201·48 ± 20·99	248·38 ± 12·46	<0·005
Resistance to oxidation (hr)	S1	21·57 ± 0·68	15·75 ± 0·95	0·000
	S2	15·34 ± 0·81	14·55 ± 0·46	0·064
Chlorophyll (p.p.m)	S1	14·38 ± 2·02	17·17 ± 0·74	0·010
	S2	nm	nm	

Crop year 1998-99. S1= green olives, S2= Semi-black olives. Data are expressed as means ± standard deviation (n= 6), P values shown for significance by 2-tailed test. Qualitative characteristics were quantified as described in sections 2.2.5, 2.2.6, 2.2.7, 2.2.14, 2.2.16, 2.2.18. nm= not measured.

Table 5.2b. Effect of irrigation and ripening on qualitative characteristics of virgin olive oils obtained from fruits of *cv* Koroneiki in 1999

		Irrigated	Non-Irrigated	Sig.(2-tailed)
Acidity (% total oil)	S1	0·23 ± 0·04	0·25 ± 0·00	0·04
	S2	0·33 ± 0·02	0·30 ± 0·02	0·02
Peroxide (meq O_2/kg oil)	S1	9·93 ± 1·53	13·13 ± 1·13	0·02
	S2	5·60 ± 0·12	6·30 ± 0·58	0·05
K232 (OD 232nm)	S1	1·63 ± 0·02	1·83 ± 0·06	<0·005
	S2	1·71 ± 0·06	1·84 ± 0·01	<0·005
K270 (OD 270nm)	S1	0·14 ± 0·00	0·17 ± 0·00	<0·005
	S2	0·15 ± 0·02	0·17 ± 0·01	0·110
Total phenol (ppm gallic Acid)	S1	485·88 ± 21·61	505·60 ± 23·46	0·260
	S2	403·60 ± 31·99	479·13 ± 29·87	0·010
Resistance to oxidation (hr)	S1	22·43 ± 0·15	15·48 ± 0·47	<0·005
	S2	18·43 ± 0·22	14·55 ± 0·29	<0·005
Chlorophyll (p.p.m)	S1	8·70 ± 0·23	15·80 ± 0·46	<0·005
	S2	8·30 ± 0·12	6·25 ± 0·40	<0·005

Crop year 1999-2000. S1= green olives, S2= Semi-black olives. Data are expressed as means ± standard deviation (n= 6) P values shown for significance by 2- tailed test. Qualitative characteristics were quantified as described in sections 2.2.5, 2.2.6, 2.2.7, 2.2.14, 2.2.16, and 2.2.18.

5.2.3. Effect of irrigation on the phenol composition of virgin olive oil of cv. Koroneiki

In contrast to other vegetable oils, extra virgin olive oil is obtained by physically crushing the whole fruit and, for this reason, is rich in biological antioxidants (phenolic compounds and tocopherols), which are usually removed from other vegetable oils due to, for example, solvent extractions. Furthermore, as others and we have shown, the composition and amount of phenolic compounds are

influenced by a number of factors including olive cultivar, climatic conditions, cultivation methods and technical details of oil extraction. Phenolic compounds and tocopherols reduce chemical oxidation, thus preserving high levels of the fruity flavor. The total phenol content of oils from semi-black olives for the two successive years of the experiment was higher in non-irrigated trees. However, in green olives there was much less difference and, in fact, opposite data was obtained for the two years although the differences in olives were not great (Tables 5.2a and 5.2b). During ripening, the total phenol content, as well as individual phenols as measured by HPLC, decreased. Simple phenols were relatively minor constituents of the olive oils analyzed. Instead, secoiridoid compounds were predominant. Some consistent changes in these were noted for the two seasons. The content of 3,4-DHPEA- EDA was considerably reduced in oils from ripe olives of trees following irrigation. The other components of such oils were hardly affected. On the other hand, oils from partially ripe (S1) had significantly more p-HPEA- EDA as well as the HPEA-derivative, for irrigated trees (Tables 5.3a and 5.3b).

Table 5.3a. Effect of irrigation and ripening on phenolic compounds (mg/Kg) of virgin olive oils obtained from fruits of *cv* Koroneiki in 1998.

		Irrigated	Non-Irrigated	Sig. (2-tailed)
3,4-DHPEA	S1	0·67 ± 0·37	0·55 ± 0·14	0·478
	S2	0·24 ± 0·21	0·52 ± 0·37	0·134
p-HPEA	S1	1·42 ± 0·38	0·87 ± 0·24	0·013
	S2	1·36 ± 0·28	0·88 ± 0·33	0·022
3,4-DHPEA-EDA	S1	473·35 ± 26·61	568·20 ± 39·36	<0·005
	S2	159·03 ± 43·70	370·49 ± 48·86	<0·005
p-HPEA-EDA	S1	507·94 ± 32·01	447·15 ± 41·70	0·018
	S2	219·34 ± 69·34	250·60 ± 42·88	0·370
p-HPEA-Derivative	S1	211·86 ± 26·14	133·43 ± 19·94	<0·005
	S2	100·17 ± 32·89	83·22 ± 8·93	0·251
3,4 -DHPEA -EA	S1	381·57 ± 35·73	384·57 ± 50·28	0·907
	S2	162·41 ± 38·60	234·36 ± 33·97	0·006

Olive crop year 1998-99. Data are expressed as means ± standard deviation (n= 6).
P values shown for significance by 2-tailed test. Phenolic compounds were quantified as described in section 2.2.15. Phenolics are abbreviated as shown in the list of abbreviations.

Table 5.3b. Effect of irrigation and ripening on phenolic compounds (mg/Kg) of virgin olive oils obtained from fruits of *cv* Koroneiki in 1999

		Irrigated	Non-Irrigated	Sig. (2-tailed)
3,4-DHPEA	S1	0·54 ± 0·10	1·18 ± 0·13	<0·005
	S2	1·58 ± 0·10	2·55 ± 0·40	<0·005
p-HPEA	S1	1·25 ± 0·24	1·33 ± 0·33	0·73
	S2	2·63 ± 0·15	1·65 ± 0·24	<0·005
3,4-DHPEA-EDA	S1	379·78 ± 42·30	394·53 ± 27.55	0·56
	S2	282·15 ± 30·20	468·15 ± 8·90	<0·005
p-HPEA-EDA	S1	431·28 ± 43·75	309·30 ± 30·25	0·02
	S2	337·73 ± 11·24	316·50 ± 53·27	0·47
p-HPEA-Derivative	S1	165·48 ± 42·54	92·50 ± 39·85	0·05
	S2	144·48 ± 6·55	111·65 ± 25·27	0·05
3,4 -DHPEA -EA	S1	396·15 ± 32·32	392·53 ± 10·29	0·837
	S2	267·28 ± 18·22	289·48 ± 28·15	0·23

Crop year 1999-2000. S1= green olives, S2= Semi-black olives. Data are expressed as means ± standard deviation (n= 6). P values shown for significance by 2-tailed test. Phenolic compounds were quantified as described in section 2.2.15. Phenolics are abbreviated as shown in the list of abbreviations.

5.2.4. Effect of irrigation on the fatty acid composition of virgin olive oils of cv. Koroneiki

The fatty acid composition of oils from fruits of non-irrigated and irrigated trees is presented in Tables 5.4a and 5.4b. Oleic acid, the major fatty acid of olive oil, was significantly higher in the oils from fruits of irrigated trees in contrast to linoleic and α-linolenic acids, which showed decreases. In contrast to these obvious alterations, these were no other consistent changes in oils obtained from the two years. Saturated fatty acids hardly changed at all and, although irrigation tended to increase the percentage of stearate, this was not significant in all cases. The decrease

in linolate and α-linoleate is well reflected in the change in total PUFA and, even more, in the MUFAs / PUFAs ratio (Tables 5.4a and 5.4b).

Table 5.4a. Effect of irrigation and ripening on the fatty acid (%) content of virgin olive oils obtained from fruits of *cv* Koroneiki.in 1998.

Fatty acid		Irrigated	Non-Irrigated	Sig. (2-tailed)
C16:0 (% total fatty acids)	S1	13·76 ± 0·15	13·41 ± 0·07	<0·005
	S2	12·75 ± 0·35	12·99 ± 0·19	0·170
C16: 1(% total fatty acids)	S1	0·86 ± 0·01	0·76 ± 0·02	<0·005
	S2	0·81 ± 0·02	1·04 ± 0·02	<0·005
C18:0 (% total fatty acids)	S1	2·80 ± 0·09	4·34 ± 0·08	<0·005
	S2	2·74 ± 0·06	3·50 ± 0·04	<0·005
C18: 1(% total fatty acids)	S1	75·73 ± 0·32	71·05 ± 0·16	<0·005
	S2	75·42 ± 0·33	71·67 ± 0·15	<0·005
C18: 2 (% total fatty acids)	S1	5·04 ± 0·12	8·20 ± 0·09	<0·005
	S2	6·50 ± 0·10	8·74 ± 0·15	<0·005
C18: 3 (% total fatty acids)	S1	0·64 ± 0·01	0·92 ± 0·02	<0·005
	S2	0·60 ± 0·01	0·82 ± 0·02	<0·005
MUFAs (% total fatty acids)	S1	76·67 ± 0·32	71·87 ± 0·17	<0·005
	S2	76·30 ± 0·34	72·78 ± 0·16	<0·005
PUFAs (% total fatty acids)	S1	5·68 ± 0·13	9·12 ± 0·11	<0·005
	S2	7·12 ± 0·10	9·56 ± 0·08	<0·005
SFA (% total fatty acids)	S1	17·25 ± 0·23	18·71 ± 0·12	<0·005
	S2	16·18 ± 0·39	17·35 ± 0·22	<0·005
MUFAs/PUFAs	S1	13·51 ± 0·36	7·89 ± 0·12	<0·005
	S2	10·72 ± 0·14	7·61 ± 0·06	<0·005

Olive crop year 1998-99. Data are expressed as means ± standard deviation (n=6) as % total fatty acids. Fatty acids were quantified as described in section 2.212. p values are shown for significance by 2-tailed test. S1= green olives, S2= Semi-black olives. Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the number afterwards giving the number of doubled bonds. PUFAs = Polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SFA= saturated fatty acids

Table 5.4b. Effect of irrigation and ripening on fatty acid content of virgin olive oils obtained from fruits of *cv* Koroneiki for 1999.

Fatty acid		Irrigated	Non-Irrigated	Sig. (2-tailed)
C16:0 (% total fatty acids)	S1	13·34 ± 0·05	12·60 ± 0·07	<0·005
	S2	12·46 ± 0·17	12·81 ± 0·03	0·01
C16: 1(% total fatty acids)	S1	0·75 ± 0·01	0·63 ± 0·02	<0·005
	S2	0·73 ± 0·02	0·80 ± 0·01	<0·005
C18:0 (% total fatty acids)	S1	2·61 ± 0·04	3·68 ± 0·16	<0·005
	S2	2·45 ± 0·51	3·48 ± 0·02	0·01
C18: 1(% total fatty acids)	S1	77·01 ± 0·28	72·44 ± 1·00	<0·005
	S2	76·51 ± 0·23	71·01 ± 0·16	<0·005
C18: 2 (% total fatty acids)	S1	4·47 ± 0·09	8·51 ± 0·94	<0·005
	S2	5·74 ± 0·05	9·80 ± 0·14	<0·005
C18: 3 (% total fatty acids)	S1	0·74 ± 0·01	0·94 ± 0·03	<0·005
	S2	0·75 ± 0·03	0·95 ± 0·03	<0·005
MUFAs (% total fatty acids)	S1	77·66 ± 0·26	73·09 ± 1·01	<0·005
	S2	77·29 ± 0·21	71·84 ± 0·18	<0·005
PUFAs (% total fatty acids)	S1	5·20 ± 0·09	9·45 ± 0·96	<0·005
	S2	6·49 ± 0·07	10·75 ± 0·16	<0·005
SFA (% total fatty acids)	S1	16·66 ± 0·27	17·03 ± 0·28	0·10
	S2	15·66 ± 0·53	17·13 ± 0·04	<0·005
MUFAs/PUFAs	S1	14·93 ± 0·29	7·80 ± 0·82	<0·005
	S2	11·92 ± 0·14	6·68 ± 0·12	<0·005

Crop year 1999-00 Data are expressed as means ± standard deviation (n=6) as % total fatty acids fatty acids were quantified as described in section 2212. P values shown for significance by 2-tailed test. S1= green olives, S2= Semi-black olives. Fatty acids are abbreviated with the number before the colon showing the number of carbon atoms and the number afterwards giving the number of doubled bonds . PUFAs = Polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SFA= saturated fatty acids

5.2.5. Effect of irrigation on triacylglycerol composition of virgin olive oil of *cv.* Koroneiki

According to Boskou (1996) in a study where he examined the fatty acid composition of olive oil more than 70 different triacylglycerols should be present in theory. Of course, some of these would be present in very small concentrations while others like the fully saturated triacylglycerols such as PPP, SSS, PSP, have never been found in olive oil. The percentage triacylglycerol molecular species composition offers a way of detecting possible adulterations of olive oil with other oils which, while having a similar fatty acid composition, have different triacylglycerol patterns. The percent triacylglycerol molecular species composition of olive oil samples from irrigated and non-irrigated trees is shown in Table 5.5. and 5.6.

As expected from the fatty acid analysis, oils from irrigated trees contained smaller proportions of those molecular species containing linoleic and /or α-linolenic acids. Moreover, the major species, OOO, was significantly greater. However, some oleate-containing species, such as SOO or POS were increased in oils from non-irrigated trees. In general, though, the triacylglycerol species results could be predicted from the alterations in the fatty acid pattern. Moreover, the pattern of triacylglycerol molecular species for irrigated trees agrees well with published data on Koroneiki cultivar grown near Falasarna (Stefanoudaki *et al.*, 1997).

Table.5.5. Effect of irrigation on triacylglycerol molecular species in virgin olive oils obtained from green olive fruits of *cv* Koroneiki in 1998.

	Irrigated	Non-Irrigated	Sig. (2-tailed)
LLL+OLLn	0·36 ± 0·15	0·57 ± 0·02	0·07
OLL	0·98 ± 0·06	2·15 ± 0·09	<0·005
OOLn	1·42 ± 0·54	1·81 ± 0·01	0·28
PLL+PoOL	0·52 ± 0·04	0·69 ± 0·05	0·01
OOL	9·41 ± 0·93	11·31 ± 0·03	0·02
POL	4·97 ± 0·43	6·65 ± 0·03	<0·005
PLL	0·55 ± 0·10	0·68 ± 0·03	0·10
OOO	42·37 ± 1·53	35·36 ± 0·22	<0·005
POO+SOL	28·07 ± 0·99	24·89 ± 0·04	<0·005
POP	3·76 ± 0·22	3·56 ± 0·04	0·20
GaOO	0·47 ± 0·00	0·53 ± 0·05	0·10
SOO	5·20 ± 0·22	7·58 ± 0·13	<0·005
POS	1·29 ± 0·09	2·13 ± 0·06	<0·005
AOO	0·77 ± 0·13	1·12 ± 0·02	0·01
SOS	0·28 ± 0·11	0·60 ± 0·04	0·01

Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. Triacylglycerols were quantified as described in section 2.2.11. P, palmitic acid; O, oleic acid; L, linoleic acid; Po, palmitoleic acid; S, stearic acid; A, arachidic acid; Ga, gadoleic acid; Ln, linolenic acid. Results shown are % in total TAGs.

Table.5.6. Effect of irrigation on triacylglycerol molecular species in virgin olive oils obtained from semi-black olive fruits of *cv* Koroneiki in 1999.

	Irrigated	Non-Irrigated	Sig. (2-tailed)
LLL+OLLn	0·28 ± 0·00	0·51 ± 0·02	<0·005
OLL	1·43 ± 0·02	2·22 ± 0·05	<0·005
OOLn	1·24 ± 0·04	1·79 ± 0·14	0·00
PLL+PoOL	0·46 ± 0·03	0·70 ± 0·05	<0·005
OOL	10·98 ± 0·12	12·74 ± 0·15	<0·005
POL	5·74 ± 0·18	7·57 ± 0·05	<0·005
PLL	0·44 ± 0·03	0·71 ± 0·03	<0·005
OOO	41·77 ± 0·21	36·18 ± 0·14	<0·005
POO+SOL	26·15 ± 0·21	24·63 ± 0·09	<0·005
POP	3·12 ± 0·09	3·16 ± 0·17	0·73
GaOO	0·49 ± 0·01	0·36 ± 0·11	0·12
SOO	5·24 ± 0·06	6·14 ± 0·12	<0·005
POS	1·24 ± 0·01	1·61 ± 0·06	<0·005
AOO	0·91 ± 0·02	0·95 ± 0·01	0·04
SOS	0·35 ± 0·03	0·44 ± 0·01	0·01

Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. Triacylglycerols were quantified as described in section 2.2.11. P, palmitic acid; O, oleic acid; L, linoleic acid; Po, palmitoleic acid; S, stearic acid; A, arachidic acid; Ga, gadoleic acid; Ln, linolenic acid. Results shown are % in total TAGs.

5.2.6. Effect of irrigation on sterol composition of virgin olive oil of cv Koroneiki.

The sterol pattern is very important for the determination of the authenticity of olive oil (Ranalli, and Angerosa, 1996; Aparicio and Aparicio-Ruiz, 2000). However, as far as the Koroneiki variety is concerned, no thorough research has been carried out. The patterns of sterols at two stages of maturation as well as following irrigation are presented in Tables 5.7 and 5.8. However the total sterol content of oils from green fruits of irrigated trees was less than 1000 ppm, which is the lower limit set by the EC norm. Total sterols were significantly higher in the oils from non-irrigated trees (Table 5.6 and 5.7). In contrast to a previous report (Inglese *et al,* 1996), β-sitosterol and campesterol were also found significantly higher in oils extracted from irrigated fruits. Another important point is that, for the green olives from irrigated trees, their campesterol content was above 4·0 ppm which is the upper limit set by the European Community norm although it was lower in semi-black fruits analyzed (Table 5.8.).

In general, the sterol pattern obtained in oils from ripe olives was only affected to a small extent by irrigation, although many of the changes were statistically significant. In contrast, the total amount of sterols was reduced considerably by irrigation (Table 5.7. and 5.8).

Table 5.7. Effect of irrigation on the % sterol content of virgin olive oils obtained from green fruits of *cv* Koroneiki in 1998.

	Irrigated	Non-Irrigated	Sig. (2-tailed)
Cholesterol	0·56 ± 0·059	0·36 ± 0·163	0·018
24-Methylene cholesterol	0·29 ± 0·010	0·27 ± 0·008	0·004
Campesterol	4·17 ± 0·072	3·58 ± 0·037	<0·005
Campestanol	0·67 ± 0·104	0·33 ± 0·005	<0·005
Stigmasterol	0·58 ± 0·072	0·39 ± 0·015	<0·005
β-Sitosterol	77·00 ± 0·722	70·02 ± 0·382	<0·005
Δ^5-Avenasterol	13·06 ± 0·394	21·47 ± 0·348	<0·005
5-24 Stigmastadienol	0·87 ± 0·084	0·77 ± 0·051	0·030
Δ^7- Stigmasterol (%)	0·36 ± 0·083	0·29 ± 0·032	0·115
Δ^7 Avenasterol (%)	0·58 ± 0·020	0·69 ± 0·020	<0·005
Total sterols (ppm)	726·71 ± 104·46	1645·28 ± 51·35	<0·005

Olive crop year 1998-99. Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. Sterols were quantified as described in section 2.2.9.

Table 5.8. Effect of irrigation on the % sterol content of virgin olive oils obtained from semi-black fruits of *cv* Koroneiki in 1998.

	Irrigated	Non-Irrigated	Sig. (2-tailed)
Cholesterol	0·36 ± 0·057	0·27 ± 0·050	0·019
24-Methylene cholesterol	0·50 ± 0·011	0·33 ± 0·011	<0·005
Campesterol	3·29 ± 0·015	2·69 ± 0·017	<0·005
Campestanol	0·47 ± 0·007	0·32 ± 0·011	<0·005
Stigmasterol	0·38 ± 0·006	0·24 ± 0·004	<0·005
β-Sitosterol	67·89 ± 0·367	66·51 ± 0·472	<0·005
Δ^5-Avenasterol	24·43 ± 0·180	26·94 ± 0·452	<0·005
5-24 Stigmastadienol	0·86 ± 0·052	0·95 ± 0·032	0·005
Δ^7- Stigmasterol	0·25 ± 0·182	0·18 ± 0·016	0·324
Δ^7 Avenasterol	0·41 ± 0·043	0·34 ± 0·062	0·040
Total sterols (ppm)	1004·50 ± 11·360	1604·20 ± 36·455	<0·005

Olive crop year 1998-99. Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. Sterols were quantified as described in section 2.2.9.

5.2.7. Effect of irrigation on the composition of aliphatic and triterpenic alcohols in virgin olive oil of cv. Koroneiki.

Mechanically extracted olive oils have much lower content of higher aliphatic alcohols than do solvent extracted oils (Tacchino and Borgoni, 1983; Gracia and Cota, 1984). This characteristic can be used for the detection of possible adulteration of virgin olive oil with olive pomace oil. Oil from fruits of irrigated trees was found to have a significantly lower content of aliphatic and triterpenic alcohols (Table 5.9 and 5.10). The biggest differences appeared to be for docosanol. These results are in agreement with those reported by Iglese *et al.*, (1995). Several alcohols appeared to decrease with maturation of the olives. Hexacosanol was the most notable of these.

Table 5.9. Effect of irrigation and ripening on the aliphatic and triterpenic alcohol composition of virgin olive oil from green fruits of cv. Koroneiki (1998).

	Irrigated	Non-Irrigated	Sig. (2-tailed)
Docosanol (mg /100g oil)	1·26 ± 0·10	10·27 ± 0·79	<0·005
Tricosanol (mg /100g oil)	0·36 ± 0·03	0·90 ± 0·07	<0·005
Tetracosanol (mg /100g oil)	6·80 ± 0·78	20·65 ± 2·18	<0·005
Pentacosanol (mg /100g oil)	0·85 ± 0·08	1·22 ± 0·15	<0·005
Hexacosanol ((mg /100g oil)	15·16 ± 2·48	19·08 ± 2·87	0·030
Heptacosanol (mg /100g oil)	0·54 ± 0·11	0·51 ± 0·12	0·668
Octacosanol (mg /100g oil)	4·52 ± 0·77	4·62 ± 0·80	0·831
Total aliphatic alcohols (ppm)	26·59 ± 7·68	54·47 ± 8·79	<0·005
Cycloartenol (mg /100g oil)	12·56 ± 0·34	24·34 ± 2·47	<0·005
24-Methylene cicloartenol	5·32 ± 0·490	10·75 ± 1·23	<0·005
Citrostadienol (mg /100g oil)	12·25 ± 0·74	16·36 ± 2·37	<0·005

Data are expressed as means ± standard deviation (n=6). . P values shown for significance by 2-tailed test. Aliphatic and triterpenic alcohols were quantified as described in section 2.2.9

Table 5.10. Effect of irrigation on the aliphatic and triterpenic alcohol composition of virgin olive oil obtained from semi-black fruits of cv. Koroneiki.(1998).

	Irrigated	Non-Irrigated	Sig. (2-tailed)
Docosanol (mg /100g oil)	3·92 ± 0·90	11·15 ± 0·59	<0·005
Tricosanol (mg /100g oil)	0·35 ± 0·01	0·75 ± 0·03	<0·005
Tetracosanol (mg /100g oil)	7·83 ± 0·64	18·52 ± 1·14	<0·005
Pentacosanol (mg /100g oil)	0·51 ± 0·03	0·72 ± 0·06	<0·005
Hexacosanol ((mg /100g oil)	9·00 ± 0·98	12·40 ± 1·34	<0·005
Heptacosanol (mg /100g oil)	0·47 ± 0·07	0·48 ± 0·02	0·818
Octacosanol (mg /100g oil)	2·67 ± 0·28	2·90 ± 0·33	0·221
Total aliphatic alcohols (ppm)	25·58 ± 2·17	46·91 ± 3·39	<0·005
Cycloartenol (mg /100g oil)	14·49 ± 0·279	18·23 ± 0·31	<0·005
24-Methylene cicloartenol	15·30 ± 0·29	9·43 ± 0·65	<0·005
Citrostadienol (mg /100g oil)	23·02 ± 0·36	12·52 ± 0·30	<0·005

Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. Aliphatic and triterpenic alcohols were quantified as described in section 2.2.9

5.2.8. Effect of irrigation on volatile compounds of virgin olive oil of *cv.* Koroneiki.

More than 20 volatile compounds were identified in the oil samples from semi-black fruits of irrigated and non-irrigated trees. The volatiles included hydrocarbons, alcohols, aldehydes and esters (Table 5.11). Aldehydes were the most prevalent of the volatiles accounting in irrigated trees for 45 % of the total volatiles and in the non-irrigated trees about 35 %. This was in agreement with previously published data for olive oil (Montedoro *et al.,* 1978). Total volatiles were significantly higher in oils from non- irrigated trees. For the 6C compounds, hexanal, 3-hexenal, *trans*-2-hexenal, *trans*-3 hexen-1-ol, *trans*-2-hexen-1-ol and hexyl acetate

showed higher concentrations in non-irrigated trees. However, significant differences were observed, only for *trans*-3-hexen-1-ol and hexyl acetate. These 6C compounds are known as green volatile compounds and are products of lipoxygenase (LOX) pathway from polyunsaturated fatty acids containing a *cis-cis* 1,4 pentadiene structure (e.g. linolenic and linoleic acids). On the other hand, the headspace analysis of olive oils shows the presence of 5C compounds in addition to 6C compounds. 5C compounds contribute also to the green odours. In fact 1-pentene –3-ol and 3-pentanone were significantly higher in oils from non-irrigated trees. These volatiles are products of anaerobic cleavage reaction of the 13-hydroperoxide of linolenic acid mediated by the lipoxyganase pathway (Sanchez and Salas, 2000). Ethyl acetate, 3-methylbutanal, acetic acid, ethyl propanoate, butyl acetate were also present. These compounds arose from the degradation of some amino acids (valine, leucine, and isoleucine) and from the fermentation of sugars naturally occurring in the fruits (Angerosa *et al.*, 1998; Sanchez and Salas, 2000; Angerosa, 2002). 3-Methyl-butanal was significantly higher in oils from irrigated trees and with the value very close to the upper limit in which high quality of oil is assured (Angerosa *et al.*, 2001).

Table 5.11. Effect of irrigation on volatile compounds of virgin olive oils obtained from semi-black fruits of *cv* Koroneiki (1999).

	Peak-area ratio (x 100)*		
	Irrigated	Non-Irrigated	Sig. (2-tailed)
Hydrocarbons			
Iso- pentane	4·68± 0·36	1·79± 0·53	<0·005
n-Hexane	9·70± 0·84	8·55± 0·75	0·15
Alcohols			
1- Pentene-3-ol	5·94± 3·51	14·70± 0·28	0·01
Iso- amyl alcohol	3·96± 3·57	4·80± 0·65	1·00
Hexan-1-ol	1·67± 0·10	1·32± 0·24	0·07
trans 3-Hexen-1-ol	2·53± 0·09	4·07± 0·40	<0·005
cis- 3- Hexen-1 -ol	2·40± 1·28	1·83± 0·35	0·50
trans -2- Hexen-1-ol	2·83± 0·59	3·54± 0·35	0·15
Aldehydes			
3-Methyl butanal	5·77± 0·59	1·35± 0·09	<0·005
Acetaldehyde	2·10± 0·02	0·72± 0·06	<0·005
Hexanal	8·87± 0·77	9·51± 0·23	0·24
3- Hexenal	4·95± 0·67	5·21± 0·19	0·54
trans -2- Hexenal	24·03± 1·29	26·36± 1·22	1·07
Octanal	1·54± 0·11	4·09± 0·13	<0·005
Ketones			
Acetone	4·24± 1·73	0·87± 0·29	0·03
3-Pentanone	2·73± 0·83	6·62± 0·43	<0·005
Esters			
Ethyl-acetate	4·83± 0·22	2·37± 0·23	<0·005
Ethyl propanoate	6·77± 1·04	11·49± 0·24	<0·005
Butyl acetate	5·12± 0·81	5·60± 0·13	0·37
Hexyl acetate	3·56± 2·35	16·66± 1·60	<0·005
Methyl nonanoate	0·73± 0·11	0·66± 0·10	0·64
Acids			
Acetic acid	0·83± 0·15	3·45± 2·19	0·11
Total volatiles	105·26± 6·30	136·43± 1·14	<0·005

Data are expressed as means ± standard deviation (n=3). P values shown for significance by 2-tailed test. Volatile characteristics were quantified as described in section 2.2.19. * Ratio of the compound peak area to the internal standard peak area, multiplied by 100.

5.2.9. Comparison of results for the two ripening stages

It is well known that the stage of fruit maturation is among several different factors (Montedoro and Garofolo, 1984), which may directly or indirectly affect the oil composition and quality (Frega *et al.,* 1991; Mariani *et al.,* 1991; Frega *et al.,* 1992; Garcia *et al.,* 1996). Ripening changes not only the external appearance of the fruit (increase in size, changes in color) but also the internal composition of the drupe where chemical transformations occur which are related to the synthesis of organic substances especially triacylglycerols (Boskou, 1996). Part of our research work was to study the influence of the fruit ripening stage in irrigated and non-irrigated olive trees of Koroneiki variety on fruit yield and qualitative characteristics of olive oil for two successive years.

Qualitative parameters of the oils, such as free acidity, peroxide value do not vary significantly since these parameters mostly depend on the physiological health of the olives. The values of K_{232} and K_{270} followed similar tendencies (Table 5.12 to 5.14). The first year of our experiment the values appeared to decrease significantly during ripening similar to the results obtained by Gutierrez *et al.* (1999) whereas in the second year the values did not change significantly. The concentration of chlorophyll decreased in all treatments similar to results obtained by Gandul-Rojas *et al.* (1999), although there was less change in the second (wetter) season (Table 5.14).

The data showed that oil stability decreased in all samples during fruit maturation. However, the percent decrease in oils from the irrigated trees was 29% and 18% for the 1998 and 1999 crop years. In contrast, for non-irrigated trees the percent decreases were only 6-7%. Similar trends to oxidative stability were followed by the total phenol content of olive oils (Table 5.12 to 5.14), with greater decreases in irrigated trees. In fact, the percent decrease in irrigated trees was about 45% and 17% for the two years, whereas in non-irrigated trees it was 27 % and 5% respectively for 1998 and 1999.

Simple phenols during the two-year of study showed different trends, especially the main phenyl alcohols of oil 3,4-DHPEA and p-HPEA did not vary significantly in the first year whereas in the second year they increased significantly, similar to the results obtained by Cimato *et al.* (1990) and Brenes *et al.* (1999). The secoiridoid derivatives, such as the dialdehydic form of elenolic acid linked to 3,4-

DHPEA or p-HPEA (3,4 DEPEA-EDA or p-HPEA-EDA respectively) and an isomer of oleuropein aglycon (3,4 DEPEA-EA) decreased during the ripening of olive fruits. However, the decrease in 1999 was significant only for 3,4 DEPEA-EA.

The percentage of monounsaturated fatty acids (MUFA) decreased but not significantly with the exception of the MUFA from non-irrigated trees of the first year, which significantly increased. The percentage of saturated fatty acids (SAFA) decreased in all treatments. However, significant differences were only observed in the first year of our experiment.

The evolution of sterols during maturation is presented in Tables 5.12 and 5.13). It is evident that most of these compounds are significantly affected by the maturation stage of the fruit. Among these campesterol, β-sitosterol and Δ^5-avenasterol are mostly affected by ripening. Campesterol and β-sitosterol decreased during ripening while Δ^5-avenasterol significantly increased.

As shown in Table 5.12, there was no change in the total aliphatic alcohols during maturation for irrigated trees. In non-irrigated trees (Table 5.13) there was a decrease but this was not statistically significant. However, there were significant differences in the composition of aliphatic alcohols of olive oils between irrigated and non-irrigated trees (Tables 5.9 and 5.10). These results are in agreement with the results obtained by Tacchino and Borgogni (1983). The content of triterpenic alcohols showed opposite trends for the different cultivation conditions. In fact, in irrigated trees the content increased whereas in non-irrigated olives they decreased. The concentrations of the volatile compounds at the two ripeness stages are shown in Table 5.16. Total content of volatiles decreased significantly during maturation. Individual volatile compounds decreased in almost all cases but changes were significant only for iso-pentane, iso-amyl alcohol, hexan-1-ol and methyl nonanoate. Hexyl acetate is an exception because it increased from green to semi-black olives. These results are in agreement with the results obtained by Aparicio and Morales (1998).

Table 5.12. Overall composition of virgin olive oils obtained from fruits of irrigated trees of *cv* Koroneiki at two maturity stages in 1998.

	Sampling 1	Sampling 2	Sig. (2-tailed)
K232 (OD 232nm)	1·70±0·05	1·44±0·04	<0·005
K270 (OD 270nm)	0·18±0·01	0·14±0·02	<0·005
Total phenols (ppm gallic acid)	366·14±14·54	201·48±20·99	<0·005
Oxidative stability (h)	21·57±0·68	15·34±0·81	<0·005
Chlorophyll	14·38±2·02	6·59±1·97	<0·005
Campesterol (%)	4·17±0·07	3·29±0·02	<0·005
β-Sitostesterol(%)	77·00±0·72	67·89±0·37	<0·005
Δ^5-Avenosterol(%)	13·06±0·39	24·43±0·18	<0·005
Total sterols (ppm)	726·71±104·46	1004·50±11·36	<0·005
Triterpenic alcohols (mg/100g)	30·13±1·56	52·81±0·92	<0·005
Total aliphatic (mg/100g)	26·59±7·68	25·58±2·17	0·764
3,4-DHPEA (ppm)	0·67±0·37	0·24±0·21	0·033
p-HPEA (ppm)	1·42±0·38	1·36±0·28	0·764
3,4-DHPEA- EDA (ppm)	473·35±26·61	159·03±43·70	<0·005
p-HPEA-EDA (ppm)	507·94±32·01	219·34±69·34	<0·005
p-HPEA-Derivative (ppm)	211·86±26·14	100·17±32·89	<0·005
3,4-DHPEA- EA (ppm)	381·57±35·73	162·41±38·60	<0·005
MUFAs (% total fatty acids)	76·67±0·32	76·30±0·34	0·084
PUFAs (% total fatty acids)	5·68±0·13	7·12±0·10	<0·005
SFA(% total fatty acids)	17·25±0·23	16·18±0·39	<0·005

Crop year 1998-99. Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. Sampling 1=green olives; sampling 2=semi-black olives.
PUFAs = Polyunsaturated fatty acids, MUFAs= monounsaturated fatty acids, SFA= saturated fatty acids. Phenolics are abbreviated as shown in the list of abbreviations.

Table 5.13. Overall composition of virgin olive oils obtained from fruits of non-irrigated trees of *cv* Koroneiki at two maturity stages in 1998.

	Sampling 1	Sampling 2	Sig. (2-tailed)
K232 (OD 232nm)	1·74±0·03	1·55±0·04	<0·005
K270 (OD 270nm)	0·19±0·03	0·158±0·02	<0·005
Total phenol (ppm gallic acid)	342·36 8·09	248·38±12·47	<0·005
Oxidative stability (h)	15·75±0·95	14·55±0·46	0·020
Chlorophyll	17·17±0·74	2·97±0·13	<0·005
Campesterol (%)	3·58±0·04	2·69±0·02	<0·005
β-Sitosterol (%)	70·02±0·38	66·51±0·47	<0·005
Δ^5avenosterol (%)	21·47 ± 0·35	26·94 ± 0·45	<0·005
Total sterols (ppm)	1645·28± 51·35	1604·20 ± 36·46	0·141
Total aliphatic alcohols (mg/100g)	54·47 ± 8·79	46·91 ± 3·39	0·078
Triterpenic alcohols (mg/100g)	51·45 ± 6·08	40·18 ± 0·89	<0·005
3,4-DHPEA (ppm)	0·55 ± 0·14	0·52 ± 0·37	0·868
p-HPEA (ppm)	0·87 ± 0·24	0·88 ± 0·33	0·912
3,4-DHPEA- EDA (ppm)	568·20 ± 39·36	370·49 ± 48·86	<0·005
p-HPEA-EDA (ppm)	447·15 ± 41·70	250·60 ± 42·88	<0·005
p-HPEA-Derivative (ppm)	133·43 ± 19·94	83·22 ± 8·93	<0·005
3,4-DHPEA- EA (ppm)	384·57 ± 50·28	234·36 ± 33·97	<0·005
MUFAs (% total fatty acids)	71·87 ± 0·17	72·78 ± 0·16	<0·005
PUFAs (% total fatty acids)	9·12 ± 0·11	9·56 ± 0·08	<0·005
SFA (% total fatty acids)	18·71 ± 0·12	17·35 ± 0·22	<0·005

Crop year 1998-99. Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. See Table 5.12 for other abbreviations.

Table 5.14. Effect of ripening on the composition of virgin olive oils obtained from fruits of irrigated trees of cv. Koroneiki in 1999.

	Sampling 1	Sampling 2	Sig. (2-tailed)
K232 (OD 232nm)	1·63 ± 0·02	1·71 ± 0·06	0·040
K270 (OD 270nm)	0·14 ± 0·03	0·15 ± 0·02	0·253
Total phenols (ppm gallic acid)	485·88 ± 21·61	403·60 ± 31·99	<0·005
Oxidative stability (h)	22·43 ± 0·15	18·43 ± 0·22	<0·005
Chlorophyll (ppm)	8·70 ± 0·23	8·30 ± 0·12	0·021
3,4-DHPEA (ppm)	0·54 ± 0·10	1·58 ± 0·10	<0·005
p-HPEA (ppm)	1·25 ± 0·24	2·63 ± 0·15	<0·005
3,4-DHPEA- EDA (ppm)	379·78 ± 42·30	282·15 ± 30·20	0·009
p-HPEA-EDA (ppm)	431·28 ± 43·75	337·73 ± 11·24	0·006
p-HPEA-Derivative (ppm)	165·48 ± 42·54	144·48 ± 6·55	0·367
3,4-DHPEA- EA (ppm)	396·15 ± 32·32	267·28 ± 18·22	<0·005
MUFAs (% total fatty acids)	77·66 ± 0·26	77·29 ± 0·21	0·067
PUFAs (% total fatty acids)	5·20 ± 0·09	6·49 ± 0·07	<0·005
SFA (% total fatty acids)	16·66 ± 0·27	15·66 ± 0·53	0·015

Crop year 1999-2000. Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. See Table 5.12 for details.

Table 5.15. Effect of ripening on the composition of virgin olive oils obtained from fruits of non-irrigated trees of cv. Koroneiki in 1999.

	Sampling1	Sampling2	Sig. (2-tailed)
K232 (OD 232nm)	1·83 ± 0·06	1·84 ± 0·01	0·819
K270 (OD 270nm)	0·17 ± 0·00	0·17 ± 0·01	0·356
Total phenols (ppm gallic acid)	505·60 ± 23·46	479·13 ± 29·87	0·213
Oxidative stability (h)	15·48 ± 0·47	14·55 ± 0·29	0·016
Chlorophyll (ppm)	15·80 ± 0·46	6·25 ± 0·40	<0·005
3,4-DHPEA (ppm)	1·18 ± 0·13	2·55 ± 0·40	<0·005
p-HPEA (ppm)	1·33 ± 0·33	1·65 ± 0·24	0·162
3,4-DHPEA- EDA (ppm)	394·53 ± 27·54	454·15 ± 32·40	0·030
p-HPEA-EDA (ppm)	309·90 ± 30·26	316·50 ± 53·27	0·876
p-HPEA-Derivative (ppm)	92·50 ± 39·85	111·65 ± 25·27	0·448
3,4-DHPEA- EA (ppm)	391·35 ± 10·29	289·48 ± 28·15	<0·005
MUFAs (% total fatty acids)	73·09 ± 1·01	71·84 ± 0·18	0·050
PUFAs (% total fatty acids)	9·45 ± 0·96	10·75 ± 0·16	0·038
SFA (% total fatty acids)	17·03 ± 0·28	17·13 ± 0·04	0·490

Crop year 1999-2000. Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. See Table 5.12 for details.

Table 5.16. Volatile compounds (ppm) in virgin olive oils obtained from fruits of irrigated trees of *cv* Koroneiki at two maturity stages in 1999.

	Peak-area ratio (x 100)*		
	Sampling 1	Sampling 2	Sig. (2-tailed)
Hydrocarbons			
Iso- pentane	29·34± 4·56	4·68± 0·36	<0·005
n-Hexane	8·14± 1·85	8·37± 2·54	0·905
Alcohols			
1- pentene-3-ol	11·52± 0·88	5·94± 3·51	0·195
Iso- amyl alcohol	18·20± 1·10	3·56± 3·57	<0·005
Hexan-1-ol	3·96± 0·08	1·67± 0·10	<0·005
trans- 3-Hhexen-1-ol	6·93± 0·95	2·53± 0·09	0·412
cis- 3- Hexen-1 -ol	3·62± 1·94	2·40± 1·28	0·348
trans -2- Hexen-1-ol	3·47± 0·86	2·83± 0·59	0·556
cis -2-Hexen-1-ol	11·83± 1·94	1·71± 1·08	0·017
Aldehydes			
3-methyl butanal	3·14± 0·64	5·77± 0·59	0·238
Acetaldehyde	3·83± 1·83	1·66± 0·76	0·129
Hexanal	10·61± 0·62	8·87± 0·77	0·055
3- Hexenal	20·18± 1·10	4·91± 0·94	<0·005
trans -2- Hexenal	21·13± 0·57	24·03± 1·29	0·496
Ketones			
Acetone	7·69± 2·11	4·24± 1·73	0·562
3-Pentanone	7·17± 1·77	2·73± 0·83	0·335
Esters			
Ethyl-acetate	5·15± 0·85	4·83± 0·22	0·071
Ethyl propanoate	5·83± 0·54	6·77± 1·04	0·017
Butyl acetate	6·09± 1·31	5·12± 0·81	0·038
Hexyl acetate	2·55± 0·05	3·56± 2·35	<0·005
Methyl nonanaate	7·66± 3·25	0·73± 0·11	<0·005
Acid			
Acetic acid	7·06± 2·75	0·83± 0·15	0·021
Total volatiles	227·44± 8·77	105·26± 6·30	<0·005

Data are expressed as means ± standard deviation (n=3). P values shown for significance by 2-tailed test. Sampling 1=green olives. Sampling 2=semi-black olives. Volatile characteristics were quantified as described in section 2.2.19. * Ratio of the compound peak area to the internal standard peak area, multiplied by 100.

5.2.10. Tocopherol variation following irrigation

The different tocopherols are well-known natural antioxidants and of these, α-tocopherol is the most prevalent in olive oil. The content of this vitamin was measured in oils from semi-black olives from two successive years (Table 5.17). In both cases, the content was markedly increased in oils from non-irrigated trees. These results are opposed to the results obtained by Tovar *et al.* (2002). This might be expected to lower the peroxide value and increase oxidation stability in such oils and, indeed, this was seen for the years 1998-9 harvest (Table 5.2a). However, the correlation was not seen for the following year (Table 5.2b),thus, emphasizing that a number of factors probably contribute to oxidative stability. Nevertheless, it is recognized generally that tocopherols and tocotrienols are often the most important natural antioxidants in fats and oils, where they act as primary or chain breaking antioxidants by converting lipid radicals to more stable products.

Table 5. 17. α-Tocopherol content of olive oils from fruits of irrigated and non-irrigated trees of *cv* Koroneiki.

Year	Irrigated	Non-Irrigated	Sig. (2-tailed)
1998-1999	173·78± 15·54	271·25± 2·27	<0·005
1999-2000	221·85± 2·29	304·45± 37·12	0· 09

Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. α-Tocopherol was quantified as described in section 2.2.17. Semi-black olives were analysed.

5.2.11. Panel test of oils.

Because our data has shown that fruit maturity, as well as whether the olive crop had been irrigated, both contribute to qualitative changes in oils we carried out a panel test. Clearly if the chemical alterations could be detected by habitual consumers (i.e. the panel tasters) then this would be an important point to consider in terms of commercial production.

The results are shown in Table 5.18. Oils from immature fruits scored less on the desirable fruity and green leaf attributes but higher on the immature bitter quality. These oils also obtained a rather poor overall score. Moreover oils from irrigated trees were also scored better on these parameters. On the other hand, the overall score from the green olive trees was low for both oils for irrigated and for the non-irrigated trees. This was because phenolic content was very high in comparison with the oils obtained from industrial extraction for Koroneiki cultivars and, as a consequence, the intensities of bitter and pungent were too strong. According to Montedoro *et al.* (1978) very high concentrations of polyphenols in oils resulted in sharp negative influence on mouth feel and flavour. Furthermore, the presence of 1- pentene-3-ol, isoamyl alcohol and acetic acid in high concentrations (Table 5.18) would give undesirable sensations during olive oil tasting (Aparicio *et al.*, 1994; Angerosa *et al.*, 1992). Moreover, some tasters noticed a slight taste of dry leaves in oils from non-irrigated trees, which may have affected their final decisions. Similarly, for the same reason although tasters did not noticed any defect in oils from semi black fruits, the scores were not very high and for non- irrigated fruits, just reached the limit for extra virgin olive oil. Thus, it seems that irrigating trees not only increased yields (Table 5.18) but also gave better quality oils, at least as judged by consumers.

Table 5. 18. Effect of irrigation and ripening on panel test results for oils obtained from fruits of *cv* Koroneiki.

Sensory attributes		Irrigated	Non-Irrigated	Sig. (2-tailed)
Fruity	S1	2·45± 0·07	2·05 ± 0·07	0·03
	S2	2·80± 0·28	2·40 ± 0·28	0·29
Cut green leaf	S1	2·10± 0·57	1·65 ± 0·21	0·40
	S2	2·35± 0·49	2·30 ± 0·14	0·90
Bitter	S1	3·60± 0·14	3·60 ± 0·14	1·00
	S2	2·85± 0·49	3·25 ± 0·07	0·38
Pungent	S1	2·85± 0·07	3·45 ± 0·07	0·01
	S2	2·65± 0·21	3·55 ± 0·35	0·09
Overall	S1	5·70± 0·14	4·80 ± 0·00	0·01
	S2	7·05± 0·35	6·50 ± 0·42	0·06

Data are expressed as means ± standard deviation (n=6). P values shown for significance by 2-tailed test. Qualitative characteristics were quantified as described in Chapter 2. Note: S1= green olives, S2= Semi-black olives,.1: barely perceptible, 2:slight 3: average, 4:great, and 5:extreme. Note that the overall score is on a scale of 1 to 9 (section 2.2.8).

5.3. Discussion

The rainfall pattern in the two years of the experiment was characterized by limited rain in May, September and October (43 and 57 mm of total rain respectively), while June, July and August there was no rainfall at all (Figure 2.2). Minimum and maximum air temperature presented typical peak values between July and August (Fig. 2.1). The data have shown that application of water during the dry season is essential for fruit growth and affects weight as well as oil content of the fruit. In fact, fruits from irrigated trees were 30% and 22 % heavier than the fruit from non-irrigated trees in the 1998 and 1999 crop years, respectively. Furthermore, oil content (expressed as % dry weight) was significantly higher in fruits of irrigated trees (12%) in the first year while in second crop year it was only 3 % higher but not significantly so (Tables 5.1a, 5.1b). The rainfall of September of the second year probably reduced the difference between the treatments. This shows that Koroneiki cultivar recovers rapidly from the effect of water stress. These results were similar to those obtained by Dettori & Rousso (1993), Inglese *et al.* (1996) and Ismail *et al.* (1997). On the other hand, oil content increased with fruit ripening in all treatments similar to results obtained by Motilva *et al.* (2000).

According to Lavee and Wodner (1991) the pattern of oil accumulation depends on environmental conditions, particular water availability and fruit yield. Fruit ripening was more advanced in the non-irrigated trees. During harvest at the end of November, the ripening index was 2.4 (green with reddish spots epicarp) for irrigated trees and 3.5 (reddish or purple areas in more than half of the fruits and the rest of the fruits had black skins and white pulp) for non-irrigated trees, respectively. Qualitative and authenticity parameters of the oil samples analyzed fell within the limits established by the European Community for classification in the extra virgin olive oil category. From a sensory point of view all the samples examined showed no defects except for the fact that some tasters noticed very slight dry leaves in oils from non-irrigated trees. However, the direct observation of the intensities of attributes detected by tasters showed that the oils studied were mainly characterized by very high intensities of bitter, pungent, fruity (green olives), and leaf attributes. These very strong sensations in green olives are due to the high content of both 6C volatile compounds (trans 2-hexenal, 3-hexenal, hexanol, trans-3-hexen-1-ol) and to secoiridoid compounds. Bitter and pungent sensation is positively related to the

amount of secoiridoid compounds (Angerosa *et al.*, 2000). It is worth mentioning that oils from semi-black olives from irrigated trees were less bitter and less pungent, while the fruity sensation was slightly more. These were the reasons that tasters gave them a higher overall score.

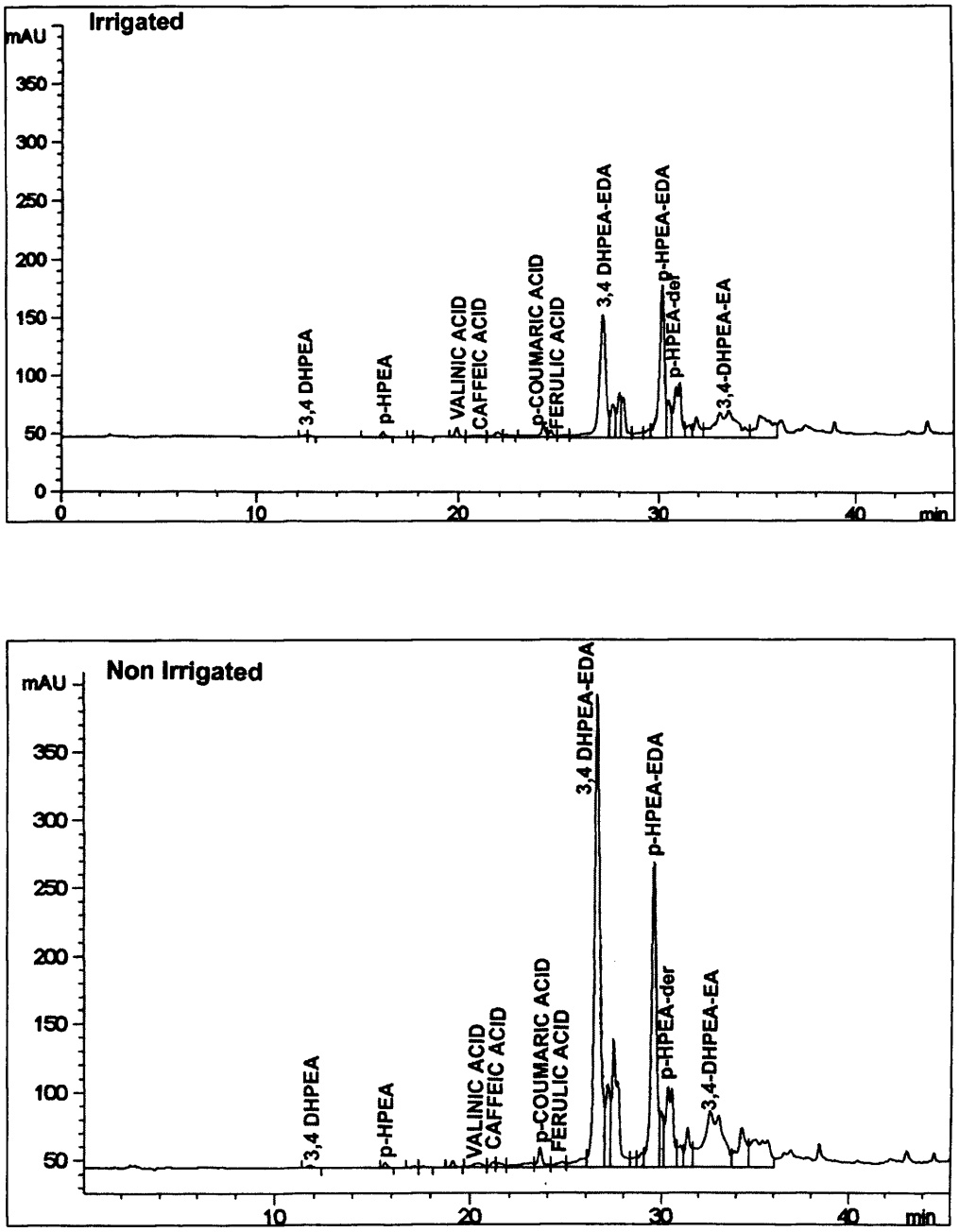


Fig. 5.1 Reverse phase high performance liquid chromatography (detector at 278 nm) of phenolic extracts from virgin olive oil samples from irrigated and non-irrigated trees.

The decrease of bitterness in oils from irrigated trees is of great importance for varieties characterized by very intense values of pungent attributes like cv.

Coratina. Our data confirms these research findings. In fact, total phenol content and derivatives of oleuropein, indicated by 3,4-DHPEA-EDA (the dialdehydic form of elenolic acid linked with 3,4-dihydroxy phenyl ethanol) and 3,4-DHPEA-EA (an isomer of oleuropein aglycon) were higher in oils from the fruits of non- irrigated trees. Moreover, the difference in the HPLC profile of phenols (Figure 5.1) between oils from fruits of irrigated and non-irrigated trees give an idea about the complicated biochemical processes that control the formation of phenols in olives. In addition, because these compounds have a scavenger effect on peroxyl radicals (Baldioli *et al.*, 1996; Blekas and Boskou, 1998) they should help to extend the shelf life of olive oil. The data shows also a positive correlation between oxidative stability and secoiridoid compounds.

The total fatty acid composition was altered by drought so that polyunsaturated fatty acids (PUFAs) were higher in non-irrigated treatments. The increase was parallel to a decrease in the % of monounsaturated fatty acids (MUFAs). These changes suggest that in non-irrigated olives further desaturation of oleate into linoleate, and then linolenate is more active (Gutierrez *et al.*, 1999). The ratio of unsaturated /saturated was also significantly lower in non-irrigated trees. This could influenced the taste of virgin olive oil because a high content of saturated fatty acids makes the oil more viscous and gives rise to a defect defined as fatty sensation (Solinas, 1990). The monounsaturated to polyunsaturated fatty acid ratio was significantly influenced by irrigation and, therefore, the intrinsic oxidative stability of the oil was altered. This explained the lower stability values in oils from non-irrigated fruit although the phenol concentration was higher. Furthermore, strong negative correlations were detected (0·80 and 0·94) between oxidative stability and PUFAs for the crop years 1998 and 1999 respectively.

Aliphatic and triterpenic alcohols, total sterols and Δ^5-avenasterol were also increased in oils from non- irrigated trees, though it is not known whether they could influence the sensory properties of olive oil significantly.

Thus, irrigation changed both quantitative and qualitative aspects of olive oils. Moreover, although irrigation increased yields and overall taste panel scores, some of the most desirable individual sensory characteristics were reduced in oils from irrigated trees.

5.4. Summary

In this chapter, the effect of irrigation and ripening stage on fruit characteristics and olive oil quality was studied. The following main conclusions could be made.

Effect of irrigation

- Irrigation increased fruit weight and oil content.
- Total phenols and secoiridoid derivatives were higher in the oil produced from the non- irrigated trees.
- Irrigation affected olive oil fatty acid composition and triacylglycerol molecular species
- Total sterols and total aliphatic alcohols were higher in the oil produced from non- irrigated trees.
- α-Tocopherol and the total content of volatiles were significantly higher in oils produced from non-irrigated trees
- The panel overall grade was higher for oils produced from semi-black fruits of irrigated trees.

While studying the effect of irrigation, olives at different stages of ripening were also studied. The data showed that:

- Oil content increased with fruit ripening
- Oil stability decreased in all samples during fruit maturation.
- Total phenols and secoiridoid derivatives decreased during the ripening of olive fruits
- Total sterols increased during maturation
- Aliphatic alcohols did not change significantly
- Total content of volatiles decreased significantly during maturation.

Chapter 6. Olive oil oxidation

6.1. Introduction

The main processes leading to the serious deterioration of olive oil are lipolysis and oxidation. Lipolysis or hydrolytic rancidity includes hydrothermal or enzymatic (lipases) hydrolysis that leads to the formation of non-esterified fatty acids and other products. Injured drupes are prone to the actions of endogenous and exogenous lipase and lipoxidase enzymes, which can modify the oxidation state and the acidity of the oil droplets (Boskou, 1996). Acylhydrolases act when the oil is still in the fruit during maturation, harvesting and extraction, while, on the other hand, oxidation takes place mainly during the extraction process and when olive oil is stored (Kiritsakis, 1990; Morales and Prybylski, 2000).

Oxidation is a complex process because of the influence of several factors, such as light, temperature, pigments, oxygen availability, enzymes, metal contamination and microorganisms (Frankel, 1991; Morales and Przybylski, 2000; Gutierrez *et al.*, 2002; Angerosa, 2002). Oxidation leads to the formation of volatile products, which not only change the initial flavor of olive oil (Morales *et al.*, 1997) but also decrease the nutritional quality and may lead to the formation of toxic products (Sanders, 1983). Even though oxidative and hydrolytic degradation processes are different, they appear to interact with each other and contribute to a reduction in the shelf life of the oil (Frega *et al.*, 1999).

Virgin olive oil is considered to be resistance to oxidation mainly due to the low content of polyunsaturated fatty acids (PUFA) and to the antioxidant activity of some of the unsaponifiable components such as α-tocopherols, or carotenoid pigments. However, the main antioxidant activity is probably exerted by a number of complex phenolic compounds, which have been identified in the polar fraction of virgin olive oil (Vanquez-Roncero *et al.*, 1975; Montedoro and Garofalo, 1984; Papadopoulos, and Boskou, 1991; Baldioli *et al.*, 1996; Berra, 1998; Aparicio *et al.*, 1999). The concentration of these compounds in the olive fruit varies a great deal according to the cultivar, the climate, the time of harvest and processing conditions (Montedoro *et al.*, 1978; Papadopoulos and Boskou, 1991). In recent years, storage stability and shelf life for fats and oils have received special attention from nutritionists, food processors, and consumers because of their influence on food quality.

During the autoxidation of olive oil, various factors with antioxidant or pro oxidant activity can act simultaneously. For this reason, it is very difficult to determine the effect of each one on the overall process of oxidation.

The current study was carried out to ascertain the effect of different storage factors on the qualitative characteristics of olive oil and to evaluate the possibility of improving stability by using nitrogen as a conditioner gas for virgin olive oil. The influence of dispersed water on the oxidation process was also studied. Such knowledge of suitable conditions for storing and packing olive oil is an important asset in order to increase competitiveness by maintaining product quality.

6.2. Effect of different storage conditions and time of storage on the qualitative characteristics of olive oil

6.2.1. Changes in non-esterified fatty acids (NEFA)

The level of hydrolysis of fat is generally ascertained by the % NEFA. Non-esterified fatty acids are of primary importance for the rate and development of rancidity. A pro-oxidant effect of fatty acids on edible oils had been demonstrated (Miyoshita and Tagari, 1986; Kiritsakis and Tsipeli, 1992b). Furthermore, Frega *et al.,* (1999) studied the effect of oleic acid, when added to virgin olive oils, on their oxidative stability and confirmed a pro-oxidant effect of non-esterified fatty acids, which was dependent on their concentration.

The acidity of the freshly extracted oils was very low and during storage it was increased slightly in almost all cases. Table 6.1 presents the changes of % NEFA during storage. In the samples stored indoors, the increase in acidity was significant only after eight to fifteen months of the storage. The increase was more obvious in the unfiltered oils where the non-esterified fatty acid release was higher. It appeared, that even after 15 months of storage, olive oil was very stable with regard to its acidity, with values far below the limit (<1%) established by EEC Regulation No. 2568/91 for extra virgin olive oil quality.

The variation in acidity, in relation to the different ways of storage was not statistically significant. Therefore, the results show that acidity was substantially independent of the storage conditions.

6.2.2 Changes in peroxide value.

Peroxide value (PV) is one of the most commonly used analytical parameters and is a valuable measurement of the initial products of oxidation. It is a measure of olive oil hydroperoxides formed through the oxidation of unsaturated fatty acids. However, the use of PV to monitor rancidity has been questioned since hydroperoxides, as high molecular weight substances, are odourless and tasteless (Frankel, 1991; Angerosa, 2002) and do not account directly for sensory changes. On the other hand they are susceptible to further oxidation or to decomposition and form secondary reaction products, such as aldehydes, ketones, acids, alcohols, hydrocarbons, lactones, furans and esters, which may be responsible for rancid attributes in olive oil.

There was a progressive increase in the peroxide value of olive oil with regard to the time and conditions of storage and packaging atmosphere. The formation of hydroperoxides followed a second order polynomial curve. The regression coefficients were high (0·87, 0·94, 0·83 and 0·78) for samples both indoors and outdoors, regardless of whether they were filtered (data not shown). The rate of peroxide increase was significantly lower in the samples with nitrogen in the headspace with regression coefficients of 0·50 and 0·60 for the samples kept indoors and outdoors respectively.

These values demonstrate clearly that nitrogen caused a reduction in peroxide values. In fact, Del Nobile *et al.* (2003) showed that in the first stages of oxidation, when oxygen concentration is closed to saturation, the rate at which hydroperoxide is consumed is lower than the rate at which hydroperoxide is produced through the autoxidation process. This leads to increased values. Moreover, the formation of hydroperoxides was more pronounced when the samples were left open to the air (Table 6.21) where more oxygen would be available. It should be born in mind, following the work of Del Nobile *et al.*, (2003) that the final peroxide values will depend on the balance of formation and degradation. Thus, small differences in the final values between samples (Table 6.2) may be due to variation in degradation. Nevertheless, the values of peroxides after 15 months of storage were all less than the limit of 20 meq O_2//kg established by the European Community (Table 6.2).

6.2.3. Change in specific absorption coefficients.

K_{232} and K_{270} are often used to estimate the oxidation stage of olive oil (Kiritsakis, 1998). K_{232}, which is a measure of conjugated dienes was significantly correlated (0.79) with PV and negatively correlated (0·69 and 0·71) with stability and PUFA respectively.

K_{232} increased during 15 months of storage in all cases (Table 6.3). Lower rates of increase were observed when nitrogen was the conditioning gas. K_{232} values increased but remained lower than the limit for extra virgin olive oil in EC regulation except for the filtered samples kept indoors for 15 months of storage. However, the lower values for those oils stored outdoors (higher temperature) suggest that further decomposition of the conjugated dienes may have taken place. K_{270} values, which correspond to conjugated trienes, increased during storage and followed similar trends to the K_{232} values. After 10 months of storage the values of K_{270} reached or exceeded the limit of 0·2 for extra virgin olive oil and only when nitrogen was used as conditioner gas were the values always below the limit. However, the increase of K_{232} and K_{270} were more intense in the samples kept in open bottles outdoors. Within two months of storage in open containers the values of K_{232} were very close to the limit for extra virgin olive oil. On the other hand, K_{270} reached the limit after only two months of storage in open containers (Table 6.21).

Table 6.1. Increase in acidity (% total oil) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outdoors		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	0·23 ± trace ^{cA}	0·20 ± trace ^{dA}	0·23 ± trace ^{bA}	0·23 ± trace ^{cA}	0·20 ± trace ^{eA}	0·23 ± 0·07^{cA}
2	0·24 ± 0·01^{cA}	0·25 ± trace ^{cA}	0·25 ± trace ^{bA}	0·24 ± 0·01^{cA}	0·24 ± 0·01^{dA}	0·25 ± trace^{bcA}
4	0·22 ± trace ^{cA}	0·25 ± trace ^{cA}	0·25 ± trace ^{abA}	0·25 ± trace ^{cA}	0·25 ± trace ^{dA}	0·25 ± trace^{bcA}
8	0·22 ± trace ^{cA}	0·25 ± trace ^{cA}	0·22 ± trace ^{bA}	0·25 ± trace ^{cA}	0·28 ± trace ^{cA}	0·25 ± trace^{bcA}
10	0·27 ± 0·02^{abB}	0·28 ± trace ^{bBA}	0·25 ± trace ^{abB}	0·28 ± trace ^{baBA}	0·31 ± trace ^{bA}	0·27 ± 0·02^{bB}
15	0·29 ± 0·01^{aB}	0·32 ± 0·01^{aAB}	0·28 ± 0·04^{aB}	0·31 ± 0·03^{aAB}	0·34 ± 0·01^{aA}	0·31 ± 0·01^{aAB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$).Trace ≤ 0·005

Table 6.2. Changes in peroxide values (meq of O_2/kg) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outdoors		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	6·44 ± 0·21^{eA}	6·42 ± 0·10^{dA}	6·44 ± 0·21^{dA}	6·44 ± 0·21^{eA}	6·42 ± 0·10^{eA}	6·44 ± 0·21^{dA}
2	8·73 ± 0·09^{dD}	10·54 ± 0·14^{cA}	8·89 ± 0·22^{cC}	9·94 ± 0·56^{dAB}	9·55 ± 0·28^{B}	9·15 ± 0·17^{bC}
4	17·34 ± trace^{bA}	14·42 ± trace^{cC}	11·73 ± 0·01^{aD}	17·03 ± 0·03^{cAB}	16·97 ± 0·05^{B}	9·53 ± 0·32^{bE}
8	16·33 ± 0·02^{cA}	16·32 ± 0·04^{bA}	9·91 ± 0·65^{bcB}	16·80 ± 0·60^{cA}	16·82 ± 0·60^{A}	9·49 ± 0·01^{bB}
10	16·27 ± 0·64^{cB}	15·94 ± 0·04^{bB}	9·60 ± 0·01^{bcD}	18·07 ± 0·04^{bA}	14·41 ± 0·04^{C}	8·58 ± 0·01^{cE}
15	19·15 ± trace^{aA}	17·75 ± 0·53^{aA}	10·73 ± 1·58^{abC}	19·12 ± 0·30^{aA}	15·71 ± 0·51^{B}	11·97 ± 0·03^{aC}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace ≤ 0·005

Table 6.3. Changes in K_{232} of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time	Indoors			Outside		
(Months)	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	1·7± trace ^{fA}	1·72± 0·71^{A}	1·70± trace^{dA}	1·7± trace^{dA}	1·72± trace^{eA}	1·7± trace^{dA}
2	1·85± 0·01^{eA}	1·89± 0·02^{A}	1·84± 0·01^{cA}	1·87± 0·03^{cA}	1·86± 0·02^{dA}	1·77± trace^{cB}
4	2·24± 0·01^{dA}	2·05± 0·01^{B}	1·87± trace^{bcC}	2·15± 0·01^{aAB}	2·10± trace^{cB}	1·75± 0·01^{cC}
8	2·37± trace ^{cA}	2·42± 0·01^{A}	1·88± 0·01^{bcB}	2·16± trace^{aA}	2·25± 0·01^{bA}	1·81± trace^{bB}
10	2·45± 0·02^{bA}	2·40± 0·01^{A}	1·95± 0·01^{aC}	2·23± 0·02^{aB}	2·17± trace^{bB}	1·85± 0·02^{aC}
15	2·80± 0·05^{aA}	2·45± 0·01^{B}	1·90± 0·06^{abE}	2·27± · 0·02^{aC}	2·16± 0·05^{aD}	1·84± 0·01^{aE}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace ≤ 0·005

Table 6.4. Changes in K_{270} of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time	Indoors			Outside		
(Months)	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	0·13± 0·01^{bA}	0·16± trace^{dB}	0·13± 0·01^{cA}	0·13± 0·01^{cA}	0·16± trace^{dB}	0·13± 0·09^{cA}
2	0·16± trace^{bA}	0·17± 0·01^{dA}	0·17± trace ^{aAB}	0·16± 0·03^{cAB}	0·14± 0·01^{eB}	0·14± 0·02^{bcB}
4	0·14± trace^{bB}	0·14± trace^{cB}	0·15± trace ^{bA}	0·15± 0·01^{cA}	0·14± trace^{eB}	0·14± 0·01^{bcB}
8	0·15± trace^{bBC}	0·16± trace^{cB}	0·14± 0·01^{bcC}	0·15± 0·03^{cB}	0·17± trace^{cA}	0·15± 0·01^{bBC}
10	0·17± trace^{bBC}	0·21± trace^{bA}	0·16± 0·01^{aC}	0·21± 0·01^{bA}	0·22± trace^{bA}	0·18± 0·01^{aB}
15	0·34± 0·04^{aA}	0·23± trace^{aC}	0·16± trace^{aD}	0·25± 0·03^{aB}	0·28± trace^{aB}	0·20± trace^{aC}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace ≤ 0·005

6.2.4. Changes in phenolic compounds

Table 6.5 shows the changes in the content of phenolic compounds during storage. It can be observed that the content of phenols decreased (with some inconsistencies) during the first 10 months of storage. Except for one sample (indoor sample under N_2) a significant reduction occurred between 10 and 15 months of storage. For the olive oil stored indoors with nitrogen, in the headspace little change was found during 15 months of storage, indicating great stability of the phenolic compounds. The reduction of total phenols is thought to be due to oxidation and to hydrolytic activity (Cinguanta *et al.,* 2001). It was noticeable that the loss of total phenols was greater in unfiltered oils for samples kept both indoors and outside. In addition, the loss of total phenols was even greater in the open samples kept outside and after only two months storage (Table 6.21).

Chemical analysis carried out by HPLC showed that 3.4 DHPEA and p-HPEA were the main simple phenols of olive oil (Table 6.6 and 6.7). Secoiridoid derivatives like the dialdehydic form of elenolic acid linked to 3.4 DHPEA or p-HPEA (3.4 DHPEA–EDA or p-HPEA- EDA) and an isomer of the oleuropein aglycon (3.4 DHPEA–EA) were also preliminarily identified (Tables 6.9 to 6.12).

Studies on the changes of phenolic compounds following 15 months of storage showed significant changes in all fractions. In particular 3.4 DHPEA and p-HPEA increased in all cases during storage. These results suggested the production of these compounds (due to the hydrolysis) from the more complex phenols like, secoiridoids, such as 3.4 DHPEA–EDA p-HPEA- EDA and 3.4 DHPEA–EA that contain 3.4 DHPEA and p-HPEA in their molecular structure (Montendoro *et al.,* 1992). The rate of hydrolysis was higher in the unfiltered oils and these results are in agreement with those obtained by Cinquanta *et al.,* (1997). According to the data, after two months storage under different conditions hydrolysis was much higher when the samples were stored outside or in open containers (Table 6.21). The percentage of hydrolysis of each oil sample (percentage of 3.4 DHPEA and p-HPEA in total phenols) is reported in Table 6.8. On the basis of these findings we can conclude that a high percentage of hydrolysis is related to aged oil, which is in an advanced oxidation state. On the other hand, fresh olive oils have low contents of 3.4 DHPEA and p-HPEA with respect to the total phenols and the ratio of percentage hydrolysis is near 1. Similar results were obtained by Cinquanta *et al.,* (1997) and Pinelli *et al.,* (2003).

Table 6.5. Changes in total phenol content (mg/Kg) of olive oil during 15 months of storage in tin containers and under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	173·95± 5·30^{aA}	170·81± 1·22^{aA}	173·95± 5·30^{aA}	173·95± 5·30^{aA}	170·81± 1·22^{aA}	173·95± 5·30^{aA}
2	163·33± 1·00^{aA}	149·06± 0·13^{cB}	167·71± 1·44^{aA}	137·54± 4·90^{cC}	133·67± 6·92^{cC}	171·79± 0·58^{aA}
4	145·89± 5·19^{bA}	157·62± 1·01^{abA}	167·92± 10·67^{aA}	142·74± 1·29^{bcA}	158·23± 9·08^{abA}	155·37± 18·60^{abA}
8	142·07± 1·20^{bB}	141·69± 4·11^{cB}	168·37± 1·62^{aA}	150·48± 2·06^{bB}	142·29± 6·07^{cB}	145·05± 4·11^{bcB}
10	140·77± 0·66^{bD}	155·48± 4·04^{bC}	178·00± 3·03^{aA}	166·29± 4·90^{aB}	158·13± 1·73^{bBC}	176·27± 4·33^{aA}
15	115·93± 7·79^{cBC}	105·63± 5·33^{dCD}	168·67± 2·17^{aA}	117·56± 3·25^{dBC}	95·54± 2·31^{dD}	125·85± 9·90^{cB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different (p <0·05). Means within rows followed by the same (upper case) letter are not significantly different (p <0·05).

Table 6.6. Changes in 3,4 DHPEA (mg/kg) of olive oil during 15 months of storage in tin containers and under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	0·35± Trace ^{dA}	0·40± Trace ^{eB}	0·35± tracee^{A}	0·35± trace^{dA}	0·4± trace^{cB}	0·33± 0·02^{cA}
2	0·82± 0·06^{cB}	0·78± 0·03^{dAB}	0·75± trace^{dAB}	0·62± 0·05^{cD}	0·7± 0·05^{bC}	0·65± trace^{bCD}
4	0·79± 0·04^{cAB}	0·79± 0·06^{dAB}	0·93± 0·11^{cA}	0·76± 0·11^{cAB}	0·83± 0·13^{bAB}	0·81± 0·05^{bAB}
8	0·98± 0·03^{bB}	1·18± 0·11^{cB}	1·05± 0·07^{cB}	1·00± 0·20^{bB}	1·58± 0·27^{aA}	1·35± 0·04^{aAB}
10	1·28± 0·33^{bA}	1·53± 0·02^{bA}	1·56± 0·12^{bA}	1·45± 0·01^{aA}	1·93± 0·69^{aA}	1·46± 0·24^{aA}
15	1·93± 0·04^{aA}	2·21± 0·05^{aA}	1·88± 0·23^{aA}	1·51± 0·23^{aB}	0·94± 0·09^{bC}	1·44± 0·02^{aB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different (p <0·05). Means within rows followed by the same (upper case) letter are not significantly different (p <0·05). Trace = <0·005

Table 6.7. Changes in p-HPEA (mg/kg) of olive oil during 15 months of storage in tin containers and under different environmental conditions.

Storage time	Indoors			Outside		
(Months)	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	$1{\cdot}22 \pm \text{trace}^{eA}$	$1{\cdot}31 \pm \text{trace}^{eA}$	$1{\cdot}22 \pm \text{trace}^{cA}$	$1{\cdot}22 \pm \text{trace}^{dA}$	$1{\cdot}31 \pm \text{trace}^{dA}$	$1{\cdot}22 \pm \text{trace}^{cA}$
2	$2{\cdot}27 \pm 0{\cdot}09^{dB}$	$2{\cdot}98 \pm 0{\cdot}35^{dA}$	$2{\cdot}32 \pm \text{trace}^{bB}$	$1{\cdot}66 \pm 0{\cdot}15^{cC}$	$2{\cdot}86 \pm 0{\cdot}28^{cdA}$	$1{\cdot}65 \pm 0{\cdot}02^{bC}$
4	$3{\cdot}29 \pm 0{\cdot}07^{bA}$	$1{\cdot}96 \pm 0{\cdot}10^{dB}$	$2{\cdot}15 \pm 0{\cdot}26^{bB}$	$3{\cdot}07 \pm 0{\cdot}37^{bA}$	$2{\cdot}75 \pm 0{\cdot}62^{cdAB}$	$1{\cdot}73 \pm 0{\cdot}43^{bB}$
8	$2{\cdot}53 \pm 0{\cdot}07^{cC}$	$6{\cdot}19 \pm 0{\cdot}50^{cA}$	$2{\cdot}44 \pm 0{\cdot}39^{bC}$	$2{\cdot}17 \pm 0{\cdot}57^{cC}$	$4{\cdot}39 \pm 0{\cdot}63^{bB}$	$3{\cdot}10 \pm 0{\cdot}15^{aC}$
10	$2{\cdot}96 \pm 0{\cdot}49^{bC}$	$7{\cdot}84 \pm 0{\cdot}06^{bA}$	$3{\cdot}26 \pm 0{\cdot}18^{aC}$	$3{\cdot}00 \pm 0{\cdot}38^{bC}$	$6{\cdot}26 \pm 0{\cdot}66^{aB}$	$3{\cdot}01 \pm 0{\cdot}27^{aC}$
15	$4{\cdot}14 \pm 0{\cdot}42^{aCD}$	$10{\cdot}95 \pm 0{\cdot}36^{aA}$	$3{\cdot}34 \pm 0{\cdot}35^{aE}$	$4{\cdot}51 \pm 0{\cdot}15^{aC}$	$5{\cdot}97 \pm 0{\cdot}25^{aB}$	$3{\cdot}49 \pm 0{\cdot}05^{aDC}$

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0{\cdot}05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0{\cdot}05$). Trace = <0· 005

Table 6.8. Changes in the ratio percentage hydrolysis (3,4 DHPEA + p-HPEA /total phenols) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time	Indoors			Outside		
Months	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	$0{\cdot}90 \pm 0{\cdot}03^{dA}$	$1{\cdot}04 \pm 0{\cdot}01^{dA}$	$0{\cdot}90 \pm 0{\cdot}03^{cA}$	$0{\cdot}90 \pm 0{\cdot}03^{dA}$	$1{\cdot}04 \pm 0{\cdot}01^{dA}$	$0{\cdot}90 \pm 0{\cdot}03^{dA}$
2	$1{\cdot}90 \pm 0{\cdot}11^{cB}$	$2{\cdot}52 \pm 0{\cdot}25^{cA}$	$1{\cdot}83 \pm 0{\cdot}01^{bB}$	$1{\cdot}66 \pm 0{\cdot}09^{cbC}$	$2{\cdot}67 \pm 0{\cdot}31^{cA}$	$1{\cdot}34 \pm \text{trace}^{cC}$
4	$2{\cdot}80 \pm 0{\cdot}12^{bA}$	$1{\cdot}65 \pm 0{\cdot}04^{cdB}$	$1{\cdot}83 \pm 0{\cdot}03^{bB}$	$2{\cdot}69 \pm 0{\cdot}30^{bA}$	$1{\cdot}94 \pm 0{\cdot}59^{cdB}$	$1{\cdot}78 \pm 0{\cdot}30^{cB}$
8	$2{\cdot}48 \pm 0{\cdot}05^{bcB}$	$5{\cdot}19 \pm 0{\cdot}28^{bA}$	$2{\cdot}07 \pm 0{\cdot}25^{bB}$	$2{\cdot}11 \pm 0{\cdot}49^{bB}$	$4{\cdot}22 \pm 0{\cdot}81^{bA}$	$3{\cdot}07 \pm 0{\cdot}04^{bB}$
10	$3{\cdot}01 \pm 0{\cdot}57^{bB}$	$6{\cdot}03 \pm 0{\cdot}13^{bA}$	$2{\cdot}71 \pm 0{\cdot}08^{aB}$	$2{\cdot}69 \pm 0{\cdot}32^{bB}$	$5{\cdot}19 \pm 0{\cdot}91^{bA}$	$2{\cdot}53 \pm 0{\cdot}23^{bB}$
15	$5{\cdot}23 \pm 0{\cdot}04^{aC}$	$12{\cdot}48 \pm 1{\cdot}01^{aA}$	$3{\cdot}10 \pm 0{\cdot}37^{aD}$	$5{\cdot}13 \pm 0{\cdot}30^{aC}$	$7{\cdot}23 \pm 0{\cdot}01^{aB}$	$3{\cdot}93 \pm 0{\cdot}36^{aD}$

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0{\cdot}05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0{\cdot}.05$). Trace = <0·005

Table 6.9. Changes in 3,4 DHPEA-EDA (mg/kg) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	$39{\cdot}94 \pm 0{\cdot}37^{aA}$	$39{\cdot}2 \pm \text{tracea}^{A}$	$39{\cdot}94 \pm 0{\cdot}37^{aA}$	$39{\cdot}94 \pm 0{\cdot}37^{aA}$	$39{\cdot}2 \pm \text{trace}^{aA}$	$39{\cdot}94 \pm 0{\cdot}37^{aA}$
2	$42{\cdot}34 \pm 3{\cdot}68^{aA}$	$32{\cdot}72 \pm 0{\cdot}83^{bB}$	$39{\cdot}10 \pm \text{trace}^{aA}$	$26{\cdot}68 \pm 0{\cdot}45^{bcC}$	$31{\cdot}43 \pm 0{\cdot}19^{cB}$	$38{\cdot}89 \pm 1{\cdot}97^{aA}$
4	$26{\cdot}35 \pm 0{\cdot}97^{bD}$	$34{\cdot}4 \pm 0{\cdot}70^{bCD}$	$42{\cdot}93 \pm 0{\cdot}57^{aA}$	$30{\cdot}39 \pm 3{\cdot}37^{bD}$	$39{\cdot}21 \pm 0{\cdot}30^{aAB}$	$35{\cdot}33 \pm 2{\cdot}21^{bBC}$
8	$29{\cdot}11 \pm 0{\cdot}03^{bC}$	$23{\cdot}78 \pm 2{\cdot}22^{cD}$	$42{\cdot}84 \pm 0{\cdot}23^{aA}$	$31{\cdot}65 \pm 1{\cdot}34^{bC}$	$36{\cdot}08 \pm 0{\cdot}11^{bB}$	$32{\cdot}57 \pm 3{\cdot}15^{bBC}$
10	$29{\cdot}35 \pm 1{\cdot}41^{bC}$	$23{\cdot}40 \pm 0{\cdot}08^{cC}$	$38{\cdot}59 \pm 2{\cdot}44^{aB}$	$29{\cdot}87 \pm 4{\cdot}19^{bC}$	$36{\cdot}21 \pm 0{\cdot}30^{bB}$	$41{\cdot}50 \pm 3{\cdot}79^{aA}$
15	$28{\cdot}85 \pm 1{\cdot}34^{bbc}$	$19{\cdot}55 \pm 0{\cdot}66^{cD}$	$35{\cdot}72 \pm 4{\cdot}23^{aA}$	$25{\cdot}30 \pm 2{\cdot}58^{cCD}$	$19{\cdot}79 \pm 1{\cdot}01^{dD}$	$31{\cdot}86 \pm 2{\cdot}20^{aAB}$

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0{\cdot}05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0{\cdot}05$). Trace = <0·005

Table 6.10. Changes of p-HPEA-EDA (mg/kg) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	$174{\cdot}37 \pm 0{\cdot}37^{bA}$	$174{\cdot}50 \pm \text{trace}^{aA}$	$174{\cdot}37 \pm 0{\cdot}37^{abA}$	$174{\cdot}37 \pm 0{\cdot}37^{aA}$	$174{\cdot}50 \pm \text{trace}^{aA}$	$174{\cdot}37 \pm 0{\cdot}37^{aA}$
2	$187{\cdot}57 \pm 13{\cdot}36^{aA}$	$167{\cdot}76 \pm 4{\cdot}01^{aA}$	$182{\cdot}78 \pm \text{trace}^{aA}$	$170{\cdot}96 \pm 12{\cdot}07^{abA}$	$132{\cdot}77 \pm 1{\cdot}61^{cB}$	$178{\cdot}46 \pm 0{\cdot}64^{aA}$
4	$151{\cdot}53 \pm 5{\cdot}10^{cB}$	$172{\cdot}78 \pm 8{\cdot}56^{aAB}$	$189{\cdot}20 \pm 7{\cdot}65^{aA}$	$161{\cdot}25 \pm 10{\cdot}25^{bB}$	$156{\cdot}27 \pm 12{\cdot}35^{bB}$	$164{\cdot}20 \pm 6{\cdot}00^{aB}$
8	$160{\cdot}62 \pm 3{\cdot}03^{bC}$	$147{\cdot}33 \pm 3{\cdot}29^{bD}$	$192{\cdot}35 \pm 3{\cdot}32^{aA}$	$150{\cdot}65 \pm 3{\cdot}74^{cD}$	$168{\cdot}90 \pm 1{\cdot}40^{aB}$	$173{\cdot}61 \pm 2{\cdot}50^{aB}$
10	$140{\cdot}22 \pm 7{\cdot}38^{cB}$	$146{\cdot}89 \pm 1{\cdot}89^{bB}$	$170{\cdot}96 \pm 7{\cdot}38^{abA}$	$148{\cdot}28 \pm 14{\cdot}96^{cB}$	$174{\cdot}23 \pm 5{\cdot}98^{aA}$	$177{\cdot}23 \pm 6{\cdot}17^{aA}$
15	$153{\cdot}06 \pm 9{\cdot}62^{cA}$	$139{\cdot}64 \pm 2{\cdot}41^{bA}$	$154{\cdot}80 \pm 10{\cdot}66^{bA}$	$152{\cdot}20 \pm 8{\cdot}79^{cA}$	$139{\cdot}7 \pm 0{\cdot}42^{aA}$	$153{\cdot}22 \pm 4{\cdot}88^{bA}$

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0{\cdot}05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0{\cdot}05$). Trace = <0·005

Table 6.11. Changes of p-HPEA derivative (mg/kg) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time	Indoors			Outdoors		
(Months)	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	77·15± 2·05^{aA}	55·37± 0·05^{aA}	77·15± 2·05^{aA}	77·15± 2·05^{aA}	55·37± 0·05^{aA}	77·15± 2·05^{aA}
2	55·00± 3·13^{bA}	58·38± 0·56^{aA}	57·63± 0·08^{aA}	52·53± 1·86^{bA}	38·92± 2·05^{aC}	49·96± 2·47^{cB}
4	48·00± 0·83^{bB}	58·28± 5·05^{aAB}	62·97± 2·51^{bA}	47·92± 2·95^{bB}	53·67± 7·59^{aAB}	55·63± 3·90^{bAB}
8	54·90 ± 1·01^{bB}	44·80 ± 3·69^{bC}	62·55± 3·61^{bA}	57·24± 9·96^{bAB}	70·90 ± 1·27^{aA}	33·30 ± 1·20^{dB}
10	38·45± 8·39^{cC}	41·30 ± 1·50^{bBC}	55·43± 3·54^{cA}	42·91± 5·04^{cBC}	38·10 ± 3·10^{bC}	53·07± 3·98^{bcAB}
15	33·27± 2·43^{cA}	31·89± 2·67^{cAB}	38·51± 4·86^{dA}	31·80 ± 2·20^{dAB}	25·92± 0·11^{cB}	36·64± 1·70^{dA}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

Table 6.12. Changes of 3,4 DHPEA-EA (mg/kg) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time	Indoors			Outdoors		
(Months)	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	148·64± 4·03^{aA}	146·18± 2·35^{aA}	148·64± 4·03^{aA}	148·64± 4·03^{aA}	146·18± 2·35^{aA}	148·64± 4·03^{aA}
2	146·84± 5·32^{aA}	135·29± 5·64^{bB}	143·75± 3·87^{aA}	130·30± 5·00^{bB}	148·01± 3·79^{aA}	137·14± 5·60^{bB}
4	147·74± 4·22^{aAB}	130·82± 1·16^{bC}	149·3± 0·99^{aAB}	141·03± 7·11^{aB}	151·27± 1·79^{aA}	143·51± 0·71^{aB}
8	137·44± 9·81^{bB}	120·46± 0·64^{cB}	150·72± 0·40^{aAB}	128·55± 2·76^{bB}	150·97± 9·43^{aAB}	156·56± 0·66^{aA}
10	130·26± 8·94^{bBC}	124·49± 2·93^{cC}	154·2± 4·21^{aA}	130·39± 8·99^{bBC}	155·6± 5·85^{aA}	149·25± 3·07^{aAB}
15	116·59± 1·48^{cAB}	113·35± 3·15^{dB}	127·84± 12·41^{bA}	128·16± 4·04^{bA}	109·65± 1·21^{bB}	118·14± 3·36^{cAB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

6.2.5. Changes in stability

Oxidative stability measured as induction time by Rancimat test is shown in Table 6.13. During the 15 months of storage the stability of the oil decreased significantly in the samples with air as conditioner gas. The reduction was more intense about 50% in the unfiltered samples kept outside compare to the samples kept inside where the reduction was very similar 24 % and 23 % for filtered and unfiltered samples, respectively. In a similar fashion to the small changes in phenolic substances, when nitrogen was used as a conditioner gas during storage, the oxidative stability did not decrease. Similar results where obtained by Di Giovacchino *et al.,* (2002) who attributed the increase of resistance to oxidation to the formation of polymeric TAGs that could increase the resistance to oxidation of oil during the forced favourable oxidation conditions that occur in the Rancimat apparatus.

During the storage the change in stability was negative correlated 3,4 DHPEA or p-HPEA, and with the ratio of C18:1/C 18:2 and a positive correlation with total phenols or with PUFAs in the filtered and unfiltered samples with air in the headspace. By contrast, in the samples with nitrogen in the headspace, very low correlation existed between the above mention parameters because they did not show any significant changes. Furthermore, in the samples kept in open containers for a two-month period the decrease in stability was 83 %, which was similar to the reduction in total phenols, which was 78 % (Table 6.21). These data showed that oxidative stability was very dependent on exposure of the oil to oxygen.

6.2.6. Changes in Chlorophylls

After 15 months of storage, all the olive oil samples appeared to show losses of chlorophyll content. The decrease was 2 to 8 % after 4 months and 8 to 18 % after 15 months of storage. It is noteworthy that the degradation rates were lower in the unfiltered samples kept indoors (Table 6.14). A higher percentage decrease appeared to occur in filtered samples. Chlorophyll and pheophytin pigments are responsible for the characteristic color of olive oil. In the presence of light, chlorophylls and their derivatives are the most active promoters of photosensitised oxidation in virgin olive oil (Kiritsakis and Dugan 1985. Rahmani and Saari Csallany, 1998). However, in

samples of olive oil kept in the dark chlorophylls exert an antioxidant activity (Gutiérrez *et al.,* 1992; Psomiadou and Tsimidou, 2001; Psomiadou and Tsimidou, 2002)

6.2.7. Changes in α-tocopherol

α-Tocopherol is an important antioxidant and its content decreased throughout storage time in most cases (Table 6.15). However, α -tocopherol loss in samples kept under different conditions did not exceed 16% and tended to be higher in samples kept outside. Moreover, much higher losses were observed in the content of α- tocopherol in the open samples because of the greater exposure to oxygen (Table 6.21). These results are in agreement with those obtained by Psomiadou and Tsimidou (2002). The reduction of α-tocopherol followed similar trends to total phenols indicating that α-tocopherol is equally susceptible to autoxidation as polar phenols are. This is relevant to their roles as anti-oxidants. Indeed by applying multivariate techniques it was found that the oil stability depends on both tocopherol and polar phenolic compound content (Blekas *et al.,* 1995).

Polar phenolic compounds are thought to be more important for the inhibition of autoxidation in the initial stages, while α-tocopherol is more effective when the activity of the polar phenolic compounds is reduced and primary products of autooxidation (peroxides) reach numbers close to the limit of 20 meq O_2 /kg set by the European Union (EC.1991). However, it should be born in mind that a slight prooxidant activity of α- tocopherol at early stages of autooxidation, depending on its concentration, has been reported (Blekas *et al.,* 1995; Blekas and Boskou, 1998).

Table 6.13. Changes of stability (h) of olive oil during 15 months of storage in tin containers under different environmental conditions with air or nitrogen in the headspace.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	8·00± 0·20^{aA}	8·27± 0·15^{aA}	8·00± 0·20^{aA}	8·00± 0·20^{bA}	8·27± 0·15^{aA}	8·00± 0·20^{bcA}
2	8·69± 0·20^{aAB}	7·80± 0·25^{bC}	9·02± 0·33^{aA}	8·56± 0·08^{aAB}	8·14± 0·48^{aBC}	9·01± 0·16^{aA}
4	7·40± 0·32^{bB}	7·97± 0·21^{abB}	9·00± 0·60^{aA}	7·17± 0·09^{cB}	8·57± 0·11^{aB}	9·23± 0·36^{aA}
8	6·85± 0·11^{bB}	6·49± 0·23^{cB}	8·65± 0·10^{aA}	7·01± 0·13^{cB}	6·87± 0·42^{bB}	8·63± 0·15^{abA}
10	6·24± 0·33^{cBC}	6·23± 0·01^{cBC}	8·4± 0·81^{aA}	7·08± 0·36^{cB}	5·78± 0·11^{bC}	8·52± 0·14^{abA}
15	6·09± 0·27^{cC}	6·39± 0·12^{cC}	8·31± 0·69^{aA}	6·87± 0·28^{cBC}	4·12± 1·04^{cD}	8·01± 0·23^{cAB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

Table 6·14. Changes of Chlorophyll (mg/Kg) of olive oil during 15 months of storage in tin containers under different environmental conditions with air or nitrogen in the headspace.

Storage time	Indoors			Outside		
Months	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	10·2	10·1	10·2	10·2	10·1	10·2
4	9·9	9·9	9·9	9·7	9·8	9·7
8	10·0	9·6	9·4	9·5	9·3	9·0
10	8·8	9·6	9·2	8·5	9·1	9·1
15	9·0	9·4	9·1	8·3	8·8	9·1

Values are means of two determinations.

Table 6·15. Changes of α- tocopherol content of olive oil during 15 months of storage in tin Containers and at different environmental conditions with air or nitrogen in the headspace

Storage time	Indoors			Outside		
Months	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	313	313	313	313	313	313
4	285	n.m.	311	283	278	n.m.
8	n.m.	n.m.	331	265	n.m.	n.m.
10	316	332	n.m.	274	282	281
15	280	284	342	264	285	274

Values are means of two determinations. n.m., not measured

6.2.8. Changes in fatty acid composition

Tables (6.18 to 6.20) present the changes in the composition of fatty acids during storage. As expected, saturated fatty acids showed little change in percentage during storage (data not shown). In contrast, the percentage of PUFAs was altered. It is known that the content of PUFAs affects the stability of the oil since the conjugated diene or triene bonds are particularly sensitive to thermal- and photo-oxidation. Consequently, the total content of polyunsaturated fatty acids gives an idea of the potential oxidative stability. This would include the content of both linoleic (C18: 2) and α-linolenic (C18:3) acids (Tables 6.17, 6.18). In the experiments, these fatty acids (C18: 2 and C18:3) remained almost constant the first two months of storage and decreased thereafter. The decreases were usually statistically significant by 4 months. The decrease was more pronounced in the unfiltered oil with decreases 7·1 % and 6·6 % following storage indoors or outdoors, respectively. Smaller decreases were found for samples stored under nitrogen, where one would expect oxidation to be lower (Table 6.20).

In contrast to the decrease in PUFAs, such as linoleate (Table 6.17), the proportion of the main fatty acid component, oleate, did not show a noteworthy change (Table 6.16). This meant that the ratio of oleate /linoleate showed significant increases on storage (Table 6.19).

The fatty acid composition and, noticeably the oleic acid/linoleic acid ratio, is thought to affect the taste of virgin olive oil (Boskou, 1996). Moreover, any increase in conjugated dienes or trienes is proportional to the oxidation extent of PUFAs. In turn, this may lower the perceived quality of the oils by affecting the taste.

Table 6.16. Changes in the % oleate in olive oils during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	75·09±0·01^{cA}	75·16± 0·07^{bcA}	75·09± 0·01^{bA}	75·09± 0·01^{cA}	75·16± 0·07^{bA}	75·08± trace^{cdA}
2	75·08±0·17^{cA}	75·06± 0·19^{cA}	75·04± 0·05^{bA}	75·07± 0·01^{cA}	75·17± 0·02^{bA}	75·00± 0·07^{dA}
4	75·28±0·07^{bA}	75·14± 0·36^{bcA}	75·24± 0·06^{aA}	75·24± 0·07^{bA}	75·36± 0·03^{aA}	75·24± 0·02^{cA}
8	75·57±0·03^{abAB}	75·63± 0·14^{aA}	75·54± 0·01^{aAB}	75·54± 0·01^{aAB}	75·43± 0·02^{aB}	75·48± 0·10^{abAB}
10	75·59±0·20^{aA}	75·58± trace^{abA}	75·47± 0·05^{aA}	75·63± 0·10^{aA}	75·42± 0·03^{aA}	75·50± 0·11^{aA}
15	75·27±0·12^{bAB}	75·31± 0·04^{abcAB}	75·04± 0·32^{bB}	75·22± 0·07^{bB}	75·11± 0·12^{bB}	75·66± 0·04^{aA}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

Table 6.17. Changes in the % of linoleate in olive oils during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	5·84± 0·02^{aA}	5·86± 0·02^{aA}	5·84± 0·02^{aA}	5·84± 0·02^{aA}	5·86± 0·02^{aA}	5·85± trace^{aA}
2	5·84± 0·04^{aA}	5·83± 0·05^{aA}	5·84± 0·02^{aA}	5·83± 0·01^{aA}	5·80± 0·01^{bA}	5·86± 0·02^{aA}
4	5·72± trace^{bA}	5·72± 0·02^{bA}	5·74± 0·01^{bA}	5·73± 0·02^{bA}	5·74± 0·02^{bA}	5·75± trace^{bA}
8	5·69± trace^{bcB}	5·71± 0·01^{bAB}	5·75± trace^{bA}	5·74± 0·02^{bA}	5·72± 0·01^{bA}	5·74± 0·02^{bA}
10	5·63± 0·03^{cC}	5·61± 0·02^{cC}	5·71± 0·01^{bA}	5·65± trace^{cB}	5·64± 0·01^{cC}	5·68± 0·01^{cAB}
15	5·55± trace^{dAB}	5·51± 0·01^{dB}	5·65± 0·08^{cA}	5·47± 0·02^{dB}	5·42± 0·01^{dB}	5·51± trace^{dB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0.05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

Table 6.18. Changes in the % of α-linolenate in olive oils during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	0·91±trace^{aA}	0·91± trace^{aA}	0·91± trace^{aA}	0·91± trace^{aA}	0·91± trace^{aA}	0·91± trace^{aA}
2	0·90±0·01^{aA}	0·9± 0·01^{aA}	0·91± trace^{aA}	0·9± 0·01^{aA}	0·90± trace^{bA}	0·90± trace^{aA}
4	0·89±0·02^{abA}	0·91± 0·02^{aA}	0·89± trace^{aA}	0·89± 0·01^{bA}	0·90± 0·01^{abA}	0·91± 0·01^{aA}
8	0·86±trace^{bB}	0·88± 0·01^{abAB}	0·89± 0·01^{aA}	0·88± 0·01^{bAB}	0·90± 0·01^{abA}	0·90± trace^{aA}
10	0·85±0·02^{cAB}	0·84± trace^{cB}	0·88± 0·01^{bA}	0·86± trace^{cAB}	0·85± 0·01^{cAB}	0·87± 0·01^{aA}
15	0·81±trace^{cB}	0·78± 0·01^{cB}	0·85± trace^{bAB}	0·82± 0·02^{cAB}	0·80± 0·01^{dAB}	0·83± 0·03^{aA}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

Table 6.19. Changes in the ratio of oleate/linoleate in olive oils during 15 months of storage under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	12·86± 0·04^{cA}	12·84± 0·04^{dA}	12·86± 0·04^{cA}	12·86± 0·04^{dA}	12·84± 0·04^{dA}	12·83± trace^{dA}
2	12·86± 0·12^{cA}	12·89± 0·15^{dA}	12·84± 0·06^{cA}	12·87± 0·01^{dA}	12·97± 0·01^{cdA}	12·79± 0·04^{dA}
4	13·16± 0·01^{bA}	13·15± 0·01^{cAB}	13·10± 0·02^{bAB}	13·15± 0·04^{cAB}	13·14± 0·04^{bAB}	13·09± 0·01^{cB}
8	13·30± 0·02^{abA}	13·26± 0·01^{cAB}	13·14± trace^{bC}	13·16± 0·03^{cC}	13·19± 0·04^{bBC}	13·14± 0·06^{bcC}
10	13·43± 0·11^{aAB}	13·49± 0·05^{bA}	13·22± 0·03^{bC}	13·37± trace^{bAB}	13·39± 0·01^{bAB}	13·31± 0·04^{aBC}
15	13·61± 0·04^{aA}	13·73± 0·03^{aA}	13·32± 0·23^{aB}	13·57± 0·01^{aAB}	13·68± 0·01^{aA}	13·48± trace^{aAB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·005$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

Table 6.20. Changes of PUFA (%) of olive oil during 15 months of storage in tin containers under different environmental conditions.

Storage time (Months)	Indoors			Outside		
	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + $N2_2$
0	6·76± 0·02^{aA}	6·77± 0·02^{aA}	6·76± 0·02^{aA}	6·76± 0·02^{aA}	6·77± 0·02^{aA}	6·76± 0·64^{aA}
2	6·75± 0·05^{aA}	6·73± 0·06^{aA}	6·75± 0·03^{aA}	6·74± 0·01^{aA}	6·70± 0·01^{bA}	6·77± 0·02^{aA}
4	6·61± 0·01^{bA}	6·63± 0·01^{bA}	6·64± 0·01^{bAB}	6·61± 0·03^{bA}	6·64± 0·01cA	6·66± 0·01^{bA}
8	6·55± 0·01^{bcC}	6·59± 0·01^{bcB}	6·64± 0·01^{bA}	6·62± 0·01^{bA}	6·62± trace^{cA}	6·64± 0·01^{bA}
10	6·49± 0·05^{cBC}	6·44± 0·03^{dC}	6·59± 0·02^{bA}	6·52± 0·01^{cB}	6·49± 0·01^{eBC}	6·55± 0·01^{aAB}
15	6·36± 0·01^{cC}	6·29± 0·01^{dC}	6·50± 0·08^{bA}	6·39± 0·01^{bAB}	6·32± 0·03^{dBC}	6·44± 0·03^{aAB}

Means ± standard deviations (n=3) are shown. Means within columns followed by the same (lower case) letter are not significantly different ($p < 0·05$). Means within rows followed by the same (upper case) letter are not significantly different ($p < 0·05$). Trace = <0·005

Table 6·21. Physicochemical characteristics of olive oil samples after storage for 2 months outside in tin containers under different environmental conditions.

	Filtered	Unfiltered	Filtered + N_2	Filtered open*
K_{232}	1·87±0·03^{b}	1·86±0·02^{b}	1·77±trace ^{b}	2·40±0·05^{a}
K_{270}	0·16±0·03^{ab}	0·14±0·01^{b}	0·15±trace^{ab}	0·19±0·01^{a}
Total Phenols (mg/kg)	137·54±4·9^{b}	133·67±6·92^{b}	171·79±0·58^{a}	38·28±1·47^{c}
Acidity (% Oleic acid)	0·24±0·01^{a}	0·24±0·01^{a}	0·25±trace ^{a}	n.m
Peroxide values (meq O2/kg)	9·94±0·56^{b}	9·55±0·28^{b}	9·15±0·17^{b}	136·26±18·18^{a}
Chlorophyll (mg/kg)	9·69±trace	9·84±trace	9·75±trace	n.m
Stability (hr)	8·56±0·08^{ab}	8·14±0·48^{b}	9·01±0·16^{a}	1·40±0·04^{c}
α- tocopherol (mg/kg)	315·40 1·52^{a}	312·85 2·5^{a}	320·32 3·40^{a}	22·79±1·41^{b}
3,4 DHPEA (mg/kg)	0·62±0·05^{b}	0·70±0·05^{b}	0·65±trace ^{b}	2·51±0·05^{a}
p-HPEA (mg/kg)	1·66±0·15^{c}	2·86±0·28^{b}	1·65±0·02^{c}	8·09±0·23^{a}
3,4 DHPEA- EDA (mg/kg)	26·68±0·45^{b}	31·43±0·19^{b}	38·89±1·97^{a}	12·31±1·95^{c}
p-HPEA-EDA (mg/kg)	139·25±9·50^{b}	132·77±1·61^{b}	167·78±14·93^{a}	53·15±3·61^{c}
p-HPEA deriv (mg/kg)	40·83±1·86^{b}	38·92±2·05^{b}	49·96±2·47^{a}	9·90±0·57^{c}
3,4 DHPEA- EA (mg/kg)	120·30±2·5 ^{a}	120·01±3·79^{a}	127·14±22·60^{a}	24·07±5·78^{c}

Means ± standard deviations (n=3) are shown. Means within rows followed by the same letter are not significantly different according to Duncan's range test at p = 0·05 values are shown for significance. *Loosely sealed. Qualitative characteristics were measured as described in sections2.2.5, 2.2.6, 2.2.7, 2.2.14, 2.2.15, 2.2.16, 2.2.17, and 2.2.18. Phenolics are abbreviated as shown in the list of abbreviations.

Table 6·22. Changes of organoleptic assessment (score) of olive oils during 15 months of storage in tin containers under different environmental conditions.

Storage time	**Indoors**			**Outside**		
Months	Filtered	Unfiltered	Filtered + N_2	Filtered	Unfiltered	Filtered + N_2
0	7·5	7·5	7·5	7·5	7·5	7·5
4	7·0	6· 8	7·5	6·5	6·0	6· 5
8	6·8	6·5	7·2	5·2	5·0	5·5
15	6·0	5·5	7·0	4·0	4·2	5·1

See section 2.2.8 for overall scores.

6.2.9. Changes in volatile compounds

As expected, during storage, there were obvious reductions in olive oil quality as perceived by the Panel Test (Table 6.22). For samples indoors, filtration seemed to preserve quality better and there was an obvious benefit to having N_2 in the headspace to reduce oxidation. The samples outside tended to deteriorate more rapidly and N_2, although beneficial, was less effective.

In order to correlate these changes in perceived quality (Table 6.22) we analyzed the volatiles, which were produced, and which are thought by different workers to be responsible for the different sensory perceptions (good or bad) in olive oils.

Table 6.23 shows the changes of the volatile components after olive oils were stored under different conditions for 2 or 15 months. As it is shown in the Table, after two months of storage inside, most of the 6C volatile compounds, which are common contributors of the green aroma of olive oil, were present. 6C volatile compounds are enzymatically produced from polyunsaturated fatty acids containing a cis-cis 1,4 pentadiene structure through the so-called lipoxygenase (LOX) pathway (Hatanaka 1993; Vick & Zimmerman 1987; Morales and Aparicio 1999; Angerosa *et al.*, 2001).

During 15 months of storage the volatiles hexanal, hexan-1-ol and hexyl acetate increased. These volatile compounds originate from linoleic acid through the lipoxygenase pathway and are responsible for the green sweet aspects of the global green flavour (Morales and Aparicio, 1999). The increase of hexanal was more pronounced in the samples kept outside for 15 months. A similar tendency was followed by hexyl acetate while hexan-1-ol increased only in the samples kept inside for 15 months. Hexanal is one of the most important contributors to the olive oil flavour because of its low odor threshold (0·32 mg/kg) (Grosh, 1993).

These results fit with the expected biochemistry since there was a reduction of about 5·65 % in the linoleic acid percentage in the samples kept outside for 15 months. The low value of hexanal in the sample kept in an open container may be due to further decomposition of hexanal at advanced oxidation stages and at elevated temperature. Moreover, volatile compounds will, of course, evaporate from an open container. *cis*-3 Hexenyl acetate was detected in the oil samples only after two months of storage inside. *cis* 3 Hexen-1-ol increased during oxidation, while, on the other hand, cis-3-hexenal was not detected after 15 months of storage. These three

compounds are responsible for the main green perception. They come from linolenic acid metabolized in the LOX pathway.

Other compounds formed further down in the LOX pathway and which are considered as the bitter astringent aspects of the green sensory perception are as follows. In the indoor sample, the concentration of *trans*-2-hexenal and 6C alcohols such as *tran*-3-hexen-1-ol decreased during storage. For *trans*-2-hexenal the decrease was more intense in the samples kept outside. *trans*-2-Hexen-1-ol decreased during storage and after 15 months of storage (either in indoor or outdoor) it was not detected.

Our results are in agreement with those of Di Giovacchino *et al.,* (2002) who found that the content of hexanal increased during oxidation. However, contrary results were obtained concerning *trans*-2- hexenal. In the samples *trans*-2-hexenal decreased during storage contrary to what they found in the samples kept at 40°C where *trans*-2- hexenal and total volatiles first increased during the first 5 or 10 months of storage and only after did the content of these compounds diminish. Part of the differences in the results may, therefore, be due to the time points assessed.

The ratio of *trans*-2-hexenal / hexanal decreased during storage of olive oil and, it should be noted that the value of this ratio is often taken to indicate the quality of the oil. (Angerosa *et al.,* 1992) 1-Pentene-3-ol increased during storage and was somewhat more concentrated in the samples kept outside after 15 months of storage. This compound is produced by β- scission of the n-6 (S)- hydroperoxy linolenic acid formed by lipoxygenase activity. *Iso*-pentane increased during oxidation in closed tin containers but its concentration in an open container was very low which means that this compound decomposed or evaporated to the atmosphere due to the higher temperature. The similar concentrations after 2 and 15 months in containers outside tend to agree with the former explanation. 3-Pentanone increased during oxidation to a greater extent in the sample kept outside. In contrast, isoamyl alcohol, that is correlated with fustiness (Angerosa *et al.*, 1990) remained at the same levels.

n-Hexane, acetaldehyde and octane, were all markedly increased in the open containers implying that they were formed by oxidation. These results are in agreement with previous research work by Snyder *et al.,* (1985). In addition, Barro Perez-Cerezal *et al.,* (1981) found an inverse relationship between quality of olive oil and octane concentration.

In other experiments, Morales *et al.,* (1997) applied a thermo oxidation process to extra virgin olive oil in order to study the evolution of volatile compounds responsible for off-flavors of olive oil. According to them, hexanal, which is usually connected with oxidation in refined vegetable oils, cannot be used as an adequate marker in oxidation of olive oils because it is present in the initial olive oil flavor. As a result, they proposed to monitor the oxidation stages of virgin olive oil through the concentration of nonanal. Nonanal is absent or only present in traces in fresh virgin olive oil and the ratio of hexanal/nonanal was found to be an appropriate way to be used to distinguish oxidized from good –quality olive oil. In our experiments nonanal was never detected (Table 6.23).

A direct relationship exists between rancidity and *trans*–2 pentenal, hexanal and *trans*-2-heptenal (Solinas and Angerosa, 1988; Solinas *et al.,* 1987a). These authors suggested using trans-2 heptenal as marker of oxidation rather than *trans*-2-pentenal or hexanal since these two compounds are present in virgin olive oil. Almost all these volatiles are responsible for olive oil off-flavors because their threshold levels for odour detection by humans (mg/kg) are very low. In our analysis (Table 6.23) only hexanal was formed in significant quantities and, as expected its concentration increased during storage in parallel with the decrease in quality (Table 6.22).

Most of the methods carried out to measure the evolution of volatile compounds produced during olive oil oxidation utilized accelerated thermo-oxidation techniques. It is widely known that at high temperatures, the rate of reaction is exponentially related to temperature. During the accelerated oxidation process the initial flavor of olive (mainly produced by compounds deriving from the LOX pathway and responsible for pleasant flavours) disappears and the oxidation process starts producing large amounts of other volatile compounds, some of which are present in the initial flavor (Morales *et al.,* 1997). Consequently, there are doubts whether it is justified to correlate high temperature oxidation experiments with the lower temperature shelf-life conditions of storage.

Table 6.23. Changes of volatile compounds of olive oil after 2 and 15 months of storage in tin containers under different environmental conditions.

	Peak-area ratio (x 100)*				
	Indoors		**Outside**		
	2 months	15 months	2 months	15 months	2- months open
Iso- pentane	0·95 ± 0·04b	5·19 ± 1·04a	5·42± 0·91a	5·11± 0·86a	0·53± 0·04b
n-Hexane	5·16 ± 0·49	n.d.	23·75± 2·65	4·04± 1·29	249·37± 6·84
Octane	n.d.	1·30 ± 0·31	n·d	2·74± 0·56	4·51± 2·20
Iso- amyl alcohol	5·25 ± 0·90a	4·64 ± 0·47a	4·58 ± 0·85a	4·45± 0·17a	5·13± 0·71a
Hexan-1-ol	1·22 ± 0·19b	7·34 ± 0·40a	1·54 ± 0·36b	0·54± 0·13c	1·24± 0·18b
trans-3-Hexen-1-ol	6·05 ± 0·86bc	3·64 ± 2·31c	9·07 ± 1·36a	6·77± 0·97ab	5·35± 0·60bc
cis-3-Hexen-1-ol	1·34 ± 0·26b	2·67 ± 0·90ab	2·57 ± 1·36ab	3·49± 0·31a	3·20± 0·57a
trans-2-Hexen-1-ol	2·56 ± 0·21	n.d.	1·67 ± 0·11	n.d.	1·88± 0·99
2-octanol	2·36 ± 0·59	n.d	2·07 ± 0·13	0·57± 0·04	1·57± 0·29
Acetaldehyde	0·99 ± 0·11b	1·39 ± 0·53b	2·22 ± 0·53b	1·27± 0·16b	24·13± 2·73a
Hexanal	9·75 ± 0·55c	12·53 ± 0·42b	10·50 ± 0·35c	22·82± 0·43a	12·20± 0·30b
3-Hexenal	8·65 ± 1·01	n·d	10·04 ± 1·25	n·d	0·90± 0·01
3-Methyl butanal	0·89 ± 0·20c	0·87 ± 0·21c	1·07 ± 0·21b	1·80± 0·49a	1·05± 0·15b
trans -2- Hexenal	44·48 ± 14·01a	34·98 ± 4·96a	42·47 ± 4·30a	42·61± 0·53a	15·69± 0·32b
Ethyl-acetate	2·08 ± 0·27b	1·51 ± 0·07c	1·61 ± 0·13c	1·55± 0·07c	4·19± 0·30a
Ethyl propanoate	4·57 ± 0·56c	4·36 ± 0·79c	4·73 ± 0·23c	14·74± 3·54a	7·19± 0·65b
Butyl acetate	5·34 ± 0·55b	7·30 ± 2·32bb	5·91 ± 0·75b	22·35± 0·50a	11·57± 1·28b
Hexyl acetate	2·04 ± 0·13a	2·23 ± 0·89aa	2·33 ± 0·59a	3·14± 2·54a	2·81± 0·49a
cis-3-hexenyl acetate	4·33 ± 0·72	n.d.	n.d.	n.d.	n.d.
Methyl nonanoate		0·19 ± 0·01	n.d.	0·8± 0·09	n.d.
3-Pentanone	2·49 ± 0·26b	2·76 ± 0·26ab	4·49± 1·57a	5·88± 0·39a	4·61± 0·92a

Means ± standard deviations (n=3) are shown· Means within columns followed by the same letter are not significantly different (p <0·05). Means within rows followed by the same letter are not significantly different (p <0·05). Volatile characteristics were quantified as described in section 2.2.19. * Ratio of the compound peak area to the internal standard peak area, multiplied by 100.

6.2.10. Conclusions

The experimental results reported in this chapter revealed that oxidation is an inevitable process but the rate of which is a function of both the time and the various conditions of storage. The quality indices, peroxide value and the specific extinction coefficients K_{232} and K_{270} and acidity always increased during oxidation. When the oil was kept for 15 months indoor and with nitrogen in the headspace the qualitative parameters acidity, peroxide values (PV), K_{232} and K_{270} were all within the official limit for extra virgin olive oil set by the European Community. This showed the advantage of using N_2 to slow oxidation.

There was a reduction in total and in the complex phenolic compounds during storage. The degree of reduction varied according to the various conditions of storage. However, content of 3,4 DHPEA and p-HPEA, which are the main phenolic alcohols in virgin olive oil, increased in all cases during storage, probably as more complex derivatives, such as 3,4 DHPEA-EDA and 3,4 DHPEA-EA, were hydrolysed. Thus, the percentage of hydrolysis of each oil sample (percentage of 3,4 DHPEA and p-HPEA compared to total phenols) was increased during storage. The % hydrolysis as well as the content of 3,4 DHPEA and p-HPEA can be used as an index for the aging olive oil since a high percentage of hydrolysis is related to oil in advanced oxidized conditions. The α-tocopherol content decreased throughout the storage time in all cases.

Again, these changes would be expected under oxidizing conditions. This means that, after a time in storage, olive oil will be more prone to oxidation simply because of the reduction in anti-oxidants such as α- tocopherol or effective phenolics.

During oil storage, the original volatile composition, mainly formed by compounds deriving from the LOX pathway and responsible for pleasant properties, changes. The concentrations of 6C aldehydes, especially *trans*-2-hexenal, and 6C alcohols produced from linolenic acid, which contribute to the green flavor of virgin olive oil and esters contributing to fruity flavour, were reduced. Instead, these were replaced by less desirable volatiles.

Overall, the results showed that virgin olive oil keeps its qualitative characteristics under the category of extra virgin olive oil for 15 months if it is stored in tin containers indoors at room temperature and with nitrogen in the headspace. Furthermore, we can also conclude that the presence of water in the unfiltered samples made some difference to the degree of deterioration with respect to certain

quality parameters. Such olive oils tend to go out of the extra virgin olive oil category within 8-10 months of storage. Furthermore, the quality deteriorates very fast (in less than a two month period) if the oil is kept outside and in loosely capped containers.

From the data, it can be concluded that a suitable way to inhibit the deterioration process in olive oil is for the oil to be stored in the dark at room temperature with an inert gas like nitrogen in the headspace. However, it should also be born in mind from this data, (as well as those from others) that is very difficult to exclude oxygen so that oxidation may still proceed at slow rates. In fact, other reactions may also occur which produce deterioration and which do not require oxygen. Nevertheless, storing oils in tin cans under the above conditions should, undoubtedly, reduce deterioration to acceptable levels

6.3 Summary

Olive oil generally has a relative long life due to its fatty acid content and antioxidant compounds. From the experiments described in this chapter, it can be concluded that the oxidative stability of olive oil is affected by the:

- Time of storage
- Temperature
- Amount of oxygen in the head space
- Filtration

According to the data, the best way to reduce olive oil oxidation is to store filtered oil in the dark at room temperature with an inert gas like nitrogen in the headspace.

Chapter 7. General Discussion

7.1 Introduction

Olive oil at the present time represents only a small share (about 3%) of the world's vegetable oil market. However, recently its status has been increasing due to the increasing popularity of the Mediterranean diet and its production and, particularly, its consumption started to spread in new countries and continents such as the United States, Canada, Australia, South America and Japan.

Due to the long procedure and high cost of production, olive oil is expensive in comparison to other vegetable oils. For this reason, it is important to guarantee its quality and authenticity in order to protect it from adulteration. Most of the quality and authenticity characteristics are described in detail in the International Olive Oil Council, in the *Codex Alimentarius* standards and the European Union Regulation 2568/91 and its amendments. These standards have established limits to detect adulteration and to protect the genuineness and quality of olive oil. The limits of certain chemical components and quality factors in virgin olive oils show quite wide range, since they take account of the oil's characteristics from all producing countries. This means that the adulteration can be carried out undetected as long as the limits prescribed are not exceeded.

An essential aspect to obtain best quality oil and fulfill the expectation of consumers for this expensive product (and at the same time protect it from adulteration) is to understand the contribution of each factor that may have an impact on quality throughout the production chain. Although a lot of research work has been carried recently there are several topics in which existing scientific knowledge cannot be considered adequate. Moreover, due to the diversity of the environmental conditions under which olives are grown and the existing large genetic variability, consideration of these factors needs to be increased. In the present thesis work important parameters that might affect quality (cultivar / agricultural methods / weather /processing /storing) have been examined in order to evaluate their possible effects on standard quality tests and consumer appreciation.

The analytical methods used for the evaluation of the unique olive oil composition, quality and authenticity, which are affected during the production process, are presented in chapter 2. Official and more detailed analytical techniques were used in order to isolate and identify important natural components of olive oil. In fact, the following determinations were carried out: Non-esterified fatty acid, peroxide value and specific extinction coefficients (K_{232}, K_{270}) were determined according to the official methods of European Community Regulation 2568/91; more detailed analysis of fatty acids, sterols and aliphatic alcohols was carried out using GLC. Triacylglycerol molecular species, phenolic compounds and tocopherol determinations were carried out using specially developed HPLC techniques. In addition, oxidative stability was determined using the Rancimat apparatus (Methrohm). Sensory quality (Panel Test) was determined according to EC Regulation 2568/91. However, the number of descriptors of the official profile sheet was increased to allow a more careful description of "green" perceptions. Furthermore, sample handling and problems that can arise while performing each analysis were highlighted at different parts of the thesis.

Olive fruits (*Olea europaea L.*) of two Greek cultivars (Koroneiki, Mastoidis) and one important Italian variety (Coratina) were used for the experiments. However, most of the experiments were done on cv Koroneiki which is the main oil-producing cultivar in Crete.

7.2. Effect of cultivar

As it mentioned previously, the two cultivars Koroneiki and Coratina were chosen because they represent major cultivars used for Greek and Italian olive oils, respectively. These two cultivars were grown in different geographic origins but they were harvested at the same maturation stage (semi-black olives). The data show that there are significant differences in the composition of fatty acids, sterols, aliphatic and triterpene alcohols and phenolic compounds. The differences in chemical characteristics among cultivars may be due to genetic and environmental factors (Montedoro and Garofolo, 1984; Montedoro, 1989; Fontanazza, 1993; Inglese, 1994; Angerosa et al., 1999). However, because the crops were grown under very similar agricultural conditions, the differences are most likely due to cultivar. Furthermore, the results of

sensory profile showed that oils of Koroneiki cultivar are characterized by high intensities of green leaf or twig and green olives attributes. In contrast, oils of Coratina cultivars are characterized by high intensity of the bitter almond and pungent attributes. The differences in sensory attributes between the two oils are probably due to different contents of volatiles and phenolic compounds. In fact, previous work has shown that the phenolic compounds mainly give a bitter and pungent taste to the oil (Mondedoro *et al.*, 1978; Gutiérrez *et al.*, 1992; Morales, M. T. and Tsimidou, M., 2000; Visioli *et al.*, 2002). In addition, the volatile composition of olive oil depends on cultivar the levels and activity of the enzymes involved in the various pathways (Mondedoro *et al.*, 1978; Angerosa *et al.*, 1999). Other factors that influence the volatile content of olives and olive oil are climate and soil type (Ranalli, *et al.*, 1999), although as mentioned above, these factors probably had a minimal influence on the results presented in this thesis.

7.3. Processing of olive fruits

2-phase and 3-phase extraction methods

The influence of 2-phase and 3-phase decanters on qualitative characteristics and sensory attributes of virgin olive oil and oil yields was studied. Olive fruits of varieties Koroneiki or Coratina at a particular stage of ripening (50% turning black under standardized conditions) but from different origin (Greece and Italy respectively) were used in these experiments.

The content of phenolic compounds of virgin olive oils were higher in the 2-phase method. The extent of influence seems to be related to variety since significant differences were only observed in Coratina. Similarly, Cert *et al.* (1999) found higher content of phenolics in 2-phase decanter in comparison with 3-phase decanter in cv Hojiblanca whereas no differences where observed in cv Picual. They attributed this discrepancy to the influence of varieties. Such a conclusion fits with the fact that the phenolic compounds dissolved in the oil are mainly secoiridoids derivatives of DHPEA and p-HPEA. These compounds are produced as mentioned before by enzymatic degradation of oleuropein and ligstroside in the olives. Consequently, the concentration in the paste depends on the extent of the enzymatic processes that occur during malaxation. Enzyme activity will, of course, be influenced by the olive variety.

Moreover, the different quantities of water added to the olive paste, (water addition, is prerequisite for the operation of 3-phase decanter) alter the partition equilibrium between the liquid phases and reduce the phenol concentration in the oily phase through dilution in the aqueous phase (Ranalli and Angerosa, 1996; Di Giovacchino *et al.*, 2002; Ranalli *et al.*, 2001; Gimeno *et al.*, 2002). This will mean that 2-phase extraction methods would be expected to give rise to higher phenolic concentrations, as observed.

The sensory analysis showed that oils produced by 2-phase decanter showed an improved organoleptic quality compared to oils produced with 3-phase decanter. In fact oils obtained using the 2-phase decanter were characterized by an increase of bitter, pungent, green leaf, and bitter almond tasting attributes in comparison to oils produced by 3-phase decanter. Again significant differences were observed for bitter and pungent attributes mainly for cultivar Coratina.

Concerning oil yield the data showed that 2-phase decanter methods in comparison to 3-phase decanter was effective in olive processing and obtained a small increase (but not statistically significant) in yield. This agreed with results obtained by Di Giovacchino (1996) for the cultivars Leccino and Dritta. In contrast, Cert *et al.*, (1999) reported slightly lower oil yields when the 2-phase method was used. In any case, both methods were satisfactory so far as yield was concerned and the better sensory properties and lower wastewater achieved by the 2-phase method makes it a technique of choice.

Malaxation

Paste malaxation is a basic step (following crushing) of the mechanical olive oil extraction process (Fig. 1.16). Most of the previous studies have been carried out at a laboratory level and available results are not consistent. In our research work, laboratory as well as industrial-scale experiments were done. Moreover, for the important Koroneiki variety no thorough previous research had been carried out so that it was important to study the effect of kneading time and temperature on the composition of the resulting oil.

The findings showed that both the olive paste malaxation time and temperature markedly influenced the analytical characteristics of oil. Total phenol content of olive

oil samples decreased during the malaxation of the olive paste. However, in 3-phase decanter the decrease occurred at different times after 30 min and 45 min respectively of malaxation for the Koroneiki and Coratina cultivars. Individual phenols followed analogous trends to that of total phenols with the exception of 3,4 DHPEA and p-HPEA which increased from 0 min to 30 min of malaxation and declined afterwards. As discussed in the experimental chapters, the endogenous oxidoreductases such as POD, PPO and LOX as well as the contact of the olive paste with air (oxygen) are probably responsible for the changes in phenolic compounds during malaxation. The oxidative stability of olive oil with malaxation time followed the same trend as the phenolic contents. On the other hand, total aliphatic alcohols and chlorophyll extaction increased as the malaxation time proceeds. The intensities of sensory attributes perceived by tasters for oils from cv. Koroneiki and cv. Coratina showed some impact of malaxation time (but not significantly).

Together with previous studies on volatile compounds in olive oils, the trends in sensory attributes suggested by the results in this thesis show agreement with those components thought to give rise to desirable attributes such as "green notes".

Effect of malaxation temperature

The specific absorption coefficients (specific extinction) in the ultraviolet region, K_{232} and K_{270} did not change significantly. The peroxide value, on the other hand, increased significantly when olive paste temperature was 42°C. Chlorophyll content increased significantly as paste temperature increased. Furthermore, total phenol content and the content of secoiridoids increased significantly with increasing paste temperature. Oxidative stability followed the same trend as total phenols. However, contradictory results have been reported in the literature concerning phenol compounds. In some research papers, a reduction in phenol content with temperature was reported (Servili *et al.*, 1994; Angerosa *et al.*, 2001). In other studies, just the opposite was found (Di Giovacchino, 1991; Parenti *et al.*, 2000; Ranallli *et al.*, 2001). On the other hand, Amirante *et al.* (2001) correlated both the mixing temperature and paste dilution with the content of phenolic compounds. They found that low dilutions of paste (19%) and temperature close to 32 °C enabled a more efficient extraction of phenols than at

temperature 27 °C. However at 35°C and at any dilution both phenolic compounds and induction time were lower. Rodis *et al.*, (2002) studied the partition coefficient (Kp) of phenolic compounds of olive fruit between aqueous and olive oil phases. Based on the UNIFAC prediction model, they showed a relative improvement of the partition of antioxidants at higher process temperatures. Thus, the antioxidant properties of phenols would be important when their content increased with temperature, such as observed.

In addition, increasing malaxation temperature decreased the intensities of sensory attributes. Tasters generally perceived a weakening of flavour at 30 min of malaxation and by raising malaxation temperature from 15°C to 42°C. The overall score award by tasters to olive oils was inversely proportional to olive paste malaxation temperature. Therefore, higher temperatures result in the flattening of olive oil aroma. These results can be explained by the inactivation of LOX and HPL, the first two enzymes in the lipoxygenase pathway due to high temperature (Salas and Sanchez, 1999). The lower activity of these enzymes would then limit the generation of important sensory volatiles by the LOX pathway.

Industrial yield

As mentioned above, during the extraction process the total oil extraction from fruits cannot be obtained, because there are oil losses in the solid and liquid effluents (olive cake and vegetation water). Depending on the quantity of extracted oil from the fruit, olive cultivars can produce "difficult pastes" which are characterised by emulsion formation, non-effective kneading, and difficulties in solid–liquid separation and this leads to significant oil losses in the various by-products. Koroneiki is considered to be an easy olive from which to extract oil because the oil yield is well above 80% (Figure 3.7). The results showed that the temperature and the duration of malaxation influence not only oil quality and its sensory characteristics but may also affect the oil yield. Increasing mixing temperature from 30°C to 45°C caused an increase (but not statistically significant) in oil extraction yields for both the 2- phase and the 3-phase extraction system. Furthermore, extraction yield increased with malaxation time in both the 2-phase and the 3-phase extraction systems studied. In addition, laboratory experiments indicated that the increase in oil yield of Koroneiki followed a polynomial

curve with a very high correlation coefficient. Oil yield increased up to about 40-45 minutes of kneading time followed by a decrease. The optimum malaxation time for Koroneiki for oil extraction was 30 min. In contrast, for Coratina the optimum malaxation time was 45 min.

Consequently, it is very important to use the most suitable extraction conditions with reference to the characteristics of the particular olive cultivar since the aim should be always of achieving both high yields and high quality olive oils. During the extraction process the presence of volatiles with pleasant perceptions should be promoted as far as possible. Since, virgin olive oil must be extracted only by using mechanical means, the amount of each volatile in the final product, can be varied by changing either the temperature or the time of the malaxation process. According to Morales *et al.* (1999). low temperature and medium malaxation times are the best extraction conditions for promoting the formation of green volatiles responsible for desirable sensory perceptions.

To summarize, the analytical quality, aroma and oxidative stability of the oils depend on cultivar, the extraction method used and the time and temperature of malaxation. In some cases, the processing techniques may have to carefully balance quantitative yields against the quality of the final oil.

7.4. Agricultural methods

In the Mediterranean region, cultivated plants often face water stress and stress caused either by salt accumulation in the soil or through irrigation with saline water. In many regions the groundwater contains high concentration of NaCl and farmers have no alternative but to use this water for irrigation. The olive tree is considered as being moderately tolerant to salinity (Mass and Hoffman, 1977; Rugini and Fedeli, 1990) and its tolerance to salinity appears to be cultivar-dependent (Tattini *et al.*, 1992; Tattini *et al.*, 1994; Charzoulakis *et al.*, 2002)

The two main oil-producing cultivars (Koroneiki and Mastoidis) of Crete were used for the experiments. The data showed a clear reduction of fruit weight and oil content with increasing salinity levels in both cultivars. In addition, there was an increase in moisture content of olive fruits, analogous, to the increase in concentration of Na^+ and Cl^- in the olive fruits (Charzoulakis *et al.*, 2004). Furthermore, changes took

place in the composition of fatty acids. In particular, oleate decreased while linoleate and linolenate increased. This suggested that Δ^{12}- and Δ^{15} -desaturase activity was increased by salinity stress. In addition, concentrations of total phenols and secoiridoid derivatives were higher when more concentrated saline solutions were used for irrigation. Changes were different for each variety with more intense alterations found in Koroneiki, which is considered less tolerant. In agreement, Chartzoulakis *et al.* (2002) found that the decrease in photosynthesis was less in Mastoidis. Moreover, the two cultivars differ in their ability to reject Na^+ or Cl^- ions. In fact, Koroneiki showed lower concentation of Na^+ or Cl^- ions in the roots and higher in the leaves whereas, the opposite was observed for Mastoidis and as a result this cultivar was less affected by saline irrigation (see Chartzoulakis *et al.*, 2002).

Salt stress changes the water relations of higher plants and salt tolerance often depends on the ability of the plant to also tolerate drought (Greenway and Munns 1980, Flowers and Yeo 1986). As salinity levels increase, plants extract water less easily from soil, which aggravates water stress conditions. Thus, plants (such as cv Mastoidis), which can tolerate the imposed water stress, will also be able to grow better with saline water.

Olive (*Olea europaea, L.*) is usually cultivated without irrigation in regions with annual rainfall 400-600 mm, but it is also found in regions with only 200 mm of rain. In addition, olives are grown on a wide variety of soils, many too poor to support cultivation of other crops. Our findings concerning irrigation confirm the above-mentioned observations that salinity will also cause osmotic stress. Thus, our data from the salinity stress and the drought stress experiments showed similar results concerning fruit size and oil content, phenolic contents (especially secoroidoid derivatives) and fatty acid composition.

Thus, irrigation exerted a positive effect on fruit weight and oil content. Furthermore, phenolic compounds and, in particular, secoiridoids derivatives (3,4-DHPEA-EDA, p-HPEA-EDA, and 3,4-DHPEA-EA) increased under water stress conditions. Our findings agree with previous reports (Salas et al., 1997; Patumi *et al.*, 1999; Andria *et al.*, 1999; Tovar et al., 2001). However, contrasting results concerning the content of total phenol were found by other authors (Dettori and Russo, 1993;

Inglese *et al.,* 1996; Ismail *et al.,* 1999). Some of these differences may reflect the cultivars used, as well as the exact experimental conditions.

The composition of fatty acid was affected by drought and resulted in a higher % of PUFAs in non-irrigated treatments. As expected (given their metabolic connection) the increase was parallel to a decrease in the % of oleate. Thus, the monounsaturated to polyunsaturated fatty acid ratio was significantly influenced by irrigation and was similar to results were obtained by Salas *et al.* (1997). Again, like the results for phenolics, contrasting results have been reported in previous studies for the influence of irrigation on the fatty acid composition and triacylglycerol species of olive oil. Thus, some authors did not observe any significant differences ((Patumi *et al.,* 1999; Patumi *et al.,* 2002; Motilva *et al.,* 2000) in these parameters following irrigation or drought stress.

The results cannot be attributed to the more advanced ripening degree of olive fruit in non-irrigated trees because previous work by Stefanoudaki *et al.* (2003) on the effect of environmental conditions on olive fruit Koroneiki during development and ripening on the fatty acids profiles showed increases in oleic acid content while polyunsaturated fatty acids were decreased up till September followed by an increase. Opposite trends in polyunsaturated fatty acids were followed by saturated fatty acid proportions. These results were similar to the effect of irrigation and ripening on fatty acid composition in the results obtained by some workers but not by others. Thus, an increase in content of oleic acid at the expense of palmitic acid was found by Esti *et al.* (1996) and by Stefanoudaki *et al.* (2003). In contrast, no such change was found by others (Nergiz and Engez, 2000; Salvador *et al.,* 2001). As mentioned before, ripening is undoubably affected by cultivar and this may explain at least some of the differences reported in the literature.

Aliphatic and triterpenic alcohols, total sterols and Δ^5 avenasterol were increased in oils from non-irrigated trees. In addition, the content of total volatiles and phenolic compounds were significantly reduced in oils from irrigated trees. The 6C compounds, produced by the lipoxygenase pathway (hexanal, 3-hexenal, *trans*-2-hexenal, *trans*-3 hexen-1-ol, *trans*-2-hexen-1-ol and hexyl acetate) responsible for the " green note" of olive oil showed higher concentrations in non-irrigated trees. However, the presence of higher concentrations of 1- pentene-3-ol, and isoamyl alcohol which give undesirable

sensations in olive oil (Aparicio *et al.*, 1994: Angerosa *et al.*, 1992) may influence the overall score of oil from non-irrigated trees. Furthermore, the high concentrations of polyphenols in such olive oils resulted in a sharp negative influence on mouth feel and flavour (Montedoro *et al.*, 1978).

However, the relationships between the individual hydrophilic phenols of virgin olive oil and sensory characteristics are not clearly defined (Servili and Montedoro, 2002). In the first sampling olives were immature and the phenol concentration was very high, so this was probably the reason for the very low sensory score for both oils. In addition, in oils from non-irrigated trees, some tasters noticed a slight dry leaves' sensation. Irrigation changed both quantitative and qualitative aspects of olive oils. Moreover, although irrigation increased yields, some of the most desirable sensory characteristics were reduced in oils from irrigated trees.

Thus, in conclusion, irrigation affected yields and quality of olive oil. Although irrigation led to decreased contents of some undesirable sensory qualities, some intense green notes were reduced. Thus, the effects on quality were complex and may be appreciated more, or less, depending on the consumer's particular needs.

7.5. Oxidation and deterioration of olive oil during storage

The high oxidative stability of olive oil is mainly due to its fatty acids composition (being low in PUFAs) and to its content of minor components with antioxidant activity. However, extrinsic factors such as oxygen, light, temperature and relative moisture can alter the rate of olive oil oxidation and, thus, its overall quality. The standardization and packaging of olive oil, which is a traditional product, is a very important issue for its protection during long-term storage and for maintaining its nutritional value and at the same time satisfying consumer preference. In the present study, commercial packaging and storage conditions were simulated. They are, of course, very important for the whole period from processing til the product reaches the consumer's table. Complementary experiments were also carried out with closed or with loose fitting covers for samples kept outside. Because in recent years virgin olive oil is often stored and consumed without filtering in some of the production countries, we decided to include unfiltered olive oil in our experiments. At the same time an approach

was made to evaluate the possibility of improving the shelf life of virgin olive oil with the utilization of nitrogen as a conditioner gas. The reason that we choose tin containers for our study was that it is a traditional material that has been used for long time in olive oil packaging. The tin container still remains one of the best means of olive oil packaging, because it has many advantages. These include robustness (and, therefore, minimal distorted containers), suitability for lithographic labeling and imperviousness to oxygen, light and humidity. Moreover, they are not very expensive. Regarding the storage environmental conditions, the largest difference was in the temperature variations indoors and outdoors, being 23°C± 5°C for indoors but 0°C to 20°C in the winter and from 20°C to 40 °C in the summer outdoors. Under the experimental conditions most, if not all, oxidation that occurred in the tinplated container could be ascribed as simple autoxidation.

Unfiltered oil samples

Freshly extracted virgin olive oil contains suspended and dispersed solids and micro-drops of vegetation water in quantities, which vary according to the variety, the ripeness of the fruit and the oil process used. These suspended materials through decantation slowly migrate towards the bottom of the container and form a sediment. Recently it was published by some authors that water and dispersed particles that constitute the turbidity of fresh virgin olive oil may act as antioxidants and as a buffer against acidity (Lecker *et al.,* 1994; Frega *et al.,* 1999; Ambrosone *et al.,* 2002). However, this sediment contains sugars, proteins and enzymes. After a few months of preservation and under suitable temperature conditions, the sediment can ferment and give rise to the production of unpleasant compounds responsible for the typical muddy sediment defect of olive oil (Angerosa, 2002).

On the other hand, oxidizing enzymes activities such as PPO and LOX were found in virgin olive oil (Georgalaki *et al.,* 1998; Georgalaki *et al.,* 1998). After filtration the activity of these enzymes increased indicating that some inhibitory components were removed.

The results showed that, after 15 months of storage, the increase in acidity was more pronounced in the unfiltered oil indicating small hydrolytic activity (Lecker *et al.,*

1994; Frega *et al.*, 1999; Ambrosone *et al.*, 2002) but the value kept very low and within the limits of European Community regulations for extra virgin olive oil. The variation in acidity, in relation to the different ways of storage, was not statistically significant.

Peroxide values and the indices K_{232} and K_{270} followed parallel trends during storage and up till 8 months of storage there were not any significant differences between unfiltered and filtered oils. The peroxides as well as the indices K_{232} and K_{270} were very unstable and after 8 months of storage (which correspond to summer months) the temperature indoors, reached 28°C on some days. Consequently, the increase in temperatures affected the rates of formation as well as degradation rates of these compounds. Moreover, lower rates of increase were observed in peroxide value, and in the indices K_{232} and K_{270} when nitrogen was the conditioning gas. These, results are in agreement with those obtained by Di Giovacchino *et al.*, (2002).

There was a reduction in total phenols and in the secoiridoid derivatives according to the various conditions of storage. The reduction was more evident after 10 months of storage and especially in the unfiltered oils. On the other hand, the content of 3,4 DHPEA and p-HPEA increased in all cases during storage due to the hydrolysis of secoiridoid derivatives and, in particular, the decrease in 3,4-DHPEA-EDA was inversely proportional to the increase. Similar results were obtained by Cinquanta *et al.* (1997), Cinquanta *et al.* (2001) and Lavelli (2002).

For the olive oil stored indoors with nitrogen in the headspace, little change was found during 15 months of storage, indicating great stability for the phenolic compounds. The content of DHPEA, and p-HPEA was lower in the oils kept outdoors in closed containers due to faster oxidation rates at the higher temperatures and to the activities of endogenous oxidoreductases such as polyphenol oxidase (PPO) and lipoxygenase (LOX) present in olive oil (Georgalaki *et al.*, 1998). These enzymes may promote oxidation of phenolic compounds (Vierhuis, 2002). In addition, the loss of total phenols and secoiridoid derivatives in the open samples was very high and after only two months of storage their concentration was very low. The antioxidant activity of phenolic compounds is due to their reaction with lipid radicals to form stable products, which break up the propagation stage of the oxidative chain reaction. This caused a slow consumption of phenolic compounds during the induction period of storage. The

antioxidant activity in extra virgin olive oil with low degradation rates (high content in 3,4-DHPEA-EDA and low in 3,4-DHPEA) was 3-5 times more efficient as 2,2-diphenyl-1- picrylhydrazyl radical (DPPH) scavengers and 2 times more efficient as inhibitors of the XOD – catalysed reaction than oils with intermediate or advanced degradation level (Lavelli, 2002). α-Tocopherol reduction in samples kept under different conditions did not exceed 16% and tended to be higher in samples kept outside. It seems that in the initial states of autoxidation polar phenolic compounds are more active. In contrast, α-tocopherol was much more effective in the open samples where the phenol content was reduced and peroxide values were very high. In this case, after two months of storage in open samples, the α-tocopherol content was very low (Blekas *et al.*, 1995; Blekas and Boskou, 1998).

The shelf life of packed virgin olive oil (as for other vegetable oils) is limited because of autoxidation of unsaturated fatty acids and the production of hydroperoxides which are not stable and decompose to produce a range of non-volatile and volatile products.

During the oxidation process the initial pleasant sensory characteristics of virgin olive oil eventually change to unpleasant sensory attributes (Morales *et al.*, 1997). After two months of storage, most of the 6C volatile compounds, which are common contributors of the green aroma of olive oil, are still present. However, the concentration of these compounds when the oil was stored outside in open containers was reduced.

Hexanal increased during storage in parallel with the decrease in quality. The increase was more pronounced in the samples kept outside for 15 months in closed containers. These results are in agreement with those reported by Solinas *et al.*, (1988) and by Morales and Przybylski, (2000). The ratio of *trans*-2-hexenal / hexanal decreased during the storage of olive oil and, it should be noted that the value of this ratio is often taken to indicate the quality of the oil (Angerosa *et al.*, 1992) indicating deterioration in the sensory quality of the oil. The lower value was observed in the samples kept outside. Furthermore the percentage of *trans-2*-hexanal in relation to total carbonyl compounds was very low for the oils samples kept outside, demonstrating again low quality oils.

The results showed that during the storage of olive oil oxidation is an inevitable process, the rate of which depends on many factors. However, useful interactions can be

made in two ways. One is to try to make a good quality product with high content of antioxidants by selecting the appropriate cultivars and by optimizing each production step, from the tree to the supermarket. The other is to ensure excellent packing materials and then to use the most favorable storage conditions.

The use of N_2 flushing caused a reduction in the oxygen present in the headspace of the olive oil containers. Due to this reduction in O_2 concentration the rate of oxidation was reduced although not eliminated. Of course, it is very difficult to eliminate all oxygen from the mass of olive oil. In fact, oxygen concentrations of no more than 2.1% are sufficient to initiate lipid oxidation and, thereby, rancid tastes (Jan, *et al.,* 1988). In addition, the absence of oxygen does not stop the progress of oxidation because some forms of LOX can oxidize fatty acids without the presence of oxygen thus forming free radicals (Sanchez and Salas, 2000). According to Georgalaki *et al.* (1998) LOX activity was found in virgin olive oil during storage. One would predict that this might be more of a problem in unfiltered oils. When the oil was kept for 15 months either indoors or even outside at elevated temperatures but with nitrogen in the headspace, the qualitative parameters acidity, peroxide value, K_{232} and K_{270} and panel test scores were all within the official limit for extra virgin olive oil of the European Community. Thus, one can say in conclusion that the best conditions for ensuring good quality olive oil are to use filtered oils stored indoors (where temperature variations are less) and with nitrogen in the headspace. However, because olive oil does not easily deteriorate then all but the most extreme conditions provide satisfactory oils during a year's storage or possibly, longer.

7.6. General conclusions

In this thesis, results have been produced which show that all stages of olive oil production can have a significant influence on the quality of the product. These influences begin with the choice of cultivars and the growing conditions and whether and how to irrigate. Within the processing, the choice of extraction method and the length and temperature of malaxation are important. Finally, inadequate storage conditions can have a detrimental effect even when the processed oil is of good quality.

We have focused on the Koroneiki cultivar but similar considerations are, no doubt, of relevance to other varieties. These data emphasized the complicated nature of olive oil and provide plenty of reasons why the best quality oils are much prized by consumers and cost many times that of much lower quality olive oils. Not only is olive oil nutritionally beneficial, it also provides subtle flavours and aromas to any food with which it is used. These factors will ensure that olive oil remains as popular on the kitchen table as it has been for thousands of years.

7.7. Possible areas for further research

The research reported here presents a first attempt at a comprehensive look at factors influencing olive oil quality in a single variety. These are some obvious extensions of the work:

a) Do the results obtained for Koroneiki apply to other varieties? There were some indications of both similarities and differences with Coratina and Mastoidis but the data are not sufficient for generalizations. Furthermore, varieties giving oils with more extreme characteristics might be usefully considered.
b) How did the LOX enzymes contribute to quality characteristics altered during malaxation. Assays with malaxation mixtures might be useful here.
c) The precise role of individual anti-oxidant could be explored by addition of such chemicals or possibly, by selective removal of some (if methodology permitted this).
d) The results on irrigation effects were partly inconsistent, probably because of the marked differences between the two years examined. Further work is needed to confirm the data.
e) The link between drought- resistance and salinity –resistance could be explored further, possibly by using more olive cultivars with proven drought resistant characteristics.
f) Could enzymes play any role in the oxidative deterioration during storage? This aspect could be explored by assaying for enzymic activity, use of inhibitors and by the addition of exogenous enzymes.

Chapter 8. References

Aggapio, M., Dettori, S. and Azzena, M. (1983) L'irrigazione dell'oliva da Mensa in Sardegna. *Instituto di coltivazione arboree dell. Unversita di Sassari*, 1-19

Ambrosone, L., Angelico, R., Cinelli, G., Di Lorenzo, V. and Ceglie, A. (2002) The role of water in the oxidation process of extra virgin olive *J. Am. Oil Chem. Soc.* **79,** 577-582

Amiot, M.J., Fleuriet, A. and Macheix, J.J. (1986) Importance and evolution of phenolic compounds in olive during growth and maturation. *J. Agric. Food Chem.* **34,** 823-826

Amiot, M.J., Fleuriet, A. and Macheix, J.J. (1989) Accumulation of oleuropein derivatives during olive maturation. *Phytochemistry* **28,** 67-69

Amirante, R., Cini, E., Montel, G.L. and Pasqualone, A. (2001) Influence of mixing and extraction parameters on virgin olive oil quality. *Grasas y Aceites* **52,** 198-201

Amirante, R., Di Renzo, G.C., Di Giocvacchino, L., Bianchi, B. and Catalano, P. (1993) Evoluzione tecnologica degli impianti di estrazione dell'olio di oliva. *Olivæ* **48,** 43-53

Andria, R., Morelli, G., Giorio, P., Patumi, M., Vergari, G. and Fontanazza, G. (1997) Yield and oil quality of young olive trees grown under different irrigation regimes. *Acta Hort.* **474,** 185-188

Angerosa, F. (2002) Influence of volatile compounds in virgin olive oil quality evaluated by analytical approach and sensor panel. *Eur. J. Lipid Sc. Technol.* **104,** 639-660

Angerosa, F. and Di Giacinto, L. (1995) Crushing influence on the quality characteristics of virgin olive oil. Nota II. *Riv. Ital. Sost. Grasse* **72,** 1-4

Angerosa, F., Basti, C. and Vito, R. (1998) Biogeneration of volatile of compounds in virgin olive oil: Their evolution in relation to malaxation time. *J. Agric. Food Chem.* **46,** 2940–2944

Angerosa, F., Basti, C. and Vito, R. (1999) Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some Italian cultivars. *J. Agric. Food Chem.* **47,** 836-839

Angerosa, F., d'Alessandro, N., Konstantinou, P. and Di Giacinto, L. (1995) GC-MS evaluation of phenolic compounds in virgin olive oil. *J. Agric. Food Chem.* **43,** 1802-1807

Angerosa, F., Di Giacinto, L. and Solinas, M. (1990) Influence of olive oil storage in pile on the flavor of resulting oils. Evaluation of the 'fusty' defect by HPLC and HRGC analyses of volatile compounds. *Riv. Merceol.* **29,** 275-294

Angerosa, F., Di Giacinto, L. and Solinas, M. (1992) Influence of *Dacus Oleae* infestation on flavor of oils, extracted from attacked olive fruits, by HPLC and HRGC analysis of volatile compounds. *Grasas y Aceites* **43,** 134-142

Angerosa, F., Mostallino, R., Basti, C. and Vito, R. (2000a) Virgin olive oil odour notes: their relationships with volatile compounds from lipoxygenase pathway and secoiridoid compounds. *Food Chem.* **68,** 283-287

Angerosa, F., Mostallino, R., Basti, C. and Vito, R. (2001) Influence of malaxation temperature and time on the quality of virgin olive oils *Food Chem.* **72,** 19-28

Angerosa, F., Mostallino, R., Basti, C., Vito, R. and Serraiocco, A. (2000b) Virgin olive oil differentiation in relation to extraction methodologies. *J. Sci. Food Agric.* **80,** 2190-95

Angerosa, F., Solinas, M. and Di Giacinto, L. (1989) Influence du stockage des olives sur la flaveur de l'huile. *Proceedings International Congress* "Chevreul" Angers. 571-577

AOAC (1984) Official Methods of analysis (Section 27.00 B. Nut and Nut Products). *Association of Official Analytical Chemists,* Washington, D.C., 14th edn, 501.

AOCS (1977) Official and Tentative Methods Vol 62, edited by *AOCS* technical committee, Champaign, IL, Method Cc 13c- 50

Aparicio R. and Aparicio-Ruiz, R. (2000) Authentication of vegetable oils by chromatographic techniques. *J. Chromatography A* **881,** 93–104

Aparicio R., Alonso M.V., Morales, M.T. and Calvente J.J. (1994) Relationship between the COI test, and other sensory profiles by statistical procedures. *Grasas y Aceites* **45,** 26-41

Aparicio R., Ferreiro, L. and Alonso, M.V. (1994) Effect of climate on the chemical composition of virgin olive oil. *Analytica Chimica Acta* **292,** 235-241

Aparicio R., Morales, M.T. and Alonso, M.V. (1996) Relationship between volatile compounds and sensory attributes of olive oils by sensory wheel. *J. Am. Oil Chem. Soc.* **73,** 1253-1264.

Aparicio, R. (2000) Characterization: Mathematical procedures for chemical analysis. *Handbook of Olive Oil. Analysis and Properties.* Eds. J. Harwood, R. Aparicio, Aspen Publication, Gaithersburg, MD (USA), 285-351

Aparicio, R. and Luna, G. (2002) Characterisation of monovarietal virgin olive oils *Eur. J. Lipid Sc. Technol.* **104,** 614-627

Aparicio, R. and Morales, M.T. (1998) Characterisation of olive ripeness by green aroma compounds of virgin olive oil. *J. Agric. Food Chem.* **46,** 116-1192

Arjona, R., Garcia, A. and Ollero, P. (1999) The drying of alpeorujo, a waste product of the olive oil mill industry. *J. Food Engin.* **41,** 229-234

Badal Garcia, E. (1990) Aportaciones de la anthracologý'a al estudio del paisaje vegetal y su evolucio'n en el cuaternario reciente, en la costa mediterranea del Paý's Valenciano y Andalucý 'a (18000–3000 B.P.). Ph.D. Thesis, Universidad de Valencia, Facultad de Geografia y Historia, Valencia

Baeten, V., Hourant, P., Morales, M.T. and Aparicio, R. (1998) Oil and fat classification by FT-Raman spectroscopy. *J. Agric. Food Chem.* **46,** 2638–2646

Baeten, V., Meurens, M., Morales, M.T. and Aparicio, R. (1996) Detection of virgin olive oils adulteration by Fourier Transform Raman spectroscopy. *J. Agric. Food Chem.* **44,** 2225–2230

Baldioli, M., Servili, M., Perretti, G. and Montedoro, G.F. (1996) Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil *J. Am. Oil Chem. Soc.* **73,** 1589-1593

Ballesteros, E., Gallego, M. and Valcarcel, M. (1995) Simultaneous determination of sterols in edible oils by use of a continuous separation module coupled to the gas chromatograph. *Analytica Chimica Acta* **308,** 253-260

Barrio Perez-Cerezal, M., Gutierrez Rosales, F. and Gutierrez Gonzalez-Quijano R. (1981) Gas Liquid chromatography application. A headspace technic to olive oils atrojado problem. *Grasas y Aceites* **32,** 155- 161

Bartolozzi, F. and Fontanazza, G. (1999) Assessment of frost tolerance in olive *(Olea europaea L.) Sci. Horti.* **81,** 309-319

Baeten, V., Hourant, P., Morales, M.T. and Aparicio, R. (1998) Oil and fat classification by RT- Raman spectroscopy. *J. Agric. Food Chem.* **46,** 2638-2646

Baeten, V., Meurens, M., Morales, M.T. and Aparicio, R. (1996) Detection of virgin olive oil adulteration by Fourier transform Raman spectroscopy. *J. Agric. Food Chem.* **44,** 2225-2230

Benlloch, M., Arboleda, F., Barranco, D., Fernadez-Escobar, R. (1991) Response of young olive trees to sodium and boron excess in irrigation water. *HortScience* **26,** 867-870.

Bernstein L. (1964) Effect of salinity on mineral composition and growth of plants. *Proc. 4th Int. Coll Plants analysis and fertilizers problems*, Bruxelles, Belgium, **4,** 25-45

Berra, B., Montorfano, G., Adorni, P., Zoppi, S., Nazzali, P. and Papelli, S. (1997) The effects on lipids and serum and red blood cell membranes caused by different diets supplemented with EFA. *Riv. Ital. Sost. Grasse* **74,** 539-544

Besnard, G., Baradat P., Chevalier, D., Tagmount, A. and Bervillé, A. (2001) Genetic differentiation in the olive complex (*Olea europaea*) revealed by RAPDs and RFLPs in the rRNA genes. *Genet. Res. Crop Evol.* **48,**165–182

Besnard, G., Baradat, P. and Bervillé, A. (2001) Genetic relationships in the olive (Olea europaea L.) reflect multilocal selection of cultivars. *Theoret. Appl. Genet.* **102,** 251–258

Bianchi, B. and Catalano, P. (1996) Power consumption and quality features in the hammer crushing process for olive oil production. *Grasas y Aceites.* **47,** 136-141

Bianchi, G. (2003) Lipids and phenols in table olives *Eur. J. Lipid Sc. Technol.* **105,** 229–242

Blee, E. and Schuber, F. (1993) Biosynthesis of Cutin monomers; Involvement of lipoxygenase/ peroxygenase pathway. *Plant J.* **4,** 113-123

Blekas, G. and Boskou D. (1998) Antioxidative activity of 3,4-dihydroxyphenylacetic acid and a-tocopherol on the triglyceride of olive oil. Effect of acidity. *Grasas y Aceites* **49,** 34-37

Blekas, G., Tsimidou, M. and Boskou, D. (1995) Contribution of α tocopherol to olive oil stability. *Food Chem.* **52,** 289-294

Bongi, G. and Loreto, F. (1989) Gas exchange properties of salt-stressed olive (*Olea europaea L*) leaves. *Plant Physiol.* **90,** 1408-1416.

Bosabalidis, A.M. and Kofidis, G. (2002) Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Science* **163,** 375-379

Boskou, D. (1996). *Olive Oil; Chemistry and Technology.* AOCS Press, USA

Bramley, P.M., Elmadfa, I., Kafatos, A., Kelly, F.J., Manios, Y., Roxborough, H.E., Schuch, W., Sheehy, P.J.A, and Wagner K.H. (2000) Vitanin E (Review) *J. Sci. Food Agric.* **80,** 913-938

Brenes, M., García, A., García, P., Rios, J.J. and Garrido, A. (1999) Phenolic compounds in Spanish olive oils. *J. Agric. Food Chem.* **47,** 3535-3540

Briante, R., Patumi, M., Limongelli, S., Febbraio, F., Vaccaro, C., Di Salle, A., La Cara, F. and Nucci, R. (2002) Changes in phenolic and enzymatic activities content during fruit ripening in two Itaalian cultivars of *Olea europaea L. Plant Science* 1-8

Browse, J. and Somerville, C.R. (1991) Glycerolipid synthesis; Biochemistry and regulation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42,** 467-506

Campeol, E., Flamini, G., Chericoni, S., Catalano, S. and Cremonini, R. (2001) Volatile compounds from three cultivars of Olea europaea from Italy. *J. Agric. Food Chem.* **49,** 5409- 5411

Campos, M. and Civantos, L. (2000) Cultural practices in olive and impact on pests and diseases. *J. Agric. Food Chem.***84,** 40- 45

Caponio, F., Alloggio, V. and Gomes, T. (1999) Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chem.* **64,** 203-209

Caponio, F., Gemez, T., Summo, C. and Pasqualone (2003) Influence of the type of olive-crusher used on the quality of extra virgin olive oils. *Eur. J. Lipid Sc. Technol.* **105,** 201–206

Carlsson D.J., Suprunchuk, T. and Wiles, D.M. (1976) Photooxidation of unsaturated oils: Effects of singlet oxygen quenchers. *J. Am. Oil Chem. Soc.* **53,** 656-659

Catalano, P., Caponio, F. and De Leonardis, T. (1995) Olio d' oliva vergine di qualita. Influenza delle macchine per la frantumazione delle drupe. *Rivista di Ingegneria Agraria* **18,** 761-773

Cert, A., Alba, M., Perez-Camino, C., Ruiz-Gomez, A., Hidalgo, F., Moreda, W., Moyano, M.J., Martinez, F., Tubaileh, R. and Olias, J.M. (1999) Influence of extraction methods on the characteristics and minor components of extra virgin olive oil. *Olivae,* **79,** 41-50

Chartzoulakis, K., Loupassaki, M., Bertaki, M. and Androulakis, I. (2002) Effects of NaCl salinity on growth, ion content and CO2 assimilation rate of six olive cultivars. *Sci. Horti.* **96,** 235–247

Chartzoulakis, K., Michealakis, N. and Tzompanakis, I. (1992) Effect of water amount and application date on yield and water utilization efficiency of Koroneiki olives under drip irrgation. *Adv. Hor. Sci.* **2,** 82-84

Chartzoulakis, K., Patakas, A. and Bosabalidis, A.M. (1999) Changes in water relations, photosynthesis and leaf anatomy induced by intemittent drought in two olive cultivars. *Env. Exp. Bot* **142,** 113-120

Chartzoulakis, K., Psarras, G., Bemmos, S., Loupassaki, M. (2004) Effect of salinity and potassium on photosynthesis, water relations and Na, Cl, K and carbohydrate concentration, of two olive cultivars. *Agricultural Research* 27, 75-84 (in Greek)

Chimi, H., Cillard, J., Cillard, P. and Rahmani, M. (1991) Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. *J. Am. Oil Chem. Soc.* **68,** 307–312

Christopoulou, E., Lazaraki, M., Alexiou, Synouri, S. and Frangiscos, E. (1996) Influence of certain factors on the composition of olive- pomace oils. Part II Sterols, triterpenic dialcohols and aliphatic alcohols. *Riv. Ital. Sost. Grasse* **73,** 201-211

Christopoulou, E., Lazaraki, M., Komaitis, M. and Kaselimis, K. (2004) Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. *Food Chem.* **84,** 463-474

Cimato, A. (1990) Effect of agronomic factors on virgin olive oil quality. *Olivæ,* **31,** 18-24

Cinquanta, L., Esti, M. and Di Matteo, M. (2001) Oxidative stability of virgin olive oils. *J. Am. Oil Chem. Soc.* **78,** 1197-1202

Cinquanta, L., Esti, M. and La Notte, E. (1997) Evolution of Phenolic Compounds in virgin Olive Oil During Storage. *J. Am. Oil Chem. Soc.* **74,** 1259-1264

Cirio, U. (1997) Agrochemicals and environmental impact in olive farming. *Olivæ,* **65,** 32-39

Civantos, L. (1996) Production techniques. In: *World Olive Encyclopaedia*, Ed, International Olive Oil Council, Madrid, 207

Civantos, M. (1995) Development of integrated pest control in Spanish olive orchards *Olivae* **59,** 75-81

Codex Alimentarius Commission (1995) Report of the Fourteenth Session of the Codex Committee on Fats and Oils. *Alinorm* 95/17. FAO/WHO, Rome (Italy)

Commission Regulation (EEC) 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of Europ. Communities.* No L **248**

Cortesi, N., Azzolini, M. and Rovellini, P. (1995) Dosaggio dei componenti minori polari (CMP) in oli vergini di oliva. *Riv. Ital. Sos. Grasse* **72,** 333-337

Cortesi, N., Azzolini, M., Rovellini, P. and Fedeli, E. (1995) I componenti minori polari degli oli vergini di oliva: ipotesi di struttura mediante LC-MS. *Riv. Ital. Sost. Grasse* **72,** 241-251

Cortesi, N., Fiorino, P. and Rovelini, P. (1997) The role of the different anatomic parts of the drupe on the chemical composition of olive oil. *Riv. Ital. Sost. Grasse* **74,** 391-393

Cortesi, N., Rovellini, P. and Fiorino, P. (1999) The role of drupe different anatomic parts on chemical olive oil composition. *Acta Hort.* **474,** 643-648

Cresti, M., Ciampolini, F., Tattini, M. and Cimato, A. (1994) Effect of salinity on productivity and oil quality of olive (*Olea europea* L.) plants. *Adv. Hort. Sci.* **8,** 211–214

D'Angeli, S., Malho, R. and Altamura, M.M. (2003) Low-temperature sensing in olive tree: calcium signalling and cold acclimation. *Plant Science* **165,** 1303-1313

Del Nobile, M.A., Bove, S., La Notte, E. and Sacchi, R. (2003) Inffuence of packaging geometry and material properties on the oxidation kinetic of bottled virgin olive oil. *J. Food Engin.* **57,** 189–197

Dettori, S. and Russo, G. (1993) Effect of cultivar and water regime on the quantity and quality of olive oil produced. *Olivæ* **49,** 36-43

Di Giovacchino, L. (1991) Olive oil extraction by direct centrifugation of olive pastes. Note I: Influence of the kneading process. *Riv. Ital. Sost. Grasse* **68,** 413-420

Di Giovacchino, L. (2000) Technological aspects *Handbook of Olive Oil. Analysis and Properties.* Eds. J. Harwood, R. Aparicio, Aspen Publication, Gaithersburg, MD USA. 17-59

Di Giovacchino, L., Costantini, N., Serraiocco, A., Surricchio, G. and Basti, C. (2001) Natural antioxidants and volatile compounds of virgin olive oils obtained by two or three-phases centrifugal decanters *Eur. J. Lipid Sc. Technol.* **103,** 279–285

Di Giovacchino, L., Mucciarella, M.R., Costantini, N., Ferrante, G. and Surricchio, G. (2002) Use of nitrogen to improve stability of virgin olive oil durng storage. *J. Am. Oil Chem. Soc.* **79,** 339-344

Di Giovacchino, L., Sestili, S. and Di Vincenzo, D. (2002) Influence of olive processing on virgin olive oil quality. *Eur. J. Lipid Sc. Technol.* **104,** 587-601

Di Giovaccino, L. (1996) Influence of extraction systems on olive oil quality. *Olivæ* **63,** 52-63

Doorenbos, J. and Pruitt, W.O. (1977) Guidelines for Predicting Crop Water Requirements; Irrigation and Drainage paper no 24. FAO: Rome

El-Agaimy, M.A., Neff, W.E., El Sayed, M. and Awatif, I.I. (1994) Effect of saline irrigation water on olive oil composition. *J. Am. Oil Chem. Soc.* **71,** 1287-1289

El-Antari, A., El Moudni, A., Ajana, H. and Cert, A. (2003) Lipid composition of two fruit parts (flesh and kernel) of six varietie of olive tree cultivated in Morocco. *Olivae* **98,** 20-28

Escudero, A., Montilla, J.C.,Garc|ia, J.M., Sanchez-Quevedo, M.C., Periago . J.L., Esti, M., Cinquanta, L. and La Notte, E. (1998). Phenolic compoundsin different olive varieties. *J. Agric. Food Chem.* **46,** 32-35.

Fakourelis, N. (1985) Effects of pigments on the oxidation stability of olive oil. Ph. D. Thesis, Ohio State University.

Fakourelis, N., Lee, E.C. and Min, D.B. (1987) Effects of chlorophyll and β-carotene on the oxidation stability of olive oil. *J.Food Sci.* **53,** 234-235

FAO/WHO. (1977) Dietary Fats and Oils in Human Nutrition. Report of an Expert Consultation. Rome: FAO

Ferreiro L and Aparicio R, (1992) Influencia de la altitud en la composicion quimica de los aceites de oliva virgenes de Andalucia. Ecuaciones matematicas de clasificacion. *Grasas y Aceites* **43,** 149–156

Firestone, D., Carson, K. and Reina, R. (1988) Update on control of olive oil adulteration and misbranding in the US *J. Am. Oil Chem. Soc.* **65,** 788-792

Fitter, A.H. and Hay, R.K.M. (1987) Environmental Physiology of Plants. *Academic Press*, London

Flath, R.A., Forrey, R.R. and Guadagni, D.G. (1973) Aroma components of olive oil. *J. Agric. Food Chem.* **21**, 948-952

Flowers, T.J. and Yeo, A.R. (1989) Effects of salinity on plant growth and crop yield. In: Cherry, J.H. (Ed.), and Environmental Stress in Plants. *Biochemical and Physiological Mechanisms,* NATO ASI Series. Springer, Berlin, pp. 101–119

Fontanazza, G. (1994) Olive farming systems developments. *California growers.* **18,** 10-11

Fontanazza, G., Patumi, M., Solinas, M. and Serraiocco, A. (1993) Influence of cultivars of the composition and quality of olive oil. *Acta Hort.* **356,** 358-361

Frankel, E.N. (1991) Recent advances in lipid oxidation. *J. Sci. Food Agric.* **54,** 495–511

Frega, N., Bocci, F. and Lercker, G. (1991) Composizione lipidica della drupa di olivo di due cultivars della zona del Chianti in funzione della maturazione. Nota I: trigliceridi ed acidi grassi. *Riv. Ita. Sost. Grasse* **68,** 69-74

Frega, N., Bocci, F. and Lercker, G. (1992) Lipid Composition of two Olive Cultivars in Connection with the Ripening Degree. Note II: Unsaponifiable Fraction. *Riv. Ital. Sos. Grasse*, **69**, 77-81

Frega, N., Mozzon, M. and Lecker, G. (1999) Effect of free fatty acids on oxidative stability of vegetable oil. *J. Am. Oil Chem. Soc.* **76**, 325-329

Gandul-Rojas, B. and Mínguez-Mosquera, M.I. (1996) Chlorophyll and carotenoid composition in virgin olive oil, from various Spanish varieties. *J. Sci. Food Agric.* **72**, 31-39

Gandul-Rojas, B., Roca López-Cepero, M. and Mínguez-Mosquera, M.I. (1999) Chlorophyll and carotenoid patterns in olive fruits, Olea europaea cv. arbequina. *J. Agric. Food Chem.* **47**, 2207-2212

Garcia J.M., Seller, S. and Perez-Camino, C. (1996) Influence of fruit ripening in olive oil quality *J. Agric. Food Chem.* **44**, 3516-3520

Gracian, J. and Cota, J. (1984) Estudio cualitativo y cuantitativo de la fraccion de alcohols grasos de los aceites de oliva y de orujo. *Grasas y Aceites* **35**, 358-363

Garcia, J.M., Gutierrez, F., Barrera, M.J. and Albi, M.A. (1996) Storage of mill olives on an industrial scale. *J. Agric. Food Chem.* **44**, 590-593

Gatto, L. (1986) Influence of the seasonal irrigation volumes on the yield of drip-irrigated oil olives (cv. Coratina); First results. *L' Iirrigazione* **4**, 187-190

Georgalaki, M.D., Bachmann, A., Sotiroudis, T.G., Xenakis, A., Porzel, A. and Feussner, I. (1998) Characterization of a 13-lipoxygenase from virgin olive oil and oil bodies of olive endosperms. *Fett/ lipid* **100**, 554-560

Georgalaki, M.D., Sotiroudis, T.G. and Xenakis, A. (1998) The presence of oxidizing enzyme activities in virgin olive oil. *J. Am. Oil Chem. Soc.* **75**, 155-159

Gimeno, E., Castellote, A.I., and Lamuela- Raventos, R.M. (2002) The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherols, and β- carotene) in virgin olive oil. *Food Chem.* **78,** 207-211

Goldhamer, D.A., Dunai, J., Ferguson, L.F., Lavee, S. and Klein, I. (1994) Irrigation requirements of olive trees and responses to sustained deficit irrigation. *Acta Hort.* **356,** 172–175

Gomez, T. (1992) Oligopolymers, diglyceride and oxidised trglyceride contents as measures of olive oil quality. *J. Am. Oil Chem. Soc.* **69,** 1219-1223

Greenway, H. and Munns, R. (1980) Mechanisms of salt tolerance in non-halophytes. *Ann. Rev. Plant Physiol.* **31,** 149–190

Grosh, W. (1993) Determination of potent odourants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **4,** 68-73

Gurr, M.I. (1996) Dietary fats with *trans* unsaturation. *Nutr Res Res* 9, 259-279

Gurr, M.I. (2000) The role of lipids in human nutrition *Handbook of Olive Oil. Analysis and Properties.* Eds. J. Harwood, R. Aparicio, Aspen Publication, Gaithersburg, MD (USA)

Gurr, M.I., Harwood, J.L. and Frayn, K.N. (2002) Lipid Biochemistry: An Introduction, 5th ed. London

Gutfinger, T. (1981) Polyphenols in olive oils. *J. Am. Oil Chem. Soc.* **58,** 966-968

Gutierrez Gonzalez-Quijano, R., Janer del Valle, C., Janer del Valle, M.L. and Gutierrez Rosales, F. (1977) Relationship between polyphenols and the quality and stability of the virgin olive oil. *Grasas y Aceites* ***28,*** 101-107

Gutierrez Rosales, F., Perdiguero, S., Gutierrez, R. and Olías, J.M. (1992) Evaluation of the bitter taste in virgin olive oil. *J. Am. Oil Chem. Soc.* **69,** 394-395

Gutierrez, F. and Fernandez, J.L. (2002) Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of "extra" quality. *J. Agric. Food Chem.* **50,** 571-577

Gutierrez, F., Jiménez, B., Ruíz, A. and Albi, A. (1999) Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties picual and hojiblanca and on different components involved. *J. Agric. Food Chem.* **47,** 121–127

Gutierrez, F., Villafranca, M.J. and Castellano, J.M. (2002) Changes in the main components and quality indices of virgin olive oil during oxidation. *J. Am. Oil Chem. Soc.* **79,** 669-676

Gutierrez-Rosales, F., Garrido-Fernández, J., Gallardo-Guerrero, L., Gandul-Rojas, B. and Mínguez-Mosquera, M.I. (1992) Action of chlorophylls on the stability of virgin olive oil *J. Am. Oil Chem. Soc.* **69,** 866-871

Haralampidis, K., Milioni, D., Sánchez, J., Baltrusch, M., Heinz, E. and Hatzopoulos, P. (1998) Temporal and transient expression of stearoyl-ACP carrier protein desaturase gene during olive fruit development. *J. Exp. Bot.* **49,** 1661-1669

Hartmann, H.T. and Panetssos, C. (1962). Effect of soil moisture deficiency during floral development on fruitfulness in olives. *Proc. Am. Soc. Hort. Sci.* **78,** 209-217

Harwood, J.L. (1988) Fatty acid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39,** 101-138

Harwood, J.L. (1996) Recent advances in the biosynthesis of plant fatty acids. *Biochim. Biophys. Acta* 1301 7-56

Harwood, J.L. and Page, R.A. (1994) Biochemistry of oil synthesis. In *Designer Oil Crop,* pp 165-194. Edited by D.R. Murphy, Weinheim, Germany: VCH.

Harwood, J.L. and Sánchez, J. (2000) Lipid biosynthesis in olives. In: *Handbook of Olive Oil.* Eds. J. L. Harwood, R. Aparicio. Aspen Publishers, Gaithersburg, MD (USA) 61-67

Harwood, J.L. and Yaqoob, P. (2002) Nutritional and healthy aspects of olive oil. *Eur. J. Lipid Sc. Technol.* **104,** 685-697

Hatanaka, A. (1993) The biogeneration of green odour by green leaves. P*hytochemistry* **34,** 1201-1218

Hermoso, M., Uceda, M., Garcia, A., Morales, B., Frias M.L. and Fernandez, A. (1991) Elaboracio n de aceite de oliva de calidad. *Junta de Andalucia. Consejería de Agricultura y Pesca.* 84-87564-54-2, 173

Inglese, P. (1994) Influence of variety on the quality characteristics of Olive Oil. *Olivæ,* **54,** 42-47

Inglese, P., Barone, E. and Gullo, G. (1996) The effect of complementary irrigation on fruit growth ripening pattern and oil characteristics of olive (*Olea europea L.*) cv. Carolea. *J. Hort. Sci.* **71,** 257-263

IOOC (1994) Trade Standard applying to olive oil and olive pomace oils. (COI T15/NC No 2) *International Olive Oil Council.* Madrid, Spain

Ismail, A.S., Stavroulakis, G. and Metzidakis, J. (1999) Effect of irrigation on the quality characteristics of organic olive oil. Proc. 3rd ISHS Symp. On Olive Growing, *Acta Hort.* **474,** 687-690

IUPAC standard method No.2.432 (1992) Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. In first supplement to the *7th Edition of Standard Methods for the Analysis of Oils, Fats and Derivatives,* edited by A. Dieffenbacher and W.D. Pocklington, Blackwell, Oxford, United Kingdom

Jacotot, B. (1994) Olive oil; A food and Medicine in one. *Olivae* **54,** 40-41

Jan, M., Langerak, D.I., Wolters, T.G., Farkas, J., Kamp, H.J.V.D., Muuse, B.G. (1988) The effect of packaging and storage conditions on the keeping quality of walnuts treated with disinfestations doses of gamma rays. *Acta Aliment.* **17,** 13-31

Kapoulias, V.M. and Andrikopoulos, N.K. (1987) Detection of virgin olive oil adultaration with refined oils by second derivative spectophotometry. *Food Chem.* **23,** 183

Katerji, N., Van Hoorn, J.W., Hamdy, A. and Mastrorilli, M. (2003) Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. *Agricultural Water Management* **62,** 37–66

Kennedy, E.P. (1961) Biosynthesis of complex lipids. *Fed. Proc. Am. Soc. Exp. Biol.* **20,** 934-940

Keys, A. (1980) Seven countries: A multivariate analysis of death and coronary heart disease. Harvard University Press, Cambridge (USA)

Keys, A. (1995) Mediterranean diet and public health: personal reflections. *Am. J. Clin. Nutrition* **61,** 1321S - 1323

Keys, A., Menotti, A., Karvonen, C., Aravanis, M.J., Blackburn, H., Buzina, R., Djordjevic, B.S., Bontas, A.S., Fridanza, F. and Keys, M.H. (1986) The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol.* **124,** 903- 915

Kimpe, K., Jacobs, P.A. and Waelkens, M. (2001) Analysis of oil used in late Roman oil lamps with different mass spectrometric techniques revealed the presence of predominantly olive oil together with traces of animal fat. *J. Chromatography A* **937,** 87–95

Kiritsakis, A. and Tsipeli, A. (1992a) Hydrolysis and oxidation of olive oil during the time that olive fruit remain on the tree. *Riv. Ital. Sost. Grasse* **69,** 453-456

Kiritsakis, A. and Tsipeli, A. (1992b) Relationship of the acidity of olive oil to its resistance to oxidation. *Riv. Ital. Sost. Grasse* **69,** 513-515

Kiritsakis, A. K., Nauos, G. D., Polymenoupoulos, Z., Thomai, T. and Sfakiotakis, E. Y. (1998) Effect of fruit storage conditions on olive oil quality. *J. Am. Oil Chem. Soc.* 75, 721-724.

Kiritsakis, A.K. (1990) Olive Oil Handbook. AOCS Press, Champaign (USA)

Kiritsakis, A.K. (1998) Flavor components of olive oil a review. *J. Am. Oil Chem. Soc.* **75,** 673–681

Kiritsakis, A.K. and Dugan, L.R. (1985) Studies in photooxidation of olive oil. *J. Am. Oil Chem. Soc.* **62,** 892- 896

Klein, I., Ben-Tal, Y., Lavee, S., De Malach, Y., and David, I. (1993) Saline irrigation of cv. Manzanillo and Uovo de Piccione Olive Trees. *Acta Hort.* **356,** 176-180

Koutsaftakis, A. and Stefanoudaki, E. (1995) Results obtained on using dual-phase decanters to extract olive oil. *Olivæ* **56,** 44-47

Koutsaftakis, A., Kotsifaki, F. and Stefanoudaki, E. (1999). Effect of the extraction system stage of ripening and kneading temperature on the sterol composition of virgin olive oils. *J. Am. Oil Chem. Soc.* **76,** 1477-1481

Lavee, S. (2003) Intensification of olive cultivation. A hazard or benefit to environments in traditional and new growing regions. *Proceedings of International Symposium of olive tree and environment* (in press)

Lavee, S. and Wonder, M. (1991) Factors affecting the nature of oil accumulation in fruit of olive (*Olea europea L.*) cultivars *J. Hort. Sci.* **66,** 583-591

Lavee, S. and Wonder, M. (2004) The effect of yield, harvest time and fruit size on the oil content in fruits of irrigated olive trees (*Olea europaea*), cvs. Barnea and Manzanillo. *Sci. Hort.* **99,** 267–277

Lavee, S., Nashef, M., Wodner, M. and Harshemesh, H. (1990) The effect of complementary irrigation added to old olive trees (*Olea europaea* L.) cv Souri on fruit characteristics, yield and oil productions. *Adv. Hort. Sci.* **4,** 135–138

Lavelli, V. (2002) Comparison of the antioxidant activity of extra virgin olive oils. *J. Agric. Food Chem.* **50,** 7704-7708

Leon-Camacho, M. (1993) Determination de trigliceridos en aceites vegetales comestibles por HPLC. *Aliment Equip. Technol.* **3,** 45-49

Lercker, G., Frega, N., Bocci, F. and Servidio, G. (1994) "Veiled" extra- virgin olive oils: Dispersion response related to oil quality. *J. Am. Oil Chem. Soc.* **71,** 657-658

Liphschitz, N., Gophna, R., Hartman, M. and Biger, G. (1991) The beginning of olive olive (*olea europaea L.*) Cultivation in old world; a reassessment. *J. Archaeol. Sci.* **18,** 441-453

Lipworth, L., Martinez, M.E., Angell, J., Hsieh, C.C. and Trichopoulos, D. (1997) Olive oil and human cancer: an assessment of the evidence. *Prevent. Med.* **26,** 181–190

Litridou, M., Linssen, J., Schols, H., Bergmans, M., Posthumus, M., Tsimidou, M. and Boskou, D. (1997) Phenolics compounds in virgin olive oils: fractionation by solid phase extraction and antioxidant activity assessment *J. Sci. Food Agric.* **74,** 169-174

Los, D.A. and Murata, N. (1998) Structure and expression of fatty acid desaturases. *Biochim. Biophys. Acta* **1394,** 3-15

Loupassaki, M.H., Chartzoulakis, K.S., Digalaki, N.B. and Androulakis, I.I. (2002) Effect of salt stress on concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium in leaves, shoots, and roots of six olive cultivars. *J. Plant Nutrition* **25,** 2457-2482

Loupassaki, M.H., Perica, S. and Androulakis, I. I. (1993) Seasonal changes in the olive fruits and the effects of summer applied nitrogen and potassium. *Adv. Hort. Sci.* **7**, 65-68

Luchetti, F. (2002) Importance and future of olive oil in the world market – an introduction to olive oil. *Eur. J. Lipid Sc. Technol.* **104**, 559–563

Maas, E.V. and Hoffman, G.J. (1977) Crop salt tolerance-current assessment. *J. Irrig. Drain. Div.,* ASCE **103,** 115–134

Mariani, C., Fedeli, E., Grop, K. and Artho, A. (1991) Variation in the free and esterified minor components of oils obtained from olives in connection with maturation and storage. *Riv. Ital. Sost. Grasse* **68,** 179-187

Marin, L., Benlloch, M. and Fernandez-Escobar, R. (1995) Screening of olive cultivars for salt tolerance. *Sci. Hort.* **64**, 113–116

Martinez Moreno, J.M. and Pertuccioli, G. (1975) Oil extraction: *Manual of olive oil technology.* Edited within the framework of the project " Centre for improvement and demostration of olive production techniques" FAO, 31-46

Martinez Moreno, J.M., Gomez Herrera, C. and Janen del Valle, C. (1957) Estudios fisico- quimicos sobre las pastas de aceitunas molidas. Afinidades entre las fases solidasss y liquidas. *Grasas y Aceites* **8,** 155-161

Mendoza, J.A. (1975) Milling, and Malaxation*: Manual of olive oil technology.* Edited within the framework of the project "Centre for improvement and demostration of olive production techniques" FAO, 24-30

Mensink, R.P. and Katan, M.B (1992) Effect of dietary fatty acids on serum lipids and lipoprotein. *Arteriosclerosis thrombosis, and vascular biology.* **12,** 911-919

Mensink, R.P. and Katan, M.B. (1990) Effect of dietary trans fatty acids on high-density lipoprotein cholesterol levels in healthy subjects, *N. Eng. J. Med.* **323,** 439–445

Michelakis, N. (1990) Yield response of table and oil olives varieties to different water use levels under drip irrigation. *Acta Hort.* **286,** 271-274

Michelakis, N. (1995) Effect of water availability on the growth and yield of olive trees *Olivæ,* No 56, 29-39

Michelakis, N., Vouyoukalou, E., and Clapaki, G. (1995). Plant growth and yield response of olive tree cv. Kalamon for different levels of soil water potential and methods of irrigation. *Adv. Hort. Sci.* **9,** 136–139

Milella, A. and Dettori (1987) Rigimi idrici ottimali e parziali per giovani olivi da mensa . *Rivista di Frutticoltura* **8,** 65-69

Minchas P.S and Cupta. (1992) Quality of irrigation water. Assessment and managements. Information and publication section ICAR, p102

Minguez-Mosquera, M.I. and Garrido-Fernaandez, J. (1989) Chlorophyll and carotenoids presence in olive fruit (*olea europaea*). *J. Agric. Food Chem.* **37,** 1-7

Minguez-Mosquera, M.I., Rejano-Navarro, L., Gandul-Rojas, B., Sanchez-Gomez, A.H. and Garrido-Fernaandez, J. (1991). Colour-pigment correlation in virgin olive oil. *J. Am. Oil Chem. Soc.* **68,** 332-336

Miyashita, K. and Tagari, T. (1986) Study on the oxidative rate and prooxidant activity of free fatty acids. *J. Am. Oil Chem. Soc.* **63,** 1380-1384

Montedoro and Garofalo, (1984) The Qualitative Characteristics of Virgin Olive Oils. The Influence of Variables such as Variety, Environment, Preservation, Extraction, Conditioning of the finished Product. *Riv. Ital. Sost. Grasse* **61,** 157-168

Montedoro, G. F., Servili, M., Baldioli, M., Selvaggini, R., Perretti, G., Magnarini, C., Cossignani, L., & Daminai, P. (1995). Characterization of some Italian virgin olive oils in relation to origin area. *Riv. Ital. Sost. Grasse*, **72**, 403–404

Montedoro, G.F. (1989) Oil: Variety and technology: Two factors affecting quality. *Olivae* **29,** 28-30

Montedoro, G.F. and Cantarelli, C. (1969) Indagini sulle sostanze fenoliche presenti negli oli di oliva. *Riv. Ital. Sost. Grasse* **46,** 115-124

Montedoro, G.F. and Servili, M. (1992) Olive oil quality parameters in relationship to agronomic and technological aspects. *Riv. Ital. Sost. Grasse* **74,** 563-573

Montedoro, G.F., Bertuccioli, M. and Anichini, F. (1978) Aroma analysis of virgin olive oil by head space (volatiles) and extraction (polyphenols) techniques. In Charolambus, and Inglett, *Flavor of foods and beverages: chemistry and technology* 247-279 Academic Press Inc., NY

Montedoro, G.F., Garafolo, L. and Bertuccioli, M. (1986) Factors shaping the quality characteristics of an olive oil. *Industrie Alimentari* **25,** 549–555

Montedoro, G.F., Servili, M., Baldioli, M. and Miniati, E. (1992) Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolyzable fraction. *J. Agric. Food Chem.* **40,** 1577-1580

Montedoro, G.F., Servili, M., Baldioli, M., Selvaggini, R., Miniati, E. and Macchioni, A. (1993) Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivates. *J. Agric. Food Chem.* **41,** 2228-2234

Monteleone, E., Caporale, G., Carlucci, A. and Pagliarini, E. (1998) Optimization of extra virgin olive oil quality. *J.Sci.Food Agric.* **77,** 31-37

Morales, M. T., Calvente, J.J. and Aparicio, R. (1996) Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. *Flavour and Fragrance Journal* **11,** 171-178

Morales, M.T. and Aparicio, R. (1999) Effect of extraction conditions on sensory quality of virgin olive oil. *J. Am. Oil Chem. Soc.* **76,** 295-300

Morales, M.T. and Leon-Camacho, M. (2000) Gas and Liquid Chromatography: Methodology Applied to Olive Oil. *Handbook of Olive Oil. Analysis and Properties.* Eds. J. Harwood, R. Aparicio, Aspen Publication, Gaithersburg, MD (USA), 159-207

Morales, M.T. and Tsimidou, M. (2000) The role of volatiles compounds and polyphenols in olive oil sensory quality. *Handbook of Olive Oil. Analysis and Properties.* Eds. J. Harwood, R. Aparicio, Aspen Publication, Gaithersburg, MD (USA), 393-458

Morales, M.T., Alonso, V., Ríos, J.J. and Aparicio, R. (1995) Virgin olive oil aroma: relationship between volatile compounds and sensory attributes by chemometrics. *J. Agric. Food Chem.* **43,** 2925-2931

Morales, M.T., and Przybylski, R. (2000) Olive oil Oxidation. *Handbook of Olive Oil. Analysis and Properties.* Eds. J. Harwood, R. Aparicio, Aspen Publication, Gaithersburg, MD (USA), 459-490

Morales, M.T., Angerosa, F. and Aparicio, R. (1999) Effect of the extraction conditions of virgin olive oil on the lipoxygenase cascade: chemical and sensory implications. *Grasas y Aceites* **50,** 114-121

Morales, M.T., Aparicio, R. and Rios, J.J. (1994) Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *J. Chromatography A* **668,** 455-462

Morales, M.T., Rios, J.J. and Aparicio, R. (1997) Changes in the volatile composition of virgin olive oil during oxidation: Flavors and off- flavors. *J. Agric. Food Chem.* **45,** 2666-2673

Morello, J.R., Motilva, M.J, Ramo, T. and Romero, M.P. (2003) Effect of freeze injuries in olive fruit on virgin olive oil composition *Food Chem.* **81,** 547–553

Motilva, M.J., Tovar, M.J., Romero, M.P., Alegre, A. and Girona, J. (2000) Influence of regulated deficit irrigation strategies applied to olive trees (Arbequina

cultivar) on oil yield and oil composition during the fruit ripening. *J. Sci. Food Agric.* **80,** 2037-2043

Moure, A., Cruz, J.M., Franco, D., Dominguez, M., Sineiro, J., Dominguez, H., Nunez, M.J. and Parajo, J.C. (2001) Natural antioxidants from residual sources. *Food Chem.* **72,** 145-171

Mousa, Y.M., Gerasopoulos, D., Metzidakis, I. and Kiritsakis, A. (1996) Effect of altidute on fruit and oil quality characteristics of 'Mastoides' olives. *J Sci Food Agric.* **71,** 345-350

Murata, N. and Tasaka, Y. (1997) Glycerol-3-phosphate acyltansferase in plants. *Biochim. Biophys. Acta* **1348,** 10-16

Murphy, D.J. (1993) Structure, function and biogenesis of storage lipid bodies and oleosin in plants. *Prog. Lipid Res.* **32,** 247-280

Murphy, D.J. (1998) Mechanisms of lipid body formation, targeting and accumulation in plants and animals In: Sanchez, J., Cerda-Olmedo, E. and Martinez-Force, E., Editors, *Advances in plant lipid research.* Secretariado de publicationes, Universidad de Sevilla, 683-688

Nergiz, C. and Engel, Y. (2000) Compositional variation of olive fruit during ripening. *Food Chem.* **69,** 55-59

Noggle, G.R. and Fritz, G.J. (1983) Introductory Plant Physiology. Prentice-Hall, Englewood Cliffs, NJ

Olias, J.M., Pérez, A.G., Rios, J.J. and Sanz, L.C. (1993) Aroma of virgin olive oil: biogenesis of the "green" odor notes. *J. Agric. Food Chem.* **41,** 2368-2373

Orgaz, F., Pastor, M., Vega, V., Castro, J. (1996) Necesidades de aqua del olivar. In: Pastor, *Manejo del olivar con riego por goteo. Serie Informaciones Tecnicas* 41/96. Consejeria de Agricultura y Pesca. Seville, 11-44

Owen, R.W., Giacosa, A., Hull, W.E., Haubner, R., Spiegelhalder, B. and Bartsch (2000) The antioxidant /anticancer potential of phenolic compounds isolated from olive oil. *Eur. J. Cancer* **36,** 1235-1247

Owen, R.W., Giacosa, A., Hull, W.E., Haubner, R., Würtele, G., Spiegelhalder, B. and Bartsch (2000) Olive-oil consumption and health: the possible role of antioxidants. *The Lancet Oncology* 107-112

Paganuzzi, V. (1980) Distribuzione dei componenti alcolici dell' insaponificabile nella drupa dell' olivo. Nota v : alcoli alifatici. *Riv. Ital. Sost. Grasse* **57,** 341-345

Pannelli, G., Famiani, F., Servili, M. and Montedoro G.F. (1989) Agro-climatic factors and characteristics of the composition of olive oils. *Acta Hort.*, **286,** 447-480

Pannelli, G., Servili, M., Selvaggini, R., Baldioli, M. and Montedoro, G.F. (1994) effect of agronomic and seasonal factors on olive (Olea europaea L.) production and on the qualitative characteristics of the oil. *Acta Hort.* **356,** 239-243

Pansiot, E.P. and Rebour, H. (1961) Improvement in olive production, FAO, Rome, 249

Papadopoulos, G. and Boskou, D. (1991) Antioxidant effect of natural phenols on olive oil. *J. Am. Oil Chem. Soc.* **68,** 669-671

Passaloglou-Emmanouilidou, S. (1990) A Comparative study of UV spectrophotometric methods for detection of olive oil adulteration by refined oils. *Z. Lebensm, Unters. Forsch.* **191,** 132

Patumi, M., D'Andria, R., Fontanazza, G., Morelli, G., Giorio, P. and Sorrentino, G. (1999) Yield and oil quality of intensively trained trees of three cultivars of olive (Olea europaea L.) under different irrigation regimes. *J.Hortic. Sci. Biotechnol.* **74,** 729-737

Patumi, M., D'Andria, R., Marsilio, V., Fontanazza, G., Morelli, G. and Lanza, B. (2002) Olive and olive oil quality after intensive monocone olive growing (Olea europaea L., cv. Kalamata) in different irrigation regimes. *Food Chem.* **77,** 27–34

Pereira, J.A., Casal, S., Bento, A. and Oliveira M.B.P.P. (2002) Influence of olive storage period on oil quality of three Portuguese cultivar of *Olea europea,* Cobrancosa, Madural, and Verdeal Transmontana *J. Agric. Food Chem.* **50,** 6335-6340

Perez-Camino, M.C., Garcia, J.M. (1992) Polar compound concentrations in virgin olive oils from stoered cultivars Picual olive fruits *J. Agric. Food Chem.* **40,** 2260-2262

Perry, H.J. and Harwood, J.L. (1994) The carbon flux to triacylglycerol in maturing oilseed rape embryos. *Biochem. Soc. Trans.* **22,** 203S

Perry, H.J., Bligny, R., Goutb, G. and Harwood, J.L. (1999) Changes in Kennedy pathway intermediates associated with increased triacylglycerol synthesis in oil-seed rape. *Phytochemistry* **52,** 799-804

Petroni, A., Blasevich, M., Salami, M., Papini, N., Montedoro, G.F, and Galli, C. (1995) Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb. Res.* **78,** 151–160

Pinelli, P., Galardi, C., Mulinacci, N., Vinccieri, F.F., Cimato, A. and Romani, A. (2003) Minor polar compounds and fatty acid analysies in monocultivar virgin olive oils from Tuscany. *Food Chem.* **80,** 331-336

Planchairs, N. (1982) Palynologie lagunaire de l'Etang de Mauguio. Pale´ oenvironnement ve´ge´tal et e´volution anthropique. *Pollen Spores* **24,** 93–118

Psomiadou, E. and Tsimidou, M. (1998) Simultaneous HPLC determination of tocopherols, carotenoids and chlorophylls for monotoring their effect on virgin olive oil oxidation *J. Agric. Food Chem.* **46,** 5132-5138

Psomiadou, E. and Tsimidou, M. (2001) Pigments in Greek virgin olive oils: occurrence and levels. *J. Sci. Food Agric.* **81,** 640-647

Psomiadou, E. and Tsimidou, M. (2002) Stability of virgin olive oil 1, autooxidation studies. *J. Agric. Food Chem.* **50,** 716-721

Rahmani, M. and Saari Csallany, A. (1998) Role of minor constituents in the photoxidation of virgin olive oil. *J. Am. Oil Chem. Soc.* **75,** 837-843

Ranalli, A. (1998) Influence of paste stone size on the output of olive oil extraction process. Note II: Centrifugation system. *Riv. Ital. Sost. Grasse vol.* **65,** 13-19

Ranalli, A. and Angerosa, F. (1996) Integral centrifuges for olive oil extraction. The qualitative characteristics of products. *J. Am. Oil Chem. Soc.* **73,** 417–421

Ranalli, A. and Martinelli, N. (1995). Integral centrifuges for olive oil extraction at the third millennium threshold. Transformation yield. *Grasas y Aceites* **46,** 255–263

Ranalli, A., Cabras, P., Iannucci, E. and Contento, S. (2001) Lipochromes, vitamins, aromas and other components of virgin olive oil are affected by processing technology. *Food Chem.* **73,** 445-451

Ranalli, A., Contento, S., Schiavone, C. and Simone, N. (2001) Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. *Eur. J. Lipid Sc. Technol.* **103,** 228-238

Ranalli, A., De Mattia, G., Patumi, M. and Proietti, P. (1999) Quality of virgin olive oil as influenced by origin area. *Grasas y Aceites* **50,** 249–259

Ranalli, A., Pollastri, L., Contento, S., Di Loreto, G., Iannucci, E., Lucera, L. and Russi, F. (2002) Acylglycerol and fatty acid components of pulp, seed, and whole olive oils. Their use to characterize fruits variety by chemometrics. *J. Agric. Food Chem.* **50,** 3775-3779

Renfrew, J.M. (1973) Palaeoethnobotany. *The food plants of the near East and Europe*. Columbia Univ. Press, New York, NY, 248

Roche, H.M., Gibney, M.J., Kafatos, A., Zampelas, A. and Williams, C.M. (2000) Beneficial properties of olive oil. *Food research International* **33,** 227-231

Rodis, P., Karathanos, V. and Mantzaninou, A. (2002) Partitioning of olive oil antioxidants between oil and water phases. *J. Agric. Food Chem.* **50,** 596-601

Rose, D.P. and Connolly, J.M. (1999) Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacol. & Ther.* **83,** 217-244

Rovelini, P., Cortesi, N. and Fedeli, E. (1997) Lipid Oxidation. Note 1: *Riv. It. Sost. Grasse* **74,** 181-189

Rugini, E. and Fedeli, E. (1990) Olive (*Olea europaea L.*) as an oil seed. In: Bajaj, Y.P.S. (Ed.), *Biotechnology in Agriculture and Forestry. Legumes and Oilseed Crops I*, Vol. 10. Springer, Berlin, 593–641

Ruiz-Gutierrez, V., Morgado, N., Prada, J.L., Perez-Jimenez, F. and Muriana, F.J. (1998) Composition of human VLDL triacylglycerols after ingestion of olive oil and high oleic sunflower oil. *J. Nutrition* **128,** 570–576

Ruiz-Gutierrez, V., Muriana, F.J., Guerrero, A. and Villar, J. (1997) Olive oil and high oleic sunflower oil on human plasma and erythrocyte membrane lipid. *National Research* **17,** 1391-1399

Rutter, A.J., Sánchez, J. and Harwood, J.L. (1997) Glycerolipid synthesis by microsomal fractions from *Olea europaea* fruits and tissue cultures. *Phytochemistry* **46** 265-272

Ryan and Robard (1998) Phenolic compounds in olives. *Analyst* **123,** 31R-44R

Ryan, D., Robards, K. and Lavee, S. (1999) Determination of phenolic compounds in olives by reversed-phase chromatography and mass spectrometry. *J. Chromatography A,* **832,** 87–96

Saija, A., Trombetta, D., Tomaino, A., Lo Cascio, L., Princi, P., Uccella, N., Bonina, F. and Castelli, F. (1998) In vitro' evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. *Int. J. Pharm.* **166,** 123–133

Salas, J., Pastor, M., Castro, J. and Vega, V. (1997) Influencia del riego sobre la composicion y caracteristicas organolepticas del aceite de oliva. *Grasas y Aceites* **48,** 74-82

Salas, J.J. and Sanchez, J. (1998a). Hydroperoxide lyase from olive (Olea europaea) fruits. In J. Sanchez, E. Cerda-Olmedo, & E. Martinez-Force, *Advances in plant lipid research.* Spain: Secretariado de publicationes, pp. 300-302. Universidad de Sevilla

Salas, J.J. and Sanchez, J. (1998b). Alcohol dehydrogenases from olive (Olea europaea) fruit. *Phytochemistry* **48,** 35-40

Salas, J.J. and Sanchez, J. (1998c). Lipoxygenase activity from the pulp tissues of olive (Olea europaea) fruits. In J. Sanchez, E. Cerda Olmedo, & E. Martinez-Force, *Advances in plant lipid research* (pp.297-299). Spain: Secretariado de publicationes, Universidad de Sevilla

Salas, J.J. and Sanchez, J. (1999) The decrease of virgin olive oil produced by high malaxation temperature is due to inactivation of hydroperoxide lyase. *J. Agric. Food Chem.* **47,** 809-812

Salas, J.J., Sanchez, J., Ramli, U.R., Manaf, A.M., Williams, M. and Harwood, J.L. (2000) Biochemistry of lipid metabolism in olive and other oil fruits. *Prog. Lipid Res.* **39,** 151-180

Salas, J.J., Williams, M., Harwood, J.L. and Sanchez, J. (1999) Lipoxygenase activity in olive (*Olea europaea*) fruit. *J. Am. Oil Chem. Soc.* **76,** 1163-1168

Salivaras, E. and McCurdy, A. R. (1992) Detection of olive oil adulteration with canola oil from triacylglycerol analysis be reversed phase high-performance liquid-chromatography. *J. Am. Oil Chem. Soc.* **69,** 935–938.

Salvador, M.D., Aranda, F. and Fregapane, G. (2000) Influence of fruit ripening on 'Cornicabra' virgin olive oil quality A study of four successive crop seasons. *Food Chem.* **73,** 45-53

Salvador, M.D., Aranda, F., Gomez-Alonso, S. and Fregapane, G. (2001) Cornicabra virgin olive oil: a study of five crop seasons. Composition, quality and oxidative stability. *Food Chem.* **74,** 267-274

Sanchez, J. and Harwood, J.L. (1992) fatty acid synthesis in soluble fractions from olive (*Olea europea*) fruits. *J. Plant Physiol.* **140,** 129-134

Sanchez, J. and Harwood, J.L. (2002) Biosynthesis of triacylglycerols and volatiles in olives. *Eur. J. Lipid Sc. Technol.* **104,** 564-573

Sánchez, J. and Salas, J.J. (2000) Biogenesis of olive oil aroma. In: *Handbook of Olive Oil: Analysis and Properties.* Eds. J. L. Harwood, R. Aparicio, Aspen Publishers, Gaithersburg, MD. (USA), 79-99

Sánchez, J., del Cuvillo, M.T. and Harwood, J.L. (1992) Glycerolipid biosynthesis by microsomal fractions from olive fruits. *Phytochemistry* **31,** 129-134

Sanchez-Muniz, F.J, Oubina, P., Benedi, J., Rodenas, S. and Cuest, C. (1998) A preliminary study on platelet aggregation in postmenopausal women consuming extra- virgin olive oil and high –oleic acid sunflower oil. *J. Am. Oil Chem. Soc.* **75,** 217-223

Sanders, A. B. (1983) Nutritional significance of rancidity. *In rancidity in foods,* pp 59-66. Edited by J.C. Allen and R.J. Hamilton. London: Applied Science Publishers

Scaramuzzi, F. and Roseli, G. (1986) Olive genetic improvement. *Olea* **17,** 21-25

Servili, M., and Montedoro, G.F. (2002) Contribution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sc. Technol.* **104,** 602-613

Servili, M., Baldioli, M. and Montedoro, G.F. (1994) Phenolic composition of virgin olive oil in relationship to some chemical and physical aspects of malaxation. *Acta Hort.* **396,** 331-336

Servili, M., Baldioli, M., Maiotti, F. and Montedoro, G.F. (1999) Phenolic composition of olive fruit and virgin olive oil: distribution in the constitutive parts of fruit and evolution during oil mechanical extraction process. *Acta Hort.* **474,** 609-613

Servili, M., Baldioli, M., Selvaggini, R., Macchioni, A. and Montedoro, G.F. (1999) Phenolic compounds of olive fruit: One- and two- dimentional nuclear Magnetic Resonance Characterization of Nüzhenide and its distribution in the constitutive parts of the fruit. *J. Agric. Food Chem.* **47,** 12-18

Servili, M., Baldioli, M., Selvaggini, R., Mariotti, F., Federici, E. and Montedoro, G.F. (1998) Effect of malaxation under N2 flush on phenolic and volatile compounds of virgin olive Oil. In J. Sanchez, E. Cerda Olmedo, & E. Martinez-Force, *Advances in plant lipid research* (pp. 307-310). Spain: Secretariado de publicationes, Universidad de Sevilla

Servili, M., Piacquadio, P., De Stefano, G., Taticchia, A. and Sciancalepore, V. (2002) Influence of a new crushing technique on the composition of the volatile compounds and related sensory quality of virgin olive oil. *Eur. J. Lipid Sc. Technol.* **104,** 483–489

Sibbett, G. and Osgood, J. (1994) Site selection and preparation, tree spacing and design, planting, and initial training. In: Ferguson, L., Sibbett, G.S., Martin, G.C. (Eds.), *Olive: Production Manual*, Publication 3353. University of California, Davies, CA, 31–37

Snyder, J.M., Frankel, E.N. and Selke, E. (1985) Capillary gas chromatographic analysis of headspace volatiles from vegetable oils. *J. Am. Oil Chem. Soc.* **62,** 1675-1679

Soler-Rivas, C., Espin, J.C. and Wichers, H.J. (2000) Oleuropein and related compounds *J Sci Food Agric* **80,** 1013-1023

Solinas, M., Angerosa, F. and Camera, L. (1988): Evoluzione ossidativa di oli vegetali durante la frittura: determinazione dei componenti volatili mediante HRGC e HPLC. *Riv. It. Sost. Grasse* **65,** 567-574

Solinas, M., Angerosa, F., Cucurachi, A. (1987a) Relation between the autoxidation of oils and fats and the rising of Rancidity in organoleptic Evaluation. Note 2 *Riv.Ital. Sost. Grasse.* **64,** 137

Solinas, M., Giovacchino, L. and Mascolo, A. (1987c) The polyphenols of olives and olive oils. Note III. Influence of temperature and kneading time on the oil polyphenol content. *J. Am. Oil Chem. Soc.* **55,** 19–23

Solinas, M., Marsilio, V. and Angerosa, F. (1987b) Behaviour of some components of virgin olive oils flavor in connection with the ripening of the olives. *Riv.Ital. Sost. Grasse* **64,** 475–480

Stefanoudaki, E. and Koutsaftakis, A. (1992) Studies on total polyphenols and Chlorophyll content of olivo oil during the ripening of olive fruits in the area of Crete. *"Olive Oil Quality"* Firenze, 381-383

Stefanoudaki, E., Kotsifaki, F. and Koutsaftakis, A. (1997) The Potential of HPLC Triglyceride Profiles for The Classification of Cretan Olive Oils *Food Chem.* **60,** 425-432

Stefanoudaki, E., Koutsaftakis, A. and Papamanolioudaki, A. (2003) Effect of environmental conditions on olive fruit development and on the triacylglycerol and fatty acids profiles. *Proccedings of International Symposium of olve tree and environment (in press)*

Stefanoudaki. E., Kotsifaki, F. and Koutsaftakis, A. (1999) Classification of Virgin Olive Oils of the Two Major Cretan Cultivars Based On Their Fatty Acid Composition. *J. Am. Oil Chem. Soc.* **76,** 623-626

Sultana, N., Ikeda, T. and Itoh, R. (1999) Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Env. Exp. Bot.* **42,** 211-220

Tacchino, E. and Borgoni, C. (1983) Indagine sul contenuto di alcoli alifatici dell'olio d'oliva di pressione e di estrazione. *Riv.Ital. Sost. Grasse* **60,** 575-581

Tattini, M. (1994) Ionic relations of aeroponically-grown olive plants during salt stress. *Plant and Soil.* **161,** 251–256

Tattini, M., Gucci, R., Coradeschi, M.A., Ponzio, C. and Everard, J.D. (1995) Growth, gas exchange and ion contentin Olea europaea plants during salinity stress and subsequent relief. *Physiol. Plant* **95,** 203–210.

Tattini, M.P., Bertoni, P. and Caselli, S. (1992) Genotypic responses of olive plants to sodium chloride. *J. Plant Nutr.* **15,** 1467–1485

Terral, J.F. (1996) Wild and cultivated olive (*olea europaea L.*): a new approach to an old problem using inorganic analysis of modern wood and archaeological charcoal. *Review of paleobotany and Palynology* **91,** 383-392

Terral, J.F. (2000) Exploitation and management of the olive tree during Prehistoric Times in mediterranean France and Spain. *J. Archaeol. Sci.* **27,** 127–133

Therios, I.N., Misopolinos, N.D. (1988) Genotypic response to sodium chloride salinity of four major olive cultivars (*Olea europaea* L.). *Plant and Soil* **106,** 105–111

Tiscornia, E., Fiorina, N. and Evangelisti, F. (1982) Chemical composition of olive oil and variations induced by refining. *Riv.Ital. Sost. Grasse* **59,** 519-556

Tombesi, A. (1994) Olive fruit growth and metabolism. *Acta Hort.* **356,** 225-232

Tombesi, A., Michelakis, N. and Pastor. (1996) Recommendations of working group on olive farming production and productivity. *Olivæ* **63,** 38-51

Tous, J., Romero, A., Plana, J., Guerrero, L., Diaz, I. and Hermoso, J.F. (1997) Caracteristicas quimico-sensoriales de los aceites de oliva « Arbequina » obtenidos en distintas zonas de Espana. *Grasas y Aceites* **48,** 415–424

Tovar, M.J., Motilva, M.J. and Romero, M.P. (2001). Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea L.* cv. Arbequina) grown under linear irrigation strategies. *J. Agric. Food Chem.* **49,** 5502–5508

Tovar, M.J., Romero, M.P., Alegre, S., Girona, J. and Motilva, M.J. (2002) Composition and organoleptic characteristics of oil from Arbequina olive (*Olea europaea L*) trees under deficit irrigation. *J. Sci. Food Agric.* **82,**1755–1763

Triat-Laval, H. (1982) Pollen-analyse de sédiments quaternaires récents du pourtour de l'étang de Berre. *Ecol. Mediterr.* **84,** 98–136

Trichopoulou, A. and Lagiou, P. (1997) Healthy traditional Mediterranean diet: an expression of culture, history and lifestyle. *Nutr Rev.* **55,** 383–389

Trichopoulou, A., Katsouyanni, K., Stuver, S., Tzala, L., Gnardellis, C., Rimm, E. and Trichopoulos, D. (1995a) Consumption of olive oil and specific food groups in relation to breast cancer risk in Greece. *J. Natl. Cancer Inst.* **87,** 110–116

Trichopoulou, A., Kouris-Blazos, A., Vassilakou, T., Gnardellis, C., Polychronopoulos, E., Venizelos, M., Lagiou, P., Wahlqvist, M.L. and Trichopoulos, D. (1995b) Diet and survival of elderly Greeks: a link to the past *Am. J. Clin. Nutr.* 1995 **61**, 1346S-1350

Trichopoulou, A., Vasilopoulou, E., Hollman, P., Chamalides, Ch., Foufa, E., Kaloudis, Tr., Kromhout, D., Miskaki, Ph., Petrochilou, I., Poulima, E., Stafilakis, K. and Theophilou, D. (2000) Nutritional composition and flavonoid

content of edible wild greens and green pies: a potential rich source of antioxidant nutrients in the Mediterranean diet. *Food Chem.* **70,** 319-323

Tsimidou, M. and Boskou, D. (1997) Phenolics compounds in virgin olive oils: fractionation by solid phase extraction and antioxidant activity assessment *J. Sci. Food Agric.* **74,** 169-174

Tsimidou, M., Papadopoulos, G. and Boskou, D. (1992) Determination of phenolic compounds in virgin olive oil by reversed-phase HPLC with emphasis on UV detection. *Food Chem.* **45,** 141-144

Ulberth, F. and Buchgraber, M. (2000) Authenticity of fats and oils *Eur. J. Lipid Sc. Technol.* **102,** 687–694

USDA, (2002). Vegetable oil production and consumption

Valasco, J and Dobarganes C. (2002) Oxidative stability of virgin olive oil. *Eur. J. Lipid Sc. Technol.* **104,** 661-676

Valenzuela A., King, J. and Nieto, S. (1995) *Trans* fatty isomers from hydrogenated fats: The controversy about health implications. *Grasas y Aceites* **46,** 369-375

Valenzuela, A. and Nieto, S. (1996) Synthetic and natural antioxidants: food quality protectors. *Grasas y Aceites* **47,** 186-196

Vazquez Roncero, A., Janer Del Valle, C. and Janer Del Valle, L. (1976) Componentes fenòlicos de la aceituna. III Polifenoles del aceite. *Grasas y Aceites* **27,** 185-191

Vick, B.A. and Zimmerman, D.C. (1987) The lipoxygenase pathway. In: Stumpf, P.K., Mudd, J.B. and Nes, W.D., Editors, 1987. *The metabolism structure and function of plant lipids*, Plenum Press, New York and London, 383-390

Vierhuis, E. (2002) Structural characteristics of polysaccharides from olive fruit cell walls in relation to ripening and processing. Ph.D. thesis Wageningen University

Vierhuis, E., Servili, M., Baldioli, M., Schols, H.A., Alphons, Voragen, A.G. J. and Montedoro, G.F. (2001) Effect of Enzyme Treatment during Mechanical Extraction of Olive Oil on Phenolic Compounds and Polysaccharides. *J. Agric. Food Chem.* **49,** 1218-1223

Visioli, F. and Galli, C. (1998) Olive oil polyphenols and their potential effects on human health *J. Agric. Food Chem.* **46,** 492 –4296

Visioli, F., Bellomo, G., and Galli, C. (1998) Free radical-scavenging properties of olive oil polyphenols. *Biochem. Biophys. Res. Comm.* **247,** 60–64

Visioli, F., Bellomo, G., Montedoro, G.F. and Calli, C. (1995) Low density Lipoproteine oxidation is inhibited in vitro by olive oil constituents *Atherosclerosis* **117,** 25-32

Visioli, F., Borsani, L. and Galli, C. (2000) Diet and prevention of coronary heart disease: the potential role of phytochemicals. *Cardiovascular Res.* **47,** 419-425

Visioli, F., Galli, C. and Caruso, D. (2002) Biological activities and metabolic fate of olive oil phenols. *Eur. J. Lipid Sc. Technol.* **104,** 677–684

Visioli, F., Poli, A. and Galli, C. (2002) Antioxidant and Other Biological Activities of Phenols from Olives and Olive Oil. *Med. Res. Rev.,* **22,** 65-75

Wartburg, U., Kratz, M. and Cullen, P. (2002) Mediterranean diet, olive oil and health. *Eur. J. Lipid Sc. Technol.* **104,** 698-705

Weisburger (1997) Dietary fat and risk of chronic disease: Mechanistic insights from experimental studies. *J. Am. Diet. Assoc.* **97,** S16-S19

West, D.W., Hoffman, G.J., Fisher, M.J., (1986) Photosynthesis, leaf conductance and water relations of cowpea under saline conditions. *Irrig. Sci. J.* 183–193.

Wiesman, Z., Itzhak, D. and Ben Dom, N. (2004) Optimization of saline water level for sustainable Barnea olive and oil production in desert conditions. *Sci. Hort.* **100,** *257-266*

Willett, W.C., Sacks, F., Trichopoulou, A., Drescher, G., Ferro-Luzzi, A., Helsing, E. and Trichopoulos, D. (1995) Mediterranean diet pyramid: a cultural model for healthy eating. *Am. J. Clin. Nutr.* **61,** 1402S-1406

Xyloyiannis C., Dichio, B., Nuzzo, V. and Celano, G. (1999) Defence strategies of olive against water stress. *Acta Hort.* **474,** 423 -426

Yanishieva-Maslarova, N. (1985) Differences in the kinetics and mechanism of autoxidation of stearic acid and tristearin. *Grasas y Aceites* **36,** 115-119

Yeo, A.R., Caporn, S.J.M., Flowers, T.J., (1985) The effect of salinity upon photosynthesis in rice (Oryza sativa L.). Gas exchange by individual leaves in relation to their salt content. *J. Exp. Bot.* **36,** 1240–1248.

Zarrouk, M., Marzouk, B., Ben Miled Daoud, D. and Cherif, A. (1996) Oil accumulation in olives and effect of salt on their composition. *Olivæ* **1,** 41-45

Zock, P.L. and Katan, M.B. (1992) Hydrogenation alternatives: effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in human. *J Lipids Res* **33,** 399

Zohary, D. and Hopf, M. (1993) Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley, 2nd ed. Clarendon Press, Oxford, UK

Zohary, D. and Spiegel-Roy, P. (1975) Beginning of fruit growing in the Old World. *Science* **187,** 319–327