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Review

Flavor Chemistry of Virgin Olive Oil: An Overview

Alessandro Genovese [1,*], Nicola Caporaso [1,2] and Raffaele Sacchi [1]

[1] Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici NA, Italy; sacchi@unina.it
[2] Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK; nicola.caporaso3@unina.it
* Correspondence: alessandro.genovese@unina.it; Tel.: +39-081-2539352

Abstract: Virgin olive oil (VOO) has unique chemical characteristics among all other vegetable oils which are of paramount importance for human health. VOO constituents are also responsible of its peculiar flavor, a complex sensation due to a combination of aroma, taste, texture, and mouthfeel or trigeminal sensations. VOO flavor depends primarily on the concentration and nature of volatile and phenolic compounds present in olive oil which can change dramatically depending on agronomical and technological factors. Another aspect that can change the flavor perception is linked to the oral process during olive oil tasting. In fact, in this case, some human physiological and matrix effects modulate the flavor release in the mouth. The present review aims to give an overview on VOO flavor, with particular emphasis on the mechanisms affecting its production and release during a tasting.

Keywords: extra virgin olive oil; phenolic compounds; volatile compounds; aroma release; virgin olive oil off-flavor; human saliva; oral process; panel test

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1. Introduction

Virgin olive oil (VOO) is a staple ingredient in the Mediterranean diet [1–3], and it is a food providing great nutritional properties due to its balanced fatty acid composition and presence of phenolic compounds, as well as unique sensory quality [4–7].

VOO is obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration [8]. Among the many commercial categories existing for olive oil, extra-virgin olive oil (EVOO) has particularly high standards in terms of composition as well as sensory characteristics assessed by recognized panels [8]. From a sensory and chemical point of view, it is a complex food mainly composed by triacylglycerols, which account to 98% of the total composition, and a series of minor constituents that are of paramount importance for its health significance and sensory implication [9]. This minor fraction comprises free fatty acids, phenols, tocopherols, sterols, phospholipids, waxes, squalene, other hydrocarbons and volatile compounds. VOO fatty acid composition is known to vary according to environmental and agronomical conditions, with oleic acid ($C_{18:1}$) being the most abundant fatty acid, representing usually 60-80% total fatty acid composition [10].

Several polyphenols in VOO are hydrolysis products of oleuropein and ligstroside and at least 30 different compounds have been identified so far [11]. They affect VOO taste in terms of its bitterness and pungency [12], the antioxidant properties of VOO [13], and they are known to play positive roles on human health [14]. Olive oil phenolics may inhibit oxidation of low-density lipoproteins, which are the most atherogenic ones, and several other positive health effects have been associated to their consumption [15–17]. Volatile compounds with 5 and 6 carbon atoms are the most abundant classes in VOO

aroma, contributing to its typical "green" fruity odor [5]. Aldehydes such as hexanal, *trans*-2-hexenal, *cis*-3-hexenal, and *trans*-2-pentenal, both with alcohols (*cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, and 1-hexanol) and 1-penten-3-one contribute strongly to the typical green notes. The different nuances of VOO are related to the level and composition of the volatile fraction, which is affected by wide possible variations in olive oil production techniques and the starting raw material [4,18–20]. In addition to the lipoxygenase pathway, various other reactions can lead to the formation of other volatile compounds which thus increase the olfactory complexity of the oil. However, when these reactions prevail, they can lead to the appearance of odor defects (off-flavor) [4].

VOO is highly appreciated for its sensory properties, and sensory assessment is compulsory for every lot of VOO that is commercialized, in accordance with the national legislations of many countries and the standards of the International Olive Council (IOC) regulations on the trade standard of this product and its sensory assessment guide. In fact, olive oil that is to be classified as "extra-virgin" must not have any presence of unpleasant aroma defined as off-flavor, while lower categories can present a slight level of sensory defects which is categorized and quantified. The presence of off-flavors or absence of fruity aroma can therefore change the commercial category of a VOO, and when a defect is too strong, even if the chemical parameters are within acceptable ranges, it will result in a product that is not-marketable, unless refined [21]. VOO is therefore a highly regulated food both at national or supranational level, e.g., by the EU, and international level. In addition, its quality may be defined by a number of parameters assessed by analytical tests, but the sensory perception of odor is the ultimate determinant [22]. Sensory analysis is still the most effective tool to evaluate VOO quality. The method established by the current EU legislation to assess the organoleptic characteristics of VOO is through sensory analysis, specifically the so-called "panel test" conducted according to the IOC method [8].

Tasting virgin olive oils is a multisensory experience that involves the visual, olfactory, gustatory and tactile senses, whereas the latter is less relevant in VOO tasting. Smell, taste and mouth-feel sensations are defined with only word "flavor". Investigating the factors that influence VOO flavor is of importance for the production and appreciation of a successful product.

2. VOO Flavor Perception

Flavor perception is mainly based on two modalities, i.e., olfaction and taste. The delicate and unique flavor of VOO can be perceived during inhalation, when olive oil odorants released from liquid into the air (headspace) pass through the external nostrils to stimulate the olfactory receptors in the nasal cavity (ortho-nasal route). In this case, the term "odor" is used. Subsequently, when the VOO is put into the mouth other sensations take place. Different chemical stimuli are dissolved in the mouth and make contact with several types of sensory receptors on the tongue. These chemical stimuli are responsible for the taste of VOO, particularly bitterness sensation but also sweetness. Other sensations in the mouth occur by the free endings of trigeminal nerve stimulation. In this case, we refer to pungency, astringency and metallic attributes of VOO [19].

Moreover, olive oil odorants can interact with the odor receptors by moving from the mouth to the nasal cavity via the nasopharynx (retro-nasal route). In this case, the term "aroma" is used. Olive oil aroma, similarly to other foods and drinks, is significantly affected by oral processing. For this reason, the odor (ortho-nasal odor) and aroma (retro-nasal odor) perception could be different, even though the same olfactory sense is involved [23–27]. Some compounds such as phenolic compounds (VOO non-volatile matrix) stimulate the tasting receptors and trigeminal nerve while volatile compounds (VOO volatile matrix) stimulate the olfactive receptors and are responsible for VOO odor and aroma. Another taste sensation during VOO taste is the viscosity, i.e., the measure of its resistance to gradual deformation by shear stress or tensile stress.

The bitterness taste sensation is more intensely perceived in the back and side of the tongue thanks to the interactions between the polar molecules (polyphenols) and the taste

buds present on the tongue [19]. The spicy or pungent sensation resembles a burning feeling; however, it is not generated by high temperatures but to the tactile stimulation of the heat receptors in the oral cavity, in particular on the mucous membranes. Finally, the sensation of astringency, sensation of dryness, roughness and lapping can be perceived not only on the tongue but throughout the oral cavity thanks to the interaction of phenolic compounds with the proline-rich proteins present in the saliva. Several studies have shown that biophenols, in particular the aglycones of the secoiridoids, are the most responsible for the bitter and spicy attributes in the oil [11,28].

The volatile compounds, of which more than 180 compounds have been identified so far in virgin olive oils, are instead responsible for the different nuances of odor that characterize the different oils, especially in relation to the cultivar. The final flavor perception is eventually codified and interpreted by the human brain (Figure 1).

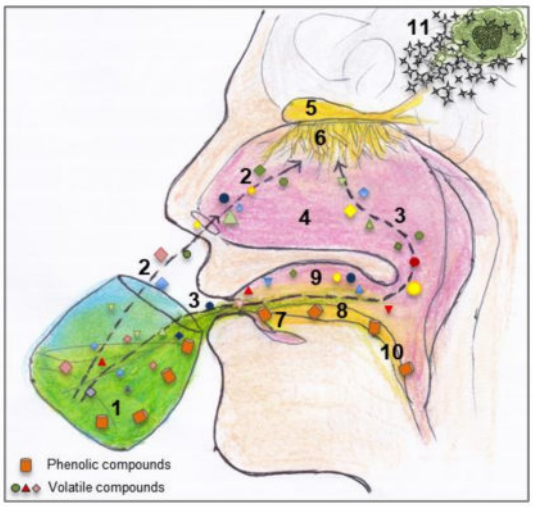


Figure 1. Schematic diagram representing virgin olive oil flavor perception. Legend: (1) extra virgin olive oil glass, (2) odor (through ortho-nasal route), (3) aroma (through retro-nasal route), (4) nasal cavity, (5) olfactory bulb, (6) olfactory epithelium (sense of smell), (7) tongue (sense of taste), (8) taste buds (bitterness perception), (9) oral cavity, (10) trigeminal nerve (chemesthesis perception: pungency), and (11) signal in the brain and the recognition of sensory perception (adapted from Genovese and Sacchi [29]).

When a stimulus reaches the olfactory or taste or trigeminal receptor, it triggers a cascade of enzymatic reactions that generate second messengers. The intracellular second messenger activates a series of electrical events, when it reaches the ion channels, increasing the cell membrane permeability to certain ions. This sequence of biochemical and electrical events is called transduction, and this is the only mechanism available to the neuroreceptors to report to the brain their successful activation. The number of possible combinations of stimulus-receptor is very large, as the number of neuroreceptors is large, with consequent high number of signals that the brain interprets [30].

Garcia-Gonzalez and coauthors [31] were the first who applied functional magnetic resonance imaging (fMRI) to evaluate the brain activity of human smelling VOO headspace volatiles. Oil samples of different quality were analyzed by Solid-phase micro extraction coupled with Gas-Chromatography/Mass-Spectrometry approach (SPME–GC/MS) to characterize their volatile composition and verify the differences. The results of the fMRI obtained showed different hedonistic values of the olfactory perception of a related oil in relation to its quality and therefore its pleasure for every single individual (Figure 2). The zones with the highest activity were the orbitofrontal, the frontal and the temporal lobes which correspond to Brodmann areas 6, 10, 11, 20 and 47. The bilateral activations BA 10, 11 and 47 were associated to the olfactory process which explains their activation in response to both pleasant and unpleasant samples. An increase in cerebral blood flow in

the BA 11 is also associated with the familiarity of odors, which would explain the high activation area seen in subjects who were regular users of virgin olive oils. Details of the application of functional magnetic resonance imaging (fMRI) and the aspects related to experimental design, data acquisition and data processing are described in Marciani and coauthors [32].

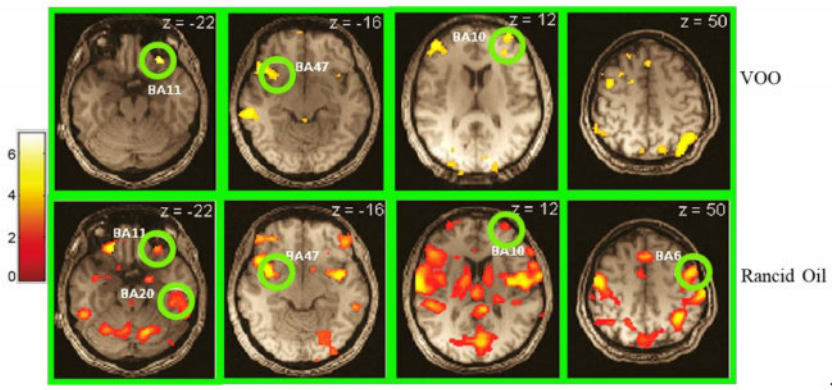


Figure 2. Results of functional Magnetic Resonance Imagining (fMRI) on the human brains of subjects exposed to rancid olive oil and extra virgin olive oil. Brodmann areas (BA) are marked with circles (Reproduced with permission from García-González, D.L.; Vivancos, J.; Aparicio, R., J. Agric. Food Chem.; published by ACS Publications, 2011).

One can hypothesize that some olive oil odorants might be able to mislead the brain. It can also be hypothesized that the "green" flavor of VOO exerts a positive effect on the food intake and satiation. In fact, German scientists reported that yogurt containing an aromatic extract of VOO modulates the cerebral blood in frontal operculum, inducing brain activation more similar to that induced by the high-fat yogurt [33]. Using a fat-free yogurt, two groups of assessors tasted a product added with an olive oil aroma extract in comparison to a control product. The group who ate the plain yogurt showed a drop in serotonin levels a hormone associated with satiety. Thus, this group reported less satiation after eating it. They also did not cut back on other calories to compensate; instead, their intake increased an average of 176 calories a day. On the contrary, the group eating the olive-oil flavored yogurt reduced their calories from other foods and showed better responses when given glucose tolerance tests, which measures the blood sugar control. Abrupt swings in blood sugar are part of what drives hunger and satiety. The sense of gratification and/or reward linked to VOO intake might be attributed to the herbaceous odor of molecules such as hexanal, *trans*–2–hexenal, and *cis*–3–hexenol.

These molecules are the most abundant volatiles in VOO, and previous research has shown that they are able to stimulate the release of dopamine in the brain [34]. This was also confirmed by the study by Kobayashi and coauthors [35], which verified an increase in the release of dopamine in the presence of herbaceous odors such as that of hexanal, explaining that this molecule could induce an increase in the intracellular concentration of Ca^{2+} and the dephosphorylation of phosphorylated proteins, phenomena required for a greater release of dopamine. The herbaceous smell of VOO, therefore, could influence and regulate the stimulation of dopamine release and thus increase the sense of gratification of the food consumed.

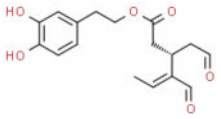
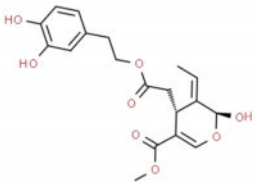
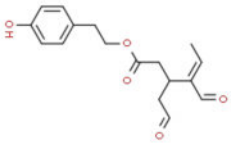
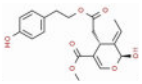
2.1. Phenolic Compounds

Many reviews that focused on the chemical, sensory, nutritional and health properties of VOO phenolic compounds have been published in recent years [36–48].

Bitterness and pungency are mainly ascribable to the quali-quantitative phenolic profile of the olive oils and are responsible of the different health functions [14]. Phenol concentrations lower than 220 mg kg^{-1} correspond to non-bitter oils or with almost imperceptible bitterness; slight bitterness corresponds to 220–340 mg kg^{-1}; bitter oils have

phenol contents ranging from 340 to 410 mg kg^{-1}; phenol contents higher than 410 mg kg^{-1} corresponds to quite bitter or very bitter oils [49]. In particular, a positive correlation was observed with secoiridoids content, like oleuropein aglycon [50]. The derivatives of oleuropein such as the dialdehydic form of decarboxymethyl elenoic acid linked to hydroxytyrosol (3,4–DHPEA–EDA or "oleacein",) and the aldehydic form of elenoic acid linked to hydroxytyrosol (3,4–DHPEA–EA or "oleuropein aglycon") are the main responsible of VOO bitterness. In contrast, the dialdehydic form of decarboxymethyl elenoic acid linked to tyrosol (p–HPEA–EDA or "oleocanthal") and the deacetoxy–ligstroside aglycon (p–HEPA–EA or "ligstroside aglycon") seem to be the main compounds responsible for the pungency of VOO [12] (Table 1).

Table 1. Some of the main phenolic compounds responsible for the bitterness, astringent, and pungent sensation of VOO.

Chemical Compound	Chemical Structure	Sensory Property
3,4–DHPEA–EDA (oleacein)		bitter, astringent and burning (mostly on tongue)
3,4–DHPEA–EA (oleuropein aglycon)		very bitter, very astringent
p–HPEA–EDA (oleocanthal)		strong burning/pungent (mostly at the back of throat); slightlybitter and astringent
p–HEPA–EA (ligstroside aglycon)		dry mouth, burning/pungent, and not bitter

Source: Andrewes and co-workers [12]; Servili and co-workers [40]. Abbreviations: 3,4–DHPEA–EDA, dialdehydic form of decarboxymethyl elenoic acid linked to hydroxytyrosol; 3,4–DHPEA–EA, aldehydic form of elenoic acid linked to hydroxytyrosol; p–HPEA–EDA, dialdehydic form of decarboxymethyl elenoic acid linked to tyrosol; p–HEPA–EA, deacetoxy–ligstroside aglycon.

It is interesting to note that the strong burning/pungent sensation produced by oleocanthal is perceived in the back of the throat. At this oropharyngeal area, Peyrot des Gachons and coworkers [51] have identified the receptor that oleocanthal selectively activates, i.e., Transient Receptor Potential cation channel, subfamily A, member 1 (TRPA1).

2.2. Volatile Compounds

VOO volatile compounds were reviewed in the last years mainly in relation to factors affecting their production [20], oil quality [4,5,19], and to explain sensory perception of virgin olive oil [52,53]. Overall, about 180 volatile compounds belonging to the chemical classes of aldehydes, alcohols, esters, hydrocarbons, ketones, furans and terpenes have been identified in VOO. Among these, 5 or 6 carbon atoms, volatile compounds generated during a complex enzymatic process named "lipoxygenase pathway" [54] generate the majority of volatile compounds in VOO (Figure 3).

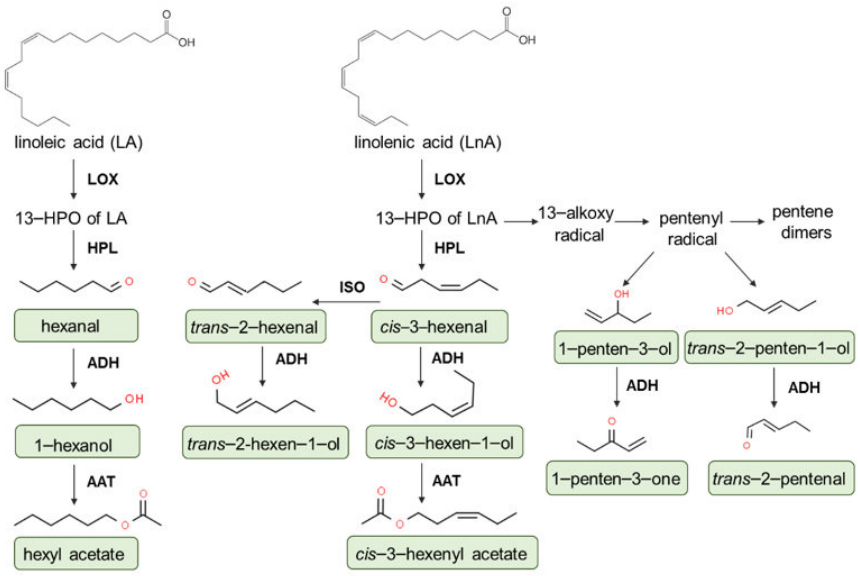


Figure 3. Lipoxygenase pathway (LOX) involved in the production of VOO volatile compounds. Abbreviations: AAT, alcohol acyltransferase; ADH, alcohol dehydrogenase; 13–HPL, 13–hydroperoxide lyase; ISO, isomerase.

These enzymatic reactions start from polyunsaturated fatty acids and in the presence of enzymes such as lipoxygenases (LOX), hydroperoxydelyase (HPL) and isomerase. These enzymes are naturally found in the olive fruit and are active since the fruit is crushed during the olive milling phases. LOX is a non-heme enzyme containing iron, which has the function of catalyzing the oxidations of the 1,4–pentadiene sequence of polyunsaturated fatty acids to produce the corresponding hydroperoxides. From the sequential action of lipases and lipoxygenases and together with the combined action of HPL, aldehyde compounds with six atoms and nine carbon atoms are formed. Subsequently, by reduction of the aldehydes through alcohol-dehydrogenase (ADH) activity, the respective alcohols are obtained. From these, in turn, the respective esters can be formed by the action of alcohol acetyl transferase. The fatty acids most involved in the lipoxygenase cascade are linoleic acid (LA) and linolenic acid (LnA). The LOXs lead to the formation, preferentially, of hydroperoxides in position 13, whose decomposition, catalyzed by hydroperoxide–lyase, determines the formation of aldehydes with six carbon atoms, including hexanal (green notes) and *cis*–3–hexenal (notes of tomato leaf) [55]. Subsequently, the *cis*–3–hexenal isomerizes, giving rise to *trans*–2–hexenal (notes of grass, almond), which usually represents the most abundant volatile compound of good quality EVOOs, giving the oil the herbaceous and pleasant aroma. The ADH subsequently have the function of transforming the aldehydes into the corresponding alcohols, making the herbaceous notes less aggressive. The subsequent esterification by alcohol–acetyl–transferase (AAT), gives rise to the production of esters with a sweet odor impact, typical of ripe fruits [19]. An additional pathway to the lipoxygenase pathway is activated when the substrate is linolenic acid. LOX could catalyze, in addition to the formation of hydroperoxides, also their cleavage through a donation of an alkoxy radical group for the stabilization of the 1,3–pentene radical. These latter compounds can dimerize leading to the formation of ten-carbon hydrocarbons (C_{10}), known as pentenes dimers, or they can join with hydroxy radicals present in the medium, producing five-carbon alcohols, which can then be enzymatically oxidized to the corresponding compounds with five carbon atoms [19].

The essential role played by some volatile compounds originated from the LOX pathway on the green attributes, particularly for hexanal and *trans*–2–hexenal, was confirmed by Angerosa and coauthors [56] by means of a Linear Regression Analysis (LRA). In addition, higher concentrations of 1–penten–3–one and phenolic compounds cause the increase of

leaf odor, and an increase in the phenolics concentration causes the increase of walnut husk odor.

Few studies were focused on the possible contribution of VOO volatiles also to its taste perception. Caporale and coauthors [57], in a model olive oil, by studying the taste-smell interaction between bitterness and cut grass odor (*cis*–3–hexen–1–ol), showed that the green odor note has a positive significant effect on the perception of bitterness. The presence of grass odor enhances the bitterness perception. On the other hand, the perception of pungency in VOO seems to be influenced not only by phenolic compounds, and particularly by deacetoxy–ligstroside aglycon as the key contributor to this sensory note [12], but also by alcohols and other compounds. Inarejos-García and coauthors [58] determined positive correlation of the sensory characteristic pungent with C_6 alcohol, i.e., hexanol, while, Angerosa and coauthors [56] indicated that 1–penten–3–one is in positive correlation with the pungent and bitter taste.

2.3. Off-Flavors

Parallel to the LOX pathway, processes such as possible sugar fermentation, metabolism of some amino acids (leucine, isoleucine and valine) or oxidative processes lead to the formation of other volatile compounds which, if in excess, can generate off-flavor. The onset of organoleptic defects depends on several factors: health status and ripeness degree of the olives, methods of harvesting and post-harvesting, storage conditions, technology used for the extraction, storage, decanting and filtration of the product. Indeed, each phase of the production process can influence the composition of volatile substances and the appearance of off-flavor.

The sensory detection of even a single negative attribute (defect) by an official panel of tasters downgrades the product from EVOO to any lower category, depending on its intensity [21].

The "mold-humidity" defect is the characteristic aroma of oils from olive fruits in which fungi and molds have developed, caused by a long period of post-harvest storage in humid and poorly ventilated environments. The most present species are Penicillium and Aspergillus which oxidize free fatty acids, with the formation of methylketones such as 2–heptanone and 2–nonanone (Table 2). Yeasts, such as *Candida* and *Saccharomyces*, in parallel, are able to reduce carbonyl groups thanks to esterification, contributing to the production of an oil with a damp-mold defect. The volatile molecules that most contribute to this defect are 1–octen–3–one, 1–octen–3–ol and 2–heptanol (Table 2).

Table 2. Some of the chemical compounds responsible for the main sensory defects detected in VOO and their odor descriptors.

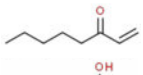
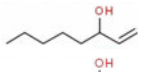
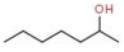
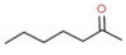
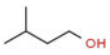
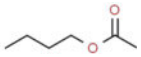
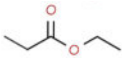
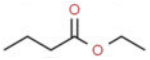
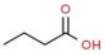
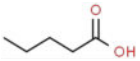
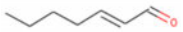
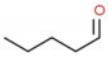
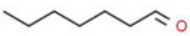
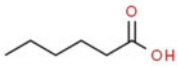
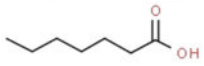
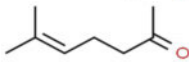
Sensory Defect	Chemical Compound	Chemical Structure	Odor Property
Mold-humidity	1–octen–3–one		mushroom, mold, pungent
	1–octen–3–ol		mold, earthy
	2–heptanol		earthy, sweety
	2–heptanone		sweet, fruity, cinnamon
Winery-vinegar	ethanol		alcohol
	acetic acid		sour, vinegary
	ethyl acetate		sticky, sweet
	3–methylbutanol		woody, whiskey, sweet

Table 2. *Cont.*

Sensory Defect	Chemical Compound	Chemical Structure	Odor Property
Fusty	butyl acetate		green, fruity, pungent
	ethyl propanoate		fruit, strong
	ethyl butanoate		sweet, fruity
	propanoic acid		pungent, sour
Muddy-sediment	butanoic acid		rancid, cheese
	pentanoic acid		unpleasant, pungent
Rancidity	*trans*–2–heptenal		oxidized, pungent
	trans–2–octenal		herbaceous, spicy
	trans–2–decenal		fishy, fatty
	pentanal		woody, bitter, oily
	hexanal		fatty, strong, green
	heptanal		oily, fatty, woody
	octanal		fatty, sharp
	nonanal		fatty, waxy, pungent
	hexanoic acid		rancid, pungent
	heptanoic acid		rancid
	6–methyl–5–hepten–2–one		herbaceous, pungent

Source: Morales and co-workers [59].

Winery-vinegar is the typical defect of oils obtained from non-fresh olives that have undergone alcoholic and acetic fermentation. The main bacteria involved in this process are the lactic (*Lactobacillus*) and acetic ones, which give rise, in the absence of oxygen, first to ethanol and then to acetic acid and ethyl acetate, volatile compounds which are the main responsible for this defect together with 3–methylbutanol [59,60] (Table 2).

The "fusty" defect is the aroma originated in olives stored for a long time in thick layers or in bags, which have undergone various fermentations, the main one being lactic one.

A similar defect, which for practicality is grouped together but has a very different biochemical origin, is the "muddy-sediment". This defect is the characteristic aroma of oils that have remained in contact with their own muddy sediment for a long time, which have undergone an anaerobic fermentation, mainly butyric. The main microorganisms identified in oils affected by heating are part of the Enterobacteriaceae family, whose proliferation starts at the beginning of the storage phase, and Pseudomonas, *Clostridium* and *Serratia*, whose growth takes place later. Ethyl butanoate, ethyl propanoate and butyl acetate are generally found at higher levels in the fusty VOO.

The high concentration of 6–methyl–5–hepten–2–one can be explained by the presence of *Pseudomonas* that degrades terpene alcohols. Instead, the abundance of butanoic and propanoic acids is due to the metabolism of *Clostridium* and propionic bacteria (Table 2).

"Rancidity" is an off-flavor resulting from the oil oxidation, a phenomenon promoted by long exposure to air, exposure to light and relatively high temperatures. The main compounds present in a rancid oil are the aldehydes (2–heptenal, 2–octenal, 2–decenal, hexanal, nonanal, octanal, pentanal, heptanal) generated during the fatty acid oxidation. When acids are also found (such as butanoic, hexanoic, heptanoic), it means that the oxidation process is very thorough, as acids are generated by the oxidation of aldehydes [59,60] (Table 2).

The sensory assessment of negative notes, however, is not performed by our nose and tongue in an independent and selective way with respect to positive ("olive fruity", "bitter", "pungent" and "green") ones. The intensity of some sensory notes, in fact, can be masked or enhanced by others as recently demonstrated by Genovese and coauthors [61], particularly in VOOs rich in phenolic compounds. The authors suggested how these effects can explain the discrepancy among panels sometimes observed during extra virgin olive oil sensory analysis over olive oil storage. In particular, a 'fusty' defect was detected in some one-year old VOOs due to the phenolic compounds natural decrease over time (auto-oxidation), while in the fresher product its higher level contributed to mask this off-flavor.

2.4. Triglycerides and Fatty Acids

The main constituents of VOOs are triglycerides (glycerol esters), diglycerides and few free fatty acids. The latter, when found in large quantities, contribute to increasing the acidity value with the consequent higher oxidation rate of the oil. The percentage composition of fatty acids in extra virgin olive oil varies according to climatic and agronomic conditions, as well as the olive drupe variety; however, oleic acid (C18:1*n*–9) is the most abundant (monounsaturated) fatty acid present in extra virgin olive oil in a range of 60–80% [10]. Oleic acid, like other fatty acids present in the lipid fraction of olive oil as constituents of the triglyceride molecules, does not exert a direct flavour impact.

Oils and fats have been shown to reduce cortical response in several brain areas related to flavor processing and reward [62]. Viscosity, greasiness and food moisture level are initially the main factors responsible for fat sensory impact. Texture perception of a creamy or fat food is strongly correlated to lipid chemic-physical properties [63,64].

It has been reported that the level of fat can be perceived sensorially, and it was suggested that this might be related to the greasiness, a tactile attribute of fats [65]. These characteristics can be linked to triglycerides, which are the predominant form of dietary fat. However, they are of such size and structure that binding to cell surface receptors or passageway through the cell membrane channels is quite unlikely [66]. However, there is now more and more evidence supporting an important role of the taste system in the perception of fat [67–69]. Functional magnetic resonance showed that the neural representations in the brain of the properties of fat differ significantly with taster status [70].

Sensory studies have highlighted the important influences of sensory signals in the identification of fat taste in terms of free fatty acids [68]. The results of the tests, performed using different fatty acids, reported that linoleic acid, oxidized linoleic acid, oleic acid, and stearic acid are detectable in the oral cavity, with retro-nasal perception thresholds greater than the ortho-nasal one. Free fatty acids are main factors responsible for fat taste perception, once the triacylglycerols have been hydrolyzed by the salivary lipase. This has been demonstrated through the use of orlistat, a tasteless substance and potent inhibitor of lipases [71]. A recent study suggested that the lipolytic activity of the human lipase is able to produce fatty acids within the necessary range of concentration necessary to activate the oral sensors [72]. There are different classes of putative receptors of fat food that have been identified in rodents [73–75], including the glycoprotein CD36 [76].

Recently, the long fatty acid receptors CD36 and GPR 120 on the surface of the circumvallate and foliate taste buds have been found in humans [77]. This indicates that long-chain fatty acids (LCFAs) play an important role, as primary taste, in the oral fat sensitivity, in addition to their potential role in the regulation of metabolic needs, as recently discovered but still to be fully investigated [78].

Fat sensitivity has been linked by some authors to fat intake, which eventually might affect the calory intake and thus the body mass index (BMI) of humans. In fact, Stewart and coauthors [69,72] determined that subjects hyposensitive to fatty acids perception consumed significantly higher amounts of fat and energy, as well as highly saturated fats like butter, meat and dairy products compared to hypersensitive subjects. It thus appears that the inability to perceive low concentrations of fats in foods is associated with a higher consumption of fatty foods [69]. The hyposensitive subjects had a genetic variant in the FA translocate gene CD36 and lipase inhibition [79,80] that could explain their lower sensitivity to fatty acids.

3. Factors Affecting the Flavor of VOO

De Roos [81] suggested that two main factors regulate the rate of release of aroma compounds from foods, i.e., the volatility (thermodynamic factor) and the resistance to mass transfer from the product to the air (kinetic factor). From this point of view, the mechanism of the release, as well as the retention of aroma compounds from olive oil, depends primarily on the concentration and nature of volatile compounds present in olive oil. The nature and the level of aroma compounds in olive oil are more affected by different agronomical and technological factors [7,20,53].

The second mechanism that affects the aroma release of VOO is the properties of aroma compounds such as their molecular size, shape, volatility, and polarity that could determine their higher or lower availability to the olfactory receptors [82–85].

The third mechanism relates to the aspects linked to the oral process [86,87]. During olive oil tasting, factors such as salivation, mouth size, breathing and temperature are able to change the volatility of olive oil odorants and consequently VOO aroma. In addition, another mechanism that can happen is the potential interaction between the matrix components and the volatile compounds that could reduce the aroma release and change the odor perception of food [88,89].

3.1. Botanical, Agronomical and Technological Process

3.1.1. Variety and Ripening Degree

The impact of botanical characteristics, agronomical practices and technological processing of the olives on olive oil quality has been extensively reported in the literature; therefore, the present section only reports some examples and general conclusions [20,53]. The olive oil variety has a dramatic effect on the final flavor of VOO. Several studies have been performed over the last decades investigating oils obtained from single varieties of olives, to understand the effect of the genetic factors, the environmental factors (such as growing location and agronomic practices) and the processing factors [90–93].

For example, comparing typical Italian olive varieties and Tunisian ones, it was found that cv. Chetoui is characterized by a low content of C_6 aldehydes, responsible of the sensory notes "green" and "fruity" [94]. According to Tura and coauthors [95], the major volatile compounds affected by the olive cultivars are the following ones: ethanol, 2–methyl propanol, pentanol, *cis*–2–penten–1–ol, *cis*–3–hexenol, and octanol.

Different varieties might be different in their fatty acid composition and the resulting flavor because of different levels of enzymes, genetically defined, leading to different levels of volatile compounds. For example, the Spanish cultivars Arbequina, Hojiblanca and Picual show great differences in their phenolic composition and fatty acid composition [96].

Important differences are observed also within the same variety depending on the drupe maturity degree or the growing location. It is well known that the harvesting moment is particularly important as the final VOO can be dramatically affected [19]. During the olive ripening, the aroma notes of "fruity-grassy" are significantly lower in oils obtained from greener, less ripe, olives. The sensory notes of bitter-pungent and other positive aromatic notes decrease with increasing ripening degree [97]. More ripe olives give rise to increased concentrations of 1–penten–3–ol and lower content of hexanal, *trans*–3–hexenol, *cis*–3–hexen–1–ol and *cis*–2–hexenol [98]. Other researchers reported that the content of

trans–2–hexenal as well as many other compounds, except *trans*–2–hexenol and hexyl acetate, decrease as maturity progressed [99]. Some triterpenic alcohols have been be proposed as markers of fruit maturity. During maturity, the concentration of phenolic compounds decreases, with consequent decrease of the bitter-pungent notes [100].

3.1.2. Environmental and Climatic Conditions

Factors such as soil composition, water availability to the plant, temperature and biotic or abiotic stress can have effects on the final VOO aroma. For example, the water availability was shown to significantly affect volatile compounds such as *trans*–2–hexenal, *cis*–3–hexen–1–ol and hexanol, in the sense of their decrease with increasing irrigation volume [101]. However, other researchers reported that VOO produced from irrigated plantations had lower values of 1–penten–3–ol and 1–penten–3–one for cv. Leccino but not for cv. Cornicabra, in which there were no significant modifications. In general, however, irrigated orchard results in oils with lower content of volatile compounds, especially C_5 compounds and lower bitter-pungent notes. There seems to be a general agreement about some volatile compounds which are dependent on the geographical area of origin, i.e., hexanal, hexanol, *trans*–2–hexenal, *cis*–3–hexenal, *trans*–3–hexenol, *cis*–3–hexenol and *trans*–2–hexenol [102].

3.1.3. Harvest and Olive Crushing System

The harvest, post-harvest practices and the olive milling have a significant impact on the composition of VOO and therefore its flavor. All stages of the extraction process modulate the volatile composition of olive oil [103]. The crushing step is of paramount importance as it leads to breaking the drupe cells such that the enzymes (Beta–glycosidase, LOX, PPO, esterase, etc.) can act. The type of milling system thus influences the final oil quality, both in term of biophenols and volatiles, with the main difference being from the traditional stone mill and the metallic hammer crushers with higher rotation rate [13,104].

3.1.4. Malaxation

Malaxation is by far the most important step as its temperature and time profile can affect the biochemical reactions, especially the LOX pathway, and the exchange of compounds from the continuous phase to the lipid phase, i.e., into the VOO. The reader can refer for this topic to the exhaustive review of Clodoveo [105].

Differences between open and closed malaxing technologies influence on analytical parameters related to VOO quality, healthy and organoleptic characteristics. Machinery evolution, from the most traditional open malaxers to the newest closed designs, leads to ensure the best extraction yield with lower damage in sensory quality and flavor. In open malaxers, increasing malaxation time leads to a higher concentration of volatile compounds, in particular C_6 and C_5 alcohols, hexanal and *trans*–2–hexenal and lower content of total phenolic compounds. High malaxation temperature (37–40 °C) also plays a major role, both in higher release of secoiridoid aglycons by beta–glycosidase, their oxidative degradation, and the decrease of C_6 volatile compounds, *cis*–3–hexenol and C_5 metabolites released by the LOX pathway [106].

Optimization of the time and temperature of malaxation is therefore required to enhance the flavor, and this must be carried out according to specific olive varieties and ripening stages.

3.1.5. Oil Separation Process

The separation of the liquid phase and solid particles from the olive paste is usually performed using two major systems, i.e., pressure or centrifugation. The old pressure system, dating back centuries, can lead to the formation of fermentative defects, while the centrifugation system separates the phases according to their density in a fast manner. However, in relation to the age of the technological plant and technology applied, it can

require the addition of some water, the amount of which can influence the final content of phenolic compounds and overall quality of VOO [104,107–109].

Finally, the filtration step and oil storage-bottling are of considerable importance to define VOO quality, characteristics and shelf-life. In particular, the lack of filtration can more easily produce defects of "muddy sediment" due to permanence of solid particles and water in the VOO, while the storage at inadequate temperature, in the presence of light and air exposure, can promote oxidation, thus leading to the "rancidity" defect. Generally, after eight months of storage, several volatile compounds are lost, and a decrease of total phenolic compound was reported, leading to the loss of important sensory attributes [103]. Filtration can also slightly influence the flavor and biophenols in filtered oils [90,110].

3.2. Physico-Chemical Properties of Volatile Compounds

Generally, high molecular weight flavor compounds are retained in the food matrix more than low molecular flavor compounds. Moreover, the chain length of flavor compounds has an impact on their perception, as long chain length molecules will be retained more than short chain molecules. Haar and co-workers [111] verified that the release of aldehydes and diacetyl in an oil-in-water model system is influenced by the length and unsaturation of compounds. Particularly, the shape, position, and nature of the functional groups of volatile compounds seem to have a strong and significant effect on the nature and strength of the bond with the food matrix and olfactory receptors.

The hydrophobicity of a molecule, expressed as log P, could regulate the mass transfer from two different liquid phases; therefore, it could be a useful means to understand the retention and/or the release of volatile compounds from olive oil emulsion produced in mouth with saliva. A high log $P_{o/w}$ value indicates that a compound tends to preferentially partition into organic phase rather than water, and it is inversely related to the solubility of a compound in water. Van Ruth and co-workers [112] studied a model system of sunflower oil and reported a "salting out" effect for some volatile compounds after the addition of artificial saliva (salts, mucin and α–amylase). The authors stated that this effect was mainly due to the hydrophilicity of the molecules.

3.3. Human Physiological Factors Affecting Flavour Perception

Physiological factors such as temperature, saliva, saliva pH, saliva flow, chewing, the speed at which the food is mixed during chewing and the size of the bolus are all factors that could affect VOO perception, and the differences in the sensory impact depending on the individual, as humans show wide variation in these parameters [113]. In addition, the respiration, as well as the dilution effect of the saliva, makes the oral processing a "dynamic process", which causes a continuous change in terms of volume, composition and viscosity of the foods. This is the reason for which VOO tasters frequently aspirate the olive oil in the mouth. In fact, this procedure promotes the volatilization by increasing the surface area contact and enhances retro-nasal detection.

Saliva is the first digestive fluid that enters in contact with foods in the digestive tract [114], and it exerts several functions [115,116]. Its action is closely correlated with chewing and swallowing. In humans, the secretion of saliva ranges from 0.5 mL/min without stimulation to over 2 mL min^{-1} during meals [117]. The total amount of saliva produced daily is on average around 1–1.5 L, including the moments close to the meals, in which saliva is produced at a higher rate [115].

Several publications have stressed the fact that saliva may partly influence the sensory perception of a food [118,119]. Salivary amylolytic activity is widely known, but also proteolytic and lipolytic activity has been reported for the saliva [120,121].

The salivary amylase and mucin play an important role in the phenomena of flocculation and coalescence that occur during the consumption of oil-in-water emulsions. These changes in the structure of the emulsion also have an impact on the product's perception [121]. It is known that mucins have binding sites available to trap volatiles [122],

as they can bind specific aroma compounds, mainly aldehydes [122,123], possibly to form Schiff bases.

Studies carried out by Pagès-Hélary and co-workers [124] demonstrated that large concentrations of α–amylase can lead to a lower flavor release, especially esters, aldehydes and ketones. Mucin influences the release of aroma compounds probably through bonds of a hydrophobic nature. In fact, mucin contains several hydrophobic domains and overall has a negative charge, due to the presence of some sulphate groups. However, also other types of non-covalent bonds cannot be excluded.

The presence of other enzymes in the saliva may also be involved in phenomena of oxidation, thus affecting the aroma release especially for high-fat food [121]. Some enzymes involved in the oxidation process of polyunsaturated fatty acids (PUFA), such as acyl hydrolases (AH), lipoxygenase (LOX), hydroperoxide lyases (HPL) and alcohol dehydrogenase (ADH), might be active in the mouth [119] and could lead to the formation of mainly C_6 aldehydes [125].

A possible effect of these enzymes was observed in a study that simulated the conditions of the mouth by using a retro-nasal aroma simulator (R.A.S.), which simulates the aroma perceived by the retro-nasal route. The result showed an increase in hexanal and *trans*–2–pentenal in the presence of human saliva [126]. Therefore, PUFAs are detectable in humans by multiple sensory systems: gustatory, olfactory and somatosensory signals [68,121]. Other molecules, mainly esters and linalool, showed a reduction in aroma compounds only in the presence of polyphenols, possibly to be attributed to a direct action of salivary constituents in the saliva-polyphenol interaction [126].

However, saliva may also have an indirect effect on aroma release in the mouth by modifying the rheological properties of food, especially viscosity and spreadability for high-fat foods. It has been reported in an in vitro study that the addition of artificial saliva to dressings increases the viscosity of food changing the aroma release. The authors explained their finding stating that saliva increases the surface exchange with the gas phase [127]. Some other authors also confirmed these results via in vivo studies on a model cheese [128].

The flow rate and composition of the saliva varies between subjects and for each individual subject may vary within a day [116,120]. The degree of hydration, body position, exposure to light, smell, smoke, previous stimulation, age, climatic conditions, cardiac, and respiratory rhythms are all factors responsible of salivary variability [129].

The inter-individual variability of saliva is the cause of different perceptions of different individuals for the same foods [130,131] or also of different preferences [69,72]. Neyraud and co-workers [120] suggested a possible link between perception and preference for fatty foods, indicating a potential enzymatic activity of saliva in "guiding" the sensory perception of the fat. In particular, they evaluated the intra- and inter-variability of salivary enzymatic activity on foods, by linking this activity to fat perception and preference. To do this, they only included the biochemical variables which showed a good stability over time, such as flow, lipolysis, lipocalin, proteolysis and the total antioxidant status.

3.4. Interaction Between Food Matrix and VOO Volatile Compounds

It has been reported that non-volatile compounds present in food, such as proteins, carbohydrates and phenolic compounds, may interact with saliva proteins and volatile compounds affecting the aroma release. These interactions may alter the food-air partitioning (volatility) of the aroma compounds [132–135].

VOO phenolic compounds, whose total content is typically around 300 mg kg^{-1}, have been recently shown to affect the release of aroma compounds in saliva-in-oil emulsion with tendency of a lowest headspace release of almost all volatile compounds (significatively for esters, linalool and *trans*–2–hexenal) [126,136]. In another study, eight volatile compounds were monitored under dynamic conditions, both in vitro and in vivo, in a refined olive oil enriched with phenolic compounds (593 mg kg^{-1}) extracted from VOO, in comparison to a blank refined oil without phenolic addition [136]. In the in vitro study it was found that

phenolic compounds can interact with molecules such as hexanal, linalool and 1-hexanol, possibly through non-covalent but reversible bonds. The in vivo study showed that, after swallowing, in the presence of phenolic compounds, the release of 1–penten–3–one, *trans*–2–hexenal, *cis*–3–hexenyl acetate, and ethyl butanoate is lower than the one in the blank sample (Figure 4), thus confirming the results obtained in the previous study [126].

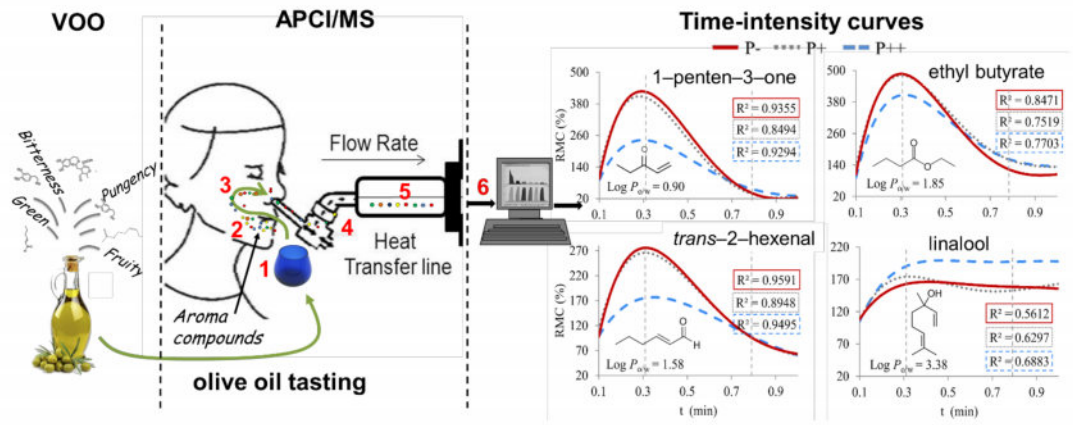


Figure 4. The principle of in vivo study by APCI/MS analysis of the aroma release (time-intensity) in mouth. Legend: (1) VOO aspiration in mouth (without ortho-nasal route), (2) VOO odorants interaction with saliva in the oral cavity, (3) retro-nasal route (aroma after interaction with saliva), (4) aroma expulsion, (5) transfer line, (6) APCI/MS mass spectrometer with registration of time-intensity curves for extra virgin olive oil aroma compounds. P− = VOO without phenolic compounds, P+ = with 354 mg kg^{-1} of phenolic compounds, P++ = with 593 mg kg^{-1} of phenolic compounds (adapted from Genovese and co-workers [136]).

Among the above-mentioned volatile compounds, 1–penten–3–one and *trans*–2–hexenal are the most important active odor aroma compounds contributing to VOO fruity note [19]. 1–penten–3–one is important for its low odor threshold while *trans*–2–hexenal for its usual high concentration in VOO [137,138].

In fact, in another study, it also emerged that a concentration of polyphenols of 511 mg kg^{-1} is responsible for the reduced perception of the positive fruity attribute of about 39% compared to an oil with a lower concentration of polyphenols, of 298 mg kg^{-1} [61]. In this sensory study, the higher concentration of phenolic compounds simulated a very bitter VOO while the lower a slightly bitter VOO [49]. Regarding the VOO off-flavors, it has been reported that "fusty" defect is perceived less (23%) in an oil with more biophenols. On the contrary, no important effect of VOO phenolic compounds on the perceived intensity of the "rancid" defect has been reported although a positive trend has been found in VOO with a higher concentration of polyphenols.

The explanation of this effect is attributed to the formation of VOO phenolic compounds-PRPs (Proline Rich Proteins) complexes that could retain some volatile compounds in the hydrophobic cavities and therefore, modifying their release into the mouth cavity. Most of the studies focused on interaction between phenolic compounds and saliva proteins concern tannins, which are responsible of astringency perception [139]. For instance, salivary PRPs have a high affinity for tannins [140]. In addition, smaller polyphenols (propyl gallate and epicatechin) can bind with one phenolic ring stacked against each proline residue, whereas larger polyphenols occupy two or three consecutive prolines [141]. The result of these interactions in wine could be similar to what occurs for VOO perception. In fact, sensory studies showed that the intensities of "fruity" and "floral" aromas is lower in wine when the level of polyphenols is increased [142]. However, it is expected that the sensory impact of this interaction might be lower compared to the effect of tannins, due to the relatively weak affinity of olive oil phenolics for the different food proteins compared to tannic acid [143].

On the contrary, for linalool and 1–hexanol, it has been reported a "salting-out" effect. These two volatile compounds can interact with VOO polyphenols in in vitro test. Other volatile compounds, on the contrary, resulted in a lower release in vivo and they are not able to interact with VOO phenolic compounds. In the presence of saliva, the interaction between phenolics and salivary proteins could attenuate the activity of the phenolics against volatile compounds and consequently cause a greater release in the oral cavity. This could explain why from a sensory point of view, other VOO descriptors increase like the "wine-vinegar" defect [61].

Another important aspect of VOO phenolics is the possible formation of polyphenol–mucin complexes that could affect the bioavailability and antioxidant capacity of phenolic compounds during VOO digestion [144].

Finally, it is currently unknown whether VOO phenolic compounds are able to induce a change in the secretion of saliva both in term of composition and flow that could further influence the VOO aroma release. However, these taste stimuli are known to strongly affect salivary gland functionality and therefore could affect saliva composition [145] and aroma release.

4. Conclusions

This review summarizes the factors involved in VOO flavor formation, analysis and detection with an in-depth focus on the mechanisms affecting its perception. The initial composition of the VOO influences volatile and non-volatile compounds release as well as its flavor perception, which are interdependent because of their modulation by the oral process. Phenolic compounds were also shown to affect the release of aroma compounds during the consumption of VOO. The quality classification of olive oils, defined by several chemical indices and the official sensory assessment ("panel test"), could be influenced by these effects, which can modify the score given by a panel test during a VOO organoleptic assessment, especially in "extreme" situations in which the sensory evaluation could be decisive for the commercial classification of VOOs. For the olive oil mill industry, thus, the understanding of these factors affecting flavor delivery remains of critical importance for a successful product development and to ensure a long shelf-life to botted EVOO. Therefore, the accurate instrumental measurement of the volatile molecules exerting an odor impact, together with the investigation of the impact of biophenols and of taste-smell interactions, will help to define in a more precise manner the identity and sensory quality of VOOs.

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