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Influence of genetic and technological factors on the quality parameters of vegetable oils

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SOMMARIO

Il progetto di dottorato consiste nella determinazione dei fattori genetici (cultivar), tecnologici (tecniche di estrazione) e di raffinazione che influenzano la qualità dell'olio di oliva e di altri oli vegetali. Nella prima parte di questo progetto saranno messi a confronto 11 oli extravergini di oliva monovarietali, evidenziando le differenze in termini di composizione chimico-fisica, sensoriali e verificando come la cultivar possa influenzare per il 50%, assieme ad altri fattori, la composizione chimica e sensoriale nonché il contenuto di sostanze bioattive.

La seconda parte consiste nella determinazione dei principali costituenti dell'olio extravergine di oliva tradizionale, denocciolati e denocciolati con la successiva aggiunta di nocciole. In particolare, acidità, perossidi, tempo di induzione attraverso Rancimat, tocoferoli e composti organici volatili sono stati analizzati in tre campioni di olio sopra descritti al momento dell'imbottigliamento e dopo 3, 6 e 12 mesi, verificando l'evoluzione dei parametri sopra indicati per ottimizzare il processo produttivo e per verificare la qualità dell'olio.

Nella terza parte del progetto, i parametri di acidità, perossidi, tempo di induzione attraverso Rancimat, tocoferoli e tocotrienoli sono stati analizzati in diversi oli vegetali (girasole, girasole alto oleico, mais, semi d'uva, soia) e in diverse fasi di raffinazione (olio greggio, essiccato, raffinato, sbiancato, deodorato, neutralizzato). In questo modo sono stati esaminati l'effetto della fase di raffinazione sui vari oli e la qualità. I risultati ottenuti potranno servire a migliorare i processi produttivi degli oli di oliva e vegetali, sia in termini di qualità che di shelf-life del prodotto.

ABSTRACT

The PhD project consists in the determination of genetic (cultivars), technological (extraction techniques) and refining factors that influence the quality of olive oil and other vegetable oils. In the first part of this project, 11 monovarietal extra virgin olive oils will be compared, highlighting the differences in terms of chemical-physical composition, sensory and verifying how the cultivar can influence for 50%, together with other factors, the chemical and sensory composition as well as the content of bioactive substances.

The second part consists in the determination of the main constituents of traditional extra virgin olive oil, pitted and pitted with the subsequent addition of kernels. Acidity, peroxides, induction time through Rancimat, tocopherols and volatile organic compounds were analyzed in three oil samples described above at the time of bottling and after 3, 6 and 12 months, verifying the evolution of the above parameters to optimize the production process and to verify the quality of the oil.

In the third part of the project, the parameters of acidity, peroxides, induction time through Rancimat, tocopherols and tocotrienols were analyzed in different vegetable oils (sunflower, high oleic sunflower, corn, grape seeds, soybeans) and in different refining phases (crude oil, dried, refined, bleached, deodorized, neutralized). In this way, the effect of the refining phase on the various oils and the quality were examined. The results obtained will be used to improve the production processes of olive and vegetable oils, both in terms of quality and shelf-life of the product.

SUMMARY

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1. INTRODUCTION

Olive tree belongs to the tribe *Oleae* from the family *Oleacea*e, including around 600 specious and some 25 genera, including *Olea*, which has an economically important European olive tree known as *Olea europaea L*. As such, commercial olives belong to the *Olea europaea L*., one of the twenty species found in tropical and subtropical areas. *Olea europaea L*. is the only one able to produce edible fruit (Sibbett & Ferguson 2005).

The plantation of *Olea europaea L*. takes about six years to reach the fully productivity. The plant is characterized by small, white and feathery flowers with ten cleft calyx and corolla, two stamens and bifid stigma. Flowers are constituted in racemes springing from the axils of the leaves. The fruit is a small drupe 1-2.5 cm (0.39–0.98 in) long. Olives are harvested in a green and a purple stage. The drupe has a seed commonly referred to as a pit, rock or stone. The main crop variables are climatic and environmental conditions, orchard design, pruning, fertilization, irrigation, plant health protection, harvesting and finally the cultivar. Suitable areas for the olive growing are characterized by minimum temperatures of -6/-7 °C. Temperatures of -3 or -4 °C can promote negative impact on olives, also leading to negative repercussions on drupe quality. The best areas for the olive cultivation are characterized by mild winters, temperatures rarely under 0° C and dry, sizzling summer. Rainfalls should be between 400 and 1000 mm and well distributed during the year (Ouhmad-Sbitri & Serafini 2007). About the soil, the presence of concretion calcareous, ferruginous and tophaceous can limit the development of root system. A well-balanced soil texture in terms of sand, silt and clay is the best substrate for the olive cultivation. Anyway, the olive is a hard tree capable of growing and producing fruit even in adverse environmental conditions. In the past, traditional orchards were planted on a square 6 m x 6 m with a density of 236 plants per hectare. Recently, high density cropping systems (also called super-intensive orchards) have allowed to reach till 1700 plants per hectare taking on a square of 3 m x 1.5 m. To achieve the best orchard condition, the pruning practices play a fundamental role. Pruning forms are tightly related with orchard characteristics and management (e.g., level of meccanization, agronomical practice), environmental conditions and long-standing traditions. Anyhow, the main aim of pruning consists in enhancing productivity, promoting regular and economically relevant fruiting. By considering the requirements of trees, the fertilization must guarantee the right amount of nutrients for correct development of the olive orchard. The nutritional needs of olive orchards should be satisfied avoiding, or at least cut down, the environmental impact. The best fertilization practices should enable to reach high quality production and yield with minimal use of fertilizer.

Due to its evergreen nature, olive is characterized by the presence of nutrient reserve organs, for this reason it has lower nutritional requirements than other plants. The principal nutrients for the culture are nitrogen (N), potassium (K), iron (Fe), boron (B) and calcium (Ca). Their deficiency leads to imbalances, which can negatively affect the harvesting. Considering water requirements of olive, the plant is characterized by high water stress resistance. As such a series of physiological mechanisms allow to keep the vital functions of olive tree also in presence of significant water stress. Nevertheless, under such conditions a decrease in quality production and olive yield can be often seen. Irrigation is traditionally used to promote quality on table olives, while olives for oil are traditionally rainfed.
The healthiness of plant is a further factor able to hardly affect olive yield and quality. As such, during the last fifty years the protection methods have been continuously changed. As well as for the other crops, plant protection of the olive started from blind chemical control (calendar based) to reach "integrated production" (Boller et al., 2004) and "organic production" (Council regulation 1991). Despite the first method, the newest one is taking in huge consideration not only the olive production but also the environmental equilibrium. Due to its botanical features, dietary benefit, attractiveness of its products as well as fruit and oil, *Olea europea L.* is a relevant crop, getting on its value all around the global market. During the last years, the International Olive Council, with the aim to guarantee future sustainable growth and balanced supply and demand, organized campaigns to promote olive and its products all around in the world. It is well known that the most important production zones of olives are located in the Mediterranean area and their consumption is expanding, due to the increasing interest for Mediterranean diet (Ryan & Robards, 1998); (Soler-Rivas et al., 2000); (Vinha et al., 2005).

ECONOMIC IMPORTANCE AND PRODUCTION OF EXTRA VIRGIN OLIVE OIL

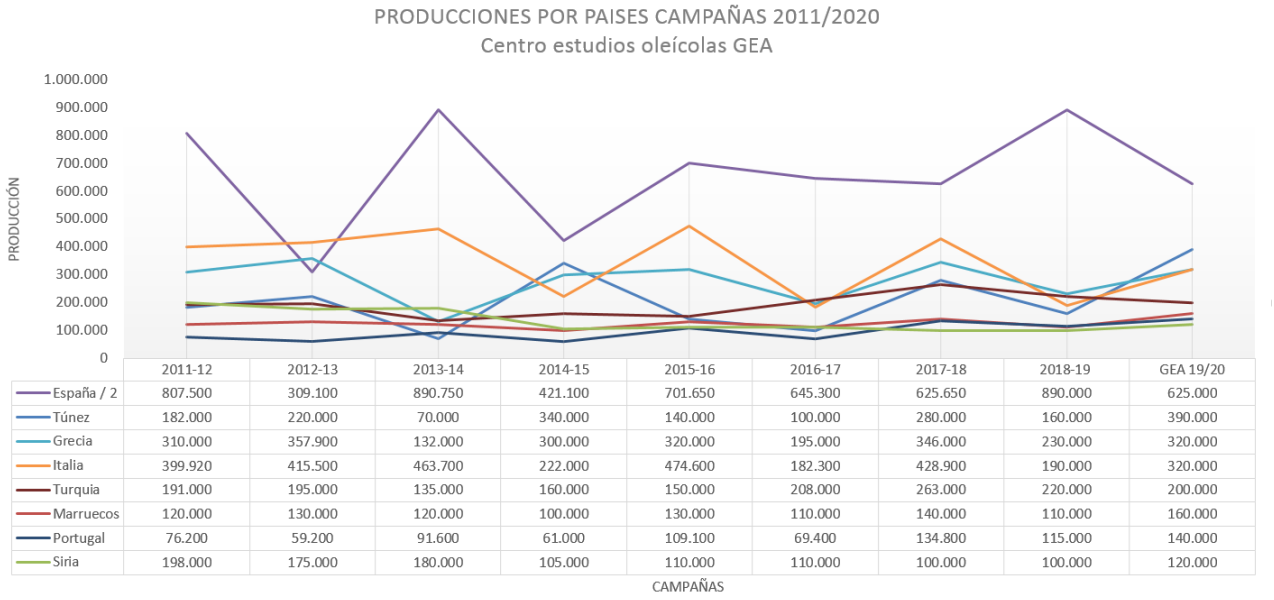
The cultivation area of the olive tree develops from the 30th to the 45th parallel of North latitude, in a temperate climate range, called precisely "olive region", and in the Mediterranean region, where about 94% of the total world oil production is concentrated. This area includes countries such as Italy, the south of Spain and France, Greece and some Middle Eastern countries bordering the eastern Mediterranean, however currently the olive tree is also cultivated in the temperate-warm regions of America, Africa and Australia.
World olive oil production reached 3,222,090 tons in the 2019/2020 marketing year (study carried out by the Department of Olive Studies of the Center of Excellence of Olive Oil of GEA). Spanish production is falling, while countries such as Tunisia, Italy and Greece are significantly increasing their share compared to the previous year.

Spain achieves a production of 1,250,000 tons, or 38.8% of world production. Tunisia is the second-largest producer country with a record of 390,000 tons (12% of world production) of olive oil. Greece shares the world's third-largest position with Italy, reaching 320,000 tons of olive oil (10% of world production). Italy reaches 320,000 tons (10% of world production).

Turkey, which is in a position immediately following Italy and Greece, will maintain a similar production to the previous year, with 200,000 tons of olive oil (6% of world production).

Morocco, whose production is estimated at 160,000 tons (5% of world production), followed. Portugal is immediately on the world stage, increasing production to 140,000 tons. Finally, Syria closes the group of the eight largest producing countries, which reaches 120,000 tons, through which it represents 3.7% of the world's production.

These eight major world producers contribute a total of 87.3%. As you can see in the graph, there is a lot of equality in production between Italy, Greece and Tunisia. One can also see equality in production between Morocco and Portugal, which in recent years alternate with the sixth and seventh positions in the ranking.



	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	GEA 19/20
España / 2	807.500	309.100	890.750	421.100	701.650	645.300	625.650	890.000	625.000
Túnez	182.000	220.000	70.000	340.000	140.000	100.000	280.000	160.000	390.000
Grecia	310.000	357.900	132.000	300.000	320.000	195.000	346.000	230.000	320.000
Italia	399.920	415.500	463.700	222.000	474.600	182.300	428.900	190.000	320.000
Turquia	191.000	195.000	135.000	160.000	150.000	208.000	263.000	220.000	200.000
Marruecos	120.000	130.000	120.000	100.000	130.000	110.000	140.000	110.000	160.000
Portugal	76.200	59.200	91.600	61.000	109.100	69.400	134.800	115.000	140.000
Siria	198.000	175.000	180.000	105.000	110.000	110.000	100.000	100.000	120.000

Table 1: world production of olive oil (from website https://www.agrodigital.com/2018/09/11/espana-producira-mas-del-50-del-aceite-de-oliva-mundial-en-la-proxima-campana/)

By considering the latest available FAO database collections, there were 42 countries, which were able to count a production of olives across the world in the 2017 (FAO, 2017). In this contest, Spain, Italy, and Greece were the top three contributors, respectively, with 6,549,499, 2,576,891, and 2,720,488 tons/year. Their amount of olives accounted for more than 50% of the world's production. Noteworthy among the main olive producers Turkey and Morocco represented respectively 11% and 10% of the global production, whereas, far from the Mediterranean basin, Argentina and Peru

followed by Chile were the major olive contributors in South America, and the United States joined the olive producing countries as unique producer in North America (FAO, 2017). It is also possible to divide the total production of olives into two sets. In effect, considering the properties of drupes, such as oil content, size, taste, etc., the olives may be preferred for olive oil production or for eating as table olives. Nevertheless, many cultivars are classified as dual-purpose. Currently, there are more than 1200 cultivars characterized and noted in 52 countries (Bartolini, 2007). Some popular olive cultivars, their features and utilization are reported in *Table 2:*

Cultivar	Main feature	Utilization	Origin
Arbequina	Early start of bearing and crop volume	Oil extraction	Spain
Koroneiki		Oil extraction	Greece
Manzanilla		Table olive	Spain
Maurino		Oil extraction	Italy
Picual		Oil extraction	Spain
Arbequina	Oil quality	Oil extraction	Spain
Frantoio		Oil extraction	Italy
Moraiolo		Oil extraction	Italy
Picual		Oil extraction	Spain
Dolce Agogia	Cold resistance	Oil extraction	Italy
Orbetana		Dual-purpose	Italy
Leccino		Oil extraction	Italy
Nostrale di Rigali		Dual-purpose	Italy
Cobrancosa	Lime tolerance	Oil extraction	Portugal
Galega		Table olive	Portugal
Hojiblanca		Dual-purpose	Spain
Lechın de Granada		Oil extraction	Spain
Lechın de Sevilla		Table olive	Spain
Picudo		Oil extraction	Spain
Arbequina	Salinity tolerance	Oil extraction	Spain
Lechın de Sevilla		Table olive	Spain
Picual		Oil extraction	Spain
Canivano		Dual-purpose	Spain
Ascolana tenera	Tolerance of Spilocaea oleagina	Table olive	Italy
Leccino		Oil extraction	Italy
Lechın de Sevilla		Table olive	Spain
Maurino		Oil extraction	Italy
Arbequina	Tolerance of Verticillium dahliae	Oil extraction	Spain
Cipressino		Oil extraction	Italy
Frantoio		Oil extraction	Italy

Cultivar	Main feature	Utilization	Origin
Cordovil de Serpa	Tolerance of Bacterium savastanoi	Oil extraction	Portugal
Dolce Agogia		Oil extraction	Italy
Galega vulgar		Dual-purpose	Portugal
Gentile di Chieti		Oil extraction	Italy
Gordal sevillana		Dual-purpose	Spain
Leccino		Oil extraction	Italy
Orbetana		Dual-purpose	Italy
Picholine marocaine		Dual-purpose	Morocc

Table 2:Some popular olive cultivars, their main feature, use, and origin (World Catalague of Olive Varities, 2007; Olive Germplasm, 2012)

THE OLIVE TREE

The olive tree probably originated in the South Caucasus area (12,000 a.c.) however it has set very well in the Mediterranean basin, in 1208 olive cultivars present in 52 countries and preserved in 94 collections of olive germplasm (Muzzalupo et al., 2011). The cultivated olive tree, *Olea europaea sativa* (*Figure 1*), is an evergreen tree, whose fruit, called olive, is a drupe whose pulp is rich in oil and components bioactive.

Figure 1: Olea europaea sativa (from website http://www.seedvendor.com/50oltrseoleu.html?viewfullsite=1)

The olive tree is characterized by a remarkable longevity, which can reach a few hundred years, however it is subject to numerous adversities both (it is essentially afraid of the cold) than of a biological type, due to the damage agents (insects, in particular the oil fly) and disease (fungi or bacteria). The oil fly (*Bactrocera oleae*) is a diptera found in all the areas of cultivation of the olive tree and present in all Italian olive groves, therefore in the regions where it is present it is considered a of the most important adversities borne by the olive tree, going so far as to condition significantly the size and quality of production in most of the cultivation area. The damage caused by the fly is

essentially related to trophic activity larvae, which develop inside the olive, feeding on the pulp of the fruits, within which they dig tunnels. Baccate olives can be invading by microorganisms that cause rot, with consequent falls. In addition, the olive fly is responsible through the ovo deposition stings of the transmission of the olive wood (*Pseudomonas savastanoi*). From the damaged olives you get a poor-quality oil, more acidic than the normal and with completely compromised aroma, for the smell of mold (the defect is called worm). The fight against the Moscow of the olive tree is chemical; however, it uses agronomic measures and biological control techniques that are also use non-specific entomophages, in addition to reducing the damage caused by autumn attacks it is possible to carry out an early harvest of the olives.

1.1. OLIVES

1.1.1. THE DRUPE

The fruit of the olive tree is a drupe. The olive, which weighs between 2 and 6 grams, consists of:

• *Epicarp*: external part, which makes up 1.5-3.5% of the weight of the drupe.

• *Mesocarp*: pulp, which makes up 70-80% by weight.

• *Endocarp*: hazel, (15-25% weight) and almond or seed (2.5- 4.0%) in turn consisting of endosperm, perisperm and embryo.

The percentage composition of the drupe, pulp, core and seed is on average represented by the constituents shown in *Table 3*.

	Drupe	*Pulp*	*Core*	*Seed*
Water	50.0	59.0	15.0	35.0
Oil	21.0	25.0	0.5	28.0
Nitrogen substances	1.5	2.0	3.0	8.0
Nitrogen extracts	18.0	7.0	38.5	24.0
Raw fibre	8.0	6.0	40.0	4.0
Ashes	1.5	1.0	3.0	1.0

Table 3:average percentage composition of the macro-constituents of the olive (Sciancalepore, 1998)

In the olive at physiological maturation, numerous constituents can be identified, which, from a quantitative point of view, can be divided into main (water and fatty substance (triglycerides)) and secondary (phosphatides, disterols, waxes, organic acids, carbohydrates, protids, phenolic substances, mineral salts, pigments, enzymes, vitamins, etc.). While the main constituents are located predominantly in the mesocarp and endosperm, secondary constituents abound in the endocarp and epicarp. About 8% of nitrogenous substances are present in the seed: this protein fraction is the enzyme charge necessary for sustenance of the embryo and, therefore, for the possible subsequent

development of a new olive tree. The ripening of the fruits takes several months, and its duration depends on multiple factors, such as cultivar, pedoclimatic conditions and agronomic practices. During maturation there are changes in the physiology of the fruit, including a weight increase of the drupe, the pulp/core ratio as well as important variations in the chemical composition, related to the main parameters that determine the qualitative characteristics of an oil, its acidic composition, the accumulation of phenolic and aromatic substances. These phenomena occur in step with the oversaturate, that is, the variation in the color assumed by the drupe that tends to take on purple and brown, black colors from green. As the ripening period progresses, an accumulation of carbohydrates in the mesocarp is initially observed, the concentration of which tends to decrease when the synthesis of the components of the oil that runs mainly within the mitochondria (inoliation process) becomes more and more relevant. When the olive reaches physiological maturation, the oil practically occupies 80% of the intracellular space and is substantially stored within a vacuolar structure (available or free oil); the remaining part, equal to about 15-20% of the total, is instead distributed in the cytoplasmic structure (bound oil). The first, easily extractable, is clearly separated from cytoplasmic content by a membrane system, which prevents its interaction with cellular enzymes (lipase). As maturation progresses, these barriers tend to degrade and enzymes can meet the oil, accelerating its kinetics of souring and therefore also of rancidity. The oil is less protected due to the decrease of substances with antioxidant activity (phenolic compounds) and therefore more susceptible to degrading processes. As the ripening progresses the drupe tends to dehydrate so apparently it is enriched in oil (increase in the "apparent" or percentage yield) but the shriveling of the fruit will lead to the rupture of the vacuoles that contain the oil, favoring its contact with degrading enzymes (increase in acidity). In fact, even the absolute amount of oil begins to decrease, in fact, in the autumn season, due to the lower intake of sunlight, photosynthetic activity decreases while aerobic respiration tends to increase because of increased energy demand (ATP) this leads to a decrease in the oil reserve. The fruit is at this stage exposed to degrading processes related to the action of enzymes that lead to an increase in free fatty acids (acidity) by the action of lipases and their subsequent enzymatic rancidity promoted by lipoxidases. The maturation of drupe is in different eras (autumn-winter in general) depending on the cultivar (genetic variability) and the climatic trend of the year considered (environmental variability). "Technological" maturation is achieved when drupe accumulate within them the maximum amount of oil. Instead, an early collection compared to the technological one if on the one hand it leads to a lower yield in oil, on the other it provides richer oils in phenols, therefore characterized by more bitter, astringent and spicy flavors, but certainly more stable to oxidation and therefore more easily preserved. In addition, there are also the methods of harvesting and storing the fruit, which play a far from negligible role in conditioning the chemical composition of the oil and therefore its resistance

to degrading processes. Harvesting systems, if carried out in an unsuitable manner, are one of the most important causes of the production of poor oils. Mechanical damage (glare, etc.) that is not always easily avoidable, can induce the rupture of the membranes of oil vacuoles facilitating their contact with enzymes, so it becomes important to limit the time between harvesting and subsequent processing. Manual harvesting would generally be preferred to obtain good quality oils, however high costs and long periods of time can make it more convenient to use mechanical harvesting. The time and methods of storing olives are very important for the purposes of the organoleptic quality of the oil produced and its shelf life. The olives maintain their metabolic activity (aerobic respiration) even in post-harvest, so they reduce the reserve in carbohydrates and therefore go against a weight reduction accentuated by the migration of water that by perspiration spreads into the surrounding atmosphere (withering of the fruit). If this fruit is stored in oxygen deficiency because it is buried inside a heap of olives, not being able to promote aerobic respiration it would begin to ferment (alcoholic fermentation) and then accumulate inside a series of metabolites that would see ethanol as the most representative. These compounds are responsible for the so-called "heating" an off flavor that denounces a mis conservation of the product before its subsequent processing (extraction of oil). It is good practice that the transport and storage of olives does not exceed 7 days from harvest, when the temperature of the fruits does not exceed 25 °C while the olives must be kept in thin layers (20-30 cm), inside perforated boxes, to avoid crushing by pressure, in dry and well-aerated rooms. In general (under optimal conditions), there is a linear (inversely proportional) relationship between oil stability to accelerated aging and olive preservation time (Lercker, 2005).

1.1.2. BIOACTIVE COMPOUNDS IN OLIVES

The foods are not exclusively a source of energy for the performance of normal metabolic processes of the body, but also the unique source of bioactive compounds, such as polyphenols, vitamins, phytosterols, among others. These compounds contribute to “maximize” the human health status and to “minimize” the risk of occurrence of diseases. In detail, bioactive compounds are high value, extra nutrition constituents that typically occur in small quantities in foods, both from plant and animal origin. Many of them have antioxidant properties, and their favourable effects on human health are well-known. Olive fruit is a rich source of valuable and bioactive nutrients, which can be considered of medicinal and therapeutic interest since they have shown a positive activity on blood platelet aggregation, chronic inflammation, joint health, oxidized low-density lipoprotein (LDL) cholesterol, neurodegenerative disorders, and skin conditions (Kris-Etherton et al., 2002). The chemical composition of olives, as well as the amount of bioactive compounds, depends on parameters including variety, cultivation practices, geographical origin, and the level of maturation. Fruit average

composition includes water (50%), protein (1.6%), oil (22%), carbohydrate (19.1%), cellulose (5.8%), inorganic substances (1.5%), and phenolic compounds (1%-3%). Other important compounds present in olive fruit are pectin, organic acids, and pigments (Boskou, 2006). The most interesting and investigated olive bioactive compounds can be divided into two main categories: nonphenolic and phenolic compounds. The group of the nonphenolic compounds includes chloroplastic pigments, phytosterols, tocopherols and triterpenoids, while the olive phenolic matter is formed by phenolic acids, phenolic alcohols, flavonoids, and secoiroidoids. All these compounds are distributed in olive pulp, skin, and stone.

1.1.2.1. CHLOROPHYLLS AND CAROTENOIDS

Chloroplastic pigments are lipophilic products including chlorophylls and carotenoids (Fernandez-Orozco et al., 2011). Chlorophyll pigments are highly appreciated as functional components in fruits and vegetables, both for their green coloring properties and their health benefits for human consumption (Ferruzzi & Blakeslee, 2007). Chlorophylls and pheophytins (metal-free chlorophyll derivative) have been reported to possess antimutagenic and antioxidant activity by breaking radical chain reactions caused by autoxidation of vegetable edible oils (stored in the dark) via a hydrogen donating mechanism (Sözgen et al., 2013). The term “chlorophylls” is related to green pigments found in the chloroplasts of algae and plants. The basic structure of a chlorophyll molecule is a porphyrin ring, coordinated to a central atom of magnesium. There are two main types of chlorophyll, named a and b (*Figure 2)*.

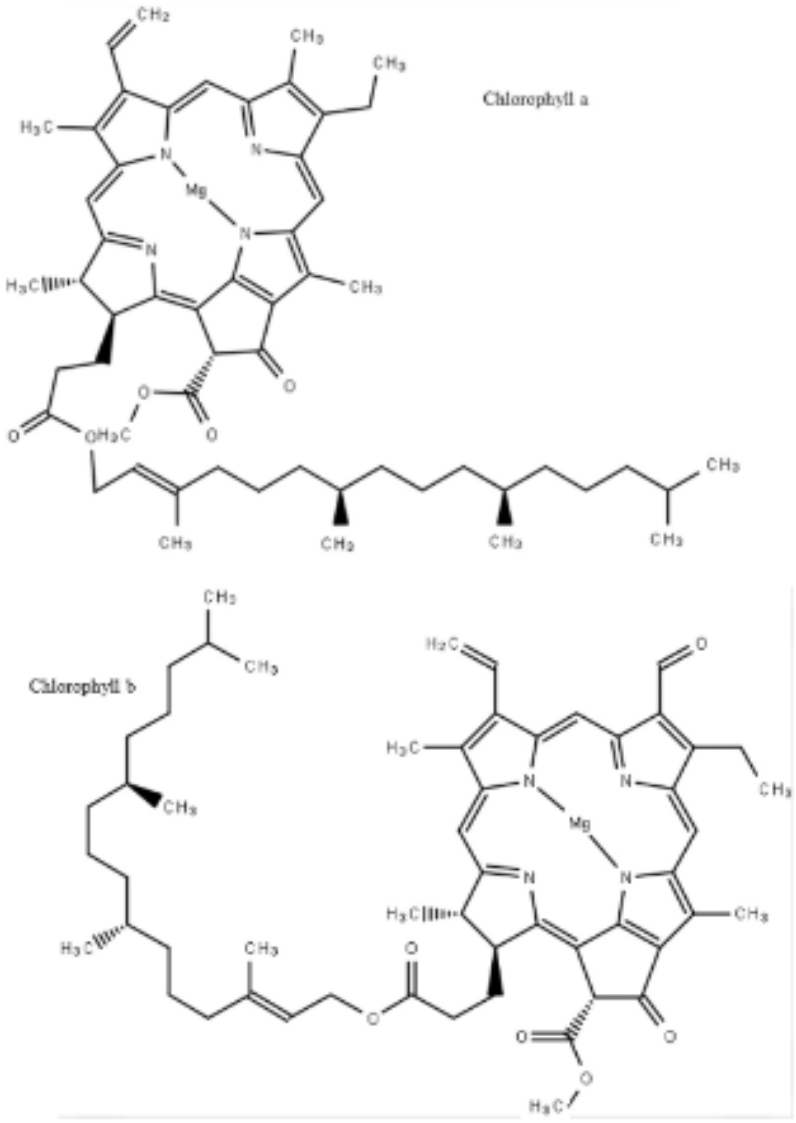


Figure 2: Chemical structures of chlorophyll a and b.

In olive fruit, chlorophylls handle the characteristic green color of the olive drupe as it begins to ripen, as such ripening notably influences the presence of chlorophylls in olive fruit (1.8–13.5 mg/100 fresh pulp). In fact, when ripening proceeds, chlorophylls in the skin decrease as anthocyanins are progressively replacing them. These phenomena can turn the fruit's color to violet or purple at the end of the maturation process (Roca & Mínguez-Mosquera, 2003). Chlorophylls are highly unstable and could be affected by light, acids, or oxygen, and their degradation is associated with aging. Considering the chlorophyll pigments distribution between peel and pulp of the fruit, they are associated with the thylakoid membranes of the chloroplasts, found in the photosynthetic tissues of the drupe, epicarp, and in pulp in a quantity that is proportional to the activity of photosynthesis. Particularly in the olive fruit, due to the higher number of chloroplasts per unit in skin than in the pulp, most of the chlorophyll is contained in the skin rather than in the pulp (Movsumov et al., 1987; Roca & Mínguez-Mosquera, 2003). Besides chlorophylls, the olive chloroplasts and chromoplasts

have other organic pigments, such as carotenoids. They are a group of over 600 fat soluble plant pigments, including xanthophylls (i.e., lutein, zeaxanthin) and carotenes (i.e., α-carotene, β-carotene, lycopene). In olive fruit, the total carotenoid content can vary between 0.6 and 2.5 mg/100 g of fresh olive pulp. The carotenoid matter is formed by lutein, zeaxanthin, β-carotene, violaxanthin, neoxanthin, antheraxanthin, and β-cryptoxanthin (Boskou, 2015). The lutein, zeaxanthin, β-carotene, and violaxanthin represent the whole carotenoids content in olive and their chemical structures are reported in *Figure 3*:

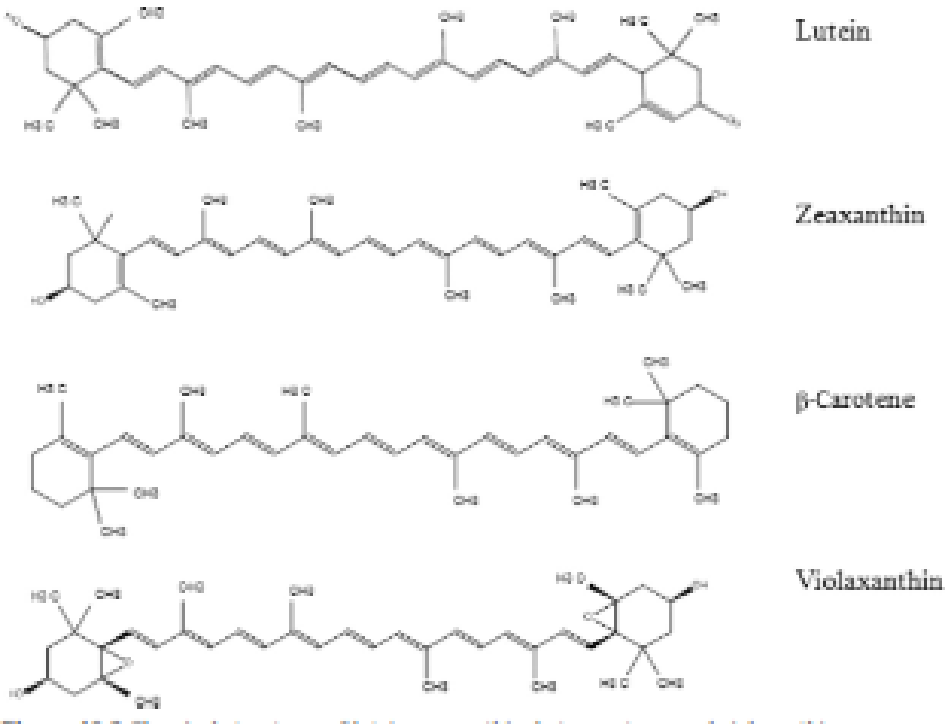


Figure 3: Chemical structures of lutein, zeaxanthin, beta-carotene, and violaxanthin.

A study conducted on olive drupe cv. Arbequina showed that the pigment concentration in the pulp was 1/10 that in the skin. Moreover, the proportion of the lutein was lower in the pulp, while the proportions of β-carotene, violaxanthin, antheraxanthin, lutein-epoxide, and the esterified xanthophylls were significantly higher. The proportions of neoxanthin and β-cryptoxanthin did not differ between the skin and pulp (Gandul-Rojas et al., 1999).

1.1.2.2. PHYTOSTEROLS

Phytosterols or "plant sterols" are lipophilic compounds that are biosynthetically derived from squalene and from a group of triterpenes (Goodwin, 1980). They regulate the fluidity of plant membranes and play a role in the adaptation of membranes to temperature (Piironen et al., 2000). Scientific evidence supports the phytosterols beneficial activity to promote some anticancer effects in colon, breast, and prostate (Awad & Fink, 2000), anti-inflammatory properties (Quilez et al., 2003),

and act as immune system modulators (Wilt et al., 1999). In olives, phytosterols represent a major part of the unsaponifiable matter. Four classes of sterols can be found: common sterols (4α-desmethylsterols), 4α-methylsterols, triterpene alcohols (4,4-dimethylsterols), and triterpene dialcohols. The most abundant sterols are β-sitosterols (1480.5±133.2 mg/kg oil from olive pulp) and Δ 5 -avenasterol (168.7±16 mg/kg oil from olive pulp), followed by stigmasterol (63.7±5.6 mg/kg oil from olive pulp) and campesterol (43.4±3.4 mg/kg oil from olive pulp) (Ranalli et al., 2002). Their chemical structures are shown in *Figure 4.*

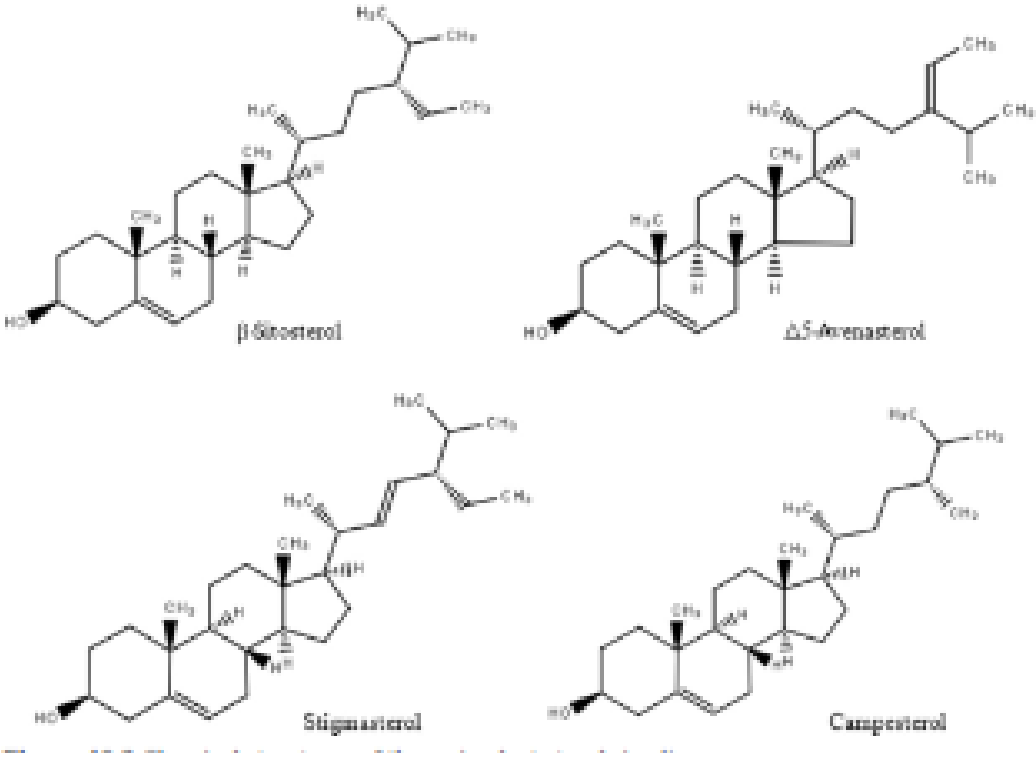


Figure 4: Chemical structures of the main plant sterols in olive

Anyway, the olive sterolic composition and the sterol content are influenced by many variables, such as cultivar, crop year, degree of fruit ripeness and geographical factors. Moreover, the distribution of the sterols changes according to the various parts of the olive. Ranalli et al. (2002) investigated the sterolic composition of the oils obtained from seed (not-woody part of the kernel), pulp (mesocarp plus epicarp), and whole olive fruit. They found that seed oil has a higher concentration of total 4-desmethylsterols (more than twofold higher), sitosterol, campesterol, cholerosterol, Δ5–24-stigmastadienol, Δ7-stigmastenol, and Δ7-avenasterol compared to other oils. Pulp and whole olive fruit oils had the same amounts of 4-desmethylsterols. The seed oil presented the lowest content of 4,4′-dimethylsterols, cycloartenol, and 24-methylenecycloartanol and the highest content of β-amyrin and butyrospermol. In general, pulp and whole olive fruit oil have the same concentration of 4,4′-dimethylsterols.

1.1.2.3. TOCOPHEROLS

Tocopherols are a class of organic compounds well-known also as “E-vitamers.” They are the most important lipid-soluble natural antioxidants, which allow the prevention or limiting of lipid peroxidation by scavenging radicals in membranes and lipoprotein particles (Esterbauer et al., 1991). Considering the tocopherols’ effects on human health, although their contribution is not yet completely understood, they are involved in many beneficial activities. α-tocopherol is able to defend the body against free radical attacks (Cheeseman & Slater, 1993; Kamal-Eldin & Andersson, 1997). Tocopherols prevent skin disorders, cancer, and arteriosclerosis (Armstrong et al., 1997; Caruso et al., 1999; Nicolaïew et al., 1998). In addition, some researchers proved a synergic antioxidant activity between phenolic and tocopherol compounds (Hudson and Lewis, 1983). In olive fruit, four types of tocopherols are found: α-, β-, γ-, and δ-tocopherol. Their chemical structures are shown in *Figure 5.*

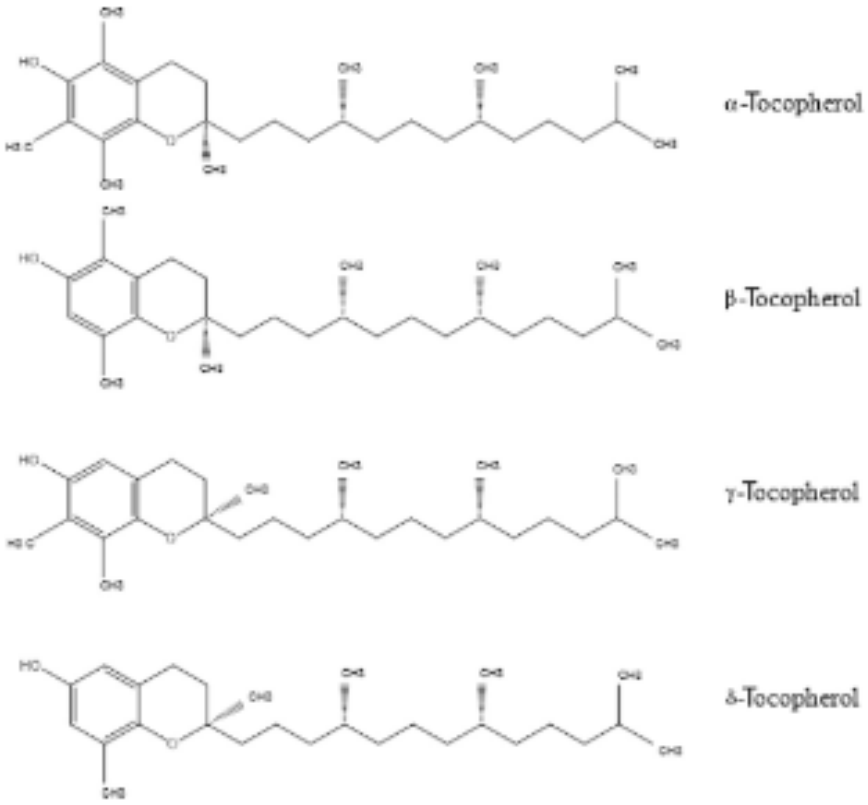


Figure 5:Chemical structures of olive tocopherols

The α– homologue is 90% of the total tocopherol content. The tocopherols content in olive closely depends on the potentiality of cultivar and technological factors. During the fruit ripening the tocopherols level decreases (Boskou, 2015). The natural table olive process does not affect the concentration of tocopherols, on the contrary lower concentrations are found in alkaline-treated olives. α-tocopherol values reported for processed olives range between 1 and 9 mg/100 g flesh. In edible flesh of green Spanish-type olives α-tocopherol accounts for 3.5 mg/100 g (Lopez et al., 2014).

1.1.2.4. TRITERPENOIDS

Triterpenoids in *O. europaea L*. are represented by pentacyclic triterpenic acids, as oleanolic and maslinic acids (Guinda et al., 2010). Their chemical structures are reported in *Figure 6*. Minor levels of pentacyclic triterpenic diols, such as erythrodiol and uvaol, have been also revealed.

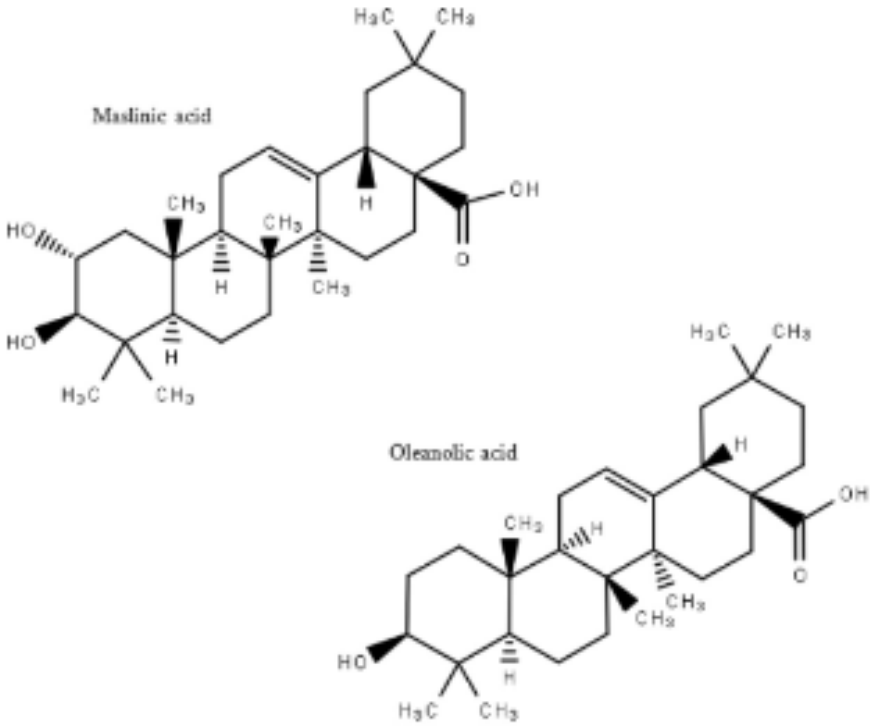


Figure 6: Chemical structures of olive tocopherols

Although triterpenoids are distributed in the olive skin (Bianchi et al., 1992; Caputo et al., 1974), they have also been reported in the stone (Ranalli et al., 2002). After the olive oil production, a major part of triterpenoids is lost, however considerable amounts of them are present in the paste (Allouche et al., 2009; Cañabate-Díaz et al., 2007; Perez-Camino & Cert, 1999). Maslinic acid is the main triterpenoid in olive fruit, its amount in the pulp consists of 25 mg/kg, whereas triterpenoids concentration reaches values up to 400 mg/kg in the skin of the olive and the concentration is around 25–50 mg/kg or even up to 200 mg/kg in virgin olive oil (Cañabate-Díaz et al., 2007; Ghanbari et al., 2012; Perez-Camino & Cert, 1999). The triterpenoids content in olive oil depends on olive variety, processing temperature, and time. Szakiel et al. (2012) revealed differences between green and black olives in the content of the dihydroxy alcohols, erythrodiol, and uvaol, which were present in substantial amounts (14%) in the wax of green olives, but only in traces in that of black olives.

1.1.2.5. PHENOLS

Phenolic compounds can be synthesized naturally by plants as a response to stress conditions, such as infection, wounding, and UV radiation (Naczk & Shahidi, 2004). They can be divided into two main groups called simple phenols and polyphenols. The first group have been characterized by the simplest chemical structure, which is also called carbolic acid (C_6H_5OH), while the polyphenols group is based on the number of phenol units in the molecule (Khoddami et al., 2013). The olive phenols handle the extent of browning in the fruit, and they also greatly contribute to sensory and aromatic characteristics of the olive as well as impart pharmaceutical and physiological benefits (Bianchi, 2003; Covas et al., 2006). In olive fruit, the phenolic fraction reaches values ranging between 1% and 3% of the fresh pulp weight. This fraction is characterized by the large number of chemical constituents: anthocyanins (cyaniding glucosides), flavonols (quercetin-3-rutinoside), flavones (luteolin and apigenin glucosides), phenolic acids (hydroxybenzoic, hydroxycinnamic, etc.), phenolic alcohols (tyrosol, hydroxytyrosol, 3,4-dihydroxyphenylglycol), secoiridoids (oleuropein, demethyloleuropein, ligstroside, nuzhenide), verbascoside (a hydroxycinnamic acid derivative), lignans, and oleoside-11-methylester (Alagna et al., 2012; Boskou, 2009; Franco et al., 2014; Kanakis et al., 2013; Lama-Muñoz et al., 2013; Silva et al., 2006; Vinha et al., 2005). The chemical structures of the principal phenols from olive fruit are shown in *Figure 7, Figure 8 and Figure 9.*

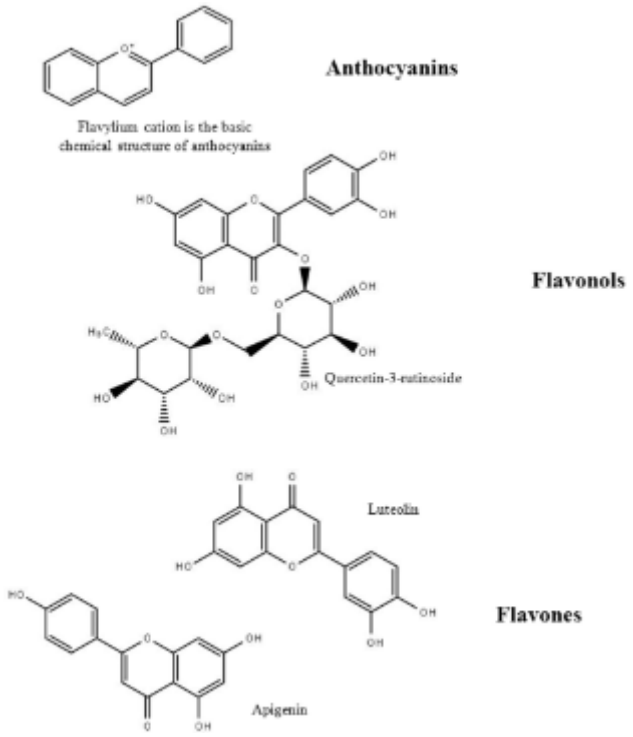


Figure 7:Main anthocyanins, flavonols, and flavones in olive fruit.

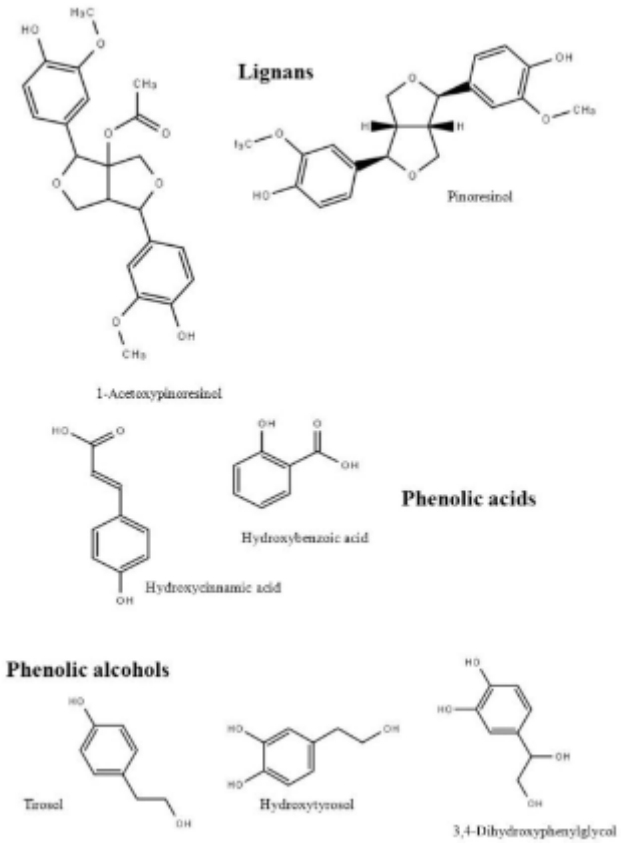


Figure 8: Main lignans, phenolic acids, and phenolic alcohol in olive fruit

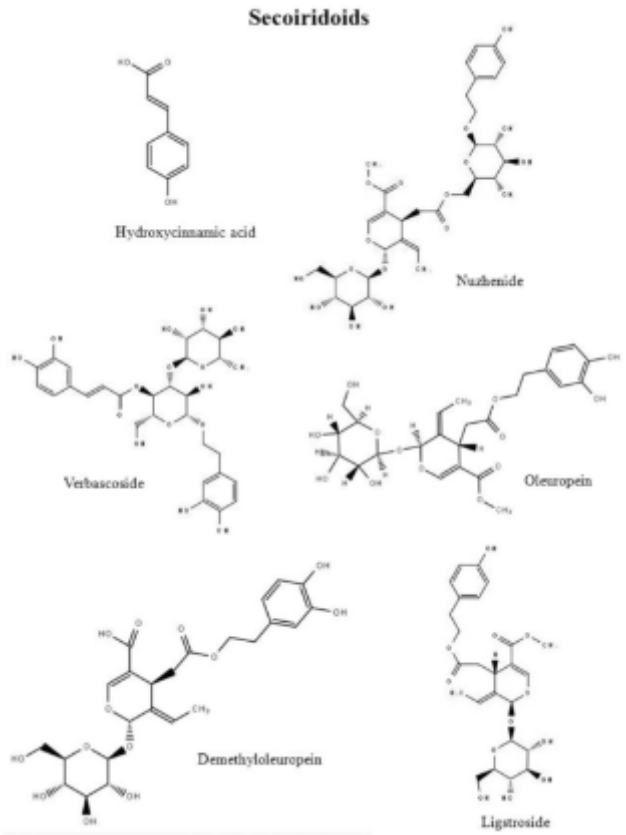


Figure 9: Main secoiridoids in olive fruit

The main phenolic compounds in olive fruit are oleuropein, verbascoside, and phenolic glycosides of elenolic acid with hydroxytyrosol and tyrosol (ligstroside). Moreover, notably levels of hydroxytyrosol and tyrosol have been revealed. Kountouri et al. (2007) revealed hydroxytyrosol and tyrosol contents of 76.73 and 19.48 mg/100 g olives, respectively. However, the amount and type of phenolic compounds in olives depend obviously on the cultivar and maturity of the fruit, climatic conditions, storage time, and processing technique (Fiorentino et al., 2003). Cecchi et al. (2015) highlighted the influence of the olive cultivar on the oleuropein level. They found 588, 1001, and 379 mg/kg olive for Frantoio, Moraiolo, and Leccino cultivars, respectively. During the ripening period, a decrease of total phenolic compounds is seen, and the olive phenolic profile is characterized by a domination of secoiridoids at the beginning of ripening and by a domination of simple phenols and flavonoids in the end. The oleuropein amounts up to 14% of the dry weight in unripe olives but during maturation undergoes hydrolysis and yields several simple molecules like hydroxytyrosol and oleuropein aglycone. Thus, the oleuropein is almost undetectable when the fruit darkens, while hydroxytyrosol, tyrosol, and verbascoside increase (Soler-Rivas et al., 2000). Elenolic acid glucoside and 3,4-dihydroxyphenylethanol are also considered indicators of maturation for olives. In fact, as the olives ripen, their tenor increases, while oleuropein decreases. Additionally, the distribution and concentration of phenolic compounds vary widely among the olive tissues. For example, nuzhenide and salidroside (a glucoside of tyrosol) are only seen in the olive seed; the flavonoids luteolin-7-glucoside, rutin, and quercetin are exclusively present in the fruit peel, while verbascoside, oleuropein, and demethyloleuropein were found in all three olive matrices. The concentration of the latter two phenolics is greatest in olive pulp. In *Table 4 and Table 5* the main phenols and their distribution in olive pulp, skin, seed and oil are shown. It should also be noted how a large amount of phenols remains in the pomace and vegetable water during the production of virgin olive oil.

Phenol	References			
	Pulp	Seed	Skin	Oil
Apigenin	[1]			[1]
Apigenin-7-glucoside	[2]			
Apigenin-7-glycosides	[3], [4]			
Apigenin-7-rutinoside	[2]			
Benzoic acid				[5]
Caffeic acid	[3], [6], [7]		[7]	[8–12]
Cinnamic acid				[9], [11–13]
2-Coumaric acid	[14]			[10], [9]
4-Coumaric acid	[3], [6], [14], [15]			[10–13], [15], [16–18]
Cyanidin-3-glucoside	[2], [4]			
Cyanidin-3-glycosides	[3], [4]			
Cyanidin-3-rutinoside	[2], [4]			
Demethyloleuropein	[2], [3], [7], [19–20]	[7]	[7]	
3,4-DHPEA-AC 4-(Acetoxyethyl)-1,2-dihydroxybenzene				[5]
3,4-DHPEA–EDA	[7]	[7]		
Elenolic acid	[3]			
Elenolic acid glucoside	[19], [20]			[9]
Ferulic acid	[14], [21]			[12], [9], [17]
Gallic acid	[14]			[22]
Hesperidin	[3]			
Homoorientin (luteolin-6-glucoside)	[2]			
Homovanillic acid	[21]			[8]
p-Hydroxyphenylacetic acid	[23]			[8], [10]
4-Hydroxybenzoic acid			[7]	[10], [9]

Table 4: Presence of phenolic compounds in pulp, seed, skin and oil of olive fruit according to the literature data.

Phenol	References			
	Pulp	Seed	Skin	Oil
Hydroxytyrosol	[2], [3], [7], [15], [19], [24]	[7]	[7]	[8], [10], [12], [9], [16], [17], [22], [25], [26]
Ligstroside	[21], [27]			
Luteolin	[1], [2]			[1]
Luteolin-7-glucoside	[2–4], [6], [15], [19], [20]			
Luteolin-7-glycoside			[7]	
1-(3-Methoxy-4-hydroxy) phenyl-6,7-dihydroxy-isochroman				[28]
Nuzhenide		[7], [29]	[7]	
Nuzhenide oleoside		[31]		
Oleuropein	[2–4], [7], [15], [19], [20], [21], [24], [27], [30], [31]	[7]	[7]	[9]
Oleuropein aglycon	[2]			[9]
Protocatechuic acid				[10], [9]
1-Phenyl-6,7-dihydroxy-isochroman				[28]
Quercetin	[32]			
Quercetin-3-rhamnoside (quercitrin)	[4], [21]			
Rutin (quercetin-3-rutinoside)	[2], [4], [19], [20], [24]		[7]	
Salidroside		[29], [30]		
Sinapic acid				[12], [13]
Syringic acid	[14], [21]			[10–13], [9], [17], [26]
Tyrosol	[2], [7], [14], [21], [24], [30]	[7]		[8], [10–13], [9], [18], [22], [25]
Vanillic acid	[2], [7], [14], [16]			[10–13], [9], [16–18], [22]
Verbascoside	[2], [7], [15], [20], [21], [24]	[7]	[7]	

Table 5: Presence of phenolic compounds in pulp, seed, skin and oil of olive fruit according to the literature data. (2)

[1] Rovellini et al. (1997); [2] Romani et al. (1999); [3] Baldi et al. (1995); [4] Vlahov (1992); [5] Bianco et al. (2001); [6] Brenes et al. (1995); [7] Servili et al. (1997); [8] Akasbi et al. (1993); [9] Montedoro et al. (1993); [10] Tsimidou et al. (1992); [11] Vacca et al. (1993); [12] Poiana (1997); [13] Mincione et al. (1996); [14] Mousa (1996); [15] Brenes-Balbuena et al. (1992); [16] Zunin et al. (1995); [17] Nergiz & Unal (1991); [18] Evangelisti et al. (1997); [19] Esti et al. (1998); [20] Amiot et al. (1986); [21] Ryan et al. (1999); [22] Mannino et al. (1993); [23] Balice & Cera (1984); [24] Brenes-Balbuena et al. (1992); [25] Cimato et al. (1989); [26] Litridou et al. (1997); [27] Kubo & Matsumoto (1984); [28] Bianco et al. (2002); [29] Maestro-Duran et al. (1994); [30] Limiroli et al. (1996); [31] Amiot et al. (1989); [32] Movsumov & Aliev (1987).

1.2. OLIVE OIL

1.2.1. LEGAL REQUIREMENTS OF THE VARIOUS TYPES OF OLIVE OIL

In accordance of step 1 of Annex of regulation 136/66/CE, the extra virgin olive oils are obtained from the fruit of the olive tree only through mechanical processes or other physical processes, under conditions, in particular thermal, that do not cause alterations of the oil, and which have not undergone

any treatment other than washing, decanting, centrifugation and filtration, excluding oils obtained by solvent or with riesterification processes and any mixture with other oils. The acidity, expressed as oleic acid, must be less than 0.8% for extra virgin olive oil. The regulation 2568/91/CE shows the features of EVO who, as regards the parameters analysable in gas chromatography and following steps 1 and 2 of Annex 1, must be as follows:

- Methyl esters of fatty acids <30 mg/kg
- Waxes C42-C44-C46<150 mg/kg
- 2 gliceril monopalmited <0.9% (if total palmitic acid <14%)
- Stigmastadienes <0.05 mg/kg
- Mystic acid <0.03%
- Linolenic acid<1%
- Arachic acid<0.6%
- Eicosenoic acid<0.4%
- Beenic acid, lignoceric acid <0.2%
- Sum of transoleic isomers<0.05%
- Sum of transoleic-translinolenic isomers<0.05%
- Cholesterol<0.5%
- Brassicasterol<0.1%
- Campesterol<4%
- Stigmasterol<campesterol
- Apparent β - sitesterol>93%
- δ-7 stigmastenol<0.5%
- Total sterols>1000 mg/kg
- Erythrodiol+uvaol<4.5%
- Single halogen volatile solvent <0.1 mg/kg
- Total halogen volatile solvent <0.2 mg/kg

The *Table 6, Table 7, Table 8 and Table 9* summarizes the characteristics of olive oils, classified as extra virgin, virgin, lampante, composed of refined olive oils and virgin olive oils, of crude pomace, of refined pomace, of pomace. In summary:
Refined olive oil: olive oil obtained from the refining of virgin olive oil.

Olive oil – composed of refined olive oils and virgin olive oils: olive oil obtained from the cut of refined olive oil with virgin olive oil.

Crude olive pomace oil: olive oil obtained from pomace by solvent treatment or physical processes

Refined olive pomace oil: olive oil obtained from refining of crude pomace oil.

Olive pomace oil: oil obtained from the cut of refined pomace oil and virgin olive oil other than lampante oil.

Category	Acidity (%) (*)	Peroxide value (mEq O_2/kg)	K_{232}	K_{268} or K_{270}	Delta-K	Organoleptic evaluation		Fatty acid ethyl esters (mg/kg)
						Median of defect (Md) (*)	Fruity median (Mf)	
1. Extra virgin olive oil	≤ 0,80	≤ 20,0	≤ 2,50	≤ 0,22	≤ 0,01	Md = 0,0	Mf > 0,0	≤ 35
2. Virgin olive oil	≤ 2,0	≤ 20,0	≤ 2,60	≤ 0,25	≤ 0,01	Md ≤ 3,5	Mf > 0,0	—
3. Lampante olive oil	> 2,0	—	—	—	—	Md > 3,5 (1)	—	—
4. Refined olive oil	≤ 0,30	≤ 5,0	—	≤ 1,25	≤ 0,16		—	—
5. Olive oil composed of refined olive oil and virgin olive oils	≤ 1,00	≤ 15,0	—	≤ 1,15	≤ 0,15		—	—
6. Crude olive-pomace oil	—	—	—	—	—		—	—
7. Refined olive-pomace oil	≤ 0,30	≤ 5,0	—	≤ 2,00	≤ 0,20		—	—
8. Olive-pomace oil	≤ 1,00	≤ 15,0	—	≤ 1,70	≤ 0,18		—	—

Table 6:features of olive oil (from Reg. 2568/91/CE)

Category	Fatty acid composition (1)						Total transoleic isomers (%)	Total transa-linoleic + transalinolenic isomers (%)	Stigmasta-dienes (mg/kg) (2)	Difference: ECN42 (HPLC) and ECN42 (theoretical calcu-lation)	2-glyceryl monopalmitate (%)
	Myristic (%)	Linolenic (%)	Arachidic (%)	Eicosenoic (%)	Behenic (%)	Lignoceric (%)					
1. Extra virgin olive oil	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,20	≤ 0,20	≤ 0,05	≤ 0,05	≤ 0,05	≤ |0,20|	≤ 0,9 if total palmitic acid % ≤ 14,00 % ≤ 1,0 if total palmitic acid % > 14,00 %
2. Virgin olive oil	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,20	≤ 0,20	≤ 0,05	≤ 0,05	≤ 0,05	≤ |0,20|	≤ 0,9 if total palmitic acid % ≤ 14,00 % ≤ 1,0 if total palmitic acid % > 14,00 %
3. Lampante olive oil	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,20	≤ 0,20	≤ 0,10	≤ 0,10	≤ 0,50	≤ |0,30|	≤ 0,9 if total palmitic acid % ≤ 14,00 % ≤ 1,1 if total palmitic acid % > 14,00 %
4. Refined olive oil	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,20	≤ 0,20	≤ 0,20	≤ 0,30	—	≤ |0,30|	≤ 0,9 if total palmitic acid % ≤ 14,00 % ≤ 1,1 if total palmitic acid % > 14,00 %
5. Olive oil composed of refined olive oil and virgin olive oils	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,20	≤ 0,20	≤ 0,20	≤ 0,30	—	≤ |0,30|	≤ 0,9 if total palmitic acid % ≤ 14,00 % ≤ 1,0 if total palmitic acid % > 14,00 %

Table 7: features of olive oil (from Reg. 2568/91/CE)

Category	Fatty acid composition (1)						Total transoleic isomers (%)	Total translinoleic + translinolenic isomers (%)	Stigmastadienes (mg/kg) (2)	Difference: ECN42 (HPLC) and ECN42 (theoretical calculation)	2-glyceryl monopalmitate (%)
	Myristic (%)	Linolenic (%)	Arachidic (%)	Eicosenoic (%)	Behenic (%)	Lignoceric (%)					
6 Crude olive-pomace oil	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,30	≤ 0,20	≤ 0,20	≤ 0,10	—	≤ |0,60|	≤ 1,4
7 Refined olive-pomace oil	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,30	≤ 0,20	≤ 0,40	≤ 0,35	—	≤ |0,50|	≤ 1,4
8 Olive-pomace oil	≤ 0,03	≤ 1,00	≤ 0,60	≤ 0,50	≤ 0,30	≤ 0,20	≤ 0,40	≤ 0,35	—	≤ |0,50|	≤ 1,2

(1) Other fatty acids content (%): palmitic: 7,50-20,00; palmitoleic: 0,30-3,50; heptadecanoic: ≤ 0,40; heptadecenoic ≤ 0,60; stearic: 0,50-5,00; oleic: 55,00- 83,00; linoleic: 2,50-21,00.
(2) Total isomers which could (or could not) be separated by capillary column.

Category	Sterols composition						Total sterols (mg/kg)	Erythrodiol and uvaol (%) (**)	Waxes (mg/kg) (**)
	Cholesterol (%)	Brassicasterol (%)	Campesterol (1) (%)	Stigmasterol (%)	App β–sitosterol (2) (%)	Delta-7-stigmastenol (3) (%)			
1 Extra virgin olive oil	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5	$C_{42} + C_{44} + C_{46} \leq 150$
2 Virgin olive oil	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5	$C_{42} + C_{44} + C_{46} \leq 150$
3 Lampante olive oil	≤ 0,5	≤ 0,1	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5 (3)	$C_{40} + C_{42} + C_{44} + C_{46} \leq 300$ (3)
4 Refined olive oil	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5	$C_{40} + C_{42} + C_{44} + C_{46} \leq 350$

Table 8: features of olive oil (from Reg. 2568/91/CE)

Category	Sterols composition						Total sterols (mg/kg)	Erythrodiol and uvaol (%) (**)	Waxes (mg/kg) (**)
	Cholesterol (%)	Brassicasterol (%)	Campesterol (1) (%)	Stigmasterol (%)	App β-sitoste-rol (2) (%)	Delta-7-stigmaste-nol (3) (%)			
5. Olive oil composed of refined olive oil and virgin olive oils	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5	$C_{40} + C_{42} + C_{44} + C_{46}$ ≤ 350
6. Crude olive-pomace oil	≤ 0,5	≤ 0,2	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 2 500	> 4,5 (4)	$C_{40} + C_{42} + C_{44} + C_{46}$ > 350 (4)
7. Refined olive-pomace oil	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 800	> 4,5	$C_{40} + C_{42} + C_{44} + C_{46}$ > 350
8. Olive-pomace oil	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 600	> 4,5	$C_{40} + C_{42} + C_{44} + C_{46}$ > 350

Table 9:features of olive oil (from Reg. 2568/91/CE)

1.2.2. CHEMICAL COMPOSITION OF EXTRA VIRGIN OLIVE OIL

The *saponifiable fraction* (Cappelli et al., 2000) is made up almost entirely of triglycerides, accompanied by small amounts of diglycerides and monoglycerides, especially in the most acidic oils (for example in lampante oils about 10% and in oils extracted with solvents about 20%). 85% of the acids present in glycerides are unsaturated and of these 70 - 80% are oleic acid and about 10% are linoleic. The composition in fatty acids of olive oil is reported in *Table 10.* A good indicator of quality is the ratio of these two acids and in extra virgin olive oil the ratio of oleic acid to linoleic acid must be greater than or equal to 7. In good quality and newly produced oils there are only traces of 1.2 diglycerides; these are in fact formed as intermediates in the biosynthesis of triglycerides and partly as a product of lipolytic processes. During aging, the 1.2 diglycerides turn into 1.3 diglycerides. Thus, the increase in the content of 1.3 diglycerides is an indication of prolonged or poor storage of oil.

Fatty acid	**%**
Palmitic acid	8.0-16.0
Palmitoleic acid	0.5-2.0
Stearic acid	1.0-4.0
Oleic acid	63.0-88.0
Linoleic acid	3.0-15.0
Linolenic acid	-

Table 10:Composition in fatty acids of olive oil (from Cappelli, Vannucchi, "Chimica degli alimenti", Zanichelli 2000)

The *unsaponifiable fraction* consists of a large group of micro components that have the common characteristic of not forming soaps when treated with a strong base (NaOH or KOH) concentrated in hot. Although it is present in modest quantities (about 1%) it is very important from a nutritional point of view and, from an analytical point of view, to control the genuineness of the oil. Its main components are tocopherols, vitamins, sterols, hydrocarbons, erythrodiol, uvaol, waxes, phenols.

Sterols: They are synthesized in nature from squalene and are present in considerable quantities; their percentage composition is a specific characteristic of the botanical species and is not influenced by genetic variations and therefore has great importance for analytical purposes; Their analysis, for example, allows us to recognize the presence of modified rapeseed oil or paper added for fraud to olive oil. In olive oil 94 - 97% of the sterols are made up of β−sitosterol, lower values indicate the presence of seed oils. Sterols are compounds that play their biochemical role within cell membranes. Their composition is typical of the botanical species from which they come. The sterolic fraction can then be used as an index of oil purity.

Erythrodiol and uvaol: These compounds are triterpenes with two alcoholic features that are naturally present in olive oils obtained by pressing. They are mainly found in great concentration in the skin and core of the drupe. In oils obtained by solvent extraction their content is significantly higher thanks to the drastic action of the solvent. Thus, their presence in oils obtained by pressure may indicate a fraudulent mixing with oil obtained for solvent extraction.

Waxes: The waxes are located on the skin of the drupe and perform several functions, including a control over water loss by evaporation from the underlying tissues, water resistance, and protection against certain parasites. In addition, waxes protect the fruit from pathogenic microorganisms, both in terms of penetration and development. Waxes, present in minimal quantities (250 mg/kg), reach high values in olive pomace oils (greater than 2%) for which they are a recognition factor. These molecules are of considerable analytical interest. Waxes are molecules formed by the combination of a wing alcohol with a fatty acid. A recent fraud is to market olive pomace oil as olive oil after dewaxing it by precipitating the waxes with cold acetone and separating them by filtration or centrifugation. It is possible to identify the fraud by going to analytically determine the percentage composition of the higher alcohols (C26, C28, C30), modified by the above operations.

Phenols: Phenolic compounds are present in olive oil in varying amounts (50 – 700 mg/kg) and have an important role in: product stability, health characteristics and flavor. The phenolic composition depends on cultivar and ripening stage. It is also affected by milling process, conditions, and retention times. The major phenolic compounds are reported in *Figure 10.*

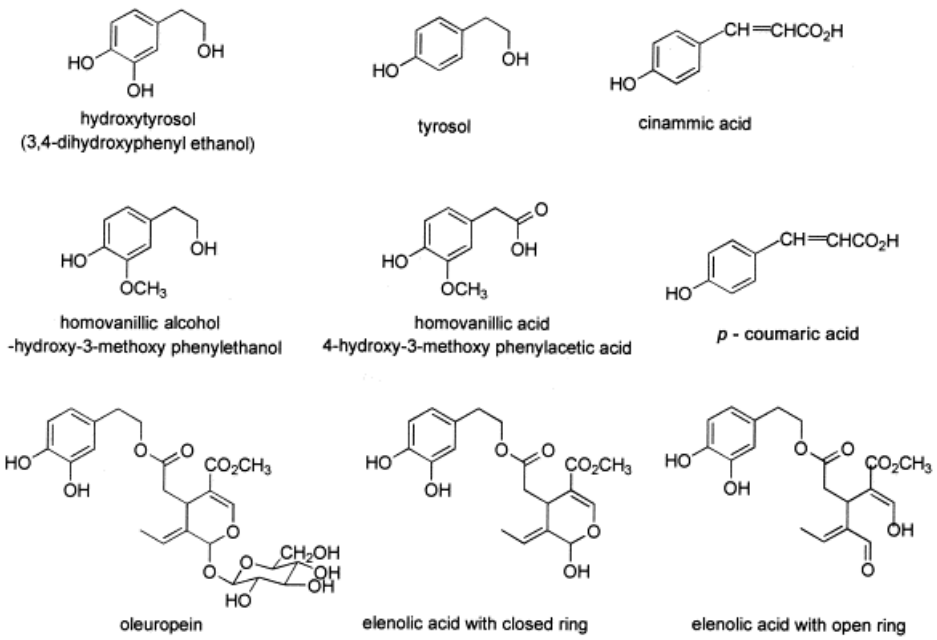


Figure 10: major phenolic compounds in olive oil

Tocopherols and vitamins: There are provitamins A (β-carotene, *Figure 11)*, vitamin F (linoleic acid - linolenic acid), vitamin E (α – tocopherol, *Figure 12*) which has a strong antioxidant action,

enhanced by the presence of phospholipids, and is present in virgin oils in quantities ranging from 150 to 200 mg/100 g of oil, absent instead in adjusted oils. Vitamin C is also present in the form of ascorbil palmitate and vitamin D. These compounds are present in olive oil at a concentration of 150 – 250 mg/kg. Normally they are present in the form α,β,γ,δ. Of these, the form α (E vit.) is the most abundant (90/95%). Tocopherols are responsible for antioxidant activity in light-exposed oils. They have high protection against UV radiation.

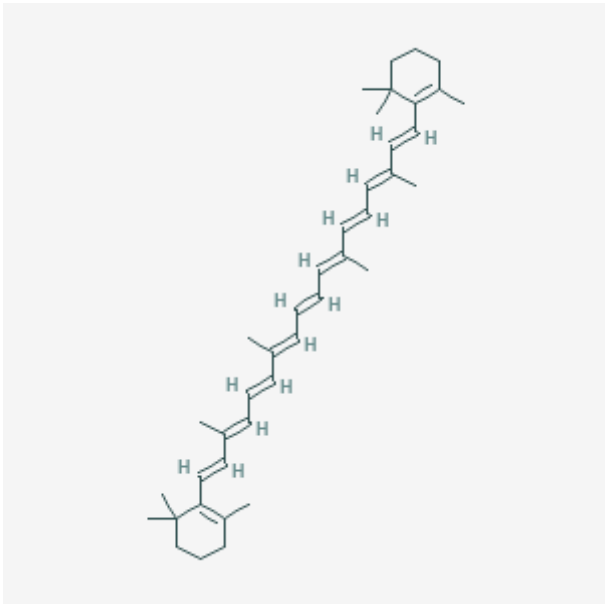


Figure 11: β-carotene

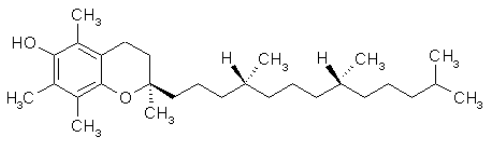


Figure 12: vitamin E

Hydrocarbons: Hydrocarbons make up about half of the unsaponifiable (30 - 40%). These molecules are composed exclusively of carbon and hydrogen atoms. Saturated ones, from C11 to C35, are linear or branched chain. The main hydrocarbon found in olive oil is squalene (C30). Squalene is an important intermediate in the biological synthesis of sterols, it is present at a concentration of about 1500 – 2000 mg/kg; the β-carotene (C40), is the precursor of vitamin A, responsible for the yellow-orange color, its concentration is 300 – 400 mg/kg. In addition, there are small concentrations of aromatic polycyclic hydrocarbons (PAH). PAHs are of anthropogenic origin, although a small component derived from biological metabolic pathways is not excluded.

Alcohols: Alcohols are found in very small amounts of aliphatic alcohols from C22 to C30; triterpenic alcohols are present in greater quantities (500 mg/l, about 25 - 30% of the unsaponifiable). There are important for recognizing olive pomace dewaxed oil.

Colored pigments: Carotenoids and chlorophyll are present as pigments; the amount of carotenoids is influenced by biological (environment) and technological factors (extraction systems, way and data of storage), but on average varies from a few mg to 100 mg/100g of oil; among carotenoids (there are about 80 compounds) the most important is β-carotene or provitamin A. The presence of chlorophyll is also variable and much depends on the degree of maturation of the olives (the olives are rich not yet invaded) and the extraction system (second-pressure oils are rich); On average in an oil of 1 - 2 months the amount of chlorophyll can vary from 1 to 10 ppm, but over time it degrades into yellow compounds, so that an oil of 7-8 months can be devoid of it.

1.2.3. ORGANOLEPTIC CHARACTERISTICS OF EXTRA OLIVE OIL

All the compounds present in the oil contribute to a different extent to determinate its organoleptic characteristics. These properties are also evaluated by chemical-physical and instrumental analysis, but above all by sensory analysis conducted by a panel, consisting of selected and properly trained tasters (Reg. CEE 2568/91). The aroma of olive oils is currently charged to more than 180 molecular species; they are low molecular weight compounds that are present in the vapor phase T environment and once reached the olfactory epithelium they bind to the receptors to trigger the odorous sensations. For volatile molecules to reach their receptors, they must show a enough degree of liposolubility to allow them to interact with lipoprotein structures, they must also have partial water solubility to interact with the water vapor that conveys them to the olfactory membrane. The volatile fraction of virgin olive oil consists of 80% aldehydes (trans-2-esenal, cis-3-esenal, hexanal), from alcohols to 6 carbon atoms (trans-2-esenol, cis-3-esenol, hexanol) and their acetyl esters (trans-2-esenilacetate, cis-3-esenilacetate, exylacetate). 70-80% of volatile compounds are released during crushing but their production continues during gramoling (20-30%). The enzymes present in the fruit are due to the development of pleasant aromas while chemical oxidation and enzymatic activity linked to the presence of microorganisms, lead to the formation of unpleasant ones.

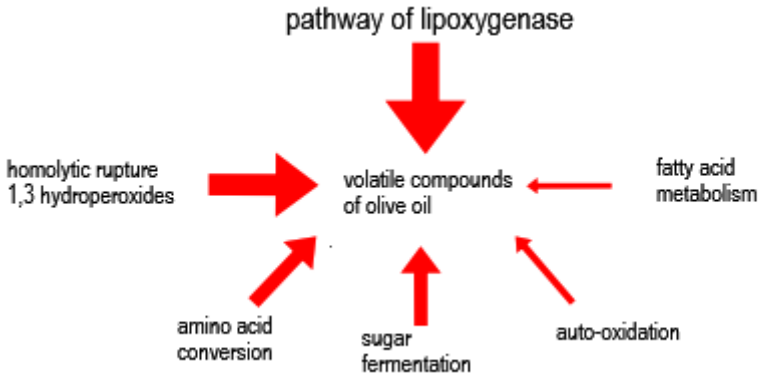


Figure 13:Chemical and biochemical pathways involved in the formation of volatile olive oil compounds; the size of the arrows is linked to the probability of following that path (Angerosa et al., 2004)

Endogenous enzymatic pathways can involve different substrates, fatty acids, amino acids, and simple sugars (mono and disaccharides). In oil, the path of lipoxygenase, which uses as a substrate the fatty acids produced by enzymatic hydrolysis of triglycerides (lipase), is the main production process of aromatic compounds and of aldehydes and alcohols with 6 carbon atoms resulting from degradation from linolenic and linoleic polyunsaturated acids in an aerobic environment by concerted action of lipoxygenase, peroxidase and alcohol dehydrogenase. Lipoxygenases act on polyunsaturated fatty acids (linoleic and linolenic acid) and on their unit 1-cis-4-cis-pentadienic, producing hydroperoxides, which tend either to accumulate inside the oil or face a process of fragmentation promoted by hydroperoxidolias leading to the formation of 6-atom carbon aldehydes. The hexanal produced by linoleic acid can be reduced to hexane (alcohol dehydrogenase) and then converted to slender acetate by alcohol acetyl transferase. The evolution of the 13-hydroperoxides of linolenic acid is more complex. Their fragmentation by hydroperoxide lipase produces cis-3-esenal, which, in part, is rapidly reduced to cis-3-esen-1-ol, by intervention of alcohol dehydrogenase, and subsequently transformed enzymatically into its corresponding ester, and therefore mostly isomerized to the more stable trans-2-esenal, subsequently reduced by catalytic activity of alcohol dehydrogenase to the corresponding alcohol. Volatile six-atom carbon aromatic compounds are responsible for the so-called "green" sensations, that is, for the fruity green and the sensations

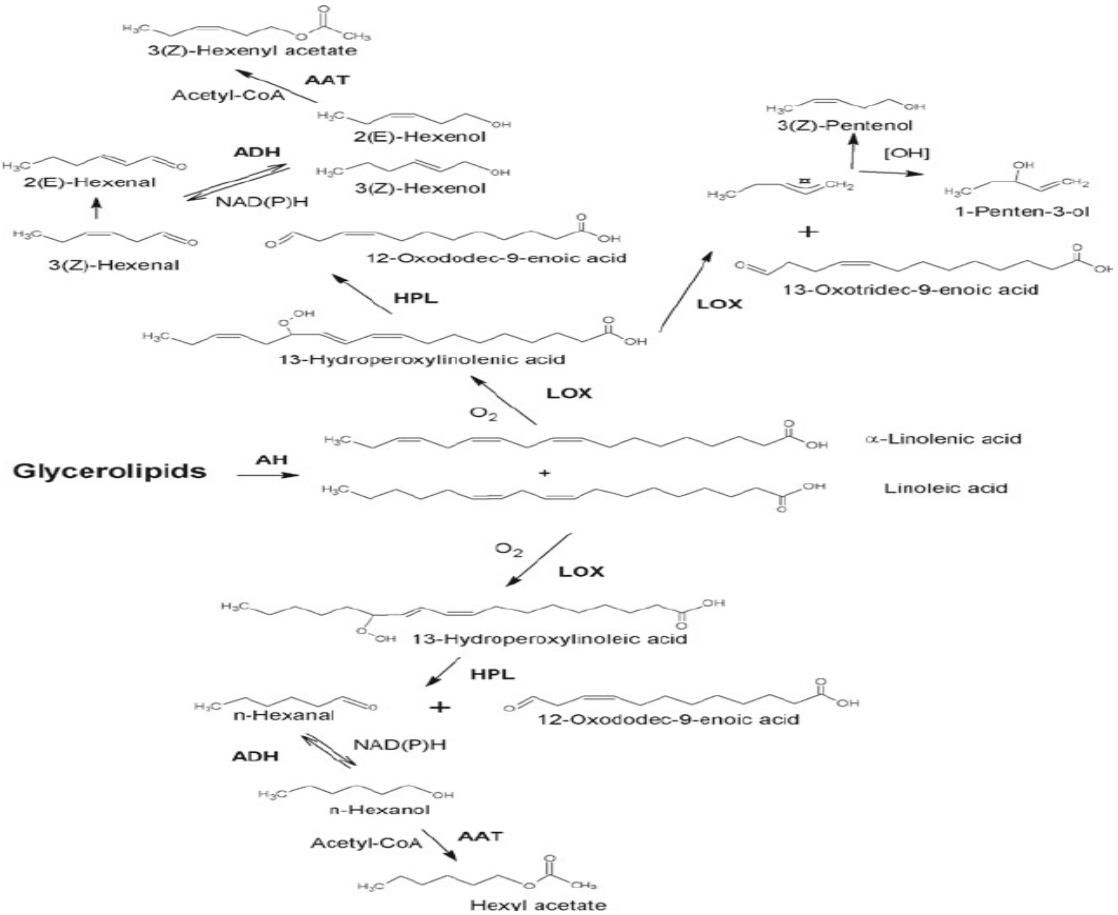


Figure 14:Production of volatile compounds c6 through the way (cascade) of lipoxygenase. Abbreviations: AH acyl hydrolase, LOX lipoxygenase, HPL hydroperoxide lyase, ADH alcohol dehydrogenase, AAT alcohol acyltransferase (Aparicio & Harwood, 2013)

leaf freshly cut grass, green fruits and vegetables (Olías et al., 1993). In particular, the concentration of these compounds plays positively or negatively in the production of the different green sensations; thus, for example, the hexanal (which plays an essential role in the formation of most green attributes) has a positive correlation with the feeling of sweet, and negative with the sensations of grass and leaf, to which contribute, respectively and in a positive and decisive way, the trans-2-esenal and the 1-penten-3-one together with phenolic compounds; on the other hand, the hexanal adversely influences the attributes of bitter and spicy, which are instead positively correlated with the content, as well as phenolic substances, of 1-penten-3-one and of cis-3-esen-1-olo, which therefore seem to have a synergistic effect in the production of such attributes (Angerosa et al., 2000). Triglycerides are responsible for the characteristics of "oiliness" and "smoothness" but are completely unsaporious and therefore almost influent from an organoleptic point of view. It is, in fact, the minor components and among them the phenolic compounds that take on an importance, as they are responsible for the feeling of bitterness typical of many olive oils. The intensity of bitterness and spicy highlighted by a virgin oil have been related to the concentrations taken by phenolic compounds resulting from the hydrolysis of oleuropein and ligstroside (two secoiridoids), which are partially fat-soluble, transfer to the oil during the extraction process. This transfer is facilitated by the action of glicosidasic enzymes that favor the detachment of the lipophilic fraction from the sugar component, accentuate its apolarity. The activity of esterases, on the other hand, frees hydroxytyrosol from oleuropein and thyrosol from ligstroside, promoting the rupture of the foreign bonds that linked them to elenoic acid. It seems that esterases also catalyze the formation of deacetoxyoleuropein-garlic and deacetoxyligstroside-garlic, compounds whose concentration combined with that assumed by the garlic of oleoeuropein, appear closely related to the intensity of bitterness perceived by tasters. The spicy attribute would be related to the deacetossiligstroside aglicone (Gutiérrez-Rosales et al., 2003); (Andrewes et al., 2003).
On the contrary, the amount of trans-2-heptenal is related to the degree of oil rancidity or to ethanol and ethyl acetate concentrations, which are related to the winey defect (Restuccia et al., 2018).

Let us now look at the relationship between the sensations of taste, smell and sight linked to the taste of extra virgin olive oil and the presence of certain substances in the oil.

The taste: Although the acidic composition, and the content of oleic acid, has an impact on "fluidity", the compounds that most affect the taste and tactile characteristics of virgin olive oils are phenolic compounds (Alfei et al., 2020). Phenolic compounds are secondary metabolites present in the olive and consequently in the oil that are among the so-called "minor compounds" and, generally, are not present in significant quantities in other vegetable oils. Virgin olive oil contains a number of classes

of different phenolic compounds: phenolic acids, phenol-alcohols, flavonoids, secoiridoids and the lignanil polyphenols (or "biophenols") of virgin olive oils belong to the classes of phenyl-acids (caffeic acid, p-coumaric acid, etc.), phenyl-alcohols (tyrosol, p-HPEA and hydroxytyrosol, 3,4-DHPEA) and aglycone secoiridoids, such as the dialdehyde form of decarboxymethyl-elenoic acid bound to 3,4-DHPEA or p-HPEA (3,4-DHPEA-EDA or p-HPEA-EDA) and the isomers of oleuropein aglycone (3,4-DHPEA-EA), and ligustroside (p-HPEA-EA). The latter substances are the most concentrated; they derive from the enzymatic conversion by the 8-glucosidases of the drupe during the mechanical extraction process, of the secoiridoids glucosides oleuropein, demethyloleuropein and ligustroside, compounds exclusive to the fruit of the olive. Other polyphenols of virgin olive oil are lignans, pinoresinol and acetoxypinoresinol. All these substances, in addition to having antioxidant activity and therefore, an important role in the prolongation of the shelf-hyphae of virgin olive oils and in the general well-being of our body (in particular, in the prevention of cardiovascular diseases and some forms of cancer), from the sensory point of view are the impact compounds for the notes of "bitter" and "spicy" and "astringent" interacting with taste cells and tactile cells, respectively, localized in the human mouth apparatus. In this regard, it has been widely demonstrated that the open-loop ligustroside derivative, p-HPEA-EDA (a molecule also known as oleocanthal), is strongly "spicy", while 3,4-DHPEA-EA and p-HPEA-EA, closed-loop compounds, would be responsible for the "bitter" sensation; 3,4-DHPEA-EDA (also known as oleacein) seems to have a marginal role in the definition of the note of "spicy" contributing, instead, to that of "bitter". These two groups of substances are present in a combined form and the two organoleptic sensations of "spicy" and "bitter" tend to coexist, although in general, the sensation of "spicy" prevails over that of "bitter". The concentration of phenolic compounds, as well as related sensory characteristics, is strictly dependent on agronomic and technological parameters. In particular, the variety, the degree of ripeness, the geographical area, the agronomic practices (especially the water status of the plant) influence the phenolic content of the drupe, while the extraction process and the related technological parameters influence the phenolic content of the oil. For this reason, the phenolic concentration of the oils has an extremely variable range from 50 to 940 mg/kg of oil. *Table 11* shows the different correlations found scientifically, among the various phenolic compounds and the typical taste/tactile sensations of virgin olive oils.

Compound	**Taste**
Tyrosol (p-HPEA)	Astringent, not bitter
p-HPEA-EA	Astringent, slightly bitter
p-HPEA-EDA=OLEOCANTHAL	Very pungent, especially in the throat, slightly bitter, astringent
3,4-DHPEA-EDA=OLEACIN	Astringent, bitter, pungent
3,4-DHPEA-EA	Very bitter, very astringent

Table 11: Correlations between taste and tactile sensations and phenolic compounds of virgin olive oil (from Alfei et al., 2020)

The aroma: The volatile substances that characterize the headspace of virgin olive oils, acting on human olfactory receptors (nerve cells of the nasal apparatus), are responsible for the aroma of this product (Alfei et al., 2020). The characteristics that make these volatile compounds such and perceptible by the olfactory receptors of the retro nasal cavity are low molecular weight (<300 Da); high volatility that allows the molecules to easily reach the olfactory epithelium; sufficient water solubility to spread into the mucous membrane covering the olfactory cells; good fat solubility to allow them to cross lipid membranes and reach receptor proteins. The volatile compounds of virgin olive oils with aromatic function are about 180 but, for most of them, it is not known exactly what the smell generated by them is. The aromatic components, which are formed during the oil extraction process, belong to different chemical classes such as: aldehydes, alcohols, esters, hydrocarbons, ketones, furans, terpenes and others not yet identified. C5 and C6 compounds, especially unsaturated and saturated linear C6 aldehydes, represent, from a quantitative point of view, the most important fraction of the volatile compounds that characterize high-quality oils. Alcohols, compared to aldehydes, contribute to a lesser extent to the sensory profile of the oil since they have a higher olfactory threshold value but still contribute to the notes of "fruity" and "green", as well as aldehydes to C5 and C6. The formation of volatile compounds, in part, takes place already inside the fruit before pressing, after the so-called climacteric ripening phase, when a series of metabolic and enzymatic changes are activated within the tissues of the drupe. The most consistent production of volatile compounds occurs, however, when the cells of the mesocarp are broken down or during the crushing phase when the pulp and the core will be reduced into fragments, that is, when the "lipoxygenase pathway" is activated, consisting of a series of sequential and concomitant reactions by four enzymes: lipoxygenase, hydroperoxide lyase, alcohol dehydrogenase and alcohol acyltransferase. The lipoxygenase cascade begins in fact, when cellular tissues are destroyed and endogenous lipases (acyl Δ -13), oxidizing them. This enzyme is thermally unstable: at 60°C its activity is reduced by 10%, in a short time. Different thermal stabilities for lipoxygenases have been reported in the literature and this can also be attributed to the existence of different isoforms. Lipoxygenase also has a greater activity against linolenic acid than linoleic acid and this favors the biogenesis of many unsaturated volatile compounds with six carbon atoms that, in fact, represent the main constituents of the aromatic profile of virgin olive oil. Subsequently, hydroperoxide lyase (HPL) catalyzes the cleavage of fatty acid hydroperoxides producing aldehydes and volatile oxyacides. The HPL enzyme can generate C6 and C12 ω-oxoacids from the 13-hydroperoxides of linolenic or linoleic acid, or C9 and ω-oxoacids C9 from 9-hydroperoxide derivatives of the same fatty acids depending on the specificity of the enzyme for the substrate. The HPL isoform that breaks down the 9-hydroperoxides is responsible for

the characteristic "cucumber" smell of some fruits and vegetables, while the isoform of the enzyme that uses the 13-hydroperoxides catalyzes the formation of C9 aldehydes responsible for the "green" aroma. The cleavage of 13-hydroperoxides, in fact, forms C6 aldehydes that include saturated (hexanal) aldehydes of linoleic acid and unsaturated ((Z)-3-hexenal) from linolenic acid. Unsaturated aldehyde (Z)-3-hexenal is rather unstable and is usually subjected to rapid isomerization in the most stable compound, E-2-hexenal, with the help of (Z)-3 (E)-2-enal isomerase. Aldehydes formed through HPL activity and isomerized with the help of (Z)-3 (E)-2-enal isomerase are further reduced to alcohols. The content and activity of HPL remains constant throughout the maturation period so the reduction of the content of volatile compounds to C6 is not due to the action of this enzyme, but to the availability of the substrates of choice of the enzyme itself or to the change in pH. Some studies have shown that HPL is particularly sensitive to heat with an optimum of action at 15 ° C with a progressive reduction around 35 ° C. Following the activity of HPL intervenes alcohol-dehydrogenase (ADH), an enzyme widespread in the plant kingdom and which contributes to the reversible reduction of aliphatic aldehydes to volatile alcohols that contribute to the aroma of many plant products. When the drupe begins to mature and the color of the epicarp turns to purple, ADH activity is reduced. The alcohols, produced by the action of alcohol-dehydrogenase, can be transformed into volatile esters that give the product positive and particularly sought-after attributes such as the hint of "fruity" and "green" but also of peculiar sensations such as those of "floral". Alcohol-acetyl transferase (ATT) catalyzes the formation of acetate esters through acetyl-CoA derivatives. In virgin olive oils, ethyl propionate and hexyl acetate are important constituents of the notes of "fruity" and "floral". ATT has less activity against short-chain alcohols such as methanol, butanol and 3-methylbutanol and this would explain the reduced content of hexyl acetate in olive oil although the concentration of its precursors ((Z)-3-hexenol and (E)-2-hexenol), among volatile alcohols, is particularly relevant. In olives the substrate of choice of alcohol-acetyl transferase is hexanol and (Z)-3-hexenol while, on the contrary, (E)-2-hexenol is not particularly used by this enzyme. The activity of the ATT presents an optimum of pH in a basic environment with a rapid decrease in the acid range, and an optimal temperature of 35 ° C. A further branching of the LPO pathway occurs when the substrate is linolenic acid, producing C5 alcohols, which can be enzymatically oxidized to the corresponding carbonyl compounds. The factors that determine the content and type of volatile compounds of virgin olive oils are many. The enzymatic activity and the level of precursors of volatile compounds depend on the variety, the degree of ripeness of the olives and the extraction techniques, determinants, along with other external factors such as climate, soil and geographical range. Another important class of volatile components with sensory impact for many food products of plant origin, are terpenes and sesquiterpenes, aromatic substances of primary

origin (that is, already present in the raw material). Terpenoids, in fact, are secondary plant metabolites biosynthesized through the mevalonate pathway. The presence of compounds such as limonene, p-cymens and alloocimens (E, Z), has been observed in national and international virgin olive oils and actively contribute to the exaltation of the notes of "fruity" and the typical nuances of variety and "geographical area" dependent. Terpenes and sesquiterpenes, are in fact considered reliable markers discriminating against the genetic and geographical origin of oils. More than 180 substances have been identified in the headspace of virgin olive oils but their correlation with aroma is not yet fully identified. albumen. Each volatile compound is characterized by an odorous note and a different olfactory threshold, and the overall sensory impact is not simply given by the sum of the odors present. Small variations in the quantitative levels and / or in the relationships between the different compounds present can in fact give rise to very different olfactory imprints and flavors. Numerous studies have been conducted to find correlations that explain the presence of positive sensations or defects in virgin olive oils. The most important sensory attribute is represented by the sensation of "fruity", a flavor that evokes olives in excellent health and harvested at the right stage of ripeness. Other pleasant sensations are the "herbaceous", reminiscent of the scent of cut or leaf grass, the "tomato", the "artichoke", the "almond", the "apple" or other fruits. The qualitative determination of the volatile compounds of these oils highlighted the predominance of the compounds at C6 and C5. Other substances of different formation, such as monounsaturated aldehydes at C7-C11 and dienals at C6-C10, branched aldehydes, alcohols and some C8 ketones, reach high concentrations, however, in the headspace of oils that have sensory defects. Such accumulation products come from possible fermentation processes, conversions of certain amino acids, enzymatic activities of mold or oxidative processes and, generally, they are related to unpleasant olfactory sensations (off-flavors) of virgin olive oils. *Table 12* summarizes the different correlations, scientifically found, between various volatile compounds and pleasant aromas and olfactory defects of virgin olive oil.

Substance	**Aroma**	**Attribute**
Aldehydes		
(Z) -2-pentenal	Herbaceous	Pleasant
(E)-2-pentenal	Green apple, floral	Pleasant
Propanal	Sweet, floral	Pleasant
Hexanal	Green apple, cut grass	Pleasant
(E)-2-hexenal	Almond, green apple, herbaceous	Pleasant
2,4 hexadienal	Cut grass	Pleasant
(Z) – hexenal	Cut grass	Pleasant
(Z)-3-hexenal	Green tomato, artichoke, herbaceous, floral, green leaf, apple, cut grass	Pleasant
Pentanal	Woody, oil	Negative

Substance	Aroma	Attribute
Heptanal	Oil, fat	Negative
(E)-2-heptanal	Oxidized	Negative
Ottanal	Fat	Negative
2,4 heptadienal	Fat, rancid	Negative
Nonanal	Oxidized	Negative
Decanal	Paper, fat	Negative
(E)-2-decenal	Paint, fish, fat	Negative
(E,E)-2,4-nonadienal	Soapy, penetrating	Negative
(E,E) 2,4-decadienal	Fried	Negative
(E,Z) 2,4 decadienal	Fried	Negative
Alcohols		
Hexan-1-ol	Fruity, aromatic, cut grass	Pleasant
(E)-2-hexen-1-ol	Herbaceous, leafy, fruity	Pleasant
Ethanol	Ride apple	Pleasant
(Z)-3-hexen-1-ol	Banana, leaf, herbaceous-fruity	Pleasant
(E)-3-hexen-1-ol	Fruity, cut grass	Pleasant
Butan-2-ol	winey	Negative
2 methyl butan-1-ol	winey	Negative
3 methyl butan-1-ol	woody	Negative
heptan-2-ol	earth	Negative
6 methyl-5-hepten-3-ol	Hazelnut	Negative
Octan-2-ol	Earth, fat	Negative
Octen-3-ol	Mold, earth	Negative
Nonanol	Rancid	Negative
Esters		
ethyl propionate	Sweet, strawberry, apple	Pleasant
Ethyl isobutyrate	fruity	Pleasant
Ethyl-2-methyl-butyrate	Fruity	Pleasant
Ethyl-3-methyl-butyrate	Fruity	Pleasant
(Z) -hexenyl acetate	Green banana, green fruity, green leaf, floral	Pleasant
Hexyl acetate	Sweet, floral, fruity	Pleasant
3-methyl butyl acetate	Banana	Pleasant
Ethyl acetate	Sticky	Negative
2-methyl-propylbutanoate	Winey, sludge	Negative
Carboxylic acids		
Acetic acid	Acid, winey	Negative
Propionic acid	Pungent, acid	Negative
Butanoic acid	Rancid, cheese	Negative

Substance	Aroma	Attribute
Pentanoic acid	Unpleasant	Negative
Hexanoic acid	Pungent, rancid	Negative
Heptanoic acid	Rancid, fat	Negative
Ketones		
Optan-2-one	Mold	Negative
1-opten-3-one	Mold, mud	Negative

Table 12: correlations between odor-positive and negative sensations and volatile compounds of virgin olive oil (from Alfei et al., 2020)

The color: Chlorophylls, pheophytins and carotenes (lutein and β-carotene) by stimulating sensory receptors for the sense of sight (cones and rods of the ocular system), give the typical colors to virgin olive oils described as "green", "yellow-green" or "yellow" depending on the prevalence of chlorophylls, pheophytins or carotenes. Another factor that can be evaluated to the eye, is the turbidity / clarity. The turbidity of virgin olive oils is given by microparticles of water that, during the centrifugation process, remain in suspension in the oil and that are not present in clear or filtered oils.

1.2.4. BIOACTIVE COMPOUNDS IN OLIVE OIL

The bioactive substances contained in olive oil can be divided into two categories: 1) non-polar bioactive substances in the unsaponifiable fraction of the oil (for example, squalene, tocopherols, sterols and triterpene compounds); 2) polar compounds characterized as "olive oil polyphenols" contained in the fraction obtained by liquid-liquid extraction or SPE. True polyphenols have two benzene rings bound by a C3. For this reason, only flavonoids such as apigenin and luteolin can be defined as polyphenols. These compounds are present in trace amounts. The other polar phenols contained in olive oil (hydroxytyrosol, tyrosol, the dialdehyde form of decarboxymethyl elenolic acid bound to tyrosol and hydroxytyrosol, glycosides and aglycones, lignants and phenolic acids) are not polyphenols. It is best to characterize these as bioactive olive oil phenols or polar phenols to better differentiate them from the other class of phenols, tocopherols, which are non-polar compounds. In addition, squalene, sterols, tocopherols, linear alcohols, and triterpene compounds are to be considered unsaponifiable, while polar phenols are saponifiable. The phenolic composition depends on various agronomic, genetic, environmental factors such as the variety of the olive tree, the region and climatic conditions, the agricultural practices applied, the stage of the maturity and harvest period, the type of extraction, the type of storage and packaging. The following substances appeared in olive oil: phenolic acids (hydroxybenzoic, hydroxyphenylacetic, hydroxycinammic); simple phenols (tyrosol, hydroxytyrosol), phenolic alcohol derivatives (tyrosol acetate, hydroxytyrosol acetate); glycosides (oleuropein, ligstroside); aglyconic derivatives of glycosides (ligstroside aglycon,

oleuropein aglycon); dialdehydic and monoaldehydic forms of decarboxymethyl elenolic acid linked to hydroxytyrosol and tyrosol; lignans ((+)-acetoxypinoresinol, (+)-pinoresinol, (+)-1-hydroxypinoresinol, syringaresinol); flavonoids (apidenin, luteolin); hydroxy-isochromans (1-phenil-6,7-dihydroxy-isochroman, 1-(3'-methoxy-4'hydroxy)phenil-6,7-dihydroxy-isochroman); other phenols (vanillin, 4-hydroxy-3-methoxy-benzaldeyde, 4-ethylphenol) and nonphenolic compounds (cinnammic acid, elenolic acid, elenolic acid glycoside). (Bendini et al., 2007; Boskou, 2009; Christophoridou et al., 2005; Franco et al., 2014, Kanakis et al., 2013; Perez et al., 2014; Saitta et al., 2010; Segura-Carretero et al., 2010). The dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA) and hydroxytyrosol (3,4-DHPEA-EDA), oleuropein and lingstroside aglycons, are the main phenols, followed by lignans, tyrosol and hydroxytyrosol, while the concentrations of phenolic acids are low. The concentrations of tyrosol and hydroxytyrosol are typically low in fresh oils but increase during oil storage due to the hydrolysis of secoiridoids, which have these phenols in their molecular structure. Basic structural characteristics of major phenols from each class of phenolic compounds reported to be present in olive oil are shown *in figs 15-20*.

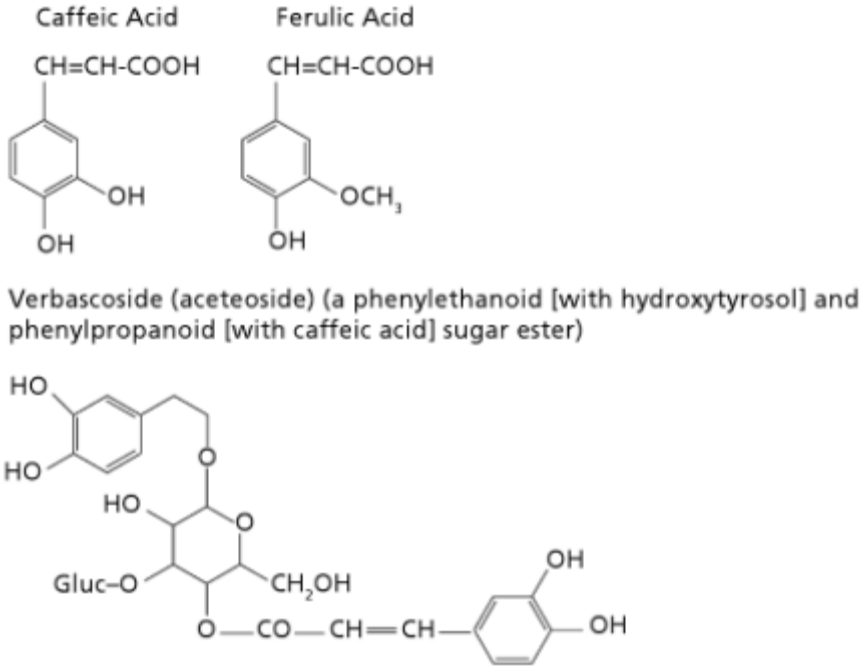


Figure 15:phenolics acids and derivatives (from Boskov, 2015)

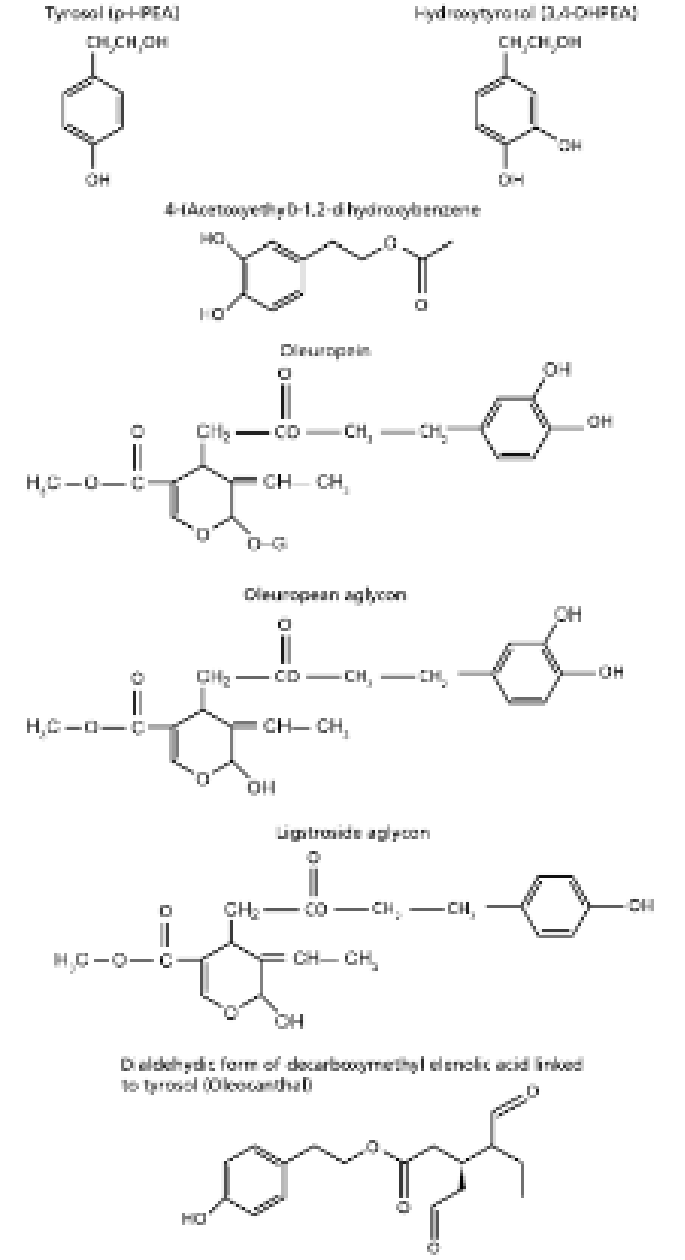


Figure 16: phenyl alcohol and derivatives (from Boskov, 2015)

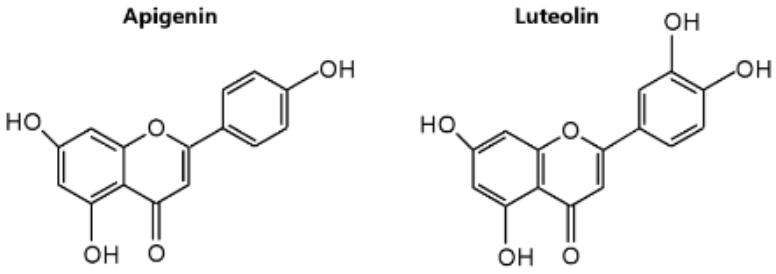


Figure 17: flavonoids (from Boskov, 2015)

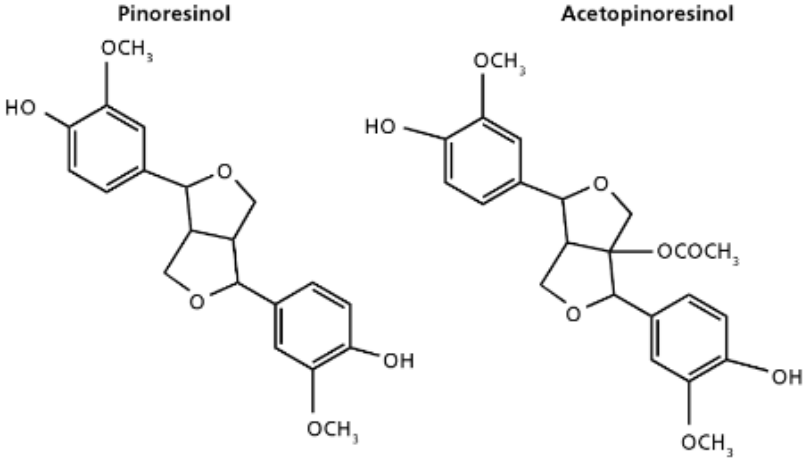
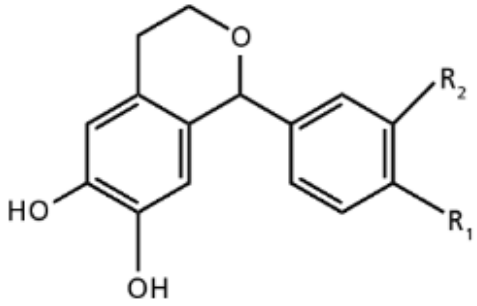


Figure 18: lignans



1-Phenyl-6,7-dihydroxychroman $R_1R_2 = H$

1-(3'-methoxy-4'-hydroxy)phenyl-6,7-dihydroxychroman $R_1 = -OH$, $R_2 = -OCH_3$

Figure 19: hydroxy isochromans (from Boskov, 2015)

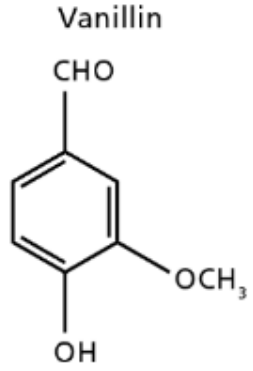


Figure 20: other phenols (from Boskov, 2015)

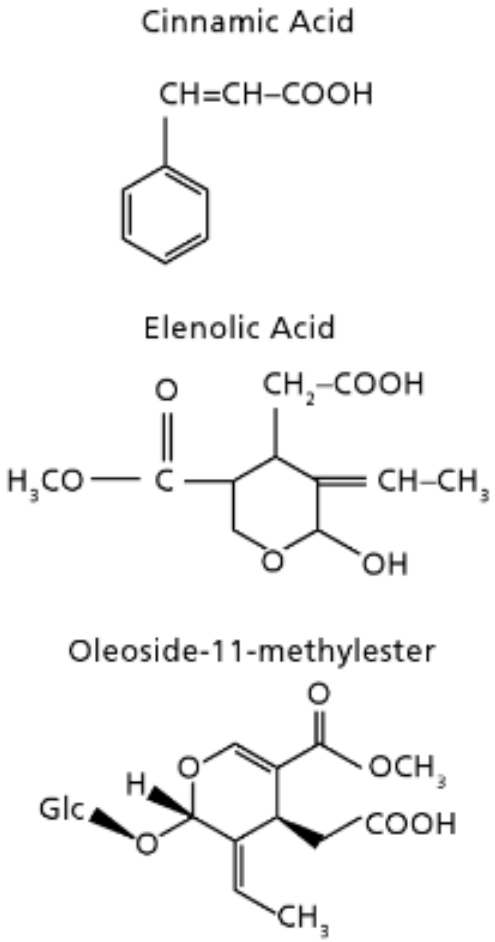


Figure 21: nonphenolic compounds (from Boskov, 2015)

1.2.4.1. TOCOPHEROLS

From the eight known "E-vitamers," the alpha-homologue comprises 90% of the total tocopherol content. Low amounts (10-20 mg/kg) are reported for homologues β-tocopherol (10 mg/kg), δ-tocopherol and γ-tocopherol (Ben-Hassine et al., 2013; Boskou et al., 2006; Cunha et al., 2006; Kalogeropoulos & Tsimidou, 2014). The levels reported for α-tocopherols content indicate a wide range that depends on the cultivar potential and technological factors. Greek oils studied by Psomiadou et al. (2000) had very high levels of α-tocopherol, ranging from 98 to 370 mg/kg. Values ranging from 93 to 260 mg/kg have been reported for the Portugeese olive oil samples (Cunha et al., 2006). Usually, high levels of tocopherols have been reported for varieties Coratina, Arbequina and Koroneiki cultivated in Egypt (above 600 mg/kg) (Benincasa et al., 2011). Variability of vitamin E in virgin olive oil by agronomical and genetic factors has been studies by Beltran et al. (2010). Olive oil can be a good source of vitamin E. The 23 g of the oil suggested per day for good health, with a mean value of 200 mg α-tocopherol/kg, provide 4.6 mg α-tocopherol, which is approximately 25%

of the recommended dietary allowance. To retain a good level of α-tocopherol, olive oil should be stored carefully (Fregapane et al., 2013; Tsimidou, 2006). The contribution of α-tocopherol to the stability of olive oil and combined autoxidation of α–tocopherol and phenols have been discussed by Baldioli et al. (1996), Bendini et al. (2006), Blekas et al. (1995), Franco et al. (2014b), Mancebo-Campos et al. (2014), Mateos et al. (2003) and Tsimidou (2010).

1.2.4.2. HYDROXYTERPENIC ACIDS

Oleanolic (3β-hydroxyolean-12-en-28-oic acid) and maslinic acid (2α,3β)-dihydroxyolean-12-en-28-oic acid) are the main terpene acids present in olive oil (see *Figure 22*). Ursolic acid (3b-hydroxy-urs-12-en-28-oic acid) and betulinic acid (3b-hydroxy-lup-20-[29]-en-28-oic acid) have also been identified. The level of triterpenic acids in olive oil range between 40 and 185 mg/kg (Boskou et al., 2006). Much higher levels are found in olives and olive pomace oil. Hydroxyterpenic acids and the triterpene dialcohols erythrodiol and uvaol *(Figure 22)* are bioactive compounds. Studies for their pharmacological potential focus on inflammation, cancer, cardiovascular pathology and vasorelaxation (Herrera et al., 2006; Rodriguez-Rodriguez & Ruiz-Gutierrez, 2010; Valero-Munoz et al., 2014).

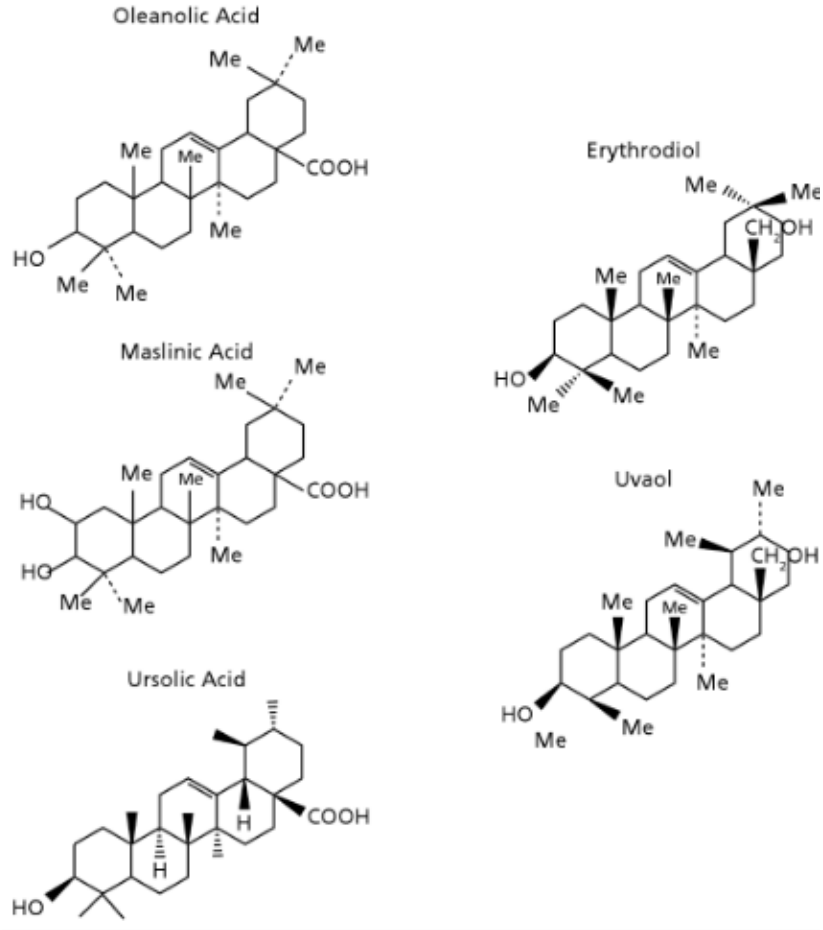


Figure 22: Hydroxyterpenic acids and triterpene dialcohols (from Boskov, 2015)

1.2.4.3. SQUALENE

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosanehexaene) is an unsatured terpene widely distributed in nature. Chemically, it is an all-trans isoprenoid having six isoprene units. It occurs in high concentrations in the liver oil of certain sharks and in smaller amounts in olive oil. It is the major constituent of olive oil unsaponifiables. Its level in olive oil may range from 200 to 7500 mg/kg, although much higher levels (up to 12,000 mg/kg) have been reported. Squalene may have a chemo preventive effect in some types of cancer, and it is beneficial for patients with heart disease and diabetes. In a 2013 report, Alvaro L. Ronco and Eduardo de Stefani stressed that it would be desirable to have higher squalene concentrations in vegetable sources such as olive oil, considering the ecological impact of capturing marine species. Squalene can be recovered from olive oil deodorization distillates.

1.2.4.4. PHYTOSTEROLS

Phytosterols are functional ingredients because they reduce the absorption of cholesterol in mammals. However, the concentration in olive oil is too low for such an effect, it is claimed that consumption of 1.5-2.0 g/day of phytosterols is needed for a hypocholesterolemic effect (commercial spreads have an elevated level of sterols or stanols [8%], in the form of esters with fatty acids). However, the possible small contribution to the effect from a natural source should not be overlooked. According to Cardeno et al. (2014), two constituents of the unsaponifiables of olive oil, β-sitosterol, and α-tocopherol have been shown to influence the reduction of reactive species as well as COX-2 activity. Four classes of sterols occur in olive oil: common sterols (4α-desmethylsterols), 4α-methylsterols, triterpene alcohols (4,4-dimethylsterols), and triterpene dialcohols. Sterol composition and total sterol content are affected by cultivar, crop year, degree of fruit ripeness, storage time of fruits prior to oil extraction and geographic factors. Olive harvesting practices and processing also affect individual sterols composition. Total sterol content and the composition of the sterolic fraction are important indices for checking olive oil authenticity.

1.2.4.5. CAROTENOIDS

Lutein, zeaxanthin and beta-carotene are plentiful in olives. Method of extraction, temperatures used during extraction, and sequence of extraction can all cause significant differences in the final carotenoid of the oil (Sanz et al., 2005). The main carotenoids present in olive oil are beta-carotene and lutein. Xanthophylls such as violaxanthin, neoxanthin and other have also been reported to occur in exceedingly small quantities. Total carotenoids in olive oil may range between 1 and 20 mg/kg,

but usually values do not exceed 10 mg/kg. Carotenoids are singlet oxygen quenchers and protect the oil photooxidation. There is a relation between carotenoids and the mode of action of polar phenols and α-tocopherol. In addition to supplying its own carotenoids, olive oil may be able to help humans absorb carotenoids from other foods.

1.2.5. OLIVE OIL ALTERATIONS

The main degrading processes at the lipid fraction are: - hydrolytic splitting (enzymatic or chemical); - chemical rancidity (self-weeding of lipids); - chetonic rancidity (enzymatic process).

1.2.5.1. HYDROLYTIC CLEAVAGE

Lipases are the main culprits for the hydrolytic splitting of glycerides as the chemical process running with much slower kinetics takes an extremely long time. This alteration represents the first stage of the process of deterioration of the quality of an oil. The enzyme, initially compartmentalized and isolated inside the intact cells of the olive, tends to mix with oil when because of crushing, cellular compartmentalization is lost so substrates and enzymes are put into contact. The detachment of fatty acids from the triglyceride promoted by lipases, proceeds in accordance with a reaction mechanism that involves the removal of only one fatty acid at a time. The concentration of fatty acids thus released constitutes the free acidity of the oil, i.e., the percentage content of free fatty acids, expressed as oleic acid in relation to the totality of the oil produced. In mechanically damaged olives, characterized by a high degree of ripening, attacked by the olive fly, or harvested using techniques that do not respect their integrity, the activity of lipase is particularly high. The extraction technology adopted will also condition its acidity, in fact it will tend to grow on the same as the other process variables, with the extraction time.

1.2.5.2. OXIDATIVE RANCIDITY

It is a very complex phenomenon that sees atmospheric oxygen interact with fatty acids produced by lipases, to create an auto catalytic process: "the self-weeding of lipids". The self-weeding of an olive oil can be established from the earliest stages related to the processing of these fruits, starting mainly during its storage. Since this is an oxidative process, it would be enough to operate in the absence of this gas to prevent its course. But traces of oxygen are enough to promote its rapid development. This alteration is facilitated by the possible competition of some environmental factors, including exposure to light and heat, the presence of peroxides, contact or presence of certain metals (iron, copper, nickel), the presence of the enzyme lipoxidase. A fundamental role is played by the presence in food, of compounds that slow down the initiation phase (antioxidants, such as tocopherols) or favor it (pro-oxidants, such as chlorophyll). From a kinetic point of view, the process is divided into three phases

characterized by a different oxygen demand. In the initiation or induction phase, connected to the development of the first radical forms, there is no absorption of oxygen that runs into the second to produce the lipid peroxides, and become exponential when the decomposition of these peroxide species leads to the auto catalytic production of increasing amounts of radical species and therefore of new hydroperoxides in a logic of exponential growth no longer easily controllable.

1.2.5.3. CHETONIC RANCIDITY

It is an altering process promoted by bacteria, yeasts and mold that can produce the enzymes necessary for its realization. Only in poorly preserved fruits and therefore exposed to the attack of these microorganisms can metabolites be accumulated responsible for this alteration of oils.

1.3. FACTORS INFLUENCING THE QUALITY OF THE OIL

1.3.1. GENETIC AND AGRONOMICS FACTORS

1.3.1.1. CULTIVAR

There are numerous varieties of olives from which the oil is obtained, and their diffusion is closely linked to the territory and each of them generates oils with different compositional characteristics (fatty acids, polyphenols, volatile components, etc.) and sensory (smells, flavors and typical flavors). This characteristic represents an effective tool for differentiating monovarietal oils from different varieties (Alfei et al., 2020). The phenolic content varies greatly depending on the genotype and its precocity. Depending on these characteristics, in fact, the processes of synthesis, polymerization and degradation of these compounds vary. The presence of oleuropein is found indiscriminately in all varieties, while the presence of verbascoside and demethyloleuropein is genotype dependent. Volatile compounds are also influenced by the genotype and their variation is predominantly quantitative. It is possible to say that the variety influences the content of the aromatic compounds present in the oil since the enzymatic activity related to their production is genetic. determined mind; for this reason, the analysis of the aromatic profile of an oil can be used for varietal recognition based on the percentage of each metabolite present. The variety, in addition to conditioning the content of the different enzymes responsible for the formation of these compounds, influences the presence, in terms of concentration, of the precursors of aromatic compounds, including those coming from the synthesis of terpenoids. Evaluating the presence and concentration of C6 aromatic compounds such as aldehydes, esters, and alcohols in oils from different varieties, but collected at the same degree of ripeness and with the same extraction operations, it emerged that their content varies in a statistically significant way both from an analytical and sensory point of view, precisely as a function of the

starting genotype. It has been observed that the volatile compounds that exhibit greater variability because of the variety are the following: ethanol, 2-methylpropanol, pentanol, cis-2-pentenol, cis-3-hexenol and octanol; considering, moreover, the sensory notes, those that vary most according to the starting genotype are those of "fruity" and "floral". By many of the chemical and sensory characteristics of virgin olive oils are influenced by genotype, the preservation and characterization of varieties play a key role in the production and marketing of quality olive oils. In Italy, for example, the market for monovarietal oils is constantly growing thanks to the increase in consumers who pay more and more attention not only to the health aspect of the product, but also to the pleasure of tasting an oil distinguishing it according to the sensory profile that characterizes it. *Figure 23* shows the significant qualitative and quantitative differences in phenolic and volatile compounds, between monovarietal oils from different Italian varieties, highlighting that these differences are high even within genotypes that traditionally come from the same range.

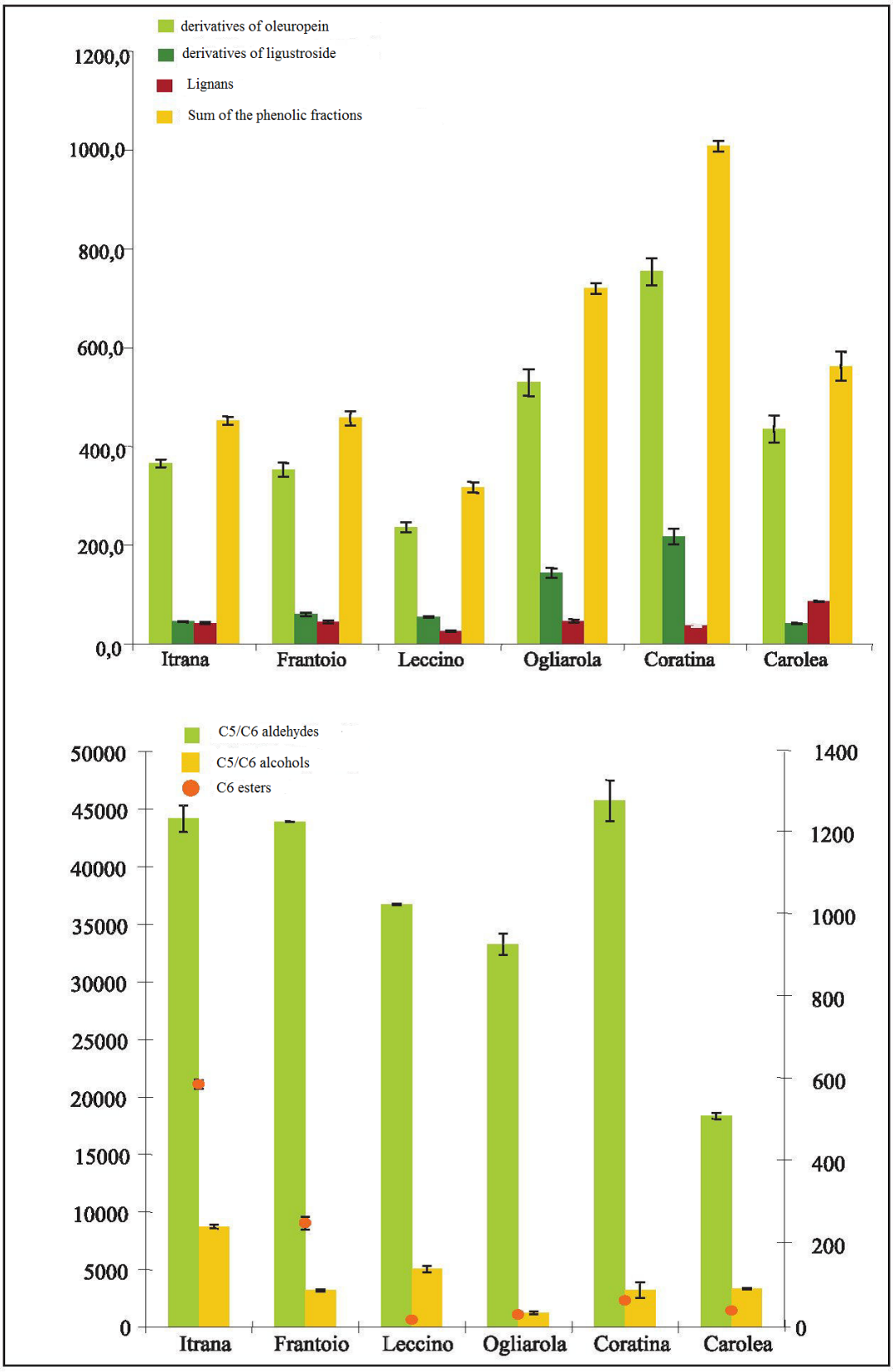


Figure 23: Concentration in polyphenols expressed as the sum of oleuropein derivatives (3,4-DHPEA, 3,4-DHPEA-EA and 3,4-DHPEA-EDA), ligustroside (p-HPEA, p-HPEA-EDA) and lignans (+) - pinoresinol, (+) - 1-acetoxypinoresinol) and sum of the different phenolic fractions (mg / kg), and of the volatile components, expressed as the sum of aldehydes, C5 and C6 alcohols; (columns) and esters a C6 (points) (u9 / kg) (From Alfei et al., 2020)

1.3.1.2. THE AREA OF ORIGIN

Even the geographical origin of virgin olive oils, especially if we consider rather distant areas, plays a fundamental role in the definition of their gustatory, tactile and olfactory characteristics. In general, European oils are characterized by a high concentration of monounsaturated fatty acids (oleic acid in particular) that make the oil more viscous, and in the case of turbid or veiled oils this is reflected in the fact that the microparticles of water responsible for this phenomenon, remain longer in suspension, thus loosening the effect of natural sedimentation (Alfei et al., 2020). Great variability is also found at the level of phenolic and volatile composition, as can be observed in *Figure 24*. The comparison between large territories allows us to have a picture of the substantial differences in phenolic constituents between Italian, Spanish, Greek and Tunisian oils, especially about the concentration of oleuropein derivatives and, secondly, of ligstroside derivatives. The lignan levels, on the contrary, remain quite similar. These results are interesting regarding the possibility of using oleuropein derivatives to monitor the geographical origin of oils. In this regard, evaluating only the data from Italy, it was observed that the variability of the concentration of oleuropein derivatives is rather homogeneous within each region but very different between regions: the ranges of values were between 250 and 600 mg / kg as the sum of hydroxytyrosol, oleacein and oleuropein aglycone. The study of the volatile component conducted on the same oils, has allowed to observe that the sums of aldehydes and alcohols at C5 and C6 and esters at C6, are characterized by large differences between the areas (*Figure 25*). The evaluation of terpenes and sesquiterpenes, which many researchers consider more stable compared to the variables in the mill, showed the same tendency to high variability, as observed above all, in the leading spaces of Italian and Greek oils (*Figure 25*).

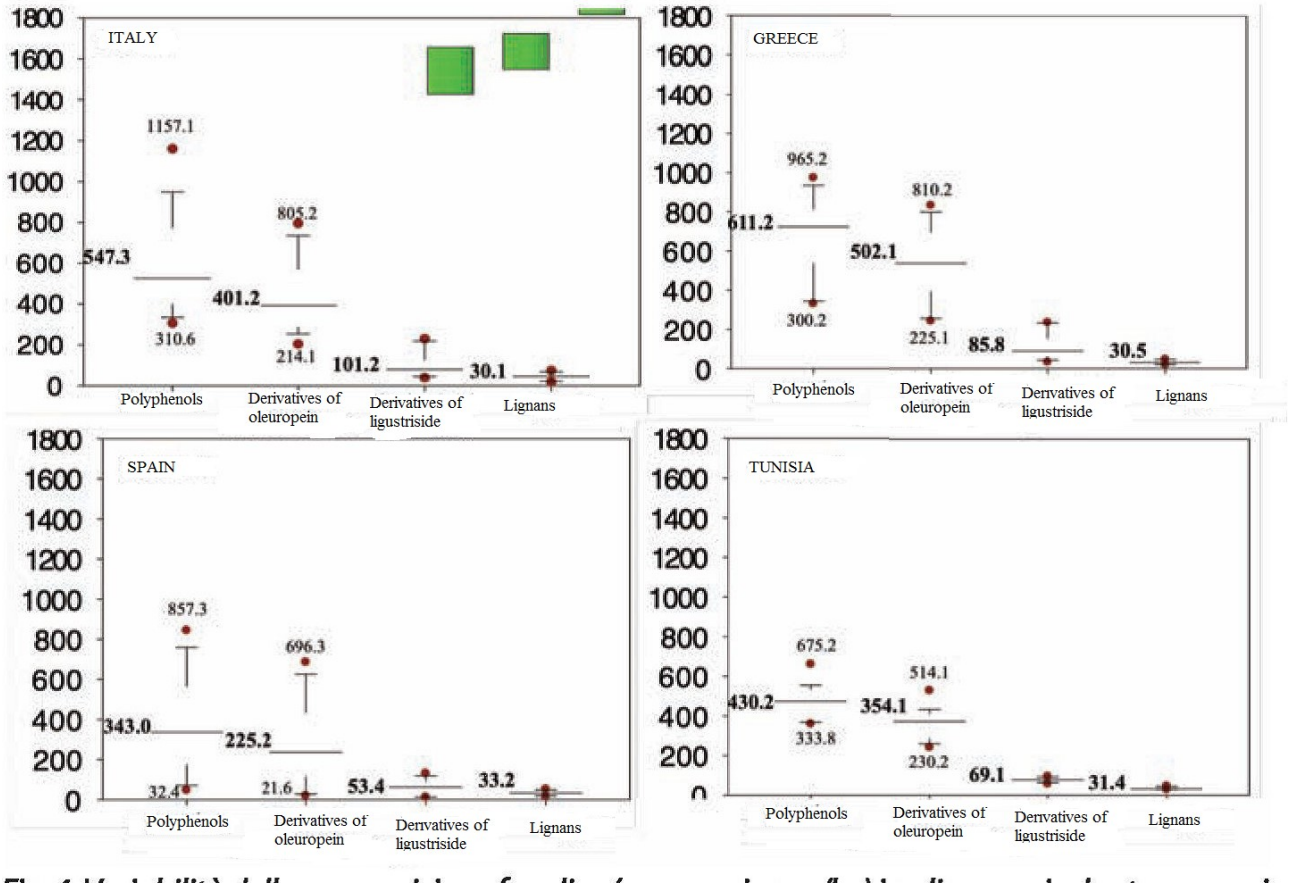


Figure 24: Variability of the phenolic composition (expressed in mg / kg) in European and extra-European oils represented by mustache boxes. Limits in percentile: box = low side 25th percentile, side high 75th percentile; whiskers = low 10th percentile mustache, high 90th percentile mustache; points = low, 5th percentile; high, 95th percentile. The line inside the boxes represents the median. (From Alfei et al. 2020)

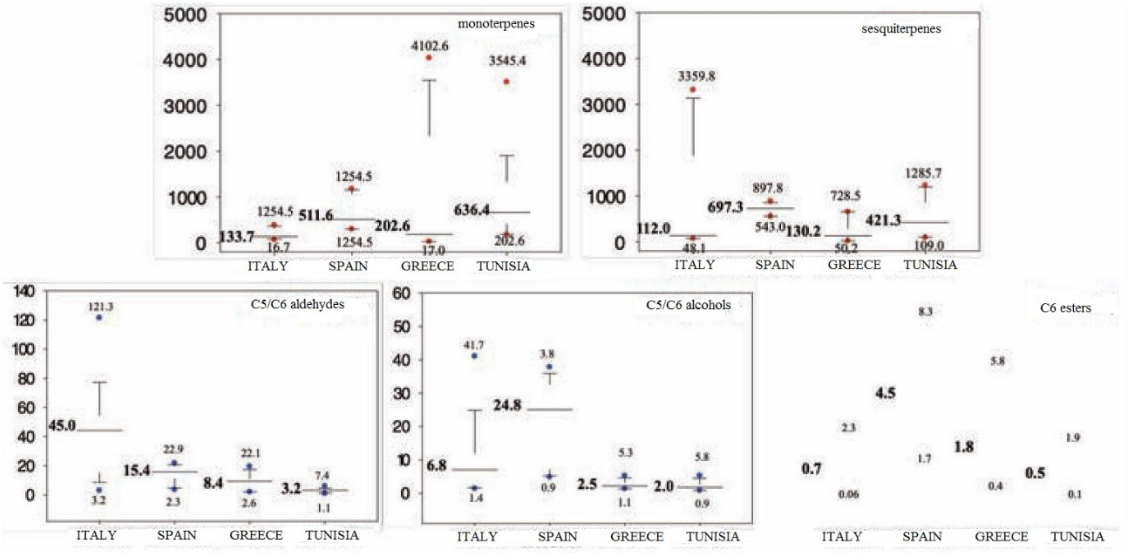


Figure 25: Variability of the volatile composition (expressed in ug / kg for compounds at C5 and C6 and in area counts for terpenes and sesquiterpenes) in European and extra-European oils represented by boxes mustache. Limits in percentile: box = low side 25th percentile, high side 75th percentile. whiskers = low 10th percentile mustache, high 90th percentile mustache; points = low, 5th percentile; tall, 95th percentile. The line inside the boxes represents the median. (From Alfei et al., 2020)

1.3.1.3. FRUIT RIPENING

During ripening, a series of changes take place inside the fruit that determines the modification of the chemical-physical characteristics of the oil: the resistance to the detachment of the drupes is progressively reduced, the synthesis of the oil is completed, the consistency of the pulp decreases and the pigmentation of the epicarp and, subsequently, of the mesocarp becomes increasingly evident, starting from the outermost layer inwards (Alfei et al., 2020). As maturation continues, the oleic acid content remains constant or increases insignificantly, while saturated fatty acids decrease. The content of palmitic acid decreases while linoleic acid increases. Another parameter that varies according to the ripening of the fruit is the ratio between monounsaturated and polyunsaturated fatty acids which, with the passage of time, tends to decrease. The content of phenolic compounds, on the other hand, increases progressively in the first stages of ripening of the fruit and then decreases rapidly with the intensification of pigmentation before the epicarp and then the mesocarp. Depending on the degree of ripeness of the drupe, the ratio between the individual phenolic compounds also varies significantly. The phenolic compounds can therefore be used not only as a marker of the cultivation area, but also as a parameter capable of defining the optimal time for harvesting and optimizing the sensory characteristics of the oil. Some studies have shown that the concentration of oleuropein aglyconic derivatives decreases with the onset of veraison, with an increase in phenolic alcohols such as tyrosol and hydroxytyrosol. The different balance between phenolic substances towards simpler forms seems to be linked to a greater activity of glucosidases and esterases during the first stages of ripening of the fruit. Even the potential content of volatile compounds reaches the maximum values in the initial stages of superficial veraison of the fruit and then decreases with its ripening precisely because of the lack of activation of endogenous enzymes related to the "lipoxygenase pathway". This scientific evidence allows us to affirm that oils produced from poorly pigmented and therefore not very ripe olives, are characterized by more pronounced sensations of "bitter", "spicy" and "fruity green", due to a greater concentration of both phenolic and aromatic compounds. The progress of maturation, yellow/green or yellow oils are obtained that tend to have a "flat" organoleptic profile. It tends to be possible to say that the phase following the superficial pigmentation of the olive constitutes a phase of profound physiological and anatomical transformation of the drupe, which translates into an overall decrease in the compounds responsible for the sensory characteristics of the relative oil. In any case, several studies argue that the reduction of these compounds, as the ripening of olives progresses, is not the same for all varieties. It has been shown, for example, that the content of hexane-1-ol decreases in The Nocellara del Belice but increases in the Coratina, or that varieties such as Coratina, Rosciola and Frantoio have high phenolic concentrations in the first period of development of the drupe, and then decrease progressively; on the contrary, the Dolce Agogia and Tendellone

varieties have extremely low oleuropein values throughout the period of development of the drupe. Choosing the period of olive harvest, especially depending on the variety, is therefore decisive for the sensory characterization of the final product.

1.3.1.4. FRUIT INTEGRITY

To obtain quality virgin olive oils it is essential to work healthy drupes. Unfortunately, sometimes it is not possible due to alterations of various kinds affecting the drupe. Among these, the dipteran *Bactrocera oleae* (olive fly), causes the greatest damage, attacking the fruits from the early summer periods until the time of harvest and causing severe damage to the pulp of the olive, starting from the larval stages up to the exit hole of the adult (Alfei et al., 2020). The damage caused to the fruit has serious repercussions on the volatile and phenolic composition of the oils and so on their sensory characteristics. The negative effects in this regard are due to a potential development of microorganisms belonging to distinct species and responsible to produce carbonyl compounds and alcohols responsible for sensory defects.

1.3.1.5. CLIMATIC CONDITIONS

The environmental conditions influence both the acidic composition of the oil and the qualitative and quantitative composition of the minor components that are decisive for the definition of the sensory profile of the oil. It is now known, in fact, how the altitude and latitude of the cultivation environment can significantly influence the chemical composition of the olive fruit. Numerous studies have shown, for example, that the ripening temperatures of the drupe, evaluated between the period of sclerification of the endocarp and the harvest, influence the acidic profile of the oil; Vintages and warm environments cause the decrease of oleic acid and the increase in the content of linoleic, palmitic and linolenic acids (Alfei et al., 2020). The effect of temperature on phenolic content is not yet clear: some studies believe that elevated temperatures negatively affect phenolic content, while others find a positive correlation between high thermal summations and the concentration in polyphenols in the final product. Temperatures and precipitation influence the course of ripening of the olives: specifically, low-rainy and particularly hot seasons accelerate the ripening process. Late frosts, typical of the Nordic regions, can negatively affect the content of carotenoids, chlorophylls and phenolic compounds and their oxidation stability, the levels of "bitter" and "spicy", as well as promoting a general worsening of the sensory characteristics of the product up to the defect of "wet wood" (frozen olives). As for the influence of the water availability of the plant, it has been scientifically demonstrated that they not only affect the qualitative and quantitative production of primary metabolites ("major" components) but also that of secondary metabolites that characterize the sensory and nutritional profile of virgin olive oil. Many studies agree in stating, in fact, that plants

grown in conditions of water stress produce fruits with a higher content of phenolic compounds. This could be related, on the one hand, to the fact that irrigation favors a consistent dilution of these compounds in the aqueous phase during the oil extraction process and, on the other hand, that in conditions of water stress, an increase in the synthesis of polyphenols within the fruit is favored through an increase in the activity of L-phenylalanine ammonia-lyase (PAL), an enzyme whose activity, is crucial in the synthesis of phenolic compounds. The water status of the plant, in fact, also influences the relationships between the individual phenolic components: fruits from plants grown under water stress have a higher content of oleuropein derivatives and a lower content of phenolic alcohols (hydroxytyrosol and tyrosol). The sensory profiles of the oils also vary significantly depending on the water status of the olive tree: oils from plants grown in dry have more important notes of "bitter" and "spicy" and, although less relevant, aromatic notes of "fresh grass" and "floral" less intense. This "aromatic reduction" seems to be associated with the decrease of some compounds related to the lipoxygenase pathway such as aldehydes and saturated and unsaturated alcohols at C5 and C6. It is possible to conclude that oils from olive trees with a good water availability have a more aromatic sensory profile but with less intense "bitter" and "spicy" sensations; on the contrary, oils from drupes grown in water stress will have a more decisive character to taste and touch but with a more limited aromatic profile. In this regard, one of the current trends in the field of olive growing is to practice irrigation in controlled deficit which consists in administering lower quantities of water than the water needs of the plant at certain times of the growing season. In this way, in addition to saving water, with a positive effect on the environment, it is possible to optimize the chemical, nutritional and sensory profile of the relative virgin olive oil.

1.3.2. TECHNOLOGICAL FACTORS

1.3.2.1. TRADITIONAL EXTRACTION TECHNOLOGIES

The production phases of olive oil are in summary: defoliation, washing, possible pitting, crushing, malaxation, extraction.

1.3.2.1.1. Preliminary operations: defoliation, washing of olives and their pitting. Any problems related to the collection methods, the preservation of drupes and the washing of olives

These preliminary stages are common to all olive processing schemes. These operations are carried out by automatic machines equipped with a suction system for the removal of leaves and a tub with forced circulation for washing olives. While the removal of foreign bodies that may be present (earth, mineral or vegetable solid residues) aims to preserve equipment furnished with mobile structures

(crushers, decanters and centrifuges), the removal of the leaves prevents the oil from being enriched with hints of freshly cut grass and bitter flavors, linked to the presence of trans-2-esenal (Di Giovacchino, 2000). These preliminary operations also include the possible pitting of the fruits, which provides for the elimination of almond, to limit the activity of polyphenolossidase in this mainly localized, which are responsible for the degradation of phenols suffered by oil during its extraction (Servili et al., 2006). This practice therefore makes it possible to obtain oils with a high content in phenolic substances, therefore with a more complex aromatic profile, with a higher health nutritional value and a shelf life longer over time (Ranalli et al., 2009).

Collection methods: nowadays the olive harvest is conducted mechanically and less and less frequently by hand directly from the plants. It is not yet clear the effect on oil quality that different harvesting methods can have. It can be said, however, that mechanization allows to speed up the harvest itself and therefore to concentrate it in the optimal ripening period of the fruit (Alfei et al., 2020). The mechanized harvesting, moreover, using shakers, allows to reach quantities suitable for processing without the need to store the fruits after harvesting while waiting to reach a certain quantity. the use of shakers seems to reduce the damage to the fruit induced by the harvest and therefore limit all the alterations of the fruit that would also compromise the sensory quality of the final product. The damage to the fruit, in fact, causes a significant reduction in phenolic and aromatic compounds in the final product, a phenomenon emphasized by a further subsequent conservation of the olives. Olives can be damaged significantly by harvesting operations causing the breakdown of tissues and consequently of cells promoting the premature activation of endogenous enzymes. Some studies claim that the use of trunk shakers and digging machines cause more substantial damage to the fruit than manual harvesting. In some production areas where the trees are excessively tall with a trunk diameter not suitable for mechanical harvesting, the olives are left on the plant until they reach overripening and spontaneously fall on the ground. The olives are then harvested using brushes and vacuum cleaners at regular intervals until the end of spring. The headspaces of the relative oils generally present a considerable concentration of alcohols and carbonyl compounds which results in unpleasant sensations of "mold" and "earth" at the same time, off-flavors typical of a prolonged contact of the fruits with the soil.

The preservation of drupes: After harvesting, another aspect of fundamental importance for the determination of the sensory quality of virgin olive oils is represented by the times and methods of conservation of the olives. The fruit during storage can undergo deterioration also due to microorganisms that can find in the drupe a favorable environment for their development: the chemical composition of the fruit, the high humidity, the possible damage to the epicarp (whose integrity allows to offer an antimicrobial barrier) and the field temperatures of the drupes (which, in

recent years, due to early harvesting and earth overheating are increasing), they are all factors that favor the proliferation of microorganisms (Alfei et al., 2020). It is therefore preferable to process the fruits in the shortest possible time because, while maintaining the optimal storage conditions (low temperatures, reduced humidity level, etc.), the concentration of volatile compounds and of (E)-2-hexene decreases compared to oils produced from processed fruits immediately after harvesting. To limit the deterioration of the fruit during storage, it is important to use perforated plastic boxes (food grade) to allow its ventilation, since, like all fruits, olives also "breathe" producing heat that favors degradation processes. Even the thickness of the layers of olives in the boxes, considering the consistency of these fruits, should not exceed 10-15 cm to reduce their damage by crushing. Even today, the storage of olives is still carried out using plastic bags that cause negative repercussions on the final product. The preservation in bags favors in fact, the overheating of the drupes and the non-aeration of the same, synergistically promoting the autolysis of the organic material, the rotting of the fruits and the uncontrolled development of microorganisms. The production of different metabolites, depending on the type of microorganism that has differentiated (depending on the temperature and humidity conditions that have been reached), in conjunction with the decrease in the presence of compounds deriving from the lipoxygenase pathway, causes the worsening of the sensory and nutritional properties of virgin olive oil. Oils produced from olives stored in bags are often characterized by the defect of "screwed" due to the growth and proliferation of yeasts and bacteria such as *Acetobacter,* which produce ethanol and ethyl acetate. Other microorganisms that often find optimal conditions for their development are belonging to the genera *Pseudomonas*, *Enterobacteriaceae* and *Clostridia* that determine the formation of volatile compounds (acetic and butyric acid) that exceed the threshold of olfactory perception and that, consequently, are also detected by sensory analysis with the defect of "heating". Most of these compounds are branched aldehydes, branched alcohols and their respective acids. Furthermore, prolonged storage, associated with the maintenance of unsuitable temperature, humidity and storage conditions, favors the development of molds (in particular *Aspergillus and Pennicillium*) whose enzymes interfere with those of the olive fruit involved in the LPO pathway causing, according to the extent of the attack, both a reduction of C6 compounds and the formation of C8 compounds, common metabolites from the LPO of molds, giving the relative oil, the defect of "mold". The complete rotting of the drupes, moreover, due to the important fungal pectolytic activities, explains the high concentrations of propan-1-ol, 2-methylpropan-1-ol, 3-methyl-butan-1-ol and their corresponding acids and esters. The prolonged and / or incorrect storage of olives, moreover, may be responsible for an increase in the free acidity in the relative oils, because of hydrolysis of fatty acids by exogenous lipases of microbial origin. In addition to the commodity decay, this can also lead to a rapid loss of turbidity in oils

deliberately kept "veiled" and form deposits faster; this could be because free fatty acids, having a polar part, can bind to microparticles of water in suspension (and responsible for the "veiled" effect), forming large and heavy aggregates that then precipitate. Lampante oil, in fact, which have an acidity of more than 2%, tend to lighten and form precipitates very quickly. Numerous studies are underway to evaluate what may be the best strategies to promote the preservation of fruits by compromising as little as possible the oxidative stability of the oil and its nutritional and sensory characteristics. Some studies recommend keeping the storage temperature at 5 ° C, to minimize the activity of microorganisms and have the possibility of storing the olives for up to 30 days without causing a deterioration in the quality of the finished product. On the contrary, temperatures close to 0 ° C are to be excluded in the conservation of drupes since they cause alterations in the chemical structures of secoiridoids. In another research, the variation in the phenolic content and enzymatic activities of olives preserved for a month at 20°C and 4°C was evaluated. Oils obtained from fruits stored at 4°C showed similar characteristics to those obtained from freshly harvested processed olives, while oils obtained from olives stored at 20°C had a lower phenolic content. After three weeks of storage at a temperature of 20 ° C there was a drastic decrease in β-glucosidase responsible for the release of the aglycone secoiridoids in the paste. In a recent study, 5 different storage conditions were tested by varying the temperature and concentrations of oxygen and carbon dioxide in the atmosphere, to determine their influence on the quality of the related oils. It has been observed that the reduction of the storage temperature to 5°C prevents the rapid alteration of the fruit, while the use of the controlled atmosphere does not seem to have any effect either on the oxidative stability or the sensory quality of the final product. In this regard, further studies are underway to optimize the gaseous composition that best allows to preserve the quality of olives and their oils in a refrigeration regime in a controlled atmosphere.

The washing of olives: the washing operation is always recommended by technologists and is especially important when the drupes, after several days of rain, appear dirty from mud splashes or have attached pieces of earth. Hot water washes, however, could cause changes in the aromatic profile of the future oil, caused by the decrease of C6 aldehydes and C5 compounds, probably due to a partial deactivation of the LPO/HPL enzyme system, while C6 alcohols and esters remain quantitatively constant suggesting that the activities of alcohol dehydrogenase are weakly affected by relatively high temperatures (Alfei et al., 2020). Sometimes, especially when the olives are quite ripe, they are processed avoiding the washing phase to reduce the potential oil losses due to the possible detachment of pieces of pulp during the phase: the corresponding oils will have a smell of "earth" not yet attributable to any specific volatile compound or group of volatile substances.

1.3.2.1.2. Crushing or milling olives and consequences on the quality of the oil

The droplets of oil, the size of a few microns in diameter, are in the pulp cells of the intact fruit and more specifically inside the vacuole. The crushing of the fruits is therefore a mandatory step to dilacerate the oleaceous cells allowing the oil to escape and therefore the formation of a more cohesive oil phase easily separable from the water present in the fruits. During the crushing with the rupture of the oleaceous cells and the lipoprotein membrane that coats it, the oil comes out of the vacuole and disperses into the cell juice in the form of small diameter droplets that, being partly related to the colloidal structure of the cytoplasm, are held in the solid part of the dough inside channels and bags. Interactions between vegetation water and oil (both the one originally dispersed in the cytoplasm and partly also that from the vacuous) lead to the formation of emulsions. The size reached by the fragments of pulp and core, because of the crushing, affect not only the efficiency of the extraction but also the chemical-physical and organoleptic characteristics of the oil produced. A paste consisting of large particles leads to a lower yield and a lower extraction of phenolic components and chlorophyll pigments, on the other hand a paste formed by particles that are too small interferes negatively with the yield due to colloids phenomena, which determine the formation of emulsions and make subsequent extraction phases more difficult. In general, a crushing leading to the release of solid fragments (core) of 2÷3 mm is considered optimal. The crushing also promotes the exchange of minor components and the activation of enzymatic processes with the possible consequent neoformation of some compounds characteristic of olive oil (free fatty acids, peroxides, volatile compounds, glucoside garlic, etc.). The distribution of the minor components runs according to their different affinity for the phases involved, so with the progress of time the ratio of the concentrations assumed by each component in the phases tends to a constant value. This constant takes the name of a distribution coefficient and depends only on temperature, while the values of the individual concentrations can also vary substantially depending on the operating conditions adopted (addition of water at the separation stage of the oily must). In olive oil stored in the vacuole of oleaceous cells, there are small quantities of volatile compounds (derived from fatty acid metabolism or the conversion of certain amino acids), the production of which becomes significant because of the activation of a series of enzymatic reactions that occurs when the loss of cellular compartmentalization promotes contact between enzymes and substrates. The synthesis of these precious components is strictly dependent on the cellular destruction of the drupe and is therefore established during the crushing phase to continue during the malaxation, but still runs because of any other traumatic mechanical event (wounds, dents, heating, shriveling, over maturation, etc.). Normally the crushing systems are

distinguished according to the force applied and its consequent effect. The violence of the apparatus used in the crushing induces a thrust production of micro-droplets that, assuming ever lower values, will require increasingly long malaxation times to join until they reach such dimensions as to be extracted from the paste (>0.03 mm) (Martinez Moreno et al., 1957). The decisive rupture of the almond, with the consequent release into the dough of the enzymatic heritage in this enclosed, turns out to be directly proportional to the disruptive force applied during the crushing. The violence of the crushing system adopted, and therefore the resulting emulsifier effect, tend to increase as implementation times decrease grinding mill < roller crusher < disc crusher < knife crusher < movable hammer crusher < fixed hammer crusher. Among the crushing systems, the most common are:

• TRADITIONAL SYSTEM IN MULLERS, consisting of a tank with granite base, equipped with an opening for the unloading of olive paste and inside which move in the rotational direction of the stone wheels (the grinding wheels), varying both in weight (2÷4 t) and in number (2÷4) *(Figure 26).*

Figure 26: Mullers (by website https://www.elicriso.it/it/olio/estrazione_olio_olive/)

It is certainly a discontinue system, generally combined with the traditional press extraction system, but also used in continuous extractive processes, to obtain oils with harmonic and balanced organoleptic characteristics. However, the high cost, the large space occupied, and the discontinuity caused to the whole process represent major limitations of this extraction mode.

• CONTINUOUS ROLLER MILLING, consisting of a pair of counter-rotating stone rollers, followed by a disc finisher. It produces harmonic oils, due to a lower extraction of bitter substances than the hammer crusher; has a high working capacity combined with a reduction in emulsions; the footprint is limited, as well as the costs, however a large limit is the heavy wear and frequent breakage due to the presence of foreign bodies.

• HAMMERS MILL, fixed or articulated, is usually present in continuous olive processing plants and provides for olives to be crushed against a fixed or rotating perforated grill *(Figure 27).*

Figure 27: Hammer mill (from website www.alfalaval.com)

This system produces a violent crushing, which involves, especially in older models, the shredding of oil droplets and the formation of emulsions, requiring long malaxation times to make the oil drops coalesce until the minimum value of the threshold measure is exceeded to drain from the paste into the separator. In the face of a greater extraction of phenols and chlorophyll pigments, linked to violent milling, marked organoleptic characters with higher hints of bitterness are detected in the oil. In addition, the shredding and strong heat produced by the mill (12-15°C more than the environment T) cause an increase in the speeds of enzymatic reactions, which are also active for longer periods.

• TOOTHED DISC CRUSHERS, fragile in the presence of foreign bodies, operate an optimal crushing, thanks to the absence of emulsions, guaranteed by rotation speeds within 1400 rpm, and the good extraction of phenols and chlorophylls *(Figure 28):*

Figure 28: Toothed disc crusher (P. Amirante et al., 2010)

• KNIFE BREAKER, recently introduced on the market of oil machinery, manages to selectively break the constituent parts of the drupe, operating an efficient degradation of the pulp and a reduced breaking action both on the peel and on the almond *(Figure 29).*

Figure 29: Knife breaker (from website https://www.clementeindustry.com/frangitore/)

Comparison tests between hammer breakers, knives and low-rpm crushers have shown an interesting effect both in the reduction of bitter notes and in the exaltation of the aromatic characteristics of virgin olive oils. On the other hand, low-rpm breakers as well as knife crushers reduce the extraction of chlorophylls from the peel and the consequent release of the green color in the oil, thus obtain oils on the one hand more harmonious at the sensory level and less bitter, but poorer in color than those obtainable with a hammer crusher. Concluding the advantages of the most modern breakers compared to traditional mills, they reside in a small footprint, a reduced capital to invest, a high working capacity resulting from continuous operation, in the rupture of many oleaceous cells, in a high concentration of chlorophyll and phenols, which give greater oxidative stability. On the other hand, stone crushers have the advantages of a crushing of fruits at low temperature, almost zero time of malaxation due to a reduced formation of emulsions, the absence of costs for wear of rotating parts at high speed, the absence of supplies of pro-oxidizing metals and the formation of a more balanced flavor characterized by more intense olfactory notes and more attenuated sensations of bitter and spicy (Angerosa, 2005). The oil extraction process is fundamental for the definition of the nutritional and sensory quality of the product. The content of volatile and phenolic compounds in virgin olive oil, in fact, is closely related to the activity of endogenous enzymes that are activated during pressing. The pressing is an operation that consists in the crushing of the fruit of the olive tree and all its parts, including the stone, determining the formation of the "crushed olive paste" containing a water-oil emulsion. During this phase, β-glucosidases and endogenous esterases are activated, catalyzing the hydrolysis of secoiridoids in their aglyconic derivatives (characterized by a greater degree of hydrophilia), favoring the solubility of these compounds in the oil (Alfei et al., 2020). The breakdown of cellular compartments favors the release and subsequent activation of other "positive" endogenous enzymes such as those involved in the "lipoxygenase pathway", and "negative" ones such as polyphenol oxidases (PPO) and peroxidases (POD) which, in the subsequent kneading phase, can catalyze the enzymatic oxidation reaction of phenolic substances, in the presence of oxygen. These enzymes have

a different compartmentalization in the drupe: POD is mainly present in the almond, PPO and glucosidases, in the mesocarp, while LPO is contained in all parts of the fruit. As for the aromatic compounds, these originate because of the rupture of the cellular structure in the pressing phase, which promotes the disintegration of the cell wall and membranes through mechanical actions that cause the escape of cellular juices, containing the enzymes involved in the "lipoxygenase pathway", and of the oil in the form of an emulsion with vegetation water. During this operation, the most consistent production of aromas occurs compared to all the other phases of the extraction process. Since oxygen is a co-substrate of the lipoxygenase cascade, its presence during the pressing phase influences the production of volatile compounds. The different concentration of oxygen in pressing is the subject of recent studies that have scientifically demonstrated how the variability of this cofactor can significantly modify the relative aromatic and phenolic component of the fruit. If on the one hand, in fact, the higher concentration of O_2 guarantees a greater production of aromatic substances linked to the lipoxygenase pathway, on the other hand it may be responsible for a greater loss of phenolic components by PPO and POD, in the next phase of kneading. The pressing, therefore, can be managed by reducing the oxidation process borne by the polyphenols without negatively affecting the production of volatile compounds, appropriately modulating the concentration of oxygen in the crushers, also by virtue of the different varieties processed. Considering also the different enzymatic compartmentalization within the different parts of the fruit it is possible to limit the release (and consequently, their activity), of oxidative enzymes through pitting (elimination of the endocarp at the same time as the pressing phase) or by means of crushers with a differentiated effect. Pitting limits the presence, and therefore the activity, of the oxidoreductase enzymes (in particular of the POD) contained in the core and this allows to reduce the enzymatic oxidation of the phenolic compounds in the kneading phase and to obtain at the same time, oils characterized by sensory profiles with marked sensations of "bitter" and "spicy" but also of "green fruity" as it has been observed that the volatile component of pitted oils does not seem to be affected by the loss of the fraction of LPO located in the endocarp of the drupe. Such an aspect has been observed on several varieties. Another valid alternative to pitting, aimed at compensating for the significant loss in yield that this entails, is represented by the "differentiated effect pressing" which allows an effective rupture of the cellular structures of the pulp and the woody parts of the almond (which acts as a solid/liquid draining element), together with a slight degradation of the seed integuments and therefore a more contained release of the POD. In this regard, some types of crushers have been tested where the percussion effect (typical of hammer crushers, the first to be used instead of grinders) is associated with the cutting effect. In this way, the rupture of the almond integument is reduced, limiting the activation of unwanted enzymes, and favoring an increase in phenolic content. The crushers that practice this effect

are those with knives, teeth, and double grid. The use of knife crusher blades, for example, positively influences the concentration of phenolic and volatile compounds by promoting an increase in aldehydes such as hexanal and (E)-2-hexenal, esters such as hexyl acetate and 3-hexyl acetate and a reduction in alcohols, such as 1-hexanol. The relative sensory analysis allowed to evaluate that the oils obtained with the knife crusher were characterized by an increase in the sensory notes of "floral" and "cut grass". The pressing also influences the color of the oil: products with higher levels of chlorophyll are generally observed on oils from the hammer crusher, given its strong impact on the skin of the olive containing this pigment. Hammer crushers, however, can promote an excessive increase in temperature, such as to reduce the activity of HPL (enzyme involved in the "lipoxygenase pathway"), thus causing the decrease in the aroma of the relative oil. In this regard, the new hammer crushers, characterized by small holes and higher rotation speed of the grid, allow to obtain oils with a higher final phenolic content and more decisive "bitter" and "spicy" sensations accompanied by aromatic profiles typical of the variety to which they belong.

1.3.2.1.3. Gramoling of pasta and consequences on the quality of the oil

The gramoling phase favors the extraction of the oil, inducing the coalescence of the oil drops produced by the crushing, through a slow and continuous reshuffle of the pastes coming from the crushing of the olives, with the aim of breaking the oil-water emulsion produced during the crushing of the fruits and favoring the assembly of the oil droplets in larger diameter drops. The kneaders generally consist of tanks coated with stainless steel, equipped with a heating shirt and longitudinal or vertical metal agitator, equipped with inclined pallets, which allows the slow shuffling of the dough and the advancement of the same *(Figure 30).*

Figure 30: Kneader (by website https://www.elicriso.it/it/olio/estrazione_olio_olive/)

Gramoling is also indispensable for the genesis of the characteristic aromas of olive oils, which under optimal conditions will be able to condition their organoleptic evaluation (Angerosa et al., 2001); (Angerosa, 2002). In fact, the lipoxygenase cycle leads to an increase in the formation of several

volatile components, in particular quantitative proportions (Olías et al., 1993); (Lercker et al., 1999); (Servili & Montedoro, 2002); (Servili et al., 2003). However, initial peroxidative mechanisms are able to promote a number of collateral oxidative chemical transformations, including the destruction of the most lay phenolic compounds (Servili et al., 1994, 1999; Servili et al., 2003). It is therefore during the gramoling that, by virtue of enzymatic activities and distribution phenomena, the flavor of the oil takes its definitive structure. On the one hand, a "refinement" of the organoleptic characteristics is obtained, with a decrease in bitter, spicy, and astringent taste, on the other the heritage of the antioxidants contained in the oil is reduced. Olives rich in compounds phenolics may undergo a longer malaxation, as it will remain a phenolic charge enough to ensure a valid preservation of the oil, while poor olives in antioxidant phenolic compounds are intended to produce more unstable oils over time. In other words, there will be an optimal time of malaxation of the paste, the extent of which will also vary according to the type of crushing adopted, to produce with high yields more stable oils and therefore more easily preserved. It has been observed that, by increasing the malaxating time, the yield in oil (percentage of oil extracted compared to the quantity initially present in the fruit) tends to increase until a limit value is reached that is less than the totality of the potentially extractable oil but determined by the extraction system adopted. the extension of the malaxation time leads to a decrease in the content of antioxidants, which results in a reduction in the oxidative stability of the extracted oil (Di Giovacchino et al., 2002). Another parameter that plays a decisive role in the gramoling phase is temperature, in fact using lower values (18- 20°C) results in unsatisfactory extraction yields, and the extracted oils are poor in phenolic components. As the temperature increases (22-28°C) both the extraction yield and the activity of the enzymes responsible for the development of the flavor are increased. Exceeding these optimal temperatures (maximum 35°C) the oxidative processes become significant and therefore the accumulation of compounds from these products in the extracted oil while decreasing the content in phenolic components (Toschi et al., 2004).

Kneading is one of the most important extraction phases of the oil because it influences its yield and quality. During this operation, the breakdown of the emulsions that are formed during the pressing process is promoted, thus promoting the phenomenon of the coalescence of the lipid droplets which will consequently favor the final separation of the oil from the other components. During this phase the endogenous enzymes that have been activated because of the crushing of the fruit catalyze a series of reactions that greatly influence the characteristics of the final product. Aromatic compounds continue to form following the continuation of the activity of the enzymes of the "lipoxygenase pathway" and, at the same time, the oxidoreductase enzymes (PPO and POD) that catalyze the oxidation of phenolic compounds also remain active (Alfei et al., 2020). During kneading, therefore, the content of aglyconic secoiridoids decreases both due to the migration of these hydrophilic

phenolic compounds in the aqueous phase in relation to their solubility, and due to the triggering of oxidative enzymatic reactions. To optimize the activity of "useful" enzymes (lipoxygenase, esterase and β-glucosidase) and limit that of POD and PPO, it is essential to control the technological parameters involved in the kneading phase, namely the kneading time, the temperature of the pastes and the concentration of oxygen in contact with the latter. The time and temperature of kneading are the parameters that have always been studied with the aim of obtaining a quality product. Since the introduction into the mills of confined kneading (closed or controlled gas exchange), the effects on polyphenols and volatile components of time and temperature, have significantly changed. The most recent studies show, in fact, a positive correlation between the increase in kneading temperature in confined kneading (controlled gas exchange) and the content of phenolic compounds in virgin olive oils and this can be explained by considering both the limited activity of oxidoreductases under such conditions (as inhibited by the reduced level of oxygen in kneading, and from the same temperatures, far from their optimum of activity), both the greater activity of hemicelullase and polygalattunorases that at higher temperatures, favor a greater release of polyphenols in the oily phase. On the contrary, several studies, while finding no correlation between the content of volatile compounds and the reduced presence of oxygen in the headspace of the kneadings, show that, although the pool of LPO enzymes is already active in the crushing phase, high kneading temperatures, can inhibit the further activity of enzymes such as HPL and, consequently, reduce the release into the headspace of aromatic substances responsible for the "green" notes of the related oils. Therefore, if on the one hand the amount of oxygen that remains inside the dough after crushing is sufficient to guarantee the activity of the LPO enzyme complex, temperatures even above 25 ° C can lead to a decrease in the catalytic activities of the enzymes of the LPO pool more sensitive to high temperatures, even if this sensitivity is strongly dependent on the variety to which they belong. Reaching high temperatures during kneading means promoting the fall in concentrations of esters and (Z)-3-hexen-1-ol and the accumulation of hex-1-ol and (E)-2-hexen-1-ol concentrations, both considered by different authors, substances that evoke unpleasant sensations. High kneading temperatures can also activate the conversion of amino acids with the production of considerable amounts of substances such as 2-methyl-butanal and 3-methyl-butanal related to some off-flavors of the oil. The final report that emerges from the numerous studies focused on the effect of kneading on the sensory quality of the relative oils, led to the conclusion that the parameters time, temperature, and oxygen concentration must be defined according to the agronomic variables (varieties, in particular) characterizing the chemical and biochemical structure of the raw material. In this regard, studies have been underway for several years that aim to identify, depending on the variety to which they belong, the optimal level of oxygen concentration and temperature to be used in closed kneading, to optimize the quality of the

relative oils. In *Figure 31*, the temperature and oxygen concentration pairs to be used in the kneading phase, observed for four different Italian varieties, "building" RSM (Response Surface Modelling) models based on the optimization of the quantitative levels of specific phenolic and volatile components previously selected and characterizing the different varieties.

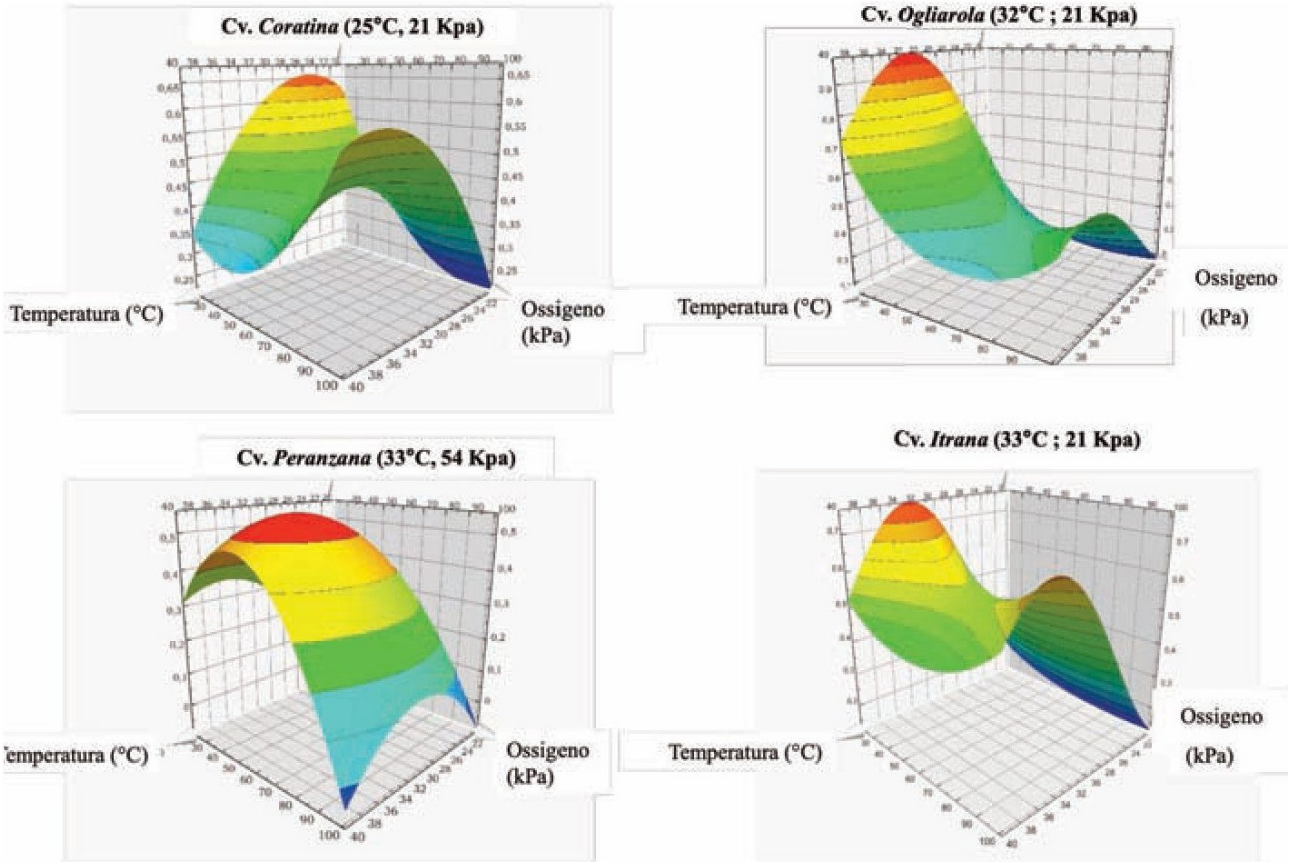


Figure 31: Simulations through response surfaces (RSM, Response Surface Modeling) Built to optimize the levels of tampering and oxygen concentration in the kneading, in four Italian cultivars. (From Alfei et al., 2020)

1.3.2.1.4. Oil separation and consequences on the quality of the oil

During this phase, the separation of the oil fraction from the vegetation waters and the pomace (solid fraction) is involved. The efficiency of this process is mainly conditioned by the quantity of non-constitutive water present in the fruit, the duration of the process and the operating temperature adopted (Di Giovacchino et al., 1994; Di Giovacchino et al., 2002). The choice of the most suitable equipment is strongly conditioned by the need to reduce oil-water contact times and therefore the effects induced by enzymatic reactions. The different extractive technologies that can be used can be identified according to the physical principle used to promote this separation: use of pressure, the use of the different percolation speeds and separation by centrifugation.

THE PRESSURE SYSTEM: the pressed olive paste, tends to reduce its volume by expelling its liquid phase, oily must (an uneven liquid consisting of oil and vegetation water). A press, generally

hydraulic, exerts the necessary pressure (up to 400 atm) for the olive paste, previously placed on draining surfaces (mats, *Figure 33)* encompassed by metal discs, to be separated from the oily must. There is therefore the retention of the solid phase (pomace) and the drainage of the liquid phase (oily must), an operation where the draining action of the peanut present in the paste can play an important role *(Figure 32*). The oily must is then conveyed to a vertical centrifugal separator that divides the oil from the water, and from the impurities and mucilage in this dissolved. The limitations of this technology are essentially linked to the discontinuity of the system and the impossibility of carrying out a complete cleaning of the draining surfaces (tax authorities), with the possible consequences that this can induce on the sensory quality of the finished product.

Figure 32: Extraction by pressure with presses (by website https://www.elicriso.it/it/olio/estrazione_olio_olive/)

Figure 33:Mats (by website https://www.elicriso.it/it/olio/estrazione_olio_olive/)

PERCOLATION (SYNOLEA SYSTEM). Since the interface tension between the oil and a steel foil is lower than that between the same foil and the water, the metal surface immersed in the paste will tend to cover itself with an oily layer that can thus be separated from the vegetation waters. This system allows to recover up to 60-70% of the potentially extractable oil, therefore it is usually coupled to a pressure extraction process or centrifugation to recover the large amount of residual oil (30-40%) *(Figure 34)*.

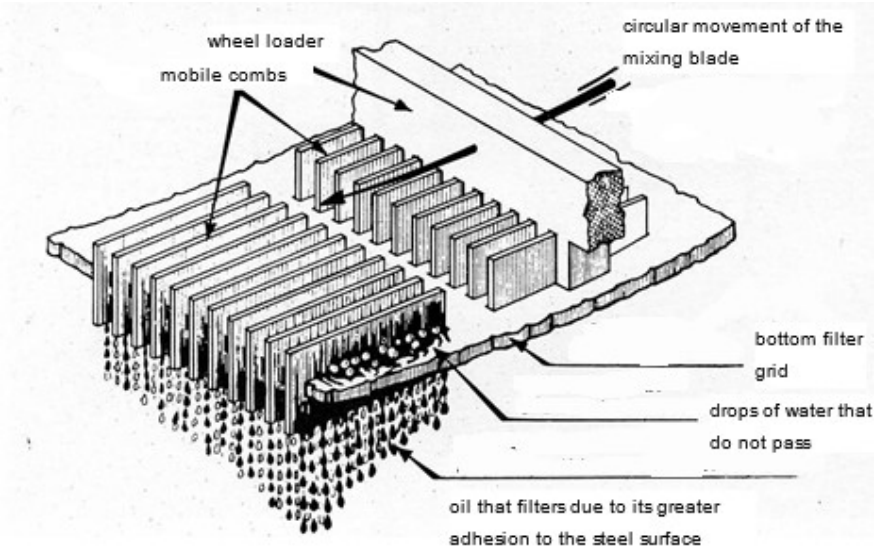


Figure 34: Synolea system (from Bastianin-Ceresa "Industrie agroalimentari" Lucisano 2010)

This system requires very long malaxation times and sometimes high temperatures, as the interface tension depends on both the temperature and the size of the oil droplets in the paste. The high oil-foil contact surface promotes the evaporation of the volatile components responsible for the flavor of the oils by reducing their intensity.

CENTRIFUGATION exploits the different force of gravity that is exerted on immiscible liquids and characterized by different density values. The paste and therefore its three macro components when subjected to a centrifugal acceleration greater than 3000-3500 times that of gravity, tend to separate quickly: the solids will layer on the walls of the centrifugal separator followed by vegetation waters while the oil, the phase characterized by the lower density, will occupy the inner layer. The decanter consists of a horizontal axis conical drum inside which is a cylinder with helical foils, which rotates at a speed slightly lower than that of the drum. The slight difference between the speeds of the drum and cylinder pushes the pomace from one part of the centrifuge, while the oil and water will head in the opposite direction. Some "exit routes", called touchers or nozzles, suitably positioned will allow the recovery of the oil separated from the vegetation water. Usually, the separation between the two liquid phases is refined and completed within vertical centrifuges with automatic discharge. While the first decanters placed on the market were triphasic *(Figure 35)* and led to the separation/production of: oil, vegetation waters and solid phase (pomace).

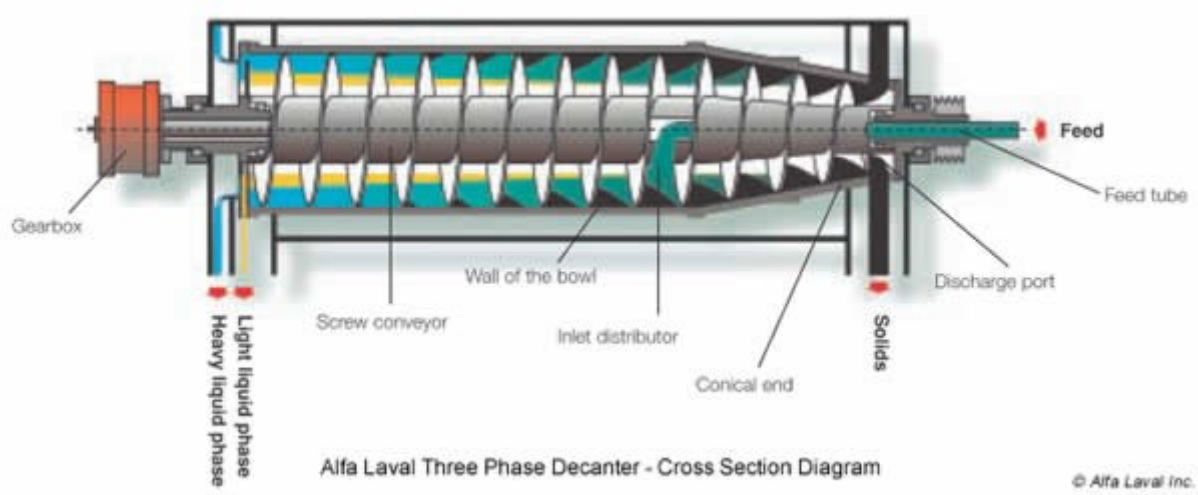


Figure 35: Three phase decanters (from website www.alfalaval.com)

A technology that in addition to requiring high gramoling times requires the addition of large quantities (added water weight equal to about that of olives water for the fluidization of olive pastes). An addition that dilags the phenolic component by impeasing its oil, a particularly serious effect in products with little antioxidant components. It was therefore thought to add the vegetation waters deriving from previous extractions. But these, in addition to high quantities of residual phenols, were rich in the products of their oxidation particularly available to transfer to the oil phase. The increase in these components in the oil decreases the future stability of the oil itself and is responsible for possible organoleptic alterations that are not appreciated by the consumer. Biphasic decanters were later introduced, separating the oil from vegetation water and pomace, which are discharged together. Using biphasic decanters *(Figure 36),* it is not necessary to dilute the paste coming out of the knead with water and this reduces the volume of processing by-products. In addition, wet pomace is sent directly to the processor together with vegetation waters that no longer represent a waste from problematic disposal. The oil coming out of the biphasic decanter is richer in phenolic substances and therefore has a greater antioxidant power.

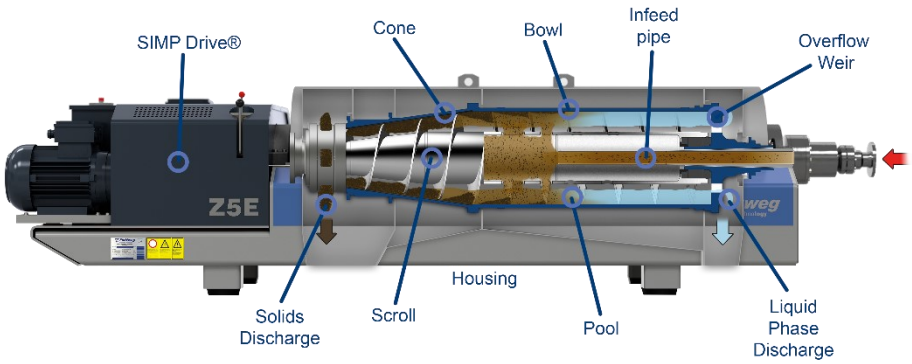


Figure 36: two phases decanter

More modern systems, called "TWO AND A HALF PHASES", lead to the separation of the three fractions and require the addition of small quantities of water varying according to the characteristics exhibited by processed olive paste. The actual separation phase therefore has a relatively relative impact on the composition of the flavor of the oils, except for those produced using the three-step decanter. In the oil separated from the vegetation waters, enzymatic transformations and diffusional processes that regulate the distribution of the different constituents between the phases involved are inactivated.

The definitive volatile and phenolic profile of a virgin olive oil also depends on the type of fruity extraction for oil recovery. The most common systems for the separation of oil from kneaded olive pastes are centrifugation and pressure system. Compound losses depend on the importance of the interactions between oil and solid parts on the one hand and oil and vegetation water on the other, which are minimized when pressure extraction systems are adopted; but it is important to underline that to obtain quality oils from a pressure extraction system, it is necessary to work drupes in good health and continuously, to prevent possible fermentation processes and / or degradation phenomena of the pulp and vegetation water on the filter diaphragms, which could give rise to defined defects (Alfei et al., 2020). Adding hot water to reduce the viscosity of olive pastes for extraction from oil in the three-step centrifugal system may explain the decrease of polyphenols and alcohols to C6, hexen-1-ol and trans-2-hexen-1-ol. The most significant variations concern, in this context, the aglycone derivatives of oleuropein and demethyloleuropein, while lignans do not seem to have significant variations in relation to the added dilution water. The introduction of the new types of centrifuges has made it possible to overcome the obstacles related to the use of traditional three-phase centrifuges and to reduce the leaching of phenols due to the addition of vegetation water. A new extraction system, in fact, do not provide for such additions, except in very limited quantities. Studies on the impact of these on the quality of the oils, have shown non-significant differences with regard to the concentration of free fatty acids and the spectrophotometric constants, while they have found a significant correlation between the type of decanter and the phenolic content, is the different differential speeds between drum and auger and the quali-quantitative composition of the glycoidoid secoiridoids in the relative oils: a lower content of the latter in particular has been observed in decanters sent at a lower speed and this, probably, due to a greater leaching of the phenols promoted by prolonged contact times between the aqueous phase (olive vegetation water) and the oily phase.

1.3.2.1.5. Oil filtration

At the end of the extraction phase, the oil obtained appears as an opalescent and turbid liquid. The reasons for this lie in the presence of solid particles in suspension, coming from plant material and

micro-droplets of water, whose presence can compromise the quality of the oil because, being an important substrate for the growth of microorganisms, it facilitates hydrolysis and fermentation processes. The impact from a qualitative point of view concerns phenolic compounds, as the water not removed is a vehicle of enzymes responsible for the oxidation of phenols, as well as the hydrolysis of chlorophyll (Alfei et al., 2020). The presence of microorganisms is also related to the onset of many sensory defects, such as "heating / sludge" and "heating", in particular. Although a group of consumers currently associates the turbidity of the oil with a greater "genuineness" of the product, several studies have shown that the filtration process allows to increase the shelf-life of the final product both in terms of product quality and nutritional and sensory. Innovations in this field include the introduction of the crossflow filtration system (with oil flow parallel to the filter membrane rather than perpendicular to it), the use of filter bags (usually polypropylene) or flows of an inert gas, such as nitrogen or argon, introduced to the center of the mass of oil to be filtered, thus ensuring greater stability of the oil to lipid oxidation.

1.3.2.1.6. Olive Oil Conservation

To preserve its nutritional and organoleptic characteristics, the oil obtained must be properly preserved, preventing it from taking unpleasant odors or encountering degrading processes (rancidity). The deterioration of this product can be induced by the following three main causes:

1. contact with inadequate materials, metal containers or deteriorating materials for which it is stored in glass or stainless steel.
2. prolonged contact with aqueous impurities, which tend to separate from the lipid phase to settle on the bottom of containers used in the storage of an oil. These sediments consist mainly of residual vegetation waters and contain sugars, enzymes and protein substances. In the aqueous phase thus formed, fermentations can be established that lead to the formation of components responsible for olfactory defects, with hints of mud and putrid. To avoid this type of problem it is necessary to move or filter the oil to quickly separate it from such sediment.
3. oxidation, which cannot be completely avoided, but it is possible to delay it by taking some special precautions: minimize the volume of tank occupied by air, the use of closures that ensure a high degree of hermeticity, avoid exposure to the light of the finished product.

The sensory profile of virgin olive oils changes during its preservation due to the simultaneous and drastic reduction of compounds derived from the LPO pathway and the neo-formation of some volatile compounds responsible for very common defects known as "rancid" and "cucumber". The newly formed compounds result from the fragmentation of tasteless and odorless hydroperoxides that occurs during the secondary phase of lipid oxidation. The defect of "rancid" is radically produced by

the processes of oxidation of lipids, which depend on various factors such as light, temperature, the presence of metals, pigments, composition in unsaturated fatty acids, concentration in sterols, quantity, and type of natural antioxidants (Alfei et al., 2020). The most important compounds in this sense, due to their low threshold of perception, are the unsaturated aldehydes whose concentration increases with the prolongation of the storage time, but other chemical species, such as saturated aldehydes, ketones, acids, alcohols, hydrocarbons, lactones, furans, and esters, contribute to the complete definition of the typical unwanted sensations. Among saturated aldehydes, the nonanal increases in parallel with the oxidation process. During storage, the degradation of the quality of virgin olive oils is inevitable although its speed depends on the composition of the oil, mainly on the content of fatty acids and minor compounds with antioxidant activity, as well as on storage conditions. The light, the temperature, the presence of oxygen in the headspace of the package, the content of natural antioxidants and oxygen dissolved in the oil at the time of packaging, as well as the type of packaging used, are the factors that most influence the level of oxidation of the product. Light, due to the phenomenon of photo-oxidation that induces, accelerated by photo-activators such as chlorophylls and pheophytins (naturally present in the oil), is the main factor of qualitative degradation during the preservation of the oil, and it has been scientifically demonstrated that the rate of decay is inversely proportional to the initial content in aglycons secoridoids (especially of oleuropein derivatives such as hydroxytyrosol, oleacein and oleuropein aglycone). Of course, the concentration of oxygen in the oil before packaging, the partial pressure of oxygen in the headspace, the oxygen permeability of the packaging material are additional factors that significantly affect the lipid deterioration, quality, and shelf life of the product. The packaging can also directly affect the quality of olive oil by protecting the product from both exposure to light and contact with oxygen. The materials currently used for packaging are glass (clear, green, amber, UVA grade), chrome band, plastic (PET, PVC, and PP bottles) and polyamines plastic/paper/aluminum (tetra Brik type packages and bag in box). Although there is a large bibliography on the effect of packaging on the shelf life of extra virgin olive oil, there are very few who have faced real time shelf-life studies and at the same time also consider the evolution of sensory quality. In this regard, a recent study, which involved oils different in phenolic and volatile composition and packaged in three different packaging (green glass, UVA grade glass and polyamines plastic / aluminum / paper), allowed to confirm that both UVA and visible rays can quickly trigger photo-oxidative phenomena and that the latter are all the slower the higher the initial content of secoiridoid derivatives of oleuropein. On the contrary, it was observed that a packaging able to completely counteract the absorption of light radiation, such as polyamine plastic / aluminum / paper, allowed to preserve for a significantly longer time (especially compared to green glass) the oils, delaying / limiting the accumulation of the secondary products of oxidation

(aldehydes to C7-C11 above all), the loss of the pleasant sensations of "fruity" "bitter" and "spicy" and the onset of the typical defect linked to the secondary phase of lipid oxidation, i.e. the "rancid" *(Figure 37).* Similar studies have also been obtained with the use of bag in box, opening a possible new scenario of distribution of oils to the consumer, who, however, by applying a rapid sensory analysis at the time of purchase, currently associates glass with a higher level of quality of the product contained.

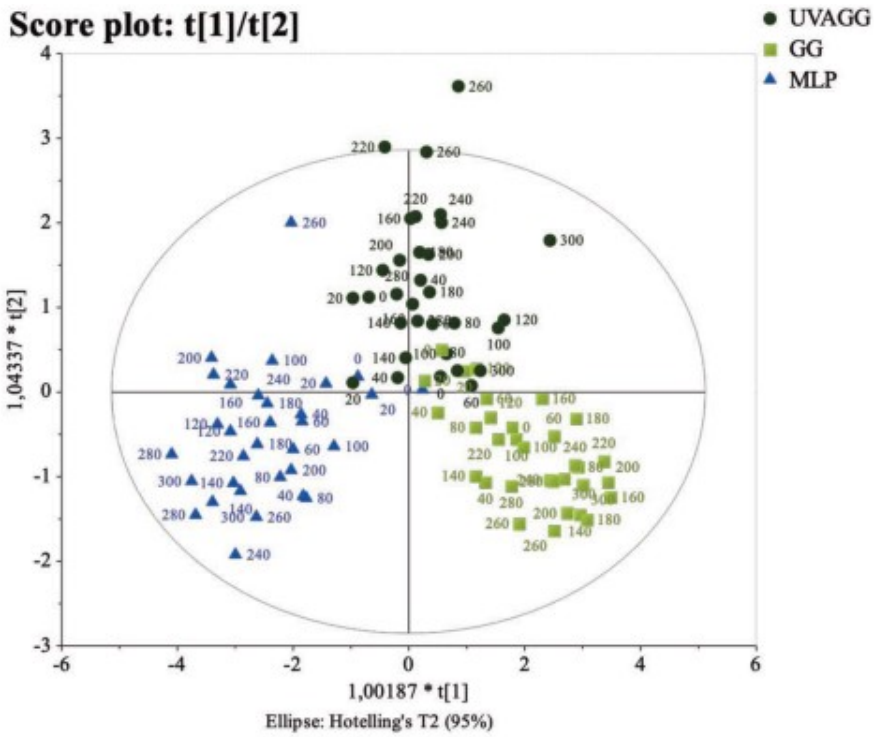


Figure 37 Effect of packaging (GG, green glass; UVAG, UVA grade green glass; MLP, plastic / aluminium / paper polyamines) on the evolution of the quality of preserved virgin olive oils simulating supermarket sales conditions (LED light exposures for 11 hours a day for 300 days at room temperature). Model O-PLS-DA built considering how latent variables the three types of packaging and all parameters as independent variables products, health and sensory products collected from 0 to 300 days of oil analysis. (From Alfei et al., 2020)

1.3.2.2. INNOVATIVE EXTRACTION OF VIRGINE OLIVE OIL

Considerable efforts have been devoted to the search for alternative processes that can preserve food quality attributes, while being environmentally friendly and low-cost. As a result, several new and emerging technologies have been developed and applied to meet the growing consumer demand for more natural products with fewer additives and preservatives that also offer practicality, freshness and safety (Cavallo et al., 2020). These methods have been designed for their ability to improve food attributes, such as color; texture and flavor (Perez et al., 2021); the content of phenolic compounds, carotenoids and vitamins; and also the availability of bioactive compounds (Garcia-Oliveira et al., 2021). Many innovative processes have been developed to increase yields and improve quality without creating additional energy costs (Amirante & Clodoveo, 2017). These processes include the

application of ultrasound (Us), microwaves, pulsed electric fields, and Us combined with a heat exchanger (Clodoveo, 2019). The Us method focuses on improving the gramoling stage during the olive oil extraction process. This method was originally developed in the laboratory and adapted for industrial commercial use. It is based on avoiding undesired temperature increases in the olive paste and on improving the extractability of minor compounds without causing changes in the quality indices (Amirante & Clodoveo, 2017); (Clodoveo, 2019). Few studies have been published on the enrichment of phenolic compounds in olive oil using Us-assisted extraction technology on phenols from olive mill wastewater (Jerman Klen & Mozetič Vodopivec, 2011).

1.3.2.2.1. Use of pitted olives

The production of pitted oil is very ancient and was carried out by grinding olives already without kernels. This oil boasts superior organoleptic properties and a lower level of acidity than normal extra virgin olive oil. Thanks to the process of separating the pulp from the olives, the tannic substances typical of the kernel are eliminated. Pitted oil and its properties are a source of numerous health benefits, in fact there is a greater amount of antioxidant substances while the presence of polyunsaturated acids is reduced to prove to be a less acidic and more resistant product to oxidation. The taste is sweeter, and the bitter and wooden aftertaste characteristic of the common extra virgin olive oil is less intense. Pitted oil also has a better durability over time thanks to a resistance superior to the oxidation process. Numerous research have shown that the quality of oils extracted from pitted olive pastes is higher than in products that are processed from whole fruits. Mechanical extraction of oil from pitted pastes can improve the phenolic concentration and oxidative enzymatic actions that occur in olive paste during the extraction process. Two enzymes, polyphenol oxidase (PPO) and peroxidase (POD), are highly concentrated in the olive kernel. PPO and POD can oxidize phenolic compounds resulting in a reduction in the phenolic concentration of the oil. The destoning process, excluding olive seed before gramoling, partially removes peroxidase activity in pastes. This results in an increase in oxidative stability and nutritional value of virgin olive oil. Polyphenolossidase, in fact, are mainly located in almond and catalyze the degradation of antioxidants of a phenolic nature during the mechanical extraction of virgin olive oils. The de-stoner (Restuccia et al., 2018) is a mechanical system allowing a selective crushing of the fruit flesh excluding the stone from the olive paste. The machine works by a screw feed assembly that conducts the olives towards a suitable perforated container. The olives are then expulsed by a rotating mixer where the pulp is collected, and the stones are expelled unbroken and clean. The pulp crosses the basket holes and drops into the hopper underneath. A screw then moves the product from the hopper to a pump that feeds the gramoling machine (*Figure 38*).

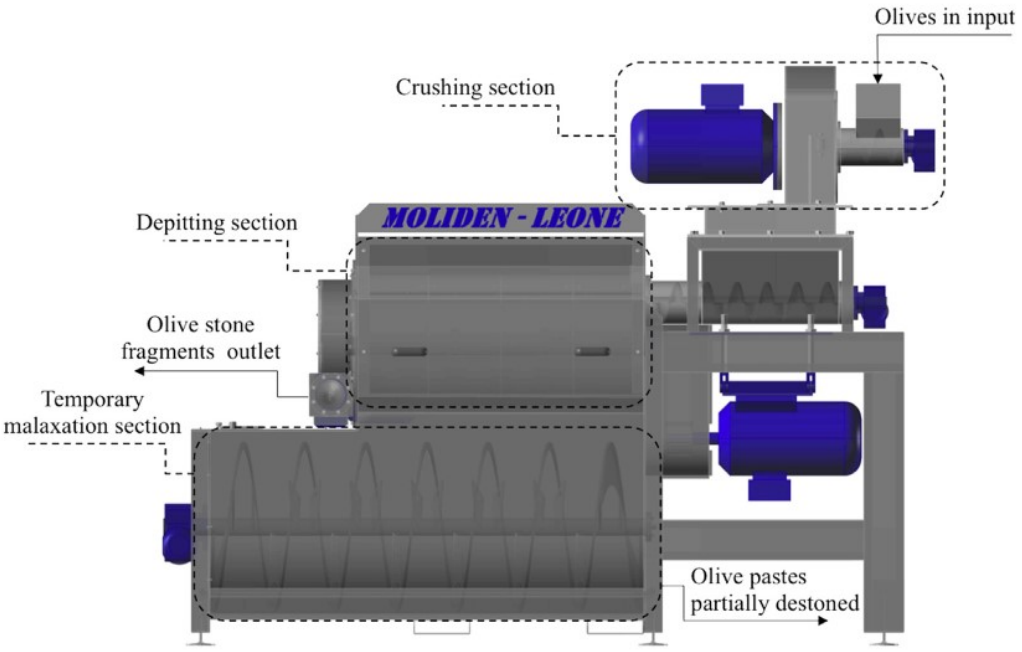


Figure 38: A de-stoner machine (Leone et al., 2015)

Just as the method of extracting oil from olives can have consequences on the composition of the oil, the organoleptic characteristics, and the period of storage of the oil, the use of pitted olives can also have positive consequences on the polyphenol content and organoleptic characteristics of the oil. It is very important to avoid the total crushing of the olive tree due to the formation of undesirable smells and flavors. In recent study (Amirante et al., 2010) it has been seen that the use of hammer crushers leads to an increase in polyphenols, greater resistance to self-oxidation and a more spicy and bitter taste. In addition, extraction by centrifugation or hammer crushers does not substantially change the content of fatty acids, peroxides, ultraviolet signals, or organoleptic characteristics, while crushers with hammers lead to the production of an oil richer in polyphenols, probably due to the more "violent" action obtained from hammers. However, hammers result in higher operating temperatures that reduce the shelf-life of the product. Compared to disc mills there is not only a shorter shelf-life, but also an increase in the probability of self-oxidation. Considering the oil from pitted olives, there is a reduction in yield, quantifiable in about 1.5 kg of oil from 100 kg of olives compared to the traditional process. This defect can be corrected with a heat exchanger, which also increases the efficiency of the extraction phase. Compared to the process carried out with traditional olives, no changes are observed on acidity, peroxide index, or signals in ultraviolet. However, there are several studies (Restuccia et al., 2018) that claim there is a reduction in acidity of up to 20% in pitted oils. On the other hand, there is a clear increase in polyphenols, volatile compounds C5 and C6 (responsible of the bitter taste and the pungent sensations, correlated to the “green” sensory notes and to the prickly taste, decreasing oil bitterness at the same time). In de-stoned olive oil phenol content was independent from the ripening stage. At the same time the de-stoning process increased the

content of tyrosol, hydroxytyrosol, and p-hydroxybenzoic acid. The oil obtained from pitted olives, a greater presence of secoiridoid derivatives and no difference in the presence of lignants was detected. This could be explained considering that de-stoning technology induces a decrease in the thermal phenol oxidation processes, followed by quinonization and polymerization of the oxidized compounds. This leads to reduced activity in oil from pitted olives of the enzyme polyphenol oxidase and peroxidase (with less peroxide production) (De Luca et al., 2016). There are contrasting effects of pitted oil on carotenoids and tocopherols, as evidenced by numerous studies. There is also a negative effect on chlorophylls, carotenoids, and various pigments. The presence of aldehydes C6 insature increases in pitted oil, with a related feeling of oiliness, while the crushed seed was richer in C6 alcohols. According to a recent study (Sakouhi et al., 2020), the composition in volatile organic compounds and fatty acids of the olive pulp and kernels of some cultivars was examined, highlighting that there is a greater presence of aldehydes, alcohol, esters, ketones and terpenes in the pulp than in the core. These compounds are responsible for the aroma of olive oil, 2-hexenal, (Z)-3-hexen-1-ol, 1-hexanol and (Z)-3-hexenyl acetate. As for the composition in fatty acids, the predominant acids are, in order, oleic acid (60-78%), linoleic acid (5-20%), palmitic (12-15%), stearic acid, linolenic acid, gadoleic acid, palmitoleic acid and arachidonic acid. Their presence is very low in kernels, while more than 80% of the above volatile substances are contained in the olive pulp. For this reason, pitted oil contains most of the substances that give aroma to the oil. Considering also the degree of ripening of the olive tree and other parameters, in another study (Katsoyannos et al., 2015) it was highlighted how the degree of acidity (from -0.01% to -0.09%) and the presence of peroxides tend to decrease in pitted oil (from -0.4 to -1.4 meqO2/kg). The yield in pitted oil, on the other hand, tends to decrease in ripe olive oil (from -4.02% to -5.17%), because in green olive oil the trend is inverted (from +0.14% to +1.03%). In contrast, K232 and K270, signals in the ultraviolet tend to increase in pitted oil (from +0.06% to +0.18% K232, from +0.01% to +0.03% K270). Acidity tends to increase (+0.04%) as the degree of maturation increases, probably because the maturation process makes the fruit more sensitive to mechanical damage and caused by pathogens, with increased activity of enzymes, including lipolytic enzymes. Peroxides (from +0.5% to +1.7%), K232 (from +0.09 to +0.15) and K270 (from +0.01 to +0.04) also increase as maturity increases, due to the increased presence of unsaturated acids. Yield increases as the degree of maturity of the fruit increases (from +3.85% to +10.05%). Green olives have a higher content of polyphenols (from +48.66 to +60.89 mg/kg oil) and tocopherols (from +20.8 to +179.29 mg/kg oil) than ripe olives. It has also been detected that the content of polyphenols and the degree of maturation influence the organoleptic characteristics and shelf-life of extra virgin olive oil. Pitted oil also has a higher polyphenol (from +22.83 to +76.96 mg/kg oil) and tocopherol content (from +42.73 to +109.2 mg/kg oil) than conventional oil, with

positive repercussions on the quality and antioxidant activity of pitted oil. This also leads to better organoleptic qualities and greater shelf life of the latter oil. The reasons have previously been stated: in the pitting process, the stones are removed at the beginning of processing and therefore, the enzymes (lipoxygenases, peroxidases) contained in the seeds do not influence the pulp composition and phenols are not enzymatically degraded thus improving their concentration and oil oxidative stability. The paste preparation technique did not affect the main fatty acid proportions of the variety olive oil. No significant changes were observed in the sums of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids or in their ratios.

1.3.2.2.2. Ultrasound

Ultrasound (US) is a promising emerging technology that has already found application in the food industry due to its significant effects on the processes, such as higher product yields, shorter processing times, reduced operating and maintenance costs, improved taste, texture, flavor and color (Clodoveo et al., 2017). The thermal effect occurs when kinetic energy of the ultrasound waves is converted into the thermal energy due to the turbulence increment in the matter. The mechanical effect is due to the cavitation phenomena. In other words, when ultrasound is applied on a continuum fluid, it produces sinusoidal acoustic waves and tiny gas bubbles grow within the fluid when the local pressure falls below the vapor pressure of the liquid. If the bubble growth reaches a critical size, it implodes causing the phenomenon of cavitation, the most important effect in high-power ultrasound. In the case of the olive paste, cavitation, by means of ultrasounds, promotes the disruption of tissue structures freeing the trapped oily phase. Thus, the application of ultrasound-waves to olive paste can effectively enhances the release of soluble compounds from the plant tissue and improves mass transfer also in the olive tissues. Moreover, ultrasound can increase the hydrophobic effect, improving the kinetic of the coalescence phenomena by enhancing the probability of particles collision leading to an increase of coalescence and oil recovery. Currently, the mechanical methods used to extract virgin oils from olives is generally made up of a mechanical crusher, a few gramoling and horizontal (decanter) and vertical-axis centrifugal separators. The mechanical crusher and the centrifugal separators operate continuously, while the gramoling is a batch machine, which works between continuous devices. For this reason, the gramoling represents the bottleneck of the continuous extraction process. Moreover, the gramoling is an inefficient heat-exchanger due to a not favorable ratio between its large volume and small surface.

It has recently been proposed (Del Coco et al., 2021) a combined extraction method based on ultrasound and thermal conditioning combined extraction method based on ultrasound and thermal

conditioning. This method leads to an increase in the yield and quality of extra virgin olive oil, thanks to a higher yield in phenols. This continuous method can be summarized as follows:

1. Olive harvesting and washing
2. Crushing phase, oil release
3. Kneading phase in steel cylinder with rotating arm shaft and steel blades in a device incorporating a heat exchanger and an ultrasound generator. At this stage the olive paste is mixed at controlled temperature (20-25°C) and the smaller droplets are grouped into larger droplets (coalescence)
4. Moving the olive paste from the bottom by pump and horizontal centrifuge that separates the oily phase from the liquid and solid phase of olive paste
5. Passage of the oil phase in vertical centrifuge, clarification with tap water

The following design *(Figure 39)* outlines such a device with the main processing steps (Amirante et al., 2017):

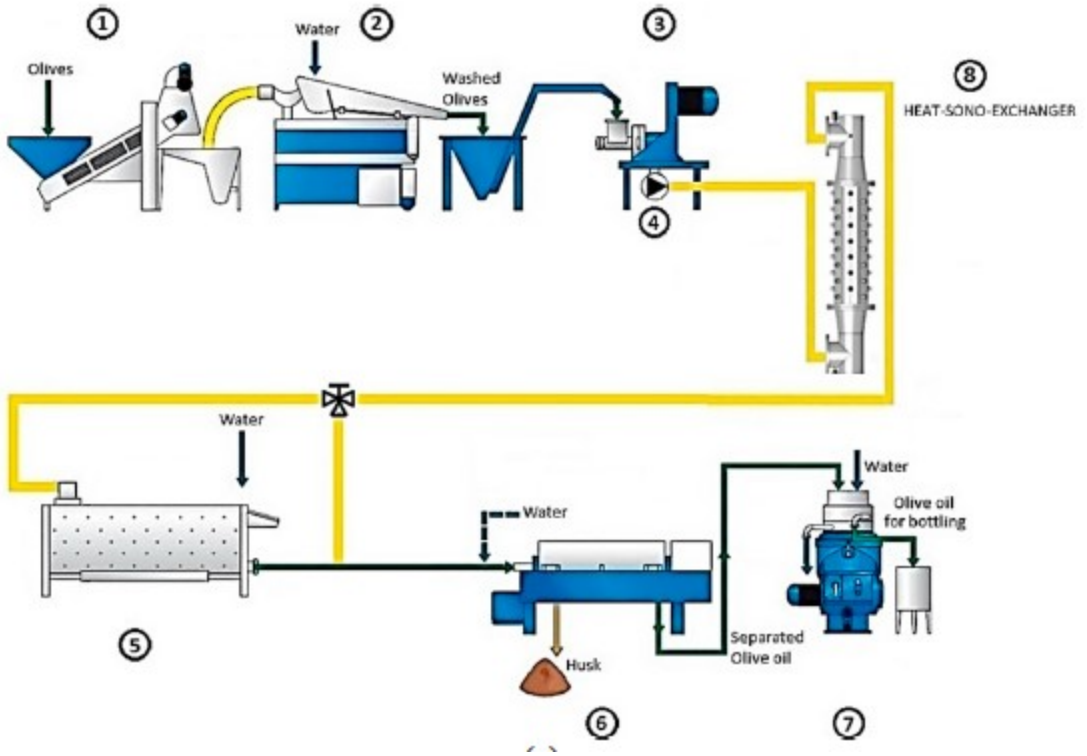


Figure 39: steps of combined extraction method based on ultrasound and thermal conditioning.

Steps: 1-Reception 2- Washing 3- Crushing 4- Pump 5- Gramoling 6- Separation 7- Clarification 8- SHE

The ultrasonic device would serve to promote the agitation phase without breaking the oil cells and also no air bubbles would form (Clodoveo et al., 2017). Heat exchange and ultrasound passage are also maximized. In a recent study (Del Coco et al., 2021) it was shown that ultrasound results in an increase in yield of up to 7.6% and an increase in the biophenols contained up to 39.6% compared to traditional extraction. This increase is higher in the green olive tree and not yet ripe rather than in the

ripe olive tree. In addition, the parameters acidity, peroxides, ΔK not significantly modified. These results confirm those obtained from another study (Cecchi et al., 2019), which shows that process times are shorter as well as energy usage. In addition, the yield is increased by 5.5%, the quality of the oil is maintained but with a higher yield in phenols and with a maintenance of organoleptic characteristics. Another study (Lammi et al., 2020) show the substantial equivalence of two extra virgin olive oil samples extracted from the same batch with (OMU) or without (OMN) using ultrasound technology, by performing chemical, biochemical, and cellular investigations. The volatile organic compounds compositions and phenolic profiles were very similar, showing that, while increasing the extraction yields, the innovative process does not change these features. The antioxidant and hypocholesterolemic activities of the extra virgin olive oil (EVOO) phenol extracts were also preserved. Finally, taking into account the ripening index (Taticchi et al., 2019), a comparison was made between traditional extraction and ultrasound. The composition in volatile organic compounds is not altered by the extraction method, with reference to aldehydes (which tend to decrease as the ripening index increases), alcohols (which decrease), esters (which decrease) and ketones (which have a maximum and then decrease). Phenols tend to increase both as extraction time increases (+ 12% from 10 to 30 minutes of extraction) and by switching from traditional extraction to ultrasound (+ 11%). The content of tocopherol tends to a maximum (20 minutes) and then decreases if an increasing extraction time is considered, while ultrasound guarantees a higher content of this compound than a traditional extraction (+ 12%). The content of aldehydes, alcohol and esters tends to have a maximum (20 minutes) and then decrease as the extraction time increases, while ketones have a different pattern. About the type of extraction, however, there are no significant variations.

1.3.2.2.3. High power ultra-sound

In the olive oil industry, ultrasound is one of the most promising technologies because of its powerful mild mechanical and thermal effects (Amirante et al., 2010). In recent years it has been found that by using stronger ultrasound (>1 W / cm^2) at a lower frequency (generally around 20-50 kHz), (high-power ultrasound (HPU)), they are physically effective in altering the properties of a substance or inactivating microorganisms. The application of high-power ultrasound in olive oil extraction was first performed under discontinuous conditions. In these studies, on the effects of direct and indirect ultrasound, it was found that direct sonication provided better extractability in olives with high humidity (> 50%), while greater extractability was achieved by indirect sonication in low-moisture olive fruits (<50%) (Maria et al., 2021). The treatment did not affect the quality parameters (free acidity, peroxide value, K270 and K232) of EVOO produced from sonicated pastes, while the content of tocopherols, chlorophylls and carotenoids increased. The enrichment of olive oil with the main

phenols in olive leaves by ultrasound has been studied by many researchers (P. Amirante et al., 2010). Ultrasound was also used to enrich olive oil with oleuropein both on a laboratory and pilot plant scale. The ultrasonically assisted extraction method greatly facilitated the enrichment of VOO into phenolic compounds compared to conventional processes. It turned out that tyrosol and hydroxytyrosol, the main phenolic compounds found in olive oil, were not significantly degraded by sonication. (Amirante et al., 2010). Other researchers tested HPU treatments at three different frequencies (20, 40 and 80 kHz) and EVOOs were extracted after two treatments: HPU application and centrifugation, with or without kneading. The results showed that HPU treatments had no apparent effect on the fatty acid composition and phenolic content of EVOO.

The olive oil extraction process has gained increasing attention in recent years and several studies have confirmed that kneading is a fundamental step in this process because physical, chemical, and biochemical phenomena result from it. Effective kneading (optimal condition for extraction) extracts the maximum yield in high-quality EVOO oil while maintaining antioxidant compounds and favorable sensory characteristics. Kneading is a mixing operation, in which time, temperature and butt space conditions are key factors in determining the efficiency of extraction and the resulting quantity and quality of EVOO (Angerosa et al., 2001). Previous studies have reported an improvement in oil yield when mixing time was prolonged and olive paste temperatures exceeded 29°C (± 1°C). However, this practice in the presence of oxygen is known to activate the lipoxygenase (LOX) pathway, thus generating volatile compounds that affect the taste of EVOO, as well as altering the desirable sensory properties associated with the final product. (Clodoveo & Hachicha Hbaieb, 2013). The combination of HPU and control of O_2 concentration in the kneading headspace could contribute to the further improvement of EVOO quality such as chemical indices, antioxidant compounds and sensory attributes.

1.3.2.2.4. Microwave

MWs are non-ionizing electromagnetic waves of frequency between 300 MHz and 300 GHz. MW is a technology applied in many food processes that enables to reduce processing times (Thostenson & Chou, 1999). However, MW has not been applied before in the VOO extraction process. MW heating is the conversion of electromagnetic energy to thermal energy through direct interaction of the incident radiation with the molecules of the target material. As MW can penetrate materials and deposit energy, the volume of the vegetable tissue increases and, in this way, cells explode releasing their content into the liquid phase. When the liquid absorbs the MW, the kinetic energy of its molecules increases, and consequently, the diffusion rate increases too (Mandal et al., 2007). Considering these observations, it is reasonable to hypothesize that the MW can reduce the length of

gramoling improving the oil releasing. In a study (Clodoveo & Hachicha Hbaieb, 2013) land ultrasound and microwave techniques were combined with a device that predicts schematic phases in the following drawing and in the following flowsheet (*Figure 40, Figure 41*):

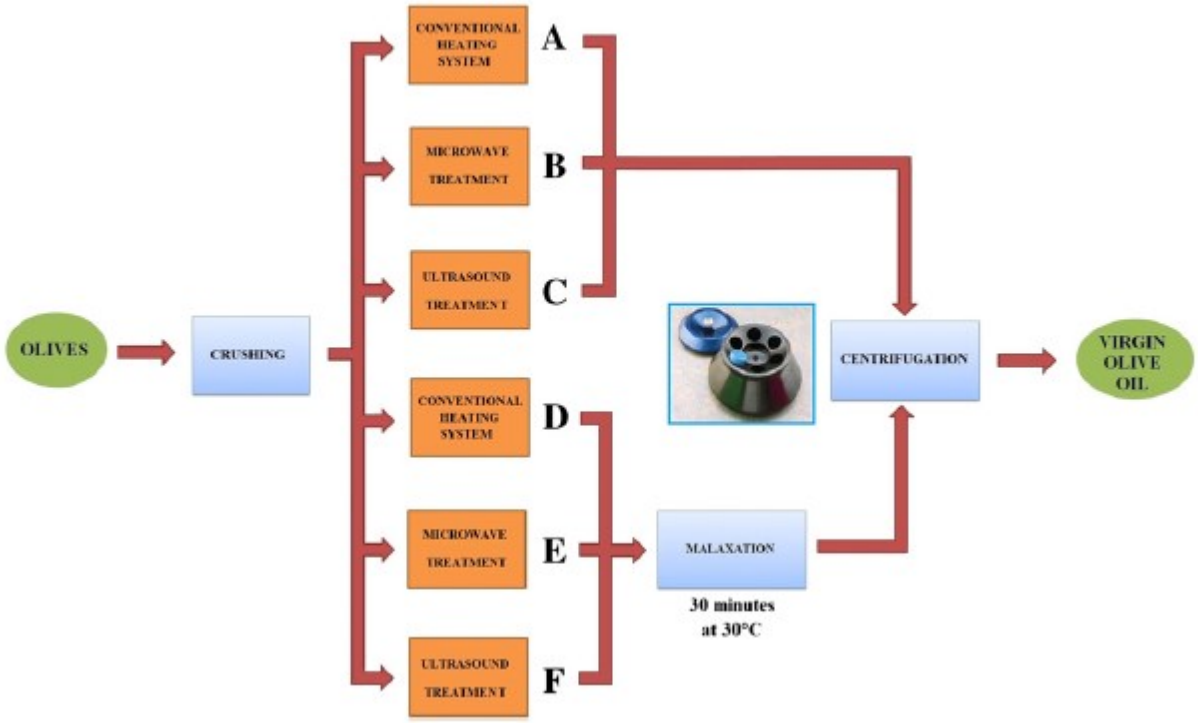


Figure 40: schematic description of combined extraction and microwave system extraction

Figure 41:flowsheet of combined extraction and microwave system extraction

The main parameters legally established (acidity, peroxide value, and specific extinction coefficients (K232 and K270) to evaluate VOO quality were not affected by the US and MW treatments. Moreover, US and MW processes significantly reduced the length of the gramoling and improved the extraction yield as compared with the control when the oils were extracted from the paste without gramoling. In another recent work (Caponio et al., 2019) microwave (MW), ultrasound (US) and heat exchange (HE) techniques were combined, verifying the composition of volatile organic substances using traditional extraction (MM) and a combination of the above techniques. Checking the main

analytical parameters, it can be noted that traditional extraction maximizes the alcohol content (21.56%) compared to other methodologies (from 13.83% HE to 19.44% HE+MM), the aldehyde content is maximum for HE+MW (56.38% versus 46.44% HE+US+MW – 53.46% HE) and finally the esters are most present in HE+MW (5.94% versus 4.21% MM- 5.58% HE+MM).

Compared to the traditional process, innovative technologies based on the heat exchanger led generally to a decrement involatile lipoxygenase (LOX) alcohol linked to alcohol dehydrogenase activity and, conversely, to a slightly increase in volatile LOX esters. Aldehydes from the same pathway were not significantly affected. However, an industrial combined plant constructed from a heat exchanger, low-frequency ultrasound device and microwave apparatus determined the highest 'fruity' intensity perceived by panelists, in accordance with the highest value of total volatiles, with values significantly higher than heat exchanger alone, which, instead, had the lowest levels of hexanal and LOX alcohols. The pungent taste showed the same trend observed for 'fruity' intensity, whereas bitter taste did not show significant differences among trials. The introduction of ultrasound, coupled with heat exchanger and microwave, seemed not to modify the behavior of enzymes of the LOX pathway, and the obtained virgin olive oils showed volatiles and organoleptic characteristics not significantly different from those obtained by the traditional olive oil extraction process. In similar devices and other works (Tamborrino et al., 2019) these were the differences for other types of substances. Comparing the same extraction methods, a maximum phenol content (22.35 mg/kg) is observed in the HE+US+MW method, while the others range from 12.32 mg/kg (HE) to 21.62 mg/kg (traditional extraction). The total tocopherol content is maximum with traditional extraction (273.64 mg/kg) while it ranges between 255.23 (HE+MM) and 262.46 mg/kg (HE) by other methods. About carotenoids, the maximum content is HE+MW (8.98 mg/l) and for the others it ranges between 6.45 (HE+MM) and 8.55 mg/kg (traditional extraction). Finally, about chlorophylls, there is a maximum of 5.18 mg/l (HE+US+MW), while it ranges between 3.79 (HE+MW) and 4.23 mg/kg (HE) in other cases.

1.3.2.2.5. Use of solid carbon dioxide

The direct addition of carbon dioxide to olives in the solid state before milling represents a fundamental step which characterizes this innovative extraction system (Zinnai et al., 2016). At room temperature conditions solid carbon dioxide evolves directly into the air phase (sublimation), and the direct contact between the cryogen and the olives induces a partial solidification of the cellular water inside the fruits. Since the volume occupied by water in the solid state is higher than that in the liquid state, the ice crystals formed are incompatible with the cellular structure and induce the collapse of the cells, besides promoting the diffusion of the cellular substances in the extracted oil, which is thus

enriched with cellular metabolites characterized by a high nutraceutical value. Furthermore, a layer of CO_2 remains over the olive paste to preserve it from oxidative degradation. The addition of solid carbon dioxide to processed olives induced a statistically significant increase in oil yield and promoted the accumulation of tocopherols in the lipid phase, whereas a not significant increase in the phenolic fraction of the oil occurred. This could slightly increase the production cost; this innovative technology is able to produce a high-quality oil characterized by a strong link with the olives used as well as their production area. The main process steps followed by this micro-oil mill can be summarized as follows: olives, properly cleaned and washed, were poured into the receiving hopper where a screw fed a crusher equipped with a hollow knife impeller. The produced paste fell into a lower mixer, where a helicoid shaped stirrer promoted its malaxation. The temperature reached by the paste was maintained in the desired range by a thermal regulation system (a temperature sensor put inside the olive paste, connected with a heat exchanger). The suitable flow of olive paste was then sent to a biphasic decanter by a pump equipped with a speed change gear. The decanter helps the separation of oil from the olive pomace mixed with water (vegetable water plus the water potentially added through a flowmeter to induce a more efficient separation). The separation efficiency of the decanter can be modulated by a suitable regulation of the nozzles, which determines the point of oil picking and then also the degree of its contamination by vegetable water.

At the end of the extraction process, the decanter was washed with an amount of water measured by a flowmeter to ensure the total spillage of the oil coming out of the olive fruits.

These phases are summarized in *Figure 42:*

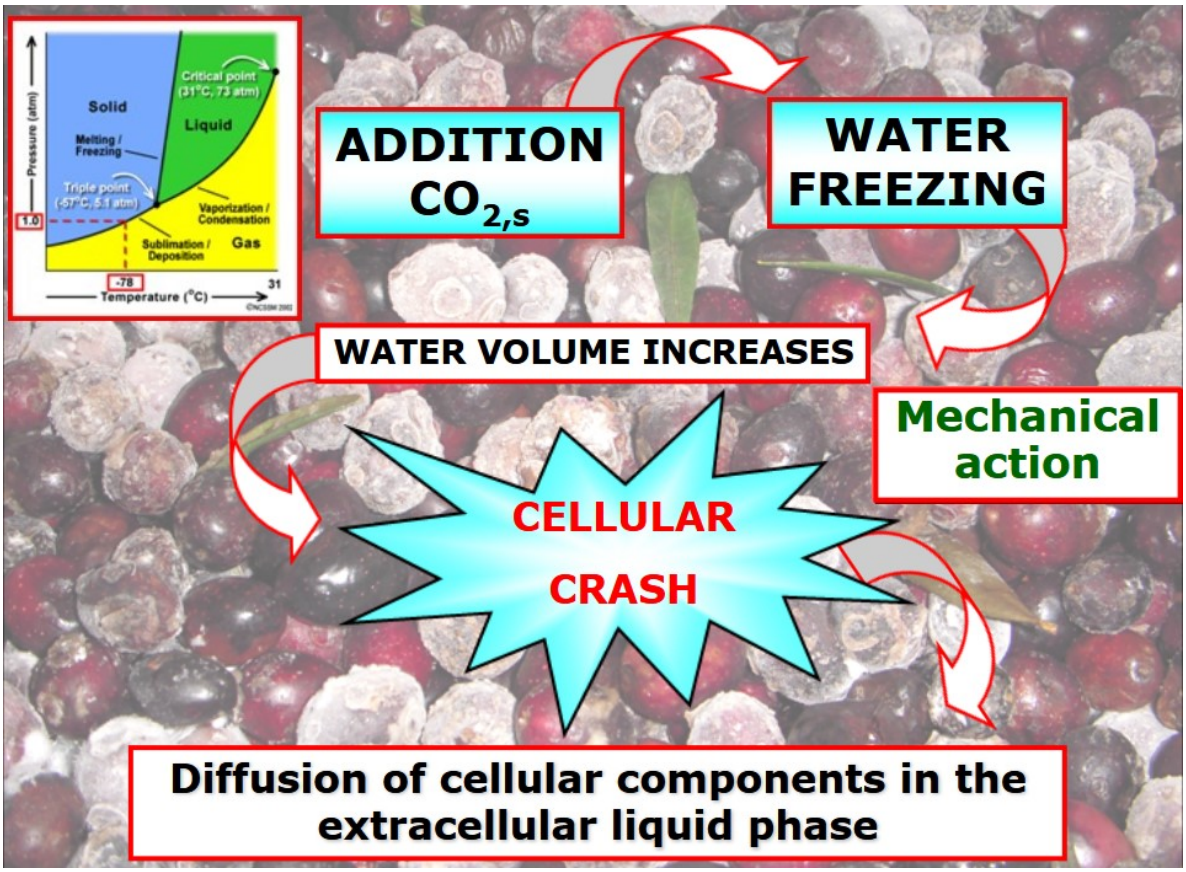


Figure 42: steps of use of solid CO2 in olive oil (form web presentation "The Utilization of Solid Carbon Dioxide in the Extraction of Extra virgin Olive Oil" Venturi F., Andrich G., Sanmartin C., Zoani C., Zappa G., (2016)

Experimentally, you can see a better yield with CO_2 (from +2.2 to +23.5%). In addition, the acidity, peroxide, K232 and K270 parameters are not substantially modified, on the contrary, in some cases there is a reduction in peroxides. Finally, there is a greater presence of tocopherols (from +0.4% to +15.4%) and phenols (to + 50%).

1.3.2.2.6. Pulsed electric field

One of the most important industrial handicaps of virgin extra olive oil production is the low efficiency of current extraction techniques. Typically, only 80% of the oil present in the fruit is easily released. The application of pulsed electric field (PEF) is an emerging physical technology that has been proposed for improving mass transfer processes in the food industry. The method (Puértolas & De Marañón, 2015) is based on the formation of pores in cell membranes due to their exposure to low-moderate external electric fields of adequate strength and duration. This electroporation mechanism increases the permeability of the vegetable cells, enhancing the diffusion of solutes through their membranes. In the present case, an increase in yield of 2.7% was observed. The impact of PEF on oil recovery could be explained by the well-known cell membrane electroporation mechanism, and the consequent improving of mass transfer phenomena. PEF acts as other technologies, like ultrasound or enzymes, assisting the release of oil from lipo-vacuoles of mesocarp

cells that have not been disrupted by crushing. A PEF treatment of olive paste could disrupt the lipoprotein membrane, favoring the release of oil (*Figure 43)*.

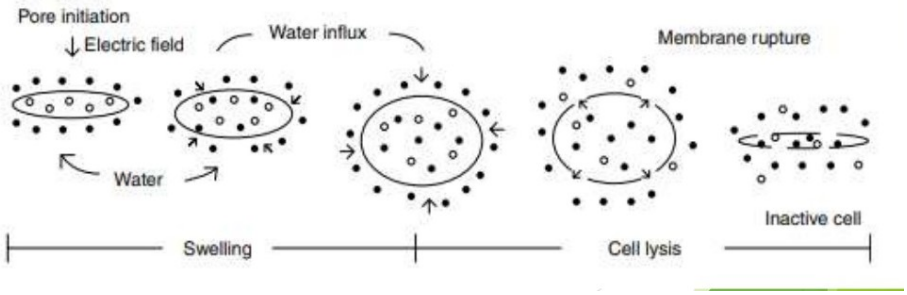


Figure 43: Mechanism of membrane rupture (Kumar et al., 2015)

PEF effect on olive oil yield could be explained by a double mechanism: the improvement of oil extraction from olive tissue, and the release of olive oil trapped in oil-vegetable water emulsions. In general, 20% of the oil is trapped in pomace, and this percentage can be reduced to 10 % using this and other techniques. There is also a slight increase in free acidity after treatment (+0.03%), while parameters K232, K270 and peroxides remain almost unchanged. The potential benefits of PEF have been demonstrated in recent research. Compared to heat treatment, PEF treatments save energy and time. High-intensity PEFs are an alternative to conventional food preservation techniques. The ability of high-intensity PEF to obtain stable liquid foods on the shelf with a high nutritional value has been demonstrated (Perez et al., 2021). On the other hand, moderate intensity PEF permeabilizes tissue structures, thus improving the extraction of intracellular metabolites and increasing drying efficiency. PEF applications have the potential to increase EVOO phytonutrient content and healthy properties. An increase in the presence of total phenols has also been observed compared to conventional oil, which depending on the conditions can be from 4 to 48%, as well as an increase in phytosterols (+140 mg/kg of oil). Total tocopherols also increased (+2.8 mg/100 g oil), with reference to α-tocopherol (+2.9 mg/100 g oil) and γ-tocopherol (+0.11 mg/100 g oil). Organoleptic characteristics remain unchanged, so this extraction method increases the quality and yield of the oil. In another study (Abenoza et al., 2013) it was seen how increasing the extraction temperature (from +0.12% to +6.49%), extraction time (from +1.13% to 6.49%) and intensity of treatment with pulsed electric fields (from +1.09% to 1.5%) increases yield. However, the yield increase is not so high from 15 to 26°C (from +0.12% to +0.93%). Excessive temperature increase could compromise oil quality. In addition, the acidity, peroxide, K232 and K270 parameters remain almost unchanged.

In addition, the composition in fatty acids does not vary and the organoleptic characteristics are not altered, while in PEF oil a lower presence of pigments (-1.19 mg pheophitins/kg, -0.42 mg luthein/kg) and total phenols (-36.7 mg gallic acid/kg) is observed.

1.3.2.2.7. Others emerging techniques

High voltage electrical discharges (HVED): A more effective extraction technology of macromolecules (for example: proteins), than PEF, requires the application of more powerful mechanical cell wall disintegration, which could be provided by high voltage electrical discharge (HVED) technology (Roselló-Soto et al., 2015). This electrotechnology is used in aqueous solutions, usually to extract oil and high added value compounds from plant matrices. In fact, it enhances the extraction of soluble molecules by the application of electrical breakdown, leading to bubbles division and improving the treatment efficiency. HVED treatment inactivates the microorganisms present in the product and leads to the discoloration of dyes. All these features are associated with the occurrence of different phenomena in water (for example: ultraviolet radiation, actives species, and shock), which are required for enhancing the kinetic and the quantity of molecules extracted in aqueous solutions, for the pretreatment of biological products, and finally for particle crushing.

Pressurized liquid extraction (PLE): Pressurized liquid extraction (PLE) is considered an advanced extraction technique that uses particular extraction conditions in which the extracting solvents are heated at high temperatures and maintained at high pressures in order to keep their liquid state during the whole extraction procedure (Roselló-Soto et al., 2015). In this way, the solubility of the analytes is enhanced, water diffusity is improved and water viscosity decreases, allowing a better penetration into the matrix and an increase of mass transfer rate, which results in an improved extraction yield. Therefore, several advantages are provided by this extraction technique, such as higher selectivity, shorter extraction times and lower consumption of toxic organic solvents (water and ethanol are the most used solvents). Additionally, this extraction technique is characterized by a reduced operational cost and controlled extraction conditions, which provides consistent qualitative and quantitative composition of the extract. These non-conventional extraction technologies have great potential to produce extracts with improved and novel properties, with lower costs, and with reduced environmental impacts, due to their different mode of action, like non-thermal (or lower thermal inputs) conditions and faster mode of action. Subcritical fluids could be considered as pressurized liquids, used for valuable compounds recovery. In fact, by increasing one parameter, either the pressure or the temperature, above the critical point generates subcritical fluids. Due to its low cost and environmentally friendly solvent; water represents the ideal solvent for industrial extraction of high-added value compounds from plants. However, regarding its poor extraction efficiency for most of the organic compounds, its use remains limited. Enhanced water extraction features (polarity, surface tension, viscosity, and disassociation constant) were obtained by using subcritical water, having similar chemical properties of organic solvents. In fact, when one of the parameters

(temperature or pressure) is below 374 °C or 22.1 MPa: the critical points, water is considered in a subcritical state. Consequently, subcritical water can solubilize polar (at lower temperatures) and non-polar (at higher temperatures) organic compounds.

High pressure processing (HPP): The application of HPP can cause structural changes in food, including cell deformation and membrane damage, which can increase permeability to solvents in cells and the spread of secondary metabolites, as shown in the *Figure 44*. HPP treatments stimulate mass transfer through the membrane due to differential pressure between the inside and outside of the cell, followed by a rapid restoration of a balanced concentration. There are few references to HPP technology applied to increase EVOO yields.(Andreou et al., 2017) studied the effect of HPP (200 and 600 MPa, 25 °C for 1 and 5 min) used before gramoling (30 min to 30 °C) on three different varieties of olive fruits (Tsounati, Amfissis and Manaki) and found an increase in extraction yield of up to 16%. Storage durability tests indicate that the quality of oil from thermally pre-treated olives varies depending on the conditions used, but the oil produced from HPP-treated olives had greater oxidative stability than the control samples. Therefore, HPP could potentially be applied to produce higher quality EVOO with increased yields. The combined application of filtration and high hydrostatic pressure on veiled EVOO has been studied. The resulting oil was not very susceptible to enzymatic and non-enzymatic phenomena, as it had no microbial contamination, low water content and low water activity, the opposite of when only high hydrostatic pressure was applied.

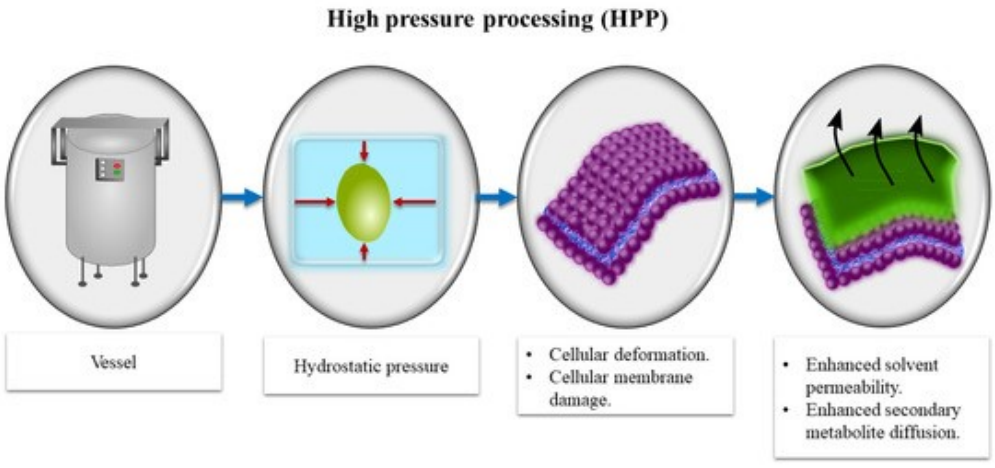


Figure 44: High pressure processing

1.3.3. EFFECT OF REFINING OF OLIVE AND VEGETABLE OILS TO QUALITY OIL

1.3.3.1. COMPOSITION OF THE MAIN VEGETABLE OILS

The acidic and sterolic composition of seed oils varies both qualitatively and quantitatively according to the botanical family to which it belongs, the soil in which the plant is grown and climatic conditions (Cappelli et al., 2000). The *Table 13* show the percentage compositions of seed oils in terms of fatty acids and sterols.

Fatty acid (%)	Palm	Palmists	Olive	Peanut	Rape	Sunflower	Seeds	Soy	Corn
capronic	/	1	/	/	/	/	/	/	/
Caprylic	/	4-6	/	/	/	/	/	/	/
Capricus	/	4-5	/	/	/	/	/	/	/
Lauric	Tr	50-55	<0.1	/	/	/	/	/	/
Myristic	0.5-2	14-16		<0.1	<0.1	<0.1	<0.2	<0.1	<0.1
Palmitic	30-50	6-7	8-17	6-15	1.5-4	3-10	7-9	7-13	8-19
Stearic	3-6	1-2	<3.5	1.5-6.5	0.5-2	2-6	3-5	2-5.5	1.5-3
Arachic	Tr	Tr	0.3-3	1-2.5	0.5-1	/	<0.5	<1	<1
Behechicus	/	/	<0.2	1.5-5	0.3-0.8	0.5-1	<0.2	<0.5	<0.5
Lignoceric	/	/	/	1-2.5	0.2-0.5	/	/	/	<0.5
Palmitoleic	Tr	Tr	0.3-3	<1	<0.5	<1	<0.5	<0.5	<0.5
Oleic	33-35	9-13	>65	35-72	10-32	14-65	15-20	19-30	19-50
Gadoleic	/	/	<0.2	0.5-2	7-11	<0.5	<0.2	<1	<0.3
Erucic	/	/	/	/	25-52	/	/	/	/
Linoleic	9-13	1-2	<13.5	13-45	11-20	20-75	60-75	48-58	34-62
Linolenic	<0.5	/	<1.5	<0.1	6-12	<0.5	<0.5	4-10	<1

Fat of (%)	Sterols	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β–sitosterol	Δ–5 avenasterol	Δ–7 stigmasterol	Δ–7 avenasterol
Olive	0.1-0.3	<0.5	/	<4	<3	>93	/	<0.5	/
Peanut	0.2-0.3	<1	/	15	9	64	8	3	1
Rapeseed	0.3-0.5	<1	10	25	Tr	58	2	5	/
Sunflower	0.3-0.5	<0.5	/	8	8	60	4	15	4
Corn	0.2-0.3	<0.5	Tr	23	6	66	4	1	Tr
Soy	0.2-0.4	<0.5	Tr	20	20	53	3	3	1
Coconut	/	<1	Tr	8	13	58	14	6	/
Palm	/	<1	Tr	14	8	74	2	1	/
palmists	/	<3	Tr	9	11	70	6	1	tr

Table 13:fatty acids and sterols composition of vegetable oils (From Cappelli et al., 2000)

1.3.3.2. REFINING OF VEGETABLE OILS

The percentage of acidity frees the marked color the presence of unpleasant odors and flavors from the appearance of alteration products prevent the direct sale of the oils both for lack of the legal requirements provided and for the unacceptable organoleptic characteristics (Cappelli et al., 2000). In this case, the products must undergo a series of treatments which, removing the causes of the alterations, make them suitable for use. In addition to olive oil with a high degree of free acidity, pomace oil, oils extracted from seeds, hydrogenated fats and margarine are always rectified. With the rectification operation, however, not only negative chemicals deriving from the degradation processes of the oil are removed, but also natural chemicals (for example tocopherols or vitamin E, vitamin A and polyphenols) that are important as they give the product the organoleptic characteristics that distinguish it. For this reason, rectification must be understood as a real correction of the oil, which if one part has its merits but on the other also has limits of use.

The grinding treatments can be summarized as follows:

1. Depuration
2. Degumming
3. Neutralization
4. Bleaching
5. Deodorization
6. Demarginating

In *Figure 45* is showed the general flow sheet in the process of refining crude vegetable oils:

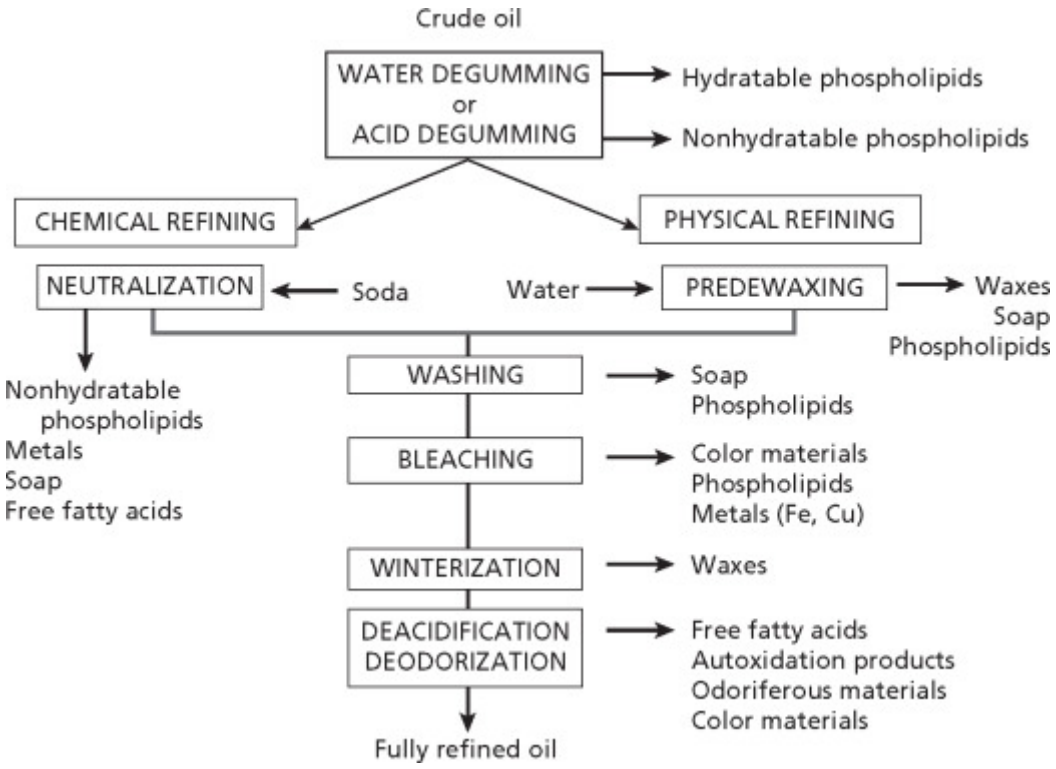


Figure 45:General flow sheet in the process of refining crude vegetable oils (from Monoj K. Gupta, in Practical Guide to Vegetable Oil Processing (Second Edition), 2017)

1.3.3.2.1. Depuration

It is a pre-rectification or pre-refining operation and consists in subjecting the oil to filtration and / or centrifugation processes, to remove any solid and coarse substances present in the product (for example oily deposits or sludge).

1.3.3.2.2. Degumming

It is carried out to eliminate insoluble substances: mucilage, phospholipids (about 90%, including lecithins, in fair quantities in soybeans oil, flax, rapeseed), resins, sugars, protein substances, traces of metals, present in total in quantities from 0.3 to 3% (Cappelli et al., 2000). The removal of mucilage can be carried out with hydration as phosphatides hydrate easily and can subsequently be removed by centrifugation. The non-hydrable part of mucilaginous can be eliminated by the addition of an acid, generally phosphoric or citric, at 60-80 °C for 5-30 minutes, followed by centrifugation and final neutralization. Another way is to add to the mixture of oil and water, held in agitation at 90-110°C, active earths, which absorb phosphatides and metals. In the case of soybean oil, with a decent lecithin content, it is advisable to carry out the treatment in two stages: a preventive hydration, which allows the recovery of lecithins, and a subsequent addition of acid with which non-moisturizing phosphatides are removed.

1.3.3.2.3. Neutralization

It is the main and most important operation of the refining processes, which allows to remove the excessive acidity of the oil (due to the presence of free fatty acids) and together with it also the pigments (for example chlorophylls and carotenoids) in addition to the phospholipids present as in the case of olive oil that is not subjected to demucillation (Sicheri, 1998, Vitagliano 1976, Viviani, 2003).

This operation is adopted for oils with a degree of acidity greater than 4% (for example lampante olive oil and crude pomace oil).

The technologies that are adopted to achieve deacidification are:

- Neutralization with alkalis by caustic soda (NaOH).
- Deacidification with selective solvents.
- Neutralizing distillation.
- Esterification of fatty acids with glycerin.

- Neutralization with alkalis

This system of deacidification using sodium hydroxide or caustic soda (NaOH), is the oldest and most widespread process. The system consists in treating the oil with sodium hydroxide (15-30%), to transform free fatty acids into soap through the chemical process of saponification. These soaps, being poorly soluble, can be easily removed from the oil in the form of a soapy mass that has neutralized oil equal to 1.2 – 2 times the volume of soapy paste.

Saponification

R-CH2-CH2-CH2-CH3COOH (fatty acid) + NaOH + elevated temperature → R-CH2-CH2-CH2-CH2-CH3Na (soap) + glycerin

The amount of caustic soda to be used must be calculated in a stoichiometric manner, previously deciding the acidity to be neutralized.

Deacidification using alkalis can be:

a) Discontinuous deacidification.
b) Continuous deacidification.

a) Discontinuous deacidification

This type of deacidification is carried out inside metal containers, in which the oil is heated to 60 – 80 ° C. Then sodium hydroxide is added to the mass at a concentration varying between 1 and 6 N (N = normality or concentration of the solution) keeping the product in slow agitation.

The added sodium hydroxide decides the salification of free fatty acids and their saponification. Soaps formed being insoluble can be removed by centrifugation. Through this process, however, other substances such as pigments, aromatic substances, vitamins, and antioxidants are also removed.
The oil at this point is washed with hot water at 80 – 90 ° C, which is also eliminated by centrifugation recovering the deacidified product.

b) Continuous deacidification

This type of deacidification, on the other hand, is used for oils that have a high acidity of up to 10%. It consists in using even more concentrated caustic soda solutions (10 – 20 N) to minimize the contact time of the substance with the oil and reduce its losses. This is because for every gram (g) of neutralized free acidity there is a loss of at least 1 g of oil.

- Deacidification with selective solvents

This method also known as the De Smet system, consists in solubilizing the oil in a volatile solvent (for example hexane, acetone, methanol, isopropanol, etc.) and then treating it with a solution of caustic soda added to propanol in order to form a hydroalcoholic solution that in the presence of the solvent forms an azeotropic mixture that is separated in three different phases depending on the temperature.
What occurs initially is a neutralization of free acidity and solubilization of soaps in the mixture. The mixture by letting it rest is divided into three layers:

Top layer
Consisting of neutral oil + solvent.
Middle layer
Consisting of mucilage and other impurities in emulsion.
Bottom layer
Consisting of soap in hydroalcoholic solution.

The top layer is first separated from the remaining two layers, and the neutral oil is separated from the solvent simply by distillation.
The same process of separation of the hydroalcoholic solution by distillation, consists instead in adding sulfuric acid (H2SO4) that salifies instead of free fatty acids, propanol which in turn can be easily recovered by exchange from the residual fraction.

Another system, *Vaccarino*, is based on the use of only one solvent, acetone, and on the lower solubility that this presents towards aqueous solutions compared to oily ones (Cappelli et al., 2000)

- Neutralizing distillation

This method of deacidification consists in treating the oil by distillation under a high vacuum followed by the removal of free fatty acids by superheated steam current exploiting the different specific weight of the substances. This method adopted for very acidic oils also allows to remove any foul-smelling substances (smell of rancid).

- Esterification of fatty acids with glycerin

Deacidification by esterification consists in artificially combining free fatty acids with glycerin in the oil. However, before esterification, the oil must undergo further rectification to remove other substances and their degradation products.

The operation is carried out in special equipment under high vacuum (5 mm of Hg = mercury), at a temperature of 180 – 220 ° C, in the presence of catalysts based on zinc Zn and copper Cu or in the form of chlorides (for example zinc chloride ZnCl2 or copper chloride CuCl2), adding synthetic glycerin to the oil.

This method is now performed in plants that create an even more intense vacuum allowing to work at even lower temperatures of the order of 50-60 ° C.

This system is often used to obtain oils for zootechnical use.

1.3.3.2.4. Bleaching.

This operation is carried out to deprive the oil of the oxidation product of metals, traces of soap, sulphur compounds and partly from pigments and their degradation products that could alter the normal color of the finished product (Sicheri, 1998, Vitagliano 1976, Viviani, 2003). The discoloration of the oil takes place by treating the product with an activated carbon or with activated earths / clays (for example bentonite or Spanish soil) in granular or powdery form. The powdery shape ensures a greater bleaching action than the granular one thanks to the greater adhesion surface. The activation of coal or earth takes place by treatment with inorganic acids (for example sulfuric acid H2SO4, hydrochloric acid HCl and nitric acid HNO3) that go to cut the positive ions (+) of the bleaching substance followed by washing with water. To perform this operation, the oil is kept in agitation at a temperature of about 90 ° C for 20 minutes under a pushed vacuum or in the presence of inert gas (for example nitrogen N2, Argon Ar etc.). At this point the bleaching substance is

dispersed in the oil and is left to allow the adsorption of pigments. At the end, a filtration with a filter press is carried out to remove the deposit or bleaching material from the oil.

1.3.3.2.5. Deodorization

It consists in the removal of all volatile substances that give the oil unpleasant smell (Cappelli et al., 2000). These include traces of free fatty acids (many already eliminated by neutralization), intermediates of fat oxidation, unsaturated hydrocarbons, proteins (partly removed by degumming). Deodorization is carried out by distillation in the current of vacuum steam pushed and at high temperature (about 200 ° C). Superheated and finely divided steam is injected at a pressure of 133-798 Pa, minimizing the oil's permanence at high temperatures, to prevent alterations to the product. In addition to the elimination of odorous substances, the partial removal of tocopherols, tocopherol, both free and esterized disterols, the elimination of pesticide residues, organic chlorine, and mycotoxins, is achieved. Both continuous and discontinuous methods are used for bleaching and deodorization. This operation unfortunately also removes other constituents of the oil that are part of the unsaponifiable fraction, important for human nutrition such as: monoglycerides, diglycerides, sterols, hydrocarbons (for example squalene), tocopherols, polyphenols, sterol esters (Sicheri, 1998, Vitagliano 1976, Viviani, 2003). For this reason, deodorization makes the oil less preservable over time, as it destroys antioxidant substances. In fact, it is often not performed if the earlier deacidification took place by distillation.

1.3.3.2.6. Demargarination (winterization)

This operation, also known as winterization, is the last one supported by the oil rectification process. It is practiced on those oils that tend to become cloudy during the cold season, because they are stored in rooms where the temperature drops between 8 and 10 ° C (Sicheri, 1998, Vitagliano 1976, Viviani, 2003). The oils that often give problems of turbidity are those richest in saturated fatty acids especially those obtained in the southern regions of Italy where there is usually a warmer climate. The operation therefore aims to cut solid triglycerides or that solidify easily giving the oil a too greasy taste of fat, less fluidity and greater turbidity. The operation is carried out by keeping the oil in slight agitation, in refrigerated rooms at a temperature not lower than 0 ° C and on average at 7 ° C. At this temperature solid triglycerides (oleomargarine) tend to crystallize because they have a higher melting point than liquid triglycerides. Once the crystals have separated and by decantation are deposited at the bottom of the vessel, they can be easily separated from the liquid mass by filtration and/or centrifugation. In this way you will get an oil that can remain clear even at temperatures of 10 ° C.

The size of the crystals and so the success of the operation also depends on the speed of cooling and the speed of agitation of the oil.

1.3.3.3. CHARACTERISTICS OF RECTIFIED OILS

Rectified oils (especially olive oils) have quite different chemical-physical characteristics from virgin oils. Chemically, rectified oils have an average amount of dienes (fatty acids with 2 double bonds) and trienes (fatty acids with 3 double bonds) two to three times lower than virgin oils. In addition, they are depleted of coloring substances (carotenes and xanthophylls), chlorophyll and partly of tocopherols and polyphenols (Sicheri, 1998, Vitagliano 1976, Viviani, 2003). The pomace oil, also made edible through the rectification process, is even more depleted of these chemical constituents due to solvent extraction and treatment carried out in very harsh conditions. In these oils, in fact, the unsaponifiable fraction can reach 3%, while in virgin oils it reaches a maximum of 1.5%. Furthermore, it should be noted that while in natural esterification, fatty acids occupy a preferential position within the molecule, in artificial esterification this mechanism occurs randomly or randomly. In this way it is possible to recognize the re-esterified oil simply by analysing the fat. In the case of olive oil subjected to even partial re-esterification, the amount of palmitic acid (C16:0) in the central position of the molecule is more than 2%. From a physical point of view, on the other hand, rectified oils have a lighter yellow color, a less persistent smell and a less aromatic and fruity taste than virgin oils. In conclusion, it is possible to recognize from the chemical point of view an oil rectified by a virgin oil by resorting to the measurement of spectrophotometric absorption in the ultraviolet U.V, or to gas chromatography.

1.3.3.4. POMACE OLIVE OIL

The pressure residue is called pomace (Cappelli et al., 2000) (it corresponds to about 40% of the initial olives) and still contains a certain amount of oil. This is extracted by solvent. The quality of the oil obtained varies according to the time spent taking and transporting the pomace from the oil mill to the pomace oil extractor and for any storage before extraction. In fact, the longer this interval is prolonged, the more significant the transformations at the expense of the product: in particular, there is an increase in free acidity and oxidative processes and water evaporation start. The solvent most used for extraction is hexane, which is particularly selective towards oil, while it is inert to the other components of the pomace. The fatty acid composition of pomace oil is like that of olive oil; increases the percentage of linoleic acid (from 9.5 to 15.5%), due to the destructive action of the solvent even on endocarp fragments particularly rich in unsaturated fatty acids. Elaidinic acid, the trans isomer of the oleic, is also found in pomace oil, in quantities of less than 0.2%. Elaidinization

occurs when the pomace has been dried in iron appliances, which catalyzes the reaction, which can also occur under other conditions:

- During bleaching with active land over 90 °C
- During synthetic esterification
- Following hydrogenation.

Pomace oil represents a particularly high free acidity, from 5 to 40% and, at the limit, also 80%, in relation to the state and storage time of the pomace. It cannot therefore be used as such for feeding but must necessarily be rectified.

1.3.3.5. CHANGES IN THE COMPOSITION OF OILS ACCORDING TO THE REFINING STEP

1.3.3.5.1. Effects of refining on total compounds

The *Figure 46* highlights the effect of the various refining phases on oils of plant origin and in particular highlights which classes of substances are removed at various stages:

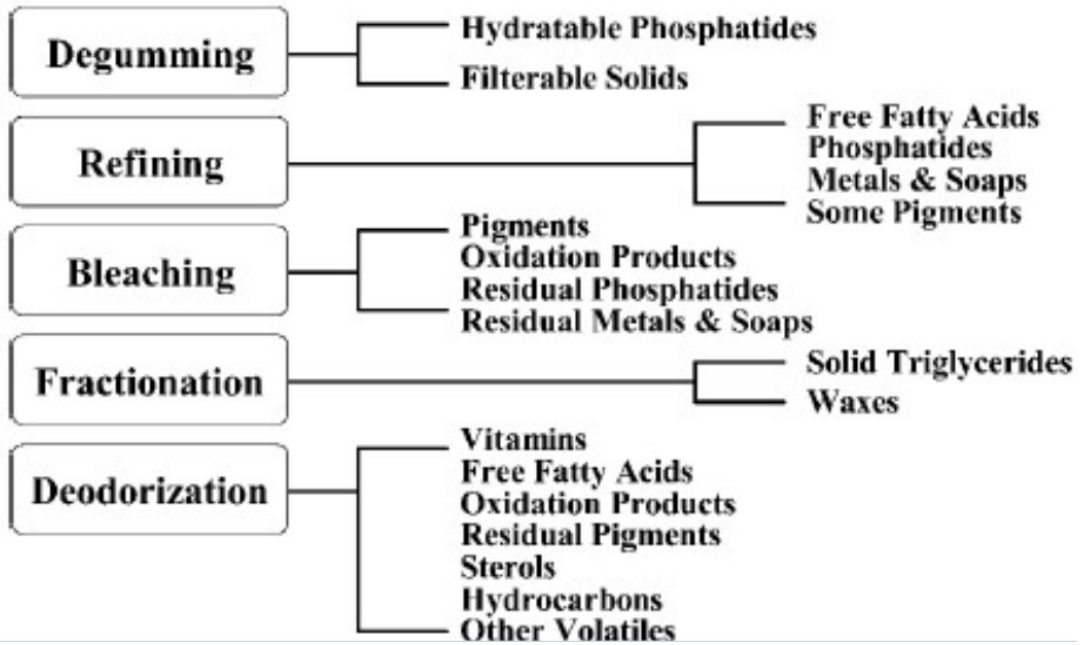


Figure 46:Classes of substances removed in the various stages of refining (by web https://oilpalmblog.wordpress.com/2015/12/26/physical-refining-degumming/, Oil palm knowledge base)

1.3.3.5.2. Effects of refining on total polyphenols

A recent study examined the effect of the various refining phases on polyphenols contained in olive oil and some vegetable oils (Lucci et al., 2020). The analysis of polyphenols was carried out on certain qualities of lampante olive oil, and the *Figure 47* summarized the effect of the various refining phases.

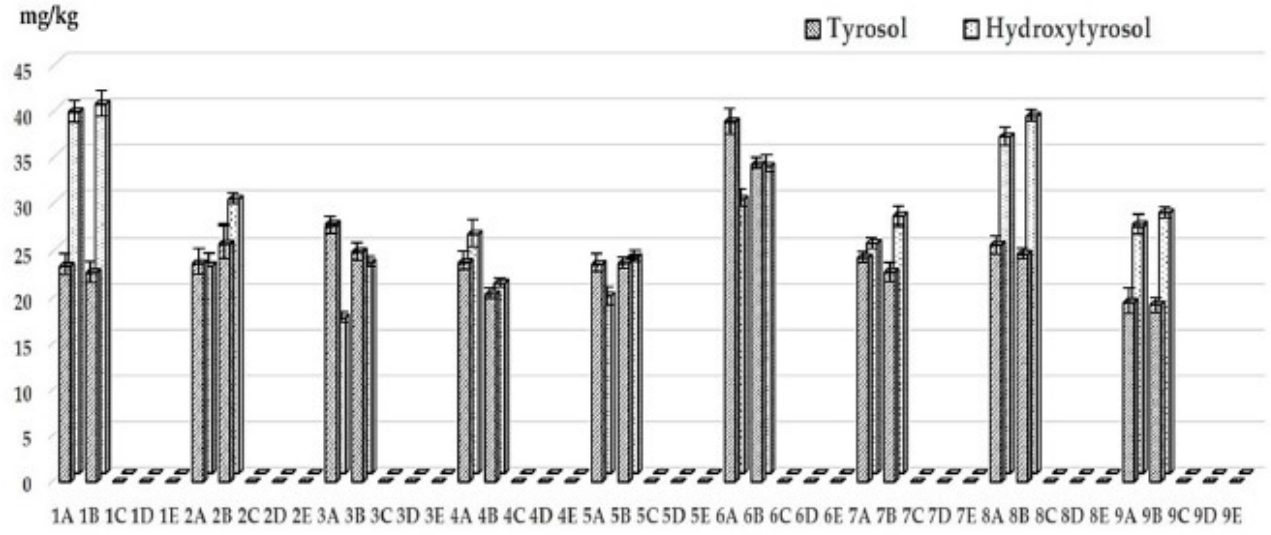


Figure 47:effect of the refining steps on the hydroxytyrosol and tyrosol content (mg/kg oil). Legend for refining steps: A: crude oil; B: degummed oil; C: neutralized oil; D: bleached oil; E: deodorized oil. (From Lucci et al., 2020)

It was observed (Kostadinovic-Velickovska & Mitrev, 2013) a reduction of the total phenolic content from 19.23 to 1.82 mg of gallic acid equivalent/10 g of oil in sunflower oil, while more recent research reported losses of 63% of polyphenolic compounds during neutralization, 16% during bleaching, and 67% during the deodorization step of rapeseed oil. In the case of lampante olive oil the hydroxytyrosol and tyrosol contend tend to decrease faster in neutralization, bleaching and deodorization steps. The speed of decreasing of polyphenols content is maximum at alkaline conditions. The experimental results (Lucci et al., 2020) show that oil samples collected for the study were characterized by initial low contents of total hydroxytyrosol and tyrosol, with values ranging from 43 to 68 mg/kg, thus very far from the fixed level (250 mg/kg) for the health claim on "olive oil polyphenols" *(Figure 47).* Regarding the evolution of these compounds during refining, a complete removal of polyphenols occurred during the process as they were not detected starting from neutralized samples. Meanwhile, no significant changes were observed between crude and degummed oil samples. In fact, o-diphenols can be easily oxidized under alkaline conditions, thus explaining their loss during this step.

1.3.3.5.3. Effects of refining on tocopherols

The content of total tocopherols has recently been studied (sum of α, β, γ tocopherol) in lampante olive oils after the various refining phases (Lucci et al., 2020), obtaining the results summarized by *Figure 48:*

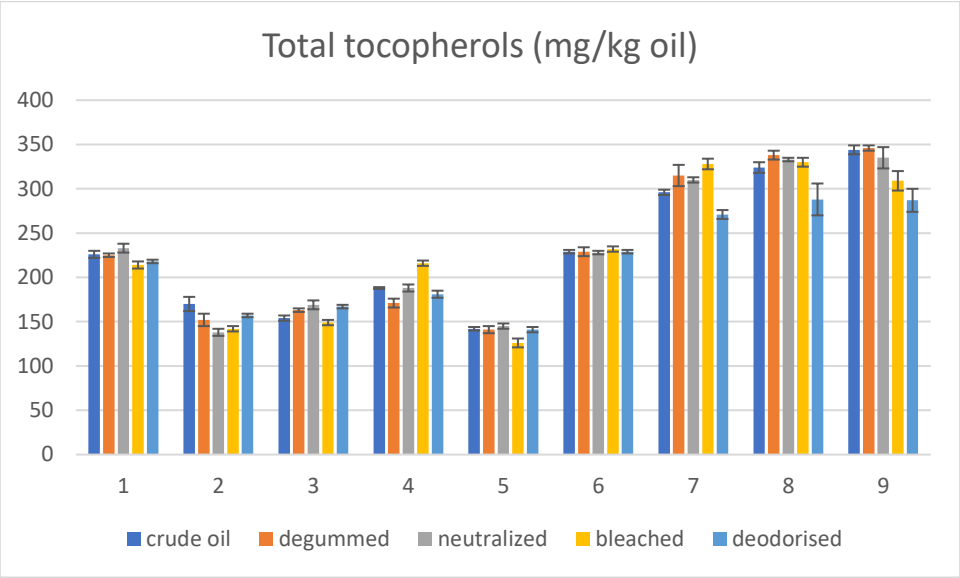


Figure 48: Effect of the refining steps on the total tocopherol content (mg/kg oil)

Data showed variations in the levels of tocopherols encountered in the nine crude oil samples, with values ranging from 142 to 344 mg/kg (Lucci et al. 2020). The α–tocopherol is the predominant form in all samples. In general, a continuous decrease of tocopherol content has been observed during the refining procedure in sunflower, rapeseed, and soybean oils. For instance, a huge decrease in total tocopherol content was observed in soybean oil (45.5%), with a considerable reduction in individual and total tocopherol levels at almost every stage of refining. A gradual but not statistically significant decrease of tocopherol content (14.0%) during the overall chemical refining process has been also reported for sunflower oil. On the other hand, other researchers reported a significant decline of tocopherols, from 750 mg/kg in crude sunflower oil to 520 mg/kg in refined samples. Major decreases have been observed during caustic neutralization because of the reduced stability of tocopherols in the presence of longer contact time with air and alkali. Other researchers revealed a reduction of tocopherol content in olive oil of 37.7% (from 172.5 mg/kg to 107.5 mg/kg) and 23.7% (from 107.5 mg/kg to 82.0 mg/kg) after the degumming–bleaching and steam distillation steps, respectively, with a total loss of 52.5%. In Lucci et al. (2020) work, however, the trends were not always clear-cut, and differences among samples behavior were found for different refining steps. Regarding the overall changes observed in tocopherol levels, the maximum decrease of about 16% was revealed for sample 9. Similar reductions were also detected for samples 8, 7, and 2, with final losses of about 11%, 8%, and 7%, respectively. It should be noted, however, that only four out of nine samples showed a statistically significant decrease ($p \leq 0.05$), while in some samples (1, 4, 5, and 6) the refining procedure did not induce any substantial decrease of total tocopherols. Moreover, in sample 3, a significant increase (8.4%) of tocopherols was encountered in the final deodorized sample. Some loss of tocopherols can also occur by evaporation during high temperature deodorization and physical refining, with the magnitude of this decrease depending on the conditions employed. For instance, in

soybean oil, after 120 min at 300 ◦C (a drastic treatment), the tocopherols almost completely disappeared, whereas the reduction during physical refining at 240 ◦C for 120 min was only about 15%–20%. The latter conditions are closer to those ones employed for the industrial refining of our lampante oil samples where deodorization at 200 ◦C and 2 mbar for about 2.5 hours was conducted, thereby explaining the reduced effect on total tocopherol contents. In some cases, this trend could be interpreted as a concentration effect: higher initial free acidity in crude oil results in a greater loss of oil mass which does not necessarily involve the tocol fraction *(Table 14).*

	Samples								
Quality Parameters	1	2	3	4	5	6	7	8	9
Free acidity (%)	4.00	5.02	4.00	4.70	5.10	3.73	2.22	2.21	2.68
Oil loss/chemical refining (%)—neutralization up to 0.2% of FFAs	3.80	4.82	3.80	4.50	4.90	3.53	2.01	2.02	2.48
Oil loss/physical refining (%)—physical flash neutralization up to 0.02% of FFAs	3.78	4.80	3.78	4.48	4.88	3.51	2.00	1.99	2.46

Table 14:Oil loss in the refining process

This aspect should be taken into consideration when examining the dynamics that influence the concentration of tocopherols of individual samples during the refining process. Therefore, besides the fact that tocopherols can suffer in the presence of oxygen and the alkaline medium, the small increases in tocols observed in our study for same samples during the degumming/neutralization phase may be associated with an increase in the tocopherol concentrations rather than their absolute values. In fact, as can be seen in *Table 14*, the loss rate of oil observed during the refining procedure is strongly related to the initial free acidity levels. As a result, a relationship between the initial free acidity of crude oil samples and the extent of decrease of tocopherols during refining could be hypothesized, as crude oil samples characterized by high free acidity resulted, except for sample 2, in refined samples with smaller changes in final tocopherol content. The refining process has a marked effect on the final content of phenolic compounds, which are eliminated at the early stages of the refining procedure, while the effect on the level of tocopherols is minimal and, in many cases, insignificant ($p > 0.05$). These results also suggest that it is not always necessary to add tocopherols to refined samples with the aim of restoring natural tocols lost in the refining process, as the threshold value fixed by the international standards would be exceeded. In fact, it should be noted that the final concentration of tocopherols is higher than 200 mg/kg in five of nine samples *(Figure 48).* Crude oils characterized by initial tocopherol values lower than 200 mg/kg, showed only a slight decrease in tocopherols in the finished products. These results are also consistent with those shown in *Figure 49*, which reports

data obtained by analyzing 11 commercial refined olive oil samples purchased at a local market. As can be seen, 8 out of 11 samples presented a total tocopherol content higher than the maximum allowed value, with 5 samples showing levels even greater than 300 mg/kg.

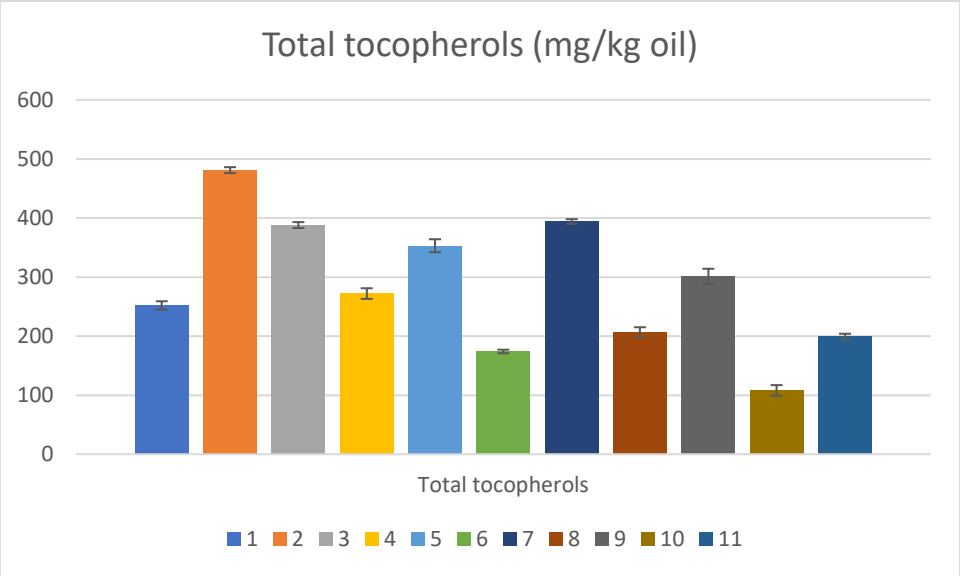


Figure 49:Total tocopherol content (mg/kg oil) of commercial olive oil samples

1.3.3.5.4. Effects of refining on other compounds

Synthesizing, we have the phospholipids and mucilaginous gums removed by the degumming followed by the removal of the free fatty acid (FFA) through the neutralization process (Marrakchi et al., 2015). Finally, bleaching reduces chlorophylls, carotenoids, while deodorization removes volatile compounds, FFA, carotenoids and tocopherols. The degumming phase tends to reduce the presence of phospholipids, compounds that give a dark color to oils and unpleasant odors. The effects of degumming tend to decrease as the concentration of phosphoric acid, the H3PO4/oil ratio, the concentration of sodium hydroxide, the use of bleaching land and the time of the bleaching phase increase. Neutralization is important to remove unwanted free fatty acids that could increase oxidation. An increase in the H3PO4/oil ratio would lead to a reduction in the presence of oleic acid. Degumming reduces the presence of free fatty acids, but under hydration conditions the opposite effect occurs. A small excess of sodium hydroxide inhibits saponification, with beneficial effects on bleaching and oil quality. At the bleaching stage the presence of trans fatty acids would be unchanged, while that of unsaturated fatty acids would increase slightly. Regarding the presence of undesirable secondary oxidation compounds, it is very important to optimally regulate the various refining phases. It has been studied how the increase in bleaching temperature, bleaching phase dosage, H_3PO_4/oil ratio, bleaching time, and the presence of phosphoric acid contribute to increased secondary oxidation in the oil. Phosphoric acid would turn conjugated dienes into trienes. When increasing ratio

H_3PO_4/oil, NaOH excess, earth clay dosage, bleaching residence time, and temperature, the oil becomes more and more colorless.

During the step of bleaching some changes occur in the oil (Cappelli et al., 2000):

- Decrease in the number of peroxides (formed during fat oxidation) as these are first transformed to aldehydes and ketones, then eliminated by deodorization.
- Formation of conjugated dienes and trienes, avoidable using activated carbons, which, however, costs more than land.
- Increase in the acidity of the oil, in case very acidic land is used in the presence of water.
- Isomerization cis - trans at the expense of oleic acid when operating at temperatures higher than 110 °C in the presence of high amounts of land.

Among the alterations that manifest themselves in a deodorized oil, there is a reduced geometric isomerization and position at the expense of unsaturated fatty acids. Linear and cyclic dimers and polymers can also be formed.

1.3.3.5.5. Applications on rectified oils

The grinding phases help to remove the polyphenols contained in raw oil, thus obtaining an oil particularly susceptible to oxidation. An attempt has been made to remedy this by implementing synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ). However, these synthetic compounds can be dangerous to human health, so an attempt has been made to replace these artificial antioxidants with natural ones. In the last few years, the use of olive cake and mill wastewater as a natural source of phenolic compounds has been widely considered, and several studies have focused on the development of new extraction methods, as well as on the production of functional foods enriched with natural antioxidants (Venturi et al., 2017). Oil-in-water emulsions formulated with stabilizers and enriched with phenolic compounds extracted from olive mill wastewater have recently been studied for the realization of emulsion-based food products with enhanced health properties. For example, the phenolic extracts were obtained from the wastewater taken at the physical refining plant outlet after the preliminary water degumming step before the steam distillation. The wastewater is extraordinarily rich in polyphenols. To maximize the recovery of the phenolic compounds from wastewater collected during physical refining, we studied different extraction processes by utilizing two different solvent solutions: ethanol and ethanol: diethyl ether (1:2 v/v). The wastewater collected during olive oil physical refining showed very high levels of phenolic compounds, in most part represented by non-flavonoid compounds; therefore, they can be considered as a potential antioxidant source. In particular, the extract obtained with pure ethanol (99.8%) as an extraction solution, showed the

highest total phenolic amount (0.131 g/kg gallic acid). The values of the quality parameters determined in both phenol-enriched oils were within the range that makes them edible (free acidity=0.12%, peroxide value 5.47 meq O_2/kg, K270=0.57, $\Delta K<0.15$, K225=from 0.15 to 0.18, bitter index from 1.20 to 1.52, total phenol content to 0.105 to 0.131 g/kg gallic acid).

The antioxidant capacity of both the enriched oils was significantly higher than that shown by the control oil: in control oil TEAC (Trolox equivalent antioxidant capacity) is 0.8 mmol Trolox/l, versus 2.2 for ethanolic extract and 2.8 for ethanol: diethyl ether (1:2 v/v) extract.

2. PURPOSE OF THE THESIS

The PhD project consists of the determination of genetic (cultivars), technological (extraction techniques) and refining factors that influence the quality of olive oil and other vegetable oils. In the first part of this project, 11 monovarietal extra virgin olive oils will be compared, highlighting the differences in terms of chemical-physical composition, sensory and verifying how the cultivar can influence for 50%, together with other factors, the chemical and sensory composition as well as the content of bioactive substances.

In the second part of this work, the differences in the main chemical components (acidity, peroxides, induction time with Rancimat, tocopherols, volatile organic compounds) between traditional EVO, pitted and pitted with the addition of kernels, in various storage phases (in the bottling phase and after 3,6 and 12 months) were examined. In this way the higher quality of the pitted oil, its greater shelf-life, its organoleptic characteristics like the traditional and the worst quality of the pitted with subsequent addition of kernels were highlighted. Likely, the cause is due to the presence of oxidizing substances. In addition, two enzymes, polyphenol oxidase (PPO) and peroxidase (POD), are highly concentrated in the olive kernel. PPO and POD can oxidize phenolic compounds resulting in a reduction in the phenolic concentration of the oil. The pitting process, excluding the olive seed before kneading, partially removes the peroxidase activity in the pastes and thus oxidation.

In the third part of the project, the effects of various refining phases (oil as it is, refining, drying, discoloration, deodorization, neutralization) on various seed oils (sunflower, high oleic sunflower, corn, grape seeds, soybeans) were highlighted. This is through the analysis of the parameters acidity, peroxides, induction time with Rancimat apparatus, tocopherols and tocotrienols. In general, it has been seen how refining reduces acidity and peroxides of vegetable oils, as well as making them edible as per current legislation. It also helps to increase the shelf life of the same, as you can see from the induction time. With this research we will try to improve the innovative production technologies of the oil.

3. EFFECT OF CULTIVAR ON OLIVE OIL QUALITY PARAMETERS

3.1. INTRODUCTION

Olive oil markets are changing rapidly. Monovarietal extra virgin olive oil (MEVOOs) are gaining increasing interest allowing for further segmenting the market and creating now trends in high market niches (Cacchiarelli et al., 2016),(García-González & Aparicio, 2010). MEVOOs are defined as oils obtained by the transformation of olives from only one variety. Traditionally, EVOOs are made of blends of all the olive varieties present in each farm but more recently, the milling technology and machinery allow for milling separately even small quantities of olives (Carbone et al., 2018). In 2017/2018 Italy was the second European olive oil producer after Spain (IOOC 2019) and it is currently the first country for cultivars biodiversity, accounting for over 800 varieties (Rotondi et al., 2013). Since MEVOOs are products reflecting the characteristics of a country beyond the genetics, their systematic sensory and chemical characterization has a pivot role to identify quality oils with remarkable diversity and clear identity. A detailed description of the chemical and/or sensory traits of MEVOO produced with olive cultivars among the most spread in Italy, such as Frantoio, Leccino and Moraiolo was reported by several authors (Blasi et al., 2019) (Portarena et al., 2015) (Rotondi et al., 2010) (Klikarová et al., 2020). Additionally, the features of MEVOO from cultivars typical of different Italian regions producing appreciated oils were also highlighted (Campus et al., 2013) (Rotondi et al., 2017). However, a frequent limitation in studies on MEVOOs is that cultivars often come from different geographical areas (Piscopo et al., 2016) (Laincer et al., 2016) (Lukić et al., 2018)(Rissato et al., 2007), therefore other variables like the pedoclimatic characteristics may introduce bias in the characterization. In fact, it is known that the same cultivar grown in different pedoclimatic conditions (altitude, latitude, climatic conditions, soil composition etc.) shows different values in fatty acid composition, phenolic content, and oxidative stability (Rissato et al., 2007) (Vichi et al., 2007). Since pedoclimatic aspects, olive ripeness, harvesting time, and the extraction system strongly impact on the chemical composition and sensory properties of oils (Campestre et al., 2017) (Bruno et al., 2019), it is recommended to control these factors when studying characteristics of MEVOOs. Within the heritage of Mediterranean diet products, MEVOOs represent precious contributions, whose sensory and healthy properties are explained by chemical compositional peculiarities, in many cases not yet investigated. Thus, the present study aimed to perform a chemical and sensory characterization of eleven different MEVOOs. Some of the cultivars investigated (Leccino, Frantoio, Maurino, Moraiolo, Pendolino) are well known and widely cultivated in several

Italian areas having adequate pedoclimatic conditions, while other cultivars are less diffused and present only in their native regions (Carboncella, Coratina, Marzio, Piantone di Falerone, Rosciola, Sargano di Fermo). Beyond the characterization of MEVOOs from minor cultivars never investigated before, an important outcome of this study, is the contribution to understand the effect that the genetic background of the fruit (effect of cultivar) plays on the chemical composition and sensory properties of the oil. In fact, in the present study all the other parameters are the same for all the cultivars investigated, i.e. olives are grown in the same experimental olive orchard and under the same conditions (fertilization, irrigation), and processed with the same technology.

3.2. MATERIALS AND METHODS

3.2.1. STANDARD, REAGENTS AND SOLVENTS

The Folin-Ciocalteu reagent and gallic acid were obtained from Merck & Co. Inc. (Darmstadt, Germany). The fatty acid methyl esters, triacylglycerols, pyridine, 1,1,1,3,3,3-hexamethylsiloxane and chloroxilane were purchased from Sigma-Aldrich Inc. (St. Louis MO, USA). The phenols p-hydroxyphenylethanol (p-HPEA), 3,4-dihydroxyphenylacetic acid, vanillic acid vanillin, oleuropein, luteolin, apigenin, were purchased from Extrasynthése (Genay, France), Sigma (St. Louis, MO, USA) and Fluka (Buchs, Switzerland). HPLC grade solvents were purchase from Merck. All the solvents and solutions were filtered through a 0.45 μm PTFE filter (Supelco, Bellefonte, PA, USA).

3.2.2. OLIVE OIL SAMPLES

MEVOOs obtained from eleven Italian cultivars were studied. Olive fruits, collected in the crop year 2018/2019, were all provided from an experimental farm of Ancona, Italy, where the olive trees were cultivated under identical agronomic and pedoclimatic conditions. However, some of the varieties investigated are currently cultivated on national scale (Leccino, Frantoio, Maurino, Moraiolo, Pendolino), while others are autochthonous of three Italian regions: Marche (Carboncella, Piantone di Falerone, Rosciola, Sargano di Fermo), Tuscany (Marzio) and Apulia (Coratina). The healthy fruits were harvested by handpicking at the same maturity index (values around 3.5, based on the color and texture of the olive drupe under Jaen index). After harvest, the olive fruits were processed by continuous system technology. For each cultivar, approximately 350-400 kg of olives were collected, and each batch were processed in a three-way continuous plant (P. Barigelli & C, Cingoli, Italy). The olive fruits were defoliated and washed prior to crushing, and then processed by hammer crusher and malaxer. The temperature of the pulp in the malaxer was set at 26°C. The olive oil was separated by B/D 400 decanter (P. Barigelli & C, Cingoli, Italy) and poured into green sealed glass bottles (0.25

mL each) and the headspace was approximately 10 mL. The EVOO bottles were stored in the dark and at room temperature (20°C±1°C) and were opened after five months for the analysis. Each analysis performed in triplicate.

3.2.3. DETERMINATION OF LEGAL QUALITY PARAMETERS

The free acidity (FA, g oleic acid in 100 g of oil), peroxide value (PV, mg eq O2 kg-1 of oil) and UV spectrophotometric determinations were carried out for each oil sample according to the EEC Reg. n. 2568/1991 and later modifications. Spectrophotometric determinations K232, K270 and ΔK were carried out using a UV-Vis-Nir Cary 5000 Varian spectrophotometer (Leiní, Italy).

3.2.4. SENSORY EVALUATION ACCORDING TO THE PANEL TEST

The sensory evaluation was performed by a trained panel (O.L.E.A. Organizzazione Laboratorio Esperti Assaggiatori, Pesaro, Italy) accredited by the Ministry of Agricultural, Food and Forestry Policies (MIPAAF) and according to the procedure reported in the EEC Reg. n. 2568/1991 and in its subsequent modifications. Panelists used a profile sheet adapted from the International Olive Council (IOC) method for Designation of Origin (IOOC 2005). The vocabulary included 12 positive attributes: nine descriptors for volatile sensations perceived by retro-olfaction (fruity, greenly fruity, ripely fruity, olive leaf, grass, artichoke, tomato, almond, apple), two tastes (bitter, sweet), and one chemesthetic sensation (pungency). Trained assessors could also mark defects if perceived. Samples (15 mL) were served in standard glass (IOOC 2007) and codified with random three-digit codes. Samples were assessed in three evaluation sessions and served in balanced and randomized order across panellists.

3.2.5. DETERMINATION OF FATTY ACID COMPOSITION

To determine fatty acid composition, fatty acid methyl esters (FAMEs) were obtained with 1M KOH in methanol (Christie, 1998) and analysed using a gas chromatograph (GC) HRGC Mega 2 series Model MFC 800 (Fisons Instruments, Milan, Italy). The GC instrument was equipped with a flame ionization detector (FID) and a fused silica capillary column coated with poly (80% biscyanopropyl/20% cyanopropylphenyl siloxane (SP 2330, 60m length x 0.25mm i.d. 0.2m film thickness, Supelco, St. Louis, MO, USA). The carrier gas was helium (2 mL min-1); the splitting ratio was 1:80. The injector and detector temperatures were set at 250°C; the temperature program started at 150°C and raised to 220°C at rate of 3°C min-1 and was held for 30 min. The FAMEs were identified by comparison with known standards.

3.2.6. TRIACYLGLYCEROL DETERMINATION

After the addition of the internal standard (triundecanoin), the trimethylsilyl derivates were obtained according to Sweeley et al.(Manufacturing, 1964). The silylated sample was injected into a gaschromatography (HRGC Model 5300, Carlo Erba, Milan, Italy) equipped with a flame ionization detector (FID) and a fused-silica capillary column coated with a 50% phenyl-/50% methylpolysiloxane (CP-TAP, 60m x 25mm x 0.25mm i.d., film thickness 0.1mm, Varian Walnut Creek, CA, USA). The chromatographic method was set according to (Boselli et al., 2008). Peak identification was carried out by comparison of the relative retention time with those reported in the literature and with the retention times of the standard substances. Quantitative analyses were performed with triundecanoin as the internal standard and adopting the corrected area normalization method.

3.2.7. DETERMINATION OF THE OXIDATIVE STABILITY

The oxidative stability of the oils was determined by Rancimat apparatus (Metrohm model 679, Herisau, Switzerland), measuring the induction time in response to force oxidation (induction period) of 5 g sample heated at 110°C under an air flow of 20 L h-1. The induction period (expressed in hours) was determined by drawing the two tangents of the time–conductivity curve and projecting the intersection onto the time-axis.

3.2.8. PHENOLS DETERMINATIONS BY FOLIN-CIOCALTEU ASSAY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) COUPLED WITH DIODE ARRAY DETECTOR (DAD)

Phenolic compounds were extracted three times following the procedure described by (Boselli et al., 2009). The phenols extract for Folin-Ciocalteu assay was resuspended in 1 mL methanol and the total phenols content was determined at 765 nm according (Singleton et al. 1965). The results were expressed as gallic acid equivalents (mg kg-1 oil) based on a calibration curve (R2 = 0.993). Phenols were also quantified by HPLC coupled with a diode array detector (DAD) and 3,4-dihydroxyphenylacetic acid solution was used as internal standard. After the extraction procedure, the dry extracts were resuspended in 1 mL methanol and the solutions were filtered through 0.2 mm regenerated cellulose filters (Schleicher & Schuell, Dassel, Germany). Phenolic compounds were separated by Chromspher C18 (5 ¼m particle size, 25 cm x 4.6 mm i.d. column, Chrompack Middelburg, Netherlands), using a Varian 9010 ternary pump (Walnut Creek, CA, USA). The sample was injected in a 20 mL loop and the mobile phase flow rate was 0.7 mL min-1. The gradient elution

was carried out according to (Fiori et al., 2014). A Varian Prostar PDA 330 was used as detector to acquire phenolic acids, phenyl ethyl alcohols and secoiridoids at 280 nm, while flavones were detected at 350 nm. The data were acquired using Varian Star 6.3 software. 3,4-Dihydroxyphenylethanol (3,4-DHPEA), p-hydroxyphenylethanol (p-HPEA), vanillic acid and vanillin were quantified using their respective standards (R2=0.998, 0.999, 0.996 and 0.998, respectively). Secoiridoids were quantified with oleuropein (R2= 0.999), while luteolin and apigenin were quantified with their standard (R2= 0.998, R2= 0.996, respectively). For structural elucidation, the HPLC system was coupled online to an LCQ ion-trap mass spectrometer (Thermoquest, San José, CA, USA) as reported by (Boselli et al., 2009)(Boselli et al., 2009).

3.2.9. DATA ANALYSIS

Two-way ANOVA models were separately conducted on intensity values given to each sensory attribute from panel descriptive data (fixed factors: cultivar, assessors) to estimate the effect of the cultivar on the perceived intensity of the sensations, followed by Tukey's pairwise test ($p<0.05$). Correlations among variables were tested with Pearson coefficient (R) ($p<0.05$). A Principal Component Analysis (PCA) was conducted on significant chemical and sensory variables, to exploratorily study the relationships among variables and cultivars. Analyses were conducted with XLStat 2019.1.1, Addinsoft, Boston, USA.

3.3. RESULT AND DISCUSSION

3.3.1. LEGAL PARAMETERS: ACIDITY, PEROXIDES, ΔK

The *Figure 50, Figure 51 and Figure 52* show the values of acidity, peroxides and Δk for all eleven EVOs considered, depending on the variety. The *Table 15* summarizes all analytical data.

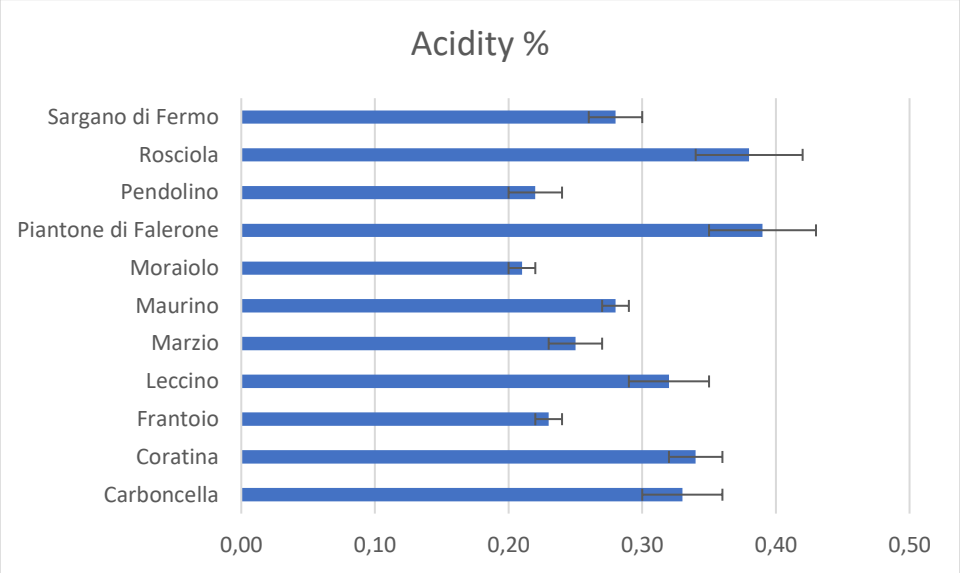


Figure 50: Acidity (%) of 11 MEVOO

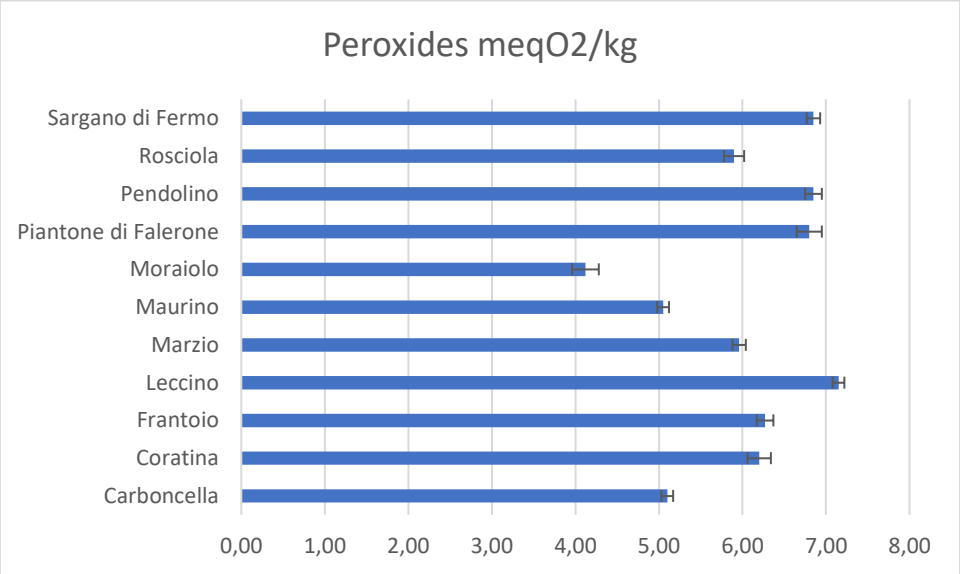


Figure 51:peroxides (meq O2/kg oil) of 11 MEVOO

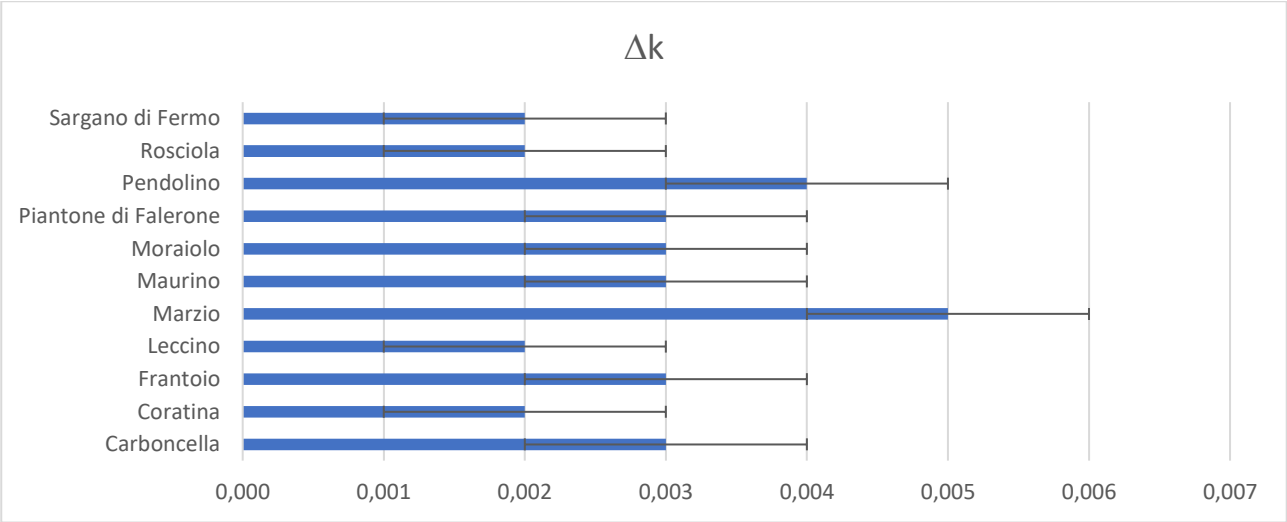


Figure 52: Δk for 11 MEVOO

Cultivar	Free acidity [x]	Peroxide value [z]	K_{232}	K_{270}	ΔK
Carboncella	0.33 [abcd] ±0.03	5.10 [d] ±0.07	1.73 [a] ±0.13	0.12 [a] ±0.002	0.003 [a] ±0.001
Coratina	0.34 [abc] ±0.02	6.20 [bc] ±0.14	1.63 [a] ±0.12	0.13 [a] ±0.003	0.002 [a] ±0.001
Frantoio	0.23 [defg] ±0.01	6.27 [b] ±0.10	1.59 [a] ±0.11	0.10 [a] ±0.002	0.003 [a] ±0.001
Leccino	0.32 [bcde] ±0.03	7.15 [a] ±0.07	1.83 [a] ±0.19	0.15 [a] ±0.005	0.002 [a] ±0.001
Marzio	0.25 [g] ±0.02	5.96 [bc] ±0.08	1.86 [a] ±0.16	0.14 [a] ±0.004	0.005 [a] ±0.001
Maurino	0.28 [cdef] ±0.01	5.05 [d] ±0.07	1.77 [a] ±0.11	0.16 [a] ±0.006	0.003 [a] ±0.001
Moraiolo	0.21 [fg] ±0.01	4.12 [e] ±0.16	1.65 [a] ±0.19	0.13 [a] ±0.004	0.003 [a] ±0.001
Piantone di Falerone	0.39 [a] ±0.04	6.80 [a] ±0.15	1.73 [a] ±0.24	0.11 [a] ±0.002	0.003 [a] ±0.001
Pendolino	0.22 [efg] ±0.02	6.85 [a] ±0.10	1.76 [a] ±0.18	0.15 [a] ±0.003	0.004 [a] ±0.001
Rosciola	0.38 [ab] ±0.04	5.90 [c] ±0.12	1.76 [a] ±0.17	0.13 [a] ±0.004	0.002 [a] ±0.001
Sargano di Fermo	0.28 [cdef] ±0.02	6.85 [a] ±0.08	1.64 [a] ±0.21	0.10 [a] ±0.001	0.002 [a] ±0.001

Table 15: Olive oil quality parameters of the eleven monovarietal oils investigated Results are expressed as mean ± standard deviation (n=3).; x g Oleic acid in 100 g of oil; z mg eq O2 kg-1 of oil; K232, K270: UV Absorption at ʎ= 232 and 270 nm.; Different letters in the same column indicate significantly different values (p <0.001).

Considering the parameters FA, PV, K232, K270 and ΔK, all MEVOOs samples complied with limits required for extra virgin olive oil category (par. 1.2.1). FA ranged from 0.22 to 0.39 % (g oleic acid per 100 g of oil), much lower than 0.8% set for EVOOs, denoting a good quality and healthy status of olives, which were immediately transformed after harvesting. PV and UV spectrophotometric indices are the two main parameters indicating the oil rancidity progress state. Values for the peroxide and UV indices were lower than the legal limits (PV<20 meq O2 per kg of oil -meq O2/kg-; K232 <2.5, K270 < 0.22 and ΔK < 0.01). The low PV levels ranged between 4.0 and 7.2 meq O2/kg, while UV indices did not show significant differences across the samples.

3.3.2. SENSORY EVALUATION ACCORDING TO THE PANEL TEST

As expected from the optimal quality of olive fruits and the technological practices, no sensory defect was detected. From two-way ANOVA models on the intensity of positive attributes *(Table 16*), five attributes significantly differed across oils from different varieties: fruity ($F=2.8$, $p<0.03$) greenly fruity ($F=3.12$, $p<0.01$), bitter ($F=5.67$, $p<0.001$), sweet ($F=5.89$, $p<0.001$) and pungency ($F=4.30$, $p<0.001$). Secondary descriptors (olive leaf, grass, artichoke, tomato, almond, apple, ripely fruity) were occasionally perceived but at low intensities (≤2.5) and without significant differences ($p>0.05$) among the oils. The lack of significant differences across varieties did not allow a clear diversification probably due to the low intensity values of these secondary descriptors. Other reports similarly showed that secondary notes slightly differ across cultivars (Rotondi et al., 2013) (Cantini, 2012) as compared to major attributes as e.g. bitterness, pungency, and they are perceived at modest/low intensities.

Cultivar	Fruity	Bitter	Pungency	Greenly fruity	Sweet
Carboncella	2.6 ^{ab} ±0.3	3.0 ^{abc} ±0.3	2.9 ^{bc} ±0.3	1.7 ^{b} ±0.3	2.4 ^{c} ±0.3
Coratina	3.1 ^{ab} ±0.3	3.6 ^{ab} ±0.3	3.1 ^{abc} ±0.3	1.9 ^{ab} ±0.3	2.7 ^{bc} ±0.3
Frantoio	2.9 ^{ab} ±0.3	1.9 ^{c} ±0.3	3.1 ^{abc} ±0.3	2.0 ^{ab} ±0.3	4.0 ^{ab} ±0.3
Leccino	2.5 ^{b} ±0.3	1.9 ^{c} ±0.3	2.1 ^{c} ±0.3	1.8 ^{ab} ±0.3	4.5 ^{a} ±0.3
Marzio	3.5 ^{a} ±0.3	4.1 ^{a} ±0.3	4.1 ^{a} ±0.3	3.2 ^{ab} ±0.3	2.3 ^{c} ±0.3
Maurino	3.1 ^{ab} ±0.3	2.3 ^{bc} ±0.3	3.2 ^{abc} ±0.3	2.2 ^{ab} ±0.3	3.5 ^{abc} ±0.3
Moraiolo	3.3 ^{ab} ±0.3	3.0 ^{abc} ±0.3	3.1 ^{abc} ±0.3	3.8 ^{a} ±0.3	3.4 ^{abc} ±0.3
Piantone di Falerone	3.3 ^{ab} ±0.3	3.0 ^{abc} ±0.3	3.1 ^{abc} ±0.3	2.5 ^{ab} ±0.3	2.9 ^{bc} ±0.3
Pendolino	3.1 ^{ab} ±0.3	2.7 ^{bc} ±0.3	3.2 ^{abc} ±0.3	2.5 ^{ab} ±0.3	2.9 ^{bc} ±0.3
Rosciola	2.9 ^{ab} ±0.3	3.2 ^{abc} ±0.3	3.5 ^{ab} ±0.3	2.8 ^{ab} ±0.3	2.7 ^{bc} ±0.3
Sargano di Fermo	3.2 ^{ab} ±0.3	3.0 ^{abc} ±0.3	3.6 ^{ab} ±0.3	3.2 ^{ab} ±0.3	3.8 ^{ab} ±0.3

Table 16: Sensory evaluation and perceived intensity (average score) of main sensory attributes in the investigated monovarietal oils. Different letters in the same column show significantly different values: fruity ($p<0.03$), greenly fruity ($p<0.01$), bitter ($p<0.001$), sweet ($p<0.001$), and pungency ($p<0.001$).

As an example, (Cantini, 2012) reported a maximum intensity of 3.0 for secondary notes such as artichoke or almond in 57 cultivars investigated. Instead, bitter, pungency and greenly fruity seemed more related to the cultivar and less linked to agronomical and pedoclimatic influences (Bendini et al., 2012). Marzio MEVOO was characterized by the significantly highest greenly fruity, bitterness and pungency. Moraiolo was also characterized by a high fruity and greenly fruity, with a pronounced pungency and bitterness. Leccino was the sweetest, and had the significantly lowest greenly fruity, bitterness and pungency intensities. These results agree with previous reports, describing Moraiolo as significantly more pungent and bitter than Leccino (Rotondi et al., 2013). Sensory similarities were found with some attributes that did not significantly differ across cultivars, such as the intensity of pungency, similar in Marzio, Sargano di Fermo, Rosciola, Piantone di Falerone, Maurino, Frantoio,

Moraiolo, and Pendolino and bitterness, at the same intensity in Marzio, Coratina, Rosciola, Pendolino, Moraiolo, Carboncella and Sargano di Fermo.

3.3.3. FATTY ACIDS

The *Figure 53, Figure 54, Figure 55 and Figure 56* show the main fatty acids. The *Table 17* show all analytics results.

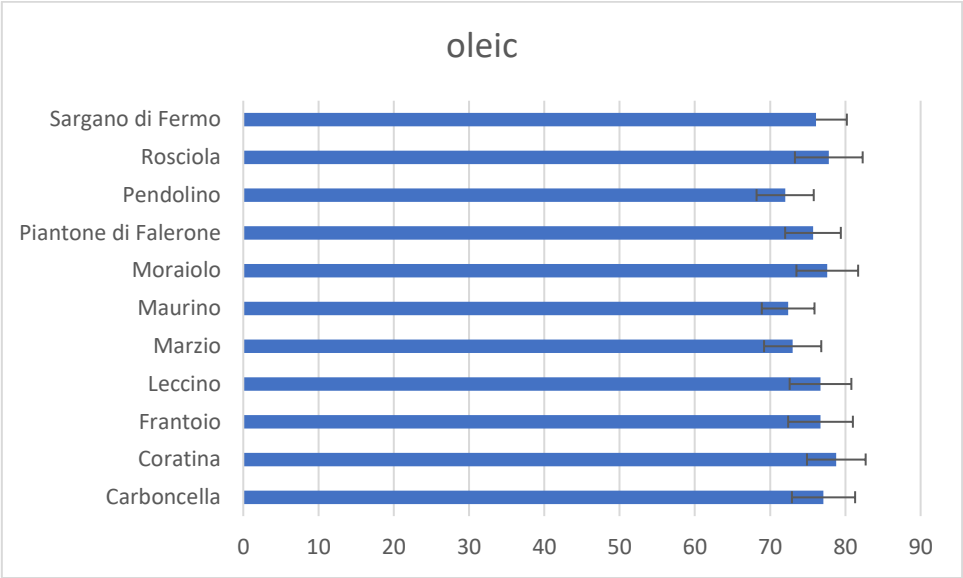


Figure 53: oleic acid (%) in 11 MEVOO

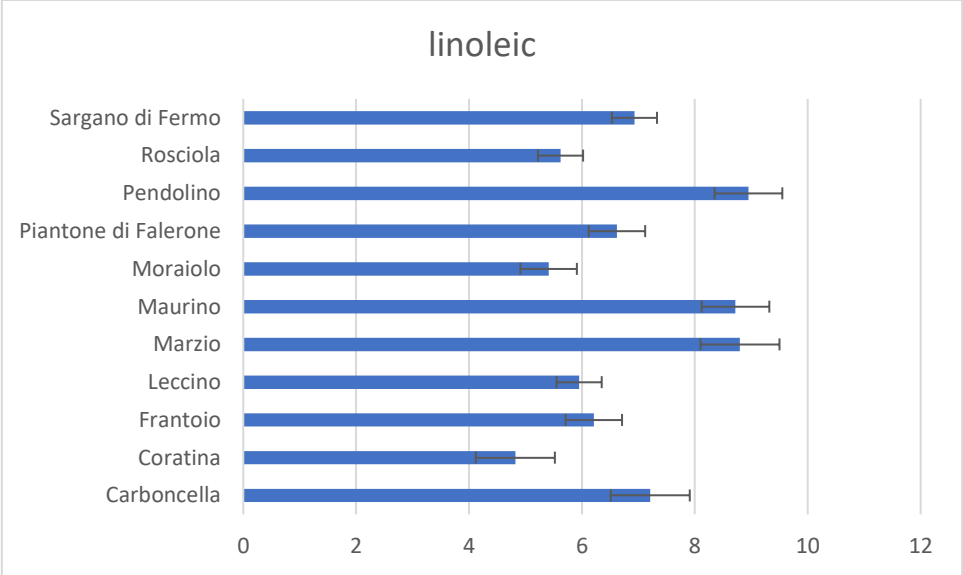


Figure 54: linoleic acid (%) in 11 MEVOO

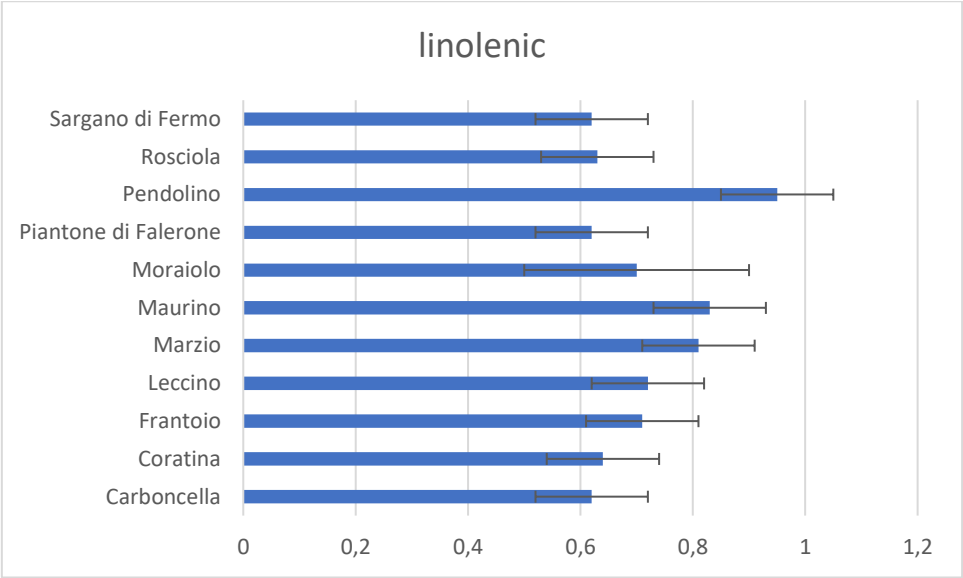


Figure 55: linolenic acid (%) in 11 MEVOO

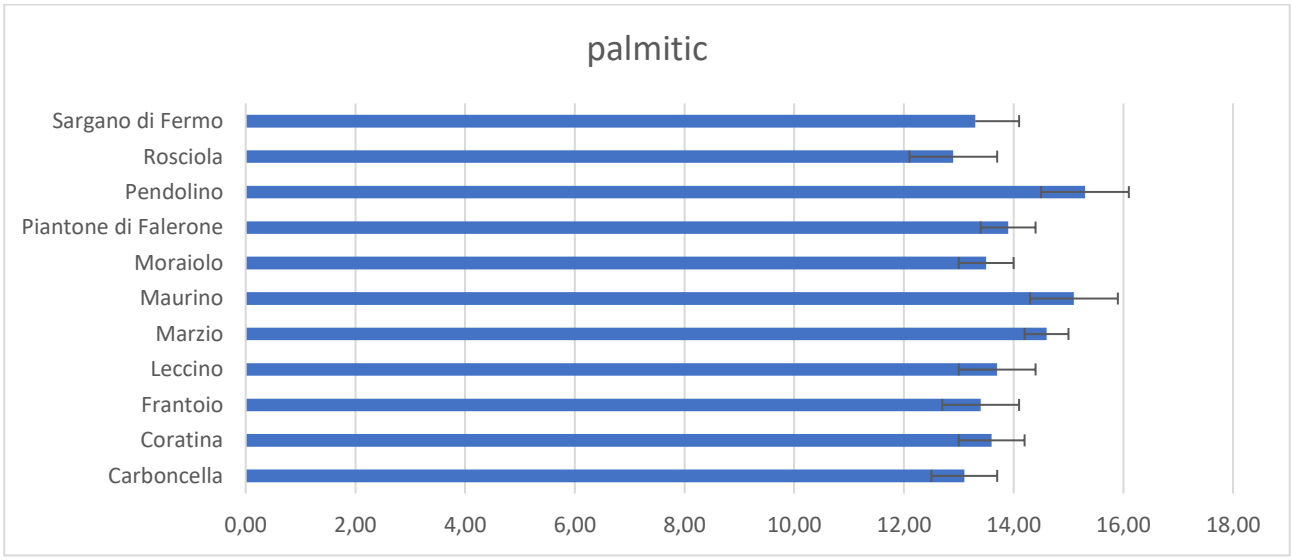


Figure 56: palmitic acid (%) in 11 MEVOO

	Carboncella	Coratina	Frantoio	Leccino	Marzio	Maurino	Moraiolo	Piantone di Falerone	Pendolino	Rosciola	Sargano di Fermo
Fatty acids (%)											
Palmitic acid	13.1 ^{c} ±0.6	13.6 ^{c} ±0.6	13.4 ^{c} ±0.7	13.7 ^{bc} ±0.7	14.6 ^{a} ±0.4	15.1 ^{a} ±0.8	13.5 ^{bc} ±0.5	13.9 ^{bc} ±0.5	15.3 ^{a} ±0.8	12.9 ^{c} ±0.8	13.3 ^{bc} ±0.8
Palmitoleic acid	0.98 ^{b} ±0.1	1.17 ^{a} ±0.3	1.08 ^{a} ±0.2	1.26 ^{a} ±0.2	0.81 ^{b} ±0.1	1.26 ^{a} ±0.1	0.94 ^{b} ±0.1	1.12 ^{a} ±0.1	1.10 ^{a} ±0.2	0.97 ^{b} ±0.1	1.04 ^{ab} ±0.1
Stearic acid	1.97 ^{a} ±0.2	1.91 ^{a} ±0.3	1.83 ^{b} ±0.4	1.69 ^{b} ±0.2	1.92 ^{a} ±0.3	1.68 ^{b} ±0.2	1.89 ^{a} ±0.2	1.94 ^{a} ±0.1	1.71 ^{b} ±0.1	2.03 ^{a} ±0.2	1.85 ^{b} ±0.2
Oleic acid	77.1 ^{a} ±4.2	78.8 ^{a} ±3.9	76.7 ^{a} ±4.3	76.7 ^{a} ±4.1	73.0 ^{bc} ±3.8	72.4 ^{bc} ±3.5	77.6 ^{a} ±4.1	75.7 ^{ab} ±3.7	72.0 ^{c} ±3.8	77.8 ^{a} ±4.5	76.1 ^{a} ±4.1
Linoleic acid	7.21 ^{b} ±0.7	4.82 ^{f} ±0.3	6.21 ^{cd} ±0.5	5.95 ^{de} ±0.4	8.80 ^{a} ±0.7	8.72 ^{a} ±0.6	5.41 ^{e} ±0.5	6.62 ^{bc} ±0.5	8.95 ^{a} ±0.6	5.62 ^{e} ±0.4	6.93 ^{bc} ±0.4
Linolenic acid	0.62 ^{c} ±0.1	0.64 ^{c} ±0.1	0.71 ^{c} ±0.1	0.72 ^{c} ±0.1	0.81 ^{ab} ±0.1	0.83 ^{ab} ±0.1	0.70 ^{c} ±0.2	0.62 ^{c} ±0.1	0.95 ^{a} ±0.1	0.63 ^{c} ±0.1	0.62 ^{c} ±0.1
Oleic acid/linoleic acid	10.6 ^{c} ±0.8	16.3 ^{a} ±1.1	12.3 ^{bc} ±0.9	12.9 ^{bc} ±0.8	8.25 ^{d} ±0.5	8.30 ^{d} ±0.6	14.5 ^{b} ±1.0	11.4 ^{bc} ±0.9	8.08 ^{d} ±1.1	13.9 ^{b} ±0.8	10.9 ^{c} ±0.8
Insaturation index^{z}	161.1 ^{b} ±11	139.6 ^{cd} ±9	151.6 ^{c} ±11	151.6 ^{c} ±12	178.6 ^{a} ±13	177.4 ^{a} ±12	146.2 ^{c} ±9.7	155.5 ^{c} ±10	181.2 ^{a} ±15	147.1 ^{c} ±9.8	161.2 ^{b} ±12

Table 17: Fatty acid composition, oleic acid:linoleic acid ratio, and insaturation index of the eleven monovarietal extra virgin olive oils investigated.

In all the MEVOOs, fatty acids percentages were compliant with the legal limits imposed by EEC Reg. n. 2568, 1991. Moreover, the investigated samples showed fatty acid compositions that are well in the average value ranges reported in literature for various Italian monovarietal oils (T. Cecchi et al., 2011) (Pacetti et al., 2020) (Bianchi et al., 2001). Overall, our findings corroborated the hypothesis that the oil fatty acid profile is strongly under genetic control. (Ayed & Rebai, 2019) noticed that oleic acid amount in olive oil can be strongly related to the polymorphisms of fatty acid-related genes, such as the stearoyl-acyl carrier protein desaturase gene (SAD). TT-SAD.1 genotype was found to be associated with a higher proportion of monounsaturated fatty acids, mainly oleic acid, as well as with lower proportion of palmitic acid, thus making olive varieties with this genotype producing more monounsaturated fatty acids, namely oleic acid, than saturated fatty acids. Indeed, we found clear differences among the MEVOOs fatty acid compositions, mainly in terms of palmitic, oleic, linoleic and linolenic acid contents. Since the investigated oils were obtained with olives cultivated in the same growing conditions and were processed with the same operative conditions, the highlighted differences were related to the olive cultivar. In detail, the oleic acid content, ranging from 71.55% to 78.42%, clustered the oils into two groups: the first composed of eight varieties (Coratina, Rosciola, Sargano di Fermo, Frantoio, Carboncella, Moraiolo, Leccino, Piantone di Falerone), with the significantly highest values ($p<0.001$), ranging from 76.2 to 78.4%, and the second with Pendolino, Maurino and Marzio oils, with the lowest values (72.0-73.0%). Simultaneously, Pendolino, Maurino and Marzio MEVOOs presented the significantly highest levels of palmitic, linoleic and linolenic acids. Thus, Pendolino, Maurino and Marzio oils stood out from the rest for their unfavorable oxidative stability parameters, such as the highest unsaturation index and lowest oleic: linoleic ratio. It is important to notice that high unsaturation index, low percent content of oleic acid and high percent content of linoleic acid bound in the acylglycerol backbone, make olive oil weakly resistant toward oxidation. Our findings were in line with previous studies reporting the comparison of fatty acid composition in some of the MEVOOs investigated by us; e.g., Bianchi et al. (2001) studied the fatty acid profile of Frantoio, Coratina and Moraiolo oils from olives harvested in different Italian regions (i.e. Apulia, Tuscany) revealing that these oils were similar on the basis of oleic acid content. (Blasi et al., 2019) did not underline significant differences, in terms of overall fatty acids composition, among Frantoio, Leccino and Moraiolo MEVOOs purchased from producers located in central Italy regions. However, the relative fatty acid composition found in the different MEVOOs is different as compared to that reported by (Portarena et al., 2015) for the same varieties. They also investigated MEVOOs processed with the same plant and obtained from olives cultivated in same areal (Perugia, Italy). Differently from our results, they found that Moraiolo differed from Frantoio and Leccino oils in terms of oleic and linoleic acids percentages. These different outcomes can be

related to the different environmental conditions thus suggesting a synergistic effect of genetic and environmental factors on the fatty acid composition. (Mousavi et al., 2019) investigated several olive cultivars, including Frantoio, Leccino, Coratina and Moraiolo, and demonstrated as the fatty acid profile of the oil was regulated by the interaction of environmental and genotype factors. It has been shown that both temperature and light play a role in modulating oleic acid content and the oleic acid / (palmitic + linoleic acids) ratio in the oil.

3.3.4. TRIACYLGLYCEROLS

The *Figure 57, Figure 58 and Figure 59* show the main triacylglycerols, while the *Table 18* show the total results:

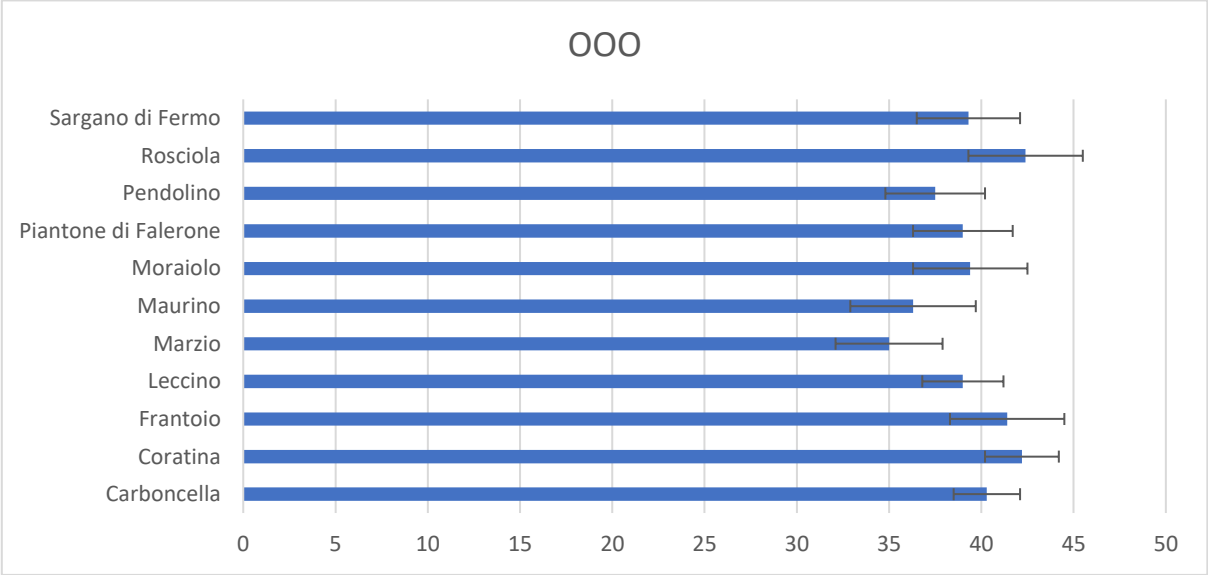


Figure 57: OOO (g of fatty acid methyl ester 100 g-1 of oil)

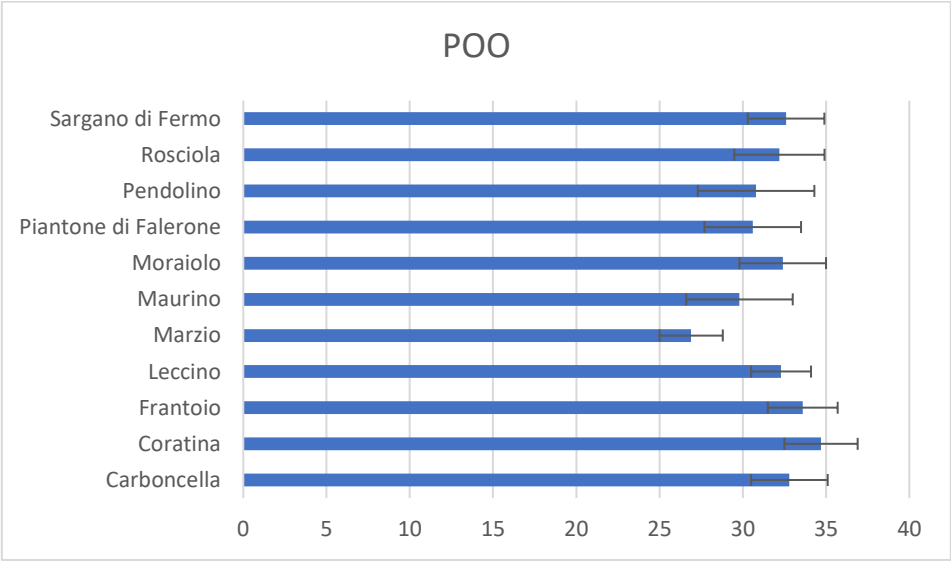


Figure 58: POO (g of fatty acid methyl ester 100 g-1 of oil)

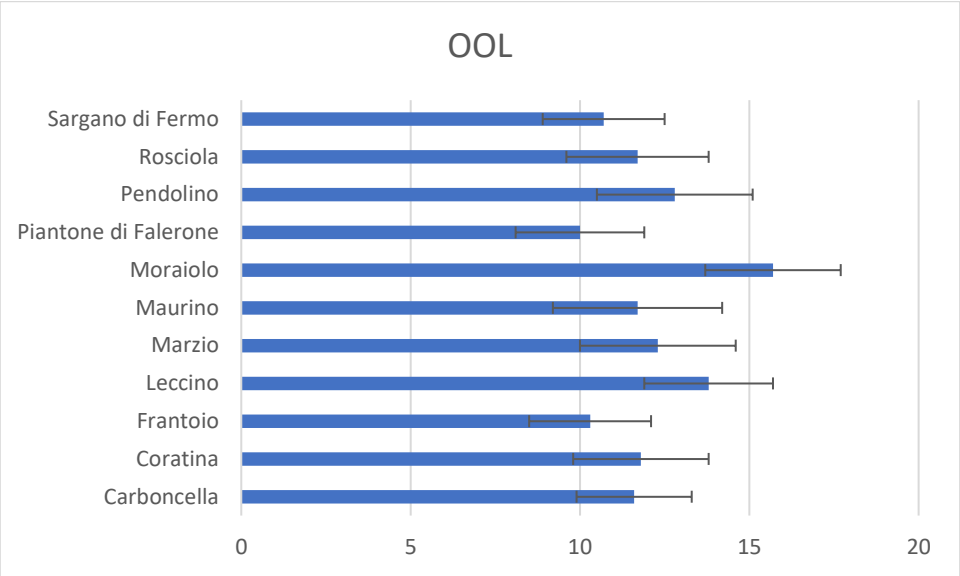


Figure 59: OOL (g of fatty acid methyl ester 100 g-1 of oil)

Triacylglycerols (%)											
PPO	5.6 ^{b} ±0.5	3.0 ^{c} ±0.4	2.9 ^{c} ±0.4	6.0 ^{ab} ±0.5	7.1 ^{a} ±0.5	5.6 ^{b} ±0.5	7.1 ^{a} ±0.7	3.3 ^{c} ±0.5	3.4 ^{c} ±0.4	4.2 ^{c} ±0.6	5.1 ^{b} ±0.4
PPL+OPPo	2.1 ±0.3	2.5 ±0.3	1.8 ±0.4	3.1 ±0.2	2.3 ±0.4	2.3 ±0.4	2.9 ±0.5	1.9 ±0.3	2.1 ±0.4	2.2 ±0.5	2.0 ±0.3
POS	2.2 ^{a} ±0.4	2.1 ^{a} ±0.3	1.6 ^{b} ±0.3	2.6 ^{a} ±0.3	2.0 ^{a} ±0.2	2.9 ^{a} ±0.5	2.3 ^{a} ±0.6	2.3 ^{a} ±0.4	2.7 ^{a} ±0.5	2.3 ^{a} ±0.3	2.2 ^{a} ±0.4
POO	32.8 ^{ab} ±2.3	34.7 ^{a} ±2.2	33.6 ^{ab} ±2.1	32.3 ^{ab} ±1.8	26.9 ^{b} ±1.9	29.8 ^{b} ±3.2	32.4 ^{ab} ±2.6	30.6 ^{ab} ±2.9	30.8 ^{ab} ±3.5	32.2 ^{ab} ±2.71	32.6 ^{ab} ±2.3
POL + OOPo	6.5 ^{bc} ±0.9	4.8 ^{c} ±0.5	5.3 ^{c} ±0.8	7.9 ^{ab} ±0.8	8.9 ^{a} ±0.7	6.2 ^{c} ±0.8	9.5 ^{a} ±0.6	4.3 ^{c} ±0.9	5.6 ^{c} ±0.5	5.2 ^{c} ±0.4	5.2 ^{c} ±0.9
PLL + PoOL	0.6 ^{b} ±0.2	0.5 ^{b} ±0.3	0.4 ^{b} ±0.1	0.3 ^{b} ±0.1	0.5 ^{b} ±0.2	0.3 ^{b} ±0.1	0.9 ^{a} ±0.2	0.4 ^{b} ±0.1	0.5 ^{b} ±0.2	0.3 ^{b} ±0.1	0.3 ^{b} ±0.1
SSO	0.2 ±0.0	0.4 ±0.1	0.3 ±0.1	0.3 ±0.1	0.3 ±0.1	0.4 ±0.1	0.5 ±0.2	0.3 ±0.1	0.4 ±0.1	0.4 ±0.1	0.3 ±0.1
SOO	4.9 ^{b} ±0.6	4.4 ^{b} ±0.7	2.8 ^{c} ±0.9	5.4 ^{b} ±0.8	4.2 ^{b} ±0.8	4.7 ^{b} ±0.6	4.3 ^{b} ±0.8	4.9 ^{b} ±1.1	6.0 ^{a} ±0.6	5.2 ^{b} ±0.7	4.9 ^{b} ±0.8
OOO	40.3 ^{ab} ±1.8	42.2 ^{a} ±2.0	41.4 ^{a} ±3.1	39.0 ^{ab} ±2.2	35.0 ^{c} ±2.9	36.3 ^{c} ±3.4	39.4 ^{ab} ±3.1	39.0 ^{ab} ±2.7	37.5 ^{bc} ±2.7	42.4 ^{a} ±3.1	39.3 ^{ab} ±2.8
OOL	11.6 ^{b} ±1.7	11.8 ^{b} ±2.0	10.3 ^{b} ±1.8	13.8 ^{b} ±1.9	12.3 ^{b} ±2.3	11.7 ^{b} ±2.5	15.7 ^{a} ±2.0	10.0 ^{b} ±1.9	12.8 ^{b} ±2.3	11.7 ^{b} ±2.1	10.7 ^{b} ±1.8
OLL	0.2 ±0.0	0.2 ±0.0	0.1 ±0.0	0.2 ±0.0	0.6 ±0.2	0.2 ±0.0	0.4 ±0.1	0.2 ±0.0	0.1 ±0.0	0.2 ±0.1	0.2 ±0.1
LLL	0.6 ^{a} ±0.2	0.7 ^{a} ±0.2	0.4 ^{ab} ±0.2	0.6 ^{a} ±0.1	0.5 ^{a} ±0.2	0.5 ^{a} ±0.2	0.5 ^{a} ±0.2	0.3 ^{b} ±0.1	0.5 ^{a} ±0.2	0.5 ^{a} ±0.2	0.4 ^{a} ±0.1
AOO	0.4 ±0.1	0.4 ±0.1	0.3 ±0.1	0.5 ±0.2	0.4 ±0.1	0.5 ±0.2	0.3 ±0.1	0.3 ±0.1	0.4 ±0.2	0.3 ±0.1	0.3 ±0.1

Table 18:Total acilglycerols (g of fatty acid methyl ester 100 g-1 of oil)

Results express as g of fatty acid methyl ester 100 g^{-1} of oil.

Different letters in the same raw indicate significantly different values (p <0.001). ^{z}Calculated as Σ [% monounsaturated + (diunsaturated x 10) + (triunsaturated x 20)] /100. Triacylglycerols molecular species abbreviations*:* 1,2-dipalmitoyl-3-oleylglycerol (PPO); 1,2-dipalmitoyl-3-linoleylglycerol (PPL); 1-oleyl-2-palmitoyl-3-palmitoleylglycerol (OPPo); 1-stearoyl-2-palmitoyl-3-oleylglycerol (POS); 2,3-dioleyl-1-palmitoylglycerol (POO); palmitoyl-2-oleyl-3-linoleylglycerol (POL); 1,2-dioleyl-3-palmitoleylglycerol (OOPo); 1-palmitoyl-2,3-dilinoleylglycerol (PLL); 1-palmitoleyl-2-oleyl-3-linoleylglycerol (PoOL); 1,3-distearoyl-2-oleylglycerol (SSO); 1-stearoyl-2,3-dioleylglycerol (SOO); 1,2,3-trioleylglycerol (OOO); 1-oleyl-2,3-dilinoleyglycerol (OLL); 1,2-dioleyl-3-linoleyglycerol (OOL); 1,2,3-trilinoleylglycerol (LLL); 1-arachidil-2,3-dioleylglycerol (AOO).

Although triacylglycerols (TAGs) have been widely utilized as markers of varietal and geographical origin of EVOO (Bajoub et al., 2016) (Ruiz-Samblás et al., 2012), the investigation of TAG profile in Italian MEVOO is still limited (Blasi et al., 2019), (Vichi et al., 2007) (Giuffrè, 2013) (Cerretani et al., 2006).. Significant variations in terms of TAG composition were found across the samples. In all the oils, the most abundant TAG was OOO, followed by POO and OOL. They made up to 80-90% of the total TAG profile. The remaining part of TAG matter was mainly formed of PPO, POL and SOO species, whose sum accounted for about 10-15% of TAG profile. Considering the most abundant TAGs, OOO ranged from 35.0±2.9 to 42.4±3.1 %, POO from 26.9±1.9 to 34.7±2.2 %, OOL from 10.0±1.9 to 13.8±1.9 %. These results are comparable to those reported for some Italian monovarietal olive oils, including Coratina, Leccino and Pendolino (Giuffrè, 2013) (Cerretani et al., 2006). The POO and OOL levels weakly changed across the cultivars. Coratina oil presented the significant highest POO level (34.7±2.2 %), Marzio and Maurino the lowest one (26.9 ±1.9 and 29.8 ±3.2 % respectively), the remaining oils had comparable POO amount accounting for about 30.6 – 32.8 %. Similarly, all the samples presented comparable OOL content (from 10.0 to 13.8%), except than Moraiolo oil, having significantly higher level (15.7 ±2.0 %) than all the other samples. Unlike POO and OOL, OOO level strongly varied among the oils. Although Marzio, Maurino and Pendolino showed similar OOO amount, only Marzio and Maurino differed from all the other samples. They presented significantly ($p<0.001$) lowest OOO level. Conversely, Pendolino was not different form all the other samples, except than Coratina and Leccino, that presented the highest OOO levels. Our results are in good agreement with those of (Giuffrè, 2013) who found higher OOO level in Coratina than in Pendolino oils. Congruently to what observed for oleic acid content, the OOO level enables the discrimination of Marzio and Maurino oils from all the others. Anyway, based on OOO levels it was not possible to differentiate Pendolino from the other oils. These outcomes lead us to suppose that the investigation of TAG prolife provides more restrictive information on oil discrimination than that deriving from analysis of total fatty acid profile. In fact, variation on TAG profile among MEVOOs could better reflect the specific metabolic behavior of each cultivar. The biosynthesis of TAG in the olive fruit involves additional pathways with respect to the biosynthesis of fatty acids. This assumption can be also reinforced by considering the variation of TAG species formed by the combination of oleic acid and the most abundant saturated fatty acids, such as palmitic and stearic acids. Although the stearic acid clustered the oils into two groups (Carboncella, Coratina, Marzio, Moraiolo, Rosciola vs the other MEVOOs), the level of the main molecular species containing stearic, SOO and POS, enabled the discrimination of Frantoio oil from all the others, since Frantoio oil showed the lowest SOO and POS amounts. Similarly, although the highest level of palmitic and the

lowest level of oleic acids distinguished Marzio, Maurino and Pendolino oils, the highest PPO level differentiated Marzio and Moraiolo from the rest of the oils.

3.3.5. OXIDATIVE STABILITY WITH RANCIMAT APPARATUS

Figure 60 reports the oxidative stability with Rancimat.

The induction time of the oils ranged from 17.5 (Leccino) to 29.5 (Coratina) hours. Coratina and Rosciola showed the significantly highest stability.

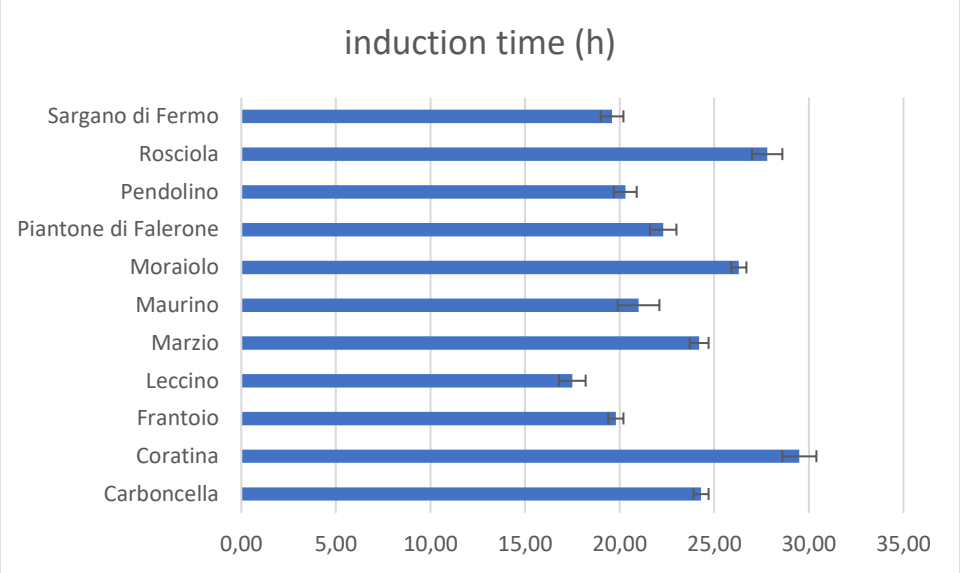


Figure 60: Induction time (h) in 11 MEVOO

3.3.6. TOTAL PHENOLS

The *Figure 61* show the total phenols in 11 MEVOO:

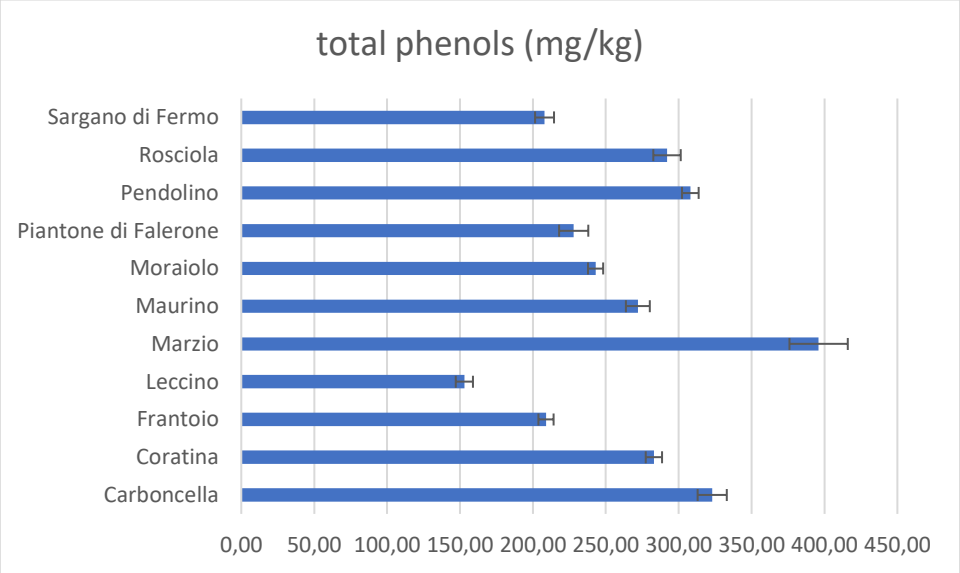


Figure 61: total phenols (mg acid gallic/kg oil)

The total amount of phenols determined by Folin-Ciocalteu assay ranged from 153 to 396 mg kg-1. Marzio oil showed the significantly highest content of phenols (396 mg kg-1) followed by Carboncella (323.1 mg kg-1) and Pendolino (307.6 mg kg-1). Leccino showed the significantly lowest amount of phenols (153 mg kg-1), while Rosciola, Coratina and Maurino oils showed comparable phenolic content. The total phenol content values agree with the one reported by other studies conducted on MEVOOs. (Baiano et al., 2009) reported values between 133 and 322 mg kg-1 for olive orchards located in the north of Apulia region, (Negro et al., 2019) presented values between 138 and 278 mg kg-1 for oils produced in the Province of Lecce (Apulia, Italy), whereas (Ninfali et al., 2001) reported values in the range of 50-236 mg kg-1 for plants cultivated in the center of Italy.(Ricciutelli et al., 2017) indicated values ranging from 136 to 437 mg kg-1 for commercial EVOOs. (Klikarová et al., 2020) showed an average total phenolic content around of 350 mg kg-1 for the Italian cultivars they analysed (including Frantoio, Coratina, Leccino and Moraiolo varieties). However, it is to be reminded that the total phenol content is strictly related to many factors, such as the olive harvesting time, oil extraction techniques or quantification methodologies (Olmo-García et al., 2019). Many studies, indeed, showed that the pedoclimatic and technological aspects are the main parameters influencing the total phenol content in EVOOs (Klikarová et al., 2020) (Di Vaio et al., 2013) (Ripa et al., 2008). The genotype may also highly influence the oil phenolic content. (Negro et al., 2019) indicated that genotype may be responsible for about the 50%. The phenolic content is usually related to the shelf-life and the oxidative stability of olive oil, although polyphenols are also responsible for the olive oil flavor related to bitterness, astringency and pungency. Bitterness in olive oil is strictly due to the content of oleuropein glucoside and its aglycon (Aparicio & Luna, 2002). Oils obtained from olive fruits rich in polyphenols, for example Marzio MEVOO, are expected to be more bitter and pungent than the others.

3.3.7. PHENOLIC PROFILE

The *Table 19* show the phenolic profile of 11 MEVOO:

Phenolic compounds (mg/kg oil)											
3,4-DHPEA	15.1 ^{b} ±0.8	10.5 ^{c} ±0.5	3.3 ^{fg} ±0.1	18.5 ^{a} ±1.4	6.1 ^{e} ±0.1	4.9 ^{ef} ±0.2	4.7 ^{ef} ±0.1	8.2 ^{d} ±0.3	1.8 ^{g} ±0.1	15.7 ^{b} ±0.9	4.5 ^{ef} ±0.1
p-HPEA	3.1 ^{ef} ±0.1	8.2 ^{c} ±0.3	4.4 ^{def} ±0.6	13.6 ^{b} ±1.3	6.0 ^{e} ±0.5	2.8 ^{ef} ±0.2	2.2 ^{f} ±0.3	5.1 ^{de} ±0.3	4.3 ^{def} ±0.5	16.8 ^{a} ±1.1	3.5 ^{def} ±0.5
Vanillic acid	0.29 ^{c} ±0.3	0.52 ^{c} ±0.1	1.1 ^{a} ±0.1	0.92 ^{b} ±0.1	0.82 ^{b} ±0.1	1.34 ^{a} ±0.2	nd	nd	0.49 ^{c} ±0.1	0.63 ^{bc} ±0.1	0.2 ^{c} ±0.01
Vanillin	0.56 ^{d} ±0.1	1.48 ^{b} ±0.2	1.3 ^{b} ±0.2	3.2 ^{a} ±0.2	1.32 ^{b} ±0.2	0.81 ^{c} ±0.1	0.92 ^{c} ±0.1	1.63 ^{b} ±0.3	0.95 ^{c} ±0.2	1.23 ^{b} ±0.2	0.50 ^{d} ±0.1
3,4-DHPEA-EDA	86.4 ^{c} ±7.6	103 ^{b} ±8.2	30.9 ^{g} ±2.8	21.0 ^{h} ±3.1	72.9 ^{d} ±6.2	37.2 ^{ef} ±2.1	80.1 ^{cd} ±8.6	74.4 ^{d} ±4.9	50.8 ^{e} ±3.1	120 ^{a} ±9.6	41.9 ^{ef} ±3.9
p-HPEA-EDA	67.3 ^{cd} ±5.5	64.4 ^{cd} ±3.5	31.2 ^{e} ±1.8	16.7 ^{f} ±2.3	56.8 ^{d} ±4.8	24.8 ^{ef} ±2.8	82.3 ^{b} ±6.8	71.8 ^{bc} ±5.8	32.6 ^{e} ±1.4	105 ^{a} ±6.9	35.5 ^{e} ±3.8
3,4-DHPEA-EA	53.5 ^{cd} ±3.6	32.4 ^{f} ±2.4	23.2 ^{g} ±3.6	3.8 ^{h} ±0.3	106 ^{a} ±9.2	57.1 ^{c} ±3.9	20.5 ^{g} ±2.3	43.2 ^{e} ±4.1	72.5 ^{b} ±2.8	46.6 ^{de} ±2.9	22.1 ^{g} ±1.6
p-HPEA-EA	16.0 ^{b} ±0.1	11.6 ^{bc} ±1.8	14.7 ^{b} ±0.7	2.9 ^{d} ±1.2	23.3 ^{a} ±2	11.5 ^{bc} ±1.2	5.5 ^{d} ±0.9	16.4 ^{b} ±2.1	11.6 ^{bc} ±1.6	14.7 ^{b} ±0.5	7.1 ^{cd} ±1.1
Luteolin	8.3 ^{a} ±1.1	5.45 ^{c} ±0.5	6.9 ^{b} ±2.3	nd	5.75 ^{c} ±0.8	nd	5.45 ^{c} ±1.1	2.65 ^{d} ±0.3	4.25 ^{c} ±0.6	nd	6.24 ^{b} ±0.5
Apigenin	2.65 ^{a} ±0.1	2.05 ^{b} ±0.3	1.9 ^{b} ±0.4	1.76 ^{b} ±0.2	2.03 ^{b} ±0.2	1.85 ^{b} ±0.3	1.87 ^{b} ±0.2	0.95 ^{c} ±0.2	0.84 ^{c} ±0.1	3.00 ^{a} ±0.4	2.08 ^{b} ±0.1

Table 19: phenolic profile of 11 MEVOO

Polyphenols abbreviation: 3,4-DHPEA: 3,4-dihydroxyphenylethanol; *p*-HPEA: *p*-hydroxyphenylethanol; 3,4-DHPEA-EDA: dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA; *p*-HPEA-EDA: dialdehydic form of decarboxymethyl elenolic acid linked to *p*-HPEA; *p*-HPEA-EDA-Ox.: *p*-HPEA-EDA oxidized; 3,4-DHPEA-EA: oleuropein aglycon; *p*-HPEA-EA: ligstroside aglycon. Different letters in the same raw indicate significantly different values (p <0.001).

The EU legislation about the health claim on olive oil polyphenols requires accurate measurements of the level of specific phenolic compounds in olive oil. In this work, twelve phenolic compounds were also specifically identified and quantified using HPLC coupled to DAD and mass spectrometry instrument. 3,4-DHPEA and p-HPEA were the main phenolic alcohols found in the MEVOOs. Their concentration is usually low in the fresh oils, but increases during storage (Boselli et al., 2009) (Fiorini et al., 2018) (M. Servili et al., 1997) due to the lysis of the secoiridoids, such as the dialdehydic forms of decarboxymethyl elenolic acid linked to 3,4-DHPEA (3,4-DHPEA-EDA) and to p-HPEA (p-HPEA-EDA), and oleuropein aglycon (3,4-DHPEA-EA) that release 3,4-DHPEA and p-HPEA, respectively. The content of 3,4-DHPEA ranged between 1.8 to 18.5 mg kg-1 for Pendolino and Leccino respectively, while p-HPEA ranged between 2.2 to 16.8 mg kg-1 for Moraiolo and Rosciola. Other studies conducted on Leccino MEVOOs reported values of 13.8 mg kg-1 (Ragusa et al., 2017) and 0.72-1.37 mg kg-1 (Gambacorta et al., 2012) for 3,4-DHPEA content and 20.2 mg kg-1 (Ragusa et al., 2017) and from 1.08 to 2.22 mg kg-1 for p-HPEA, indicating a certain variability for these phenolics. (Ricciutelli et al., 2017) have identified a mean value of 9.9 mg/kg oil and 13.4 mg/kg oil for 3,4-DHPEA and p-HPEA, respectively, in commercial oils EVOOs. Among phenolic alcohols, 3,4-DHPEA is worthy of investigation for its nutraceutical properties (Robles-Almazan et al., 2018), so the cultivars that showed the highest contents (mainly Leccino, Rosciola and Carboncella) are worthy of interest to obtain EVOO blends with increased nutraceutical properties. Then 3,4-DHPEA has been indicated by (Carrasco-Pancorbo et al., 2005) as the main contributor among polyphenolic compounds for oxidative stability of olive oils. Vanillic acid and vanillin were found at very low concentrations in all samples (0.2-1.34 mg kg-1 and 0.50-3.2 mg kg-1 of oil respectively), with small differences among studied oils. Only in Moraiolo and Piantone di Falerone vanillic acid was not detected. These values are in accordance with the ones found by (Gambacorta et al., 2012) in MEVOOs investigated (including Coratina, Frantoio and Leccino varieties) and also (Ricciutelli et al., 2017) indicated an average value for vanillic acid of 0.3 mg kg-1 for commercial EVOOs. The secoiridoid compounds are in general the most abundant phenolic compounds present in fresh oils but during the shelf life their content decreases (Boselli et al., 2009). Rosciola showed the highest content for 3,4-DHPEA-EDA and p-HPEA-EDA (120 and 105 mg kg-1, respectively). Other varieties such as Carboncella, Coratina, Moraiolo and Piantone di Falerone showed a good content of both the phenols compared to the other ones. Coratina, one of variety appreciated for the high phenols content, showed 103 and 64.4 mg kg-1 of 3,4-DHPEA-EDA and p-HPEA-EDA, respectively. Frantoio, Marzio, Maurino, Pendolino and Sargano di Fermo cultivars showed an average content of these two compounds compared to Coratina, while Leccino presented the lowest content (21 and 16.7 mg kg-1 for 3,4-DHPEA-EDA and p-HPEA-EDA, respectively). p-HPEA-EDA deserves great attention

because of its several nutraceutical properties reported by many studies and reviews (Francisco et al., 2019) (Pang & Chin, 2018) . It showed wide concentration ranges in olive oils. (Bakhouche et al., 2013) reported values from 3.3 to 4.6 mg kg-1 for Spanish oils, (Fuentes et al., 2018) from 25 to 77 mg kg-1 for Chilean oils, (Negro et al., 2019) indicated values from 4.3 to 103.4 mg kg-1 for Apulian varieties. Considering the important role of p-HPEA-EDA in the nutraceutical properties of EVOO, mainly Rosciola and Moraiolo genotypes represent an excellent source. In all varieties, the dialdehydic form of ligstroside aglycon (DLA) was coeluted with the oxidised form of p-HPEA-EDA and the highest content was found in Marzio (147 mg kg-1) followed by Pendolino and Sargano di Fermo (126 and 101 mg kg-1, respectively), while Leccino and Coratina showed lower content (49.3 and 51.2 mg kg-1, respectively). The last two secoiridoids in terms of elution time were 3,4-DHPEA-EA and p-HPEA-EA found in all varieties. 3,4-DHPEA-EA was higher in Marzio (106 mg kg-1), followed by Pendolino (72.5 mg kg-1), Maurino (57.1 mg kg-1) and Carboncella (53.5 mg kg-1), while Leccino variety showed the lower content (3.8 mg kg-1). For some varieties (Negro et al., 2019) reported higher values, in the range of 33.8-152.3 mg kg-1, while similar values were reported in the oils analysed by (Ragusa et al., 2017). Normally 3,4-DHPEA-EA tends to decrease from drupes to malaxation paste and to the final oil (Negro et al., 2019). p-HPEA-EA was the last secoiridoid quantified, its content ranged between 2.9 mg kg-1 and 23.3 mg kg-1, for Leccino and Marzio, respectively. In this case p-HPEA-EA content is similar to the values reported by (Negro et al., 2019) and slightly lower than the ones reported by (Ragusa et al., 2017). The flavonoids that usually can be found in EVOO extracts are luteolin, apigenin and sometimes methoxyluteolin. This class of compounds is known to have many beneficial biological effects including anti-inflammatory, antioxidant and estrogenic activity (García-Martínez et al., 2016). Methoxyluteolin was found in traces only in Moraiolo variety, while luteolin ranged between 2.65 and 8.3 mg kg-1 for Piantone di Falerone and Carboncella, respectively. Similar values were found by (García-Martínez et al., 2016) in Spanish EVOOs (1.66-6.21 mg kg-1) and by (Tuberoso et al., 2016) in varieties from Sardinia region (Italy) (0.2-7.1 mg kg-1). Luteolin was not detected in Leccino and Maurino varieties. Finally, apigenin was found with an average content of about 2 mg kg-1, in accordance with the values reported for EVOOs in several other studies (Fiorini et al., 2018) (García-Martínez et al., 2016) (Tuberoso et al., 2016). Six varieties (Marzio, Carboncella, Pendolino, Rosciola, Coratina, and Maurino) out of the 11 investigated complied with the content required to acknowledge the health claim (250 mg kg-1) (Reg. 432/2012 EU).

3.3.8. SENSORY PROPERTIES OF OILS AND RELATIONSHIP BETWEEN SENSORY SENSATIONS AND CHEMICAL COMPOSITION

The biplot from PCA illustrates the mutual relationships between samples and discriminating chemical and sensory variables.

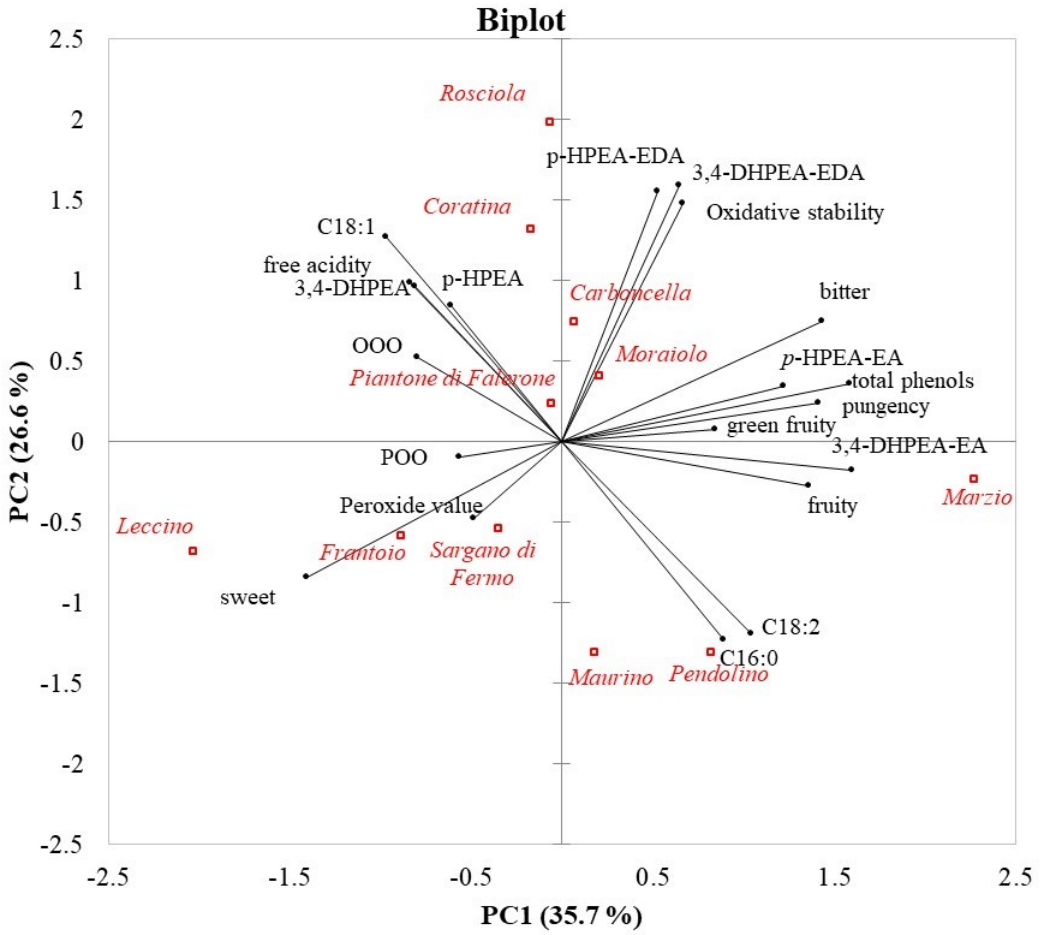


Figure 62: Bi-plot from principal component analysis (PCA) reporting principal components 1 and 2 (PC1 and PC2 respectively) with the loadings of selected chemical and sensory variables and the scores (oil samples).

Legend: 3,4-DHPEA: 3,4-dihydroxyphenylethanol; *p*-HPEA: *p*-hydroxyphenylethanol; 3,4-DHPEA-EDA: dialdehydic form of decarboxymethyl elenolic acid linked to 3,4-DHPEA; *p*-HPEA-EDA: dialdehydic form of decarboxymethyl elenolic acid linked to *p*-HPEA; 3,4-DHPEA-EA: oleuropein aglycon; *p*-HPEA-EA: ligstroside aglycon; C16:0: palmitic acid; C18:1: oleic acid; C18:2: linoleic acid; total phenols (determined by Folin Ciocalteu method); OOO: 1,2,3-trioleylglycerol; POO: 2,3-dioleyl-1-palmitoylglycerol.

The first two components in the PCA accounted for 62.3 % of total variance, with the first component (PC1) explaining 35.7 %. Samples were distributed on the PC1 according to a contraposition between bitter, greenly fruity and spicy (positively correlated on PC1) and sweet (negatively correlated on PC1). Along PC1, bitter, greenly fruity and pungency showed a high correlation with total phenols compounds, p-HPEA-EA and with the amount of 3,4-DHPEA-EA. This is in agreement with previous studies clearly showing that 3,4-DHPEA-EA is crucial for the perception of bitter and pungency in

EVOOs (Bendini et al., 2007). The positive correlation observed on bi-plot between oxidative stability and total phenols has been previously documented (Boselli et al., 2009). Instead, sweet was positively correlated with peroxide index and POO; this finding maybe explained by the lower phenols content that protect from the oxidation phenomena and that contribute to pungency/bitterness, attributes lacking in sweet oils. As the correlation on PC2 positively increased, both the oxidative stability and the amount of 3,4-DHPEA-EDA and p-HPEA-EDA increased. Acidity, amount of 3,4-DHPEA, amount of p-HPEA and oleic acid (C18:1) % had high positive loadings on PC2. The content of p-HPEA and 3,4-DHPEA, that is known to increase with oil aging, strongly characterized Coratina; these phenols correlated with FA, that in fact also derives from hydrolytic processes. On PC2, palmitic acid (C16:0) and linoleic acid (C18:2) had negative loadings as also PV and POO (despite lower loadings).

3.4. CONCLUSIONS

In the present work, a chemical and sensory characterization was conducted on eleven MEVOOs from olives grown in the same experimental olive orchard, with the same conditions (fertilization, irrigation), and processed with the same technology. Differences found across MEVOOs were attributable only to the factors related to the genetic background of the olive cultivar. The findings highlighted the impact of genetic background of the olive on fatty acid, TAG and phenolic compositions of the oils. Across the investigated oils, Marzio stood out from the rest resulting the significantly most bitter, pungent, fruity and the richest in phenolic compounds. The high phenolic level conferred it a good oxidative stability although it presented the highest unsaturation index.

4. EFFECT OF TECHNOLOGY ON OLIVE OIL QUALITY PARAMETERS

4.1. INTRODUCTION

The concept of quality has continuously evolved over time. Among the various definitions, the UNI EN ISO 8402: 1995 standard defines quality as “the set of characteristics of a product that give it the ability to satisfy the expressed or implicit needs of customers”. A further UNI EN ISO (9000:2000) definition defines quality as the “degree of which a set of intrinsic characteristics satisfy the requirements”, which are the expectations or needs of the customer. The interesting aspect of this definition, compared to the previous one, lies in the desire to make quality “quantifiable”. Consumers orient their choices according to the quality of the “extra virgin olive oil”, which depends on several of its intrinsic factors, as well as consumption habits, which, in turn, are influenced by marketing strategies, advertising and trends.

The present research aims to assess the main chemical-physical and organoleptic differences between a traditional and pitted extra virgin olive oil. In addition, a pitted oil was also compared and then added to kernels, to verify the possible effects of oxidation and modification of the organoleptic and nutritional characteristics of the kernels on the pitted oil compared to the traditional one, as well as to compare the shelf-life and the quality. The analysis was carried out at the time of bottling and after 3,6,12 months of storage, also verifying the effect of storage. In addition to the differences, a further aim is to verify whether the search for innovative extraction methods can lead to improvements in oil quality, as well as in production.

In literature, considering the degree of ripening of the olive tree and other parameters, in a study (Katsoyannos et al., 2015) it was highlighted how the degree of acidity (from -0.01% to -0.09%) and the presence of peroxides tend to decrease in pitted oil (from -0.4 to -1.4 meqO2/kg). The yield in pitted oil, on the other hand, tends to decrease in ripe olive oil (from -4.02% to -5.17%), because in green olive oil the trend is inverted (from +0.14% to +1.03%). In contrast, K232 and K270, signals in the ultraviolet tend to increase in pitted oil (from +0.06% to +0.18% K232, from +0.01% to +0.03% K270). Acidity tends to increase (+0.04%) as the degree of maturation increases, probably because the maturation process makes the fruit more sensitive to mechanical damage and caused by pathogens, with increased activity of enzymes, including lipolytic enzymes. Peroxides (from +0.5% to +1.7%), K232 (from +0.09 to +0.15) and K270 (from +0.01 to +0.04) also increase as maturity increases, due to the increased presence of unsaturated acids. Yield increases as the degree of maturity of the fruit increases (from +3.85% to +10.05%). Green olives have a higher content of polyphenols (from +48.66 to +60.89 mg/kg oil) and tocopherols (from +20.8 to +179.29 mg/kg oil) than ripe olives. It

has also been detected that the content of polyphenols and the degree of maturation influence the organoleptic characteristics and shelf-life of extra virgin olive oil. Pitted oil also has a higher polyphenol (from +22.83 to +76.96 mg/kg oil) and tocopherol content (from +42.73 to +109.2 mg/kg oil) than conventional oil, with positive repercussions on the quality and antioxidant activity of pitted oil. This also leads to better organoleptic qualities and greater shelf life of the latter oil. The reasons have previously been stated: in the pitting process, the stones are removed at the beginning of processing and therefore, the enzymes (lipoxygenases, peroxidases) contained in the seeds do not influence the pulp composition and phenols are not enzymatically degraded thus improving their concentration and oil oxidative stability. The paste preparation technique did not affect the main fatty acid proportions of the variety olive oil. No significant changes were observed in the sums of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids or in their ratios.

These oils were analyzed in the following parameters, at the time of bottling and after three, six, and twelve months:

- Acidity (expressed as oleic acid)
- Peroxides
- Induction time with Rancimat apparatus
- Volatile organic compounds
- α and γ-tocopherols

4.2. MATERIALS AND METHODS

4.2.1. SAMPLING

The olive cultivar was Sargano and come from an organic farm of Fermo. 125 kg of olives were pressed, corresponding to about 9.5 kg of oil and 20 kg of kernels. The system consists of a pitting machine, a two-phase decanter and filtration on cartons. The traditional oil was milled on 20/11/2020. 125 kg of oil was pressed, corresponding to 8 kg of oil. The production system is the "Leopard" of the Pieralisi company of Jesi, consisting of a two-and-a-half-phase kneader and decanter. For the preparation of the samples of pitted oil of 9.5 kg of oil, 5 kg of oil were obtained in 20 bottles of 250 ml labeled, while the remaining 4.5 kg in bag in box. Instead, for the preparation of samples of traditional normal oil, 8 kg of oil were obtained from 5 kg of oil in 20 bottles of 250 ml and the remaining 3 kg in bag-in-box.

The equipment is completed by 1 bag of whole 10 kg kernels and 2 bags of crushed kernels of 10 kg.

From a quantitative point of view, from 1 q of olives 10 liters of continuous oil are obtained, 5 are put in canisters and 5 bottled. From the same amount of olives are obtained 10 liters of pitted oil, of which 5 go in a tank and 5 in a canister in which kernels are added. The first is bottled by the manufacturer in 20 bottles of 0.25 l, the second is bottled in the present thesis work in another 20 bottles of 0.25 ml. The analyzes are done at bottling and after 3,6,12 months. Sample 1 comes from 20 bottles of oil, like 2 and 3. To see the photooxidation, it is necessary that the oils are stored at 25 ° C, a brightness of 500 lux, for 12 hours in 12 months every now and then the bottles open and shake. To see the self-oxidation and therefore the presence of the hint of rancid, it is necessary to verify the presence of trans 2 decenal and trans 2.4 decadenal.

Pitted and pitted oil + kernels was prepared with the following procedure.

500 ml of pitted oil were weighed and put in a pan. In a calibrated aluminum tray are inserted 1 kg of freshly pulled hazelnuts out of the fridge, after which they are inserted into the pan. A mixing should be carried out for 20 minutes before filtration. The aluminum tank and all operations should be repaired by light, as the EVO degrades due to light. A vacuum flask wrapped in aluminum with Buckner without filter should be prepared. Insert into the Buckner a layer of about 1 cm of uniform kernels and strain by putting the vacuum into operation. Before filtration, a plastic lid should be inserted to increase the vacuum. After filtration, the whole core should be transferred to an aluminum-covered glass container. Before completing each filtration operation and when the oil no longer passes through the Buckner it is necessary to mix and make another vacuum, repeating the procedure until the oil filtration speed is almost nothing. After filtration, the core is translated into the aluminum tray, another core is put into the Buckner, and the procedure described above is repeated. All this must be repeated throughout the core, adding about 2 tablespoons of wezel in phase. When in some stages the filtration speed appears too low, try uncorking the Buckner holes with a thin iron made from a staple. The filtration procedure should be continued until the desired level of oil is reached. If this level is not reached, the core is recycled. At the end of the procedure, the oil is leaks into a dark, calibrated bottle and the oil weighs. Labelling is carried out.

The following samples of extra virgin olive oil were analyzed *(Table 20, Figure 63)*, in the bottling phase and after three, six and twelve months:

Number of samples	**Type oil**
1	Traditional EVO
2	Pitted EVO
3	EVO pitted and additioned of kernels

Table 20: EVO oils in experimental part

Figure 63: EVO analyzed

4.2.2. ACIDITY

"KIT" METHOD: the fatty acids of the sample, under pH < 7,0 conditions, react with a chromogen by developing a color whose optical density, measured at 630 nm, is proportional to the concentration of fat acidity, expressed as % of oleic acid. The method used is the AOCS Official Method Ca 5a-40. A Foodlab Junior MLB 242 CDR Kit was used for analysis.

OFFICIAL METHOD (Reg. EC 2568/91): the oil concerned was dissolved in a mixture of 95% ethyl alcohol and ethyl ether, in a proportion of 1:2, and then titrated, until phenolphthalein (ethanol solution 1%) used as an indicator, with a solution of NaOH 0.1 N.

Acidity, expressed as % by mass of oleic acid, is given by:

Acidity = V • c • M/1000 • 100/m = V • c • M / (10 • m)

Where:

V = volume (mL) of the NaOH solution used

c = concentration (moles/L) of the NaOH solution used

M = molar weight of the acid adopted for the expression of the result (oleic acid: PM = 282 g/mol)

m = weight (g) oil

The acid content of edible fats is given by the quantity of free fatty acids deriving from the hydrolytic rancidity of triglycerides. As this alteration occurs in unsuitable conditions for the processing and preservation of fats, acidity represents a basic indicator of the genuineness of the product. The test is particularly important during the refining of oils and fats, for the assessment of the processing cycle and for the definition of product categories.

4.2.3. PEROXIDES

"KIT" METHOD: R-O-O-R peroxides oxidize Fe^{2+} ions. The Fe^{3+} ions resulting from oxidation are grouped and form a red complex. Its colorimetric intensity, measured at 505 nm, is directly proportional to the concentration of peroxides in the sample. Results are expressed as meqO_2/kg. A Foodlab Junior MLB 242 CDR Kit was used for analysis. The method shows a very good correlation with AOCS Official Method Cd 8-53.

OFFICIAL METHOD (Reg. EC/2568/91): The determination was made by titration with sodium thiosulfate solution 0,1 N until the blue-purple color of the starch weld indicator disappeared (Reg. EC/2568/91). The value of the number of peroxides, expressed in milliequivalents of active oxygen per kg, is given by:

number of peroxides = [(V • T) / m] • 1000

Where:

V = volume (mL) of the known sodium thiosulfate solution used in the analysis

T = normality of the sodium thiosulfate solution used

m = mass (g) of the substance to be analyzed

The amount of peroxides of fats indicate the degree of primary oxidation and therefore its likeliness of becoming rancid. A lower number of peroxides indicates a good quality of oil and a good preservation status. Unsaturated free fatty acids react with oxygen and form peroxides, which determine a series of chain reactions that generate the production of smelling volatile substances. Those reactions are accelerated by high temperature and by light and oxygen exposure.

4.2.4. OXIDATION STABILITY BY RANCIMAT

The oxidative stability of the oils was determined by Rancimat apparatus (Metrohm model 679, Herisau, Switzerland), measuring the induction time in response to force oxidation (induction period) of 5 g sample heated at 110°C under an air flow of 20 L h-1 (Di Lecce et al., 2020). The induction period (expressed in hours) was determined by drawing the two tangents of the time–conductivity curve and projecting the intersection onto the time-axis. The Rancimat method is an accelerated aging test. Air is passing through the sample in the reaction vessel at constant elevated temperature. In this process fatty acids are oxidized. At the end of the test volatile, secondary reaction products are formed, which are transported into the measuring vessel by the air stream and absorbed in the measuring solution (deionized water). The continuously recorded electrical conductivity of the measuring solution is increasing due to the absorption of the reaction products. Thus, their appearance can be detected. The time until secondary reaction products are detected is called induction time. It characterizes the oxidation stability of oils and fats. The tests were conducted with the Metrohm 679

Rancimat. The measuring vessel is filled with 60 mL deionized water and placed on the Rancimat together with the measuring vessel cover (Manual Metrohm 679 Rancimat, Laubli et al., 1986). It must be ensured that the electrode is immersed into the measuring solution at any time. Then sample is weighed directly into the reaction vessel. For liquid samples and for samples that melt at elevated temperatures a sample size of 5.0 g is used. The reaction vessel is closed with a reaction vessel cover assembled with an air inlet tube. Before the determination can be started, the temperature of the heating block must be stable. The two tubings between Rancimat and reaction vessel and between reaction vessel and measuring vessel are connected. Then the reaction vessel is placed in the heating block and the measurement is started immediately. The measuring temperature depends on the oxidation stability of the sample. For the sample types described in this document, usually temperatures between 80 and 160 °C are appropriate. 50 to 220 °C are possible.

4.2.5. TOCOPHEROLS

A sample of olive oil is loaded on a UPLC Acquity H-Class system (Waters Corporation, Milford, MA, USA) (Orlando et al., 2020) equipped with a fluorometric detector (FLD) and Ascentis Express HILIC (15 cm × 2.1 mm i.d., particle size 2.7 μm, Merck, Darmstadt, Germany) column set up at 30 ◦C. An isocratic elution (8 min) of n-hexane (95.5%), isopropanol (0.4%) and acetic acid (0.1%) at 0.3 mL/min was performed. FLD was set with an excitation and emission wavelength of 290 and 330 nm, respectively. Tocopherols were identified by comparison of retention time with pure standards and quantified with external calibration. For the quantification, seven standard stock solutions of each tocopherol (α-T, γ-T, δ-T) in isopropanol were prepared in the range 3.5–100 μg/mL and analyzed to obtain the calibration curve (R2 = 0.9836–0.9965).

4.2.6. VOLATILE ORGANIC COMPOUNDS

The determination of volatile organic compounds is of great importance to verify the organoleptic and sensory characteristics of monovarietal EVOs, as the presence of some of them determines their taste and smell. However, it is the coexistence of several compounds that gives smells and flavors. The *Table 21* shows the relationship between some volatile organic compounds and their smell/taste. These 18 substances have been defined as standards for the analysis of VOCs in EVO.

VOC	FLAVOR
Octane	alkane, sweet
Ethyl acetate	pineapple, ethereal, sweet, aromatic, sticky, fruity
Ethanol	alcoholic, apple, sweet, floral
Ethyl propanoate	strawberry, fruity, sweet, apple, strong
Hexanal	green, fruit, cut grass, apple, leaf, sweet, at low conc; tallow, fat, lawn, sebaceous, oil at high conc
3-methyl-1-butanol	sweet, herbal, green, malty, whiskey, burnt, woody, yeast
(E)-2-hexenal	bitter almond, green, cut grass, leaf, fruity, apple, sharp, sweet, astringent, bitter, fat

VOC	FLAVOR
(Z)-3-hexenyl acetate	banana, fruity, green, floral, leaves, olives, sweet
(E)-2-heptenal	soap, fat, bitter almond, green, greasy, pungent, oxidized, wood, tallow, grass
6-methyl-5 hepten-2-one	mushroom, rubber, pepper, green, grass, pungent, banana, fruity, herb
1-hexanol	resin, floral, green, grass, fruit, aromatic, banana, alcoholic, rough, astringent, soft, sweet, tomato
Nonanal	fat, citrus, grass, green, rancid, wax, pungent, soap, tallow
(E,E)-2,4-hexadienal	green, floral, cut grass, fresh, fat, solvent, citric
1-octen-3-ol	earth, mushrooms, mold
Acetic acid	sour, vinegary, pungent
Propanoic acid	pungent, aromatic, rancid, soy, sour, sweat, fruity, mold
(E)-2-decenal	tallow, fat, soapy, paint, fish, green, wax, earthy, orange
Pentanoic acid	putrid, pungent, sweat, rancid, old, sharp, fruity

Table 21: relationship between VOC and flavor (from Cecchi, Migliorini, Mulinacci 2021)

The volatile organic compounds present in the oil were examined by the following method: (Casadei et al., 2021).

Preparation of the internal standard solution: refined olive oil (15 g) was weighed in a vial, and 0.1 g of 4-methyl- 2-pentanol (internal standard, IS) was added and more refined olive oil was added to reach 20 g (IS approximate concentration of 5000 mg/kg). Exact weights (balance precision of 0.001 g in all measurements) were noted for calculation of concentration. This was considered the stock standard solution of the internal standard. Next, refined olive oil (5 g) was weighed in a vial and 0.1 g of the above-mentioned stock standard solution was added. Finally, refined olive oil was added to reach 10 g (approximate concentration of 50 mg/kg). Exact weights were noted for calculation of concentration. In all the described steps, a rapid preparation was highly advisable to avoid evaporation of IS and reduce errors.

Sample preparation and extraction of volatiles: working at controlled room temperature (20–25 °C) due to the high volatility of the standard, 1.9 g of sample was weighed in a 20 mL glass vial and 0.1 g of 4-methyl-2-pentanol standard solution was added as IS (approximate concentration 2.5 mg/kg, although exact concentrations were considered in all calculations). Next, the vial was hermetically closed with a polytetrafluoroethylene septum. The sample was left for 10 min at 40 °C under agitation (250 rpm) to allow for equilibration of the VOCs in the headspace. After that, the septum covering each vial was pierced with a solid phase microextraction (SPME) needle and the fiber was exposed to the headspace for 40 min at 40 °C. The SPME fiber (length 1 cm, 50/ 30 μm film thickness) was endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Merck KGaA, Darmstadt, Germany). The fiber was previously conditioned by following the instructions of the supplier. After exposition to the sample headspace, the fiber was then inserted into the injector port of the GC. The volatiles adsorbed by the fiber were thermally desorbed in the hot injection port of GC instruments Varian 430 GC for 5 min at 250 °C with the purge valve off (split less mode) and transferred to a capillary column (polar phase based on polyethylene glycol of a gas chromatograph equipped with an FID. The carrier gas was helium or hydrogen at a flow rate of 1.5 mL/min. The oven temperature was held at 40°C for 10 min and then

programmed to rise by 3 °C/min to a final temperature of 200 °C. A cleaning step was added by all participants (20 °C/min to 250 °C for 5 min) to ensure that the column was ready for the next analysis. The temperature of the FID was set at 260°C.

Method developed in our studies: injection and analysis of individual standards for training in the identification of the 18 volatile compounds for SPME-GC-FID.

For each one of the 18 standards, there are 18 VOC in a 2 ml vial (18 vials in total) containing a dilution of each standard in refined olive oil (stock mixture), at an approximate concentration of 200 mg/kg. Those matrices can be optionally used to train and ensure a correct identification of the 18 selected volatile compounds. You exactly weight 1.650 ± 0.001 g of refined olive oil in a 20 ml glass vial and add 0.250 ± 0.001 g of the stock mixture that was provided in the 2 ml vial. Finally, add 0.100 ± 0.001 g of 4-methyl-2-pentanol standard solution as internal standard (IS approximate concentration = 2.5 mg/kg). Take note of exact weights (± 0.001 g) for calculation of the concentration of each standard, which shall be 25 mg/kg approx. Close hermetically the vial with polytetrafluoroethylene septum (PTFE). Shake the vial manually, but very gently and softly (never spread the oil through the vial walls or the septum). Analyze the content of the vial in the same manner than an olive oil sample. When observing the corresponding chromatogram and comparing it with a refined olive oil's one, the peak of each standard (and, in consequence, the retention time of the compound) should be well-identified.

Standards have been identified, including retention time *(Table 22):*

VOC	Time retention	Volatile organic compound
VOC_001	9.30	Octane
VOC_002	12.50	Ethyl acetate
VOC_003	14.90	Ethanol
VOC_004	15.90	Ethyl propanoate
VOC_005	22.90	Hexanal
VOC_006	29.70	3-methyl-1-butanol
VOC_007	30.70	(E)-2-hexenal
VOC_008	35.40	(Z)-3-hexenyl acetate
VOC_009	36.00	(E)-2-heptenal
VOC_0010	36.50	6-methyl-5 hepten-2-one
VOC_0011	36.9	1-hexanol
VOC_0012	39,00	Nonanal
VOC_0013	40,00	(E,E)-2,4-hexadienal

VOC	Time retention	Volatile organic compound
VOC_0014	41,20	1-octen-3-ol
VOC_0015	41,40	Acetic acid
VOC_0016	45,10	Propanoic acid
VOC_0017	49,7	(E)-2-decenal
VOC_0018	52,90	Pentanoic acid
Internal Standard (IS)	27,50	4-methyl-2-pentanol

Table 22:List of "standards" in VOC

4.3. RESULT AND DISCUSSION

4.3.1. ACIDITY, PEROXIDES, OXIDATIVE STABILITY WITH RANCIMAT

For acidity, peroxides and oxidative stability with Rancimat these are the analytical results *(Table 23, Table 24, Table 25),* the same experimental data are represented in graphs in *Figure 64, Figure 65 and Figure 66:*

Type of oil	Shelf-life	Sample code	Acidity %		
Traditional EVOO	0 month	TRAD 0	0,06	±	0,02
	3 months	TRAD 3	0,12	±	0,01
	6 months	TRAD 6	0,15	±	0,02
	12 months	TRAD 12	0,08	±	0,01
Pitted EVOO	0 month	DEN 0	0,21	±	0,03
	3 months	DEN 3	0,27	±	0,03
	6 months	DEN 6	0,26	±	0,01
	12 months	DEN 12	0,18	±	0,03
Pitted EVO + kernels	0 month	RIN 0	0,50	±	0,02
	3 months	RIN 3	1,10	±	0,00
	6 months	RIN 6	1,00	±	0,14
	12 months	RIN 12	0,91	±	0,09

Table 23: Experimental acidity (%) (mean of three replicates ± standard deviation.) (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels)

Type of oil	Shelf-life	Sample code	Peroxides meq O2/kg
Traditional EVOO	0 Month	TRAD 0	2,53
	3 Months	TRAD 3	6,54
	6 Months	TRAD 6	11,81
	12 months	TRAD 12	15,49
Pitted EVOO	0 Month	DEN 0	1,99
	3 Months	DEN 3	5,18
	6 Months	DEN 6	9,08
	12 months	DEN 12	11,82
Pitted EVOO + kernels	0 Month	RIN 0	2,48
	3 Months	RIN 3	4,72
	6 Months	RIN 6	2,48
	12 months	RIN 12	20,14

Table 24: Experimental peroxides (meq O2/kg oil) in EVO oils (one single replicate) (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels)

Type of oil	Shelf-life	Sample code	Rancimat h		
Traditional EVOO	0 month	TRAD 0	13,0	±	0,2
	3 months	TRAD 3	12,5	±	0,1
	6 months	TRAD 6	10,5	±	0,1
	12 months	TRAD 12	10.4	±	0,2
Pitted EVOO	0 month	DEN 0	15,2	±	0,1
	3 months	DEN 3	14,9	±	0,2
	6 months	DEN 6	12,0	±	0,4
	12 months	DEN 12	13.4	±	0,1
Pitted EVOO + kernels	0 month	RIN 0	7,4	±	0,4
	3 months	RIN 3	8,9	±	0,3
	6 months	RIN 6	5,4	±	0,4
	12 months	RIN 12	6,7	±	0,1

Table 25: Experimental induction time with Rancimat (h) in EVO oils (mean of two replicates ± standard deviation) (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels)

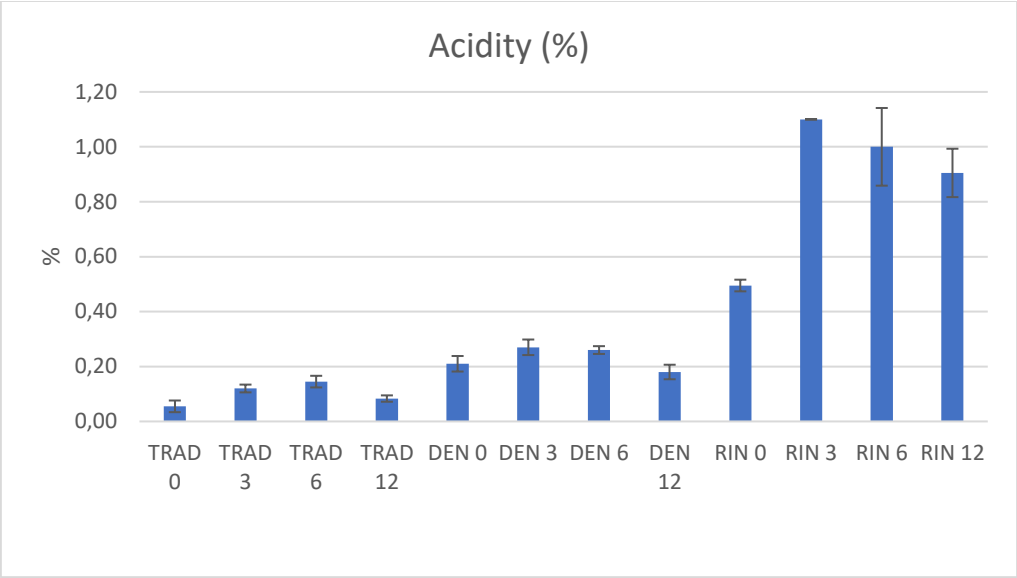


Figure 64: Graphical experimental data of acidity (%) in EVOO (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels) (TRAD 0=traditional oil in bottling time TRAD 3= traditional oil three months after bottling…)

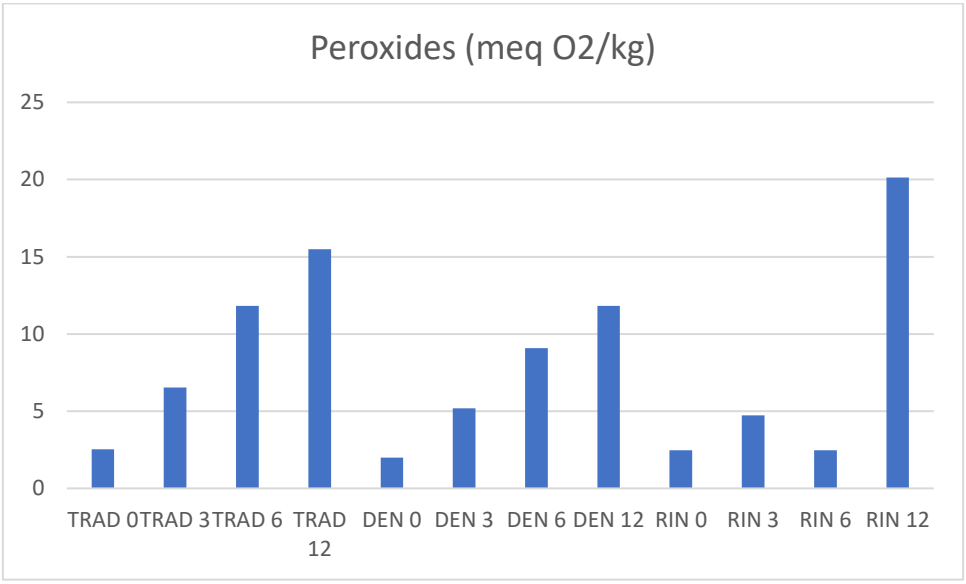


Figure 65: graphical experimental data of peroxides (meq O2/kg oil) in EVO (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels) (TRAD 0=traditional oil in bottling time TRAD 3= traditional oil three months after bottling…)

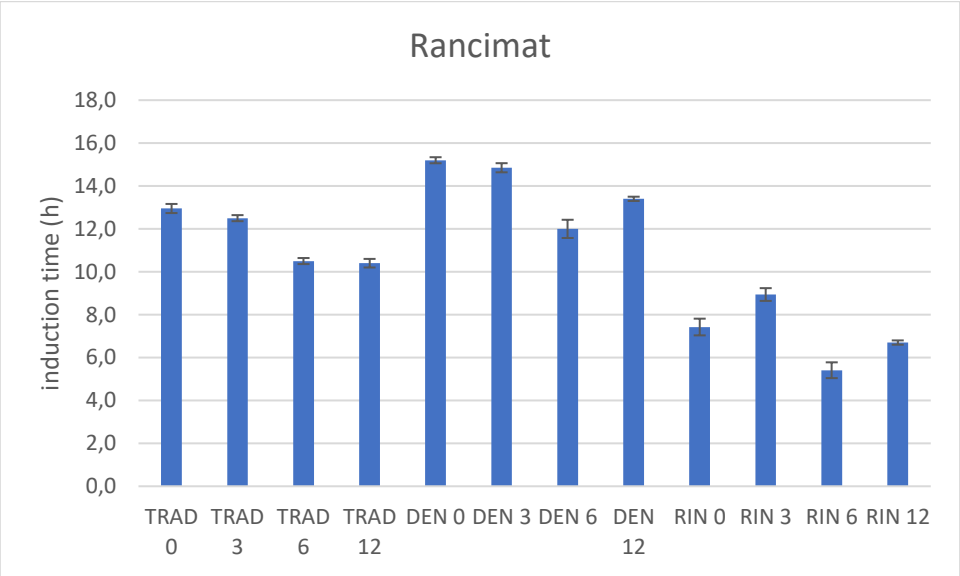


Figure 66: graphical experimental data of induction time with Rancimat (h) in EVOO (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels) (TRAD 0=traditional oil in bottling time TRAD 3= traditional oil three months after bottling…)

From the experimental data of *Table 23* and *Figure 64*, it can be noted that **the acidity** in traditional oil with the passing of the months tends to increase while remaining below the threshold limit, highlighting that with the passage of time, the stored oil tends to decrease its integrity. Pitted oil maintains values ranging in the range of 0.21-0.27%, with a slight decrease after the sixth month of production, indicating that the level of preservation of the pitted oil and its organoleptic characteristics remain practically unchanged for a long time. The pitted oil + kernels have an initial amount of free fatty acids of 0.51% that tend to increase considerably in the months following production, to exceed the limit of acceptability established by European legislation; after 3 months and even after 6 months the acidity exceeds 1%. It can also be added that traditional and pitted oil maintain acidity levels in line with European directives even after 6 months, while in pitted oil + kernels this situation has not occurred. The increase in acidity between the traditional and the pitted goes from 71% to bottling, to 55% after 3 months up to 42% after 6 months, highlighting an initial large increase in acidity that then stabilizes. In relation to the literature, the acidity values are around 0.3%, which increase, after more than 7 days of storage, to 0.56%. In other works the acidity goes from 0.70% to 0.73% after three months, to 0.76% after 6 months and to 0.95% after a year of storage in black bottles at 22 ° C, reproducing the same experimental trend of the thesis work (Lolis et al., 2020). This results are comparable to (Katsoyannos et al., 2015), because acidity tends to increase (+0.04%) as the degree of maturation increases, probably because the maturation process makes the fruit more sensitive to mechanical damage and caused by pathogens, with increased activity of enzymes, including lipolytic enzymes. It should be noted that after 12 months, regardless of the type of oil, a reduction in acidity

is observed compared to the same oil after 6 months, a sign of a progressive reduction in the oxidation process and a greater stabilization due to a degradation of the product.

From the *Table 24 and Figure 65* it can be deduced that there is a progressive increase over time of **peroxides** in traditional and pitted EVO, with a lower incidence on the pitted one which will therefore have a greater shelf-life, in accord to (Katsoyannos et al., 2015) (the presence of peroxides tend to decrease in pitted oil (from -0.4 to -1.4 meqO2/kg), peroxides (from +0.5% to +1.7%) increase as maturity increases, due to the increased presence of unsaturated acid), while this trend does not appear in the pitted oil + kernels. Pitted oil has a progressive increase in the degree of oxidation which, however, at 6 months does not exceed even half of the maximum threshold established by law. In traditional oil, on the other hand, a quantity of peroxides was found, after 6 months from production, equal to 11.81, greater than the oils produced with the other two extraction techniques. The increase in peroxides between the pitted and the traditional goes from 21% to bottling, to 20% after 3 months up to 23% after 6 months, highlighting an almost constant trend. The literature values found range from 6.96 at the time of bottling to 9.56 after more than 7 days of storage (Rotondi et al., 2021). In other works, it goes from 12.31 to 13.95 after three months, 14.12 after 6 months and 13.93 after 1 year of storage in dark bottles at 22°C, reproducing the same experimental trend of the present thesis work (Lolis et al., 2020). Also in this case, an increase in peroxides is observed, regardless of the type of oil, passing from 6 months to 12 months, a clear sign of an acceleration of the secondary oxidation process. It is interesting to note that only after 12 months and only in the case of pitted oil + kernels the legal limit of 20 meq O2 / kg oil is exceeded.

Comparing the values reported on *Table 25 and Figure 66* the can be highlighted the greater shelf-life and greater **antioxidant capacity** of the pitted oil, followed by the traditional and finally by the pitted. In traditional and pitted oils, it is also possible to notice a decreasing trend over time, contrary to the pitted oil + kernels which shows a fluctuating trend, as well as the smallest antioxidant capacity. In all cases, an induction time is observed that quickly decays after 6 months. The increase in **induction time** (h, Rancimat) between the traditional and the pitted goes from 14% to bottling, to 16% after 3 months up to 12% after 6 months, highlighting an almost constant trend. Regarding the results of the literature, induction times were found, at the bottling time, which depending on the quality of the oil are in the range of 64-180 h, but with 3.5 g of sample instead of 5, 100 ° C instead of 160 ° C and with a halved flow (10 l / h instead of 20). Beyond the different conditions there is a comparable trend (Alvarruiz et al., 2020). Regardless of the type of oil, after 12 months a slight increase in induction time is observed, except for traditional oil. This could be explained because the

primary oxidation, responsible for the decrease in induction time, is over and at the same time the secondary oxidation has begun. In traditional oil this process is less evident, while in the oil pitted and then added to the kernels, the secondary oxidation process was evident from after 6 months from bottling.

In general, with regard to the parameters **acidity**, **peroxides** and **induction time**, in pitted oil with the addition of kernels there is a previously increasing trend in the first three months, explainable by the primary oxidation of these compounds. Then there is a decrease in the second two months due to the secondary oxidation of these compounds, and therefore a progressive decrease in peroxides, acidity and induction time, closely related parameters, and a progressive rancidity of the oil. Finally, after 12 months, an increase in induction time and peroxide parameters and a reduction in acidity are seen.

4.3.2. VOC (Volatile organic compounds)

The *Table 26* shows the experimental results of the 18 volatile organic compounds, defined as standard in the method mentioned in section 4.2.6, in the various EVO (in the bottling and after 3,6 months) and expressed in mg/kg of oil. The same data are expressed in graphic form in *Figure 67.*

mg/kg oil	RIN 0	RIN 3	RIN 6	DEN 0	DEN 3	DEN 6	TRAD 0	TRAD 3	TRAD 6
1) Octane	<LOD	<LOD	0,04	0,03	0,03	0,04	<LOD	<LOD	0,03
2) Ethyl acetate	0,05	0,06	0,05	0,05	<LOD	<LOD	0,08	0,27	0,05
3) Ethanol	0,37	0,45	0,21	1,09	0,70	0,65	0,22	0,32	0,11
4) Ethyl propanoate	<LOD	<LOD	<LOD	<LOD	0,05	0,02	0,05	0,05	0,06
5) Hexanal	0,60	0,75	1,46	1,24	1,41	1,39	0,20	0,60	0,62
6) 3-Methyl-1-butanol	0,14	0,08	0,12	0,26	0,24	0,27	0,27	0,19	0,21
7) E-2-hexenal	5,93	7,13	6,20	18,27	17,21	20,69	19,19	13,62	12,86
8) Z-3-hexenyl acetate	0,17	0,10	0,13	0,40	0,37	0,43	0,80	0,15	0,16
9) E-2-Heptenal	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
10) 6-methyl-5-hepten-2-one	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
11) 1-hexanol	0,36	0,21	0,36	0,30	0,29	0,38	0,76	1,61	3,39
12) Nonanal	0,60	0,97	1,77	0,73	0,93	2,05	<LOD	3,34	<LOD
13) 1-octen-3-ol	<LOD	<LOD	0,50	<LOD	<LOD	<LOD	<LOD	0,26	<LOD
14) E,E-2,4-hexadienal	0,28	0,31	0,00	0,88	0,76	0,87	0,89	0,72	0,66
15) Acetic acid	<LOD	0,11	0,12	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
16) Propanoic acid	0,16	0,12	0,19	0,21	0,15	<LOD	<LOD	0,13	0,15
17) E-2-decenal	2,08	0,00	0,00	<LOD	<LOD	<LOD	12,84	18,10	19,50
18) Pentanoic acid	<LOD	<LOD	0,53	<LOD	<LOD	0,46	<LOD	<LOD	<LOD

Table 26: experimental data of VOC in EVOO (mg/kg oil) (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels) (TRAD 0=traditional oil in bottling time TRAD 3= traditional oil three months after bottling…)

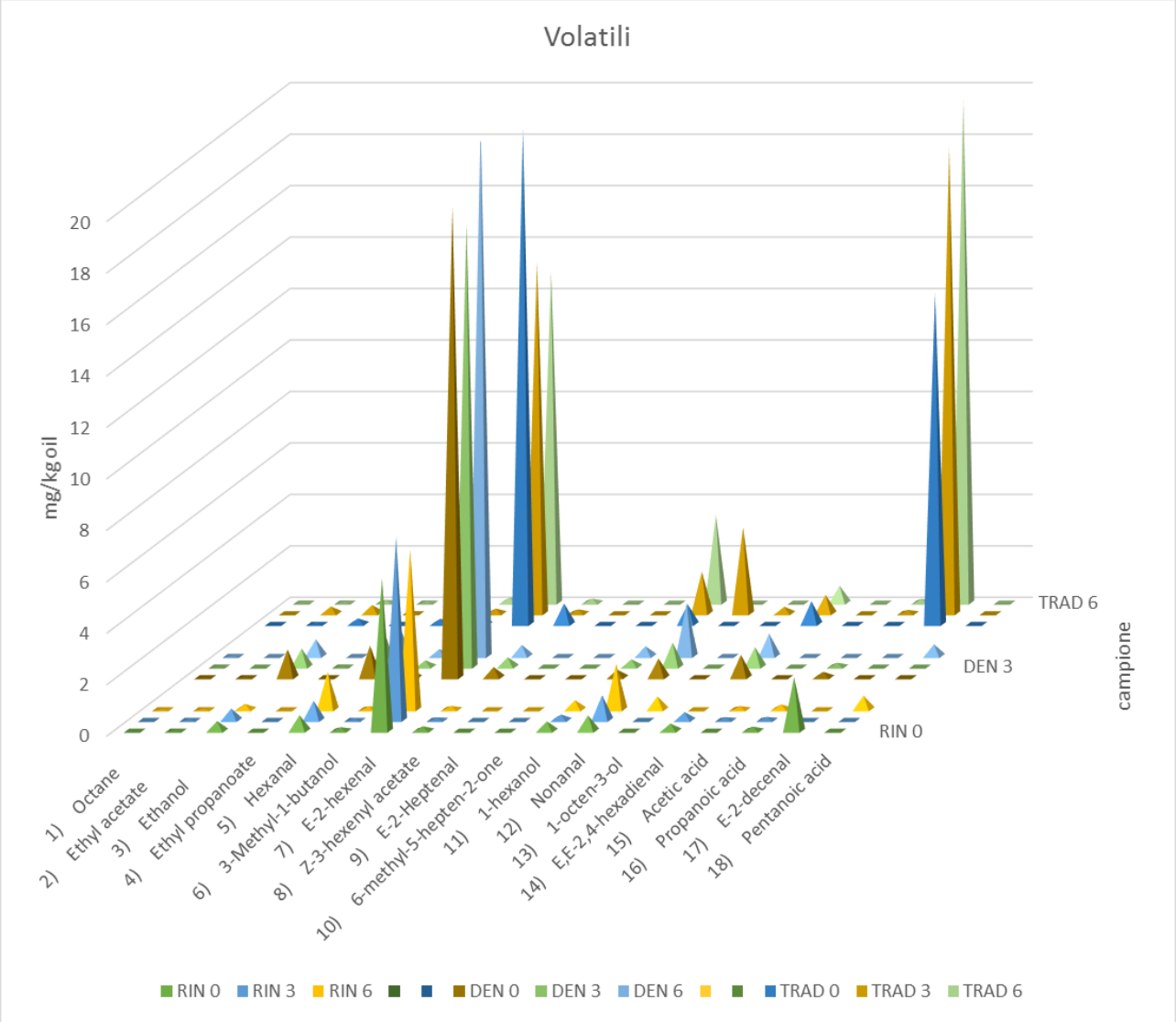


Figure 67: graphical representation of VOC in EVOO (mg/kg oil) (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels) (TRAD 0=traditional oil in bottling time TRAD 3= traditional oil three months after bottling…)

As stated in some studies (Yan et al., 2020), the odor of those compounds (acetaldehyde, 2-propenal, propanal, acetic acid, dimethyl sulfide, propanoic acid, dimethyl sulfoxide, butanoic acid) are considered to contribute strongly to the odor of the oils. Surprisingly, most of those compounds are associated with odor defects. Regarding the identified C5 compounds, trans-2-pentenal is associated with green-fruity odor note. Regarding the identified C6 compounds, trans-2-hexenal is associated with a green-fruity odor. Hexanal is associated with a green-sweet odor note. Butyl acetate, ethyl butyrate, and ethyl isobutyrate are associated with a green-sweet-fruity note. Therefore, trans-2-pentenal, trans-2-hexenal, hexanal, butyl acetate, ethyl butyrate, and ethyl isobutyrate might be the relevant contributors to the green odor notes of EVOO. Moreover, trans-2-hexenal and hexanal are most likely the most important contributors to the green odor notes of the EVOO. It is reported that a great amount of the VOCs associated with the green odor notes have been found in high-

quality/grade OO (EVOO). Therefore, the odor of those compounds most likely contributes to the differences in perception of the green odor notes between the premium grade EVOO and the lower grades of OOs. Although the identified C7–C15 compounds have relatively low concentrations in OOs compared to the identified C1–C6 compounds (2,4 heptadienal, trans-2-heptenal, heptanal, heptan-2-one, trans-2-octenal, 3-octen-2-one, octanal, 1-octen-3-ol, octan-2-one, trans, trans-2,4-nonadienal, trans-2-nonenal, nonanal, nonan-2-one, trans, trans-2,4-decadienal, trans-2-decenal, decanal). However, most of those compounds are associated with odor defects. Hexyl acetate is associated with a green-fruity note and this compound might not be a relevant contributor to the green odor notes of OOs. Summarizing, the identified C5–C6 compounds mainly possess the green odor notes, while the identified C1–C4 and C7–C15 compounds are mainly associated with odor defects. Consumers' preference in OOs is mainly related to the odor descriptors qualified with the 'green' note. Therefore, the green notes are important sensory traits. Trans-2-hexenal, hexanal, butyl acetate, ethyl butyrate, and ethyl isobutyrate are expected to contribute to the green odor notes of OOs. EVOO was present with consistently higher concentrations of these compounds with green notes.

In relation to the experimental data obtained, the following can be said:

- The presence of octane, ethyl acetate, ethylpropanoate, E-2-heptenal (oil defect), 6-methyl-5-hepten-2-one, 1-oct-3-ol (oil defect), acetic acid and propanoic acid, E-2-decenal and pentanoic acid were not considered present in quantities such as to carry out statistical evaluations.
- The presence of 3 methyl 1 butanol, Z-3-hexenylacetate, E, E-2,4-hexadienal is fluctuating in the various oils and it is not possible to make statistical evaluations.
- Ethanol has a fluctuating trend in traditional and pitted oils + kernels, while it decreases with increasing ripening time in pitted oil. The quantities in pitted oil range from 1.09 to 0.70 mg/kg (-35.8%), after 3 months, to 0.65 mg/kg (-7.1%), after 6 months, showing a progressive "stabilization" in the decrease after 6 months. Pitted oil is also the one with the highest ethanol content, which gives a positive connotation to the aroma. In fact, its aroma is, as well as alcoholic, also apple and floral (L. Cecchi et al., 2021).
- Hexanal is more present in pitted oil, and in general its presence grows with the passing of maturation (from 1.24 to 1.41 mg/l). In traditional oil there is a growth that becomes quite evident after 6 months, coming to exceed the amount present in the pitted oil (1.46 mg / kg after an increase of 71% in the last 3 months). Its presence in large concentrations takes on a negative connotation, on the contrary positive in small concentrations. Its aroma is green, fruit, cut grass, apple, leaf, sweet, low conc content; segus, fat, lawn, sebaceous, oil with a high content of conc (L. Cecchi et al., 2021).

- E-2-hexenal is most present in pitted oil (from 17.21 to 20.69 mg/kg), followed by the traditional (from 12.86 to 19.19 mg/kg), and then in the pitted oil + kernels (from 5.93 to 7.13 mg/kg). In pitted oil + kernels the trend is constantly decrescent, but the speed of this decrement is high after 3 months (-30%). The aroma is bitter almond, green, cut grass, leafy, fruity, apple, sharp, sweet, astringent, bitter, fatty (L. Cecchi et al., 2021). Together with the hexanal they contribute to make the quality of the pitted oil greater than the others and to increase the aroma of green, apple, herbaceous.
- Hexanol is more present in pitted oil + kernels, with a crescent trend (+52.8% after 3 months, doubles after 6 months), and this takes on a positive connotation. The other oils contain small amounts of hexanol and with fluctuating trend. Its aroma is resin, floral, green, grass, fruit, aromatic, banana, alcoholic, rough, astringent, soft, sweet, tomato (L. Cecchi et al., 2021).
- The nonanal is more present in pitted oil (from 0.73 to 2.06 mg/l) followed by the traditional oil (from 0.60 to 1.77 mg/l), in general an increase in the presence is observed as the degree of ripeness increases (+ 21.5% after 3 months, +54.6% after 6 months for pitted, +38% after 3 months, +45% after 6 months for the traditional), speed increases with the passage of time. Its presence takes on a negative connotation (oxidized). Its aroma is fat, citrus, grass, green, rancid, wax, pungent, soap, segus (L. Cecchi et al., 2021).
- With reference to other literature works, the same trend projected for the substances ethylacetate, ethanol, hexanal, nonanal and 2.4 hexadienal was found after 9 and 18 months of storage. In particular, they increase hexanal and nonanal (Lolis et al., 2020).

4.3.3. TOCOPHEROLS

Finally, the *Table 27 and the Figure 68* show the experimental results of α and γ tocopherols:

Type of oil	Shelf-life	Sample code	a-T mg/kg oil	g-T
Traditional EVOO	0 month	TRAD 0	248 ± 1	18 ± 1
	3 months	TRAD 3	212 ± 6	15 ± 1
	6 months	TRAD 6	116 ± 6	5 ± 0
Pitted EVOO	0 month	DEN 0	262 ± 6	8 ± 0
	3 months	DEN 3	234 ± 4	6 ± 0
	6 months	DEN 6	127 ± 3	2 ± 0
Pitted EVOO + kernels	0 month	RIN 0	262 ± 3	11 ± 0
	3 months	RIN 3	208 ± 4	8 ± 0
	6 months	RIN 6	95 ± 3	3 ± 0

Table 27: α and γ tocopherols in EVO (mg/kg oil) (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels) (TRAD 0=traditional oil in bottling time TRAD 3= traditional oil three months after bottling…)

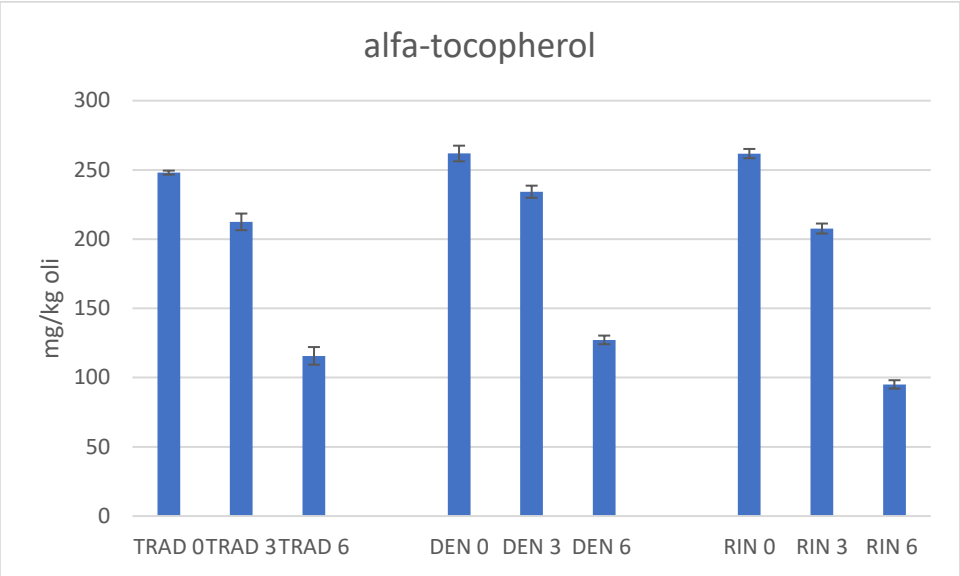


Figure 68: α-tocopherols in EVO (mg/kg oil) (TRAD=traditional oil DEN=pitted oil RIN=pitted oil plus kernels) (TRAD 0=traditional oil in bottling time TRAD 3= traditional oil three months after bottling…)

A decrease in the presence of α-tocopherol is observed in all cases with increasing storage period, with a slightly greater presence of tocopherols in pitted and pitted oil + kernels at bottling. The speed of decrease is maximum for pitted oil + kernels (from 262 to 95 mg/kg, -20.6% after 3 months, -54% after 6 months), followed by the traditional (from 248 to 116 mg/kg, -14.5% after 3 months, -45% after 6 months) and the pitted oil (from 262 to 127 mg/kg, -10.7% after 3 months, -45% after 6 months). After 6 months it is the pitted oil that shows the highest levels of α-tocopherol, and always after 6 months the fastest decrease is obtained for all oils. The presence in the core of enzymes that contribute to oxidize different components of the oil explain the greater tendency of pitted oil and then added kernels to "destroy" α-tocopherols, with lower antioxidant capacity. In the literature, values of α–tocopherol of 184 mg/kg oil which after more than 7 days decreases to 158 mg/kg oil (Rotondi et al., 2021). A similar trend is also found for γ–tocopherol, but in this case, it is the traditional oil that has the highest levels, even after 6 months (from 5 to 18 mg / l). For γ–tocopherol almost constant values of 8 mg/kg oil were found, with a very slow decreasing trend (Rotondi et al., 2021). In another study, (Katsoyannos et al., 2015), all experimental results are congruent with results of this work (Green olives have a higher content of tocopherols (from +20.8 to +179.29 mg/kg oil) than ripe olives. It has also been detected that the content of polyphenols and the degree of maturation influence the organoleptic characteristics and shelf-life of extra virgin olive oil. Pitted oil also has a higher tocopherol content (from +42.73 to +109.2 mg/kg oil) than conventional oil, with positive repercussions on the quality and antioxidant activity of pitted oil. This also leads to better organoleptic qualities and greater shelf life of the latter oil. The reasons have previously been stated: in the pitting

process, the stones are removed at the beginning of processing and therefore, the enzymes (lipoxygenases, peroxidases) contained in the seeds do not influence the pulp composition and phenols are not enzymatically degraded thus improving their concentration and oil oxidative stability).

4.4. CONCLUSIONS

From the experimental results it emerged that the pitted oil has a lower peroxide content than the others, a greater conservation as the induction time is greater, but a slightly higher acidity. These considerations remain valid both in the bottling phase and after 3.6 months. After 12 months, the beginning of secondary oxidation and the end of primary oxidation begins to be observed, which involves a reduction in acidity and an increase in induction time. This behavior is also observable for traditional oil, while the presence of kernels, which contain substances with oxidizing action and enzymes that reduce the content of polyphenols (with antioxidant capacity), make the behavior of the pitted and added kernel oil completely different from the others.

About volatile organic substances, pitted oil contains a greater amount of hexanal and 2-hexenal, which contribute to improving the organoleptic qualities of the latter, thanks to the green aromas, cut grass, fruity etc. Even the nonanal, more present in pitted oil, is responsible for aromas. In pitted oil + kernels, on the other hand, hexanol is more present, with its resin, floral, green, grass, fruit, aromatic, banana, alcoholic, rough, astringent, soft, sweet, tomato aroma. Tocopherols are substances with antioxidant action and in general tend to decrease with increasing storage period. A greater initial quantity of α-tocopherols is observed in pitted and pitted oil with kernels, but their decrease is particularly evident, with increasing storage period, in pitted + kernels due to the presence of enzymes in the core that destroy α-tocopherol. After 6 months it is the pitted oil that shows the highest levels of a-tocopherol, and always after 6 months the fastest decrease is obtained for all oils. γ-tocopherol is instead more present in traditional oil and in this case a decreasing trend is observed in all oils as the storage period increases. The decrease is especially noticeable in traditional oil after 6 months.

Ultimately, pitted oil has a higher quality and better shelf life, superior organoleptic qualities but on the other hand a slightly higher acidity. In addition, production technologies have lower yields and higher costs.

5. EFFECT OF REFINING ON VEGETABLE OILS

5.1. INTRODUCTION

The effects of various refining phases (oil as it is, refining, drying, discoloration, deodorization, neutralization) on 22 seed oils (sunflower, high oleic sunflower, corn, grape seeds, soybeans) were highlighted. This is through the analysis of the parameters acidity, peroxides, induction time with Rancimat apparatus, tocopherols and tocotrienols. With this research we will try to improve the innovative production technologies of the oil and to verify changes in the quality parameters of vegetable oils. The quality of vegetable oils depends not only on chemical parameters such as acidity, peroxides, polyphenols, etc., but also its organoleptic characteristics. The free acidity (FA, g oleic acid in 100 g of oil) and peroxide value (PV, mg eq O_2 kg-1 of oil) was carried out for each oil sample according to the EEC Reg. n. 2568/1991 and subsequent modifications (see paragraph 1.2.1). For example, the acidity limit provided for by current legislation on refined seed oils is 0.5% expressed as oleic acid. As reported in other works (Marrakchi et al., 2015), (Cappelli et al., 2000) the discoloration phase removes, among the VOC, the tocopherols, the neutralization removes the free fatty acids. A small excess of sodium hydroxide inhibits saponification, with beneficial effects on discoloration and oil quality. Discoloration reduces the presence of peroxides and increases the acidity of the oil.

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5.2. MATERIALS AND METHODS

5.2.1. SAMPLING

These vegetable oils at various levels of refining were analyzed *(Table 28, Figure 69):*

Number of samples	Type oil	Farm
1	Bleached sunflower oil	Marseglia
2	Deodorized sunflower oil	Marseglia
3	Crude sunflower oil	Marseglia
4	Bleached grape seed oil	Marseglia
5	Deodorized grape seed oil	Marseglia
6	Crude grape seed oil	Marseglia
7	Refined soybeans oil	Zucchi
8	Bleached soybeans oil	Zucchi
9	Neutral soybeans oil	Zucchi
10	Crude soybeans oil	Zucchi
11	Refined corn oil	Zucchi
12	Bleached corn oil	Zucchi
13	Dried corn oil	Zucchi
14	Crude corn oil	Zucchi

Number of samples	Type oil	Farm
15	Refined sunflower oil	Zucchi
16	Bleached sunflower oil	Zucchi
17	Neutral sunflower oil	Zucchi
18	Crude sunflower oil	Zucchi
19	Refined high oleic sunflower oil	Zucchi
20	Bleached high oleic sunflower oil	Zucchi
21	Dried high oleic sunflower oil	Zucchi
22	Crude high oleic sunflower oil	Zucchi

Table 28: vegetable oils in experimental part

Figure 69: Vegetable oils analyzed

Oil samples were stored at room temperature and in the dark. These oils come from two different farms, and there are two types of sunflower oil and one type of high oleic sunflower oil.

These parameters were analyzed:

- Acidity (% oleic acid)
- Peroxides (meq O2/kg oil)
- Oxidative stability with Rancimat apparatus (h)
- Tocopherols (mg/kg)
- Tocotrienols (mg/kg)

5.2.2. ACIDITY

The oil concerned was dissolved in a mixture of 95% ethyl alcohol and ethyl ether, in a proportion of 1:2, and then titrated, until phenolphthalein (ethanol solution 1%) used as an indicator, with a solution of NaOH 0.1 N (Reg. EC/2568/91).

Acidity, expressed as % by mass of oleic acid, is given by:

Acidity = V • c • M/1000 • 100/m = V • c • M / (10 • m)

Where:

V = volume (mL) of the NaOH solution used

c = concentration (moles/L) of the NaOH solution used

M = molar weight of the acid adopted for the expression of the result (oleic acid: PM = 282 g/mol)

m = weight (g) oil

The acid content of edible fats is given by the quantity of free fatty acids deriving from the hydrolytic rancidity of triglycerides. As this alteration occurs in unsuitable conditions for the processing and preservation of fats, acidity represents a basic indicator of the genuineness of the product. The test is particularly important during the refining of oils and fats, for the assessment of the processing cycle and for the definition of product categories. The acidity limit provided for by current legislation on refined seed oils is 0.5% expressed as oleic acid.

5.2.3. PEROXIDES

The determination was made by titration with sodium thiosulfate solution 0,1 N until the blue-purple color of the starch weld indicator disappeared (Reg. EC/2568/91). The value of the number of peroxides, expressed in milliequivalents of active oxygen per kg, is given by:

number of peroxides = [(V • T) / m] • 1000

Where:

V = volume (mL) of the known sodium thiosulfate solution used in the analysis

T = normality of the sodium thiosulfate solution used

m = mass (g) of the substance to be analyzed

The amount of peroxides of fats indicate the degree of primary oxidation and therefore its likeliness of becoming rancid. A lower number of peroxides indicates a good quality of oil and a good preservation status. Unsaturated free fatty acids react with oxygen and form peroxides, which determine a series of chain reactions that generate the production of smelling volatile substances. Those reactions are accelerated by high temperature and by light and oxygen exposure. The legal limit for refined vegetable oils is set at 10 meq O2/kg of oil.

5.2.4. OXIDATION STABILITY BY RANCIMAT APPARATUS

The oxidative stability of the oils was determined by Rancimat apparatus (Metrohm model 679, Herisau, Switzerland), measuring the induction time in response to force oxidation (induction period) of 5 g sample heated at 110°C under an air flow of 20 L h-1 (Di Lecce et al., 2020). The induction period (expressed in hours) was determined by drawing the two tangents of the time–conductivity curve and projecting the intersection onto the time-axis.

5.2.5. TOCOPHEROLS AND TOCOTRIENOLS

Oil saponification phase

In a 50 ml flask 100 mg of oil are weighed, in which 5 ml of ethanol pyrogallol (6% w/v) and 0.5 ml of KOH are added (80% w/v). Homogenize to the vortex for 30 seconds, then connect the flask to a coolant, soaking the ball in a water bath at 75°C, keeping the boiling for 30 minutes. As soon as the procedure is finished, it cools down in a bath of water and ice for 5 minutes. 3 ml of distilled water and 5 ml of hexane are added. It homogenizes to the vortex for 30 seconds. It transfers everything to a vial of appropriate length and centrifuges for 10 minutes. The surface organic phase is taken in a new 50 ml flask. The first ball must be cleaned with another 5 ml of hexane and transfers everything to the vials containing the aqueous phase. Homogenization with vortex is carried out for 30 seconds, followed by centrifugation for 10 minutes. The organic phase is taken and join it to the previous one. The extraction with hexane is repeated. Once the organic phases have been combined, they are dried with rotavapor, then 0.5 ml of hexane is added, and everything is transferred to vial for subsequent analysis in HPLC.

Analysis phase

This sample is loaded on a UPLC Acquity H-Class system (Waters Corporation, Milford, MA, USA) (Orlando et al., 2020) equipped with a fluorometric detector (FLD) and Ascentis Express HILIC (15 cm × 2.1 mm i.d., particle size 2.7 µm, Merck, Darmstadt, Germany) column set up at 30 ◦C. An isocratic elution (8 min) of n-hexane (95.5%), isopropanol (0.4%) and acetic acid (0.1%) at 0.3 mL/min was performed. FLD was set with an excitation and emission wavelength of 290 and 330 nm, respectively. Tocopherols were identified by comparison of retention time with pure standards and quantified with external calibration. For the quantification, seven standard stock solutions of each tocopherol (α-T, γ-T, δ-T) in isopropanol were prepared in the range 3.5–100 µg/mL and analyzed to obtain the calibration curve (R2 = 0.9836–0.9965).

5.3. RESULTS AND DISCUSSION

As described above, there are various vegetable oils at various refining steps to which the following parameters have been analyzed: acidity, peroxides, oxidative stability with Rancimat apparatus. The following tables indicate the analytical parameters in the various oils (*Table 29, Table 30, Table 31*).

5.3.1. INDUCTION TIME WITH RANCIMAT APPARATUS

Description	Induction time (h)	Reference (Maszewska et al., 2018)
Bleached sunflower oil (Marseglia)	2,14 ± 0,42	
Deodorized sunflower oil (Marseglia)	2,55 ± 0,40	
Crude sunflower oil (Marseglia)	2,20 ± 0,33	
Bleached grape seed oil (Marseglia)	1,83 ± 0,36	
Deodorized grape seed oil (Marseglia)	2,21 ± 0,27	2.4
Crude grape seed oil (Marseglia)	1,91 ± 0,23	
Refined soybeans oil (Zucchi)	3,62 ± 0,14	
Bleached soybeans oil (Zucchi)	2,52 ± 0,01	
Neutral soybeans oil (Zucchi)	2,84 ± 0,08	
Crude soybeans oil (Zucchi)	3,27 ± 0,04	
Refined corn oil (Zucchi)	5,16 ± 0,26	4.8
Bleached corn oil (Zucchi)	2,43 ± 0,28	
Dried corn oil (Zucchi)	3,68 ± 0,16	
Crude corn oil (Zucchi)	9,74 ± 0,98	
Refined sunflower oil (Zucchi)	2,96 ± 0,30	
Bleached sunflower oil (Zucchi)	1,85 ± 0,46	
Neutral sunflower oil (Zucchi)	2,12 ± 0,42	
Crude sunflower oil (Zucchi)	2,60 ± 0,46	
Refined high oleic sunflower oil (Zucchi)	9,67 ± 0,32	
Bleached high oleic sunflower oil (Zucchi)	6,94 ± 0,39	
Dried high oleic sunflower oil (Zucchi)	6,84 ± 0,25	
Crude high oleic sunflower oil (Zucchi)	8,63 ± 0,28	

Table 29: Experimental induction time with Rancimat in vegetable oils (h) (Mean of two replicates ± standard deviation)

Higher induction times are observed in high oleic sunflower oil (from 6.84 to 9.67 h), followed by corn oil (5.16 h), soybeans (3.62 h) and sunflower (2.96 h). Crude corn oil was the oil with the highest induction time (9.74 h) among the 22 vegetable oils analyzed. In general, refined oils (from 2.96 to 9.67 h) exhibit a longer induction time than raw oils (from 1.91 to 8.63 h), except for corn oil. The induction time values of refined vegetable oils are congruent with those obtained in similar works in the literature (Maszewska et al., 2018). As reported in other works, (Marrakchi et al., 2015) the greater induction times found in general in refined oils are linked to the reduction of acidity that is worked especially in the neutralization phase, and also in the discoloration phase, where the lower possibility that you can have saponification favors the discoloration and the quality of the oil.

5.3.2. ACIDITY

Description	Acidity (%)	Reference . (Konuskan, Arslan, & Oksuz, 2019), (Osawa, Gonçalves, & Ragazzi, 2007).
Bleached sunflower oil (Marseglia)	0.14 ± 0.01	
Deodorized sunflower oil (Marseglia)	0.28 ± 0.02	
Crude sunflower oil (Marseglia)	0.85 ± 0.04	0.81
Bleached grape seed oil (Marseglia)	0.42 ± 0.03	
Deodorized grape seed oil (Marseglia)	0.14 ± 0.01	
Crude grape seed oil (Marseglia)	2.12± 0.04	
Refined soybeans oil (Zucchi)	0.14 ± 0.02	0.02-0.04
Bleached soybeans oil (Zucchi)	0.14 ± 0.01	
Neutral soybeans oil (Zucchi)	0.28 ± 0.01	
Crude soybeans oil (Zucchi)	1.13 ± 0.04	0.17-1.40
Refined corn oil (Zucchi)	0.14 ± 0.01	0.05-0.07
Bleached corn oil (Zucchi)	0.14 ± 0.01	
Dried corn oil (Zucchi)	0.28 ± 0.02	
Crude corn oil (Zucchi)	3.96 ± 0.04	2.04-3.30
Refined sunflower oil (Zucchi)	0.14 ± 0.01	0.03-0.06
Bleached sunflower oil (Zucchi)	0.14 ± 0.02	
Neutral sunflower oil (Zucchi)	0.28 ± 0.02	
Crude sunflower oil (Zucchi)	1.13 ± 0.04	0.81
Refined high oleic sunflower oil (Zucchi)	0.14 ± 0.01	
Bleached high oleic sunflower oil (Zucchi)	0.14 ± 0.01	
Dried high oleic sunflower oil (Zucchi)	0.28 ± 0.02	
Crude high oleic sunflower oil (Zucchi)	1.55 ±0.04	

Table 30: Experimental acidity of vegetable oils. (% oleic acid) (Mean of three replicates ± standard deviation.)

As can be seen, the acidity of crude oils exceeds 1%, (from 0.85% sunflower oil to 3.96% corn oil) while in the case of refined oils acidity values of less than 0.5% are always observed in any case (lower in all cases than the legal limit). The acidity values of raw and refined vegetable oils are congruent with those obtained in similar works in the literature (Konuskan et al., 2019), (Osawa et al., 2007). As reported in other works, (Marrakchi et al., 2015) the neutralization phase reduces acidity and increases the integrity and quality of the oil, making it edible.

5.3.3. PEROXIDES

Description	Peroxides (meq O2/kg oil)
Bleached sunflower oil (Marseglia)	41,3
Deodorized sunflower oil (Marseglia)	11,3
Crude sunflower oil (Marseglia)	43,7
Bleached grape seed oil (Marseglia)	18,9
Deodorized grape seed oil (Marseglia)	18
Crude grape seed oil (Marseglia)	50
Refined soybeans oil (Zucchi)	19,1
Bleached soybeans oil (Zucchi)	32,5
Neutral soybeans oil (Zucchi)	46,1
Crude soybeans oil (Zucchi)	30,3
Refined corn oil (Zucchi)	22,2
Bleached corn oil (Zucchi)	50
Dried corn oil (Zucchi)	50
Crude corn oil (Zucchi)	12,4
Refined sunflower oil (Zucchi)	50
Bleached sunflower oil (Zucchi)	50
Neutral sunflower oil (Zucchi)	50
Crude sunflower oil (Zucchi)	35,4
Refined high oleic sunflower oil (Zucchi)	24,8
Bleached high oleic sunflower oil (Zucchi)	46,8
Dried high oleic sunflower oil (Zucchi)	10,7
Crude high oleic sunflower oil (Zucchi)	4,6

Table 31:Peroxides in vegetable oils (meq O2/kg oil) (one single replicate)

In general, higher values of peroxides are observed in crude oils and lower values in refined oils at various levels. This trend is not observed in soybeans, corn, and some sunflower oils. The following experimental results showed that the peroxide content depends more on the type of oil than on storage. However, as reported in other works, (Marrakchi et al., 2015) the discoloration phase reduces the presence of peroxides.

5.3.4. TOCOPHEROLS AND TOCOTRIENOLS

The following tables are respectively indicated:

- Tocopherol levels in saponified samples in *Table 32*
- Tocotrienol levels in saponified samples in *Table 33*
- Tocopherol levels in unsaponified samples in *Table 34*
- Tocotrienol levels in unsaponified samples in *Table 35*

In the following *Figure 70* is illustrated the graphic representation of the same data by type of oil (corn, oil, grape, sunflower, high oleic sunflower).

Description	α-tocopherols	β-tocopherols	γ-tocopherols	δ-tocopherols
Bleached sunflower oil (Marseglia)	704 ± 8	21 ± 0	0	0
Deodorized sunflower oil (Marseglia)	616 ± 6	22 ± 1	0	0
Crude sunflower oil (Marseglia)	622 ± 5	16 ± 0	0	0
Bleached grape seed oil (Marseglia)	94 ± 1	13 ± 0	13 ± 0	1 ± 0
Deodorized grape seed oil (Marseglia)	103 ± 2	14 ± 0	14 ± 0	1 ± 0
Crude grape seed oil (Marseglia)	181 ± 1	32 ± 2	62 ± 1	1 ± 0
Refined soybeans oil (Zucchi)	257 ± 2	0	2000 ± 14	979 ± 9
Bleached soybeans oil (Zucchi)	317 ± 4	0	1745 ± 16	712 ± 6
Neutral soybeans oil (Zucchi)	354 ± 5	0	2512 ± 18	1380 ± 11
Crude soybeans oil (Zucchi)	386 ± 6	0	2017 ± 19	755 ± 8
Refined corn oil (Zucchi)	518 ± 9	0	1185 ± 12	46 ± 1
Bleached corn oil (Zucchi)	531 ± 7	0	1391 ± 14	64 ± 2
Dried corn oil (Zucchi)	451 ± 5	0	1013 ± 10	39 ± 1
Crude corn oil (Zucchi)	540 ± 6	0	1375 ± 11	59 ± 1
Refined sunflower oil (Zucchi)	736 ± 8	37 ± 1	0	0
Bleached sunflower oil (Zucchi)	236 ± 2	11 ± 0	0	0
Neutral sunflower oil (Zucchi)	3219 ± 21	122 ± 5	0	0
Crude sunflower oil (Zucchi)	2542 ± 13	85 ± 1	0	0
Refined high oleic sunflower oil (Zucchi)	789 ± 8	36 ± 0	0	0
Bleached high oleic sunflower oil (Zucchi)	458 ± 5	18 ± 0	0	0
Dried high oleic sunflower oil (Zucchi)	701 ± 8	75 ± 2	0	0
Crude high oleic sunflower oil (Zucchi)	426 ± 5	11 ± 0	0	0

Table 32: tocopherols (mg/kg oil) results in saponified seed oils

Description	α-tocotrienols	β- tocotrienols	γ- tocotrienols	δ- tocotrienols
Bleached sunflower oil (Marseglia)	0	0	0	0
Deodorized sunflower oil (Marseglia)	0	0	0	0
Crude sunflower oil (Marseglia)	0	0	0	0
Bleached grape seed oil (Marseglia)	57 ± 1	0	95 ± 2	1 ± 0
Deodorized grape seed oil (Marseglia)	63 ± 1	0	105 ± 2	2 ± 0
Crude grape seed oil (Marseglia)	181 ± 3	0	481 ± 5	9 ± 0
Refined soybeans oil (Zucchi)	0	0	0	0
Bleached soybeans oil (Zucchi)	0	0	0	0
Neutral soybeans oil (Zucchi)	0	0	0	0
Crude soybeans oil (Zucchi)	0	0	0	0
Refined corn oil (Zucchi)	0	0	52 ± 0	0
Bleached corn oil (Zucchi)	0	0	65 ± 1	0
Dried corn oil (Zucchi)	0	0	40 ± 0	0
Crude corn oil (Zucchi)	0	0	60 ± 1	0
Refined sunflower oil (Zucchi)	0	0	0	0
Bleached sunflower oil (Zucchi)	0	0	0	0
Neutral sunflower oil (Zucchi)	0	0	0	0
Crude sunflower oil (Zucchi)	0	0	0	0
Refined high oleic sunflower oil (Zucchi)	0	0	0	0
Bleached high oleic sunflower oil (Zucchi)	0	0	0	0
Dried high oleic sunflower oil (Zucchi)	0	0	0	0
Crude high oleic sunflower oil (Zucchi)	0	0	0	0

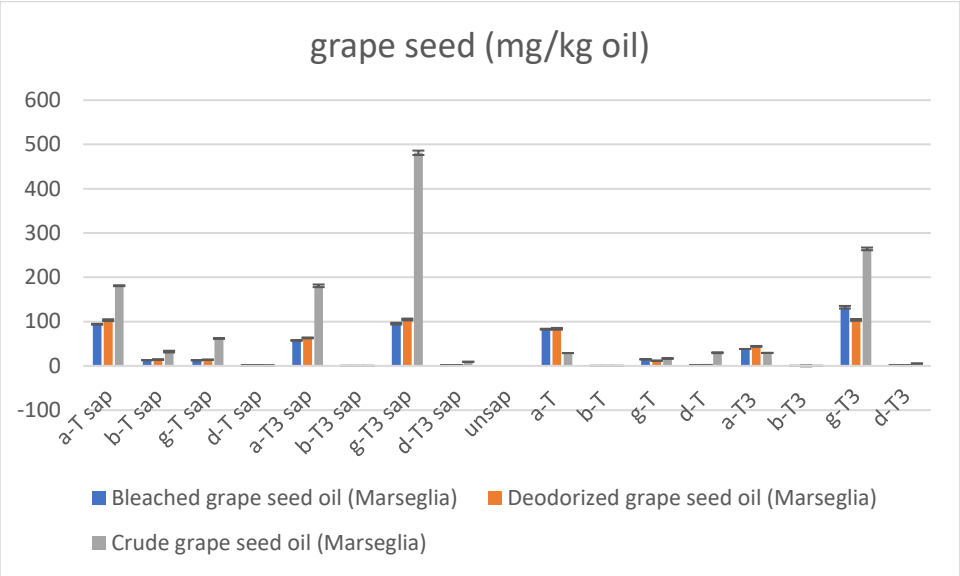
Table 33: tocotrienols (mg/kg oil) results in saponified seed oils

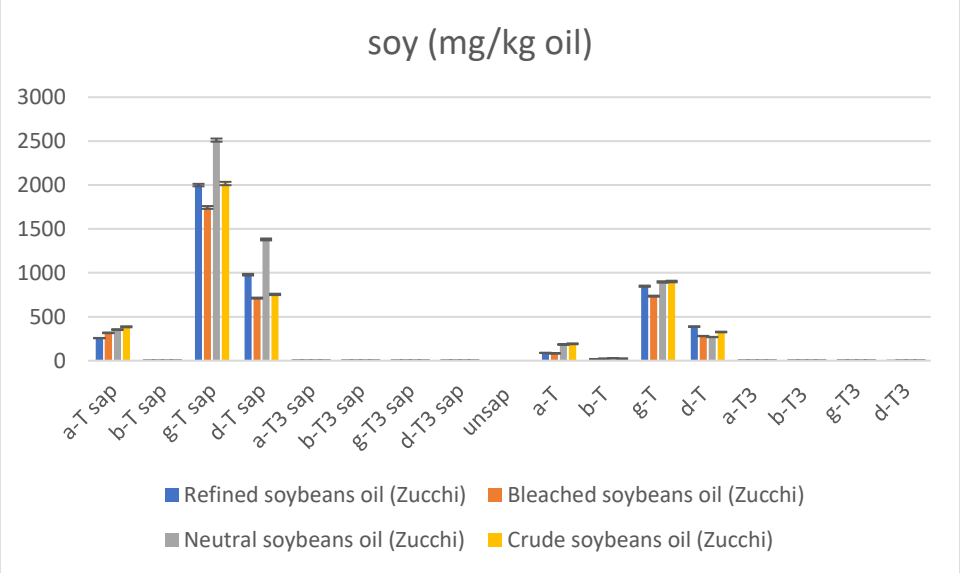
Description	α-tocopherols	β-tocopherols	γ-tocopherols	δ-tocopherols
Bleached sunflower oil (Marseglia)	569 ± 8	22 ± 0	0	0
Deodorized sunflower oil (Marseglia)	683 ± 9	24 ± 0	0	0
Crude sunflower oil (Marseglia)	863 ± 7	31 ± 1	0	0
Bleached grape seed oil (Marseglia)	83 ± 1	0	15 ± 0	2 ± 0
Deodorized grape seed oil (Marseglia)	84 ± 2	0	12 ± 0	2 ± 0
Crude grape seed oil (Marseglia)	29 ± 0	0	17 ± 0	30 ± 1
Refined soybeans oil (Zucchi)	89 ± 2	17 ± 0	848 ± 7	388 ± 3
Bleached soybeans oil (Zucchi)	83 ± 2	22 ± 0	734 ± 6	280 ± 2
Neutral soybeans oil (Zucchi)	184 ± 4	28 ± 2	896 ± 8	269 ± 2
Crude soybeans oil (Zucchi)	192 ± 5	13 ± 1	901 ± 9	326 ± 4
Refined corn oil (Zucchi)	163 ± 4	0	389 ± 4	11 ± 0
Bleached corn oil (Zucchi)	172 ± 3	0	435 ± 5	14 ± 0
Dried corn oil (Zucchi)	193 ± 4	0	627 ± 6	29 ± 1
Crude corn oil (Zucchi)	198 ± 5	0	470 ± 5	16 ± 0
Refined sunflower oil (Zucchi)	462 ± 7	0	0	0
Bleached sunflower oil (Zucchi)	427 ± 6	0	0	0
Neutral sunflower oil (Zucchi)	472 ± 8	0	0	0
Crude sunflower oil (Zucchi)	487 ± 7	0	0	0
Refined high oleic sunflower oil (Zucchi)	444 ± 6	0	0	0
Bleached high oleic sunflower oil (Zucchi)	456 ± 7	0	0	0
Dried high oleic sunflower oil (Zucchi)	456 ± 8	0	0	0
Crude high oleic sunflower oil (Zucchi)	444 ± 7	5 ± 0	0	0

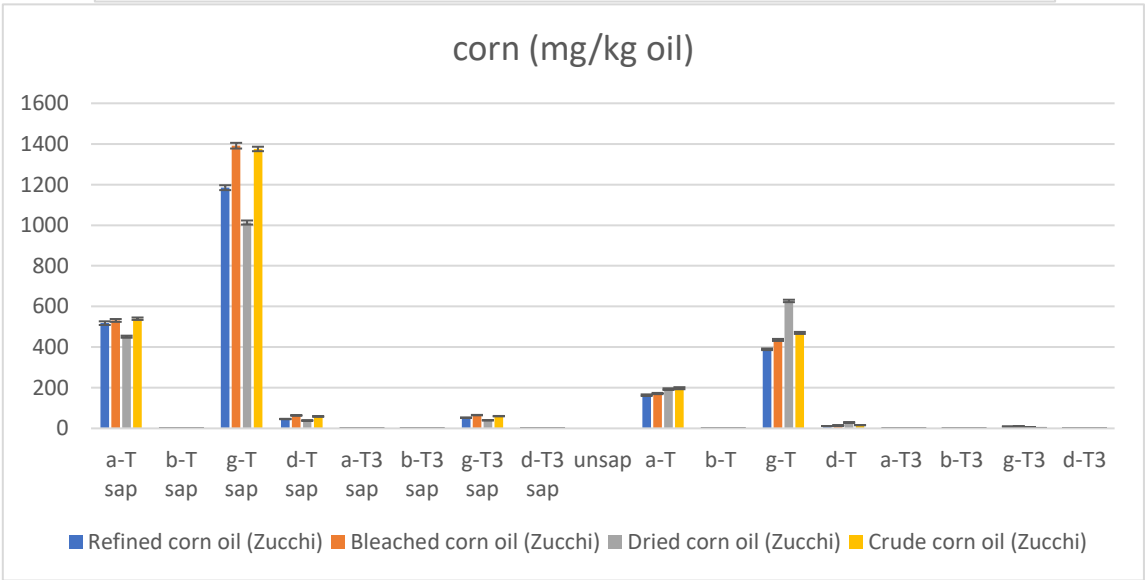
Table 34: tocopherols (mg/kg oil) results in unsaponified seed oils

Description	α-tocotrienols	β- tocotrienols	γ- tocotrienols	δ- tocotrienols
Bleached sunflower oil (Marseglia)	0	0	0	0
Deodorized sunflower oil (Marseglia)	0	0	0	0
Crude sunflower oil (Marseglia)	0	0	0	0
Bleached grape seed oil (Marseglia)	38 ± 0	0	132 ± 3	2 ± 0
Deodorized grape seed oil (Marseglia)	44 ± 1	0	104 ± 2	1 ± 0
Crude grape seed oil (Marseglia)	29 ± 0	0	264 ± 3	5 ± 0
Refined soybeans oil (Zucchi)	0	0	0	0
Bleached soybeans oil (Zucchi)	0	0	0	0
Neutral soybeans oil (Zucchi)	0	0	0	0
Crude soybeans oil (Zucchi)	0	0	0	0
Refined corn oil (Zucchi)	0	0	9 ± 1	0
Bleached corn oil (Zucchi)	0	0	9 ± 0	0
Dried corn oil (Zucchi)	0	0	5 ± 0	0
Crude corn oil (Zucchi)	0	0	2 ± 0	0
Refined sunflower oil (Zucchi)	0	0	0	0
Bleached sunflower oil (Zucchi)	0	0	0	0
Neutral sunflower oil (Zucchi)	0	0	0	0
Crude sunflower oil (Zucchi)	0	0	0	0
Refined high oleic sunflower oil (Zucchi)	0	0	0	0
Bleached high oleic sunflower oil (Zucchi)	0	0	0	0
Dried high oleic sunflower oil (Zucchi)	0	0	0	0
Crude high oleic sunflower oil (Zucchi)	0	0	0	0

Table 35: tocotrienols (mg/kg oil) results in unsaponified seed oils


grape seed (mg/kg oil)
600
500
400
300
200
100
0
-100
a-T sap
b-T sap
g-T sap
d-T sap
a-T3 sap
b-T3 sap
g-T3 sap
d-T3 sap
unsap
a-T
b-T
g-T
d-T
a-T3
b-T3
g-T3
d-T3
Bleached grape seed oil (Marseglia)
Deodorized grape seed oil (Marseglia)
Crude grape seed oil (Marseglia)


soy (mg/kg oil)
3000
2500
2000
1500
1000
500
0
a-T sap
b-T sap
g-T sap
d-T sap
a-T3 sap
b-T3 sap
g-T3 sap
d-T3 sap
unsap
a-T
b-T
g-T
d-T
a-T3
b-T3
g-T3
d-T3
Refined soybeans oil (Zucchi)
Bleached soybeans oil (Zucchi)
Neutral soybeans oil (Zucchi)
Crude soybeans oil (Zucchi)


corn (mg/kg oil)
1600
1400
1200
1000
800
600
400
200
0
a-T sap
b-T sap
g-T sap
d-T sap
a-T3 sap
b-T3 sap
g-T3 sap
d-T3 sap
unsap
a-T
b-T
g-T
d-T
a-T3
b-T3
g-T3
d-T3
Refined corn oil (Zucchi)
Bleached corn oil (Zucchi)
Dried corn oil (Zucchi)
Crude corn oil (Zucchi)

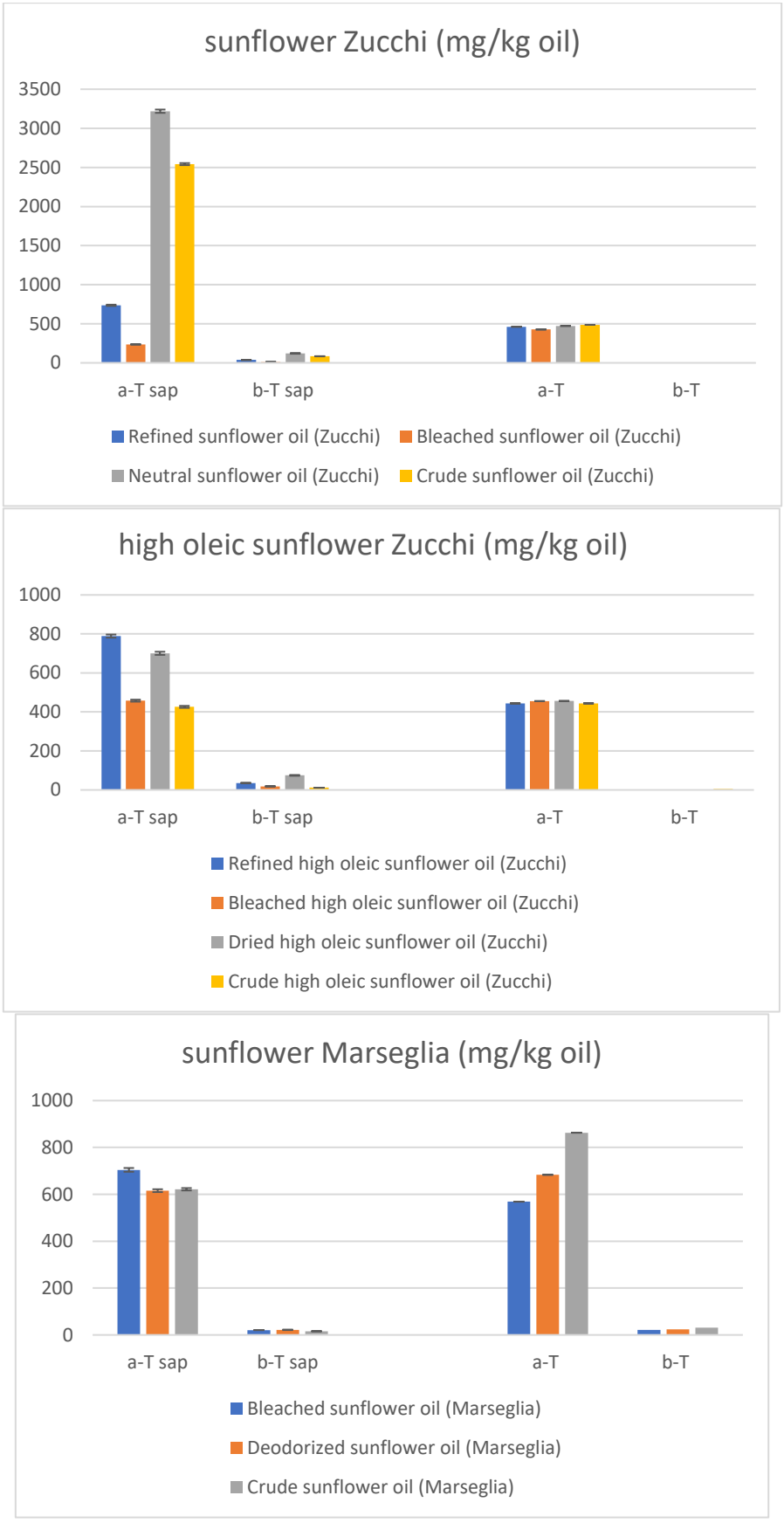


Figure 70: summary graphs of the presence of tocopherols and tocotrienols in the various seed oils (a-T= α-tocopherols b-T= β-tocopherols g-T= γ-tocopherols d-T= δ-tocopherols; a-T3= α-tocotrienols b-T3= β-tocotrienols g-T3= γ-tocotrienols d-T3= δ-tocotrienols)

As can be seen in *Figure 70,* the following can be deduced from the experimental data:

- Saponification in general increases the availability of tocopherols (94-3219 mg/kg oil α-tocopherols of saponified sample vs 29-863 mg/kg oil α-tocopherols of unsaponified sample) and tocotrienols (0-481 mg/kg oil γ-tocotrienols of saponified sample vs 0-264 mg/kg oil γ-tocotrienols of unsaponified sample).
- In crude (863 mg/l for unsaponified sample) and neutralized sunflower oils (3219 mg/l for saponified sample), the highest amounts of α-tocopherol were highlighted. In crude sunflower oil (122 mg/l for saponified sample, 31 mg/l for unsaponified sample), the highest amounts of β-tocopherol were highlighted.
- Soybean and corn oils contain greater amounts of γ and δ–tocopherol (2512 mg/kg of γ–tocopherols in neutral soybean oil and saponified sample, 1380 mg/kg of δ–tocopherol in neutral soybean oil and saponified sample, 901 mg/kg of γ–tocopherols in crude soybean oil and unsaponified sample, 388 mg/kg of δ–tocopherol in refined soybean oil and unsaponified sample).
- Various forms of grape seed oil contain significant amounts of tocotrienols in all forms (181 mg/kg α sap. sample crude grape seed oil, 481 mg/l γ sap. sample crude grape seed oil, 9 δ sap. sample crude grape seed oil, 44 mg/l α unsap. sample deodorized grape seed oil, 264 mg/l γ unsap. sample crude grape seed oil, 5 δ unsap. sample crude grape seed oil)
- The presence of tocopherols in these proportions is confirmed in samples not saponified by similar works in the literature (Celenk et al., 2018).
- As confirmed by other works (Marrakchi et al., 2015), in general all raw oils contain more tocopherols than their bleached counterparts, as discoloration reduces the content of total tocopherols.
- In corn oil, about the α-tocopherols of the unsaponified samples, a reduction of the free forms is observed, passing from raw to refined oil, with a consequent increase in esterified forms. γ-tocopherols are also present in more significant quantities in the non-saponified sample (from 1013 to 1391 mg/kg oil), and γ- tocotrienols especially in the saponified sample (from 40 to 65 mg/kg oil).
- The same thing occurs in soy oil, that is, α-tocopherols both saponified and not, and δ- tocopherols are present, especially in the saponified sample (from 712 to 1380 mg/kg oil).

- In grape seed oil there is a reduction in the presence of tocopherols and tocotrienols of saponified samples (α, β and γ) from raw to refined oil, with a reduction in free forms and an increase in esterified. For non-saponified forms there is a fluctuating trend.
- In sunflower oil and high oleic sunflower oil there is the presence of α and β tocopherols, but without trends. Only in the case of Marseglia sunflower oil, for unsaponified α-tocopherol there is a reduction passing from raw to refined, with a decrease in free forms and on the contrary an increase for esterified ones.

5.4. CONCLUSIONS

Refined vegetable oils generally exhibit a lower acidity (and therefore a lower tendency to lose integrity and above all the possible edibility of these oils) and higher induction times (and therefore a higher shelf life). The trend depends rather on the type of oil: for example, sunflower oil, in any form, has obtained induction times always greater than 6 hours, crude corn oil even greater than 9 hours. Regarding peroxides, here too the level is rather linked to the type of oil, but in general the level of peroxides is higher in raw oils than in refined ones. About tocopherols and tocotrienols, the saponification in general increases the availability of tocopherols and tocotrienols. In crude and neutralized sunflower oils, the highest amounts of α-tocopherol were highlighted. In crude sunflower oil the highest amounts of β-tocopherol were highlighted. Soybean and corn oils contain greater amounts of γ and δ–tocopherol. Various forms of grape seed oil contain significant amounts of tocotrienols in all forms. In corn oil, about the α-tocopherols of the unsaponified samples, a reduction of the free forms is observed, passing from raw to refined oil, with a consequent increase in esterified forms. The same thing occurs in soy oil, that is, α-tocopherols both saponified and not. In grape seed oil there is a reduction in the presence of tocopherols and tocotrienols of saponified samples (α, β and γ) from raw to refined oil, with a reduction in free forms and an increase in esterified. For non-saponified forms there is a fluctuating trend. Only in the case of Marseglia sunflower oil, for unsaponified α-tocopherol there is a reduction passing from raw to refined, with a decrease in free forms and on the contrary an increase for esterified ones.

6. FINAL REMARKS

The PhD project consists of the determination of genetic (cultivars), technological (extraction techniques) and refining factors that influence the quality of olive oil and other vegetable oils. In the first part of this project, 11 monovarietal extra virgin olive oils will be compared, highlighting the differences in terms of chemical-physical composition, sensory and verifying how the cultivar can influence for 50%, together with other factors, the chemical and sensory composition as well as the content of bioactive substances. In the second part of this work, the differences in the main chemical components (acidity, peroxides, Rancimat induction time, tocopherols, volatile organic compounds) between traditional EVO, pitted and pitted with the addition of kernels, in various storage phases (in the bottling phase and after 3,6 and 12 months) were examined. In this way the higher quality of the pitted oil, its greater shelf-life, its organoleptic characteristics like the traditional and the worst quality of the pitted with subsequent addition of kernels were highlighted. Likely, the cause is due to the presence of oxidizing substances. In addition, two enzymes, polyphenol oxidase (PPO) and peroxidase (POD), are highly concentrated in the olive kernel. PPO and POD can oxidize phenolic compounds resulting in a reduction in the phenolic concentration of the oil. The pitting process, excluding the olive seed before kneading, partially removes the peroxidase activity in the pastes and thus oxidation. In the third part of the project, the effects of various refining phases (oil as it is, refining, drying, discoloration, deodorization, neutralization) on various seed oils (sunflower, high oleic sunflower, corn, grape seeds, soybeans) were highlighted. This is through the analysis of the parameters acidity, peroxides, induction time with Rancimat apparatus, tocopherols and tocotrienols. With this research we will try to improve the innovative production technologies of the oil.

These were the scientific evidence of the present work regarding olive oil:

INFLUENCE OF CULTIVAR

- Differences found across MEVOOs were attributable only to the factors related to the genetic background of the olive cultivar. The findings highlighted the impact of genetic background of the olive on fatty acid, TAG and phenolic compositions of the oils. Across the investigated oils, Marzio stood out from the rest resulting the significantly most bitter, pungent, fruity and the richest in phenolic compounds. The high phenolic level conferred it a good oxidative stability although it presented the highest unsaturation index.

INFLUENCE OF PITTING

- The pitted oil has a lower peroxide content than the others, a greater conservation as the induction time is greater, but a slightly higher acidity. These considerations remain valid both in the bottling phase and after 3,6 months. After 12 months, the beginning of secondary oxidation and the end of primary oxidation begins to be observed, which involves a reduction in acidity and an increase in induction time. This behavior is also observable for traditional oil, while the presence of kernels, which contain substances with oxidizing action and enzymes that reduce the content of polyphenols (with antioxidant capacity), make the behavior of the pitted and added kernel oil completely different from the others.
- About volatile organic substances, pitted oil contains a greater amount of hexanal and 2-hexenal, which contribute to improving the organoleptic qualities of the latter, thanks to the green aromas, cut grass, fruity etc. Even the nonanal, more present in pitted oil, is responsible for aromas. In pitted oil + kernels, on the other hand, hexanol is more present, with its resin, floral, green, grass, fruit, aromatic, banana, alcoholic, rough, astringent, soft, sweet, tomato aroma.
- Tocopherols are substances with antioxidant action and in general tend to decrease with increasing storage period. A greater initial quantity of α-tocopherols is observed in pitted and pitted oil with kernels, but their decrease is particularly evident, with increasing storage period, in pitted + kernels due to the presence of enzymes in the core that destroy α-tocopherol. After 6 months it is the pitted oil that shows the highest levels of a-tocopherol, and always after 6 months the fastest decrease is obtained for all oils. γ-tocopherol is instead more present in traditional oil and in this case a decreasing trend is observed in all oils as the storage period increases. The decrease is especially noticeable in traditional oil after 6 months.
- Ultimately, pitted oil has a higher quality and better shelf life, superior organoleptic qualities but on the other hand a slightly higher acidity. In addition, production technologies have lower yields and higher costs.

EFFECT OF REFINING OF VEGETABLE OILS

- As reported in other works (Marrakchi et al., 2015), (Cappelli et al., 2000) the discoloration phase removes, among the VOC, the tocopherols, the neutralization removes the free fatty acids. A small excess of sodium hydroxide inhibits saponification, with beneficial effects on discoloration and oil quality. Discoloration reduces the presence of peroxides and increases the acidity of the oil
- Refined vegetable oils generally exhibit a lower acidity (and therefore a lower tendency to lose integrity and above all the possible edibility of these oils) and higher induction times (and therefore a higher shelf life). The trend depends rather on the type of oil: for example, sunflower

oil, in any form, has obtained induction times always greater than 6 hours, crude corn oil even greater than 9 hours.

- Regarding peroxides, here too the level is rather linked to the type of oil, but in general the level of peroxides is higher in raw oils than in refined ones.
- About tocopherols and tocotrienols, the saponification in general increases the availability of tocopherols and tocotrienols. In crude and neutralized sunflower oils, the highest amounts of α-tocopherol were highlighted. In crude sunflower oil the highest amounts of β-tocopherol were highlighted. Soybean and corn oils contain greater amounts of γ and δ–tocopherol. Various forms of grape seed oil contain significant amounts of tocotrienols in all forms. In corn oil, about the α-tocopherols of the unsaponified samples, a reduction of the free forms is observed, passing from raw to refined oil, with a consequent increase in esterified forms. The same thing occurs in soy oil, that is, α-tocopherols both saponified and not. In grape seed oil there is a reduction in the presence of tocopherols and tocotrienols of saponified samples (α, β and γ) from raw to refined oil, with a reduction in free forms and an increase in esterified. For non-saponified forms there is a fluctuating trend. Only in the case of Marseglia sunflower oil, for unsaponified α-tocopherol there is a reduction passing from raw to refined, with a decrease in free forms and on the contrary an increase for esterified ones.

The search for agronomic and genetic, technological factors and the possible refining of an oil not suitable for feeding are the requirements that allow to modify the quality parameters of the oils for the better. These factors are partly unchangeable (for example cultivars), while acting on modifiable factors it is possible to obtain an oil of higher quality and with health characteristics for human life, thanks to the content of bioactive substances. An area of active research could be the search for the best cultivars to obtain an oil with certain characteristics. Another area could be the expansion of the parameters to be analyzed to verify further properties of the oils (for example fatty acids, phenolic profile, etc.). All this is aimed at improving production technologies (better yield, lower costs, more qualitative products).

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