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*Review*

# Veiled Extra Virgin Olive Oils: Role of Emulsion, Water and Antioxidants

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**Abstract:** This review traces the current knowledge on the effects of various factors and phenomena that occur at interface, and the role of dispersed phase on the physicochemical, sensorial and nutritional characteristics of veiled extra virgin olive oil (VVOO). Since 1994 there have been numerous articles in the literature regarding the peculiar characteristic of unfiltered olive oil, so-called veiled or cloud virgin olive oil. It is a colloidal system (emulsion–sol), where the continuous lipidic phase dispreads mini droplets of milling water, fragments of cells and biotic fraction obtained from oil processing. During storage, the dispersed phase collapses and determines the quality of the virgin olive oil (VOO). The observed phenomena lead to worsening the quality of the product by causing defects such as oxidation of phenols, triacylglycerols hydrolysis and off-flavor formation. The addition of bioactive compounds, such as vitamins, on product based on VVOO, must take into account the eventual synergistic effect of individual substances. The role of the interphase is crucial to the synergic activity of bioactive molecules in improving oxidative stability, sensorial and health characteristics of VVOO.

**Keywords:** water-in-oil emulsion; olive oil; antioxidant activity; veiled olive oil

## 1. Introduction

Extra virgin olive oil (VOO) is the product obtained by milling of drupes from *Olea europea oleaster*. It is generally considered as resistant to oxidative degradation due to the low content of polyunsaturated fatty acids. Extra VOO is, indeed, characterized by a high content of monounsaturated fatty acid (oleic acid) and by the presence of minor components represented by natural antioxidant α-tocopherol and phenolic compounds like hydroxytyrosol, tyrosol, caffeic acid and others [1]. Small amounts of linoleic and linolenic acids make the oil susceptible to oxidation and to oxidative degradation. As well as resulting in the development rancid odors and flavors, lipid oxidation decreases the nutritional quality and safety of the oils [2,3]. The rate of the oxidation reactions can be controlled through different strategies.

Fresh-milled olive oils appear to the human eye as a cloudy system (colloidal systems), due to cellular fragments and small droplets of vegetation water remaining suspended in the oil matrix. Such cloudy extra VOO is also indicated as veiled extra virgin olive oil (VVOO), so appreciated for its health benefits and esteemed aromas or genuine flavors. Unfiltered VOO is unique among vegetable oils that are consumed without any refining process. VVOO presents higher resistance to oxidation than the filtered ones, indicating the significance of internal structure to the final quality of the product. Depending on the intrinsic characteristics and the storage conditions, VVOOs convert over a period of weeks to months, from straw-yellow to bright-green in color a product of limpid aspect due to the separation (precipitation) of the suspended aqueous phase.

Olive oils are mixtures of triglycerides of several fatty acids and small quantities of minor components. The extraction process affects the final characteristics of the product. Among the minor components, in addition to the natural antioxidants recalled above, there are either amphiphilic or even hydrophilic molecules that, together with some remaining water, induce colloidal association within the lipid phase. This local polar structure hosts active molecules that influence the quality and the stability of the veiled olive oils [4].

The activity and the amount of dispersed water are essential to chemical and enzymatic reactions and to microorganisms' growth. This aspect was investigated by Breschi et al. [5] who associated the degree of turbidity, the amount of solid particles, the microbial contamination and the amount of phenolic substances with a different level of VVOO degradation during storage. Furthermore, the proteins and peptides present in olive oil may contribute to its oxidative stability. The values found by Koidis et al. [6] demonstrated that cloudy olive oils have a higher phosphorus content compared to the filtered ones and were less prone to oxidation. The lower susceptibility to oxidation of the cloudy olive oils was attributed to the higher levels of polar phenols. Considering the values of phospholipids found in the filtered olive oil samples, it was deduced that the phosphatides had a small or no effect on the overall oxidation stability. Contrasting views are reported in literature concerning the role of minor constituents including phenolic and volatile compounds. Cryo-SEM investigations by Veneziani et al. [7], on veiled oils showed the presence of microdispersed water particles that did not contain apparent vegetable fragments. By the end of the storage period, the changes in the quality parameters showed no negative effects on the oxidative stability of the veiled oils compared to the filtered oils. Nevertheless, a higher phenolic concentration in VVOOs was detected at the end of the storage period compared to filtered samples. Because vegetation water is rich in polyphenols, the filtration of fresh veiled olive oil decreases the oxidative stability, correlated to a loss in total polyphenol content. Consequently, a greater increase in peroxide values (PVs) and also a faster decrease in total polar phenol content in filtered samples are expected. However, the phenolic fractions, and their evolution during storage, indicate interesting differences in the individual compounds between the filtered and veiled olive oils. It is suggested that, not only the higher content, but also the forms of individual phenols liberated in veiled oils, due to hydrolytic processes, are responsible for the high oxidative stability of VVOOs [8].

In the last three decades, the literature has reported several articles regarding the peculiar characteristic of VVOO, while colloidal science developed in parallel. This research outlines the key knowledge on the effects of various factors and phenomena that occur at interface, and the role of the dispersed phase on the physicochemical, sensorial and nutritional characteristics of VVOO.

## 2. Extra Virgin Olive Oil

During ripening, chemical and synthesis reactions of organic molecules occur in mesocarp of olives, where large vacuoles accumulate lipids. Figure 1 shows the composition of olive oils. They are predominantly composed of triacylglycerols, di- and monoacylglycerols (98–99%) and a mixture of other lipids such as free linear carboxylic acids, hydrocarbons, aliphatic alcohols, tocopherols, β-carotene, pigments, phenolic and volatile compounds (1–2%). All these molecules contribute to the valuable characteristics of the extra VOO.

### *2.1. Saponifiable Fraction*

#### 2.1.1. Triacylglycerols and Their Hydrolysis Products

Olive oils are mainly composed of triglycerides and normally their content in most oils is in the range of 95–98% *w*/*w*. Triglycerides consist of three carboxylic acid molecules esterified to one molecule of glycerol. The lengths of naturally occurring fatty acids chains in triglycerides can be variable, however those with 16, 18 and 20 carbon atoms are the most common. Hydrolysis of triacylglycerols yields fatty acids. Those present in extra VOO have the compositional limits reported in Figure 2:

palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) represent almost the totality of fatty acids. Myristic (C14:0), margaric (C17:0) and arachidic (C20:0) acids are extremely low. In edible virgin olive oil, the levels for C18:1 trans and for the sum of C18:2 trans and C18:3 trans isomers are found in a very small amounts (< 0.05% in each case). The type of fatty acids and their ratio depends on the climate, the variety, the harvest time and the ripeness of the drupe. It seems that oleic acid is formed first in the drupe and there is a strong antagonistic relationship between oleic and palmitic, palmitoleic and linoleic acids [9]. In fact, environment and cultivar characteristics involve widely varying values for most abundant fatty acids: C16:0, 7.8–18.8%; C18:1, 58.5–83.2%, C18:2, 2.8–21.1%; C18:3: 0.42–1.91%.

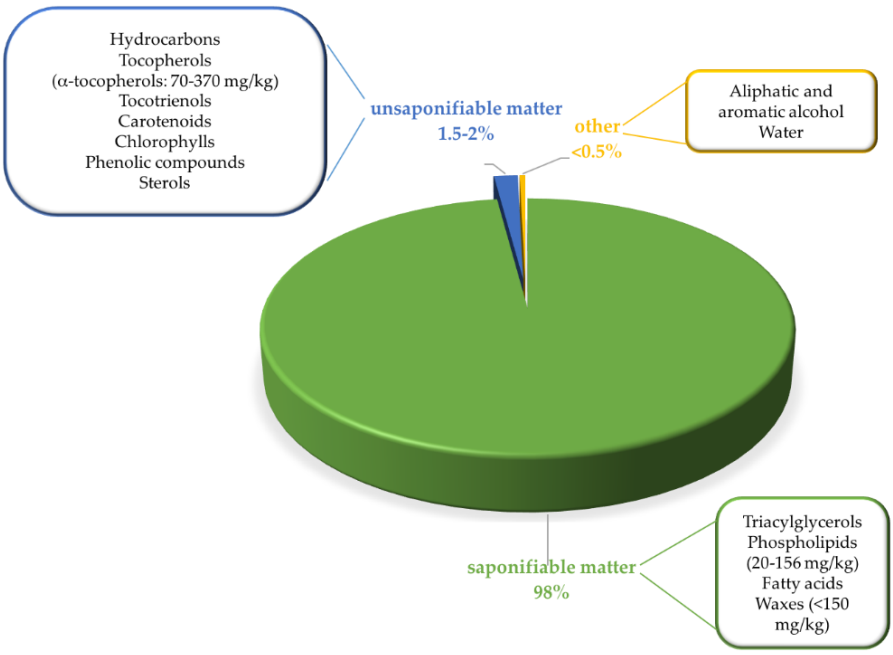


**Figure 1.** Olive oil composition.

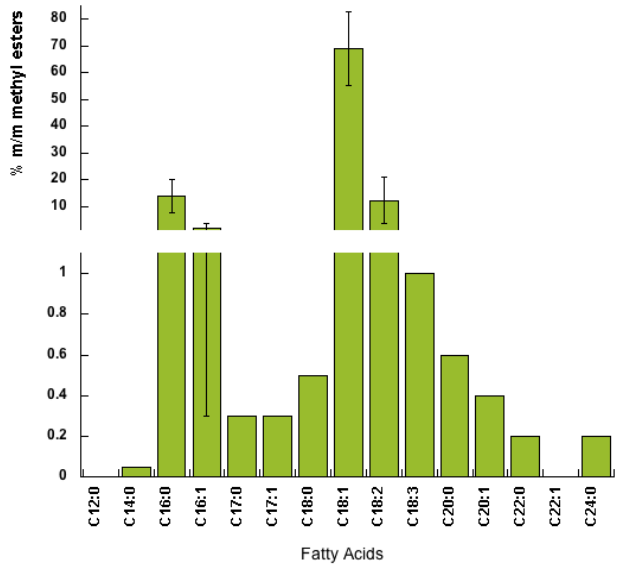


**Figure 2.** Fatty acid composition determined by gaschromatography (% m/m methyl esters) International Olive Oil Contest (IOOC) (2003).

The presence of mono- and diglycerides in olive oil is due either to partial triacylglycerol biosynthesis or hydrolytic reactions. In virgin olive oil, the concentration of diacylglycerols is lower than 2.8% [10]. Mono- and diacylglycerols have surfactant properties, in fact these molecules have a hydrophilic-lipophilic balance (HLB) of 3.4–3.8 and ≈ 1.8, respectively.

#### 2.1.2. Phospholipids

Phosphatidylcholine, phospatidylethanolamine, phosphatitylinositol and phosphatidyl-serine were reported to be the major phospholipids present in olive oil [11]. Their presence is important because these compounds can have antioxidant activity. In accordance with the studies of Pokorný et al. [12], these lipids can regenerate some antioxidants (α-tocopherol or other phenols). Nevertheless, at high levels, phospholipids seem to provoke foaming or browning during cooking. Phosphorous is found in veiled, filtered and refined olive oils (approximately at levels of 20–156 mg phospholipids/kg) [6]. The higher levels of phospholipids found in the veiled oils produce a synergistic effect with phenols against the oxidation process [8,13].

#### 2.1.3. Waxes

Waxes are high-molecular-weight esters that are constructed from carboxylic acids and alcohols with long hydrocarbon chains (C42 + C44 + C46 ≤ 150 mg/kg in virgin olive oil) that endow these compounds with high melting points as the result of extensive, intermolecular London dispersion forces between the hydrocarbon tails. The presence of waxes in olive oil contributes to the turbidity of some samples, such as those extracted of olive pomace [14].

### *2.2. Unsaponifiable Fraction*

#### 2.2.1. Hydrocarbons

The most abundant hydrocarbon in olive oils is squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22 -tetracosahexaene). It is the last metabolite preceding sterol ring formation. Its presence is regarded as partially responsible for the beneficial health effects of olive oil and its chemo-preventive action against certain cancers [15,16]. It is the major constituent of the unsaponifiable matter and is 90% of the hydrocarbon fraction [17,18]. It ranges from 200 up to 12000 mg/kg [18].

Squalene content depends on olive cultivar [19,20] and technology of oil extraction [21] and it is considerably reduced during the process of refining [18,22]. Excluding squalene, the other hydrocarbon fraction of VOO is mainly made up of di- and triterpene hydrocarbons [18].

#### 2.2.2. Tocopherols and Tocotrienols

Tocopherols and tocotrienols are monophenolic liposoluble compounds with variable antioxidant activities. In plants, there are four common types of tocopherols and tocotrienols, namely alpha (α), beta (β), gamma (γ) and delta (δ), all having vitamin E activity. In olive oil, α-Tocopherol (90% of the total tocopherol) is found in the free form. The levels reported indicate a wide range of milligrams α-tocopherol per kg oil (70–370 mg/kg) [23] that depends on the cultivar and technological factors. This fact is important considering the antioxidant properties of the α-homologue [24,25]. In addition, low amounts of the homologues β-tocopherol (~10 mg/kg), δ-tocopherol (~10 mg/kg) and γ-tocopherol (~20 mg/kg) are found.

The levels of α-tocopherol may be associated with the high levels of chlorophyll pigments and they can be involved in deactivation of singlet oxygen [26]. Tocopherol concentration seems also to depend on the ripeness and extraction system [27,28]. Refining and hydrogenation processes cause loss of vitamin activities [29,30].

#### 2.2.3. Pigments (Chlorophylls and Carotenoids)

VOO color is due to the presence of pigments (chlorophylls and carotenoids). They are influenced by olive cultivar, maturation index, production zone, extraction system and storage conditions. Light exposure decolorizes green pigments [31]: Very small amounts of chlorophyll can only be found in freshly extracted oils. The main carotenoids present in olive oil are β-carotene and lutein [32–35].

#### 2.2.4. Phenolic Compounds

The phenolic compounds found in olive oils are polar molecules commonly referred as "polyphenols" although not all of them are polyhydroxyphenyl derivatives. Furthermore, they are a complex mixture of compounds with different chemical structures obtained from the oil by extraction with methanol-water. Phenolic compounds are associated with oil stability as well as biological and health-related properties. The latter have received much attention and today numerous phenolic compounds contained in the oil, mainly tyrosol and its derivatives, are being thoroughly investigated with the aim of establishing a relationship between dietary intakes and decreasing risk of cardiovascular disease or cancer. Ongoing and completed studies in this area associate these phenols with the beneficial role of olive oil for human health. The phenolic content varies considerably and depends on cultivar and extraction and also the refining processes [36,37].

#### 2.2.5. Sterol

Sterols are important lipids related to the quality of the oil and broadly used for checking its genuineness. Vegetable sterols, known as phytosterols, are structural analogues of cholesterol, the main sterol in animal tissues. The type and amount of phytosterols (e.g., stigmasterol, β-sitosterol, campesterol) contained in natural vegetable oils vary with the source of the oil [38]. Phytosterols are poorly soluble in lipids and almost insoluble in water.

### *2.3. Other Minor Compounds*

Various minor constituents have been found only in the fresh oil and these include aliphatic and aromatic alcohol.

#### 2.3.1. Volatile and Aroma Compounds

Several studies highlight the presence of volatile compounds in olive oil [39–42], in which hundreds of them can be identified. They are hydrocarbons, alcohols, aldehydes, ketones, acids, esters, ethers, furan derivatives and thiophene derivatives, which contribute to the aroma and to the flavor of VOOs also with sensory defects [42–44].

Hexanal, (E)-2-hexenal, (Z)-3-hexenal, hexan-1-ol, (Z)-3-hexen-1-ol, hexyl acetate and (Z)-3-hex-enyl acetate are the most abundant volatile compounds, responsible for the fresh and fruity taste of the VVOO aroma. On the other hand, a greater number of volatile compounds have been found in VOOs of poorer quality. The main volatiles responsible for the "rancid" off-flavor are aldehydes that are the decomposition products of linolenic, linoleic and oleic acid hydroperoxides.

The synthesis of these compounds is affected by the cultivar, the degree of ripeness, the storage time of fruits prior to oil extraction and by the processing [39,45–51].

Other compounds that constitute the volatile fraction are ethyl esters. In fact, olive oils obtained from altered drupes or olive pomace contain high levels of ethyl palmitate, ethyl oleate and ethyl linoleate. The levels of these esters in extra VOO are low [52].

#### 2.3.2. Proteins

Different research groups have investigated the role of proteins, peptides and enzymes as minor components of VOO during the last decades [53–57]. They reported the presence of evident catalytic

activity of oxidizing enzymes and polyphenol oxidase in raw prepared olive oils, notwithstanding the hydrophobic environment.

Hidalgo et al. [58] emphasize that proteins and peptides are habitually considered among minor constituents, as impurities of olive oils. Currently, their presence is considered noteworthy due to the oxidation stability and the potential allergenicity. Nevertheless, a high percentage of proteins and peptides are lost during the extraction process and are not present in the refined commercial olive oils.

#### 2.3.3. Water

Usually, water can be found as trace amounts in raw and commercial olive oil mainly due to the extraction and refining processes. Dispersed water (below 1–2%), especially in VVOO may change during storage, can settle on the bottom and is absorbed from the environment or lost from the oil, if the container is open. Because water is practically immiscible with oil, it is probably found in association colloidal form stabilized by endogenous surfactants.

## 3. Veiled Extra Virgin Olive Oil

VVOO extraction produces an emulsion-sol suspension/dispersion system, which can persist for weeks or several months before the dispersed phase forms a muddy sediment. While in the past, the raw olive oil market was limited, nowadays it is expanding also because attentive consumers consider VVOO a natural product with higher nutritional value [59]. Fresh VOO contains a tyrosol derivative (oleocanthal), that has a similar chemical structure and pharmacological properties to Ibuprofen, a nonsteroidal anti-inflammatory compound, also related to the stinging sensation in the back of the throat (pungency) [60].

VVOOs are not traditionally filtrated, and not sedimented in steel barrels, but they are packaged and commercialized without any filtration processes or only after a mild separation to remove the coarse particles through cellulose or press filter. Sometimes, in such cases, it is possible to adjust the droplets size of the dispersed phase. There are various brands specializing in the trade of packed raw virgin olive oil with varying sensorial characteristics (e.g., spicy, bitter and fruity smell and taste, etc.), nonetheless they are generally achieved by using olives at beginning of veraison. Frequently VVOO is publicized as “first cold pressed” or “stone made” to highlight that the temperature during the production is kept at 35 °C or lower and the process is traditional, although the International Olive Oil Council states that all VOOs are first cold pressed.

Several authors have described that various compounds can act in freshly produced VOOs as natural surfactants such as partial glycerides [61], phospholipids, glycoside derivates and proteins [6,62,63].

The physicochemical properties of such compounds and the kinetically stable emulsions may allow an increase in the transfer between interfaces of hydrophilic molecules, such as phenols, which are strong antioxidants. Some studies suggest that small quantities of proteins may contribute to the higher oxidative stability of unfiltered oil [64]. Nevertheless, there is a discrepancy in the literature concerning the level of proteins present in olive oil (from 0.1 to 400 mg/kg), [53,55].

A lipoxygenase activity has been detected in freshly prepared olive oil [54]. In spite of this, the presence of a small quantity of water in the nonfiltered oils is a favorable condition for enzymatic activities—these oils have higher oxidative stabilities. The polar phenolic compounds not only act as antioxidants but also as inhibitors of oxidizing enzymes.

However, the presence of water in unfiltered oils leads to a more rapid hydrolysis of these water-associated phenolics and of the triglycerides. Enzymes present in particles of olive fruit and microorganisms such as yeasts accelerate the quality degradation of the VOO increasing the free acidity and the rancidity. The overall effect of filtration on olive oil durability is controversial, with different studies coming to different conclusions, and likely also depends on the specific systems used to filter the oil [4].

## 4. Olive Oil Emulsions

Emulsions are colloidal dispersions of two immiscible liquids, such as oil and water, in which one is present as fine droplets dispersed in the other that acts as continuous medium [65]. The interesting properties of such systems allow their application in several fields, from the use of emulsion in chemical and pharmaceutical industries [66,67] to their food and biotechnological applications [68,69]. These colloidal dispersions can be classified based on the dispersed phase as oil-in-water (O/W), when they exhibit oil droplets dispersed in an aqueous phase, or water-in-oil (W/O) when the opposite occurs. An example of VVOOs, a water-in-VOO (W/O) emulsion, with 3% *w*/*w* of added water, is represented in Figure 3.

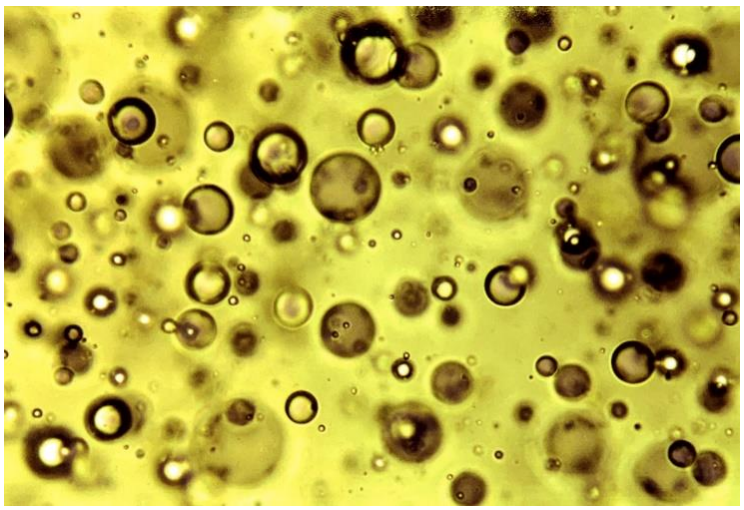

**Figure 3.** Microdroplets of a water-in-oil (W/O) extra virgin olive oil emulsion with 3% *w*/*w* of water, observed with a light microscope at a magnification of 400, emulsified with Ultra-Turrax. Droplet size distribution is a function of numerous factors, such as time/energy, temperature, type and concentration of surfactants [70].

In order to study the role of the interface on the VVOOs, VOOs can be artificially veiled stabilizing different water amounts through the addition of edible emulsifiers or surfactants [71].

Emulsion stability is related to the surfactant used. Thus, according to the type of emulsion the most suitable emulsifier can be selected based on the hydrophilic-lipophilic balance value, known as HLB [72]. Usually, high values of HLB individuate surfactant with hydrophilic characteristics, mostly used for O/W emulsions, while low values of HLB are associated with surfactant suitable for stabilizing W/O systems. The most common surfactants in the food industries are polysorbates, sugar esters, lecithins and other polymers.

The nature of the interface formed by the surface-active agent, so-called the physico-chemical properties, will have a large impact on the rate of lipid oxidation by influencing the location and reactivity of free radical scavengers, pro-oxidative transition metals, lipid hydroperoxides and metal chelators. [65].

As reported in some studies [73], it is possible to use also a combination of surfactants to obtain systems with enhanced stability. Mosca et al. [74], showed how the interaction of the hydrophobic tails of Span 80 (Sorbitan Oleate) and Tween 20 (Polyolaetilene-20-sorbitan monolaurate) can stabilize O/W emulsions and how water-soluble pro-oxidants added to the system can easily go through the oil phase. Among the hydrocolloids, sodium caseinate, characterized by good solubility and suitable surface properties when combined with a non-ionic surfactant (Tween 20) generated more oil/water interface helping to obtain stable emulsions also at acidic pH closed to caseinate isoelectric point. O/W emulsion so obtained, selected for their stability, can be used as a carrier for hydrophilic active compound that can be dispersed in the oil phase [75,76]. On the other hand W/O emulsion can also

be stable [77] and used in various applications [68,78,79]. A stable emulsion is one in which there is a uniform distribution of the dispersed globules throughout the continuous phase. Figure 4 shows major types of chemical-physical instabilities that can occur in VVOOs.

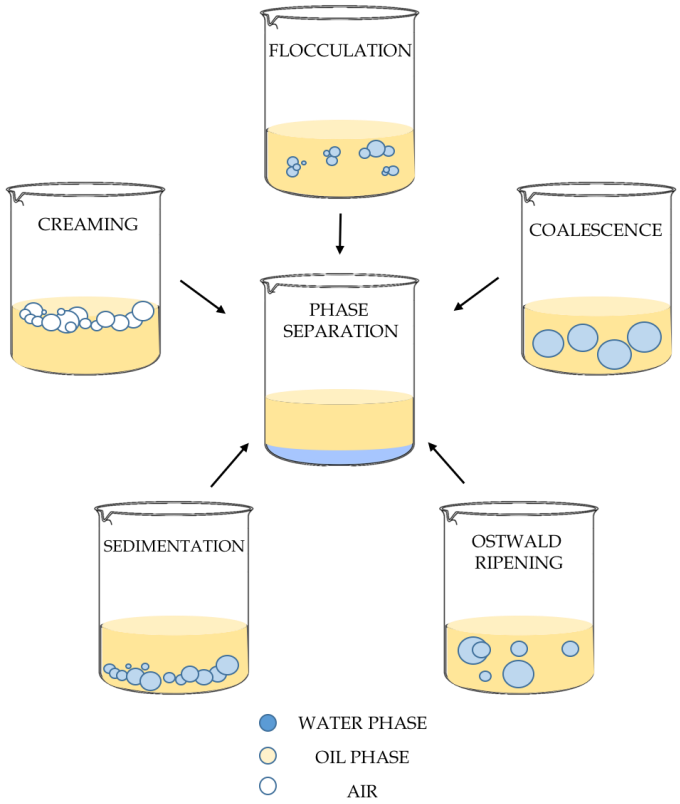


**Figure 4.** Different mechanisms of emulsion destabilization, depending on the particle size and dispersed phase concentration.

Actually, the "veiling" of VOO gives positive effects because droplets act as an antioxidant, reducing the PVs as evaluated in some studies (commented in-depth below) after the application of UV to stimulate the oxidation process in VVOO compared to filtered oil samples [80]. Thus, also the way in which water is dispersed in the oil matrix can affect the interfacial properties of the emulsion system.

In veiled oil, the importance of water droplets' dimension is fundamental for the correlation of the water/oil interface obtained, in order to see how the oxidative degradation of the oil could be affected and if water droplets have a stabilizing role in respect to oxidation [80,81].

Furthermore, thinking of practical application of emulsions, the material that "veils" the extra VOO can represent an ideal food additive, acting as an acidity buffer and antioxidant, without affecting the taste and quality of the vegetable fat [82] thanks to the low amount added. In this respect, it is possible to enhance a food matrix such as extra VOO obtaining a product characterized by higher quality and longer stability [83].

Katsouli et al. [84], observed also that the presence of phenolic acid in the aqueous phase of W/O emulsions leads to a significant decrease of the mean droplets diameter, a positive aspect as larger droplet tends to cream faster than smaller ones, so a more stable system is obtained. Together with polyphenols, olive oil endogenous amphiphiles can migrate and concentrate to the oil-water interface of emulsified systems to form a more energetically favorable structure [85].

In general, as the number of small droplets increases, the greater the interfacial area will be [86,87]. Therefore, the water phase could be enriched with natural or spiked antioxidants, pro-oxidants or other compounds, while in the lipidic phase hydroperoxides can be present. The latter are able to accumulate at the emulsion droplet interface; here the interface acts as a boundary where physicochemical processes

of great interest take place. Indeed, the formulation strategy and the partitioning of the emulsifiers are fundamental to highlight unambiguously the effect of the interface [88] because the type of surfactant for the production of VVOO will lead to the formation of a sort of physical wall, which separates the hydrophilic from hydrophobic compounds [89]. Hence, the interfacial region represents the critical area where the oxidation of the lipid phase is promoted.

Even if it was shown that VVOO presents higher oxidation stability compared to the plain one, some authors indicate that the oxidation rate of W/O emulsion can be higher due to the increase of the surface area, where interaction between lipids and water-soluble transition metal could be facilitated. [80] Therefore, the correlation between the structure of the water/olive oil emulsion and the oxidation rate is pointed out and full knowledge of the oxidation process could help to prevent negative phenomena.

## 5. Oxidations in Water in Oil

Oxidation is the major problem affecting VVOO because it causes important deteriorative modifications in its chemical and nutritional characteristics. Autoxidation and photo-oxidation occur in the presence of oxygen and when lipids oxidize, they form hydroperoxides, which may undergo further oxidation or decomposition into secondary products such as aldehydes, ketones, acids and alcohols. The relative oxidation rates depend on the degree of unsaturation, e.g., at 100 °C an 18 carbon atoms fatty acid would be: 18:3 (3000) > 18:2 (1000) > 18:1 (100) >> 18:0 [90].

Oxidation rates of unsaturated fatty acids subjected to photo-oxidation and autoxidation depend on the degree of unsaturation and generally have a ratio of $10^4$ to 1 at storage temperature (~20°), respectively [91]. Various catalytic systems and initiators facilitate the oxidation of lipids. These include light, pigments, temperature, proteins, enzymes, microorganisms and transition metals such as iron, cobalt, nickel and manganese. In these reactions free radicals or reactive oxygen species appear. The oxidation reactions can happen either in the dark or in the presence of light, showing differences in their oxidation pathway.

VVOO is resistant to oxidative degradation due to a high monounsaturated-to-polyunsaturated fatty acid ratio and to the presence of natural antioxidant components such as α-tocopherol and phenolic compounds. However, oxidative degradation in olive oil involves in particular polyunsaturated fatty acids, the most important cause of deterioration in the quality. Once started, autoxidation cannot be stopped but can be only slowed down.

The oxidation process can be distinguished in three steps:

I. Initiation. In the first step, a fatty acid radical is produced by homolytic C-H bond cleavage. The most noteworthy initiators are reactive oxygen species, such as OH• and HOO•, which combine with a hydrogen atom to make fatty acid and water radical.

$$\text{RH + initiator (oxygen, metal, etc.)} \rightarrow \text{R}^\bullet + \text{H}^\bullet \text{ (initiator)} \quad (1)$$

II. Propagation. The fatty acid radical binds quickly with $O_2$, creating a peroxyl-fatty acid radical. This is an unstable molecule that reacts with another C-H bond, producing different lipid peroxide and radical, or cyclic peroxide if it forms a bond with itself. This reaction continues until the lipid radical reacts in the same way (chain reaction mechanism).

$$\text{R}^\bullet + \text{O}_2 \rightarrow \text{ROO}^\bullet \quad (2)$$

$$\text{ROO}^\bullet + \text{RH} \rightarrow \text{ROOH} + \text{R}^\bullet \quad (3)$$

III. Termination. The radical reaction finishes when two radicals join together and generate nonradical molecules. This occurs only when the amount of radical species is high enough to allow a high probability of collision of two radicals. Dimers, ethers and peroxide lipid are formed.

$$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR \quad (4)$$

$$RO^{\bullet} + R^{\bullet} \rightarrow ROR \quad (5)$$

$$R^{\bullet} + R^{\bullet} \rightarrow RR \quad (6)$$

R = lipid alkyl; species marked with (•) are radical molecules.

When an oxidation reaction is examined in an emulsion system, it is necessary to consider, in addition to what has been supposed above, how the interface can influence the whole process. In fact, the species that are activated and the new ones dispersed in the droplets can influence the progress of the reaction.

Ambrosone et al. [80] used different emulsifying techniques to study the influence of water on the oxidative degradation of extra VOO. Several emulsions of water-in-VOO, were formed by dispersing water differently and oxidizing through $O_2$, UV radiation and air. Oil oxidation, monitored by measuring the PVs and polyphenolic content, was greater for emulsions obtained with low dispersing power. In Figure 5 PVs are reported as function of the energy supplied to form the emulsions.

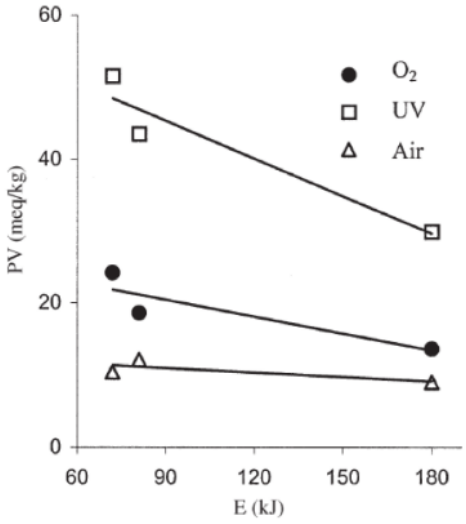


**Figure 5.** Peroxide values (PV) of emulsified extra virgin olive oil as function of mechanical energy supplied to make the emulsions. The symbols in the plot represent the oxidizing conditions. Reprinted from JAOCS, Vol 19, Issue 1. The role of water in the oxidation process of extra virgin olive oils, Pages No 577–582, Copyright (2002), with permission from Wiley and Sons [80].

In any case, there is a negative correlation between PVs and energy supplied to disperse smaller droplets. The major variation of PVs was verified in the emulsion prepared with high energy (smaller droplets) and oxidized by UV light (254 nm). The water/oil interface participates to the oxidation processes and the dispersed water seems to have an antioxidant effect on the oil. Extensive studies have shown that the emulsion structure influences the oxidation processes in emulsified olive oils [92]. The susceptibility to oxidation revealed a dependence on the specific surface area of the water dispersed phase.

Georgalaki et al. [53] in 1998 examined samples of Greek VOOs for the purpose of assessing the presence of proteins and oxidative enzyme activities. In all oil samples, detectable amounts of proteins with low molecular mass (10–40 kDa) and lipoxygenase and polyphenol oxidase activities were found. Since VVOO is an extremely complex matrix, a W/O glycerol trioleate emulsion has

been used as a model to study the effect of ascorbic acid on light-promoted oxidation of the oil [93]. The system consisted of 3% *w/w* water dispersed in oil containing 0.5% *w/w* sodium oleate/oleic acid mixture (20/80 mol/mol%) as a stabilizer. Results demonstrated that ascorbic acid activity depends on its concentration and on the characteristics of the W/O interface. In the presence of ascorbyl palmitate or sorbitan trioleate (Span 85) in the continuous phase, ascorbic acid activity increases in the first few hours of oxidation. Mosca et al. [83] identified a correlation between the composition of the dispersed phase and the emulsion kinetic and oxidative stability. The water phase contained polyphenolic extracts from olive mill wastewater or green tea leaves. Emulsion oxidative stability was examined by activating the oxidation reaction in the oil phase with the lipophilic radical initiator AMVN (2,2'-azobis(2,4-dimethylvaleronitrile)). UV-vis turbidity measurements, allowed to study the effect of antioxidant dispersions on emulsion kinetic stability. In general, antioxidant dispersions delayed the oxidation reaction differently, independent of their oxygen radical adsorption capacity (ORAC) values and their components' amphiphilicity. The results emphasize that the aqueous polyphenol extract from olives had a better action on kinetic and oxidative stability. Moreover, the main role of the interfacial properties of olive oil polyphenols was highlighted. Papadimitriou et al. [85] studied, for the first time, the structure and dynamics of the colloidal dispersions in VVOO with different scattering techniques and related them to the extraction conditions applied by the olive oil producers. Samples of discrete droplets were found but no anisotropic crystals could be observed. Finally, radical scavenging activity showed that the antioxidant capacity of the VVOO was higher than the one of the filtered oils. Results show that dual-phase oil extraction system seems to be more appropriate for the production of stable and rich-in-minor-constituents olive oils.

In Table 1 studies on oxidation and kinetics stability in W/O emulsions are summarized.

**Table 1.** Examples of oxidation and kinetics stability of W/O emulsions.

| Samples | Emulsification Technique | Oxidizing Conditions | Analyzed Parameters | Ref. |
|---|---|---|---|---|
| Natural olive oil; Filtered olive oil;Emulsified olive oil (water 3% *w/w*) | Ultra-Turrax Vortex Rotatory mixer | $O_2$ UV light Air | PVs; Polyphenol content | [80] |
| Natural olive oil;Filtered olive oil;Emulsified olive oil (1.5% *w/w* water) | Ultra-Turrax | UV light | PVs; Size distribution (optical microscopy) | [92] |
| w/o emulsion *oil phase*: glicerol trioleate (sodium oleate/oleic acid) *water phase*: Ascorbic acid, *Surfactant*: Span 85, Ascorbyl palmitate | Ultra-Turrax | UV-light | PVs; Percentage of Inhibition; Size distribution (optical microscopy) | [93] |
| w/o emulsion *oil phase*: olive oil + Span 80 *water phase*: water + Tween 80 enriched with antioxidant extract (caffeic acid, aqueous phenolic extract of olive oil, green tea leaves and olive mill waste) | Ultra-Turrax | Radical initiator AMVN (2,2-azobis(2,4-dimethylvaleronitrile) | Hydroperoxide concentration (Fluorescence spectroscopy); oxygen radical adsorption capacity (ORAC); *p*-anisidine; α-tocopherols; Turbidity | [83] |
| Refined olive oil; VOO | Three-phase extraction procedure (oil/externally added water) Dual-phase extraction procedure (no externally added water) | | Static light scattering; Dynamic light scattering; Small Angle X-ray scattering; Confocal microscopy; Radical scavenging activity | [85] |

## 6. Fortification of W/O Emulsions

Food fortification refers to the addition of micronutrients to foodstuff. Generally, this strategy can lead to fast improvements in the micronutrient status of a population, with a low cost, particularly if benefit can be taken of existing technology and local traditional foods.

Among the micronutrients, vitamins are involved in wide range of metabolic pathways in the human body and their deficiency may cause onset of oxidative stress and serious diseases. Vitamin E (α-tocopherol) is a lipid-soluble antioxidant; when introduced in diet, it protects cell membranes against oxidative damage [94] by reducing the formation of hydroperoxides [95]. Vitamin C is a water-soluble antioxidant; it preserves the oxidative-reductive potential and inactivates free radical species. The enhancement of foods with these vitamins is not easy, e.g., vitamin C is degraded by heat

treatments, light and air [96] and vitamin E is definitely oxidized by air [94]. However, when used together, these vitamins have been shown to protect each other through a synergistic mechanism [97].

The development of food matrices with peculiar structures is of primary importance in strategy to overcome micronutrient deficiencies. A heterogeneous system such as emulsion, allows the co-solubilisation of both hydrophilic and lipophilic molecules.

Recently, Cinelli et al. [98] have shown that W/O emulsions are suitable systems for enrichment formulations with antioxidant compounds found in red wine.

Although the activity of vitamins has been widely examined in solutions and in O/W emulsions, [74,99–102], little has been written about the usefulness of vitamin C in W/O emulsions.

In recent scientific literature there are also various studies regarding the synergistic effectiveness of vitamins to prevent the oxidation reactions [97,103–105]. In particular, vitamin C in micellar systems with sodium dodecyl sulfate (SDS) has a noteworthy role in the oxidation of linoleic acid by acting from the aqueous phase to regenerate the vitamin E in the micellar phase [106]. Gitto et al. [107] have studied the action of a mix of melatonin with vitamin C and E, glutathione and desferrioxamine (deferoxamine) in rat liver homogenates. Similarly, Cuomo et al. [25] characterized and investigated on the fortification of W/O emulsion of VVOO with vitamins E and C, highlighting the antioxidant property of a combination of vitamin E and C. Finally, Liu et al. [104] documented how the antioxidant effects of a combination of lycopene, vitamin E, vitamin C and β-carotene were considerably greater than the sum of the individual antioxidant effects. In Table 2 examples of emulsions enriched with active molecules are reported.

**Table 2.** Examples of W/O emulsion enriched with active molecules.

| W/O Emulsion Composition | | | Enriched Compound | Observation | Ref. |
|---|---|---|---|---|---|
| **Aqueous Phase** | **Oil Phase** | **Surfactant** | | | |
| Water (0.7%) + Tween 80 (0.7%) | Olive oil | Span 80 (0.7%) | Green tea leaves extract, polyphenols extract from virgin olive oil, extract from olive mill wastes | The incorporation of all antioxidants made emulsions more stable. The higher antioxidant effect was obtained with the incorporation of enriched compounds. | [83] |
| Water (2–3% *w*/*w*) | Extra VOO (96 and 95% *w*/*w*) | Tween 20 (2% *w*/*w*) | Vanillic acid, caffeic acid, syringic acid (0.1% *w*/*w*) | The loading of caffeic acid in the water phase (at 2%) showed the lowest droplet diameter (251 nm) with long oxidation stability (33.6 h). | [84] |
| Water (2% *w*/*w*) | Extra VOO | Tween 20 (0%, 2%, 4% and 6% g emulsifier/g final emulsion) | Gallic acid Vanillic acid Syringic acid (1% *w*/*w*) | Incorporation of olive oil endogenous compounds in the aqueous phase lowered the surface tension facilitating the nanoemulsion formation. Additionally, the addition of the acids enhanced the kinetic and oxidation stability. | [108] |
| Water (2% *w*/*w*) | Extra VOO (92, 94 and 96% *w*/*w*) | Tween 20 2%–4%–6% | Ascorbic acid Gallic acid (0.5% and 1% *w*/*w*) | Both the bioactive compound affected the surface tension of the aqueous phase. The optimal formulation was provided by 1% of the ascorbic and gallic acid and 4% of Tween. | [109] |
| Water (10 and 20% wt) | Extra VOO | Tween 20 Span 80 (8%, 10% and 12% wt) | Phenolic compound extract from *Hibiscus sabdariffa* | Systems incorporating bioactive compounds extracted from *H. sabdariffa* showed good oxidative stability during one month of storage. | [110] |
| Water (1% *w*/*w*) | Olive oil | Span 80 (1% *w*/*w*) | Wine-dried extract (0.4, 1 and 1.5 mg of extract/g of emulsion) | Increasing the content of wine extract, the oxidation process was slowed down, while parameters such as the size of the dispersed phase were not affected. | [98] |
| Water (1% *w*/*w*) | Olive oil | Span 80 (1% *w*/*w*) | Vitamin E; Vitamin C ($1.5 \times 10^{-5}$ mol and $1.05 \times 10^{-4}$ mol); Vit E + Vit C ($3 \times 10^{-5}$ mol and $2.1 \times 10^{-4}$ mol) | The co-loading of vitamins was very effective as reported by the PVs that remain stable for about 40 days of storage, while when vitamin E was added alone it acts as pro-oxidant. | [25] |

## 7. Product Maintenance

Although the first studies on VVOOs have shown greater oxidative stability compared to traditional oils, subsequently other important aspects, never taken into account, have been considered to understand how to optimize product maintenance starting from the first stages of the oil production process.

To understand the role of different amounts of water and microorganisms on the evolution of VVOO quality during storage, different types of filtration and selective effects are used (individual or combined filtration and high hydrostatic pressure treatments). Guerrini et al. [111] investigating for legal parameters, volatile organic compounds and phenolic compounds during the storage tests of veiled olive oil samples, relate the microbial contamination, with Aw > 0.6, to the formation of volatile aroma compounds, which were responsible for the development of unpleasant off-flavors and odours. Furthermore, high water activity values were associated with an increase in the hydrolytic degradation rate of the phenolic compounds. The oil turbidity had to be planned and controlled, starting from the adjustment of the water content and application of good manufacturing practices.

Zullo et al. [112] demonstrated that the storage of the freshly produced VVOO in closed containers in a fixed or periodically inverted position, influences the composition of the product and the persistence of the characteristic veiled appearance. They investigated changes in polyphenol content, acidity and PVs over a period of one year. Although in the samples of veiled oil stored statically the polyphenols were drastically reduced, in the periodically inverted samples there was only a slight concentration reduction. The free acidity and PVs were slightly increased and related with polyphenol concentration. The reduced quality of veiled oil is due, among other causes, to the activity of bacteria, yeasts and moulds (Figure 6), even if after a year, yeasts survive only in samples at higher concentrations of polyphenols. Additionally, the individual phenols released in veiled oils, due to hydrolytic processes, can be responsible for the unpredicted high oxidative stability of VVOO [61,113].

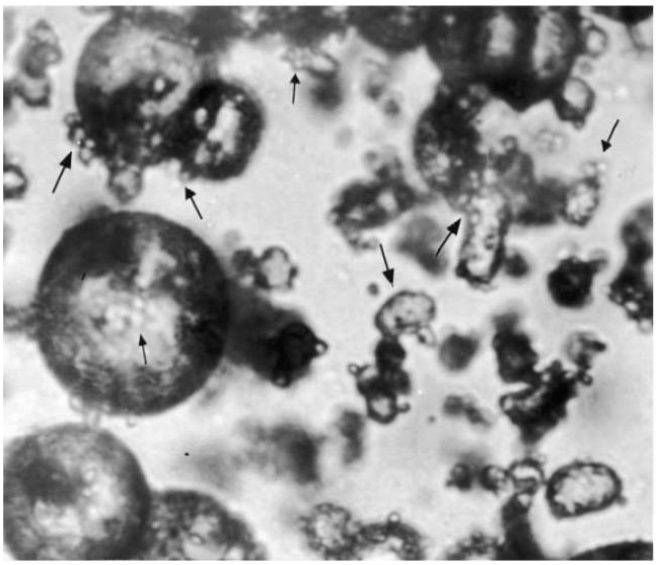

**Figure 6.** Microdroplets of vegetation water and solid particles observed with a light microscope at a magnification of 600 in newly produced olive oil (the arrows show the locations of microorganisms). Reprinted from Food Microbiology, Vol 19, Issue 1, Survival of micro-organisms in extra virgin olive oil during storage, Pages No. 105–109, Copyright (2002) with permission from Elsevier [114].

## 8. Conclusions and Perspective

Although VVOOs are perishable products, a huge number of publications regard remarkable quality and proprieties of veiled olive oils. These benefits come from the dispersed matter and involve physicochemical aspects of colloidal science. The dispersed phase solubilizes compounds that increase

the oxidative and kinetic stabilities of the oil, but on the other hand, they can also trigger hydrolytic phenomena and enzyme activity.

In the dispersed phase, yeasts, bacteria and moulds may be present, influencing the quality of VVOO. Polyphenols, dispersed in the water phase, play a fundamental role in maintaining the original characteristics of the product. The addition of antioxidant substances such as vitamins, in a fortified product based on veiled olive oil, must take into account not only the effect of the individual component, but also the synergistic effect. Briefly, veiled oil has a limited shelf life that could be extended through the addition of antioxidants such as vitamins, enhancing also oil properties for longer periods. Nonetheless, emulsion fortification can certainly improve antioxidant compounds intake to the consumer diet. Among all these considerations, the role played by the W/O interface, in the exchange of reactive species is of primary importance. Certainly it is not easy to keep under control all these parameters, considering the degree of complexity and the variability in all steps of the preparation of veiled olive oils. Many other multidisciplinary studies should be done to understand the complex mechanisms that take place and how to manipulate them to obtain the desired product. A better knowledge of the specific quality characteristics of fresh VVOO must be taken into consideration to promote a traditional product in addition to the various potentials of the novelty of the VVOO and product derived therefrom.

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## References

1. Boskou, D.; Blekas, G.; Tsimidou, M. Phenolic compounds in olive oil and olives. *Curr. Top. Nutraceutical Res.* **2005**, *3*, 125–136.
2. De Leonardis, A.; Macciola, V.; Lembo, G.; Aretini, A.; Nag, A. Studies on oxidative stabilisation of lard by natural antioxidants recovered from olive-oil mill wastewater. *Food Chem.* **2007**, *100*, 998–1004. [CrossRef]
3. De Leonardis, A.; Macciola, V.; Lopez, F. The role of virgin olive oil in the traditional Mediterranean cuisine. In *Virgin Olive Oil: Production, Composition, Uses and Benefits for Man*; De Leonardis, A., Ed.; Nova Science Publisher: New York, NY, USA, 2014; Volume 14, pp. 259–282.
4. Xenakis, A.; Papadimitriou, V.; Sotiroudis, T.G. Colloidal structures in natural oils. *Curr. Opin. Colloid Interface Sci.* **2010**, *15*, 55–60. [CrossRef]
5. Breschi, C.; Guerrini, L.; Domizio, P.; Ferraro, G.; Calamai, L.; Canuti, V.; Masella, P.; Parenti, A.; Fratini, E.; Fia, G.; et al. Physical, Chemical, and Biological Characterization of Veiled Extra Virgin Olive Oil Turbidity for Degradation Risk Assessment. *Eur. J. Lipid Sci. Technol.* **2019**, *121*, 1900195. [CrossRef]
6. Koidis, A.; Boskou, D. The contents of proteins and phospholipids in cloudy (veiled) virgin olive oils. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 323–328. [CrossRef]
7. Veneziani, G.; Esposto, S.; Minnocci, A.; Taticchi, A.; Urbani, S.; Selvaggini, R.; Sordini, B.; Sebastiani, L.; Servili, M. Compositional differences between veiled and filtered virgin olive oils during a simulated shelf life. *LWT* **2018**, *94*, 87–95. [CrossRef]
8. Tsimidou, M.Z.; Georgiou, A.; Koidis, A.; Boskou, D. Loss of stability of "veiled" (cloudy) virgin olive oils in storage. *Food Chem.* **2005**, *93*, 377–383. [CrossRef]
9. Ninni, V. A statistical approach to the biosynthetic route of fatty acids in olive oil: Cross-sectional and time series analyses. *J. Sci. Food Agric.* **1999**, *79*, 2113–2121. [CrossRef]
10. Frega, N.; Bocci, F.; Lercker, G. High-resolution gas-chromatographic determination of diacylglycerols in common vegetable oils. *J. Am. Oil Chem. Soc.* **1993**, *70*, 175–177. [CrossRef]
11. Alter, M.; Gutfinger, T. Phospholipids in several vegetable oils [olive, avocado, cotton, maize, rape]. *Riv. Ital. Delle Sostanze Grasse (Italy)* **1983**, *59*, 14–18.

12. Pokorný, J.; Korczak, J. Preparation of natural antioxidants. *Antioxid. Food: Pract. Appl.* **2001**, 311–330. [CrossRef]
13. Budilarto, E.S.; Kamal-Eldin, A. The supramolecular chemistry of lipid oxidation and antioxidation in bulk oils. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1095–1137. [CrossRef]
14. Parenti, A.; Spugnoli, P.; Baldi, F.; Masella, P.; Calamai, L.; Hattei, A. Preliminary observations on veiled olive oil turbidity with regards to wax content. *Riv. Ital. Delle Sostanze Grasse* **2008**, *85*, 221–228.
15. Rao, C.V.; Newmark, H.L.; Reddy, B.S. Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* **1998**, *19*, 287–290. [CrossRef]
16. Smith, T.J.; Yang, G.Y.; Seril, D.N.; Liao, J.; Kim, S. Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis by dietary olive oil and squalene. *Carcinogenesis* **1998**, *19*, 703–706. [CrossRef]
17. Perrin, J. Minor components and natural antioxidants in olives and olive oil. *Rev. Fr. Des Corps Gras (Fr.)* **1992**, *39*, 25–32.
18. Lanzón, A.; Albi, T.; Cert, A.; Gracián, J. The hydrocarbon fraction of virgin olive oil and changes resulting from refining. *J. Am. Oil Chem. Soc.* **1994**, *71*, 285–291. [CrossRef]
19. De Leonardis, A.; Macciola, V.; De Felice, M.A. Rapid determination of squalene in virgin olive oils using gas-liquid chromatography. *Ital. J. Food Sci.* **1998**, *10*, 75–80.
20. Manzi, P.; Panfili, G.; Esti, M.; Pizzoferrato, L. Natural antioxidants in the unsaponifiable fraction of virgin olive oils from different cultivars. *J. Sci. Food Agric.* **1998**, *77*, 115–120. [CrossRef]
21. Nergiz, C.; Unal, K. The effect of extraction systems on triterpene alcohols and squalene content of virgin olive oil. *Grasas Y Aceites (Spain)* **1990**, *41*, 117–121.
22. Bondioli, P.; Mariani, C.; Lanzani, A.; Fedeli, E.; Mossa, A.; Muller, A. Lampante olive oil refining with supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* **1992**, *69*, 477–480. [CrossRef]
23. Psomiadou, E.; Tsimidou, M.; Boskou, D. α-Tocopherol content of Greek virgin olive oils. *J. Agric. Food Chem.* **2000**, *48*, 1770–1775. [CrossRef]
24. Kamal-Eldin, A.; Appelqvist, L.Å. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* **1996**, *31*, 671–701. [CrossRef]
25. Cuomo, F.; Cinelli, G.; Chirascu, C.; Marconi, E.; Lopez, F. Antioxidant Effect of Vitamins in Olive Oil Emulsion. *Colloids Interfaces* **2020**, *4*, 23. [CrossRef]
26. Grams, G.; Eskins, K.; Inglett, G. Dye-sensitized photooxidation of. alpha.-tocopherol. *J. Am. Chem. Soc.* **1972**, *94*, 866–868. [CrossRef]
27. Psomiadou, E.; Tsimidou, M. Simultaneous HPLC determination of tocopherols, carotenoids, and chlorophylls for monitoring their effect on virgin olive oil oxidation. *J. Agric. Food Chem.* **1998**, *46*, 5132–5138. [CrossRef]
28. Beltrán, G.; Aguilera, M.P.; Del Rio, C.; Sanchez, S.; Martinez, L. Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chem.* **2005**, *89*, 207–215. [CrossRef]
29. Andrikopoulos, N.K.; Hassapidou, M.N.; Manoukas, A.G. The tocopherol content of Greek olive oils. *J. Sci. Food Agric.* **1989**, *46*, 503–509. [CrossRef]
30. Rabascall, N.H.; Riera, J. Changes in tocopherol and tocotrienol content during the extraction, refining and hydrogenation of edible oils. *Grasas Aceites (Seville)* **1987**, *38*, 145–148.
31. Psomiadou, E.; Tsimidou, M. Stability of virgin olive oil. 1. Autoxidation studies. *J. Agric. Food Chem.* **2002**, *50*, 716–721. [CrossRef] [PubMed]
32. Minguez-Mosquera, M.I.; Gandul-Rojas, B.; Garrido-Fernandez, J.; Gallardo-Guerrero, L. Pigments present in virgin olive oil. *J. Am. Oil Chem. Soc.* **1990**, *67*, 192–196. [CrossRef]
33. Ranalli, A. Carotenoids in virgin olive oils effect of technology. *Ital. J. Food Sci.* **1992**, *4*, 53–57.
34. Rahmani, M.; Csallany, A.S. Chlorophyll and β-carotene pigments in moroccan virgin olive oils measured by high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* **1991**, *68*, 672–674. [CrossRef]
35. Gandul-Rojas, B.; Minguez-Mosquera, M.I. Chlorophyll and carotenoid composition in virgin olive oils from various Spanish olive varieties. *J. Sci. Food Agric.* **1996**, *72*, 31–39. [CrossRef]
36. Colquhoun, D.; Hicks, B.; Reed, A. Phenolic content of olive oil is reduced in extraction and refining: Analysis of phenolic content of three grades of olive and ten seed oils. *Asia Pac. J. Clin. Nut* **1996**, *5*, 105–107.
37. Papadimitriou, V.; Sotiroudis, T.G.; Xenakis, A.; Sofikiti, N.; Stavyiannoudaki, V.; Chaniotakis, N. Oxida-tive stability and radical scavenging activity of extra virgin olive oils: An electron paramagnetic resonance spectroscopy study. *Anal. Chim. Acta* **2006**, *573*, 453–458. [CrossRef]

38. Verleyen, T.; Forcades, M.; Verhe, R.; Dewettinck, K.; Huyghebaert, A.; De Greyt, W. Analysis of free and esterified sterols in vegetable oils. *J. Am. Oil Chem. Soc.* **2002**, *79*, 117–122. [CrossRef]
39. Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G. Volatile compounds and phenolic composition of virgin olive oil: Optimization of temperature and time of exposure of olive pastes to air contact during the mechanical extraction process. *J. Agric. Food Chem.* **2003**, *51*, 7980–7988. [CrossRef]
40. Vichi, S.; Castellote, A.I.; Pizzale, L.; Conte, L.S.; Buxaderas, S.; Lopez-Tamames, E. Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization detection. *J. Chromatogr. A* **2003**, *983*, 19–33. [CrossRef]
41. Cavalli, J.-F.; Fernandez, X.; Lizzani-Cuvelier, L.; Loiseau, A.-M. Characterization of volatile compounds of French and Spanish virgin olive oils by HS-SPME: Identification of quality-freshness markers. *Food Chem.* **2004**, *88*, 151–157. [CrossRef]
42. Morales, M.; Luna, G.; Aparicio, R. Comparative study of virgin olive oil sensory defects. *Food Chem.* **2005**, *91*, 293–301. [CrossRef]
43. Saez, J.S.; Garraleta, M.H.; Otero, T.B. Identification of cinnamic acid ethyl ester and 4-vinylphenol in off-flavor olive oils. *Anal. Chim. Acta* **1991**, *247*, 295–297. [CrossRef]
44. Angerosa, F.; d'Alessandro, N.; Corana, F.; Mellerio, G. Characterization of phenolic and secoiridoid aglycons present in virgin olive oil by gas chromatography-chemical ionization mass spectrometry. *J. Chromatogr. A* **1996**, *736*, 195–203. [CrossRef]
45. Aparicio, R.; Luna, G. Characterisation of monovarietal virgin olive oils. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 614–627. [CrossRef]
46. Ridolfi, M.; Terenziani, S.; Patumi, M.; Fontanazza, G. Characterization of the lipoxygenases in some olive cultivars and determination of their role in volatile compounds formation. *J. Agric. Food Chem.* **2002**, *50*, 835–839. [CrossRef] [PubMed]
47. Servili, M.; Montedoro, G. Contribution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 602–613. [CrossRef]
48. Angerosa, F.; Basti, C. The volatile composition of samples from the blend of monovarietal olive oils and from the processing of mixtures of olive fruits. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 327–332. [CrossRef]
49. Benincasa, C.; De Nino, A.; Lombardo, N.; Perri, E.; Sindona, G.; Tagarelli, A. Assay of aroma active components of virgin olive oils from southern Italian regions by SPME-GC/ion trap mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 733–741. [CrossRef]
50. Luaces, P.; Pérez, A.G.; Sanz, C. Role of olive seed in the biogenesis of virgin olive oil aroma. *J. Agric. Food Chem.* **2003**, *51*, 4741–4745. [CrossRef]
51. Pérez, A.G.; Luaces, P.; Ríos, J.J.; García, J.M.; Sanz, C. Modification of volatile compound profile of virgin olive oil due to hot-water treatment of olive fruit. *J. Agric. Food Chem.* **2003**, *51*, 6544–6549. [CrossRef]
52. Pérez-Camino, M.C.; Moreda, W.; Mateos, R.; Cert, A. Determination of esters of fatty acids with low molecular weight alcohols in olive oils. *J. Agric. Food Chem.* **2002**, *50*, 4721–4725. [CrossRef] [PubMed]
53. Georgalaki, M.; Sotiroudis, T.G.; Xenakis, A. The presence of oxidizing enzyme activities in virgin olive oil. *J. Am. Oil Chem. Soc.* **1998**, *75*, 155–159. [CrossRef]
54. Georgalaki, M.D.; Bachmann, A.; Sotiroudis, T.G.; Xenakis, A.; Porzel, A.; Feussner, I. Characterization of a 13-lipoxygenase from virgin olive oil and oil bodies of olive endosperms. *Lipid/Fett* **1998**, *100*, 554–560. [CrossRef]
55. Hidalgo, F.J.; Alaiz, M.; Zamora, R. Low molecular weight polypeptides in virgin and refined olive oils. *J. Am. Oil Chem. Soc.* **2002**, *79*, 685–689. [CrossRef]
56. Saraiva, J.A.; Nunes, C.S.; Coimbra, M.A. Purification and characterization of olive (Olea europaea L.) peroxidase–Evidence for the occurrence of a pectin binding peroxidase. *Food Chem.* **2007**, *101*, 1571–1579. [CrossRef]
57. Tzika, E.D.; Sotiroudis, T.G.; Papadimitriou, V.; Xenakis, A. Partial purification and characterization of peroxidase from olives (Olea europaea cv. Koroneiki). *Eur. Food Res. Technol.* **2009**, *228*, 487–495. [CrossRef]
58. Hidalgo, F.J.; Zamora, R. Peptides and proteins in edible oils: Stability, allergenicity, and new processing trends. *Trends Food Sci. Technol.* **2006**, *17*, 56–63. [CrossRef]
59. Valli, E.; Bendini, A.; Popp, M.; Bongartz, A. Sensory analysis and consumer acceptance of 140 high-quality extra virgin olive oils. *J. Sci. Food Agric.* **2014**, *94*, 2124–2132. [CrossRef]

60. Beauchamp, G.K.; Keast, R.S.; Morel, D.; Lin, J.; Pika, J.; Han, Q.; Lee, C.-H.; Smith, A.B.; Breslin, P.A. Ibuprofen-like activity in extra-virgin olive oil. *Nature* **2005**, *437*, 45–46. [CrossRef]
61. Kiosseoglou, V.; Kouzounas, P. The role of diglycerides, monoglycerides, and free fatty acids in olive oil minor surface-active lipid interaction with proteins at oil-water interfaces. *J. Dispers. Sci. Technol.* **1993**, *14*, 527–539. [CrossRef]
62. Bianco, A.; Mazzei, R.A.; Melchioni, C.; Romeo, G.; Scarpati, M.L.; Soriero, A.; Uccella, N. Microcompo- nents of olive oil — III. Glucosides of 2 (3, 4-dihydroxy-phenyl) ethanol. *Food Chem.* **1998**, *63*, 461–464. [CrossRef]
63. Frega, N.; Mozzon, M.; Lercker, G. Effects of free fatty acids on oxidative stability of vegetable oil. *J. Am. Oil Chem. Soc.* **1999**, *76*, 325–329. [CrossRef]
64. Zamora, R.; Alaiz, M.; Hidalgo, F.J. Influence of cultivar and fruit ripening on olive (Olea europaea) fruit protein content, composition, and antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4267–4270. [CrossRef]
65. McClements, D.; Decker, E. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *J. Food Sci.* **2000**, *65*, 1270–1282. [CrossRef]
66. Branco, I.G.; Sen, K.; Rinaldi, C. Effect of sodium alginate and different types of oil on the physical properties of ultrasound-assisted nanoemulsions. *Chem. Eng. Process. -Process Intensif.* **2020**, *153*, 107942. [CrossRef]
67. Melnikov, S.M.; Popp, A.K.; Miao, S.; Patel, A.R.; Flendrig, L.M.; Velikov, K.P. Colloidal emulsion based delivery systems for steroid glycosides. *J. Funct. Foods* **2017**, *28*, 90–95. [CrossRef]
68. Cinelli, G.; Cuomo, F.; Hochkoeppler, A.; Ceglie, A.; Lopez, F. Use of Rhodotorula minuta live cells hosted in water-in-oil macroemulsion for biotrasformation reaction. *Biotechnol. Prog.* **2006**, *22*, 689–695. [CrossRef]
69. Cuomo, F.; Cofelice, M.; Lopez, F. Rheological characterization of hydrogels from alginate-based nanodispersion. *Polymers* **2019**, *11*, 259. [CrossRef]
70. Goodarzi, F.; Zendehboudi, S. A comprehensive review on emulsions and emulsion stability in chemical and energy industries. *Can. J. Chem. Eng.* **2019**, *97*, 281–309. [CrossRef]
71. Ambrosone, L.; Mosca, M.; Ceglie, A. Impact of edible surfactants on the oxidation of olive oil in water-in-oil emulsions. *Food Hydrocoll.* **2007**, *21*, 1163–1171. [CrossRef]
72. Weiss, J.; McClements, D.J. Mass transport phenomena in oil-in-water emulsions containing surfactant micelles: Solubilization. *Langmuir* **2000**, *16*, 5879–5883. [CrossRef]
73. Hong, I.K.; Kim, S.I.; Lee, S.B. Effects of HLB value on oil-in-water emulsions: Droplet size, rheological behavior, zeta-potential, and creaming index. *J. Ind. Eng. Chem.* **2018**, *67*, 123–131. [CrossRef]
74. Mosca, M.; Cuomo, F.; Lopez, F.; Ceglie, A. Role of emulsifier layer, antioxidants and radical initiators in the oxidation of olive oil-in-water emulsions. *Food Res. Int.* **2013**, *50*, 377–383. [CrossRef]
75. Perugini, L.; Cinelli, G.; Cofelice, M.; Ceglie, A.; Lopez, F.; Cuomo, F. Effect of the coexistence of sodium caseinate and Tween 20 as stabilizers of food emulsions at acidic pH. *Colloids Surf. B Biointerfaces* **2018**, *168*, 163–168. [CrossRef]
76. Cuomo, F.; Perugini, L.; Marconi, E.; Messia, M.C.; Lopez, F. Enhanced Curcumin Bioavailability through Nonionic Surfactant/Caseinate Mixed Nanoemulsions. *J. Food Sci.* **2019**, *84*, 2584–2591. [CrossRef] [PubMed]
77. Ushikubo, F.; Cunha, R. Stability mechanisms of liquid water-in-oil emulsions. *Food Hydrocoll.* **2014**, *34*, 145–153. [CrossRef]
78. Kinoe, K.; Higashi, K. Water-in-Oil Emulsion Adhesive. U.S. Patents US8822596B2, 2 September 2014.
79. Zhu, Q.; Pan, Y.; Jia, X.; Li, J.; Zhang, M.; Yin, L. Review on the stability mechanism and application of water-in-oil emulsions encapsulating various additives. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 1660–1675. [CrossRef]
80. Ambrosone, L.; Angelico, R.; Cinelli, G.; Di Lorenzo, V.; Ceglie, A. The role of water in the oxidation process of extra virgin olive oils. *JaocsJ. Am. Oil Chem. Soc.* **2002**, *79*, 577–582. [CrossRef]
81. Colafemmina, G.; Palazzo, G.; Ceglie, A.; Ambrosone, L.; Cinelli, G.; Di Lorenzo, V. Restricted diffusion: An effective tool to investigate food emulsions. In *Progress in Colloid and Polymer Science, Lipid and polymer-Lipid systems*; Nylander, T., Lindman, B., Eds.; Springer: Berlin, Germany, 2002; Volume 120, pp. 23–27.
82. Lercker, G.; Frega, N.; Bocci, F.; Servidio, G. "Veiled" extra-virgin olive oils: Dispersion response related to oil quality. *J. Am. Oil Chem. Soc.* **1994**, *71*, 657–658. [CrossRef]
83. Mosca, M.; Diantom, A.; Lopez, F.; Ambrosone, L.; Ceglie, A. Impact of antioxidants dispersions on the stability and oxidation of water-in-olive-oil emulsions. *Eur. Food Res. Technol.* **2013**, *236*, 319–328. [CrossRef]

84. Katsouli, M.; Polychniatou, V.; Tzia, C. Influence of surface-active phenolic acids and aqueous phase ratio on w/o nano-emulsions properties; model fitting and prediction of nano-emulsions oxidation stability. *J. Food Eng.* **2017**, *214*, 40–46. [CrossRef]
85. Papadimitriou, V.; Dulle, M.; Wachter, W.; Sotiroudis, T.G.; Glatter, O.; Xenakis, A. Structure and Dynamics of Veiled Virgin Olive Oil: Influence of Production Conditions and Relation to its Antioxidant Capacity. *Food Biophys.* **2013**, *8*, 112–121. [CrossRef]
86. Kralchevsky, P.A.; Danov, K.D.; Denkov, N.D. Chemical physics of colloid systems and interfaces. In *Handbook of Surface and Colloid Chemistry*, 2nd ed.; Birdi, K.S., Ed.; CRC Press: Boca Raton, FL, USA, 2003; pp. 137–321.
87. Fennell Evans, D.; Wennerstrom, H.; Rajagopalan, R. The colloidal domain: Where physics, chemistry, biology, and technology meet. *J. Colloid Interface Sci.* **1995**, *172*, 541.
88. Berton-Carabin, C.C.; Ropers, M.H.; Genot, C. Lipid Oxidation in Oil-in-Water Emulsions: Involvement of the Interfacial Layer. *Compr. Rev. Food Sci. Food Saf.* **2014**, *13*, 945–977. [CrossRef]
89. Kargar, M.; Spyropoulos, F.; Norton, I.T. The effect of interfacial microstructure on the lipid oxidation stability of oil-in-water emulsions. *J. Colloid Interface Sci.* **2011**, *357*, 527–533. [CrossRef]
90. Lipid oxidation. In *Handbook of Food Science and Technology 1: Food Alteration and Food Quality*, 1st ed.; Jeantet, R.; Croguennec, T.; Schuck, P.; Brulé, G. (Eds.) John Wiley & Sons: Hoboken, NJ, USA, 2016; pp. 99–129.
91. Mozuraityte, R.; Kristinova, V.; Rustad, T. Oxidation of Food Components. *Encycl. Food Health* **2016**, 186–190. [CrossRef]
92. Ambrosone, L.; Cinelli, G.; Mosca, M.; Ceglie, A. Susceptibility of water-emulsified extra virgin olive oils to oxidation. *JaocsJ. Am. Oil Chem. Soc.* **2006**, *83*, 165–170. [CrossRef]
93. Mosca, M.; Ceglie, A.; Ambrosone, L. Biocompatible water-in-oil emulsion as a model to study ascorbic acid effect on lipid oxidation. *J. Phys. Chem. B* **2008**, *112*, 4635–4641. [CrossRef]
94. Atkinson, J.; Epand, R.F.; Epand, R.M. Tocopherols and tocotrienols in membranes: A critical review. *Free Radic. Biol. Med.* **2008**, *44*, 739–764. [CrossRef]
95. Li, Y.-J.; Luo, S.-C.; Lee, Y.-J.; Lin, F.-J.; Cheng, C.-C.; Wein, Y.-S.; Kuo, Y.-H.; Huang, C.-J. Isolation and identification of α-CEHC sulfate in rat urine and an improved method for the determination of conjugated α-CEHC. *J. Agric. Food Chem.* **2008**, *56*, 11105–11113. [CrossRef]
96. Lešková, E.; Kubíková, J.; Kováčiková, E.; Košická, M.; Porubská, J.; Holčíková, K. Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *J. Food Compos. Anal.* **2006**, *19*, 252–276. [CrossRef]
97. Niki, E.; Kawakami, A.; Yamamoto, Y.; Kamiya, Y. Oxidation of lipids. VIII. Synergistic inhibition of oxidation of phosphatidylcholine liposome in aqueous dispersion by vitamin E and vitamin C. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1971–1975. [CrossRef]
98. Cinelli, G.; Sbrocchi, G.; Iacovino, S.; Ambrosone, L.; Ceglie, A.; Lopez, F.; Cuomo, F. Red Wine-Enriched Olive Oil Emulsions: Role of Wine Polyphenols in the Oxidative Stability. *Colloids Interfaces* **2019**, *3*, 59. [CrossRef]
99. Frei, B. Reactive oxygen species and antioxidant vitamins: Mechanisms of action. *Am. J. Med.* **1994**, *97*, 5–13. [CrossRef]
100. Tikekar, R.V.; Nitin, N. Distribution of encapsulated materials in colloidal particles and its impact on oxidative stability of encapsulated materials. *Langmuir* **2012**, *28*, 9233–9243. [CrossRef]
101. Waraho, T.; McClements, D.J.; Decker, E.A. Mechanisms of lipid oxidation in food dispersions. *Trends Food Sci. Technol.* **2011**, *22*, 3–13. [CrossRef]
102. Yi, J.; Zhu, Z.; McClements, D.J.; Decker, E.A. Influence of aqueous phase emulsifiers on lipid oxidation in water-in-walnut oil emulsions. *J. Agric. Food Chem.* **2014**, *62*, 2104–2111. [CrossRef]
103. Dai, F.; Chen, W.-F.; Zhou, B. Antioxidant synergism of green tea polyphenols with α-tocopherol and l-ascorbic acid in SDS micelles. *Biochimie* **2008**, *90*, 1499–1505. [CrossRef]
104. Liu, D.; Shi, J.; Ibarra, A.C.; Kakuda, Y.; Xue, S.J. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β-carotene mixtures on the DPPH free radical. *Lwt-Food Sci. Technol.* **2008**, *41*, 1344–1349. [CrossRef]
105. Rozman, B.; Gašperlin, M. Stability of vitamins C and E in topical microemulsions for combined antioxidant therapy. *Drug Deliv.* **2007**, *14*, 235–245. [CrossRef]

106. Barclay, L.R.C.; Locke, S.J.; MacNeil, J.M. Autoxidation in micelles. Synergism of vitamin C with lipid-soluble vitamin E and water-soluble Trolox. *Can. J. Chem.* **1985**, *63*, 366–374. [CrossRef]
107. Gitto, E.; Tan, D.X.; Reiter, R.J.; Karbownik, M.; Manchester, L.C.; Cuzzocrea, S.; Fulia, F.; Barberi, I. Individual and synergistic antioxidative actions of melatonin: Studies with vitamin E, vitamin C, glutathione and desferrioxamine (desferoxamine) in rat liver homogenates. *J. Pharm. Pharmacol.* **2001**, *53*, 1393–1401. [CrossRef] [PubMed]
108. Polychniatou, V.; Tzia, C. Evaluation of surface-active and antioxidant effect of olive oil endogenous compounds on the stabilization of water-in-olive-oil nanoemulsions. *Food Chem.* **2018**, *240*, 1146. [CrossRef] [PubMed]
109. Katsouli, M.; Polychniatou, V.; Tzia, C. Optimization of water in olive oil nano-emulsions composition with bioactive compounds by response surface methodology. *LWT* **2018**, *89*, 740–748. [CrossRef]
110. Pimentel-Moral, S.; Rodríguez-Pérez, C.; Segura-Carretero, A.; Martínez-Férez, A. Development and stability evaluation of water-in-edible oils emulsions formulated with the incorporation of hydrophilic *Hibiscus sabdariffa* extract. *Food Chem.* **2018**, *260*, 200–207. [CrossRef]
111. Guerrini, L.; Zanoni, B.; Breschi, C.; Angeloni, G.; Masella, P.; Calamai, L.; Parenti, A. Understanding olive oil stability using filtration and high hydrostatic pressure. *Molecules* **2020**, *25*, 420. [CrossRef]
112. Zullo, B.A.; Ciafardini, G. Changes in Physicochemical and Microbiological Parameters of Short and Long-Lived Veiled (Cloudy) Virgin Olive Oil Upon Storage in the Dark. *Eur. J. Lipid Sci. Technol.* **2018**, *120*, 1700309. [CrossRef]
113. Zullo, B.A.; Pachioli, S.; Ciafardini, G. Reducing the bitter taste of virgin olive oil Don Carlo by microbial and vegetable enzymes linked to the colloidal fraction. *Colloids Interfaces* **2020**, *4*, 11. [CrossRef]
114. Ciafardini, G.; Zullo, B. Survival of micro-organisms in extra virgin olive oil during storage. *Food Microbiol.* **2002**, *19*, 105–109. [CrossRef]