Effect of antimicrobial compounds from olive products on microorganisms related to health, food and agriculture

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Antimicrobial activity has recently been demonstrated in olive products. The anti-bacterial effect was correlated with the presence of olive glutaraldehyde-like compounds such as the dialdehydic form of decarboxymethyl elenolic acid either free (EDA), linked to tyrosol (TyEDA) or to hydroxytyrosol (HyEDA). Hence, the bactericidal activity of these substances is due to their dialdehydic structure, which is similar to those of the commercial antiseptics glutaraldehyde and *o*-phthalaldehyde. Antimicrobial studies have been carried out in several fields: human health, food processing and agricultural pest control. The antimicrobial activity of olive oil was tested against *Helicobacter pylori* as well as against several foodborne pathogens. It has also been found that some salt-free solutions from table olive and olive oil production processes are rich in these antimicrobial compounds, and they can exert bactericidal and antifungal activity against phytopathogenic microorganisms.

Keywords olive; phenolic; antibacterial; antifungal.

1. Introduction

The olive tree (*Olea europaea* L.) has been cultivated from ancient times in the Mediterranean area to produce table olives, olive oil and olive leaf extracts. Olive products have been employed during centuries as food, natural preservatives and in folk medicine. Moreover, during the 19th century, extracts of boiled leaves were used as a remedy in malaria patients. Nowadays, olive leaf extracts are commercialized to treat several diseases, many of them caused by microorganisms.

The presence of antimicrobial compounds in olive fruits has been suspected since the beginning of the table olive fermentation [1]. Lactic acid fermentation occasionally fails in table olive processing [2], which has been attributed to the presence of antimicrobial substances in olives [3]. The traditional treatment of olives with a NaOH solution (2–3% w/v) during the processing of Spanish-style green olives gives rise, among other consequences, to abundant growth of lactic acid bacteria (LAB), which is not observed if the concentration of the alkali is low (<1.8%) [4]. By contrast, olives put directly into brines do not permit the growth of LAB, in particular those of the Manzanilla variety [5].

Oleuropein was initially identified as the inhibitory compound of lactic acid bacteria present in the fermentation of the Spanish-style green olives [3]. It was subsequently understood that the products of oleuropein hydrolysis such as the aglycon of oleuropein and the elenolic acid were more inhibitory than their precursor [6, 7]. Although, the oleuropein hydrolysis products have not been detected in cover brine solutions, the inhibitory effect of oleuropein derivatives was confirmed *in vitro* using olive fruit extracts [8]. Additionally, a component of the oleuropein moiety, hydroxytyrosol, is the main polyphenol isolated from olive brine solutions [9, 10]. In contrast to previous results, hydroxytyrosol showed bactericidal activity against LAB. However, this polyphenol is found in brines of olives both treated [11] and non-treated with NaOH [12].

Studies on substances responsible for antimicrobial effect of olive oil are scarce [13, 14], and researchers analyzed only the minor polyphenols of the oil (simple phenols), but not the secoiridoid aglycons of oleuropein and ligustroside [15] and the lignans [16]. Likewise, there are numerous papers describing the antimicrobial activity of oleuropein [17], the main phenolic compound in olive fruits found in very low amounts in olive oil [18]. Furthermore, fatty acids and monoglycerides present in olives have been found to have a broad spectrum of microbicidal activity against bacteria and yeasts [19]. In particular, the α, β-unsaturated aldehydes from olives and olive oil flavor have been demonstrated to possess a noticeable activity against pathogens of the human intestinal and respiratory tracts [20-23]. Even though olive oil has been used for centuries as a food preservative and in folk medicine, the components of the oil responsible for this bioactivity remained unrevealed.

Antimicrobial activity has been reported in olive oil mill wastewaters [24-26] and olive leaf extracts [27, 28] as well. Their bioactivities have also been related to the phenolic compounds, oleuropein and hydroxytyrosol among others. The antimicrobial activity against foodborne pathogens such as *Salmonella enteritidis* and *Staphylococcus aureus* has been demonstrated *in vitro* when high concentrations of oleuropein were used [29,30]. Moreover, oleuropein and hydroxytyrosol exerted antimicrobial effects against pathogenic bacteria and viruses [31, 32].

2. Glutaraldehyde-like compounds in table olives and olive oil

Recently, it has been demonstrated that the main antimicrobial compounds in table olive brine solutions are the dialdehydic form of decarboxymethyl elenolic acid (EDA), EDA linked to hydroxytyrosol (HyEDA) and an isomer of oleoside 11-methyl ester (Figure 1); whereas the formerly identified inhibitors (oleuropein, its aglycon, hydroxytyrosol, and elenolic acid) were ineffective or not found in olive brines [33]. Figure 2 shows the antimicrobial activity exerted by olive derived compounds against *Lactobacillus pentosus*. Both EDA and HyEDA can explain most of the antimicrobial activity found in olive. Medina et al. stored olives of different varieties in an acidified brine under aseptic conditions for 2 months and observed a relationship between growth inhibition of *Lactobacillus pentosus* and the presence of HyEDA and EDA in the brines [34]. This could explain for the first time the differences in LAB growth reported by many researchers when brining different olive varieties [5, 35, 36]. The growth of LAB in the brines of olives non-treated with NaOH depends, among other variables, on the content in these solutions of the antimicrobial compounds HyEDA, EDA and oleoside 11-methyl ester, the concentration of them being variety dependent. Likewise, these substances are not present in raw olives but are formed during brining because of the cleavage of the glycosidic bond of oleuropein by ß-glucosidases.

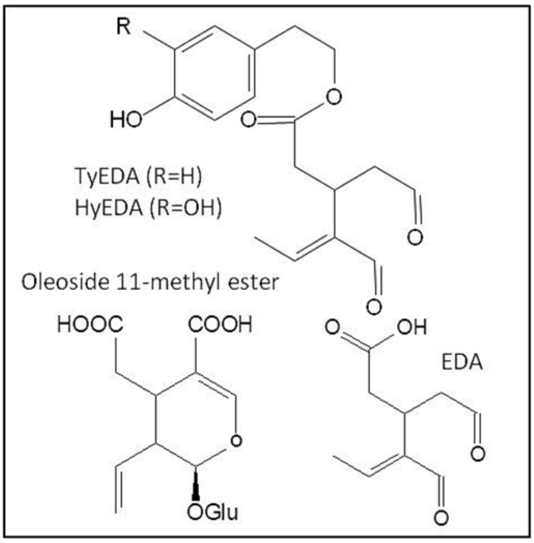

Fig. 1 Structures of the main antimicrobial compounds in olive oil and table olive. Dialdehydic form of decarboxymethyl elenolic acid either free (EDA), linked to tyrosol (TyEDA) or to hydroxytyrosol (HyEDA).

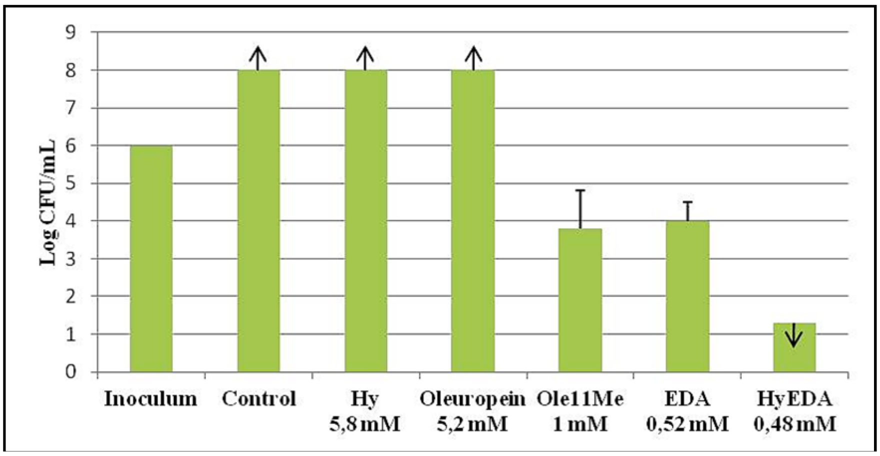

Fig. 2 Antimicrobial activity of different compounds against *Lactobacillus pentosus* ATCC 8041. Bars means standard error. Hydroxytyrosol (Hy), Oleoside 11-methyl ester (Ole11Me), Dialdehydic form of decarboxymethyl elenolic acid (EDA) and linked to hydroxytyrosol (HyEDA).

Studies were carried out to analyze the evolution of these antimicrobial compounds in an industrial preservation of natural olives, without NaOH treatment [37]. A notable concentration of HyEDA and EDA was found at the beginning of preservation, which was high enough to inhibit LAB growth. However, acidic degradation of these antimicrobials occurred after 3 months, particularly in the summer, and it allowed for the growth of lactobacilli and the formation of lactic acid. The results explained the microbial fermentation in acidified solutions of olives which are not treated with NaOH at the industrial scale. These olive solutions, which represent a significant environmental problem, have a very high concentration of HyEDA and EDA that make them ideal as a source of natural antimicrobial compounds.

Frequently, a delay or lack of lactic acid fermentation occurs during the processing of Spanish-style green olives, in particular of the Manzanilla variety, especially when an insufficient NaOH treatment is applied [4]. Many variables such as salt concentration [38], temperature [39], nutrient content [40], inhibitors [41] and others can play a role in the failure of a lactic acid fermentation. The presence of antimicrobials from Spanish-style green olives has been confirmed at a pilot plant scale [42] and could well explain such phenomenon. It has been demonstrated that an alkaline treatment with a low NaOH strength and insufficient alkali penetration allowed the presence of EDA and HyEDA in brines, which inhibited the growth of *L. pentosus*. By contrast, a more intense alkaline treatment gave rise to an abundant growth of the microorganism without any antimicrobials detected in the brine solutions. This finding will be useful for processors to better understand the table olive fermentation of Spanish-style green olives processing.

The bactericidal activity of many different types of virgin olive oils against several microorganisms has been studied *in vitro* [43]. Such study revealed that olive oil exerted a strong bactericidal action against a broad spectrum of microorganisms, this effect being in general higher against Gram-positive bacteria as compared to Gram-negative bacteria. Thus, olive oils showed bactericidal activity not only against potential harmful bacteria of the intestinal microbiota (*Clostridium perfringens* and *Escherichia coli*), but also against beneficial microorganisms such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Otherwise, most of the foodborne pathogens tested (*Listeria monocytogenes*, S*taphylococcus aureus*, *Salmonella enteritidis* sv. Enterica, *Yersinia* sp., and *Shigella sonnei*) did not survive after 1 h of contact with olive oils. This effect was not observed for other edible vegetable oils (corn, sunflower, soybean, rapeseed and cotton). The antimicrobial activity was higher in virgin olive oils, followed by olive oils and pomace olive oils, which is in accordance with their decreasing content in phenolic compounds. All olive oil polyphenols were isolated by HPLC and tested against *L. monocytogenes* and *Helicobacter pylori* [43, 44]. The most bactericidal polyphenols of olive oil were HyEDA and TyEDA, in particular the latter compound which is also known as oleocanthal [45]. No significant effect was observed with hydroxytyrosol, tyrosol, and the oleuropein and ligustroside aglycons. These findings opened up the possibility of using olive oil as a food preservative to prevent the growth of foodborne pathogens or to delay the onset of food spoilage.

The bactericidal effects of TyEDA, HyEDA and EDA, other olive compounds (nonenal, oleuropein, tyrosol), other food phenolic compounds (catechin, epicatechin, eugenol, thymol, carvacrol, and carnosic acid), and commercial disinfectants (glutaraldehyde [GTA] and ortho-phthalaldehyde [OPA]) have been tested against strains of *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli* [46]. Results demonstrated that the bactericidal activities of olive compounds (EDA, HyEDA, and TyEDA) were greater than those exerted by several food phenolic substances. Surprisingly, these olive antimicrobials were as active as the synthetic biocides GTA and OPA against the four bacteria studied. Thus, it has been proposed that the bactericidal activity of the main olive antimicrobials is primarily due to their dialdehydic structure, which is similar to that of the commercial biocides GTA and OPA. Additionally, the presence of phenolic compounds (tyrosol and hydroxytyrosol) in the basic structure (EDA) of the olive antimicrobials increases their lipophilic character and antibacterial efficacy, which was even greater than that of synthetic disinfectants (GTA and OPA) when working under conditions of high organic load.

3. Implication in human health

The antimicrobial action of olive oil compounds has been studied in more detail against *Helicobacter pylori.* This microorganism is responsible for most peptic ulcers and gastric cancers [47]. Some studies suggested that the consumption of olive oil instead of animal fats in the diet reduce the gastric ulcer in patients [48], and other authors related the olive oil consumption with the gastric juice secretion [49]. A digestion of olive oil was simulated and it was confirmed that olive oil polyphenols, in particular HyEDA and TyEDA, diffused to the aqueous phase in the first 5 minutes and remained stable during hours in acid conditions [44]. Also, they exerted *in vitro* a strong bactericidal activity against eight strains of *H. pylori*, three of them resistant to some antibiotics. Figure 3 shows the antimicrobial activity of phenolic compounds against *H. pylori*. HyEDA and TyEDA showed the strongest bactericidal effect at a concentration as low as 1.3 μg/mL, much lower than those found for phenolic compounds from tea, wine, and plant extracts.

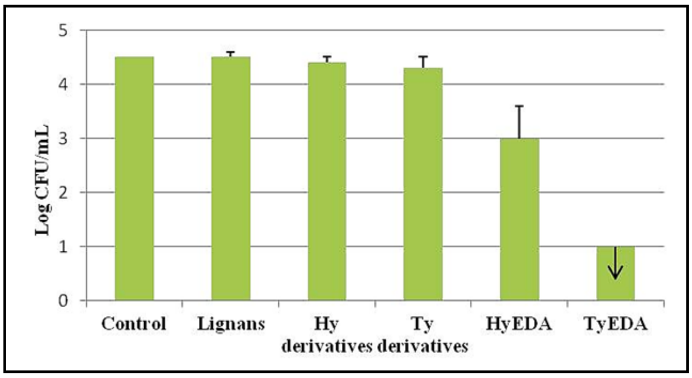

Fig. 3 Antimicrobial activity of phenolic compound against *H. pylori* after 60 min of contact. Lignans include 1-acetoxipinoresinol and pinoresinol. Hy derivatives include hydroxytyrosol acetate, hydroxytyrosol-glicol, hydroxytyrosol and oleuropein aglycon. Ty derivatives include tyrosol and ligustroside aglycon. HyEDA: Dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol. TyEDA: Dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol

The resistance of the *H. pylori* to antibiotic treatment is now found worldwide. These relevant findings open the possibility of considering virgin olive oil, a chemopreventive agent, for peptic ulcer or gastric cancer. Studies *in vivo* have recently been carried out in the Valme University Hospital [50] to confirm these *in vitro* data. Two clinical trials consisted in providing virgin olive oil to patients infected with *H. pylori* during several days. Before and after the treatment, urea breath test were carried out. In this study, the eradication rates were 11 to 23% after 1 month of intervention, but partial suppression was reached up to 19-43 % when the urea test was carried out immediately after 24-72 h from the last oil dose. Further studies are needed to confirm these findings, especially with longer periods, different administration conditions, and several types of olive oil alone or in combination with antibiotics.

4. Implication in food

Nowadays, there is still need for new methods to reduce or eliminate foodborne pathogens. Despite increased hygiene and advanced food production techniques, illness outbreaks as a consequence of consumption of contaminated foods are a serious problem in all countries. Biopreservatives from plants, natural food [51] and essential oils [52] are increasingly demanded.

It was the aim to compare the bactericidal effect of different foodstuffs against several pathogenic microorganisms such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enteritidis*, *E. coli*, *Shigella sonnei* and *Yersinia* sp [53]. Figure 4 shows that vinegar and aqueous extracts of virgin olive oil displayed the strongest bactericidal activity against all strains tested. Red and white wines also killed most strains after 5 min of contact; black and green tea extracts showed weak antimicrobial activity under these conditions, and no effect was observed for the remaining beverages (fruit juices, coke soft-drink, dairy products, coffee, and beer). The strong bactericidal activity of vinegar is well known [54] because of its high acetic acid content, but, surprisingly, the aqueous extracts of virgin olive oil exerted a similar effect to that of vinegar, which is a very new and interesting finding. This activity was related to the phenolic content of these extracts, in particular the synergistic action of TyEDA and HyEDA. At the same time, mayonnaises and salads were used as food models to confirm the results obtained on the bactericidal effect of olive oil. Virgin olive oil in mayonnaises and salads reduced the counts of inoculated *Salmonella* Enteritidis and *Listeria monocytogenes* by approximately 3 log CFU/g, whereas no effects were observed when sunflower oil was employed.

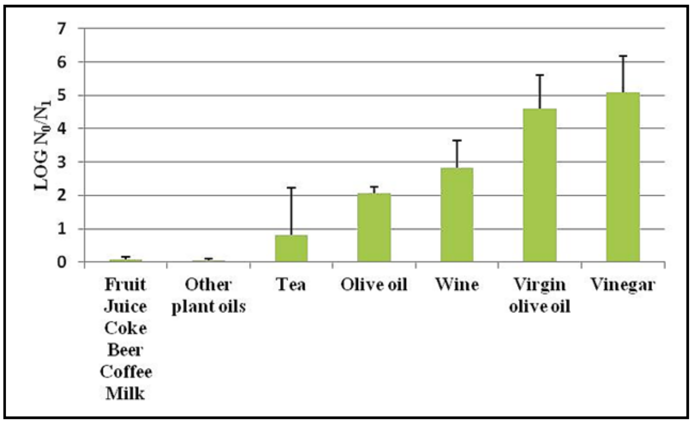

Fig. 4 Antimicrobial activity of olive oil and other foodstuffs against *S. aureus*, *L. monocytogenes*, *E. coli, S. sonnei* and *Yersinia* sp. N_0: initial inoculum $5{,}5x10^5$ – $1x10^6$ CFU/mL. N_1: CFU/mL after 5 min of treatment

Olive oil may be consumed directly on bread and in fresh salads, but it is also used in many homemade dishes (e.g., mayonnaise, cakes) and processed foods (e.g., canned tuna, fried tomato, meat foods). Hence, the bactericidal activity of olive oil polyphenols may be exerted directly on these foods, but it may also act as a hurdle component in certain contaminated meals during their ingestion.

5. Implication in agriculture

Phytopathogenic bacteria and particularly fungi can cause severe economically damaging plant diseases. Among bacteria, *Erwinia uredovora*, *Pseudomonas savastanoi*, and *Clavibacter michiganensis* are the most phytopathogenic species for plants cultivated in the Mediterranean basin. Likewise, the fungi *Alternaria*, *Botrytis*, *Pestaloptiopsis*, *Phytophthora* and *Colletotrichum* contribute to many diseases in strawberry, carrot, potato, grape, and others, and huge amounts of fungicides are used by farmers to fight against these pathogens [55-57]. At the same time, there is an increasing demand in agriculture for pesticides obtained from natural sources [58, 59]. Researchers have paid much attention in recent years to look for new bactericidal and fungicidal materials [60-62], many of them rich in phenolic compounds.

Aqueous solutions from table olives constitute a major environmental problem because of their high mineral and organic contamination, but they also contain compounds with antimicrobial properties that could be exploited for the control of plant diseases. Among them, the washing waters of alkali-treated green olives and the acidified preservation solutions of natural olives contain high amount of antimicrobial phenolic and oleosidic compounds, and they are also salt-free solutions [37, 42, 63]. The bactericidal and fungicidal activities of these solutions against several phytopathogenic micro-organisms have been investigated [64]. The storage solutions of natural olives showed noticeable bactericidal activity against species of *Erwinia*, *Clavibacter*, *Agrobacterium* and *Pseudomonas*, although the washing waters from Spanish-style green olives were less effective. In fact, natural olive storage solutions diluted to 20–30% were able to reduce the inoculated population of *Erwinia amylovora* and *Pseudomonas syringae* up to 6–7 logs. This bactericidal activity was correlated with the presence of olive glutaraldehyde-like compounds such as HyEDA and EDA in these solutions and both could explain most of the activity of the natural olive storage solutions. The minimal bactericidal concentration of these solutions could be considered high if compared with that observed for synthetic plant antibiotics, such as streptomycin and oxytetracycline [64]. Moreover, the incorporation of table olive liquids into the culture medium showed a potent antifungal activity against phytopathogenic fungi, in particular the storage solutions of natural olives. The mycelial growth of *Alternaria* spp. and *Pestalotiopsis cactorum* was almost completely inhibited by the olive storage solutions diluted up to 50%; although they were less effective against *Botrytis cinerea*, *Colletotrichum acutatum* and *Phytophthora dyospiri*. Figure 5 shows the inhibition exerted by black olive storage solutions against *B. cinerea*. In contrast, the washing waters of green olives showed low antifungal activity against all the fungi tested, except against *P. cactorum*, for which about 50% mycelia growth inhibition was obtained at 50% concentration of this olive solution.

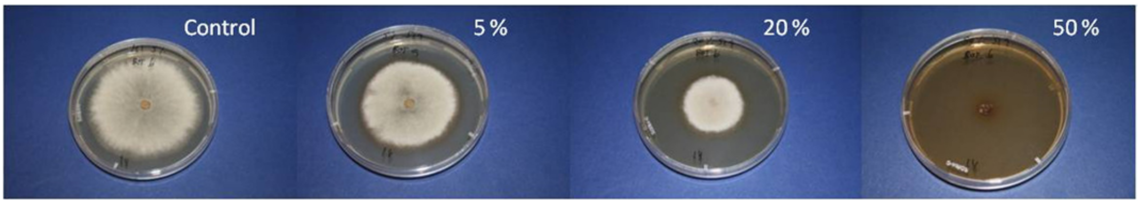

Fig. 5 Mycelial growth inhibition of *Botritis cinerea* by black olive storage solutions.

Likewise, the liquid phase originated after the processing of stored alpeorujo (OWSA) was characterized by its content in phenolic compounds and examined against phytopathogenic bacteria and fungi [65]. Studies *in vitro* indicated a clear bactericidal effect of these solutions against *Erwinia*, *Pseudomonas*, and *Clavibacter*. 10% OWSA reduced the initial population by ca. 1-3 log CFU/mL, being notable the effect with 20% for the three genera. Also, OWSA showed a dose-dependent antifungal activity. In fact, a complete inhibition of mycelium growth was only reached for *P. cactorum* with 50% OWSA in the culture medium. Less activity was detected against *C. acutatum*, *Alternaria* sp., *B. cinerea*, and *P. dyospiri*, but growth inhibition of 30-60% was obtained in most cases with 50% OWSA in the culture medium. It was found that the bactericidal effect was due to the joint action of low molecular mass phenolic compounds, but this activity cannot be assigned to a particular substance or to the polymeric fraction of this liquid. Hence, OWSA constitutes a promising natural solution to fight against plant phytopathogenic bacteria and fungi.

6. Conclusions

Both foods obtained from olives (olive oil and table olives) and byproducts from their processing (wet olive pomace or alpeorujo, olive preservation solutions, wash-waters, and others) constitute a remarkable source of substances which exhibit a great antimicrobial activity. The molecules responsible for this effect have been unveiled, and this opens a promising areas of research and applications which are now at their onsets. The dialdehydic form of decarboxymethyl elenolic acid (EDA), EDA linked to hydroxytyrosol, EDA linked to tyrosol, among other polyphenols present in olive products, can be successfully used to fight against different pathogens. Investigations have already been carried out in relation to human health (effect on *H. pylori*), food safety (effect on foodborne pathogens in vitro and in food models), and agricultural pest management (effect on bacterial and fungal plant pathogens). The fact that these compounds are naturally present in olives, opens the possibility to be also used in organic products and as biopesticides. Undoubtedly, more research is needed to disclose the best methods to obtain and apply the powerful tools that constitute the antimicrobial compounds provided by the olive fruits.

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