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Olive Leaf: Antimicrobial and Antioxidant Properties, Food Applications, and Current Studies

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Abstract

Olive leaves are an important natural source of phenolic compounds with many bioactive properties. It is important to use them in different areas instead of throwing them as waste. Its antioxidant and antimicrobial properties have been proven by many studies and have recently been used in medicine, cosmetics, pharmaceuticals and food products. In this review, the importance of olive leaves, their composition, antimicrobial and antioxidant effects and food applications are briefly explained by giving the recent studies on this subject as examples. This review is likely to be valuable to interested academic and industrial researchers in their studies and knowledge of olive leaves, natural antimicrobials and antioxidants, and innovative food formulations with functional qualities.

Keywords: Food waste, Functional foods, Natural food additives, Olive leaves, Phenolic compounds

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INTRODUCTION

Many wastes and by-products contain valuable compounds that arise during the cultivation, harvesting, and industrial processing of agricultural crops. It is extremely important in terms of economy, environment, social and human health to evaluate these products in different ways and transform resources in order to protect natural sources, minimize waste and ensure sustainability. One of the most valuable compounds in agricultural wastes and by-products are phenolic compounds. They are secondary metabolite products in plants and important in the plant's defense mechanism against insects, microorganisms, and UV radiation. Because of their antimicrobial and antioxidant activity properties, they are used in many areas for example medicine, food, and cosmetics.

The olive tree (*Olea europaea* L.), a member of Oleaceae family and a symbol of Mediterranean civilization, peace, and abundance, is widely spread plant in the world. Nearly 98% of the world's olive harvest comes from the 8 million hectares of trees that are found in countries in the Mediterranean region (Ahmad-Qasem et al., 2014). The olive fruits are used in the production of olive and olive oil, and the leftover pieces and residues developed as a result of the processing of the olive and pruning are regarded as by-products of olive (Souilem et al., 2017; Talhaoui et al., 2015). Olive mill wastewater, olive pomace, olive leaves, and olive seeds can be given as examples of these by-products, which are rich in phenolic substances. (Souilem et al., 2017; Talhaoui et al., 2015). Mostly, these are regarded as waste, and their management and disposal constitute an environmental problem. On the other hand, because they are important sources of bioactive compounds such as phenols, sterols, squalene, tocopherols, etc., they are valuable in human nutrition. The biochemical properties of these compounds have been better recognized with studies on olives, olive leaves, and olive oil, and the use of these products in daily nutrition, as ingredients in the food sector and in the pharmaceutical industry has become widespread.

Olive leaf (OL) is one of these products. It contains high amount of polyphenolic compounds and other beneficial phytochemicals, such as flavonoids, triterpenes, and chalcones, which have positive effects on health and has been used in traditional medicine for a long time for its health advantages, such as boosting the immune and cardiovascular systems, supporting energy levels, decreasing blood pressure, treating diarrhea, respiratory and urinary tract infections, stomach and intestinal diseases, eye infection, and as mouth cleanser (Borjan et al., 2020; Hashmi et al., 2015; Benavente-Garcia et al., 2000). The European Food Safety Authority (EFSA) considers that olive oil polyphenols offer protection against oxidative damage and LDL (cholesterol indicator) and recommends a minimum consumption of 5 mg/day (EFSA, 2011). In the same report, the Panel also considered that there was insufficient evidence to establish a cause and effect relationship between the consumption of olive oil polyphenols and the maintenance of normal blood HDL-cholesterol concentrations. In the scientific opinion report of EFSA on the substantiation of a health claim related to olive leaf extract (OLE), it has accepted OLE as a safe products and the positive effect of OL on health was supported by citing scientific studies, but the scientific evidence is insufficient to establish a cause and effect relationship between the consumption of OLE and an increase in glucose tolerance (EFSA, 2014).

Besides positive effects on human health, recent research has shown that it is possible to use OLE in food formulations and food packaging as an antibacterial and antioxidant additive instead of synthetic agents to increase the shelf life and safety of foods. For these reasons, studies on phenolic component recovery from OLs, the use of OLs, OLE and the main phenolic compound of the extract (oleuropein) in foods, cosmetics, and pharmaceutical industries keep increasing in the literature. The products obtained from olive leaves also have an important commercial value. Generally, you can find several OL preparations in the markets in the form of dried whole leaf as herbal tea, powders made from dried plant material, liquid extracts, tablets, capsules, and food supplements (Arslan et al., 2021; Romani et al., 2019; Özcan and Matthäus, 2017). Especially the consumption of whole dried OLs as tea attracts attention from consumers. It is also possible to find several products sold as supplements having immune stimulator, antioxidant, anti-inflammatory, anti-aging, and blood sugar-regulating effects that use OLs in their formulations. Thus, the objective of this paper is to provide an overview of the antioxidant and antibacterial properties of phenolic acids, as well as their application in the food industry.

OLIVE LEAF AND ITS PHENOLIC COMPOUNDS

OLs are discarded in large amounts as a residue during pruning, harvesting and production of olive-related products. The amount of OLs released by pruning varies between 12 to 30 kg/tree, depending on the pruning type and age of the tree. They represent 10% of the overall weight of harvested olives (Khemakhem et al., 2017; Talhaoui et al., 2015). The European Medical Agency has acknowledged OL as an approved herbal preparation with a wide range of health-promoting qualities among several plants that have been the subject of investigations (EMA Monograph., 2017). In the scientific database titled "Compendium of botanicals reported to contain naturally substances of possible concern for human health when used in food and food supplements" published by EFSA in 2016, information on plants used in food applications in European Union countries was compiled. The olive tree, and thus the other olive-related parts, are not included in the list in this database (EFSA, 2012a). Furthermore, the olive tree is included in the "Allocation List of Herbals Considered as Food" published by the Tea & Herbal Infusions Europe (THIE) association, and it is stated that the part of the plant used is the leaf (THIE, 2019).

The beneficial bioactive properties and potential health benefits of OLs like antioxidant, hypoglycemic, antimicrobial, antihypertensive, anti-inflammatory, anticarcinogenic, and antiatherosclerotic have been reported by many studies on humans and animals, and are attributed to their phenolic compound content (Qabaha et al., 2018; Difonzo et al., 2017; Liu et al., 2017; Boss et al., 2016; Wainstein et al., 2012; Lee and Lee, 2010; Sanchez et al., 2007). The major phenolic substances found in OLs are secoiridoids (oleuropein, dimethyloleuropein, verbascoside, ligtroside, and oleuroside), phenolic acid and its derivatives (vanilic acid, caffeic acid, vanillin), phenolic alcohols (tyrosol, hydroxytyrosol), flavonols (quercetin, isoramnetin, rutin), flavones (luteolin-7-glucoside, apigenin7-glucoside, luteolin, diosmetin, and diosmetin-7-glucoside), and flavon-3-ols (catechin, gallocatechin) (Jung, 2019; Vizza, 2019; Giacometti, 2018; Lockyer, 2016; Talhaoui et al., 2015).

The most prevalent phenolic compound, oleuropein, is a secoiridoid, heterosidic ester of hydroxytyrosol and elenolic acid, representing 1-14% of olive leaf weight (EFSA, 2012b). It gives a bitter taste to olives and is found in different amounts in various parts of olive tree, such as branches, leaves, roots, buds, fruit, and flowers (Benavente-Garcia et al., 2000). OLs are shown to be the richest source of oleuropein in nature and their levels can range between 10 to 140 g/kg OLs (Barbaro et al., 2014; Japón-Luján et al., 2008). The composition of commercial OLEs varies, with oleuropein normally contributing 20–40 % of the total phenolic compounds (EFSA, 2012b). The other important phenolic substances in OL is hydroxytyrosol. It is degradation product of oleuropein and has high antioxidant activity as well. Oleuropein decomposes into elenolic acid and hydroxytyrosol under the influence of β-glucosidase enzyme, light, temperature, acid, or base. Hydroxytyrosol has an amphiihilic structure, is a lipid- and water-soluble molecule and has the catechol group in its chemical structure (Rietjens et al.., 2007). It can be found in extra virgin olive oil esterified with elenolic acid or in simple phenol form. During the ripening and processing of the olive, the amount of oleuropein decrease, while the amount of hydroxytyrosol increase (Abaza et al., 2017; Özcan and Matthäus, 2017). So, olive oil contains higher amounts of hydroxytyrosol than oleuropein, and the amount of oleuropein is quite low compared to OL (Malik and Bradford, 2008).

The amount of phenolic substances and their composition in OLs are variable depending on the age of the tree, origin, proportion of branches, harvest time, climate, moisture, storage conditions, etc. It is stated in various studies that the quantity of phenolic substances in the leaves is its the highest level between November and March, which also includes the harvest and pruning periods (Di Meo et al., 2022; Abaza et al., 2017; Blasi et al., 2016; Çetinkaya and Kulak, 2016). In addition, the quantity and composition of OLE's phenolic substances depend on the drying method, such as drying in the sun or shade or in the oven, and the extraction methods. The extraction method type, temperature, pH, solvent type, and extraction time are fundamental factors affecting the quality and quantity of polyphenolic compounds recovered from olive leaves (Giacometti et al., 2021; Alcántara et al., 2020;

Ghomari et al., 2019; Rahmanian et al., 2015; Ahmad-Qasem et al., 2014). Compounds vary in their ability to be extracted using various techniques based on their chemical properties. For instance, it has been found that supercritical fluid extraction or pressurized liquid extraction are better suited for obtaining phenolics with less polarity, such as luteolin and apigenin, whereas polar compounds, such as derivatives of oleuropein, are better obtained through maceration (Taamalli et al., 2012).

ANTIOXIDANT EFFECTS OF OLs

It is stated that fruit and vegetable-rich diets have protective effects against several diseases. This has been attributed partly to the antioxidant effects of their phenolic compounds (Benavente-Garcia et al., 2000). Although synthetic antioxidants are used in many products, because of their possible mutagenic and carcinogenic effects, there is a growing interest in the use of antioxidants derived from herbal origins. Natural polyphenols found in foods have antioxidant activity and may prevent or reduce oxidative damage occurring at cellular levels. Utilizing them in many industries, such as cosmetics, pharmaceutical industries and food supplements, is becoming more popular due to their rich phenolic content. OLs are a frequently emphasized and studied product as a natural antioxidant. It has exhibited powerful antioxidant activities by preventing or delaying the oxidation of oxidative substrates. Because of their high oleuropein and hydroxytyrosol content, OLs have the highest antioxidant properties compared to other olive tree sections (Dekanski et al., 2011; Japon-Lujan et al., 2006; Benavente-Garcia et al., 2000). The amount and placement of hydroxyl groups in respect to the carboxyl functional group are often important factors in antioxidant action (Robards et al., 1999). The structure of phenolic compounds, such as the glycosylation site and positions and numbers of the hydroxyl groups on the carboxyl functional group, glycosidic moiety, is an important factor in both their metal chelating and radical scavenging action (Xie et al., 2015; Balasundram et al., 2006). Research has shown that the biological activity of aglycone part is higher than that of the glycoside portion (aglycones bound to glycones) (Oniszczuk et al., 2019).

Polyphenols (like hydroxytyrosol) and their secoiridoid derivatives (like oleuropein) in olive products react with free radicals, donate their own hydrogen to oxidants, neutralize them and scavenge free radicals, thus forming more stable variants (Quiñones et al., 2012; Saija and Uccella, 2000). They scavenge radicals such as DPPH, ABTS·+, OH and O2· –. (El and Karakaya, 2009; De la Puerta et al., 1999). They may also reduce reactive oxygen species generation by increasing the activity of antioxidant-acting enzymes or preventing enzyme activity that induces pro-oxidant activities. Another important point for antioxidant activity is the number and location of aromatic hydroxyl groups and the stability of the formed aroxyl radicals (Xiang and Ning, 2008; Benavente-Garcia et al., 2000). The aroxyl radical can delocalize electrons extensively. This is necessary in order to generate several mesomeric structures and radical stabilization. Antioxidant activity by oleuropein and hydroxytyrosol can also be associated with their hydrogen-donating and chelating metal ions like as Fe^{3+} and Cu^{2+}, which catalyze reactions that generate free radicals and have the power to block a number of inflammatory enzymes, including lipoxygenases, without having an impact on the cyclo-oxygenase pathway (Özcan and Matthäus, 2017; El and Karakaya, 2009; Andrikopoulos et al., 2002; Visioli et al., 2002). Oleuropein and hydroxytyrosol have also been found to be scavengers of hypochlorous acid and superoxide anions, two powerful oxidants formed at the inflammatory location and a key ingredient in bleaches based on chlorine that frequently contact food during production (Visioli et al., 2002).

ANTIMICROBIAL EFFECT OF OLs

OLs' antibacterial properties have long been utilized to treat various illnesses and fevers. Studies showed that OLE has antimicrobial activities against a wide range of bacteria, yeasts, molds, viruses, and other parasites including *Listeria monocytogenes,* E*scherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Yersinia enterocolitica, Salmonella typhi, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Helicobacter pylori, Vibrio parahaemolyticus, Campylobacter jejuni,* and *Candida albicans* (El-Sohaimy et al., 2021; Sánchez-Gutiérrez et al., 2021; Liu et al., 2017; Gökmen et al., 2016; Techathuvanan et al., 2014; Aliabadi et al., 2012; Keskin et al., 2012; Lee and Lee, 2010; Sudjana et al., 2009; Pereira et al., 2007; Markin et al., 2003). Although the mechanism of antimicrobial action has not yet been fully elucidated, it is reported that phenolic compounds have the ability to denature proteins, damage cell membranes, break down cell peptidoglycans, adversely affect cell membrane permeability, and thus create leakage of cytoplasmic elements such as potassium, glutamate or inorganic phosphate, which delays and inhibits the growth rate of microorganisms (Lee and Lee, 2010; Sanchez et al., 2007). The potential of hydroxyl groups in phenolic substances to attach to important enzymes' active sites and change microbial metabolism is another factor contributing to their antimicrobial effects (Gyawali and Ibrahim, 2014). The length of the saturated side-chain and position of the hydroxyl group in aromatic ring both affect the antimicrobial activity (Cueva et al., 2010). Caffeic acid, for instance, has been shown to have greater antibacterial activity than p-coumaric acid because it contains more hydroxyl groups replaced in the phenolic ring (Stojković et al., 2013).

In studies demonstrating the antioxidant and antimicrobial activities of OL, although phenolic substances show antimicrobial or antioxidant properties individually, it has been shown that they show a stronger activity when used together (Giacometti et al., 2021; Lee and Lee. 2010; Pereira et al., 2007). The level of antimicrobial effect and the sensitivity of microorganisms vary according to the type of microorganism. Pereira et al. (2007) indicated that *B.cereus* was the most susceptible microorganism among the strains tested, while *B.subtilis* was found to be the most resistant. In the same study, *E.coli* (Gram-negative bacteria) was found to be more sensitive compared to *S.aureus* (Gram-positive bacteria). Contrary to this study, it was determined that the oleuropein showed greater antimicrobial activity against *St.aureus* than its effect on *E.coli* and this was due to variations in cell structures by Sanchez et al. (2007). Markin et al. (2003) reported that OLE killed *E.coli*, *St.aureus,* *K.pneumonia,* and *P.aeruginosa* in 3 hours of exposure at 0.6% (w/v) concentration. However, due to *B.subtilis*'s ability to form spores, it was possible to inhibit when OLE was used up to 20% (w/v) concentration.

Keskin et al. (2012) also demonstrated that OLE significantly decreased the growth of all studied Gram-positive and Gram-negative bacteria, with the exception of *B.cereus* CCM 99, *Enterobacter aerogenes* ATCC 13048, and *Enterobacter cloacae* ATCC 13047. In the study by Sudjana et al. (2009), *C.jejuni*, *Helicobacter pylori*, and *S.aureus* were the most susceptible bacteria to OLE, whereas *E. coli*, *P.aeruginosa*, *B.subtilis*, *K.pneumoniae*, and S*erratia marcescens* were the least susceptible. Liu et al. (2017) found OLE, when used at a certain concentration, almost completely inhibited growth of major foodborne pathogens such as *E.coli* O157: H7, *L.monocytogenes*, and *S.enteritidis*. It reduced cell mobility in *L.monocytogenes* which corresponded with the lack of flagella, as seen by scanning electron microscopy, and inhibited biofilm formation by *S.enteritidis*. and *L.monocytogenes*.

Gökmen et al. (2014) determined the antibacterial efficacy of OLE on *L.monocytogenes* with MIC values greater than 32 mg/ml. OLE exhibited antifungal effects with a MIC of 24 mg/ml, minimal fungicidal concentration of 48 mg/ml, and 21 mm inhibitory zone diameter against *C.albicans* PTCC-5027 in the study of Nasrollahi and Abolhasannezhad (2015) and stated that it can be beneficial for treatment and prevention of infections like oral thrush caused by *Candida* spp. Zoric et al. (2016) determined the antifungal effects of OLE against *C.dubliniensis* CBS 7987 and *C.albicans* ATCC 10231 strains and found the MIC value for *C.albicans* to be 46,875 mg/mL and for *C. dubliniensis* to be 62.5 mg/mL. By Markin et al. (2003), the antifungal activity of OLE was demonstrated against *C.albicans* as well as against three dermatophytes, *Microsporum canis*, *Trichophyton mentagrophytes* and *T.rubrum*. It was suggested that it can be used in cases of fungal opportunistic infections. Unlike these studies, Shialy et al. (2015) determined that OLE did not show antifungal activity on *C.tropicalis*, *C.albicans*, *C.krusei*, and *C.glabrata*.

FOOD APPLICATIONS

Awareness of the importance of diet in health has been raised among consumers, and it has shown that foods should be considered for their active positive impacts on health as well as the quality and amount of nutrients they contain. To promote the health benefits of natural resources, research is being done on the creation and marketing of functional foods, dietary supplements, and nutraceuticals made from natural sources. In this respect, OLs products, abundant sources of phenolic compounds, have potential to be used as natural food additives and supplements in foods. In fact, OLs arise from the pruning of olive trees and are also waste products from table and olive oil production. Nowadays, considering zero-waste approaches, it is very important to reduce waste generation and transform it into other sources and their valorization. The waste or by-products of one sector can become the raw materials for another one through industrial symbiosis. Generally, several OL preparations can be found at the markets in the form of dried whole leaf as herbal tea, powders made from dried plant material, liquid extracts, tablets, and food supplements (Arslan et al., 2021; Romani et al., 2019; Özcan and Matthäus, 2017). Especially use of dried whole OLs to be consumed as tea attracts attention by consumers.

One of the most common uses of OLs in foods as an antioxidant. Oxidation of lipids can happen during food preparation and storage as a result of free radicals, light, heat, enzymes, metals, metalloproteins, and the activity of microorganisms. Oxidative balance is very important in maintaining the quality of products, especially with high oil-content foods. In food products, oxidative degradation causes rancidity, potentially toxic oxidative products, degradation of colors, sensory decay, loss of nutritional quality, and decrease in shell life. Commercial chemical antioxidants are mostly used in food industry to prevent or slow down oxidation because they are effective, cheap, and highly stable. On the other hand, it is known that they are suspected of being promoters of carcinogenesis process and consumers have recently preferred to use natural additives instead of synthetic ones (Farag et al., 2007). Due to their great antioxidant potential, OLs have drawn more attention as the significance of natural antioxidants has increased. Besides their antioxidant effects, enriching food products with OLs might also improve nutritional value and provide health benefits.

Studies have revealed that olive leaves are generally used in foods as natural food additives for enrichment with antioxidant and bioactive compounds, extending shelf life by increasing oxidation stability and producing functional products. OLE is thought to be useful in extending the shelf life of vegetable oils by improving oxidation stability and maintaining nutritional components. Ammar et al. (2017) carried out research on the phenolic profile of Tunisian cultivars' olive oil, to which olive leaves were added before extraction process. The recovered oil from the Chétoui cultivar that had been treated with 3 Oueslati variety of Tunisian olive oil has been proven to contain more polyphenols (44%), chlorophyll (about 67%), and carotenoids (about 62%) when olive leaves (3%) are added to the oil (Tarchoune et al., 2019). According to Sevim et al. (2013), adding olive leaves to olive oil may help the oil's bioactive profile after 18 months of storage. The leave-treated oils showed a significant increase in chlorophyll, alpha-tocopherols, phenolic contents, and antioxidant activity.

Studies have been done to enhance olive oils with the addition of phenolic substances as a result of the growing interest in olive oils with high phenol content in recent years. Thus, both nutritionally rich olive oil with high phenol content and high oxidation stability are obtained. In particular, studies on the addition of phenolic substances obtained from olive leaves by different extraction methods to olive oil have attracted attention (Arfaoui et al. 2022). The studies show that the enrichment of OLEs improves the virgin olive oil's oxidation by lowering the peroxide value and extinction coefficients during the storage period. Also from a sensory perspective, high overall acceptability and a better flavor and odor were observed in enriched olive oil after six months compared to the control. Arfaoui et al. (2022) incorporated OLE as a natural antioxidant into extra virgin olive oil from "Chetoui" variety to improve nutritional quality and oxidative stability. Results showed that the enrichment maintained organic standards and had no impact on the olive oil quality.

Oxidation and off-flavour can occur easier in refined vegetable oils, like soybean oil, palm oil, and sunflower oil since there are unsaturated fatty acids and low amount of antioxidant substances. For this reason, butylated hydroxytoluene (BHT) is generally added to these oils to improve their oxidative stability. As mentioned above, studies on switching to natural antioxidants from synthetic ones are among the most emphasized. Salta et al. (2007) added OLE to olive oil, sunflower oil, palm oil, and vegetable fats to enrich them with polyphenols and determined that OLE contributes to increasing the capacity to scavenge radicals and oils' oxidative stability, showing more antioxidant activity when compared to BHT. Hassan et al. (2022) assessed the impact of OLE addition at various concentrations on lipid oxidation in refined soybean oil and BHT at a concentration of 200 ppm and showed that OLE had a strong antioxidant capacity similar to BHT at 200 ppm during accelerated storage, making it possible to use it as natural antioxidant in soybean oil.

Dauber et al. (2022) compared the performance of OLEs produced by using various extraction methods in preventing oxidative damage in canola oil. During five-week storage at 60 °C, the ability of canola oil to resist oxidation with or without the addition of extract was evaluated. When OLEs were added to canola oil, the oxidation process was delayed relative to the control oil, extending the oil's shelf life. In another study, the addition of olive hydroalcoholic leaf extracts (OHE) increased the resistance of canola oil and high oleic sunflower oil to heat during frying of French potatoes. When compared to oils without extracts, oils with OHE reduced the generation of polar substances and displayed an anti-polymeric impact (Jimenez et al., 2017). In another study in which refined olive and husk oils were enriched with OLs, rich in oleuropein and hydroxytyrosol, it was determined that OLs showed significant resistance to oxidative deterioration and increased their stability and prevented deterioration during storage (Bouaziz et al., 2008). They examined the antioxidant capacity of OLs and hydrolyzed OLEs in pomace and refined olive oils and found that they could lower the production of conjugated dienes and trienes as well as lipid hydroperoxides when compared to the control, improve the quality of the pomace olive oil, and bring it closer to that of virgin olive oil. Compared to OLE itself, hydrolysate was more efficient. During storage, the two main components of enzymatic OLE hydrolysate, oleuropein aglycone and hydroxytyrosol, demonstrated the strongest antioxidant capacity and the lowest observed stability, followed by oleuropein. There are also studies showing that an extract rich in hydroxytyrosol has antioxidant effects on food lipids and increases oxidative stability (De Leonardis et al., 2008).

Malheiro et al. (2013) added different amounts of OL (1%, 2.5%, 5% and 10%; w/w) during oil extraction from olives and verified the effect of adding OLs on the quality and composition of olive oils. They determined that the OLs addition significantly improved the resistance of olive oils to oxidation and 10% addition of leaves increased the amount of vitamin E in oils by approximately 30% due to α-tocopherol in the leaves. Similarly, Kritsakis et al. (2017) determined that the adding OLE at the malaxation stage increased oxidation stability and phenol content overall in olive oils and a darker green olive oil with the desired properties was obtained due to the increase in carotenoid and chlorophyll contents. When OLE is used during olive oil extraction, the olive oil obtained has more fruity, bitter and positive properties. As a result, it was stated that OLE can be used as a natural additive to extend the shelf life of olive oils and increase their functional properties.

As for frying oil; during cooking, thermal oxidation rate is faster than autoxidation and the unstable hydroperoxides quickly break down into secondary oxidation products. Foods are heated to about 180 °C and above in frying processes and several chemical reactions take place, including autoxidation, polymerization, isomerization, and hydrolysis. Farag et al. (2007) investigated if sunflower oil obtained directly by pressing OL would increase the stability of reused oil during intermittent frying for 5 h/day for five consecutive days. During intermediate heating, the researchers discovered that sunflower oil blended with olive phenols had a greater smoke point and fewer primary oxidation products. Additionally, there was a correlation between the content of OL phenols and the beneficial effect against thermal oxidation. Accordingly, the high presence of phenolic substances in the OL resulted in low production of substances that react with thiobarbituric acid (TBA). Also, an activity against polymerization was found, as the amount of polar substances and triacylglycerol polymers, was also found to be lower in the treated oil compared to the control. Depending on the frying technique, there are differences in the amount of oil absorbed by the food. Therefore, the efficiency of phenolic transfer to fried food may vary. The addition of OLE did not considerably reduce the total polar material or the conjugated dienoic acids during the frying cycles, but it did significantly increase the oxidation stability compared to the control oil (Zribi et al. 2013).

OL and OLE can be incorporated as active components in milk and yogurt to extend thier shelf life and/or functional properties. Adding a certain amount of OL to milk and yogurt can increase the functionality of fermented milk products without influencing the survival of bacteria such as *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Str. thermophiles* (De Leonardis et al., 2008; Zoidou et al., 2014; Barukčić et al., 2022). OLE at concentrations of 0.2, 0.4 and 0.6% was applied in milk and yoghurt to investigate the effect on growth and viability of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* (Marhamatizadeh et al., 2013). OL milk and yoghurt had much more *L.acidophilus* and *B.bifidum* than control milk and yoghurt, and positive correlation was found between bacterial growth and OL concentration. The addition of OLE during the preparation of lowfat apricot yogurt considerably impacted *Str.thermophilus* count, protein, pH value, dry matter, and ash contents. The water-holding capacity values decreased throughout the storage period and antioxidant activity was higher at the end of the storage (Peker and Aslan, 2017). Barukčić et al. (2022) enriched yogurts with OLE (1.5, 3, and 5% v/v) and observed shorter fermentation and lower pH, probably due to the starter culture's stimulation to produce acids more quickly, with no negative impact on the survival of yogurt starter bacteria. Despite having slightly unfavorable sensory properties, OLE-enriched yoghurts had improved phenol content, syneresis, and antioxidant activity, whereas viscosity and water-holding capacity decreased. Taking into account all of the results, 1.5% OLE addition seemed ideal for enriched yogurt products. Pourghorban et al. (2022) compared the effects of OL on the microbiological, physicochemical, antioxidant, and sensory properties of pre- and post-fermented yogurt samples enriched with various amounts of OLP and OLE during 21 days of storage. The results of the sensory evaluation indicated that acceptability was dependent on the concentration, OLE and OLP concentrations lower than 1.5 and 5 mg/ml, respectively, were acceptable. It was found that OLP or OLE had no adverse effect on yogurt starter culture bacteria and the total phenol amount, antioxidant capacity, and shelf life of the samples with OLE or OLP added were higher. Further phenolic compound reduction was seen when OLE and OLP were introduced to the samples during the pre-fermentation stage. This may be related to the starter bacteria's activity during fermentation. Similar to how the starting bacteria might use them as a source of carbon or energy. Additionally, in the samples of enhanced yogurt, the lactic acid formation during fermentation may result in the breakdown of phenolic compounds at an acidic pH.

In bakery products such as bread, crackers, cakes and biscuits, OLP or OLE is used in formulations to both enrich the product and increase its oxidation stability. The studies show that, these products with OLE added have higher antioxidant activity and longer shelf life. In addition, it has been stated that a functional product can be produced due to its high phenolic content (Palmeri et al., 2016). To develop breadsticks that are nourishing and healthful with a long shelf life, phenolic rich extracts from OL were incorporated into gluten-free formulations. Compared to the control, fortified breadsticks exhibited increased moisture and a_w, lower hardness, same color, and significant phenolic composition changes, as shown by a decline in the insoluble/soluble polyphenol ratio and a parallel rise in polyphenol bioaccessibility and antioxidant activity. Furthermore, all reinforced breadsticks showed a notable shelf life extension (Conte et al., 2021). Cedola et al. (2020) illustrated OLE valorization as a food ingredient instead of white wine in a popular cereal-based food named "taralli". The enhanced product was usually deemed satisfactory from a sensory perspective because the control sample displayed less friability than the standard product did. According to the data, OLEs enhance the nutritional value of enriched Taralli, and cooking did not change the amount of polyphenols, antioxidant activity, or flavonoids.

OLE and OLP can be added to minced beef meat to prevent lipid and myoglobin oxidation during cold storage (Aouidi et al., 2017). OL shows a capacity to prevent lipid oxidation and myoglobin oxidation. OL also improves the meat's technological quality by reducing storage and defrosting losses. It was assessed how varying oleuropein concentrations (0.3%, 0.6%, and 0.9%) affected the antioxidant activity and storage quality of Tabaq-Maz, a well-known type of traditional fried ribs in India. Oleuropein significantly reduced the TBARS (thiobarbituric acid-reactive substances) and free fatty acid (as % oleic acid) values compared to control, indicating an impact on oxidative stability. The count of total bacteria, psychrophills, yeast and mold was found to be lower in OLE-added samples, thus it was observed that it had a significant effect on microbiological properties (Dua et al., 2015). Hayes et al. (2010) examined the effects of lutein, sesamol, ellagic acid, and OLE on total viable bacteria, TBARS value, lipid oxidation, oxymyoglobin oxidation, color, pH, and sensorial qualities of raw beef patties. All the nutraceuticals reduced total viable counts, or TBARS in food products. While sesamol addition to beef increased oxymyoglobin oxidation, OLE and lutein decreased oxymyoglobin oxidation in comparison to the control (Hayes et al., 2010).

Ranchman et al. (2021) also determined how OLE affected the physical characteristics of chicken breast sausage (CBS) and how OLE protected CBS from lipid oxidation during frozen storage. From 15 to 60 days in storage, the weight loss of control CBS without OLE upon thawing increased, but that of OLE-CBS did not change. Control CBS's viscosity, elasticity, breaking strength, and water-holding capacity reduced after being frozen, but those of OLE-CBSs remained the same. SEM analysis of CBSs revealed that, in contrast to control CBS, OLE-CBSs that were held frozen kept their unfrozen counterparts' structural similarity. Additionally, the addition of OLE to CBS prevented the oxidation of lipids, lowering of pH, and discoloration brought on by frozen storage.

Romeo et al. (2021) evaluated impact of the OLE addition on the mayonnaise's oxidative resistance during storage. It was greatly improved by enriching it with various quantities of OLEs. Individual phenolic content, oxidative stability, microbiological and sensory criteria, and antioxidant parameters were all checked to ensure its purity. The antioxidant capacity of the studied materials was functionalized and improved by hydroxytyrosol and hydroxyl acetyl derivate, as demonstrated by their connection with DPPH assay. No microbiological contamination was found, and enriched mayonnaises displayed good pH values. On the other side, using too much extract can alter the finished product's flavor and render it unpalatable to consumers.

Moudache et al. (2017) developed a packaging material with OLE that has antioxidant capacity for food and is used for fresh minced pork meat. OLEs were incorporated in an adhesive formula used to create the multilayer polyethylene film that served as the packaging material. This packaging was used to keep fresh pork minced meat in storage for 16 days at 4 °C. The study showed that active films containing 10% and 15% of OLEs effectively improved the antioxidant capacity of a fresh meat and can be used to increase the storage life of fresh minced meat for around two days (Moudache et al., 2017). It was observed that there was a development in the physical characteristics of chitosan-based composite films developed by adding OLEs and olive pomace extracts (OPEs) (Khalifa et al., 2016). The film containing 20 g/L of OLE had the lowest water solubility (17.82%). Besides, the composite films showed a stronger antifungal effect against *Penicillium expansum* and *Rhizopus stolonifier* than chitosan films as the inhibition percentage of these molds was increased by roughly two times by the addition of chitosan-20 g/L OLE and chitosan-20 g/L OPE. Chitosan-20 g/L OLE-based coatings maintained the qualities of coated products like apples and strawberries while delaying mold growth during cold storage.

The other common use of OL in foods is as an antimicrobial agent. The rise of drug-resistant bacteria around the world has begun to threaten human health. Therefore, intensive research continues to develop new effective antimicrobials. Antimicrobial drugs, which have a wide range of clinical uses, are replaced by plant-derived antimicrobial substances such as oleuropein due to their narrow antimicrobial spectrum and the resistance created by microorganisms over time, as well as causing serious side effects (Bisignano et al., 1999). Plant derived antimicrobials are generally considered safer compared to synthetic food preservatives and attract more attention as they can have beneficial impacts on human health.

In addition to prolonging the storage period of foods, natural plant antimicrobials can also benefit from their flavoring properties (Liu et al., 2017). High temperature treatments and chemical preservatives are generally used for food preservation, delaying food deterioration, and inhibiting the development of microorganisms in foods. Since high heat treatments can adversely affect physical, sensory and nutritional qualities of food, and chemical preservatives have negative effects on human health, studies on alternative preservation methods and the use of natural compounds in foods continue to increase. For this purpose, studies on the effects and usage possibilities of bioactive substances with antimicrobial activity, especially in some plants, come to the fore.

In a study conducted by Eraslan (2017), the effect of the use of commercial OLE on the microbiological properties of tomato paste was investigated as an alternative to chemical preservatives. It was determined that the total mesophilic aerobic bacteria, yeast and mold numbers in tomato paste samples decreased significantly with OLE supplementation. The researcher stated that the addition of OLE at different rates to tomato paste improves the microbiological quality and has a positive impact on the storage life.

Ahmed et al. (2014) evaluated the antimicrobial performance of OLE at different concentrations on raw, peeled shrimp samples. While *E.coli*, *P.aeruginosa*, *K.pneumoniae*, and *S.aureus* sensitive to OLE, a higher OLE concentration was needed to inhibit *B. subtilis* possibly due to its spore forming ability. The levels of total viable bacteria and total coliform bacteria in samples immersed in OLE for 3 hours at 4 °C were reduced by 1 log level and it has been stated that it is possible to use OLE as a natural preservative in seafood. The antimicrobial effect of adding OLE to meatballs at different rates on the growth of *St.aureus, S.typhimurium*, and *E.coli* O157 was investigated (Gökmen et al., 2016). In the control group meatball samples, *St.aureus*, *S.typhimurium*, and *E.coli* O157 numbers at 3 log and 6 log inoculation levels, respectively, increased in the range of 0.17-0.36, 0.05-0.40, and 0.15-0.20 logarithmic units at the end of the 7th day of storage. After the storage time, *St.aureus, S.typhimurium*, and *E.coli* O157 numbers decreased in the range of 0.23-0.94, 0.15-0.60, and 0.23-0.25 logarithmic units at 3 and 6 log inoculation levels, respectively, in the samples to which OLE was added at different rates. It was determined that the antimicrobial effect was significant. In conclusion of the study, the addition of OLE to meatballs at different concentrations enhanced the microbiological properties, had no negative effects on sensory aspects and had a positive effect on the shelf life. In this study, encouraging findings were revealed regarding the use of OLE as an antioxidant and antimicrobial in foods such as meatballs (Gökmen et al., 2016). OLE was applied to rainbow trout fillets that had been hot smoked and the changes in microbiological and sensory properties during storage at 4 ºC were examined. It was found that OLE application increased the shelf life of the product, did not cause any negative changes in sensory properties, and recommended to be used as a natural preservative in seafood (Mutlu and Bilgin, 2016). Kurt and Ceylan (2017) determined the effects of OLE at various concentrations (500, 250 and 125 ppm) on the chemical, physical, and microbiological features of Turkish dry fermented sausage. While OLE decreased the overall number of aerobic mesophilic bacteria and the number of lactic acid bacteria (LAB) during storage, it did not significantly affect the pH or color of sausage. It also decreased the level of free fatty acids and thiobarbituric acid. To assess the shelf-life extension and safety of raw minced beef during retail/display, Djenane et al. (2019) added OLE at 1 and 5% (v/w). Depending on its concentration, OLE has much stronger antibacterial and antioxidant properties. Shiga toxin-producing *E.coli* O157:H7 and *S.enterica* ser. Enteritidis pathogens levels, as well as psychrotrophic counts and TBARS valuse, were all decreased by OLE addition. In another study, the effects of jujube, blueberry extracts, and OLEs on the shelf life and quality of meatballs during storage were examined, and it was found that the highest protective effect was obtained with OL (Gök and Bor, 2012). In a study using sausages, the effects of OLE, green tea and nettle on the physicochemical, textural, microbiological, and sensory features of Frankfurter (German type) sausages were evaluated. It has been determined that it reduces at least 2 logs and does not have an adverse effect on product quality (Alirezalu et al., 2016).

Lahreche et al. (2020) studied the impact of OLE as a natural antimicrobial and antioxidant on the quality characteristics of white and dark muscles from fillets of frigate mackerel maintained at a fridge temperature (3±1°C) under vacuum packing. The OLE application extended the storage-life of muscles, initiated lipid peroxidation in white muscle at an initial stage of storage and improved the microbiological properties of both muscles by restricting bacterial growth. The effect of OLE on *L.monocytogenes*'s heat resistance was examined both in Tryptic Soy Broth (TSB) and sous vide packed ground beef (Akdemir and Kıymetli, 2021). Total reductions in TSB and sous vide packed ground beef supplemented with OLE were higher than control samples for all heating temperatures (55, 60 and 65°C). The results obtained indicate that the use of OLE in the formulation may be an extra barrier to the control of *L.monocytogenes* growth in heat-treated ground beef.

The milk fortification with OLE reduced total mesophilic bacteria and fat and lactose losses. The OLE can be used to make a novel functional milk with a longer shelf life. OLE has also a strong antibacterial effect particularly against *B.cereus* and can inhibit α-glucosidase enzyme at 0.2 mg oleuropein/ml concentration (Palmeri et al., 2019).

Aliyari and Rezaei (2021) enriched the French sauce samples with OLE and investigated the oxidative stability, the microbiological and sensory properties of samples. It was observed that OLE could enhance the samples' oxidative stability and could be applied as a preservative in place of the commercial preservatives. The French sauce sample containing OLE indicated a lower total mesophilic aerobic bacteria value than the control French sauce sample. According to the results of the microbiological tests, in addition to preventing the growth of LAB at the end of the examined shelf-life time, OLE was able to retard the growth of microorganisms during the storage period.

Recently, studies on the utilization of natural bioactive substances in the development of active and functional packaging used to increase food safety and provided a longer shelf life for foods have attracted attention. In this sense, OLE has been used especially in chitosan-based films formulation. According to the results of the study of Musella et al. (2021), chitosan films with OLE incorporated had significant antimicrobial efficacy against *C.jejuni* and *L.monocytogenes* , mainly prolonging the lag phase of the microorganisms. However, with the exception of *C.jejuni*, the OLE does not appear to enhance the chitosan's inherent antimicrobial properties. The antimicrobial impact of CH + OLE films on *L.monocytogenes* at room temperature was more obvious than it was at 37 °C. The results imply that CH + OLE may have a significant impact in preventing the growth of microorganisms in food at cold temperatures. The chitosan films with OLE exhibited increasing antioxidant activity and were more soluble in food-like substances, and had a lower water vapor permeability. Famiglietti et al. (2022) developed chitosan-based films containing dried OLE and investigated the amount of polyphenols and the antioxidant and antimicrobial activity of these CH-based-edible films entrapped this extract. The antimicrobial effect of the films was tested against *Ent.faecalis*

ATCC® 29212, *S.enteritidis* RIVM 706, and *S.enterica* subsp. *enterica* serovar Typhimurium ATCC® 14028 in vitro.

All the bacteria studied were effectively inhibited by the dried OLE component of the edible films, depending on the concentration used. It was shown that these films might behave as active bioplastics when used for hamburgers rather than traditional baking paper. Bastante et al. (2019) showed that polyethylene terephthalate/polypropylene films made by the integration of phenolic compounds from OLE by supercritical solvent impregnation have antimicrobial effects against *P.aeruginosa* and *S.enteritidis*. Additionally, gelatin films containing 5.63% OLE were successful in lowering L.monocytogenes counts in fish that had been cold-smoked (Albertos et al., 2017).

CONCLUSIONS and FUTURE PROSPECTS

Because they have valuable compounds, OL has many positive effects on both human health and food quality. For this reason, instead of being thrown away as waste, it can be used for foods to improve food storage life and to develop functional foods, as well as in the fields of medicines, cosmetics and pharmaceuticals. Different authors showed antioxidant activity of OLE in delaying oxidation in a variable food matrix and antimicrobial activity against several microorganisms in foods at certain concentrations and conditions. However, OLE concentration is an important factor and should be considered before application because of its pro-oxidant activity and bitter taste. Furthermore, OLE's stability in food products is important for achieving its intended use. On the other hand, in using OLE to produce functional foods, more *in vivo* studies should be conducted to better understand functional effects of the OLE on human health. Although several human studies have examined the bioavailability and metabolism of the compounds in OLE, the information is still limited and unable to provide a complete grasp of this subject. OL extraction methods and conditions are another important issue. A desired approach is to increase extraction effectiveness (taking into account both yield and functionality) while reducing overall cost. More extraction studies are needed to improve OL extraction, especially by green processes suitable for application in foods.

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