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Review

A Narrative Review of Metabolomic Insights into Olive Oil's Nutritional Value

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Abstract: This review explores the critical role of advanced metabolomic methodologies in interpreting the complex relationship between the bioactive compounds in olive oil and their health benefits. Olive oil, the cornerstone of the Mediterranean diet, is renowned for its numerous health benefits, including anti-inflammatory, antioxidant, and cardioprotective properties. This review begins with an overview of the importance of olive oil for nutrition and health, followed by a detailed discussion of the key metabolites that contribute to its therapeutic potential. It further explores the evolution of analytical techniques in metabolomics from traditional methods to cutting-edge technologies, such as mass spectrometry and nuclear magnetic resonance spectroscopy, which have enhanced our understanding of the complex composition of olive oil. This review highlights how these advanced analytical findings correlate with the known health properties of olive oil and presents case studies in which these methodologies have led to new insights. Finally, we address the challenges in standardizing these techniques and discuss future research directions, underscoring the significance of metabolomics in nutritional science and public health. This comprehensive analysis aims to provide a deeper understanding of the health benefits of olive oil, informed by the latest advancements in analytical methodologies.

Keywords: olive oil; bioactive compounds; health benefits; antioxidant properties; metabolite profiling

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1. Introduction

1.1. Importance of Olive Oil in Health and Nutrition

Olive trees (*Olea europaea* L.) are found in all landscapes surrounding the Mediterranean Sea, including Spain, Italy, Greece, and northern South Africa. Olive oil is a globally recognized symbol of all cultures present in this geographical area and is a testament to the harmonious relationship between the land and its inhabitants. Olive oil obtained directly from olives, the fruit of olive trees, has played a fundamental role in the history and culture of humanity for centuries. In ancient Greece, it was a symbol of a high social status. The wealthy used olive oil for cooking, lighting, and medicinal purposes. Most Greek doctors believed that it had therapeutic properties. Hippocrates mentioned and registered more than 60 uses, from treating wounds to burns. Even Greek athletes would anoint themselves with olive oil before competing, and the winners would receive a large amount as a reward [1]. During the Roman Empire, olive oil gained immense importance not only as a

food item but also for body care, cosmetics, lighting, and medicine. Interestingly, Hispania exported more than 30 million amphorae of olive oil. Most of this olive oil was produced in Andalusia, a region located south of the Iberian Peninsula. Therefore, it can be said that, in ancient times, those who possessed olive oil were considered to have a treasure not only for its economic value but also its nutritional and therapeutic benefits. As a result, it has been passed down through generations and is still highly valued in modern civilization [1].

This "golden liquid" has also contributed to the establishment of a healthy dietary pattern known as the Mediterranean diet (MD). The polyphenols present in olive oil, particularly extra virgin olive oil (EVOO), possess antioxidant, anti-inflammatory, and neuroprotective properties. Several clinical trials have demonstrated that olive oil consumption is associated with a lower prevalence of chronic diseases such as cardiovascular conditions, obesity, cancer, and even neurodegenerative disorders. The Prevención con Dieta Mediterranea (PREDIMED) study is a Spanish clinical trial that aimed to explore the preventive effects of MD on cardiovascular and aging-related disorders. The results of this study demonstrated that this particular dietary pattern, which is rich in olive oil, reduces the incidence of these disorders and even delays cognitive decline, leading to an increase in longevity [2,3]. PREDIMED demonstrated that the administration of olive oil in the context of MD consumption in patients and animal models reduced the amount of low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL), resulting in protection from the risk of cardiovascular diseases (CVDs) which, following MD, was approximately 31% lower compared with a control diet [4]. Furthermore, a meta-analysis found a protective role of olive oil consumption in the reduction of the relative risk of CVDs for an intake of >20 g/day of olive oil. In particular, there was a dose-dependent reduction of 4% for every 5 g/day increase in olive oil consumption [4]. In the same context, the Coronary Diet Intervention with Olive Oil and Cardiovascular Prevention (CORDIOPREV) study was a long-term randomized trial that compared the effects of an MD enriched with olive oil versus a low-fat diet for the incidence of cardiovascular events. The results demonstrated that a diet enriched with olive oil has a greater preventive effect against CVDs [5]. In contrast, the MICOIL pilot study compared the effect of Greek high-phenolic early harvest extra virgin olive oil (HP-EH-EVOO) versus moderate-phenolic extra virgin olive oil (MP-EVOO) and an MD in people with mild cognitive impairment. They concluded that long-term intervention with HP-EH-EVOO or MP-EVOO was associated with a significant improvement in cognitive function compared with the MD, delaying the prevalence of neurodegenerative disorders [6].

Given the significant historical importance and growing scientific evidence supporting the health benefits of olive oil, it is crucial to further investigate the metabolomics underlying its nutritional value. Metabolomics, the comprehensive examination of metabolites within a biological system, provides a unique perspective on the intricate biochemical processes influenced by olive oil consumption. By exploring the metabolomic profile of olive oil, especially EVOO, scientists can identify the compounds responsible for its protective effects against various diseases. The rich history of olive oil is now being complemented by cutting-edge research, which has revealed its complex biochemical composition and significant impact on human health. The continuation of this research not only reaffirms the cultural and historical importance of olive oil but also solidifies its role as a cornerstone of nutritional science and preventive medicine. This narrative review aims to emphasize these metabolomic insights, offering a deeper understanding of why olive oil has been and continues to be a treasured component of human civilization.

1.2. Introduction to Metabolomics

The concept of "metabolomics" was defined for the first time in 1999 as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" [7]. The main scope of omics science is to study the function and interaction of a vast quantity of biological data, with the aim of comprehending their contribution to human health. It provides an overview of the

metabolic status and global biochemical events associated with a cellular or biological system, focusing on the study of hundreds of metabolites in cells, tissues, and biofluids [8]. Spectroscopic analysis of different biosamples has been employed in metabolomics studies to collect metabolites based on specific phenotypes such as illness, condition, or exposure to treatment [9]. In the context of food and nutrition, nutrimetabolomics (or nutritional or food metabolomics) is a new concept related to nutritional science, and it is crucial to decode the link between diet and health [10]. This has significantly impacted food research, improving the sensitivity demanded by the existing food quality and safety legislation, as industry and society demand the identification of the real effect of one's diet on human health. It has also opened new opportunities for biomarker food intake discovery and the identification of new metabolite biomarkers in body fluids following the consumption of various foods, ingredients, meals, or diets [11].

A biomarker is defined as an "objective measure used to characterize the current condition of a biological system" [12]. Considering this concept, it is imperative to highlight the importance of biological interpretation of the results obtained in metabolomic studies. It can be challenging to ascertain the suitability of certain metabolites as reliable biomarkers. Some metabolites, despite being present in a diet, are normally produced by humans, which makes it difficult to determine whether the levels found in both plasma and urine are related to consumption or endogenous production. Nevertheless, thousands of diverse polyphenolic compounds, organic acids, and terpenoids have the advantage of not being synthesized in mammals and only in a given plant or plant family, and they cannot be degraded by human enzymes, being the only substrate of different fermentative bacteria that produces the degradative substances found in urine and blood. Therefore, these compounds can be regarded as credible biomarkers [8].

Nutrimetabolomics plays a fundamental role in associating food compounds with health outcomes by providing a comprehensive overview of the metabolic status of a biological system and identifying the key biomarkers linked to dietary patterns and health. However, it is fundamental to be aware of the complexity of the process, since the concentration of many metabolites is given as "relative levels", and it is complicated to establish a direct connection between these metabolites and one's health condition.

2. Olive Oil Metabolomics

The metabolites present in olive oil have been the subject of extensive research, owing to their fundamental relevance to health. Studies have identified clear benefits of this important component of the MD in protecting against chronic and degenerative diseases, which can be attributed to its metabolites [13]. Olive oil extraction involves collection, cleaning, extraction, separation, centrifugation, storage, and packaging. However, to obtain higher-quality oil, namely virgin olive oil (VOO), no heat or chemicals can be used, because it is directly obtained from the olive only through mechanical processes under specific thermal conditions that do not cause alterations. VOO is rich in phenolic compounds and has an acidity level of $\leq$2 g per 100 g [14,15]. EVOO has the highest quality and lowest acidity level of 0.8 g per 100 g [15]. The main components of olive oil are triacylglycerols, free fatty acids, mono- and diacylglycerols, and in a reduced percentage, there are highly bioactive minor compounds (Figure 1) [16]. In subsequent sections, the health benefits of the key metabolites are described.

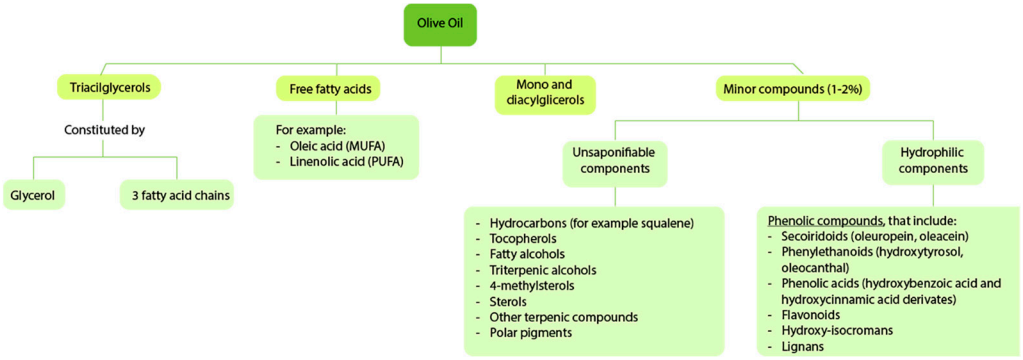

Figure 1. Graphical representation of olive oil components. MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

2.1. Oleic Acid

About 70–80% of olive oil corresponds to oleic acid (OA), a monounsaturated fatty acid (MUFA). The main health benefits of olive oil have been attributed to OA for several years. For instance, it facilitates healing of cutaneous wounds by inhibiting early production of nitric oxide (NO). The lack of NO leads to the secretion of mediators by fibroblasts and inflammatory cells and a reduction in collagen deposition at the wound [17]. Furthermore, as an MUFA, OA regulates the structure of the cellular membrane, resulting in the regulation of adrenergic receptors and, consequently, a decrease in blood pressure, highlighting its important hypotensive effect [18].

The antioxidant effect of this metabolite is notable because it regulates the synthesis and activity of several antioxidant enzymes, which may contribute to its hypotensive effect by improving endothelial dysfunction. In general, supplementation with OA can reduce the levels of C-reactive protein (CRP), an inflammatory biomarker, thereby diminishing the risk of CVDs [19,20]. Although a study analyzing data from several randomized trials concluded that there were no significant alterations in important pro-inflammatory mediators, such as interleukin (IL)-6 or tumour necrosis factor alpha (TNF-α), following OA treatment [19], some authors described a reduction in the levels of these mediators [21,22]. Even though several other anti-inflammatory effects of OA have been described, such as increased regulatory T cells and decreased nuclear factor kappa B (NF-κB) levels in macrophages, some proinflammatory effects appear to be important. For instance, OA causes increased levels of reactive oxygen species (ROS) and matrix metalloproteinase (MMP)-9 in processes mediated by phospholipase C as well as increased granule release and phagocytosis in neutrophils. ROS production following OA supplementation can increase the killing capacity and phagocytosis of neutrophils, thereby contributing to the efficiency of the immune system. Moreover, OA enhanced lymphocyte proliferation [23].

OA plays a fundamental role in the development of insulin resistance and diabetes. Insulin resistance frequently leads to the development of diabetes, both of which are caused by decreased mitochondrial function [20]. This decreased mitochondrial function might result in incomplete oxidation of fatty acids, leading to the accumulation of lipid metabolites and lower generation of adenosine triphosphate (ATP). Normally, ATP regulates the exocytosis of insulin granules in pancreatic β cells, leading to the closure of ATP-sensitive K^+ channels and, consequently, the depolarization of these cells. Subsequently, calcium channels open, allowing the secretion of granules containing insulin. Insulin resistance occurs in the absence of high ATP levels [24–26]. OA upregulates the expression of genes responsible for fatty acid oxidation and improves insulin sensitivity. Furthermore, activation of nuclear respiratory factor 1 (NRF-1) by OA upgrades mitochondrial biogenesis, potentially contributing to decreased resistance [20].

There is a correlation between OA and a lower prevalence of neurodegenerative diseases [27]. This fatty acid has shown potential for therapeutic use because it can improve amyloidosis in cellular and mouse models of Alzheimer's disease [28]. Furthermore, both

OA and linoleic acid, a polyunsaturated fatty acid (PUFA) that also constitutes olive oil, are associated with a decreased risk of Parkinson's disease. In fact, a complex containing camel α-lactalbumin and OA has shown promising results as a potential therapeutic component by decreasing oxidative stress, improving dopamine levels, enhancing the expression of silent mating type information regulation 2 homolog (SIRT1), and preventing neurodegeneration [29]. Moreover, a low concentration of free fatty acids, including OA, in plasma has been found to be a pathogenic biomarker of amyotrophic lateral sclerosis. In fact, nitro-OA can cross the blood- brain barrier, inducing a neuroprotective action, which indicates that NO_2-OA could be considered a therapeutic approach [30,31]. OA exerts remarkable anticancer effects by reducing tumor cell proliferation, suppressing oncogene overexpression, and inducing apoptosis [32].

2.2. Mono- and Diacylglycerols

Mono-and diacylglycerols, which are relatively understudied components of olive oil compared with their counterparts, have not been overlooked in certain research endeavors, where the findings highlight some of their potential health benefits. For instance, monoacylglycerols enriched in OA have demonstrated antioxidant, antiatherogenic, and antidiabetic effects, as they contribute to the inhibition of insulin levels in the blood following the ingestion of food [33]. In contrast, diacylglycerols possess the ability to activate enzymes involved in β oxidation of fat. Furthermore, they reduce the risk of CVDs by decreasing the levels of CRP, TNF-α, inducible nitric oxide (i-NOS), cyclooxygenase (COX)-2, and vascular cell adhesion molecule-1 (VCAM-1), which have antidiabetic effects. Diacylglycerols can control the production of inflammatory mediators and cytokines, highlighting their involvement in inflammation [34].

2.3. Minor Compounds

2.3.1. Phenolic Compounds

Phenolic compounds are a category of minor compounds present in olive oil (Figure 1). Numerous health benefits of olive oil have been attributed to the presence of polyphenols. These compounds are associated with high antioxidant potential, which is influenced by two factors. First, the presence of at least one hydroxyl group allows the donation of a hydrogen atom or electron to a free radical. Second, the existence of one or more aromatic rings contributes to the delocalization of unpaired electrons [35]. Phenolic compounds can be subdivided into secoiridoids, phenylethanoids, phenolic acids, hydroxy-isochromans, flavonoids, and lignans [36].

Oleuropein

The secoiridoid oleuropein (OLE) and its derivative hydroxytyrosol (HT) are two of the most important polyphenols, and their concentrations decrease during olive fruit maturation [13]. OLE exists at high concentrations in unprocessed olive leaves and olive fruit, whereas high concentrations of HT are present in olive fruit and olive oil [37].

OLE can protect cellular membranes against metal-induced lipid oxidation because of its ability to scavenge free radicals and chelate metals. Its antioxidant effect is evident from a reduction in the production of ROS and reactive nitrogen species (RNS) in in vitro cell-free systems. Furthermore, hydrogen peroxide-induced DNA damage is reduced when OLE is present, which stimulates the expression of intracellular antioxidant enzymes when cells are exposed to oxidative stress-inducing agents, such as angiotensin II [38]. In ethanol-induced oxidative stress, OLE enhances the levels of glutathione peroxidase (GPx-1), superoxide dismutase (SOD), and total glutathione (GSH) [39]. This suggests that OLE recruits antioxidant enzymes when the cell is exposed to oxidative stress caused by different compounds, thereby protecting against oxidative damage that could compromise several biomolecules.

Moreover, the cardioprotective and hypotensive effects of OLE have been demonstrated in several studies [39–42]. Administration of OLE resulted in decreased expression

of enzymes that, when present at high levels, potentially indicate heart damage, such as lactate dehydrogenase (LDH) or alanine aminotransferase. In rabbits fed a high-cholesterol diet, OLE reduced LDL and cholesterol levels and increased ATP levels, indicating its cardioprotective effects [39]. A study examining the effects of OLE in rats with acute myocardial infarction found that the animals treated with OLE had lower levels of malondialdehyde (MDA), IL-1β, TNF-α, creatine kinase-MB, troponin I, and LDH. Hence, treatment with OLE mitigates cardiac impairment because of its ability to alleviate oxidative stress and inhibit the release of proinflammatory cytokines [40]. A study conducted on human peripheral blood cells, in which OLE and HT were tested, showed that these two compounds have a protective role against oxidative DNA damage [41]. Among olive oil metabolites, OLE has shown cardioprotective effects against acute adriamycin cardiotoxicity and anti-ischemic and hypolipidemic activities. Indeed, the administration of a diet containing 7 mg/kg OLE reduced the total cholesterol and LDL oxidation levels in rabbits [42]. In rat models, the role of OLE in the reduction of oxidative stress was measured in ischemic hearts, relieving oxidized GSH and resulting in a reduction in oxidation-related damage [42].

Furthermore, olive leaf polyphenols have been shown to inhibit platelet function in vitro in the blood of 11 healthy, non-smoking males [43]. They produced significant dose-dependent suppression of ATP release by platelets and thus platelet aggregation. In addition, studies have highlighted how polyphenols, such as OLE, reduce cardiotoxicity induced by antineoplastic agents, such as doxorubicin, thereby inhibiting lipid peroxidation products and oxidative stress and reducing iNOS in cardiomyocytes. OLE also appears to promote the restoration of metabolic activity in stressed cardiomyocytes [44].

In addition, OLE seems to induce increased scavenger activity in hypertensive rats, acting on the expression of antioxidant factors such as SOD and at the molecular level for Nrf-2, leading to increased production of related antioxidative enzymes, nicotinamide adenine dinucleotide phosphate (NADPH) quinone dehydrogenase 1, and heme oxygenase-1. Furthermore, the activity of OLE against lipoxygenase (LOX) has been previously reported [45].

Nonetheless, OLE also has significant antidiabetic properties. Phenolic compounds can decrease the activity of digestive enzymes but can also inhibit glucose transporter 2 (GLUT2) in a dose-dependent manner. Since the OLE molecule contains a glucose residue, it can compete with sodium/glucose cotransporter 1 (SGLT1). Other mechanisms that contribute to this antidiabetic effect include activation of the adenosine monophosphate-activated protein kinase (AMPK) pathway, heightened incretin release, antioxidant activity, and increased testosterone levels [46].

Consistent consumption of OLE appears to be associated with a reduced likelihood of developing neural disorders. In fact, it not only induces apoptosis and autophagy but also improves the antioxidant capacity of the cerebral region and diminishes the release of inflammatory mediators in situations where they are not entirely required, thereby preventing neuroinflammation [47]. Finally, OLE has been reported to have important antimicrobial and antineoplastic effects [39].

Hydroxytyrosol

As previously mentioned, OLE is a precursor of HT which has several beneficial effects on health. In oxidative stress situations, HT is able to not only stimulate the activity of protective enzymes but also act in mitochondrial biogenesis. Furthermore, it improves endoplasmatic reticulum (ER) homeostasis and activates a pathway that follows ER stress, thereby preventing ROS accumulation. HT exhibits the capability of inhibiting copper sulphate-induced oxidation of LDL, decreasing the plasma levels of LDL and total cholesterol, and increasing the levels of HDL, thereby contributing to overall cardiovascular health [37]. Further health benefits of HT have been described, including its involvement in protection against metabolic disorders [48,49], neuroprotective effects [50,51], and impressive potential for cancer prevention and therapeutics [51].

The numerous health benefits of olives and their bioproducts can be attributed to the anti-inflammatory properties of polyphenols [52], like HT and OLE, whose such properties have been extensively studied and treated [53]. The administration of olive oil containing 5 mg/kg HT affected serum TNF-α levels and iNOS and COX-2 expression [54]. Similarly, treatment with HT-20, a preparation containing 20% HT, reduced the TNF-α and IL-1β mRNA levels in a mouse model of acute inflammation and hyperalgesia but did not influence the IL-10 levels [54]. Furthermore, THP-1 macrophages cultured with increasing doses of HT (25, 50, and 100 μM) before LPS stimulation showed a dose-dependent reduction in TNF-α, iNOS, and COX-2 protein and gene expression levels, underlying the connection between the modulation of inflammatory and oxidative processes [55]. Similar results were obtained in a model of inflammation in LPS-stimulated J774 murine macrophages, in which downregulation of iNOS and COX-2 gene expression was observed by preventing NF-κB, STAT-1α, and IRF-1 activation mediated by LPS-induced ROS generation [56]. Treatment with HT and the derivatives of LPS-stimulated human monocytes induced a reduction in the mRNA levels of COX-2 and prostaglandin 2 and increased TNF-α production, in contrast to what we have observed thus far [57]. HT also reduces the expression of IL-1β and chemokines such as macrophage migration inhibitory factor and RANTES, as well as ROS production [58]. The cells considered in these in vitro studies, such as monocytes and macrophages, are important players in atherosclerotic plaque formation and in the pathological process underlying the development of CVDs. The addition of olive oil extracts enriched in OLE to THP-1 cell cultures modulated the levels of MMP-9 and TNF-α expression, influencing the function of monocytes in the onset of vascular diseases such as atherosclerosis, in which the inflammatory component is prevalent [58]. An effort in this study was made by the administration of OLE to a myocardial infarction group of people, which reduced the TNF-α and IL-1β levels [59].

Oleocanthal and Hydroxy-Isocromanans

Interestingly, the phenolic compound oleocanthal has anti-inflammatory effects comparable to those of ibuprofen through the inhibition of COX-1 and COX-2, which are involved in prostaglandin synthesis. This mechanism of action may contribute to the prevention of CVDs and cancer [52,60]. The neuroprotective action of oleocanthal as well as its ability to enhance neuronal energy metabolism and diminish the harmful effects of protein glycation have been reported [61]. Furthermore, this compound can enhance the removal of amyloid beta protein from neurons, a key component in the pathogenesis of Alzheimer's disease, and mitigate astrocyte inflammation [62]. Hydroxy-isocromanans have also been found to exhibit anti-inflammatory activities [59].

Flavonoids and Lignans

Generally, flavonoids appear to have anti-inflammatory and antioxidant activities as well as antidiabetic, anticancer, and cardiovascular protective properties [63]. Lignans are a class of phenolic compounds that have several health benefits. In particular, (+)-pinoresinol exhibits antioxidant and anti-inflammatory activities by inhibiting NO and the mediators prostaglandin E2 (PGE2), COX-2, TNF-α, IL-1β, IL-6, and NF-κB. Additionally, it possesses the ability to be antifungal, neuroprotective, and hypoglycemic [64].

2.4. Tocopherols

Tocopherols, also known as vitamin E, are important metabolites with antioxidant properties that are found in olive ingredients [65]. They can stabilize oils during storage, with α-tocopherol being the most abundant [66], and α-tocopherol can prevent atherosclerosis by acting as a scavenger of oxidized LDL. Furthermore, it could potentially be used not only for prevention but also for treatment, because its anti-inflammatory activity allows it to modulate the activity of proteins that can degrade atherogenic lipids [67]. However, a persistent problem has been that clinical trials involving humans that have tested the efficacy of α-tocopherol in reducing atherosclerotic plaques have yielded controversial

findings. The reduction in atherosclerotic plaques could not be consistently substantiated, and even when apparent, it did not always translate into favorable outcomes in epidemiological studies. Hence, other isoforms have been investigated; γ-tocopherol has a higher antioxidant potential, and its combination with α-tocopherol shows higher efficacy than when used alone [68,69]. Additionally, because vitamin E deficiency is associated with several neurological problems, it is safe to assume that it may play a role in the prevention of neurodegenerative diseases. Vitamin E plays a protective role in neurodegeneration and is a risk factor for the development of neurological diseases such as Parkinson's disease and amyotrophic lateral sclerosis [70].

3. Techniques Used for Metabolite Profiling and Evolution

Metabolite profiling allows for the identification and quantification of metabolites. To prepare samples, the compounds of interest must normally be isolated from the matrix through chromatographic separation, and detection is performed using mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy [71]. Analysis of the different components of olive oil, particularly VOO, is essential, considering that this field allows for the discernment of potential adulteration with other vegetable oils, characterization of bioactive minor compounds, and detection of metabolites of pesticide residues, thus evaluating the overall safety of olive oil [72].

Metabolite analysis has evolved significantly over the years, from basic techniques such as chromatography to advanced methods such as MS and NMR spectroscopy. Therefore, a timeline for techniques used for phenolic compound analysis can be established. In 1973, the Folin–Ciocalteu method was commonly used to determine the total phenolic content in a sample, but it was modified because a purification step via paper chromatography or thin layer chromatography (TLC) was added (Figure 2) [73]. This method involves the reaction of polyphenols with a redox agent, namely the Folin–Ciocalteu reagent, which leads to the formation of a blue complex that can be accurately measured and quantified using visible light spectroscopy [74]. In the 1980s and the early 1990s, gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) coupled with MS emerged as new approaches for the analysis and separation of these compounds. In general, HPLC methods are more efficient, owing to the utilization of ultraviolet detection and the fact that they do not require chemical or physical modifications of the compounds before quantification. High-resolution gas chromatography (HRGC) was developed in 1987 (Figure 2) [73].

For the unsaponifiable components, different approaches took place in the late 1990s and the early 2000s, depending on whether the compound of interest was a hydrocarbon, carotene, tocopherol, linear or triterpenic alcohol, 4-methylsterol, sterol, or sterol oxidation product. Nevertheless, a saponification step, followed by TLC, was common to all components and allowed the separation of the different types. GC or HPLC can be used for hydrocarbons, although the latter separates polycyclic aromatic hydrocarbons, which originate from environmental pollution, from other hydrocarbons more efficiently. HPLC is preferred for carotenoids because GC degrades them. Tocopherols were often eluted with other compounds in TLC and were then analyzed as trimethylsilyl derivatives in non-capillary GC columns (cGCs), which have also been used for linear and triterpenic alcohols. However, it is difficult to separate them through TLC because of their higher polarity. In fact, cGCs were used for both 4-methylsterols and sterols, although TLC could separate sterols into two bands: one corresponding to 7-sterols and the other corresponding to 5-sterols. Finally, analysis of sterol oxidation products focused on cholesterol, whose separation occurred through cold saponification and enrichment by silica or aminopropyl solid-phase extraction. Among these compounds, cGCs offered better results than HPLC [75].

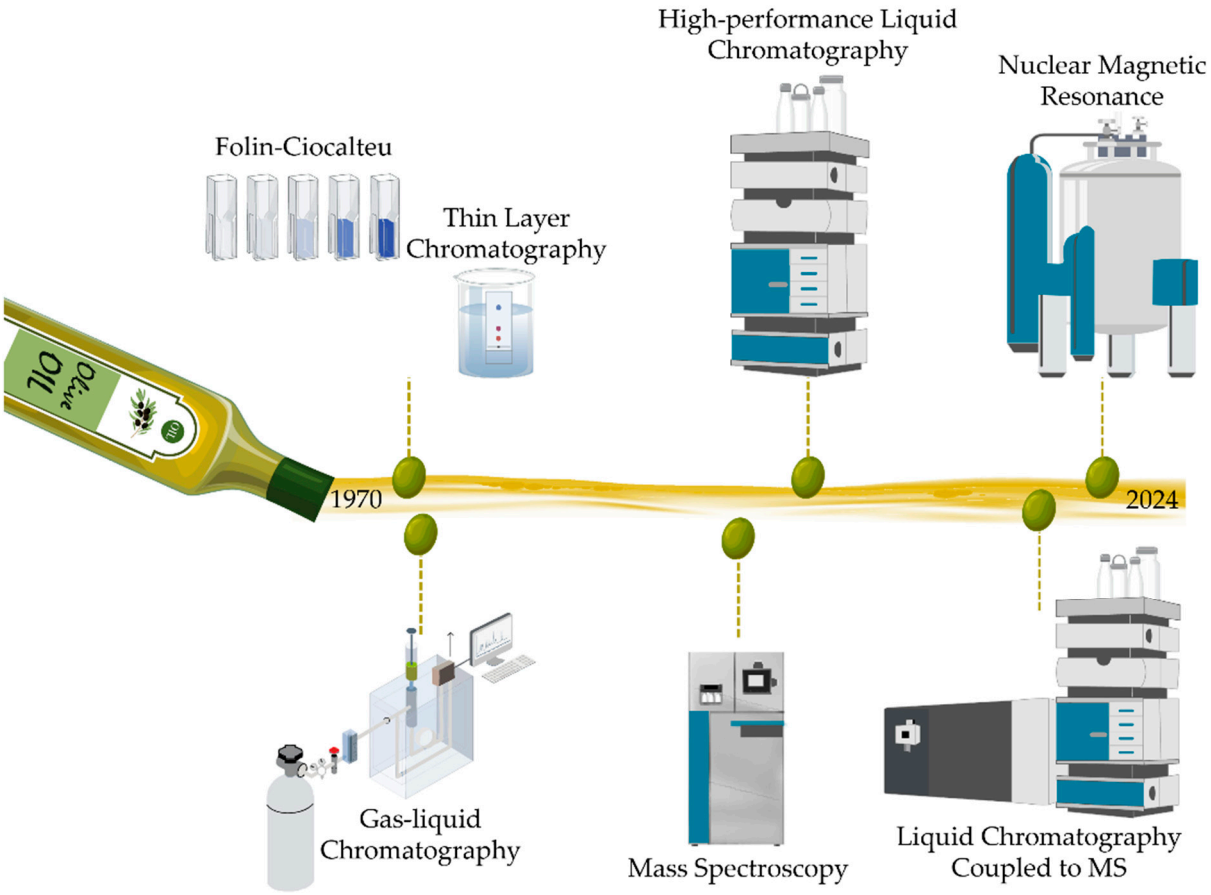

Figure 2. Schematic representation of the already-used methods for the identification and quantification of olive oil metabolites.

Currently, metabolomics can be targeted or untargeted. The first involves methods that determine a particular group of metabolites of interest, whereas the second involves measuring the maximum number of metabolites without focusing on any particular group [76]. MS and NMR are the two main types of spectroscopy used for metabolite profiling. Some crucial advantages of MS are its high sensitivity and resolution, which allow the visualization of chemical diversity over a wide dynamic range. Furthermore, it enables the determination of the molecular weight and the structure of unknown compounds. However, in NMR, there is no need to preselect the conditions to perform the analysis, which permits the generation of spectra with a high information content. Moreover, this technique allows easy quantification of metabolites, and the NMR chemical shifts are relatively stable, which guarantees that the results are reproducible under consistent experimental conditions (Figure 2) [77].

MS uses a mass spectrometer to measure the masses of different molecules in a sample of interest. This process focuses on specific signals, such as the ion current generated by molecules of a selected and specific mass. The detector in the mass spectrometer senses the relative differences in these signals, manifested as peaks or drops, and calculates them as the response ratios. These ratios are determined by comparing the chromatographical changes with the baseline signal [78]. Several analytical tools can be used for MS-based metabolomics, such as liquid chromatography (LC) coupled with MS (LC-MS), two-dimensional LC coupled with MS (LCxLC-MS), GC coupled with MS (GC-MS), two-dimensional GC coupled with MS (GCxGC-MS), direct infusion with MS (DI-MS), which involves direct introduction of the sample into the mass spectrometer, and capillary electrophoresis coupled with MS (CE-MS) [76].

Chromatography allows the separation of compounds into two distinct phases: stationary and mobile. Retention of these compounds in the stationary phase results in a lower velocity of movement through the chromatographic system [79]. In LC-MS, the mobile phase is a liquid, as in TLC and capillary electrochromatography (CEC), although TLC uses capillary forces, and CEC uses the electroosmotic flow. The stationary phase in LC-MS can be liquid (liquid-liquid chromatography) or solid (liquid-solid chromatography). In general, liquid chromatography (LC) can be divided into four categories. First, normal-phase LC uses an absorbent material in the stationary phase, which is highly polar and has a relatively nonpolar mobile phase. In reverse-phase LC, the stationary phase is nonpolar, and the mobile phase is polar. Additionally, in ion-exchange LC, the analyte interacts with the stationary phase because of the ionic interactions that result in retention in the column. Finally, size exclusion LC involves separation based on size. Molecules with larger sizes do not penetrate the porous material of the stationary phase and move faster [80].

LC-MS allows the detection and analysis of phenolic compounds and unsaponifiable components and is faster than GC-MS because it does not require derivatization prior to analysis. It is possible to integrate ionization methods that can aid in the simultaneous examination of numerous classes of metabolites. Electrospray ionization (ESI) does not allow additional extraction steps in LC-ESI-MS. Nevertheless, when required, the extraction of metabolites is a fundamental step that constitutes pretreatment and can be of distinct types, including liquid-liquid extraction, liquid-liquid microextraction, ultrasound-assisted extraction, and solid-phase extraction [76].

GC can have a liquid or solid stationary phase that influences the appearance of the system. In a GC with a liquid stationary phase, the system consists of a lengthy capillary covered with a thin layer of a relatively viscous liquid or, alternatively, a polymer with liquid-like properties. A GC with a solid stationary phase uses a capillary featuring a thin porous layer on its walls or columns filled with porous particles [80].

GC-MS allows the separation and analysis of organic compounds, fatty acids, and fatty acid alkyl esters in olive oil, which are either volatile or semi-volatile. To obtain volatile compounds, chemical derivatization can be performed prior to analysis, which increases the thermal stability, sensitivity, volatility, and detector response. Ionization methods that are also used in LC-MS, such as electron and chemical ionization, can be applied. There are several methods for sample pretreatment, including solid-phase microextraction (SPME), dynamic headspace sorptive extraction combined with thermal desorption, purge and trap extraction, and solvent-assisted flavor evaporation. SPME can be performed through headspace or liquid sampling, with the former being the most common method for analyzing olive oil metabolites because it allows a valuable level of automation and repeatability. GC-MS excels in overcoming the challenges commonly associated with LC-MS, such as matrix effects and ion suppression arising from co-eluting compounds, and it is able to provide superior chromatographic resolution. Nonetheless, this technique has a significant limitation in that it is primarily applicable for the separation and analysis of low-molecular-weight compounds [76].

CE-MS allows the profiling of polar ionized and ionizable metabolites and offers several advantages, such as the possibility of utilizing small samples and decreased reagent volumes, minimal or no organic solvent consumption, and the use of simple fused silica capillaries instead of expensive LC columns. Therefore, this study presents a sustainable and cost-effective analytical technique [54].

NMR spectroscopy is an analytical technique based on the principles of nuclear spin physics. This technique exploits the inherent magnetic properties of the atomic nuclei. In fact, it operates on the assumption that either nuclei with an odd mass number (number of protons + number of electrons) or nuclei with an even mass number but an odd atomic number generate a tiny magnetic field. They are characterized by the presence of a property called nuclear spin, a form of angular momentum, and a nuclear spin quantum number (I). For atoms whose nuclei have odd mass numbers, I assumes half-integer values such as 1/2 or 3/2, whereas for atoms with even mass numbers and odd atomic numbers, I takes

integer values. These atoms generate a magnetic field that interacts with the significant external magnetic field created by the NMR instrument, allowing researchers to study these atoms and their properties. Furthermore, atoms with I = 1/2, such as hydrogen (1H) or carbon-13 (13C), result in clear signals in the NMR spectrum, which are often used in food science. NMR can be either 1D or 2D, with the latter being a better option for complex mixtures [81]. In the context of olive oil, 1H NMR has proven to be a valuable tool for understanding the details of lipid classes as well as the composition of fatty acids and several bioactive minor compounds and the levels of unsaturation, whereas 13C NMR has provided distinctive insights into the arrangement of fatty acids on the glycerol moiety and the stereochemical aspects of unsaturation. In general, no sample pretreatment is required for these methods. However, their effectiveness is not consistently high, particularly when attempting to quantify certain minor compounds such as mono-or diacylglycerols [82].

The evolution of metabolite profiling techniques has been instrumental in improving our ability to characterize a complex mixture of compounds found in olive oil and has provided valuable insights into the factors that contribute to its unique flavor and nutritional properties. Table 1 provides an overview of the commonly employed methods for analyzing the metabolites present in olive oil. This table systematically outlines each method along with its respective advantages and disadvantages.

Table 1. Commonly employed methods for analysis of the metabolites present in olive oil, along with their respective advantages and disadvantages.

Technique for the Analysis of Olive Oil	Strengths	Drawbacks
LC-MS	Does not require derivation prior to analysis. Ionization methods (such as ESI) can be applied to allow simultaneous analysis of several classes of metabolites. Sample preparation is relatively simple. The possibility of using different analytical platforms, such as time of flight (TOF) analyzers or orbital ion traps (Orbitraps), guarantees high resolution.	Ionization methods can lead to the formation of dimers or adducts, which within a single compound amplify the spectrum of molecular characteristics. Matrix effects and ion suppression arising from co-eluting compounds are common challenges associated with this methodology. Systems using TOF have decreased resolution for lower masses. Systems with Orbitraps exhibit variations in the MS/MS spectra, depending on concentration.
GC-MS	Chemical derivation is able to increase the thermal stability, sensitivity, volatility, and detector response. The application of ionization methods can occur. Capable of overcoming the challenges associated with LC-MS, such as matrix effects and ion suppression arising from co-eluting compounds. Provides superior chromatographic resolution. It is possible to acquire high-resolution MS/MS spectra (TOF).	Requires compounds in volatile form, and thus chemical derivation can be performed before the analysis. The fragmentation caused by ionization can be too extensive. Primarily applicable for the separation and analysis of low-molecular-weight compounds. To ensure sufficient numbers of points per peak, especially in the case of GCxGC separation, fast acquisition speeds may be required.
CE-MS	Small samples and decreased reagent volumes can be used. Minimal or no organic solvent consumption. It uses simple fused silica capillaries instead of expensive LC columns. Ionization methods can be used as well (ESI or MALDI).	Salt and lipids can affect reproducibility and the possibility of comparing data, and hence sample cleanness is required. Capillary coating is needed to prevent protein adhesion and, consequently, the alteration of retention times for metabolites. The usage of MALDI is not only time-consuming but also requires fractioning on a target plate. It can be associated with loss of resolution.

Table 1. *Cont.*

Technique for the Analysis of Olive Oil	Strengths	Drawbacks
NMR spectroscopy	This allows easy quantification of metabolites. No sample pretreatment is required. There is no need to pre-select the conditions to perform the analysis, guaranteeing the generation of spectra with a high information content. The chemical shifts are relatively stable, which allows the results to be reproducible under consistent experimental conditions.	The effectiveness is not consistently high, particularly when attempting to quantify certain minor compounds such as mono- or diacylglycerols.

MALDI = matrix-assisted laser desorption/ionization; ESI = electrospray ionization; TOF = time of flight. Adapted from [83].

4. Case Studies and Applications

Examples of analytical breakthroughs have provided new insights into the health benefits of olive oil. Over the years, advanced analytical techniques have uncovered new aspects of the health-promoting properties of olive oil, possibly leading to its novel applications and therapeutic uses. According to the aim of this review, a variety of analytical techniques applied in the study of olive oil focused on how these methods assess the bioavailability and effects of specific olive oil components, particularly phenolic compounds. These findings are summarized in Table 2.

Table 2. Metabolomics studies on olive oil.

Technique	Aim	Compounds Analyzed	Results	Reference
HPLC/MS/MS UPLC-MS/MS	Identification of biomarkers of exposure to phenolic compounds	HT sulfate, homovanillic alcohol sulfate, HT acetate sulfate, homovanilic acid sulfate, HT glucuronide, and homovanilic alcohol glucuronide	HT sulfate and HT acetate sulfate could be used as biomarkers for the intake of bioactive compounds	Rubió et al. [84]
UHPLC-ESI-QqQ-MS/MS	Evaluate the bioavailability of phenolic compounds	HT, HT acetate, DOPAC, homovanilic alcohol, and tyrosol	Despite having similar levels of absorption and metabolism, HT, HT acetate, and DOPAC levels did not increase proportionally with dosage, possibly due to metabolic saturation and transporter limitations	Domínguez-Perles et al. [85]
UHPLC-ESI-QqQ-MS/MS	Evaluate whether different food matrices influenced the pharmacokinetics and bioavailability of HT and its metabolites	HT, HT acetate, and DOPAC	The food matrix and nature of the oil influenced both factors being studied, with the best option being EVOO	Alemán-Jiménez et al. [86]

Table 2. *Cont.*

Technique	Aim	Compounds Analyzed	Results	Reference
UPLC-ESI-HRMS	Assess the metabolism and bioavailability of HT and tyrosol using a gastrointestinal dialysis-colon model	HT, tyrosol, and their derivates	The spectral analysis provided insights into the derivates of HT and tyrosol, elucidating potential mechanisms for their chemical transformation in the distinct conditions along the gastrointestinal tract	Sakavitsi et al. [87]
LC-ESI-QqQ-MS/MS	Evaluate the bioavailability of phenolic compounds	HT and tyrosol	This approach allowed a quantitative and qualitative characterization of the metabolism of phenolic compounds in VOO	Luque-Córdoba et al. [88]
UPLC-MS	Evaluate the bioavailability of phenolic compounds and assess whether metabolites derived from polyphenols that are present in biological samples are related to the health benefits of polyphenols	3′-O-HT-glucuronide, 4′-O-HT-glucuronide, and 4′-O-glucuronides of tyrosol and homovanillyl alcohol	Polyphenols have low bioavailability, and conjugated metabolites derived from HT have non-significant antioxidant activities, and hence they are not responsible for the health benefits associated with olive oil	Khymenets et al. [89]
H NMR HPLC	Study the implications of a phenolic compound in the gut microbiota, potentially aiding in the prediction of bacterial distribution in the human body	S-(-)-oleocanthal	Polyphenols have effects on the levels of numerous bacteria of known importance in human health	Qusa et al. [90]
H NMR	Assess the effects of MD on urine metabolites in the context of the PRIMED trial	Several metabolites from the MD, including carbohydrates, creatine, creatinine, amino acids, lipids, and microbial cometabolites	There were clear differences in the metabolites present in the urine between the three groups (MD + EVOO, MD + nuts, and low-fat diet)	Vázquez-Fresno et al. [91]
HPLC CE-MS	Evaluate the protective effect of olive oil against tumor cells	HT, tyrosol, elenolic acid, (+)-pinoresinol, 1-(+)-acetoxypinoresinol, deacetoxy OLE aglycone, ligstroside aglycone, and OLE aglycone	Polyphenols promote proteasomal degradation of the protein HER2	Menendez et al. [92]

Table 2. *Cont.*

Technique	Aim	Compounds Analyzed	Results	Reference
nanoLC-ESI-TOF-MS	Evaluate the effects of olive oil components on colon cancer cells	HT, elenolic acid, luteolin, vanillin, OLE aglycone, HT acetate, 4-OH-benzoic acid, vanillin acid, 10-H-OLE aglycone, syringarenisol, acetoxy-pinoresinol, pinoresinol, apigenin, methyl-DOA, and DOA	Phenolic compounds have antiproliferative and pro-apoptotic effects on colon cancer cells	Fernández-Arroyo et al. [93]
HPLC with diode array detection (DAD)	Assess the beneficial health effects of isolated polyphenols	HT, tyrosol, oleocanthal, and oleacein	Oleocanthal and oleacein have not only antioxidant properties but also healthy aging-promoting effects	Nikou et al. [94]

CE-MS = capillary electrophoresis mass spectrometry; DOA = decarboxymethyl oleuropein aglycone; DOPAC = 2,3-dihydroxyphenylacetic acid; H NMR = hydrogen nuclear magnetic resonance; HPLC with diode array detection (DAD) = high-performance liquid chromatography with diode array detection; HPLC = high-performance liquid chromatography; HPLC/MS/MS = high-performance liquid chromatography tandem mass spectrometry; HT = hydroxytyrosol; LC-ESI-QqQ-MS/MS = liquid chromatography electrospray ionization triple quadrupole mass spectrometry; nanoLC-ESI-TOF-MS = nano-liquid chromatography electrospray ionization time-of-flight mass spectrometry; OLE = oleuropein; UHPLC-ESI-QqQ-MS/MS = ultra-high-performance liquid chromatography electrospray ionization triple quadrupole mass spectrometry; UPLC-ESI-HRMS = ultra-performance liquid chromatography electrospray ionization high-resolution mass spectrometry; UPLC-MS = ultra-performance liquid chromatography mass spectrometry; UPLC-MS/MS = ultra-performance liquid chromatography tandem mass spectrometry.

A randomized, double-blind, controlled crossover trial aimed to identify the biomarkers of exposure to phenolic compounds that may provide insights into the potential health effects of phenol intake. The volunteers were subjected to consistent consumption of two olive oils enriched in polyphenols over a three-week period. While the HPLC/MS/MS analytical technique allowed researchers to analyze the phenolic profile of the olive oils that were used, ultra-performance liquid chromatography coupled with tandem MS (UPLC-MS/MS) allowed thorough determination of the metabolites present in biological fluids upon treatment. In general, HT metabolites are less specific. Nevertheless, HT sulphate (sulHT) and HT acetate sulphate (sulHTAc) showed remarkable potential as biomarkers for the intake of these bioactive compounds, considering that their concentrations were significantly higher after treatment. Hence, the analytical technique used for the examination of biological samples not only allowed the identification of possible biomarkers but also proved to be reliable for phenolic biomarker profiling in nutrition studies [84].

The bioavailability of phenolic compounds is associated with their activity in the human body and, consequently, their effects on health [87]. Therefore, several studies have attempted to evaluate their bioavailability using the previously mentioned analytical techniques. For instance, a study using Sprague-Dawley rats assessed the bioavailability and metabolism after oral intake of HT, HT acetate (HTA), and 2,3-dihydroxyphenylacetic acid (DOPAC) using ultra-HPLC coupled to a mass spectrometer with an ESI chamber and a triple quadrupole mass analyzer for tandem analysis (UHPLC-ESI-QqQ-MS/MS). The results demonstrated that all the compounds were absorbed in the gastrointestinal tract, and their metabolism appeared to have several resemblances. However, bioavailability depends on numerous factors such as sex, the derivative itself, and dosage. The authors found that the concentrations of HT, HTA, and DOPAC in the plasma and urine did not increase proportionally with the dosage, potentially owing to saturation of the first-phase metabolic processes and intestinal transporters [85]. The same analysis method, UHPLC-ESI-QqQ-MS/MS, was applied in a distinct study whose purpose was to evaluate whether different food matrices influenced the pharmacokinetics and bioavailability of HT and its metabolites HTA and DOPAC. In fact, a blind study involved 20 volunteers who were administered

5 mg of HT through various food matrices. Plasma and urine samples were analyzed, and the results showed that the food matrix and nature of the oil influenced both factors being studied. According to the authors, the best matrix for delivering HT and its derivatives is EVOO [86]. Furthermore, in a distinct study, the use of an in vitro dialysis system that mimics the metabolism of the human gastrointestinal system, the gastrointestinal dialysis-colon model, allowed for the study of the metabolism and bioavailability of both HT and tyrosol. Samples were collected and analyzed by UPLC-ESI-high-resolution MS (HRMS) as the compounds went through the system, allowing the authors to uncover aspects of the pathways in which these compounds are involved [87]. Moreover, the analytical technique LC-ESI-QqQ-MS/MS and a novel strategy of solid-phase extraction allowed the determination of the levels of phenols excreted in urine, contributing to the knowledge regarding their bioavailability [88]. Nevertheless, while the health benefits of olive oil appear to be clear, one study used UPLC-MS to demonstrate the low bioavailability of phenolic compounds and, consequently, their decreased concentration as free phenolic compounds in biological fluids. Polyphenols, particularly HT, are present as conjugated metabolites in urine and plasma. Contrary to previous studies, the authors demonstrated that these conjugated metabolites have non-significant antioxidant activities, suggesting that they are not the main contributors to the numerous health benefits associated with olive oil consumption [89].

Furthermore, NMR spectroscopy has contributed to the study of the implications of the phenolic compound S-(-)-oleocanthal in the gut microbiota in a novel ex vivo mouse fecal anaerobic fermentation model that mimics the human gut environment, potentially aiding the prediction of bacterial distribution in the human body. Solid-phase extraction was performed, and H NMR and HPLC analyses allowed the achievement of 99% purity for the metabolite. These results demonstrate that polyphenols can affect the levels of several bacteria of known importance in human health [90].

Another study aimed to evaluate the effect of MD on urine metabolites in the context of the PREDIMED trial. H NMR allowed a comparison between the three dietary pattern groups, and the non-diabetic individuals were divided into MD + EVOO, MD + nuts, and low-fat diet groups. The metabolites in the urine were analyzed at baseline, a one-year follow-up, and a three-year follow-up, and evidence demonstrated clear differences between the groups [91].

As previously mentioned, olive oil has a protective effect against tumor cells, mainly owing to the antioxidant effects of phenolic compounds. In fact, in a study where HPLC allowed isolation of the phenolic compounds of EVOO, and the identification of the polyphenols was confirmed via CE-MS, polyphenols were found to promote proteasomal degradation of the protein HER2, coded by the homonymous oncogene [92]. Nanoliquid chromatography coupled with ESI time-of-flight mass spectrometry (nanoLC-ESI-TOF-MS) was the analytical technique used to isolate the phenolic fraction of olive oil in a study that provided valuable insights into the antiproliferative and pro-apoptotic effects of these olive oil components in colon cancer cells [93].

These analytical techniques are fundamental for understanding the beneficial health effects of isolated polyphenols. For instance, in a survey comparison of EVOO polyphenols where HPLC with diode array detection (DAD) was used to analyze and quantify HT, tyrosol, oleocanthal, and oleacein, the authors found, in a fly model, healthy aging-promoting effects from both oleocanthal and oleacein, along with their fundamental antioxidant properties [94].

In conclusion, the ability of advanced analytical techniques to precisely identify and quantify the key components of olive oil has contributed to ongoing efforts to harness its full potential as a valuable component of MD with several health benefits.

5. Challenges and Future Directions

Metabolomics's involvement in nutrition and food production remains relatively unexplored. Its primary application lies in defining the composition of the VOO and EVOO

compounds to facilitate commercialization. However, defining metabolomic properties is time-consuming and prone to errors, necessitating standardized techniques [76].

A critical point concerns the processing methods used to produce olive oil, such as the extraction techniques and storage conditions. This could influence the chemical composition and bioactivity of olive oil as well as its metabolic profile. Future studies can have an important role in the investigation of the effects of different processing methods on the metabolomic profile of olive oil and its health-promoting properties. Understanding how processing factors affect the stability and bioavailability of bioactive compounds can guide industrial practices to produce high-quality olive oil with maximum health benefits [95].

Metabolomics definitions are particularly useful for quality control and authentication. Metabolomic changes in response to an individual's pathophysiological status, interactions with the environment, and genetic alterations are important factors for the evaluation of individual health assessments. Consequently, the significance of defining the metabolomic composition stems from the importance of different compounds in individual health [76].

"Foodomics" examines the combination of food and nutrition using techniques typical of "omics" sciences. A typical workflow involves sampling the nutritional source, preparing an extract containing the known or unknown metabolites of interest, identifying the sample, and acquiring data. In olive oil foodomics, studying metabolites through an omics approach helps to identify phenols and other relevant compounds [76].

The identification of metabolites involves the use of several methods that were previously mentioned. MS is the most used technique for identifying targeted and untargeted metabolites, and when coupled with GC, it permits identifying volatile compounds. Specific purification assays are instead essential for targeted metabolites, whereas untargeted metabolites yield a diverse range of compounds and require appropriate statistical analyses. Principal component analysis often represents the first attempt of analysis in this case [76,96]. Various other techniques, such as UHPLC-Q-TOF-MS and LC-MS/MS, have been employed to identify compounds in olive oil, aiding authenticity evaluation [76].

The challenge in applying these techniques lies in the need to combine different methodologies to profile metabolites, and each technique presents its own set of difficulties. Furthermore, analysing these compounds is challenging, owing to their chemical diversity, variable concentrations, and enormous amounts of data generated from larger analyses, particularly untargeted ones [96].

Despite the difficulties in applying these methods and the need for workflows that are as standardized as possible, olive oil metabolomics is a promising area for future research. Metabolomics offers a transdisciplinary approach for studying the metabolites present in olive oil. It involves analytical chemistry, chemometrics, and bioinformatics to comprehensively understand olive oil's metabolome [97]. The approaches and methodologies applied varied depending on the purpose of the study. For example, single-class methodologies focus on specific classes of compounds such as phenolic compounds, pentacyclic triterpenes, tocopherols, and phytosterols. They involve sample preparation, chromatographic separation, and detection using LC-MS or GC-MS. Multi-class approaches aim to simultaneously analyze multiple classes of compounds in olive oil. They offer a comprehensive view of olive oil composition and are becoming increasingly popular in metabolomic studies [96].

In this context, integrated approaches combining metabolomics with other disciplines and longitudinal studies are needed to gain deeper insights into the health impacts of olive oil and to address challenges such as authenticity and quality assurance.

In recent years, a novel technique for metabolite profiling and analysis of their involvement in metabolic pathways has emerged: metabolic flux analysis (MFA). This method includes two common approaches, which are flux balance analysis (FBA) and ^{13}C-metabolic flux analysis (^{13}C MFA). While the former involves modeling metabolic reactions as a set of linear equations, the later uses ^{13}C-labeled substrates combined either with MS or NMR spectroscopy to analyze isotopic labeling patterns [98]. To our knowledge, MFA has not yet been applied in any studies assessing the health benefits of olive oil. Nevertheless, we

believe that it could be crucial for helping the scientific community better understand the pathways that the bioactive compounds of this fundamental ingredient of olive oil enters in the human body.

Conducting longitudinal studies to track the changes in metabolic profiles over time in response to olive oil consumption can provide valuable insights into its long-term health effects. By analyzing metabolomic data at multiple time points, researchers can identify the biomarkers associated with olive oil intake and their impact on various physiological processes such as inflammation, oxidative stress, and lipid metabolism, as described previously [99].

Integrating metabolomics with other -omics technologies such as genomics, transcriptomics, and proteomics should offer a comprehensive understanding of the molecular mechanisms underlying the health effects of olive oil. By correlating changes in metabolite levels with genetic variation, gene expression patterns, and protein profiles, researchers can identify novel pathways and biological targets influenced by olive oil consumption.

Future research on olive oil metabolomics can also employ nutrigenomic approaches to investigate how genetic variation affects individual responses to olive oil consumption through the identification of gene–diet interactions. Thus, it is possible to personalize dietary recommendations and optimize health outcomes based on genetic profiles.

Another important aspect is the implementation of clinical trials downstream of the study and development process for olive oil metabolomics. This would allow the health potential of longitudinal studies and meta-analyses to be directly highlighted, thereby capturing the real purpose of these studies to have an impact on human health. As previously mentioned, the PREDIMED trial demonstrated that the daily consumption of 50 g or more of polyphenol-rich EVOO as part of the MD could reduce CVD morbidity and mortality in high-risk individuals [100]. Several other sub-studies within PREDIMED analyzed the plasma metabolites related to CVD risk and EVOO consumption. Among the metabolites analyzed, ceramide was associated with CVD prevention after 7.4 years of treatment, demonstrating the modulation of the risk of disease. Another clinical trial regarding the role of VOO in HDL functionality reported the impact of some metabolites (i.e., phenolic compounds) of VOO on the HDL triacylglycerol levels. Finally, a postprandial clinical trial involving the consumption of 40 mL of EVOO rich in oleocanthal demonstrated the anti-aggregation properties of the olive oil metabolite [97]. Postprandial studies are interesting investigations that explore the post-meal metabolomic response to olive oil consumption, revealing differences in the serum metabolite profiles compared with other edible oils. Such studies have highlighted the potential of metabolomics to distinguish between different oil types and understand their physiological effects.

In summary, future research on olive oil metabolomics should adopt integrated approaches, including longitudinal studies, omics technologies, nutrigenomics analyses, and clinical trials, to comprehensively understand its health impacts. By unraveling the complex interplay among olive oil metabolites, genetic factors, and processing methods, researchers can advance our knowledge of the health-promoting properties of olive oil and provide evidence-based dietary recommendations for disease prevention and management.

In conclusion, although evidence supports the metabolic effects of olive oil consumption, further research using standardized methodologies is required to better understand the specific metabolites and mechanisms underlying their beneficial properties.

6. Conclusions

Olive trees and olive oil, one of its derivative products, hold significant cultural, historical, and nutritional importance across the Mediterranean region and beyond. Olive oil has transcended its role as a culinary ingredient and has become a symbol of tradition, health, and prosperity. Modern scientific research has shed light on the numerous health benefits of olive oil consumption, particularly in the MD context. Studies have consistently demonstrated its role in reducing the risk of chronic diseases such as CVDs, obesity, cancer, and neurodegenerative conditions. The rich composition of olive oil, which includes

MUFA, polyphenols, and vitamins, contributes to its antioxidant, anti-inflammatory, and neuroprotective effects, favoring its role as a protective agent against a wide spectrum of disorders.

Furthermore, the emerging field of "nutrimetabolomics" offers promising avenues for understanding the intricate relationships between olive oil components and human health. By analyzing the metabolic response to olive oil consumption, researchers have aimed to identify specific biomarkers and mechanisms underlying their therapeutic effects. This interdisciplinary approach involves collaboration among medical professionals, data analysts, nutritionists, and analytical chemists to unravel the complexities of nutritional metabolomics in the context of translational research.

Metabolite analysis techniques have played a pivotal role in comprehensively understanding olive oil and its diverse compounds. The versatility of targeted and untargeted metabolomics has broadened the scope of analysis, allowing for a more nuanced understanding of the chemical composition of olive oil and its potential health implications. MS, with its sensitivity and structural elucidation capabilities, along with NMR and its associated reproducibility and information-rich spectra have become indispensable tools for metabolite profiling. Chromatographic techniques such as HPLC and GC have facilitated the separation and quantification of various compounds, enhancing our ability to explore the health-promoting properties of olive oil. These techniques have overcome challenges such as matrix effects and ion suppression, providing superior resolution and sensitivity, particularly in the analysis of low-molecular-weight compounds.

The application of advanced analytical methods has led to significant discoveries regarding the bioavailability, metabolism, and health effects of the phenolic compounds in olive oil, identifying potential biomarkers, elucidating metabolic pathways, and highlighting the role of olive oil in human health. However, differences in the experimental design and analytical methods across studies make it challenging to identify the specific metabolites responsible for the observed health benefits.

Metabolomic studies in the realm of nutrition and food, particularly concerning olive oil, are still relatively nascent but hold significant promise. Although their primary application currently revolves around delineating the composition of VOO and EVOO compounds for commercial purposes, there is growing recognition of their potential in broader contexts. The concept of "foodomics" integrates metabolomic approaches to identify relevant compounds in olive oil, aiding authenticity assessment and quality assurance.

Having said that, challenges persist in applying metabolomic methodologies, including the need for standardized workflows, the integration of diverse techniques, and the management of vast datasets generated by comprehensive analyses.

In conclusion, combining metabolomics with other "omics" technologies, conducting longitudinal studies, and employing nutrigenomic approaches can provide deeper insights into the molecular mechanisms underlying the health effects of olive oil consumption.

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