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Antimicrobial activity of *Olea europaea* Linné extracts and their applicability as natural food preservative agents.

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Running Titel: Antimicrobial *Olea europaea* extracts

Abstract

The antimicrobial activity of phenolic compounds from *Olea* (*O.*) *europaea* Linné (L.) is part of the scientific discussion regarding the use of natural plant extracts as alternative food preservative agents. Although, the basic knowledge on the antimicrobial potential of certain molecules such as oleuropein, hydroxytyrosol or elenolic acid derivatives is given, there is still little information regarding their applicability for food preservation. This might be primarily due to the lack of information regarding the full antimicrobial spectrum of the compounds, their synergisms in natural or artificial combinations and their interaction with food ingredients. The present review accumulates available literature from the past 40 years, investigating the antimicrobial activity of *O. europaea* L. derived extracts and compounds *in vitro* and in food matrices, in order to evaluate their food applicability. In summary, defined extracts from olive fruit or leaves containing the strongest antimicrobial compounds hydroxytyrosol, oleacein or oleacanthal in considerable concentrations appear to be suitable for food preservation. Nonetheless there is still need for consequent research on the compounds activity in food matrices, their effect on the natural microbiota of certain foods and their influence on the sensorial properties of the targeted products.

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Highlights:

- Elenolic acid derivatives are the most potent antimicrobial compounds in *O. europaea*
- Olive mill waste and olive leaves are most exploitable remedies
- Hydroxytyrosol and elenolic acid derivatives might be suitable natural preservatives

Keywords:

- antimicrobial susceptibility,
- *Olea europaea*,
- Hydroxytyrosol,
- Oleuropein,
- Elenolic acid,
- food preservation

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1. Introduction

Fueled by trends towards minimally processed foods with an apparent natural and healthy character, researchers are intensively searching for plant derived natural preservative agents (Tiwari et al., 2009). These should prolong shelf-life on the one and ensure food safety on the other hand, replacing traditional preservatives (Burt, 2004). The latter, which are often rejected by consumers, do not fit the "green" character of modern, minimally processed foods as for example fresh cut vegetables or fruits (Negi, 2012).

Many attempts have been made to find and apply suitable natural preservatives from plant sources (Tiwari et al., 2009). As for example the use of crude extracts or single compounds, such as oregano essential oil (Lambert et al., 2001; Skandamis and Nychas, 2001) and its major constituent carvacrol, even in combination with physical preservation processes (Karatzas et al., 2001; Kim et al., 1995). The plant diversity estimated on earth (approx. > 250000 species (Borris, 1996)) enables extensive research regarding useable antimicrobial secondary metabolites, but also hinders the identification of the most promising candidates (Silva and Fernandes, 2010).

One of the plants whose antimicrobial potential has been scientifically known since the early 1970s is *Olea europaea* Linné, commonly known as the olive tree. Its antibacterial activity was first observed due to problems regarding olive fruit fermentation (Etchells et al., 1966), an issue which is still under scientific investigation (Medina et al., 2008). Besides, *Olea europaea* is more prevalently known for its antioxidant capacities and the associated dietary health aspects (Benavente-García et al., 2000; Brenes et al., 2007; Markin et al., 2003). It is scientifically accepted that *O. europaea* products, such as the fruits and the virgin olive oil, have beneficial health effects when they are a regular part of the human diet (Keys, 1970). The contribution of antioxidant compounds from *O. europaea* to health protective effects has been extensively studied and reviewed (Ghanbari et al., 2012; Martin-Pelaez et al., 2013; Sofi

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et al., 2008; Visioli, 2012). These scientific findings already led to health claim proposals (EFSA, 2011). But besides the exploitation of these antioxidant activities there are also intentions regarding the application of olive extracts or contained compounds as natural food preservatives (Brenes et al., 2007; Soni et al., 2006).

To develop a preservative olive extract with an overall natural character for food applications appears to be a promising approach in order to exploit the bioactivities of *Olea europaea*. This review was conducted to aggregate available scientific information upon the antimicrobial activity of crude extracts derived from *O. europaea* L. plant parts and the contained antimicrobial active phenolic constituents from secoiridoid hydrolysis. The cited reports are presented in a chronological manner, focusing on antibacterial activity. Antifungal potential is highlighted individually. Reports about application trials of antimicrobial extracts from olive plant parts in food products are also evaluated. The gathered information is assessed critically towards possible technological benefits from olive extracts and compounds in terms of shelf-life prolongation and microbiological food safety.

2. The olive tree *Olea europaea* L., products and by-products

The olive tree *O. europaea* L. is native to the Mediterranean countries. Although its cultivation is spreading globally 98 % of olive agriculture is still domiciled in the Mediterranean basin (Peralbo-Molina and de Castro, 2013). Olive trees are preferably cultivated for the production of table olives and olive oil, two of the most representative components of the Mediterranean diet (Obied et al., 2012). The fruits of the *O. europaea* tree also referred to as drupes, consist of a hard stone (endocarp) containing the seed, embedded in a cortex of soft fruity flesh (mesocarp), which is covered by a waxy skin (epicarp). They are generally composed of water (50.0 %), oil (22.0 %) and sugar (19.1 %), accompanied by cellulose (5.8 %), proteins (1.6 %) and ash (1.5 %) (Niaounakis and Halvadakis, 2006).

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Approximately 1.5 million tons of fermented table olives are produced annually (Medina, Garcia, et al., 2009).

Virgin olive oil is the most valuable product from *O. europaea*, produced from the fruits by mechanical homogenization and pressing only. Except washing, decantation, centrifugation and filtration no further processing steps are applied. The produced virgin olive oil contains high amounts of phenolic constituents with several beneficial effects on human health (Caramia et al., 2012; Cicerale et al., 2010; Ghanbari et al., 2012; Kratzb and Cullenc, 2002).

The production of olives generates a vast amount of by-products. Olive tree cultivation and the necessary pruning, lead to the accumulation of olive leaves, approximately 25 % per tree by weight (Talhaoui, Taamalli, et al., 2015). The vast amount of these remedies is usually burned or discarded otherwise (Romero-Garcia et al., 2014). The high content of bioactive phenols is consequently squandered (Bouaziz and Sayadi, 2005; Lee et al., 2009; Meirinhos et al., 2005).

Additionally, oil extraction leads to the remaining of olive press cakes and olive mill waste waters (OMWW). The waste water still contain high concentrations of phenolic components, up to 24 g/L (Niaounakis and Halvadakis, 2006). Due to the difficult disposal of these waste waters, there have been many different considerations how to exploit their phenolic content (Morillo et al., 2009; Paredes et al., 2002; Roig et al., 2006).

3. The phenolic profile of *Olea europaea* L.

The phenolic profile of olive leaves and fruits, as reviewed by Charoenprasert and Mitchell (2012) and Ye (2014), is primarily dominated by phenolic acids (e.g. ferulic, vaillic, coumaric acd), phenolic alcohols (e.g. tyrosol and hydroxytyrosol), flavonoids (e.g. luteolin-7-glucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, rutin, apigenin-7-glucoside, quercetin-3-rhamnoside, luteolin) and secoiridoids (e.g. oleuropein, ligstroside). These phenolic

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compositions of olive fruit and leaves, and therefore their products, are strongly affected by the cultivar and environmental conditions, such as region, climate and irrigation and furthermore by point of harvest, ripeness and post-harvest processing (Romero et al., 2004; Salvador et al., 2001; Vinha et al., 2005).

Regarding the antimicrobial activity of *O. europaea* L. most studies focus on secoiridoid compounds and their derivatives. The bitter tasting secoiridoids oleuropein, demethyloleuropein and ligstroside are the predominant phenolic compounds in *O. europaea* L. and are exclusive to the plants of the *Oleaceae* family (Servili et al., 2004). These phytoalexins and their precursors are accumulated during fruit and leaf maturation, acting as defense molecules against herbivores and microbial pathogens (Kubo et al., 1985).

Figure 1 Chemical structures of phenolic antimicrobial compounds in *Olea europaea*.

As shown in Figure 1 oleuropein (3,4-DHPEA-EA) consists of an ester of hydroxytyrosol (3,4-DHPEA) and elenolic acid (EA) which is additionally β-glycosylated. Ligstroside (4-HPEA-EA) on the other hand represents the ester of β-glycosylated elenolic acid and tyrosol (4-HPEA). Demethyloleuropein is another derivate of oleuropein consisting of esterified hydroxytyrosol and decarboxymethyl elenolic acid. After the hydrolytic removal of the sugar component by β –glucosidase the remaining molecules are generally referred to as aglycones.

Figure 2 Enzymatic hydrolysis of oleuropein and ligstrosid (Reprinted with permission from Charoenprasert and Mitchell (2012) Copyright American Chemical Society.

As shown in Figure 2 the consequent enzymatic secoiridoid breakdown leads to the formation of free tyrosol, hydroxytyrosol or elenolic acid (Charoenprasert and Mitchell, 2012). The emergence of the dialdehydic form of decarboxymethyl elenolic acid (EDA), either linked to tyrosol (4-HPEA) or hydroxytyrosol (3,4-DHPEA), is also favored. Oleacein is the synonym

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for 3,4-DHPEA-EDA whereas 4-HPEA-EDA is referred to as oleocanthal (see Figure 1) (Vougogiannopoulou et al., 2014). Regarding the antimicrobial activity of extracts from *O. europaea* L. plant parts the named compounds are the most often identified molecules. Table 1 gives a brief overview regarding the quantitative distribution of these molecules in *Olea europaea* L. fruit and leaves and their originating products.

Table 1 Concentrations (mg/kg) of antimicrobial molecules from enzymatic secoiridoid breakdown in *Olea europaea* L. products

4. Antimicrobial activity of phenolic compounds from *Olea europaea* L.

4.1 Oleuropein

Fleming and Etchells (1967) stated that oleuropein might be the primary inhibitory compound in olive fruit extracts, an assumption postulated first by Vaughn (1954) and confirmed by Juven et al. (1968). These authors identified oleuropein and its aglycone as major constituents in the inhibitory fraction of an ethyl acetate extract from green olives which inhibited several Gram-positive and Gram-negative bacteria (Juven et al., 1968). Continuative studies revealed a higher antimicrobial potential for the oleuropein aglycone compared to the oleuropein glycoside (Juven and Henis, 1970), which again was suspected first by Fleming et al. (1969). Regarding the theory of Etchells et al. (1966) it was concluded, that the heat treatment led to a β–glucosidase inactivation, preventing the enzymatic hydrolysis of oleuropein-glycoside into oleuropein-aglycone or smaller compounds (Fleming et al., 1973) as shown in Figure 2. Studying the antimicrobial mechanism of oleuropein on *Lactobacillus* (*Lb.*) *plantarum,* Juven et al. (1972) found first evidence on membrane associated activities, leading to ion leakage and ATP depletion.

Further research was conducted regarding the hydrolysis products of oleuropein, meaning its aglycone and also whose components, 3,4-DHPEA and EA (Figure 1) (Fleming et al., 1973).

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The four compounds were tested for their activity against *Lb. plantarum* and contradictive results were revealed. The authors found no inhibitory effects by oleuropein alone (Fleming et al., 1973), what is inconsistent with the results by Juven et al. (1972) who found minimal inhibitory concentrations (MIC) for oleuropein against *Lb. plantarum* at 330-670 µg/mL in an antimicrobial streak test. This discrepancy reappeared when Ruiz-Barba et al. (1991) showed bactericidal effects of oleuropein against nine selected strains of *Lb. plantarum*, whereas Rozes and Peres (1996) found only slight inhibitory effects of oleuropein on the growth of *Lb. plantarum.* Fleming et al. (1973) had shown antimicrobial potential of selected extract fractions containing the oleuropein aglycone (150 µg/mL) against a variety of Gram-positive and Gram-negative bacteria (paper disc assay), but none against the tested yeasts. These early studies revealed rather contradictive results regarding the antimicrobial activity of oleuropein against lactic acid bacteria. Although these discrepancies may be generally attributed to the varying methodical approaches of the authors it was nonetheless concluded, that oleuropein may act as a plant defense molecule (Fleming et al., 1973), which is activated by β-glucosidase into the oleuropein aglycone (Juven and Henis, 1970). The aglycone may act as a potent antimicrobial phytoalexin itself or can be further hydrolyzed (Kubo et al., 1985).

Purified oleuropein was found to be less active versus *Bacillus* (*B.*) *cereus* than crude extracts from green olives (Tassou et al., 1991). Treating oleuropein in alcalic conditions did not have an activity enhancing effect (Rozes and Peres, 1996) but as previously shown (Juven et al., 1968; Ruiz-Barba et al., 1991), oleuropein is heat sensitive and can be transformed into more effective bactericides by thermal hydrolysis (Rozes and Peres, 1996). Nychas et al. (1990) and Tranter et al. (1993) investigated the effect of oleuropein on *Staphylococcus* (*S.*) *aureus*. Concentrations above 0.2 $\%_{w/v}$ sufficiently delayed growth and toxin production, whereas 0.6 $\%_{w/v}$ completely inhibited both metabolic parameters (Tranter et al., 1993). The authors were the first to mention the possibility to use oleuropein as an additive for food safety

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management applications. Tassou and Nychas (1994) and Tassou and Nychas (1995) confirmed the observations by Tranter et al. (1993) and found increased antibacterial activity of oleuropein against *S. aureus* at reduced pH levels (Tassou and Nychas, 1994). Regarding *Salmonella* (*Salm.*) *enteritidis* it was assumed, that oleuropein may only have a bacteriostatic effect (Koutsoumanis et al., 1998).

Beside these studies focusing on single microorganisms, later studies on oleuropein were more comparative. As shown in Table 2 Bisignano et al. (1999) found oleuropein to be growth inhibitory at concentrations between 62.5 and 500 µg/ml against different bacteria and investigations with *Mycoplasma* species also revealed a broad concentration range necessary for inhibitory effects (Furneri et al., 2002). As these MIC concentrations in order to achieve bacteriostatic effects appear to be quite high, the results regarding bactericidal activity from Medina, Brenes, et al. (2007) become less significant. These authors performed time kill assays and found all tested species to be insensitive against oleuropein at 3.1 µg/ml. This concentration was comparably low. Nonetheless growth of *Escherichia* (*E.*) *coli* and selected *Enterobacter* (*Eb.*) species was inhibited. Growth of the lactic acid bacteria *L. mesenteroides, Lb. pentosus* and *Lb. plantarum* on the contrary were not affected at all (Medina, Brenes, et al., 2007). *Listeria* (*L.*) *monocytogenes* was also found to be unaffected by oleuropein, but again the concentration used was extremely low (0.042 µg/mL) (Medina et al., 2006).

Serra et al. (2008) showed oleuropein to be fully inhibitory against *B. cereus* at 250 µg/mL in nutrient broth, which is in line with the concentrations postulated by Bisignano et al. (1999). But in Mueller Hinton broth no inhibition was observed, even at 700 µg/mL. Similar was found for *E. coli*, which was never fully inhibited at the concentrations tested, but the growth inhibition was also greater in nutrient broth than in Mueller Hinton broth (Serra et al., 2008). The authors presumed this medium dependent discrepancy may be attributed to the reduced complexity of the nutrient broth in comparison to Mueller Hinton broth, meaning less cellular

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energy and consequently less extrusion capacity. But there were no investigations regarding the true oleuropein concentration in the cultivation media or possible concentration reductions due to complexation of oleuropein by media components. As oleuropein and especially its β-glucosidase activated glutaraldehyde like aglycone was identified to be a strong protein crosslinker (Konno et al., 1999) interactions of the molecules with medium ingredients may be expected and need to be investigated in future studies regarding the antimicrobial activity of oleuropein and its aglycone.

Eventually, results regarding the antimicrobial activity of oleuropein are arguable. Although concentration dependent activity against *S. aureus, E. coli and Salm. enteritidis* was shown, a general differentiation between Gram-negative and Gram-positive species is not possible. Lactic acid bacteria appear to be generally more resistant to oleuropein than other bacteria. But concerning this assumption there appears to be lacking information on lactic acid bacteria's abilities to develop structural or metabolic resistance towards oleuropein and its derivatives. Furthermore there is an uninvestigated interdependency between the antimicrobial activity of oleuropein and the growth media used.

4.2 Hydroxytyrosol

First reports on the antimicrobial potential of hydroxytyrosol were published by Fleming et al. (1973) who found it to be non-inhibitory against *Lb. plantarum.* A result questioned 20 years later by Ruiz-Barba et al. (1990) who found hydroxytyrosol to be the most active compound isolated from olive brines against *Lb. plantarum*, killing 2.0 10^{10} cfu/mL within 22 h at 585 µg/mL. These authors also stated a cell wall associated mode of action. Further studies by Ruiz-Barba et al. (1993) revealed similar results. Hydroxytyrosol was able to inactivate *Lb. plantarum* ATCC 8014 (2.0 10^{8} cfu/mL) within 24 h at a concentration of 350 µg/mL. The combination of hydroxytyrosol with oleuropein led to an increased bactericidal action, killing *Lb. plantarum* (2.0 10^{8} cfu/mL) in less than 2 h (Ruiz-Barba et al., 1993). Hydroxytyrosol

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isolated from olive mill waste waters was tested against the plant pathogenic bacteria *Pseudomonas* (*Ps.*) *syringae* pv. *savastanoi* (Gram-negative) and *Corynebacterium* (*Coryne.*) *michiganense* (Gram-positive) by Capasso et al. (1995). At concentrations of 154 µg/mL growth of *Ps. savastanoi* was inhibited, whereas growth of *Coryne. michiganense* was unaffected.

As previously described for oleuropein and shown in Table 2 Bisignano et al. (1999) were the first to perform comparative studies screening the antimicrobial activity of hydroxytyrosol against a broad range of bacteria. They tested five ATCC-listed bacterial strains and 44 clinical bacterial isolates against hydroxytyrosol the broth microdilution assay for MIC determination (Table 2). The compound showed high antimicrobial activity against Gram-negative and -positive bacteria, as shown in Table 2. Hydroxytyrosol appeared to be more effective than oleuropein (Bisignano et al., 1999).

Table 2 Comparative presentation of scientific studies on the antimicrobial activity of oleuropein, hydroxytyrosol and elenolic acid derivatives.

Medina, Brenes, et al. (2007) found hydroxytyrosol (0,8 µg/mL) to lack antimicrobial potential versus the lactic acid bacteria *L. mesenteroides, Lb. pentosus* and *Lb. plantarum.* But versus *E. coli* and three *Enterobacter* species it was found to be bactericidal. *L. monocytogenes* was resistant to hydroxytyrosol at 0.042 µg/mL, which is equals to the mean concentration of hydroxytyrosol in the virgin olive oils investigated by the authors (Medina et al., 2006).

The hydroxytyrosol concentrations used by Bisignano et al. (1999), Medina et al. (2006) and Medina et al. (2007) must be considered exceedingly low. As reviewed before, early studies reported active concentrations between 154 to 585 µg/ml. This is also the case for later studies. Tafesh et al. (2011) showed hydroxytyrosol to be growth inhibitory at 400 µg/mL against *Streptococcus pyogenes, S. aureus, E. coli* and *Klebsiella* (*K.*) *pneumonia* in a broth

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microdilution assay (tryptone soy broth). Combinations of hydroxytyrosol with gallic acid or ascorbic acid revealed additive effects (Tafesh et al., 2011). *Helicobacter* (*H.*) *pylori* was found to be fully resistant towards hydroxytyrosol in an aqueous medium, even at reduced pH levels (Romero et al., 2007). Using a broth based kill-time method Friedman et al. (2011) found pure hydroxytyrosol to be bactericidal for *S. aureus* within 60 min at concentrations above 150 µg/mL. Recent *in vitro* investigations regarding the antifungal potential of hydroxytyrosol against medically important *Candia* spp. revealed minimal fungicidal concentrations (MFC) values ranging between 97.6 µg/mL and 6.25 mg/mL (Table 2) (Zoric et al., 2013). For the sake of completeness it has to be noted that the author's result interpretations did not accord to established MIC or MFC definitions. Thereafter preformed fluorescent dye-exclusion based studies with *Candida* (*C.*) *albicans* revealed a membrane associated antifungal mechanism at sub-inhibitory concentrations (Zoric et al., 2013).

Altogether, Hydroxytyrosol appears to possess stronger inhibitory activity versus *E. coli, S. aureus* or *Salm. thyphii* than oleuropein. But, as for the precursor molecule, a general distinction between the effects of hydroxytyrosol on Gram-positive or Gram-negative species cannot be made. As it becomes visible from Table 2, results are inconsistent and partially contradictive, especially when methodical approaches and the used nutrition medium are taken into account. These controversial results do not allow to rate the antimicrobial activity of hydroxytyrosol. (Medina-Martínez et al., 2016) recently addressed these discrepancies and reevaluated the compounds activity against various pathogenic bacteria (see Table 2). Furthermore they focused their trials on *E. coli*, confirming strong dependencies of hydroxytyrosol's antimicrobial activity on bacterial strain, cultivation conditions and nutrition medium used in the antimicrobial assay. Similar results were previously reported by Serra et al. (2008), who found a medium and inoculum size dependent activity of hydroxytyrosol against *E. coli* and *B. cereus.* Although growth of *E. coli* was never fully inhibited at

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concentrations up to 75 µg/mL, growth was more pronounced in starch containing Mueller-Hinton broth than in a self-made nutrient broth. The same tendency was shown for *B. cereus* which was fully inhibited at 75 µg/mL in nutrient broth, but was unaffected in Mueller-Hinton broth. Medina-Martínez et al. (2016) concurrently stressed the fact that hydroxytyrosol might be quickly oxidized in complex nutrition media. This observation was previously reported by Long et al. (2010) who showed hydroxytyrosol to undergo rapid oxidation in complex media leading to H_2O_2 formation. Measuring the concentration of hydroxytyrosol after incubation in different nutrition media with and without different strains of *E. coli,* Medina-Martínez et al. (2016) found it's concentrations to be reduced by 15 to 35 % in the presence of *E. coli,* but to remain unchanged in their absence. It was not clarified if the tested *E. coli* strains truly metabolized hydroxytyrosol or possessed another defense mechanism. Nonetheless, these results highlight the importance to evaluate the stability of natural antimicrobial compounds such as hydroxytyrosol in different cultivation media, as well as the degradation or transformation by the microorganisms investigated. The need for more thorough studies using standardized methods is apparent. Furthermore, there is need for studies regarding the antimicrobial mode of action of hydroxytyrosol, as on synergistic or antagonistic behavior with other antimicrobial compounds.

4.3 Tyrosol

Although tyrosol is also a potent antioxidant compound, exhibiting several beneficial bioactivities, reported antimicrobial activity appears to be questionable. Gourama et al. (1989) investigated its antifungal activity and found it to be inhibitory against *Aspergillus* (*A.*) and *Penicillium* (*P.*) species isolates from olives. Tuncel and Nergiz (1993) stated antibacterial activity of tyrosol, in terms of MIC values about 400 µg/mL against *B. cereus* and *Salm. typhimurium* and 600 µg/mL against *S. aureus* and *E. coli*. Later studies showed the compound to be inefficient to inhibit the growth of *Coryne. michiganse*, *Ps. savastanoi*

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(Capasso et al., 1995) and *Lb. plantarum* (Ruiz-Barba et al., 1993). A comparative study with several phenolic compounds from olive oils found tyrosol (250 µg/mL) to be inactive against *L. monocytogenes* (Medina et al., 2006). Growth of *H. pylori* was also not affected by tyrosol (Romero et al., 2007). A more recent study using a broth microdilution assay found *S. aureus* to be unaffected by tyrosol, whereas *E. coli, Strept. pyogenes* and *K. pneumoniae* were inhibited at 600 µg/mL (Tafesh et al., 2011). As stated for hydroxytyrosol the results regarding the antimicrobial potential of tyrosol are partially contradictive and further investigations are necessary to exclude disruptive factors in methodology. Nonetheless available results allow to rate tyrosol the least antimicrobial active phenolic molecule from secoiridoid breakdown in *Olea europaea.* But it's synergistic or even antagonistic effects on other more active antimicrobial compounds from *O. europaea* remain to be elucidated. This will especially important regarding the possible development of a natural extract from *O. europaea* for food preservative purpose.

4.4 Elenolic acid and derivatives

The dialdehydic form of decarboxymethyl elenolic acid (EDA) which can also be linked to tyrosol (4-HPEA) or hydroxytyrosol (3,4-DHPEA), does possess very potent antimicrobial activity against various enterobacteria and lactic acid bacteria, as shown in Table 2 (Medina, Brenes, et al., 2007). In comparison to oleuropein (3.13 µg/mL) and hydroxytyrosol (0.8 µg/mL), 3,4-DHPEA-EDA (oleacein) (0.16 µg/mL) proved to possess strongest bactericidal potential. Oleocanthal (4-HPEA-EDA) on the other hand was shown to exhibit bactericidal activity against *H. pylori* at 1.3 µg/mL, whereas oleuropein, hydroxytyrosol and tyrosol did not exhibit any activity against *H. pylori* (Romero et al., 2007). Regarding the unwanted inhibition of lactic acid bacteria during fermentation it was concluded that EDA derivatives are the key molecules (Medina, Garcia, et al., 2009). Brines of Manzanilla olives were found to contain the highest amounts of elenolic acid (Medina, Brenes, et al., 2007),

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which cannot be found in pasteurized olives due to thermal β-glucosidase inactivation and lacking oleuropein hydrolysis (Medina, Garcia, et al., 2009). Elenolic acid derivatives seem to be the least investigated antimicrobial compounds found in *O. europaea* products, but they apparently possess strongest antimicrobial activities. Medina, Brenes, et al. (2009) found the dialdehydic form of decarboxymethyl elenolic acid either free or linked to tyrosol or to hydroxytyrosol to be bactericidal against *Ps. fluorescens, S. aureus*, *Enterococcus* (*Ec.*) *faecalis*, and *E. coli* at concentrations between 0.024 and 0.183 µg/mL. Their antimicrobial activities were significantly higher than those of thymol or carvacrol and comparable to common biocides such as glutaraldehyde and ortho-phtalaldehyde (Medina, Brenes, et al., 2009). The authors raised the theory that the enhanced antimicrobial effect of elenolic acid derivatives may be linked to their dialdehydic structure. This is in line with the postulations of Konno et al. (1999) who named the dialdehydic structures from enzymatic oleuropein breakdown to be strong protein crosslinkers and central molecules in the plants defense mechanisms against microbial pathogens and herbivores. On the other hand this interactivity with nitrogenous compounds can lead to reduced antimicrobial activities as shown by (Medina et al., 2010). These authors were able to diminish the growth inhibitory effect of EDA derivatives on lactic acid bacteria starter cultures in olive brines by addition of MRS broth or yeast extract, both containing nitrogenous compounds (Medina et al., 2010). Brenes et al. (2011) found storage solutions from black olives containing EDA and 3,4-DHPEA-EDA to be more bactericidal and fungicidal against plant pathogenic organisms than storage solutions lacking these compounds but having higher hydroxytyrosol concentrations. As stated by the cited authors elenolic acid derivatives appear to be the strongest antimicrobial compounds found in *Olea europaea* L. plant parts. They obviously possess a broader antimicrobial spectrum and appear to be active against lactic acid bacteria which were shown to be mostly resistant to tyrosol, hydroxytyrosol or oleuropein. Nonetheless more studies are

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necessary to broaden knowledge on the antimicrobial spectrum of EDA derivatives and to elucidate their mode of action, which has not been the center of antimicrobial studies to date.

5. Antimicrobial activity of *Olea europaea* L. products

The comparative investigations of Medina et al. (2006) and Serra et al. (2008) on the antimicrobial activity of crude olive extracts versus single phenolic compounds implicate a superior activity for natural complex mixtures of olive phenols in comparison to pure standalone compounds. A finding observed and stated by others, as presented in the following.

5.1 Fruits

The first scientific reports on the antimicrobial potential of olive extracts and their constituents date back to the 1970's. The studies were motivated by lacking insights into technological aspects of olive fermentation. Etchells et al. (1966) had found a mild heating of olives prior to their inoculation to promote an effective fermentation. This result indicated the existence of one or more potent bacterial inhibitors present in the olive's flesh or skin, at this time still unknown compounds, which may become inactive due to the preliminary heating step (Etchells et al., 1966).

Fleming and Etchells (1967) produced aqueous extracts from olives, which were able to inhibit different strains of lactic acid bacteria used in olive fermentation starter cultures. *Leuconostoc mesenteroides* appeared to be the most and *L. brevis* the least sensitive strain. The inhibitory effects could furthermore be increased by freezing and thawing the olives prior to extraction. Although no chemical characterizations of these extracts were carried out, the authors mentioned oleuropein to be known as a potent antimicrobial, found in all plant parts of *Olea europaea* L. (Fleming and Etchells, 1967). Until the early 1990s there were no further publications on the antimicrobial activity of olive extracts.

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Table 3 - Antimicrobial activity of extracts from olive fruits

Table 3 summarizes studies giving quantitative information on the antimicrobial activity of crude extracts from olive drupes. Beside the difficulties to interpret results regarding antimicrobial activity from different methods, conclusions on the activity of crude extracts are even more complicated if the extract compositions are unspecified. Nychas et al. (1990) for example gave a short report about depressive effects upon protein excretion by *S. aureus* in presence of 10 $\%_{v/v}$ of an uncharacterized crude extract from olives (Nychas et al., 1990). Investigations with *B. cereus* T spores by Tassou et al. (1991) revealed significant outgrowth inhibition by crude extracts from green olives (4 $\%_{v/v}$), which could be further increased by the addition of 1 $\%_{v/v}$ oleuropein. Another more recent study on an extract from stoned table olives revealed strong growth inhibitory potential in terms of IC_{50} against *E. coli, B. cereus, B. subtilis, S. aureus* and *K. pneumoniae*. In contrast to other studies summarized in Table 3, this extract did not contain oleuropein, tyrosol or hydroxytyrosol (Sousa et al., 2006). Furthermore, *P. aeruginosa* and the tested fungi *C. albicans* and *Cryptococcus neoformans* were found to be resistant against this luteolin-dominated extract at concentrations up to 50 mg/mL (Sousa et al., 2006). Comparative studies using extracts from olives of four different cultivars confirmed a resistance of *C. albicans* against olive extracts (up to 100 mg/mL). *E. coli* and *B. subtilis* appeared to be the most susceptible bacteria tested. Although hydroxytyrosol was the most abundant phenolic compound in all extracts tested, the study showed that cultivar and processing technology dependent changes in phenolic compositions have a considerable impact on the antimicrobial potential of crude olive extracts (Pereira et al., 2006).

Regarding the recovery of antimicrobial active phenolic compounds from *O. europaea* L: plant parts, the fruits appear to be of minor importance. As reviewed, extracts from olives showed moderate antimicrobial activity at best. This might be due to the lack of dialdehydic

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elenolic acid derived molecules. Olive fruits are mostly dominated by oleuropein and hydroxytyrosol as shown in Table 1. As reviewed before both compounds exhibit moderate antimicrobial activity. Extracts produced from *O. europaea* L. drupes might be enzymatically processed to generate EDA derivatives, but as olives are highly valued food products this approach is neglect able.

5.2 Oil

Studies on the antimicrobial activity of extracts virgin olive oil are presented in Table 4. Beside quantitative numbers regarding the bactericidal activity versus a wide variety of bacteria, three of these studies also inform on the phenolic composition of the extracts. Taking both aspects into account it becomes obvious that the natural mixture of tyrosol, hydroxytyrosol and elenolic acid derivatives exhibits remarkable bactericidal activity, exceeding the previously described activities of the compounds alone.

Table 4 Antimicrobial activity of extracts or emulsions from virgin olive oil

Medina et al. (2006) assessed the antimicrobial activity of olive oil in comparison to other plant oils against a broad variety of food associated bacteria. A virgin olive oil emulsion (50 $\%_{v/v}$) revealed bactericidal potential, mainly against Gram-positive bacteria. *S. enterica* revealed to be highly sensitive as well, whereas *E. coli* was the least affected bacterial species tested. The yeast *C. albicans* on the contrary was completely unaffected. The authors stressed the fact, that antimicrobial activity is related to the dialdehydic forms of the oleuropein and ligstroside aglycons and the simple phenols tyrosol and hydroxytyrosol (Medina et al., 2006). These molecules are highly abundant in unrefined (virgin) olive oil, as shown in Table 1. Aqueous extracts from virgin and refined olive oils were tested for antibacterial potential at concentrations of 2.5 $\%_{v/v}$ by Medina, Romero, et al. (2007). Although the total phenolic content of the extracts (Table 4) was only half the amount present in oil, both extracts exhibited strong bactericidal activity against six pathogenic foodborne bacteria. Virgin olive

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oil extract proved to be significantly more effective than the refined olive oil extract, due to its higher phenolic content (Medina, Romero, et al., 2007). Again both, Gram-positive and -negative bacteria were highly susceptible against the natural mixture of oleuropein derived phenolic molecules. Aqueous extracts of phenol rich virgin olive oil, containing high concentrations of hydroxytyrosol (3,4-DHPEA), tyrosol (4-HPEA) and oleacein (3,4-DHPEA-EDA) also showed significant bactericidal activity against *H. pylori* (Romero et al., 2007). As reviewed before *H. pylori* was resistant to oleuropein, tyrosol and hydroxytyrosl, but susceptible to oleocanthal (4-HPEA-EDA). Karaosmanoglu et al. (2010) investigated different Turkish virgin olive oils (50 $\%_{v/v}$) and found them to be bactericidal against the food borne pathogens *E. coli*, *L. monocytogenes* and *S. enteritidis*. The natural mixture of phenols in the oils appeared to be more effective than single phenolic compounds alone or in artificial combination, but except for tyrosol no oleuropein derivatives were tested (Karaosmanoglu et al., 2010).

The given results indicate the interdependency of antimicrobial activity and phenolic content or more precisely its composition. As shown in Table 1 virgin olive oil contains the comparably lowest amounts of oleuropein, ligstroside, tyrosol and hydroxytyrosol, but it does also contain significant concentrations of oleacein and oleocanthal. This distinct combination of phenolic compounds resulted in increased antibacterial effectivity. Although the exploitation of olive oil for antimicrobial phenolic compounds is clearly unsuitable as it already is a highly valuable product, its unique phenolic composition might be a template which should be scientifically exploited in terms of antimicrobial activity.

5.3 Mill Waste

As mentioned, olive mill waste waters (OMWW) contain high amounts of phenolic components (Niaounakis and Halvadakis, 2006). Table 1 shows some elevated concentrations of hydroxytyrosol and oleacein. This might due to the process dependent release of enzymes

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from olive tissue leading to a continuous oleuropein and ligstroside degradation (Morillo et al., 2009; Roig et al., 2006). Regarding the antimicrobial activity of OMWW studies are most often focused on single microorganisms. The broader screenings preferably focused on antifungal activity. Furthermore information on the quantitative phenolic composition of OMWW extracts is mostly skimped.

Paredes et al. (1986) found OMWW to inhibit spore forming bacteria from the genus *Bacillus*, but no effects on species from the family of the *Corynebacteriaceae* (Paredes et al., 1986; Paredes et al., 1987). Similar effects were reported by Rodriguez et al. (1988). β–glucosidase treated extracts from concentrated OMWW effectively inactivated *B. megaterium*. Sporulation and germination were inhibited as well (Rodriguez et al., 1988). The phenolic mixtures of these OMWW formulations are unknown. Ramos-Cormenzana et al. (1996) on the contrary found *B. pumilus*, isolated from plant remedies, to be able to grow in OMWW and to reduce its phenolic content. Hydroxytyrosol and tyrosol were the preferably oxidized molecules. The ability to degrade the phenolic composition of OMWW was also found for *C. oleophila*, oxidizing 83 % of the total polyphenol content and consequently reducing antimicrobial activity (Amaral et al., 2012).

Further studies revealed differences in activity between single phenolic components and crude aqueous methanol (80%$_{v/v}$, pH 2, HCl, 40 mL) OMWW extracts (defatted with hexanal), showing better inhibitory potentials for the latter (Obied et al., 2007). This extract from Australian OMWW was inhibitory against four different pathogenic bacteria (*S. aureus, B. subtilis, E. coli, P. aeruginosa)* at 5 mg per plate, tested in a disc diffusion assay. But the investigated fungi (*Candida albicans, Aspergillus niger*) appeared to be unaffected by the crude extract (Obied et al., 2007). The quantitative composition of the extract is not given, but tyrosol, hydroxytyrosol and oleuropein were among the present phenolic compounds. Yangui et al. (2009) enriched OMWW from Tunisia with hydroxytyrosol by hydrolysation following

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the methods of Bitler et al. (2005) and Crea (2002) through acidification and incubation. Oleuropein was completely degraded and final hydroxytyrosol concentration reached 52.7 $\%_{w/w.}$ The authors found minimal bactericidal concentrations against *Pseudomonas syringae* and *Xanthomonas campestris* of about 7.18 µg/mL using a broth dilution method. The phytopathogenic fungi *Alternaria solani* (7.18 µg/mL), *Fusarim sambinicum* (28.72 µg/mL), *Verticulum dahlia* (7.18 µg/mL) were also inactivated (>4 log) by the hydroxytyrosol-enriched extracts (Yangui et al. 2009). The same extract was found to be sporicidal for *Botrytis cincera* (4 log) at 1.25 $\%_{w/v}$ within 30 min in a dilution-neutralization assay (Yangui et al. 2010). The given bactericidal concentrations are quite low and appear to be mostly due to the high hydroxytyrosol concentration. But, as discussed before, the true antimicrobial activity of hydroxytyrosol remains a controversy. Yangui et al. (2009) stated 70.23 $\%_{w/w}$ total phenolics for their extract, wherof 52.7 $\%_{w/w}$ were identified as hydroxytyrosol. But as the acompanying phenolic compounds were not specified possible synergistic contributions phenols need to be suspected.

Studying the antifungal activity of OMWW diluted in water and ethanol (4:1), Chaves-Lopez et al. (2015) found 1.25 % of OMWW to be growth inhibitory against *Penicillium expansum, Penicillium verrucosum, Aspergillus clavatus, Eurotium amstelodami* and *Cladosporium cladosporioides. Aspergillus parasiticus* was found to be resistant. In comparison to the other studies on OMWW reviewed this extract was dominated by oleacein instead of hydroxytyrosol (Table 6).

Concerning their potential antimicrobial activities, crude OMWW extracts appear to be less investigated, but have proven to be reliable sources of phenolic compounds which are known to exhibit antimicrobial effects. Hydroxytyrosol rich OMWW possess strong bactericidal activity, although the synergistic influences of accompanying phenols is poorly investigated. The antifungal activity of certain OMWW against molds might be preferably attributed to

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oleacein (3,4-DHPEA-EDA), which can also be contained in high amounts (Table 1). Again, there is need for studies investigating the synergistic behavior of the active compounds. Regarding the application of extracts from *O. europaea* L. or contained compounds in further technological applications such as food preservation, it appears to be more reasonable to exploit the byproduct OMWW for this purpose than valuable olive products like the fruit or the oil.

5.4 Leaves

As shown in Table 1 olive leaves can contain high amounts of oleuropein and hydroxytyrosol. Elenolic acid has also been quantified (Talhaoui et al. 2014), but oleacein and oleocanthal were not reported. Studies on the antimicrobial potential of olive leaf extract (OLE) are accumulated in Table 5. Markin et al. (2003) first concluded that molecules smaller than 1000 g/mol (oleuropein: 540 g/mol) are the most effective bactericides in aqueous OLE. Most authors investigating OLE for antimicrobial activity highlight the elevated amounts of oleuropein in their extracts, but do not elucidate the complete spectrum of phenolic compounds contained. In conjunction with varying methodical approaches these gaps produce discrepancies regarding antimicrobial activity and spectrum. Pereira et al. (2007) on the one hand found *B. cereus* and *C. albicans* to be the most susceptible species (IC_{25}: 0.63 and 0.85 mg/mL) in investigations for growth inhibitory potential, whereas Markin et al. (2003) and Sudjana et al. (2009) found *C. albicans* and *B. subtilis* among the least susceptible, most resistant microorganisms. But Keskin et al. (2012) found *C. albicans* species to be resistant as well. Disregarding these discrepancies the studies reviewed in Table 5 showed the suitable antimicrobial activity of olive leaf extracts against a wide variety of bacteria. A dependency on the Gram-type however does not become apparent. *Campylobacter jejuni* and *H. pylori* were found to be very susceptible *in vitro* with MBCs of 0.78 $\%_{v/v}$, 0.31 $\%_{v/v}$ and 0.62 $\%_{v/v}$ respectively (Sudjana et al., 2009). *Salm. thyphimurium* and *Salm. enteritidis* on the contrary

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were the most resistant species with MBCs of 180 and 370 µg/mL, respectively, for an acetone based OLE (Korukluoglu et al. 2010).

Table 5 Antimicrobial activity of extracts from olive leaves.

Lee and Lee (2010) mimicked the phenolic profile of their olive leaf extract by combining oleuropein, rutin, vanillin and caffeic acid in a disc diffusion assay. They found the combination to be more inhibitory than each single compound alone. At concentrations of 800 µg per disc *B. cereus* and *Salm. enteritidis* were fully inhibited by the combination, whereas growth of *S. aureus* and *E. coli* was not affected (Lee and Lee, 2010). A similar study focusing on combinations of molecules derived from secoiridoid breakdown was not available.

In dependence on the respective extraction process, olive leaf extracts can present mixtures with high amounts of phenolic constituents, exhibiting growth inhibitory and bactericidal potential against various Gram-positive and –negative bacteria. Studies screening their mere antifungal activity are not available. Further *in vitro* studies are necessary to identify synergisms between the active compounds in order to produce optimized antimicrobial olive leaf extracts. Future investigations should consequently broaden the insight on the phenolic composition of the extracts produced to establish connections between composition and antimicrobial activity. Nonetheless, olive leaves appear to be a promising source for natural antimicrobial extracts. As shown in Table 1 leaves contain highest oleuropein contents, but same as for OMWW, they are considered to be less valuable remedies. This renders them highly interesting for alternative exploitation such as the production of phenolic extracts for food preservation or health associated applications.

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6. Application of antimicrobial extracts and compounds from *Olea europaea* L. in food

Recent findings on the antimicrobial efficiency of selected bioactive phytochemicals from *Olea europaea* L. quickly led to the idea to exploit these compounds as natural, "green" food preservatives. Available application studies are accumulated in Table 6.

First scientific trials regarding the antimicrobial effect of extracts from *O. europaea* in foods date back to 1990, to our best knowledge starting with the work of Radford et al. (1991). These authors detected elevated death rates for *Salm. enteriditis* in mayonnaise containing virgin olive oil with a high phenolic content. But no antibacterial effects could be detected in emulsions when made with sunflower oil or refined olive oil (Radford et al., 1991). Similar results were postulated by Medina et al. (2007) who found virgin olive oil to be bactericidal for *Salm. enteritidis* and *L. monocytogenes* in milk or egg based mayonnaise. In contrast to the findings of Radford et al. (1991), Medina et al. (2007) stated a pH dependency as bacteria were only inactivated when virgin olive oil and lemon juice were used in combination. In sunflower oil lemon juice had no bactericidal effect (Medina et al., 2007). In both studies the authors ascribed the bactericidal effects to the unique phenolic profiles of the virgin olive oils used.

Table 6 Results on the applicability of extracts from *Olea europaea* L. on food products and its effect on product quality.

On lettuce preparations virgin olive oil inactivated the complete population of *L. monocytogenes* (2.0 10^3 cfu/g) in less than 30 min without the addition of vinegar or lemon juice (Medina, Romero, et al., 2007). In comparable trials with lettuce Moore et al. (2011) found rinsing waters (deionized) containing (1, 3, 5 $\%_{v/v}$) an olive fruit extract (commercial, HIDROX®, CreAgri Inc., Hayward, USA) with a high hydroxytyrosol content to exhibit notable bactericidal activity against the initial bacterial load (0.3-2.3 log N_0/N), as well as artificially inoculated *Salm. enterica* (1.5-2.5 log N_0/N), on cut green leaf products (iceberg,

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romaine, adult spinach, baby spinach) (see Table 6). The authors furthermore stated that olive extract treatments were of equal or better efficiency than industrial standard decontamination processes for leafy greens based on hydrogen peroxide (Moore et al., 2011). Although the microbial results of Moore et al. (2011) are promising their study was designed to investigate the activity of residual extract, but the sensory impact of the extract remains were not investiated. Hydroxytyrosol and elenolic acid for example were found to be non-bitter, but oleuropein is known to express strong bitterness (Frank et al., 2001). The investigation of the sensory impact of a however composed phenolic extract from *O. europaea* L. on food products should therefore be mandatory for future food application studies.

Table 6 also summarizes trials with extracts *O. europaea* plant parts in order to prolong shelf-life and quality of meat products. Regarding the microbial status of fresh beef only Gok and Bor (2012) found an influence of their olive leaf extract on product microbiology. In comparison to untreated references the applied OLE concentrations of 0.5 and 1.0 g/kg delayed microbial growth about 1.95 and 2.27 log cfu/g, respectively, in a 10 day storage test at 7 °C. They furthermore stated improvements in all sensory attributes tested. Aytul et al. (2004) and Hayes et al. (2010) on the contrary reported no growth inhibitory potential for their olive leaf extracts in fresh beef preparations. Nonetheless all extracts had notably beneficial effects on lipid oxidation (Aytul et al., 2004, Hayes et al., 2010, Gok and Bor, 2012). Positive effects on color stability in terms of redness (a*-values) were also reported (Hayes et al., 2010, Gok and Bor, 2012). Another study addressing the quality of beef patties spiked with 3 $\%_{w/w}$ of an olive fruit extract (commercial, HIDROX®, CreAgri Inc., Hayward, USA) was performed by Rounds et al. (2013) who investigated its beneficial effects in low temperature cooking processes (45 °C). This cooking temperature was insufficient to inactivate preliminarily added *E. coli* (10^7 cfu/g). But the addition of 3 % olive pulp extract lead to a complete inactivation of the pathogen in combination with the heating step. The

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extract was furthermore able to significantly reduce the heat-induced formation of cancerogenic heterocyclic amines about 50 % (Rounds et al., 2013).

None of these studies with meat investigated the interaction of the phenolic compounds contained in the applied extracts with the protein in the products. This is especially necessary, as Juven and Henis (1970), Medina, Brenes, et al. (2009) and Medina et al. (2010) have found nitrogenous compounds to reduce the antimicrobial activity of oleuropein and its derivatives. Preserving effects of antimicrobial *O. europaea* L. extracts might therefore be diminished on foods with high protein contents.

Further application studies on protein rich products were performed by Tassou et al. (1996) and Chaves-Lopez et al. (2015) as shown in Table 6. A storage trial with fish fillets marinated with a mixture of virgin olive oil, lemon and oregano, under aerobic or MAP conditions at 0 °C revealed bacteriostatic and bactericidal effects on the artificially inoculated bacteria *S. aureus* and *Salm. enteritidis* as well as the natural microbiota (Tassou et al., 1996). Although this study investigated practical storage conditions and showed the principal possibility to prolong shelf-life with olive based antimicrobial compositions, the unique preservative effect of the olive phenols themselves was not investigated separately. Chaves-Lopez et al. (2015) documented the possibility to reduce the growth of mycelia from household molds on fermented sausages by dipping them in diluted OMWW (5 %). Evaluation of the accompanied sensory changes revealed increasing color intensity and chewiness, maybe due to reduced proteolytic mold activity, as well as an increase in olfactory intensity and saltiness. But the authors concluded that the OMWW treatment did not exert any undesired sensory changes (Chaves-Lopez et al., 2015).

Although in small numbers to date, the reviewed studies on the applicability of extracts from *O. europaea* L. plant parts on food products highlight the basic suitability of *O. europaea* L. derived phenolic extracts to improve the quality of certain food products. Antimicrobial

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effects were reduced on protein rich products, possibly due to interactions of the phenolic compounds with proteins, but beneficial effect on lipid oxidation and color stability were reported. It is apparent that further studies on the dependencies between active phenolic compounds and food ingredients are necessary. On the other hand olive extract preparations were shown to be suitable antimicrobial additives for leafy green washing waters. But there is no information on the change of the products sensory profile of such products treated with phenol rich extracts from *O. europaea.*

As well as traditional preservative agents *O. europaea* extracts do not possess a comprehensive antimicrobial activity independent of microorganism or species. Hence a specific evaluation of the microbiota of the targeted food product might be recommended to screen the extracts activity and to evaluate its applicability on the specific product.

7. Conclusion and future aspects

As reviewed, the antimicrobial activity of *Olea europaea* L. has been extensively investigated over the last four decades. These scientific efforts mainly focused on the overall antimicrobial activity of crude extracts against a wide variety of bacteria and fungi. Oleuropein and ligstroside have been identified as antimicrobial precursors for smaller, more active antimicrobial compounds such as their aglycones, hydroxytyrosol, tyrosol and elenolic acid. Especially the dialdehydic form of elenolic acid and its derivatives oleacein and oleocanthal have proven to possess remarkable antibacterial activity (Medina, Brenes, et al., 2009). But as the search for the single most active compound is ongoing, the comprehensive investigation of synergistic activities among the constituents of complex *O. europaea* extracts is shortened. Knowledge which might become important regarding the design of defined antimicrobial extracts to be applied on certain food products with a distinct natural microbiota. Furthermore,

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studies do not sufficiently elucidate antimicrobial modes of action or the respective resistance mechanisms, leaving room for subsequent research.

Nonetheless *O. europaea* L. appears to be a reliable source of phenolic compounds which could serve as appropriate food additives. In the scope of their extraordinary bioactivity, the technologically controlled addition of hydroxytyrosol, tyrosol or elenolic acid derivatives to food may be considered favorable, as they possess strong health beneficial effects in contrast to traditional preservative agents.

Although activities, modes of action and food applicability need to be further investigated, the existing results indicate the potential use of defined *O. europaea* extracts or contained compounds like oleuropein, hydroxytyrosol, oleacein or oleacanthal as natural preservative compounds in food products. Consequently, research is necessary to investigate the behavior of phenolic *O. europaea* constituents in food matrices and to broaden the knowledge on possible sensorial alterations.

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FIGURE CAPTIONS

Figure 1 Chemical structures of phenolic antimicrobial compounds in *Olea europaea*.

Figure 2 Enzymatic hydrolysis of oleuropein and ligstrosid (Reprinted with permission from Charoenprasert and Mitchell (2012) Copyright American Chemical Society.

TABLE CAPTIONS

Table 1Concentrations (mg/kg) of antimicrobial molecules from enzymatic secoiridoid breakdown in *Olea europaea* L. products

Table 2 Comparative presentation of scientific studies on the antimicrobial activity of oleuropein, hydroxytyrosol and elenolic acid derivatives.

Table 3 Antimicrobial activity of extracts from olive fruits

Table 4 Antimicrobial activity of extracts or emulsions from virgin olive oil.

Table 5 Antimicrobial activity of extracts from olive leaves.

Table 6 Results on the applicability of extracts from Olea europaea L. on food products and its effect on product quality.

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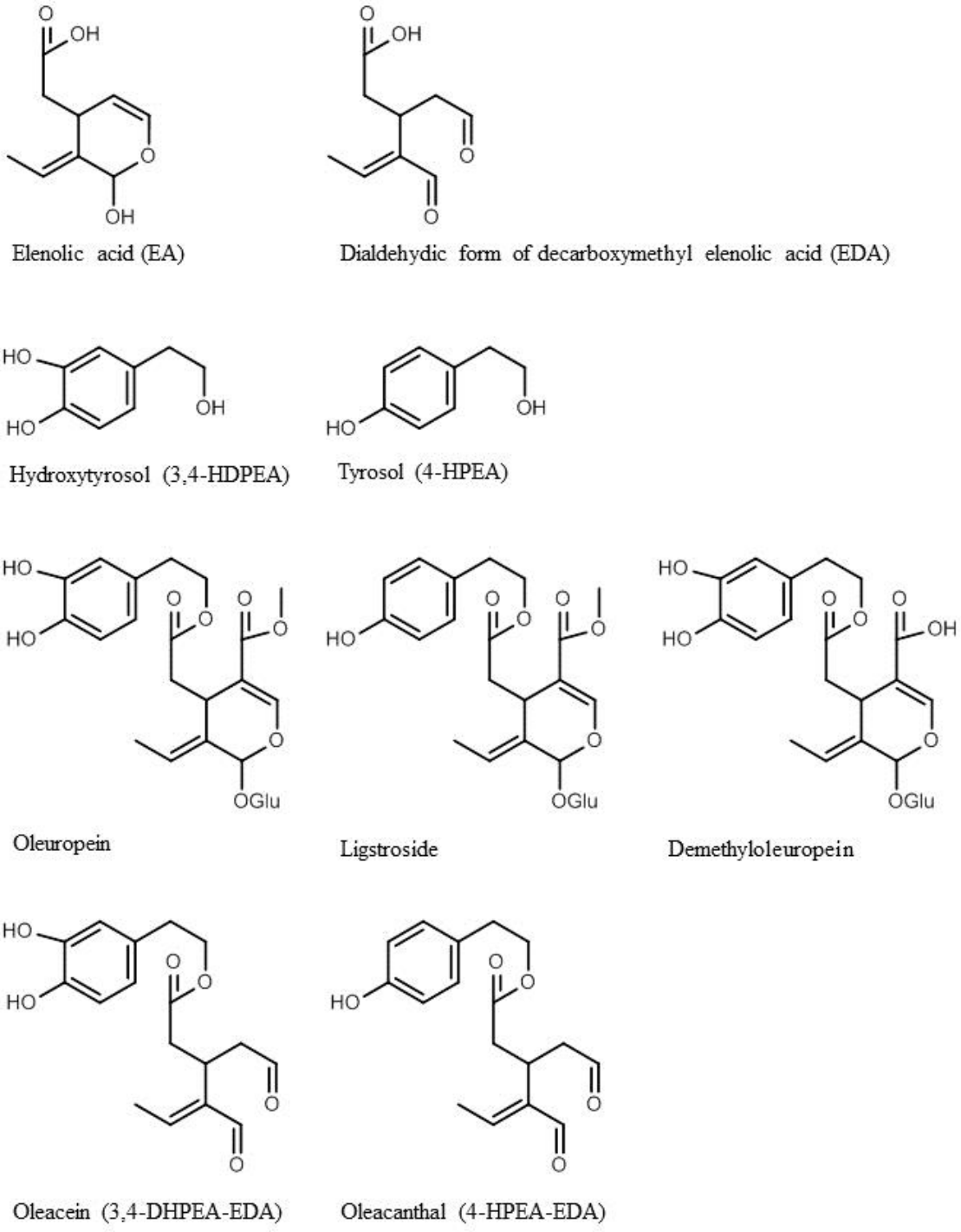

Figure 1

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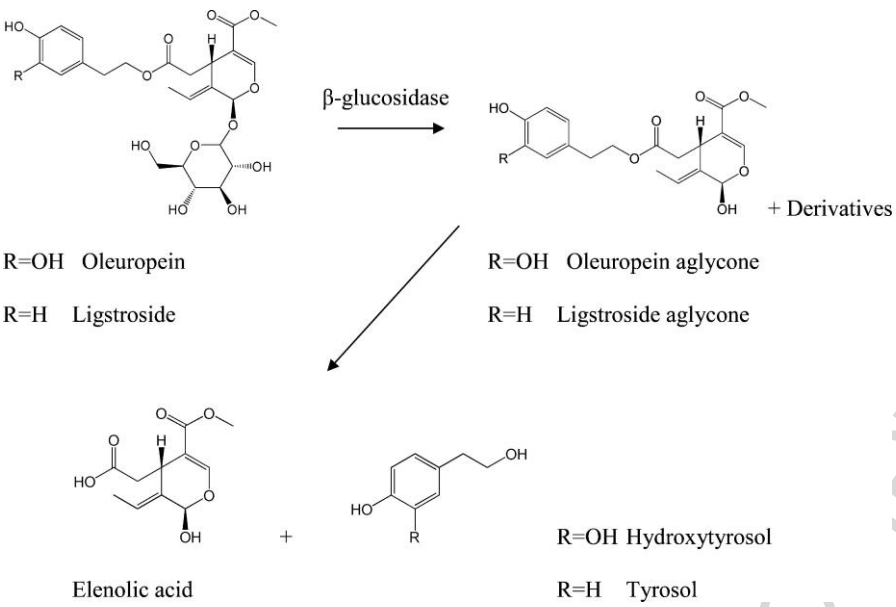

Figure 2

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Table 1 Concentrations (mg/kg) of antimicrobial molecules from enzymatic secoiridoid breakdown in *Olea europaea* L. products

	Concentration [mg/kg] in							
Phenolic compound	**Leaves**	**Reference**	**Fruits**	**Reference**	**Virgin oil**	**Reference**	**Mill waste**	**Reference**
Oleuropein	24540.0	Benavente-García et al. (2000)	1150.0	Bianchi (2003)	399.9	Medina et al. (2006)	1268.3	Servili et al. (1999)
	937.0	Ortega-Garcia and Peragon (2010)	21681.0	Vinha et al. (2005)	929.6	Romero-Segura et al. (2012)		
	539.0	Lalas et al. (2011)	13834.0	Jerman et al. (2010)				
	21653.0	Talhaoui et al. (2014)						
	35076.0	Talhaoui et al. (2015)						
Demethyloleuropein	1338.0	Talhaoui et al. (2014)	350.0	Bianchi (2003)			506.4	Servili et al. (1999)
	1230.0	Talhaoui et al. (2015)	1645.0	Pereira et al. (2006)				
			9334.0	Jerman et al. (2010)				
Oleuropein aglycone	170.0	Talhaoui et al. (2014)			85.4	Medina et al. (1999)		
	183.0	Talhaoui et al. (2015)						
Ligstroside	3251.0	Talhaoui et al. (2014)			281.0	Medina et al. (2006)	82.0	De Marco et al. (2007)
	599.0	Talhaoui et al. (2015)			624.2	Romero-Segura et al. (2012)		
Hydroxytyrosol	14600.0	Benavente-García et al. (2000)	350.0	Bianchi (2003)	0.87	Stefanoudaki et al. (1997)	121.4	Servili et al. (1999)
(3,4-DHPEA)	190.0	Ortega-Garcia and Peragon (2010)	1433.1	Romero et al. (2004)	14.7	Servili et al. (1999)	1433.4	Fki et al. (2005)
	200.0	Lalas et al. (2011)	27673.0	Vinha et al. (2005)	55.6	Medina et al. (1999)	1224.0	De Marco et al. (2007)
	826.0	Talhaoui et al. (2014)	3833.0	Pereira et al. (2006)	14.4	Owen et al. (2000)		
	1702.0	Talhaoui et al. (2015)			73.9	Medina et al. (2006)		
					2.54	Romero-Segura et al. (2012)		
					8.5	Mosele et al. (2014)		
Tyrosol	710.0	Benavente-García et al. (2000)	50.0	Bianchi (2003)	3.74	Stefanoudaki et al. (1997)	320.4	Servili et al. (1999)
(4-HPEA)	100.0	Ortega-Garcia and Peragon (2010)	192.7	Romero et al. (2004)	4.51	Servili et al. (1999)	851.0	Fki et al. (2005)
	858.0	Talhaoui et al. (2014)	139.1	Pereira et al. (2006)	85.8	Medina et al. (1999)	208.0	De Marco et al. (2007)
	461.0	Talhaoui et al. (2015)			22.1	Owen et al. (2000)		
					22.0	Medina et al. (2006)		
					3.03	Romero-Segura et al. (2012)		
					4.4	Mosele et al. (2014)		
Elenolic acid	3291.0	Talhaoui et al. (2014)						
	1013.0	Talhaoui et al. (2015)						
Oleacein					194.3	Romero-Segura et al. (2012)	18390.4	Servili et al. (1999)
(3,4-DHPEA-EDA)					269.3	Mosele et al. (2014)		
Oleocanthal					158.3	Romero-Segura et al. (2012)		
(4-HPEA-EDA)					11.4	Mosele et al. (2014)		

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Table 2 - Comparative presentation of scientific studies on the antimicrobial activity of oleuropein, hydroxytyrosol and elenolic acid derivatives.

Antimicrobial method	Microorganism	Gram type	Strain No. or Strain count	Antimicrobial activity	Oleuropein	Hydroxytyrosol	Dialdehydic elenolic acid	Dialdehydic elenolic acid + hydroxytyrosol	Dialdehydic elenolic acid + tyrosol	Reference
Broth microdilution assay,	*Haemophilus influenza*	-	ATCC 9006	MIC	500	0.97				Bisignano et al.
MIC, aerob,	*Moraxella catarrhalis*	-	ATCC 8176	(μg/ml)	500	1.92				(1999)
$N_0=10^5$ cfu/ml,	*Salmonella typhi*	-	ATCC 6539		125	3.94				
Mueller-Hinton broth,	*Vibrio parahaemolyticus*	-	ATCC 17802		62.5	0.24				
37 °C, 18 h	*Staphylococcus aureus*	+	ATCC 25923		62.5	7.85				
Broth microdilution assay,	*Mycoplasma hominis*		PG21	MIC	160					Furneri et al.
MIC, aerob,	*Mycoplasma fermentans*		PG18	(μg/ml)	20					(2002)
$N_0=10^3$-10^5 cfu/ml,	*Mycoplasma pneumoniae*		K7		20					
SP4 Mycoplsama broth,	*Mycoplasma pirum*		not given		320					
37 °C, 18 h										
Broth microdilution assay,	*Mycoplasma hominis*		20 strains	MIC		0.03-0.12				Furneri et al.,
MIC, aerob,	*Mycoplasma fermentans*		3 strains	(μg/ml)		0.25				(2004)
$N_0=10^3$-10^5 cfu/ml,	*Mycoplasma pneumoniae*		2 strains			0.5				
10-B or SP4 Mycoplsama broth,										
37 °C, 18 h										
Broth dilution assay,					at 3.13 μg/ml	at 0.8 μg/ml		at 0.16 μg/ml		Medina et al.
time kill assay,	*Enterobacter aerogenes*	-	CECT 684	logarithmic cell	n.I., no growth	>2.5		>3.5		(2007)
bacteria: $N_0=10^6$ cfu/ml,	*Escherichia coli*	-	CECT 434	count reduction	>0.3	>3.5		>3.0		
yeasts: $N_0=3.6*10^3$ cfu/ml,	*Enterococcus faecium*	+	CECT 410		n.I., no growth	n.I., no growth		>3.6		
diluted olive brine (pH 4),	*Enterococcus faealis*	+	CECT 481		n.I., no growth	>1.5		>4.0		
48 h, 32 °C	*Leuconostoc mesenteroides*	+	LM 51		n.I. growth	>0.1		>2.5		
	Lactobacillus pentosus	+	ATCC 8041		n.I. growth	n.I., growth		>4.0		
	Lactobacillus plantarum	+	ATCC 14917		n.I. growth	n.I., growth		>2.5		
	Saccharomyces cerevisiae		ATCC 9080		n.I. growth	n.I., growth		n.I., growth		
	Pichia membranaefaciens		CECT 10482		n.I. growth	n.I., growth		n.I., growth		
Broth dilution assay,	*Staphylococcus aureus*	+	CECT 239	MBC			0.30	0.057	0.024	Medina et al.
MBC>3 log,	*Enterococcus faecalis*	+	CECT 481	(μg/ml)			0.30	0.057	0.024	(2009)
$N_0=10^6$ cfu/ml,	*Escherichia coli*	-	CECT 434				0.18	0.284	0.152	
phosphate buffered saline,	*Pseudomonas fluorescence*	-	CECT 378				0.24	0.284	0.183	
5 min, 37 °C, pH 7										
Broth dilution assay, MBC	*Staphylococcus aureus*	+	not given	MBC		150				Friedman et al.
$N_0=10^2$ cfu/ml,				(μg/ml)						(2011)
phosphate buffered saline										
60 min, 37 °C, pH 7										
Broth microdilution assay,	*Streptococcus pyrogenes*	+	ATCC 19675	MIC		400				Tafesh et al.
MIC, $N_0<10^8$ cfu/ml,	*Staphylococcus aureus*	+	ATCC 25923	(μg/ml)		400				(2011)
Tryptone soy broth,	*Escherichia coli*	-	ATCC 25922			400				
37 °C, 18 h	*Klebsiella pneumoniae*	-	ATCC 700603			400				

n.I. – no inhibition/inactivation

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Table 2 - Comparative presentation of scientific studies on the antimicrobial activity of oleuropein, hydroxytyrosol and elenolic acid derivatives - continued.

Antimicrobial method	Microorganism	Gram type	Strain No. or Strain count	Antimicrobial activity	Oleuropein	Hydroxytyrosol	Dialdehydic elenolic acid	Dialdehydic elenolic acid + hydroxytyrosol	Dialdehydic elenolic acid + tyrosol	Reference
Broth microdilution assay,	*Candida albicans*		ATCC 10231	MFC		6.25				Zoric et al.
MFC<10 % recultivation,	*Candida albicans*		MFBF 11103	(mg/ml)		6.25				(2013)
$N_0=5*10^5$ cfu/ml	*Candida dubliniensis*		MFBF 10837			6.25				
RPMI 1640 broth,	*Candida parapsilosis*		ATCC 22019			1.56				
	Candida tropicalis		ATCC 750			6.25				
	Candida kefyr		ATCC 2512			1.56				
	Candida curvata		MFBF 10827			0.09				
	Blastoschizomyces capitatum		MFBF 10837			0.19				
	Saccharomyces cerevisiae		NCTY 87			6.25				
Broth microdilution assay,	*Staphylococcus aureus*	+	ATCC 51153	MIC				125		Bisignano et al.
unspecified,			ATCC 6538P	(μg/ml)				125		(2014)
CLSI-standard M100-S18,			ATCC 43300					250		
Mueller-Hinton broth	*Staphylococcus epidermis*	+	ATCC 49134					7.81		
			ATCC35984					62.5		
			ATCC 12228					15.6		
	Streptococcus pneumoniae	+	ATCC 6003					n.I.		
	Streptococcus pyrogenes	+	ATCC 19615					n.I.		
	Listeria monocytogenes	+	ATCC 7644					n.I.		
			ATCC 1392					n.I.		
	Enterococcus hirae	+	ATCC10541					n.I.		
	Moraxella catarrhalis	-	ATCC 8176					n.I.		
Broth microdilution assay,	*Erwinia carotovora*	-	CECT 225	MIC		1000				Medina-
ISO Sensitest broth,	*Klebsiella pneumoniae*	-	CECT 143	(μg/ml)		1000				Martínez et al.
undefined,	*Pseudomonas aeruginosa*	-	CECT 110			n.I.				(2016)
24 H, 30-37 °C,	*Escherichia coli*	-	CECT 4972			400				
inoculum size unspecified	*Yersinia enterolcolitica*	-	CECT 4315			n.I.				
	Salmonella typhimurium	-	NCTC 12013			n.I.				
	Aeromonas hydrophila	-	CECT 389			n.I.				
	Shigella sonnei	-	CECT 457			1000				
	Pediococcus acidilactici	+	CECT 98			1000				
	Kocuria rhizophila	+	CECT 4070			1000				
	Listeria monocytogenes	+	CECT 940			n.I.				
	Staphylococcus aureus	+	CECT 794			400				

n.I. - no inhibition/inactivation

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Table 3 - Antimicrobial activity of extracts from olive fruits

Plant material	Extract form	Prevalent phenolic compounds	Concentration [mg/kg]	Antimicrobial test method	Microorganisms	Strain number	Antimicrobial activity	References
Olive fruits	aqueous, undefined	not identified, content of active compounds described as units, authors assume oleuropein to be the active compound		paper disc ihibition zone, 0.0.5 ml/disc, MIC determination	*Lactobacillus plantarum*	FBB-12	1,5 units/ml	Fleming and Etchells (1967)
						FBB-15	no inhibition	
						FBB-68	1.5 units/ml	
						L-442	2.5 units/ml	
						WSO	2.5 units/ml	
					Pediococcus cerevisiae	FBB-39	1.5 units/ml	
						FBB-61	1.5 units/ml	
					Lactobacillus brevis	FB-50	2.5 units/ml	
						FBB-70	1.5 units/ml	
					Leuconostoc mesenteroides	FBB-42	1.0 units/ml	
						FBB-43	1.0 units/ml	
						FBB-71	1.0 units/ml	
						FBB7-3	1.0 units/ml	
Olive fruits	ethanol extraction, ethyl acetate extraction, evaporation, reconstituted in water	Oleuropein	not quantified	broth dilution, 90 min turbidity measurement, 580 nm	*Bacillus cereus* T	-	inhibition of spore germination at 4.0 % olive extract 1.0 % oleuropein	Tassou et al. (1991)
Olive fruits	aqueous, lyophilized, reconstituted in water, concentration 50 mg/ml	Luteolin, Luteolin 7-*O*-glucoside, Apigenin 7-*O*-glucoside	111.7 9.3 1.4	growth kinetic assay, 10^8 cfu/ml, nutrient broth, 24 h, 37 °C, turbidity measurement, 540 nm, IC_{50} calculation	*Bacillus cereus*	CECT 148	0.981 mg/ml	Sousa et al. (2006)
					Bacillus subtilis	CECT 498	2.445 mg/ml	
					Staphylococcus aureus	ESA 7	2.841 mg/ml	
					Escherichia coli	CECT 101	0.722 mg/ml	
					Klebsiella pneumoniae	ESA 8	0.813 mg/ml	
Olive fruits	aqueous, lyophilized, reconstituted in water, concentration 100 mg/ml	Hydroxytyrosol, Tyrosol, Verbascoside, Luteolin	672.4-3833.0 139.1-217.1 0-475.8 9.6-249.9	agar diffusion assay, 10^7 cfu/plate, 50 µl extract, 24 h	*Bacillus cereus*	CECT 148	10-25 mg/ml	Pereira et al. (2006)
					Bacillus subtilis	CECT 498	50-100 mg/ml	
					Staphylococcus aureus	ESA 7	50-100 mg/ml	
					Escherichia coli	CECT 101	75-100 mg/ml	
					Klebsiella pneumoniae	ESA 8	50-100 mg/ml	
					Pseudomonas aeruginosa	CECT 108	100 mg/ml	
					Candida albicans	CECT 1394	100 mg/ml	
					Cryptococcus neoformans	ESA 3	100 mg/ml	

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Table 4 Antimicrobial activity of extracts or emulsions from virgin olive oil.

Raw material	Extract form	Prevalent phenolic compounds	Concentration [mg/kg]	Antimicrobial test method	Microorganisms	Strain number	Antimicrobial activity	References
Virgin olive oil	aqueous emulsion (1:1), containing NaCL (0.85 %) and Tween 20 (0.25 %)	tyrosol hydroxytyrsol oleuropein aglycone	5.5 – 22.8 7.4 – 72.7 15.74 – 539.9	macro dilution assay, inactivation kinetic, 10^2-10^5 cfu/ml, 1 h, 32 °C,	*Enterococcus faecalis* *Enterococcus faecium* *Streptococcus mutans* *Listeria monocytogenes* *Lactobacillus acidophilus* *Bifidobacterium bifidum* *Staphylococcus aureus* *Salmonella enterica* *Escherichia coli* *Candida albicans* *Clostridium perfringens*	CECT 481 CECT 410 CECT 479 CECT 4031 CECT 903 CECT 870 CECT 86 CECT 4300 CECT 434 CECT 1472 CECT 376	>4.94 log >4.84 log >4.79 log >4.82 log >4.59 log >4.95 log >4.60 log >5.11 log >1.76 log <0.01 log >5.38 log	Medina et al. (2006)
Virgin olive oil	aqueous extract, 1 g in 1 ml saline (0.85 % NaCl), 1 min, room temperature, centrifuged, aqueous phase isolated	dialdehydic form of elenolic acid hydroxytyrosol tyrosol oleuropein aglycone	118.4 36.9 15.2 52.3	macro dilution assay, inactivation kinetic, 10^6 cfu/ml, 5 min	*Salmonella enterica*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*	CECT 4300 CECT 4156 CECT4031 CECT 4032 CECT 86 CECT 239 CECT 4267 CECT 5947	>4.5 log >4.5 log >6.0 log >4.0 log	Medina et al. (2007)
Virgin olive oil	aqueos extract, 1 g oil in 1 ml PBS 5 min, room temperature, aqueous phase isolated	hydroxytyrosol tyrosol oleuropein aglycon	24.5 – 73.5 7.2 – 22.0 32.16 – 235.6	macro dilution assay, inactivation kinetic, 10^5 cfu/ml, 5 min, 32 °C,	*Helicobacter pylori*	LMG 19449 LMG 18041 LMG 8775	>1.5 log >1.3 log >4.5 log	Romero et al. (2007)
Virgin olive oil	aqueous emulsion, 10 % oil, 90 % PBST with Tween 20	not identified		macro dilution assay, inactivation kinetic, $5*10^6$ cfu/ml, 5 min, 37 °C,	*Escherichia coli* *Salmonella enteritidis* *Listeria monocytoenes*	NCTC 12900 NCTC 1994 NCTC 12694	5.99 log 6.71 log no survivors	Karaosmanoglu et al. (2010)

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Table 5 Antimicrobial activity of extracts from olive leaves.

Plant material	Extract form	Prevalent phenolic compounds	Concentration [mg/kg]	Antimicrobial test method	Microorganisms	Strain number	Antimicrobial activity	References
Olive leaves	aqueous extract from ground leaves, 20 $\%_{w/v}$, 121 °C, 20 min, sieved, lyophilized	not identified		macro dilution assay, 0,6 %w/v leaf extract, 10^5-10^6 cfu/mL, 1-4 h, 37 °C,	*Escherichia coli* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Bacillus subtilis* *Klebsiella pneumoniae* *Candida albicans*	clinical isolates	>5 log, 3 h >5 log, 1 h >5 log, 2 h, >2.5 log, 4 h >5 log, 1 h >5 log, 24 h (15 $\%_{w/v}$)	Markin et al. (2003)
Olive leaves	aqueous extract from ground leaves, 2 $\%_{w/v,}$ 100 °C, 45 min, filtered, lyophilized	Oleuropein Verbascoside Luteolin-7-O-glucoside Apigenin 7-O-glucoside Luteolin 4'-O-glucoside	26471.4 966.1 4208.9 2333.1 1355.9	growth kinetic, IC_{25} calculation, 0.05-5 mg/mL, 10^7 cfu/mL, 24 h, 37 °C, turbidity at: 540 nm	*Bacillus cereus* *Bacillus subtilis* *Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Candida albicans* *Cryptococcus neoformans*	CECT 148 CECT 498 ESA 7 CECT 101 CECT 108 ESA 8 CECT 1394 ESA 3	0.63 mg/ml 4.12 mg/ml 2.68 mg/ml 1.81 mg/ml 3.22 mg/ml 3.13 mg/ml 0.85 mg/ml 3.00 mg/ml	Pereira et al. (2007)
Olive leaves	commercial, undefined	Oleuropein	4400.0	subcultures from broth micro dilution assays, MBC determination, 0.002-50 $\%_{v/v}$ extract concentration, 1-4 d, 35 °C	*Bacillus cereus (1 strain)* *Bacillus subtilis (1)* *Campylobacter jejuni (10)* *Candida albicans (2)* *Candida glabrata (2)* *Candida parapsilosis (2)* *Enterococcus faecalis (6)* *Escherichia coli (4)* *Helicobacter pylori (4)* *Klebsiella pneumoniae (3)* *Kocuria rhizophila (1)* *Lactobacillus acidophilus (1)* *Lactobacillus casei (3)* *Lactobacillus spp. (13)* *Listeria innocua (1)* *Listeria monocytogenes (8)* *Micrococcus luteus (1)* *Pseudomonas aeruginosa (4)* *Salmonella enterica (1)* *Serratia marcescens (3)* *MSSA (12)* *MRSA (17)* *Staphylococcus capitis (2)* *Staphylococcus epidermidis (4)* *Staphylococcus hominis (2)* *Staphylococcus xylosus (2)* *Streptococcus pyogenes (10)*	clinical isolates	12.5 $\%_{v/v}$ 50 $\%_{v/v}$ <0.8 >50 $\%_{v/v}$ 50 $\%_{v/v}$ 50 $\%_{v/v}$ 50 $\%_{v/v}$ 25-50 $\%_{v/v}$ <0.8 50 $\%_{v/v}$ 50 $\%_{v/v}$ 12.5 $\%_{v/v}$ 12.5 $\%_{v/v}$ 25 $\%_{v/v}$ 12.5 $\%_{v/v}$ 25-50 $\%_{v/v}$ 25 $\%_{v/v}$ 50 $\%_{v/v}$ 25 $\%_{v/v}$ 25-50 $\%_{v/v}$ 0.8-6.2 $\%_{v/v}$ 0.8-12.5 $\%_{v/v}$ 3.1 $\%_{v/v}$ 1.6-3.1 $\%_{v/v}$ 6.2 $\%_{v/v}$ 6.2-25 $\%_{v/v}$ 6.2-50 $\%_{v/v}$	Sudjana et al. (2009)

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Table5 Antimicrobial activity of extracts from olive leaves – continued.

Plant material	Extract form	Prevalent phenolic compounds	Concentration [mg/kg]	Antimicrobial test method	Microorganisms	Strain number	Antimicrobial activity	References
Olive leaves	acetone based extract from ground leaves, 40 $\%_{w/v}$, 4 h, Soxhlet	Oleuropein	6.053	MIC determination, broth microdilution assay, 0.01-2 mg/mL, $1.6*10^7$ cfu/mL, 48 h	*Bacillus cereus*	UUVF-AB12	68 µg/ml	Korukluoglu et al. (2010)
					Enterococcus faecalis	UUMF-EF01	90 µg/ml	
					Staphylococcus aureus	UUMF-SA11	55 µg/ml	
					Lactobacillus plantarum	UUFE-048	30 µg/ml	
					Lactobacillus brevis	UUFE-023	30 µg/ml	
					Lactobacillus bulgaricus	UUFE-Y09	35 µg/ml	
					Streptococcus thermophiles	UUFE-K01	37 µg/ml	
					Pediococcus cerevisiae	UUFE-P56	26 µg/ml	
					Leuconostoc mesenteroides	UUFE-009	35 µg/ml	
					Salmonella typhimurium	UUMF-ST07	110 µg/ml	
					Salmonella enteritidis	UUMF-SE02	170 µg/ml	
					Escherichia coli	UUMF-EC12	60 µg/ml	
					Pseudomonas aeruginosa	UUMF-PA03	32 µg/ml	
					Klebsiella pneumonia	UUMF-KP16	25 µg/ml	
Olive leaves	aqueos extract from ground leaves, 20 $\%_{w/v,}$ 4 h, Soxhlet	not identified		Paper disc diffusion assay, 1.0 mg/disc, yeast: 10^4 cfu/plate, 72 h, 25 °C, bacteria: 10^6 cfu/plate, 24 h, 30 °C	*Staphylococcus aureus*	ATCC 6538P	10 mm	Keskin et al. (2012)
					MRSA	ATCC 43300	12 mm	
					MRSA	MU 40	15 mm	
					Micrococcus luteus	NRRL B-4375	9 mm	
					Streptococcus faecalis	ATCC4083	11 mm	
					Bacillus subtilis	ATCC 6633	12 mm	
					Bacillus cereus	CM 99	-	
					Escherichia coli	ATCC 29998	9 mm	
						ATCC 35218	9 mm	
					Enterobacter aerogenes	ATCC 13048	-	
					Enterobacter cloacae	ATCC 13047	-	
					Solmonella typhimurium	CCM 3819	9 mm	
					Pseudomonas aeruinosa	ATCC 27853	-	
					Pseudomonas fluorescens	ATCC 12843	11 mm	
					Klebsiella pneumoniae	CCM2318	11 mm	
					Candida albicans	ATCC 10259	-	
					Candida tropicalis	ATCC 750	-	

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Table 6 Results on the applicability of extracts from *Olea europaea* L. on food products and its effect on product quality.

Raw material	Extract form	Prevalent phenolic compounds	Concentration [mg/kg]	Food product	Test setup	Tested parameters	Results	Reference
Virgin olive oil	pure	Tyrosol Oleuropein	not quantified	Mayonnaise	Self-made mayonnaise, inoculated: $5*10^4$ cfu/g, *Salm. enteritidis*, was stored at 20 °C for 72 h.	*Salmonella enteritidi*	*Salm. enteritidis* was unable to grow in all mayonnaise recipes, but was significantly inactivated in mayonnaise containing virgin olive oil: >2.5 log with virgin olive oil, 0.4 log with sunflower oil within 48 h.	Radford et al. (1991).
Virgin olive oil	pure	Hydroxytyrosol Tyrosol Oleuropein aglycone Dialdehydic form of elenolic acid	52.8 17.0 210.9 179.1	Mayonnaise	Milk or egg (150 ml) based mayonnaise was produced with 300 ml sunflower oil or virgin olive oil with (pH 4.5-5.4) or without 11 ml lemon juice (pH 7.2-7.8) and inoculated: $2*10^3$ cfu/g, *Salm. enteritidis*, *L. monocytogenes.*	*Slam. enteritidis*, *Listeria monocytogenes*	Virgin olive oil caused full inactivation of the initial *Listeria* and *Salmonella* contamination (>3.5 log) within 30 min, but only in combination with lemon juice. Virgin olive oil alone did not reduce Salmonella cell count. Sunflower oil did not cause *Listeria* or *Salmonella* inactivation independent of lemon juice addition or time.	Medina et al., (2007)
Virgin olive oil	pure	Hydroxytyrosol Tyrosol Oleuropein aglycone Dialdehydic form of elenolic acid	52.8 17.0 210.9 179.1	Lettuce	Lettuce inoculated with *L. monocytogenes* $2*10^3$ cfu/g was mixed for 30 min with a dressing made of 27.5 ml olive oil or sunflower oil, 25 ml water (0.1 %NaCl), 2.5 ml lemon juice or vinegar.	*L. monocytogenes*	Virgin olive oil (alone or in combination with vinegar or lemon juice) caused full inactivation of the initial *Listeria* contamination (>3.5 log) in 30 min. Sunflower oil did not cause *Listeria* inactivation. Lemon juice or vinegar had no significant activity (approx. 0.5 log).	Medina et al., (2007)
Olive fruits	commercial, undefined, powder	Hydroxytyrosol	60000 - 84000	Lettuce	10 g samples of lettuce, and spinach were stained with *Salm. enterica* (10^5 cfu/g). Then dried for 30 min and immersed in olive extract solutions (1, 3, 5 %) for 2 min. Treated samples and untreated (PBS) control samples were stored aerobically at 4 °C for 3 days.	Total viable count *Salmonella enterica*	5 % treatment reduced cfu/g at 3 d: 0.4-2.5 log on romaine lettuce initial log-reductions (0 d) on romaine lettuce: 1.43 (1 %), 2.3 (3 %), 1.7 (5 %) log N_0/N, final log-reduction (3 d) on romaine lettuce: 2.5 (1 %), 2.7 (3 %), 2.7 (5 %) log all effects were generally stronger on lettuce than on spinach	Moore et al., 2011
Virgin olive oil	pure + lemon juice + oregano	not identified		Marinated fresh fish fillets	Fillets were marinated in a mixture of 500 g virgin olive oil with 50 or100 g lemon juice and 1 or 2 g oregano at a pH of 4.4 or 3.9; Inoculated with *Salm. enteritidis* and *S. aureus*; 10^7 cfu/fillet, MAP: 40 % CO_2, 30 % O_2, 30 % N_2; stored at 0 °C for 30 d.	Enterobacteria Lactic acid bacteria Pseudomonads *Salm. enteritidis* *Staphylococcus aureus* *Shewanella putrefaciens* *Brochotrix thermospacta*	no difference from reference, 30 d reduced final count, 30 d lag-phase (due to MAP only), 30 d bactericidal effects, >1.0 log bactericidal effects, >3.4 log lag-phase; significantly reduced final counts; lag-phase; no final count reduction	Tassou et al., (1996)

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Table 6 Results on the applicability of extracts from *Olea europaea* L. on food products and its effect on product quality - continued.

Raw material	Extract form	Prevalent phenolic compounds	Concentration [mg/kg]	Food product	Test setup	Tested parameters	Results	References
Olive leaves	unspecified	Oleuropein	9400	Beef cubes	Beef cubes (1.5*1.5 cm) were immersed with water (reference) or solutions (1, 2, 3 %) of olive leaf extract, packed and stored for 9 d at 4 °C.	Moisture pH-level Color stability Lipid oxidation (TBARS) Total cell count Coliforms Lactic acid bacteria	no difference from reference, no difference from reference, significantly reduced, significantly reduced TBA values at day 9 (20-49 %), no significant difference from reference, no significant difference from reference, no significant difference from reference	Aytul et al. (2004)
Olive leaves	undefined	Oleuropein Verbascoside Luteolin-7-O-glucoside Apignenin 7-O-glucoside Tyrosol Hydroxytyrosol	1151.1 68.6 25.6 15.9 15.6 10.2	Ground beef patties	Freshly ground beef was spiked with 100 or 200 g/kg OLE. Patties were formed, packed aerobically or in MAP (80 % O2, 20 % CO2) and stored for 12 d at 4 °C.	Total viable count Lipid oxidation (TBARS) Color stability pH Water holding capacity Cooking loss Sensory attributes	no difference from reference, OLE reduced TBA values at day 12 (41-80 %), a*-values (red)increased: aerob:+22 %, MAP:+56%; no difference from reference, significantly increased: 6.5-12.4 %, insignificant increase: 4.8-5.4 %, all attributes indifferent from reference	Hayes et al. (2010)
Olive leaves	methanol, 0.05 g/ml, 2 h, 40 °C centrifuged, concentrated	not identified		Ground beef patties	Freshly minced beef was spiked with 500 or 1000 g/kg olive leaf extract (and other spices) and was stored aerobically at 4 °c for 10 days.	Total viable count Lactic acid bacteria Enterobacteria Pseudomonads Moisture Lipid oxidation (TBARS) Color stability Sensory attributes	day 10, reference 8.09, samples 6.14/ 5.82 log cfu/g day 10, reference 6.35, samples 5.15/4.90 log cfu/g day 10, reference 7.28, samples 6.32/6.07 log cfu/g day 10, reference 6.67, samples 5.15/4.90 log cfu/g no significantly increased moisture loss, significantly decreased: 40.8%-50.2%, 12 d a*-value increased: +23.5-31.5 %, 12 d reference scores at 0 at 10 d (8 at 0 d) samples with OLE at 5.5-6.6 at 10 d (8 at 0 d)	Gok and Bor (2012)
Olive fruits	commercial, undefined, powder	Hydroxytyrosol	60000 - 84000	Ground beef patties	Ground beef was spiked with 1 or 3 g/kg OLE and inoculated with E. coli (10^7 cfu/g). Uniform patties were formed, cooked and shock cooled.	Total viable count Amine quantification	OLE enhanced cell count reduction, at 3 % no survivors detectable, reduction of amine formation: up to 50.6 %	Rounds et al. (2013)
Olive mill waste waters	concentrate, enzymatic treatment	Hydroxytyrosol, Tyrosol Verbascoside Oleacein	100.23 21.93 135.20 500.43	Fermented sausages	During the drying process fermented sausages were dipped in olive mill waste extract solutions (2.5 %, 5 %) for 1 min at 20 °C and were continued drying.	Total viable count Lactic acid bacteria Micrococci Yeasts Molds pH Water activity Lipid oxidation (TBARS) Volatile compounds Sensory attributes	no difference from reference no difference from reference growth reduction affected volatile compound profile no significant difference from reference reduction of unwanted species and wanted species no significant difference from reference no significant difference from reference reduced values 12 % (2.5 %OLE), 38 % (5 % OLE) volatile compounds from microbial esterification and from lipid oxidation reduced redness increased significantly, chewiness slightly	Chaves-Lopez et al. (2015)