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Comparison of the Concentrations of Phenolic Compounds in Olive Oils and Other Plant Oils: Correlation with Antimicrobial Activity

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The antimicrobial activity of different edible vegetable oils was studied. In vitro results revealed that the oils from olive fruits had a strong bactericidal action against a broad spectrum of microorganisms, this effect being higher in general against Gram-positive than Gram-negative bacteria. Thus, olive oils showed bactericidal activity not only against harmful bacteria of the intestinal microbiota (*Clostridium perfringens* and *Escherichia coli*) also against beneficial microorganisms such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. Otherwise, most of the foodborne pathogens tested (*Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Yersinia* sp., and *Shigella sonnei*) did not survive after 1 h of contact with olive oils. The dialdehydic form of decarboxymethyl oleuropein and ligstroside aglycons, hydroxytyrosol and tyrosol, were the phenolic compounds that statistically correlated with bacterial survival. These findings were confirmed by testing each individual phenolic compound, isolated by HPLC, against *L. monocytogenes*. In particular, the dialdehydic form of decarboxymethyl ligstroside aglycon showed a potent antimicrobial activity. These results indicate that not all oils classified as "olive oil" had similar bactericidal effects and that this bioactivity depended on their content of certain phenolic compounds.

KEYWORDS: Olive oil; phenolic compounds; antimicrobial activity

INTRODUCTION

Among edible vegetable oils, olive oil is one of the few consumed unrefined, which means that as well as its triglyceride composition it possesses other minor bioactive components such as sterols, vitamins, escualene, polyphenols, and others. The consumption of olive oil has recently been considered healthy by the U.S. Food and Drug Administration on the basis of its high content in monounsaturated fatty acids (oleic acid), although numerous researchers and studies have indicated that the minor components may also contribute to the beneficial effects of olive oil to human health, in particular the phenolic compounds (*1*). Investigations have disclosed that these substances exert potent antioxidant activity and can reduce the oxidation of low-density lipoprotein in vitro (*2*), whereas results obtained in vivo were contradictory (*3*). A diverse range of other bioactivities has also been pointed out such as the prevention of certain cancers (*4*), anti-inflammatory action (*5*), and antimicrobials (*6*).

Although the antimicrobial activity of the polyphenols present in the olive fruit (*7*), olive oil mill wastewaters (*8*), and olive leaves (*9*) is well reported, studies on olive oil are few (*6*, *10*). Besides, researchers analyzed only the minor polyphenols of the oil (simple phenols), but not the secoiridoid aglycons of oleuropein and ligstroside (*11*) and the lignans (*12*), which are the main components (**Figure 1**). Likewise, there are numerous papers describing the antimicrobial activity of oleuropein (*13*), the main phenolic compound in olive fruits found in very low amounts in olive oil (*14*). In many cases, another drawback associated with studies on olive oil is the use of only one type of oil in the experiments. It has to be said that there are three types of "olive oil": (i) virgin olive oil, obtained directly from fresh fruits; (ii) olive oil, a mixture of virgin olive oil and refined olive oil; and (iii) pomace olive oil, a mixture of virgin olive oil and refined pomace oil. All of them have the same content of total fatty acids but not phenolic compounds, which are higher in virgin olive oil followed by olive oil and pomace olive oil (*15*). The type of olive variety also determines the phenolic composition of the virgin olive oil (*15*).

Furthermore, fatty acids and monoglycerides have been found to have a broad spectrum of microbicidal activity against bacteria and yeasts (*16*), and the α,β-unsaturated aldehydes from olives and olive oil flavor have also been demonstrated to possess a noticeable activity against pathogens of the human intestinal and respiratory tracts (*17*).

In relation to human health, much concern has been focused on phenolic compounds from plants and foods that may

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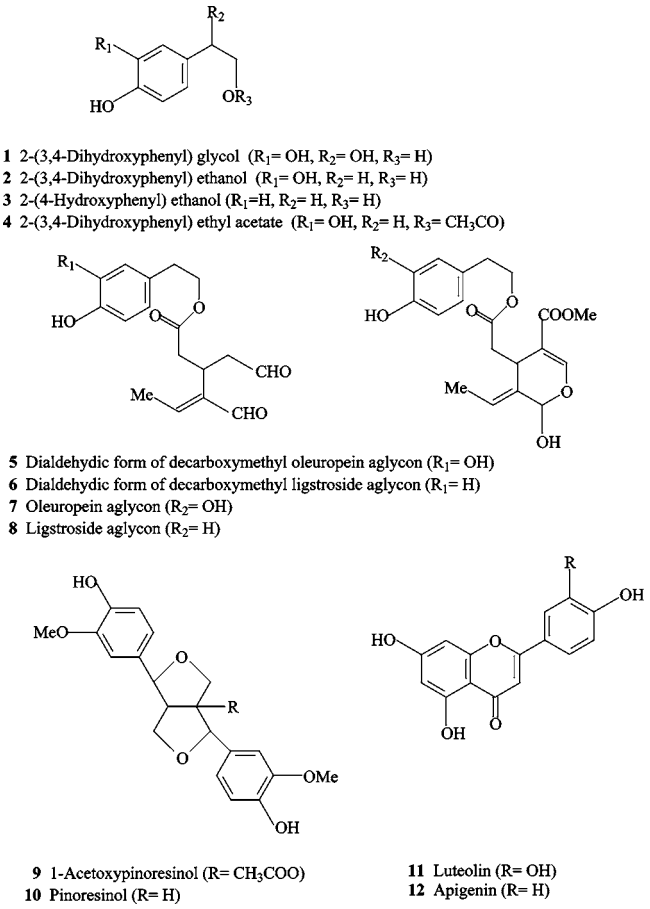

Figure 1. Structures of the studied low molecular weight phenolic compounds. Arabic numbers identify compounds.

modulate microbiota in the intestine by selectively increasing the growth of bifidobacteria and lactobacilli and decreasing that of harmful bacteria such as clostridia (*18*, *19*). The ingestion of phenolic compounds from olive oil could therefore contribute to a well-balanced microbiota of the human intestine.

There is still need for new methods for reducing or eliminating foodborne pathogens, and new biopreservatives from plants or foods (*20*) and essential oils (*21*) are requested. Olive oil may be consumed directly on bread and in fresh salads, but it is also employed in many homemade dishes (mayonnaise, cakes, others), canned tuna (*22*), salad dressing (*23*), and meat foods (*24*), in the preservation of cheese and fish (*25*), and in cosmetics. Even though olive oil has been used for centuries as a food preservative and in folk medicine, which components of the oil are responsible for this bioactivity remains undiscovered.

The aims of this work were (i) to study the phenolic composition of different olive oils and (ii) to correlate it with the antimicrobial activity of oils.

MATERIALS AND METHODS

Microorganisms and Culture Conditions. Most of the strains used in this study were obtained from the Spanish Type Culture Collection (CECT) at Burjassot, Valencia, Spain. Type strains (^{T}) *Enterococcus faecalis* CECT 481^T, *Enterococcus faecium* CECT 410^T, *Streptococcus mutans* CECT 479^T, and *Listeria monocytogenes* CECT 4031^T were grown in BHI (Oxoid Ltd., Basingstoke, U.K.). For *Lactobacillus acidophilus* CECT 903^T and *Bifidobacterium bifidum* CECT 870^T, MRS broth (Merck, Darmstadt, Germany) was used, with 0.05% L-cysteine hydrochloride (Fluka, Buchs, Switzerland) added for the latter strain. *Staphylococcus aureus* CECT 86^T, *Salmonella enterica* subs. *enterica* CECT 4300^T (formerly type strain of *S. enteritidis*), and *Escherichia coli* CECT 434 were cultured in nutrient broth containing, per liter, 5 g of "Lab-lemco" powder (Oxoid), 10 g of meat peptone (Pronadisa, Laboratorios Conda, Madrid, Spain), and 5 g of sodium chloride (Panreac, Barcelona, Spain). *Candida albicans* CECT 1472 was grown in YM broth (Difco, Sparks, MD) and *Clostridium perfringens* CECT 376^T in liver broth (Oxoid). Other bacterial strains from clinical origin were a gift from Dr. José-Carlos Palomares (Valme Hospital, Seville, Spain). These were *Yersinia* sp. 5057655, *Bacteroides* sp. 667, and *Shigella sonnei* JCP. The latter strain was cultured in nutrient broth, and the other two were cultured in BHI. All strains were preserved at −80 °C in the same medium plus 20% glycerol. For solid media, 1.5% agar (Panreac) was added to the corresponding broth except for *C. perfringens*, for which SPS Agar (Merck) was used. *B. bifidum*, *C. perfringens*, and *Bacteroides* sp. were grown anaerobically in jars with Anaerogen (Oxoid). All strains were incubated at 37 °C except *C. albicans* (26 °C) and *C. perfringens* (45 °C).

Edible Vegetable Oils. Oils from different vegetable origin, and diverse categories and varieties of olive oils, were purchased from local department stores. Fifteen virgin olive oils of the Picual (VOOP), Arbequina (VOOA), Manzanilla (VOOM), Cornicabra (VOOC), and Hojiblanca (VOOH) varieties, three olive oils (OO), three pomace olive oils (POO), two sunflower oils (SO), two corn oils (CO), two rapeseed oils (RSO), one soybean oil (SBO), and one cotton oil (CTO) were employed.

Evaluation of Oil Antimicrobial Activity. As a rule, every target strain was cultured twice in its corresponding broth, from the frozen stock, before testing, and overnight cultures were routinely used for inoculum preparation (except *B. bifidum*, for whic a 48 h culture was used). The bactericidal activity assay was based on the method reported by Friedman et al. (*26*). A model experiment was designed: 2 mL of sterilized phosphate-buffered saline with Tween 20 (PBST, pH 7.0) was inoculated with 0.1 or 0.2 mL of the refreshed target strain previously diluted with saline (0.85% NaCl) to obtain an initial inoculum between 5.0×10^4 and 1.0×10^5 colony-forming units (CFU)/mL in the case of bacteria and 1.0×10^2, 1.0×10^3, and 1.0×10^4 CFU/mL for *C. albicans*. PBST was prepared by mixing 100 mM dibasic sodium phosphate with 100 mM monobasic sodium phosphate in a 2:1 ratio, this mixture was added 1:1 to 150 mM NaCl, and Tween 20 was incorporated at 0.25% (w/w) final concentration. Subsequently, 2 mL of the assayed oil was added into the test tube containing the inoculated buffer, and the mixture was shaken in a GFL 3005 orbital shaker for 1 h at 450 rpm inside a 32 °C incubator. For anaerobic strains, buffer and oil were first mixed and the test tubes sealed with rubber stoppers. Then nitrogen gas was injected to eliminate oxygen, and finally the inoculum was added through the plug.

Culturable survivors after treatment were determined by plating these mixtures on the appropriate solid media, both spreading 0.1 mL on the surface and plating the 10^{-1} dilution (0.1% peptone water) with a Spiral Plater (Don Whitley Sci. Ltd., model WASP 2, Shipley, U.K.). Controls with no oil were also done, and all trials were carried out in duplicate. This standard test was performed with all strains against the following oils: VOOP2, VOOA2, OO2, POO1, SO1, and CO1. Apart from it, *S. sonnei* was assayed against a mixtures of oil with buffer in ratios of buffer to oil of 1:1, 2:1, and 4:1 following the same method. These experiments were carried out with both olive oil (OO2) and pomace olive oil (POO1). Also, *E. coli* was tested counter to all oils, and *S. enterica* was additionally tested against all monovarietal virgin olive oils at a ratio of buffer to oil of 4:1 (3.2 mL of buffer and 0.8 mL of oil).

Antimicrobial Effect of Buffer Extracts from Oils. The antimicrobial effect of the aqueous phase obtained after mixing oils (POO1 and OO2) and buffer against *S. enterica* was also studied. After 1 h of shaking 2 mL of oil with 2 mL of PBST, tubes were allowed to settle for 30−40 min, and the aqueous phase free of oil was collected with Pasteur pipet and inoculated with the selected strain at the same levels as before (from 5.0×10^4 to 1.0×10^5 CFU/mL). Then cells were subjected to a shaking period of 1 h, and the suspension was appropriately plated. Control assays were run with the standard mixture of buffer to oil (1:1). All experiments were run in duplicate.

Table 1. Antimicrobial Activity (Log N_0/N_1)[a] of Different Edible Vegetable Oils (Ratio of Buffer to Oil Was 1:1)

microorganism[b]	control	Picual virgin olive oil (VOOP2)	Arbequina virgin olive oil (VOOA2)	olive oil (OO2)	pomace olive (POO1)	sunflower oil (SO1)	corn oil (CO1)
L. acidophilus	<0.01	>4.59	>4.59	>4.59	>4.59	<0.01	<0.01
L. monocytogenes	0.13 (0.03)[c]	>4.82	>4.82	>4.82	>4.82	0.07 (0.01)	0.12 (0.01)
S. mutans	0.24 (0.07)	>4.79	>4.79	>4.79	>4.79	0.22 (0.01)	0.31 (0.03)
B. bifidum	0.71 (0.07)	>4.95	>4.95	>4.95	3.81 (2.08)	0.68 (0.05)	0.70 (0.06)
E. faecium	0.07 (0.02)	>4.84	>4.84	>4.84	0.71 (0.10)	0.05 (0.01)	0.10 (0.01)
E. faecalis	<0.01	>4.94	>4.94	>4.94	3.71 (0.33)	0.07 (0.01)	0.06 (0.01)
S. aureus	<0.01	>4.60	>4.60	>4.60	>4.60	<0.01	<0.01
C. perfringens	0.36 (0.01)	>5.38	>5.38	>5.38	4.28 (0.34)	0.86 (0.01)	0.12 (0.01)
S. sonnei	0.07 (0.05)	2.43 (0.13)	3.47 (0.75)	1.79 (0.01)	0.31 (0.14)	0.04 (0.01)	0.04 (0.02)
Bacteroides sp.	<0.01	>5.11	>5.11	>5.11	>5.11	0.06 (0.03)	0.19 (0.08)
Yersinia sp.	0.09 (0.01)	>4.58	>4.58	>4.58	0.08 (0.03)	0.02 (0.01)	0.04 (0.02)
E. coli	<0.01	1.76 (0.01)	1.22 (0.01)	0.72 (0.01)	0.39 (0.07)	0.15 (0.01)	0.18 (0.01)
S. enterica	0.44 (0.05)	>5.11	>5.11	2.67 (0.16)	0.59 (0.04)	0.58 (0.01)	0.56 (0.01)
C. albicans	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[a] N_0 = CFU/mL inoculated; N_1= CFU/mL after 1 h. [b] Initial inoculum was $\approx 10^5$ CFU/mL for all bacteria. *C. albicans* was 10^2 CFU/mL. [c] Standard deviation.

Polyphenol Analysis. Phenolic extracts of olive oils were obtained following the procedure described elsewhere (*12*). Briefly, 0.6 mL of olive oil was extracted using 3 × 0.6 mL of *N*,*N*-dimethylformamide (DMF); the extract was then washed with hexane, and N_2 was bubbled into the DMF extract to eliminate the residual hexane. Finally, the extract was filtered through 0.45 μm pore size and injected into the chromatograph.

The analysis of the polyphenols in the PBST buffer extract was made by directly injecting the solution into the chromatograph after filtration through a 0.45 μm pore size filter.

The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600E pump, and a Waters column heater module (Waters Inc., Milford, MA). A Spherisorb ODS-2 (5 μm, 25 cm × 4.6 mm i.d., Waters Inc.) column was used. Separation was achieved using an elution gradient with an initial composition of 90% water (pH adjusted to 3.0 with phosphoric acid) and 10% methanol. The concentration of the latter solvent was increased to 30% over 10 min and maintained for 20 min. Subsequently, the methanol percentage was raised to 40% over 10 min, maintained for 5 min, and then increased to 50%. Finally, the methanol percentage was increased to 60, 70, and 100% in 5 min periods. Initial conditions were reached in 15 min. A flow of 1 mL/min and a temperature of 35 °C were used in all of the experiments. A Waters 996 diode array detector and a Jasco FP-920 fluorescence detector (Jasco, Tokyo, Japan) were connected in series. Compounds **5** and **6** were monitored by UV at 280 nm, compounds **11** and **12** at 340 nm, the oleosidic compounds at 240 nm, and the rest of the phenolic compounds by fluorescence with an excitation wavelength at 280 nm and an emission wavelength at 320 nm. Both detectors were operated with Millenium 2015 software (Waters Inc.). Quantification of phenolic compounds was made by using and internal standard (syringic acid). Compounds **1** and **3** were purchased from Sigma Chemical Co. (St. Louis, MO) and compounds **11** and **12** from Extrasynthese (Z. I. Lyon-Nord, Genay, France). The rest of the phenolic compounds analyzed were obtained by semipreparative HPLC as described elsewhere (*12*). Polyphenol identification was also carried out by HPLC-MS.

HPLC-MS Analysis. Phenolic extracts were analyzed by LC-MS using a ZMD4 mass spectrometer (Waters Inc.) equipped with an ESI probe and working in the negative-ion mode. Cone voltage fragmentation was 20 V, capillary voltage, 3 kV, desolvation temperature, 250 °C, source temperature, 120 °C, and extractor voltage, 12 V. A constant flow of 1 mL/min was used for each analysis with a split ratio of approximately 5:1 (UV detector/MS detector).

Isolation of Phenolic Compounds. Polyphenols were extracted from a virgin olive oil by using the methanol/water method reported by Montedoro et al. (*11*). The DMF method (*12*) was not used because the solvent could be recovered in the HPLC fractions. The analytical column, mobile phases, gradient, and equipment were the same as used for polyphenol analysis except the aqueous mobile phase, which was acidified with HCl to pH 4. Fractions from 80 HPLC runs were collected peak by peak. The pooled extract for each peak (50−80 mL) was evaporated under reduced pressure close to dryness and the residue was dissolved in 1 mL of deionized water. Finally, the purity and concentration of each phenolic compound were measured by HPLC. A control run was also performed by injecting methanol and collecting all fractions of the run (75 mL). The pooled fractions were evaporated close to dryness, and the residue was dissolved in 1 mL of deionized water.

Antimicrobial Effect of Isolated Phenolic Compounds on *L. monocytogenes*. The strain *L. monocytogenes* CECT 4031 was chosen for testing the effect of each compound and their mixtures. Overnight culture in BHI was diluted in 2-fold concentrated PBS. One hundred microliters of the 10^{-3} dilution was added to an Eppendorf containing 100 μL of each isolated compound, vortexed, and left for 5 min at room temperature (25 °C). Viable count was determined by plating 90 μL directly on BHI agar and the 10^{-1} dilution in spiral as well. Colonies were counted after 24 h of incubation at 37 °C. The experiments were carried out twice at the average concentration found for each phenolic compound in the buffer extract of the virgin olive oils studied (**Table 3**). They were also tested at a concentration of 250 μM. A control test was performed with the control HPLC run extract obtained.

Statistical Analysis. Regression analysis was performed using the Statistica package software (Statistica for Windows, Tulsa, OK, 1996).

RESULTS AND DISCUSSION

Neither sunflower oil nor corn oil showed antimicrobial activity against the microorganisms tested (**Table 1**). Indeed, none of the edible vegetable oils studied had this ability except oils obtained from olive fruits, and these results led us to think that different components of olive oil other than fatty acids were responsible for the bactericidal action. Although it has been reported that fatty acids possess antimicrobial activity (*16*), the fact that only olive oils presented this activity suggested that the minor components of the oil should be involved in this biological property. It is worth noting that the bactericidal activity was higher for virgin olive oil followed by olive oil and pomace olive oil, which is also the order of decreasing content in the minor components of these oils.

Among the microorganisms tested, *C. albicans* was the only one to survive after treatment with the olive oils studied even with an initial inoculum as low as 10^2 CFU/mL. Surprisingly, a mixture of honey, olive oil, and beeswax has been proposed to treat the disease *diaper dermatitis* produced partly by this yeast (*27*), and an olive leaf extract was able to inhibit the growth of *C. albicans* after 1 day of contact (*9*). It must therefore be assumed that the active compounds of the olive leaf extract, mainly phenolic compounds, were different from those of the

olive oils employed in our experiments. However, it has to be said that the olive leaf extract was much more inhibitory against *E. coli* than against *C. albicans*. It has also been reported that virgin olive oil was effective in preserving yogurt cheese inoculated with the yeast *Kluyveromyces marxianus* (*28*), but this effect was attributed to the anaerobic conditions created by the oil because other edible vegetable oils also preserved the yogurt cheese. Hence, it seems that yeast growth is not inhibited to a large extent by olive phenolics.

With regard to bacteria, most of them did not survive after 1 h of contact with virgin olive oils of the Picual and Arbequina varieties (**Table 1**). Only the two Gram-negative bacteria, *S. sonnei* and *E. coli*, partly survived. It seemed that olive oils were more active against Gram-positive than against Gram-negative bacteria, although not as a general rule. In fact, there is some controversy over this point when natural compounds are tested against a broad spectrum of microorganisms. For example, phenolic compounds from berry extracts generally inhibited the growth of Gram-negative but not Gram-positive bacteria (*29*), and the opposite has been reported for phenolics of essential oils (*21*). Otherwise, olive oils exhibited bactericidal activity against *E. coli* and *C. perfringens*, which grow in the intestine. Many plant extracts (*19*) and foods such as tea (*18*) may improve the intestinal microbiota by inhibiting harmful microorganisms but also promoting or maintaining the beneficial ones such as lactic acid producing bacteria. Unfortunately, olive oils also exerted bactericidal activity against the beneficial microorganisms *L. acidophilus* and *B. bifidum*. Although these results have been obtained in vitro, and the inhibitory components of olive oil may be lost or transformed during ingestion, these findings suggest that intestine microbiota growth, including *Bacteroides* sp., could be influenced by olive oil consumption but, of course, this depends on the amount of oil ingested. In addition, the bioactive compounds such as polyphenols can be absorbed before they reach the colon (*30*), and they are even transformed by the intestinal microbiota (*31*).

Several food commodities have been proposed to inhibit the growth of the cariogenic bacteria *S. mutans* such as tea (*18*), and, very recently, olive oil (*32*). Researchers found that an olive oil formulation dentifrice can decrease both bacterial growth and adhesion. In our work, we observed a very strong bactericidal effect of all types of olive oils against *S. mutans* (**Table 1**); even the pomace olive oil killed all inoculated bacteria. Pretty et al. (*32*) proposed the need for long-term gingival studies to better correlate the use of an olive oil formulation dentifrice with gingival health. However, in light of our findings, we think that this study should be performed directly with olive oil because its consumption alone could influence the oral microbiota.

Another aim of this work was to assess the antimicrobial activity of olive oil against foodborne pathogens. In this sense, virgin olive oil, olive oil, and pomace olive oil showed a strong bactericidal effect against *S. aureus*, *L. monocytogenes*, and *Yersinia* sp., but a weaker effect against *S. enterica* and *S. sonnei*. These findings confirm the suspected antimicrobial properties of olive oil, which had not been scientifically demonstrated. It also opens the possibility of using olive oils as a food preservative to prevent the growth of foodborne pathogens or to delay the onset of food spoilage. To our knowledge, there is only one paper relating growth inhibition of foodborne pathogens to the use of olive oil (*10*). In this work, investigators observed a faster death rate of *S. enterica* in mayonnaise made with virgin olive oil than in that prepared with sunflower oil. They attributed this effect to the high acidity as well as the polyphenols in olive oil. Unfortunately, this work was done before it was discovered that the main phenolic compounds in olive oil are the secoiridoid aglycons of oleuropein and ligstroside (*11*) and the lignans (*12*), and they were not analyzed.

Because of the well-demonstrated antimicrobial activity of plant polyphenols (*20*, *29*), the known influence of phenol structure on this activity, and the fact that different types of olive oil showed differences in bactericidal action (**Table 1**), we tried to correlate the antimicrobial activity of olive oil with each of its phenolic compounds.

The polyphenol analysis of the different types of olive oil was undertaken by HPLC. Most of them had previously been characterized in olive oil (*11*, *12*) except compound **1**. It was first detected in the vegetation water of olive fruits (*33*) but never before in olive oil. It was a peak eluting at 4.2 min with maximum absorbance at 280 nm like compound **2** (hydroxytyrosol) and with a mass spectrum showing molecular ion species at 169 and 151 uma, working under negative mode with an ESI probe. All of these data were compared with those of an authentic standard, and they confirmed the presence of compound **1** (hydroxytyrosol glycol) in most oils.

Other compounds tentatively identified in olive oil by HPLC-MS, and with attributed antimicrobial properties (*34*), were the oleosides elenolic acids A (*m*/*z* 242) and B (*m*/*z* 242) and the oxidized elenolic acid (*m*/*z* 258). All of them showed an absorbance UV spectrum with a maximum between 230 and 240 nm. They have been previously detected in olive oil by using HPLC-MS (*35*), but their complete structural characterization is difficult because of their instability.

As could be expected, the main phenolic compounds in virgin olive oil were the aglycons of oleuropein and ligstroside (compounds **5**−**8**) (**Table 2**), followed by the simple phenols hydroxytyrosol, tyrosol, hydroxytyrosol acetate, and lignans (compounds **2**−**4**, **9**, and **10**) (*15*). The newly identified compound **1** was found in a concentration lower than 35 μmol/kg, although higher than that detected for the flavones (compounds **11** and **12**). Otherwise, great differences in phenolic composition found among olive varietys and even within the same varietys must be stressed, as well as differences among virgin olive oil, olive oil, and pomace olive oil. The pomace olive oils had a significantly lower concentration in total polyphenols than the rest of the oils (Duncan's multiple-range test), followed by olive oils, although not statistically different from their concentration in Arbequina and Hojiblanca oils. Virgin olive oils of the Manzanilla and Cornicabra varieties also had a significantly higher concentration in total polyphenols than other monovarietal olive oils. These results explain the need to specify the type of olive oil and even the phenolic composition of the oil when testing against microorganisms is performed. The bactericidal effect of all edible vegetable oils and the virgin olive oils against *E. coli* and *S. enterica*, respectively, is presented in **Figure 2**. Virgin olive oil, in particular, certain monovarietal oils, showed the highest activity, and the refined oils, which lose their phenolic compounds during refining, the lowest (sunflower, soybean, cotton, corn, and rapeseed oils). These data together with the phenolic composition of each olive oil were used to correlate each phenolic compound with the viability of *E. coli* (**Table 4**) and *S. enterica* cells (**Table 5**) by a linear regression. The determination coefficient (R^2), which represents the proportion of variance accounted for by the regression, and the probability (*p*), which is the probability of a relationship between two variables based on an *F* test of the regression analysis of variance, were calculated. Among polyphe-

Table 2. Phenolic Compounds (Micromoles per Kilogram) in the Olive Oils Studied

	compound												
oil	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	total
VOOP1	26.9	176.8	52.3	500.4	923.4	202.6	622.7	253.6	18.7	154.2	6.3	1.4	2939.3
VOOP2	31.9	147.3	75.0	51.5	437.6	157.6	783.5	454.5	8.7	109.1	9.4	2.4	2268.5
VOOP3	14.6	14.4	56.1	37.8	41.6	167.2	391.3	348.2	3.9	144.3	5.3	1.3	1226.0
VOOA1	12.3	95.4	43.4	198.7	385.7	164.7	87.4	21.1	187.1	116.7	14.7	3.7	1330.8
VOOA2	14.3	179.6	59.9	202.6	559.4	179.8	85.0	17.5	133.9	107.6	13.7	4.4	1557.8
VOOA3	32.9	44.5	26.2	440.0	443.8	48.4	84.5	13.4	269.1	132.8	29.1	5.9	1570.7
VOOM1	11.4	145.8	65.2	105.4	802.7	209.0	1217.6	644.3	6.4	101.0	8.3	3.8	3320.9
VOOM2	8.8	479.6	159.4	130.8	740.5	283.5	535.8	249.6	72.2	52.7	12.0	5.2	2730.0
VOOM3	10.1	317.2	158.4	213.7	1427.0	364.0	852.2	355.2	83.1	52.4	11.7	5.4	2850.5
VOOC1	10.8	164.1	54.1	1.5	1332.7	427.1	778.6	497.2	9.2	104.4	1.3	0.5	3381.3
VOOC2	26.0	295.4	128.6	27.7	420.2	216.9	293.7	168.8	25.5	127.7	3.5	1.3	1731.3
VOOC3	22.8	342.7	123.5	ND[a]	559.8	258.1	558.0	181.2	39.1	158.9	11.3	1.5	2256.9
VOOH1	12.9	116.6	33.6	95.4	337.7	110.4	373.9	179.5	53.3	46.0	10.2	3.9	1373.3
VOOH2	16.4	159.2	121.6	51.9	296.2	160.2	454.2	240.8	54.4	86.0	11.9	5.1	1657.9
VOOH3	2.8	87.9	34.1	64.9	358.1	101.9	309.6	145.8	52.7	76.5	8.5	2.0	1244.8
OO1	31.7	137.7	55.3	76.4	216.3	122.4	402.9	213.6	5.4	63.5	3.0	0.8	1328.8
OO2	ND	91.6	53.9	58.5	248.1	116.7	390.5	173.3	50.5	105.2	7.3	2.0	1297.7
OO3	12.6	89.9	29.3	125.5	196.8	101.7	204.9	111.6	4.5	63.0	2.5	0.8	953.3
POO1	ND	23.5	8.7	7.1	13.3	17.2	68.1	41.3	0.1	8.2	ND	ND	187.6
POO2	0.4	ND	ND	ND	ND	ND	16.8	10.1	20.8	1.9	6.2	0.9	57.3
POO3	0.4	1.2	ND	ND	ND	ND	16.2	7.9	4.6	3.4	5.4	0.9	40.0

[a] Not detected.

Table 3. Phenolic Compounds (Micromoles per Liter) in the Buffer after 1 h of Contact between Oil and Buffer (1:1)

	compound												
oil	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	total
VOOP1	31.0	314.3	58.5	380.9	657.8	74.3	179.7	28.0	7.2	32.3	6.1	0.7	1770.8
VOOP2	30.2	284.7	86.9	30.7	281.3	56.9	173.2	40.7	4.3	19.6	7.7	1.0	1017.2
VOOP3	37.2	106.3	73.3	22.8	ND[a]	54.8	96.9	31.9	1.8	27.4	4.5	0.7	457.4
VOOA1	9.6	47.7	51.6	52.6	177.7	24.6	10.7	2.4	28.9	11.3	11.8	1.3	299.1
VOOA2	10.8	164.4	30.2	105.9	290.8	47.5	18.5	4.2	35.1	18.6	10.2	1.4	751.7
VOOA3	16.1	83.5	24.7	199.5	169.0	11.0	18.4	2.1	59.9	20.4	21.0	1.9	627.5
VOOM1	21.6	449.6	92.3	62.8	571.8	74.6	313.7	58.1	4.7	20.3	7.3	1.6	1678.5
VOOM2	8.9	485.8	164.9	71.9	447.6	86.3	135.9	28.0	23.2	10.0	10.3	2.4	1457.9
VOOM3	14.5	450.6	142.7	114.7	880.9	105.3	184.5	30.8	29.3	9.7	9.7	1.7	1973.1
VOOC1	15.9	353.7	79.6	ND	1180.2	256.1	216.4	49.2	7.2	23.2	1.2	0.2	2182.8
VOOC2	18.4	338.9	127.1	16.9	346.7	114.6	98.3	42.1	10.1	27.0	3.5	0.5	1144.2
VOOC3	22.6	471.9	139.2	ND	634.9	155.1	168.5	50.1	11.0	29.8	3.5	0.4	1686.9
VOOH1	17.0	157.1	39.9	47.4	191.4	31.4	70.4	15.6	15.2	7.9	8.1	1.5	603.0
VOOH2	19.7	217.9	118.2	30.1	172.6	59.9	113.3	31.3	19.0	16.6	7.2	1.6	807.4
VOOH3	7.5	161.2	68.0	41.1	245.5	36.4	79.8	16.9	18.4	16.5	8.9	1.6	676.2
mean[b]	18.7	272.5	86.5	78.5	416.6	79.3	125.2	28.8	18.4	19.4	8.1	1.2	1142.2
OO1	23.9	148.0	41.5	30.5	94.3	30.8	71.4	18.8	2.2	8.6	1.9	0.3	472.1
OO2	9.0	136.9	46.3	24.0	133.1	27.6	69.4	14.2	12.4	16.0	4.9	0.5	494.3
OO3	9.5	91.8	22.6	55.2	74.1	15.5	37.3	11.0	1.3	8.9	1.6	0.2	329.1
POO1	1.8	31.6	7.8	2.4	ND	ND	15.7	5.2	0.5	0.1	ND	ND	66.0
POO2	ND	ND	ND	ND	ND	ND	ND	1.8	ND	NaD	ND	ND	1.8
POO3	0.8	2.1	0.3	0.8	ND	ND	1.5	1.9	ND	0.2	ND	ND	8.0

[a] Not detected. [b] Average of each compound in virgin olive oils.

nols, compounds **2**, **3**, **5**, and **6** showed statistically significant correlations with cell viability ($p < 0.01$), in particular, the last two compounds. Surprisingly, compounds **7** and **8**, which are the main polyphenols in many oils, did not correlate with cell viability, nor did compounds **4** and **9**−**12**. Neither of the oleosides, estimated as their area under the peak, correlated with cell viability (data not shown). It must be noted that two of the bactericidal compounds possess antioxidant activity (compounds **2** and **5**) but that the other two (compounds **3** and **6**) do not.

Indeed, a better correlation was found between the sum of the four compounds **2**, **3**, **5**, and **6** and the cell viability of *E. coli* ($R^2 = 0.69$) and *S. enterica* ($R^2 = 0.83$). Keceli and Robinson (*6*) tested the antimicrobial activity of phenolic extracts from virgin olive oil, but they did not relate cell viability with any single compound. In fact, they detected only compounds **2** and **3** and other minor components in the oil. Thus, this is the first time that antimicrobial activity has been attributed to the phenolic compounds **5** and **6**. For many years, researchers have focused their studies on the antimicrobial activity of oleuropein (*7*), which is present in olive oil in a very few amount (*13*), compounds **7** (*36*), and compound **2** (*37*), but never on compounds **5** and **6**. The fact that only compounds **2**, **3**, **5**, and **6** correlated with cell viability led us to think that this effect could be related to phenolic polarity and, therefore, their diffusion into the aqueous phase (PBST buffer). Several studies have demonstrated a good correlation between the partition coefficient of olive oil polyphenols, determined by the octanol/water method, and their elution time during HPLC analysis (*38*). The four mentioned polar polyphenols eluted between 15 and 30 min. In contrast, compounds **1** and **4**, which are also polar

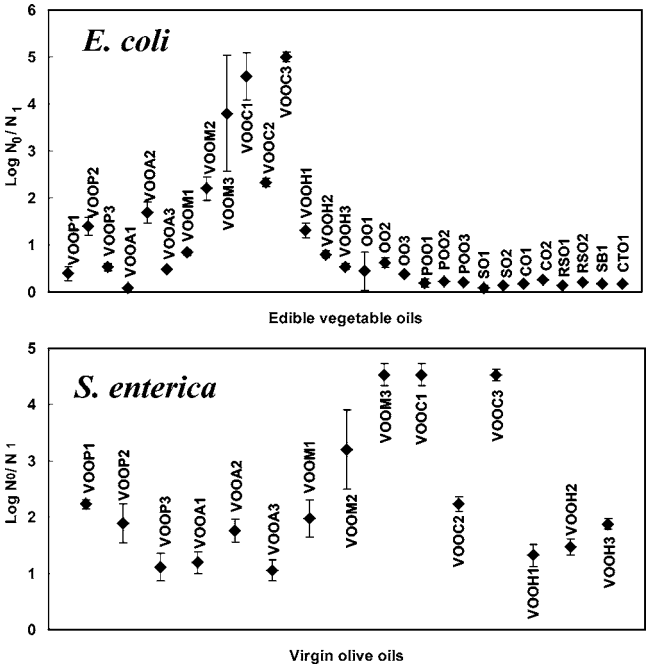

Figure 2. Effect of different edible vegetable oils on the viability of *E. coli* and *S. enterica* cells following 1 h of exposure to a mixture of buffer/oil (1:1). Bars indicate the standard errors of the mean.

Table 4. Results of the Linear Regression Analysis [Determination Coefficient (R^2) and Probability (p)] Performed among the Data of the Viability of *E. coli* Cells Reflected in **Figure 2** and the Phenolic Compounds Present in Olive Oils (**Table 2**) or Diffused into the Buffer (**Table 3**)

	polyphenols in oils		polyphenols in buffer	
compound	R^2	p	R^2	p
6	0.40	<0.01	0.58	<0.01
5	0.47	<0.01	0.64	<0.01
2	0.47	<0.01	0.55	<0.01
3	0.40	<0.01	0.49	<0.01
7	0.22	>0.01	0.27	>0.01
8	0.14	>0.01	0.39	<0.01
4	0.04	>0.01	0.02	>0.01
9	0.01	>0.01	0.01	>0.01
10	0.07	>0.01	0.15	>0.01
11	0.01	>0.01	0.01	>0.01
12	0.01	>0.01	0.02	>0.01
1	0.02	>0.01	0.03	>0.01
sum of all polyphenols	0.40	<0.01	0.58	<0.01
sum of **2**, **3**, **5**, and **6**	0.69	<0.01	0.79	<0.01

compounds, rapidly eluted from the column, but they did not correlate with cell viability. To confirm this suspicion, the phenolic composition of the buffer solution was analyzed after 1 h of contact with the different olive oils (**Table 3**). The more polar the compound, the higher the amount diffused from oil to buffer. Thus, almost all of compounds **2** and **3**, ≈40−60% of compounds **5** and **6**, and only 15−30% of compounds **7** and **8** diffused. It must be pointed out that the concentrations of **2** and **3** were even higher in the buffer than in the original oil before contact, which probably occurred because of hydrolysis reactions of the secoiridoid aglycons.

A new linear regression analysis was made with the data of the phenolic compounds in the buffer extract and cell viability, and, again, only compounds **2**, **3**, **5**, and **6** showed a good correlation with bacterial survival (**Tables 4** and **5**). In fact, the determination coefficients were higher than those obtained with the phenolic compounds of the original olive oil. The sum of

Table 5. Results of the Linear Regression Analysis [Determination Coefficient (R^2) and Probability (p)] Performed among the Data of the viability of *S. enterica* Cells Reflected in **Figure 2** and the Phenolic Compounds Present in Virgin Olive Oils (**Table 2**) or Diffused into the Buffer (**Table 3**)

	polyphenols in oils		polyphenols in buffer	
compound	R^2	p	R^2	p
6	0.77	<0.01	0.70	<0.01
5	0.60	<0.01	0.74	<0.01
2	0.46	<0.01	0.62	<0.01
3	0.33	>0.01	0.42	<0.01
7	0.24	>0.01	0.29	>0.01
8	0.12	>0.01	0.24	>0.01
4	0.06	>0.01	0.02	>0.01
9	0.10	>0.01	0.02	>0.01
10	0.01	>0.01	0.10	>0.01
11	0.10	>0.01	0.19	>0.01
12	0.05	>0.01	0.08	>0.01
1	0.03	>0.01	0.02	>0.01
sum of all polyphenols	0.54	<0.01	0.74	<0.01
sum of **2**, **3**, **5**, and **6**	0.83	<0.01	0.88	<0.01

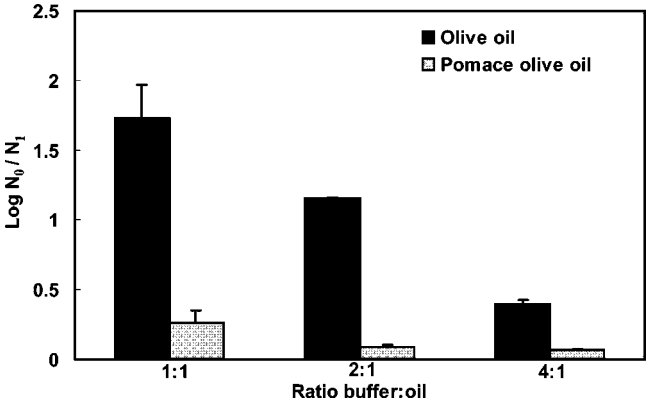

Figure 3. Effect of olive oil and pomace olive oil on the survival of *S. sonnei* cells following 1 h of exposure to different buffer/oil mixtures. N_0 = CFU/mL inoculated; N_1 = CFU/mL after 1 h. Bars indicate the standard errors of the mean.

the four compounds showed determination coefficients (R^2) of 0.79 and 0.88 for *E. coli* and *S. enterica*, respectively.

It is known that the antioxidant and antimicrobial activities of phenolic compounds in emulsions of oil/water depend on their polarity and their chemical structure (*26*). In our case, the four compounds that correlated with cell viability also diffused into the buffer phase to a large extent, giving rise to a high concentration of these polyphenols in the aqueous extract. However, there were some exceptions; for example, the concentrations of compounds **4** and **7** were in general similar to those of compounds **3** and **6**, but the former two did not correlate with cell viability, which probably implies a chemical structural influence on the antimicrobial activity of olive oil polyphenols.

Subsequently, we carried out two new experiments to demonstrate the influence of the phenolic diffusion on bacteria survival. Results from the first experiment confirmed that survival of *S. sonnei* increased with an increasing ratio of buffer to oil for both olive oil and pomace olive oil, although it was statistically significant for olive oil but not for pomace olive oil (Duncan's multiple-range test) (**Figure 3**). Similarly, the concentration of polyphenols decreased in the buffer with an increasing ratio of buffer to oil, which means that they were involved in the antimicrobial activity. In the second experiment,

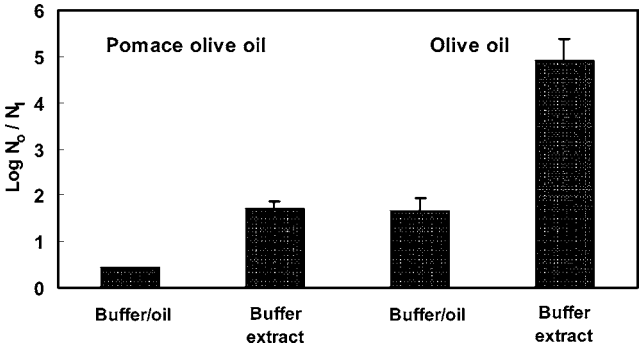

Figure 4. Comparison of the bactericidal effect of a mixture of buffer/oil and the buffer extract alone against *S. enterica*. N_0 = CFU/mL inoculated; N_1 = CFU/mL after 1 h. Bars indicate the standard errors of the mean.

Table 6. Effect of Isolated Phenolic Compounds on the Viability of *L. monocytogenes*

compound	concn[a] (μM)	log N_0/N_1[b]	concn (μM)	log N_0/N_1
1	18.7	<0.01	250	<0.01
2	272.5	<0.01	250	<0.01
3	86.5	<0.01	250	<0.01
4	78.5	<0.01	250	<0.01
7	125.2	<0.01	250	<0.01
8	28.8	<0.01	250	<0.01
9	18.4	<0.01	250	<0.01
10	19.4	<0.01	250	<0.01
5	416.6	0.12	250	<0.01
6	79.3	0.39	250	0.82
sum of **2**, **3**, **5**, and **6**	272.5, 86.5 416.6, 79.3	1.45		

[a] Mean value recorded in **Table 3**. [b] N_0 = CFU/mL inoculated; N_1 = CFU/mL after 1 h. Initital inoculum was $\approx 10^4$ CFU/mL.

a comparison of the bactericidal activity between the emulsion buffer/oil ratio and the buffer extracts, obtained after 1 h of contact with oils (olive oil and pomace olive oil), was made. Surprisingly, the survival of *S. enterica* in the buffer extract was even statistically lower (Duncan's multiple-range test) than observed in the buffer/oil emulsion (**Figure 4**).

Finally, the bactericidal effect of each isolated phenolic compound was studied against *L. monocytogenes*. Because of the wide range of concentration found for these substances in virgin olive oils, the mean concentation of each polyphenol in the buffer extracts (**Table 3**) was chosen to perform the experiments. Also, the bactericidal effect of all of them was assessed at a concentration of 250 μM. Results displayed in **Table 6** reveal that compound **6** showed the highest bactericidal effect followed by compound **5**. However, it must be stressed that the mixture of **2**, **3**, **5**, and **6** decreased the number of cells by $\approx$2 log cycles, which means that the bactericidal effect of virgin olive oil was a joint action of different phenolic compounds, mainly **2**, **3**, **5**, and **6**, in particular compound **6**, which also possesses anti-inflammatory activity (*5*). Interestingly, these results are in agreement with those reported in **Tables 4** and **5** obtained by a linear regression analysis of the bactericidal effect of phenolic compounds and their concentration in virgin olive oils and buffer extracts.

Olive oil showed a strong antibacterial activity against foodborne pathogens and, even when the polyphenol activity may be influenced by factors such as pH, proteins, or the water activity of food (*39*), virgin olive oil should be considered a potential biopreservative for foodstuffs.

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LITERATURE CITED

(1) Visioli, F.; Galli, C. Biological properties of olive oil phytochemicals. *Crit. Rev. Food Sci. Nutr.* **2002**, *42*, 209−221.
(2) Visioli, F.; Caruso, D.; Grande, S.; Bosisio, R.; Villa, M.; Galli, G.; Sirtori, C.; Galli, C. Virgin olive oil study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *Eur. J. Nutr.* **2005**, *44*, 121−127.
(3) Vissers, M. N.; Zock, P. L.; Katan, M. B. Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *Eur. J. Clin. Nutr.* **2004**, *58*, 955−965.
(4) D'Angelo, S.; Ingrosso, D.; Migliardi, V.; Sorretino, A.; Donnarumma, G.; Baroni, A.; Masella, L.; Tufano, M. A.; Zappia, M.; Galletti, P. Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. *Free Radical Biol. Med.* **2005**, *38*, 908−919.
(5) Beauchamp, G. K.; Keast, R. S. J.; Morel, D.; Ling, J.; Pika, J.; Han, Q.; Lee, C.; Smith, A. B.; Breslin, P. A. S. Ibuprofen-like activity in extra-virgin olive oil. *Nature* **2005**, *437*, 45−46.
(6) Keceli, T.; Robinson, R. K. Antimicrobial activity of phenolic extracts from virgin olive oil. *Milchwissenschaft* **2002**, *57*, 436−440.
(7) Fleming, H. P.; Walter, W. M.; Etchells, J. L. Antimicrobial properties of oleuropein and products of its hydrolysis from green olives. *Appl. Microbiol.* **1973**, *26*, 777−782.
(8) Capasso, R.; Evidente, A.; Schivo, L.; Orru, G.; Marcialis, M. A.; Cristinzo, G. Antibacterial polyphenols from olive oil mill waste waters. *J. Appl. Bacteriol.* **1995**, *79*, 393−398.
(9) Markin, D.; Duek, L.; Berdicevsky, I. In vitro antimicrobial activity of olive leaves. *Mycoses* **2003**, *46*, 132−136.
(10) Radford, S. A.; Tassou, C. C.; Nychas, G. J. E.; Board, R. G. The influence of different oils on the death rate of *Salmonella enteritidis* in homemade mayonnaise. *Lett. Appl. Microbiol.* **1991**, *12*, 125−128.
(11) Montedoro, G. F.; Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterization of the secoiridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.
(12) Brenes, M.; Hidalgo, F. J.; García, A.; Rios, J. J.; García, P.; Zamora, R.; Garrido, A. Pinoresinol and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *J. Am. Oil Chem.* Soc. **2000**, *77*, 715−720.
(13) Perri, E.; Rafaelli, A.; Sindona, G. Quantitation of oleuropein in virgin olive oil by ion spray mass spectrometry−selected reaction monitoring. *J. Agric. Food Chem.* **1999**, *47*, 4156−4160.
(14) Tranter, H.; Tassou, C. C.; Nychas, G. J. E. The effect of the olive phenolic compound, oleuropein, on growth and enterotoxin B production by *Staphylococcus aureus*. *J. Appl. Bacteriol.* **1993**, *74*, 253−259.
(15) García, A.; Brenes, M.; García, P.; Romero, C.; Garrido, A. Phenolic content of commercial olive oils. *Eur. Food Res. Technol.* **2003**, *216*, 520−525.
(16) Bergsson, G.; Arnfinnsson, J.; Steingrimsson, O.; Thomár, H. In vitro killing of *Candida albicans* by fatty acids and monoglycerides. *Antimicrob. Agents Chemother.* **2001**, *45*, 3209−3212.
(17) Trombetta, D.; Saija, A.; Bisignano, G.; Arena, S.; Caruso, S.; Mazzanti, G.; Uccella, N.; Castelli, F. Study on the mechanisms on the antibacterial action of some plant α,β-unsaturated aldehydes. *Lett. Appl. Microbiol.* **2002**, *35*, 285−290.
(18) Koo, M. W. L.; Cho, C. H. Pharmacological effects of green tea on the gastrointestinal system. *Eur. J. Pharmacol.* **2004**, *500*, 177−185.

(19) Park, B.; Kim, J.; Lee, S.; Kim, K. S.; Takeoka, G. R.; Ahn, Y.; Kim, J. Selective growth-inhibiting effects of compounds identified in *Tabebuia impetiginosa* inner bark on human intestinal bacteria. *J. Agric. Food Chem*. **2005**, *53*, 1152−1157.

(20) Draughon, F. A. Use of botanicals as biopreservatives in foods. *Food Technol.* **2004**, *58*, 20−28.

(21) Burt, S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* **2004**, *94*, 223−253.

(22) Caponio, F.; Gomes, T.; Summno, C. Quality assessment of edible oils used as liquid medium in canned tuna. *Eur. Food Res. Technol*. **2003**, *216*, 104−108.

(23) Paraskevopoulou, A.; Boskou, D.; Kiosseoglou, V. Stabilization of olive oil-lemon juice emulsion with polysaccharides. *Food Chem*. **2005**, *90*, 627−634.

(24) Kayaardi, S.; Gok, V. Effect of replacing beef fat with olive oil on quality characteristics of Turkish soudjouk (sucuk). *Meat Sci*. **2004**, *66*, 249−257.

(25) Tassou, C. C.; Drosinos, E. H.; Nychas, G. J. E. Inhibition of resident microbial flora and pathogen inocula on cold fresh fish fillets in olive oil, oregano, and lemon juice under modified atmosphere or air. *J. Food Prot*. **1996**, *59*, 31−34.

(26) Friedman, M.; Henika, P. R.; Mandrell, R. E. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica. J. Food Prot.* **2003**, *66*, 1811−1821.

(27) Al-Waili, N. S. Clinical and mycological benefits of topical application of honey, olive oil and beeswax in diaper dermatitis. *Clin. Microbiol. Infect*. **2005**, *11*, 160−163.

(28) Keceli, T.; Robinson, R. K.; Gordon, M. H. The role of olive oil in the preservation of yogurt cheese (labneh anbaris). *Int. J. Dairy Technol*. **1999**, *52*, 68−72.

(29) Puupponen-Pimiä, R.; Nohynek, L.; Meier, C.; Kähkönen, M.; Heinonen, M.; Hopia, A.; Oksman-Caldentey, K. M. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.* **2001**, *90*, 494−507.

(30) Scalbert, A.; Monrad, C.; Manach, C.; Rémésy, C. Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed. Pharmacother*. **2002**, *56*, 276−282.

(31) Simons, A. L.; Renouf, M.; Hendrich, S.; Murphy, P. A. Human gut microbial degradation of flavonoids: structure−function relationships. *J. Agric. Food Chem.* **2005**, *53*, 4258−4263.

(32) Pretty, I. A.; Gallagher, M. J.; Martin, M. V.; Edgar, W. M.; Higham, S. M. A study to asses the effects of a new detergent-free, olive oil formulation dentifrice in vitro and in vivo. *J. Dent*. **2003**, *31*, 327−332.

(33) Bianchi, G.; Pozzi, N. 3,4-dihydroxyphenylglycol, a major C_6−C_2 phenolic in *Olea europaea* fruits. *Phytochemistry* **1994**, *35*, 1335−1337.

(34) Renis, H. E. In vitro antiviral activity of calcium elenolate. *Antimicrob. Agents Chemother*. **1969**, 167−172.

(35) Rovellini, R.; Cortesi, N. Liquid chromatography−mass spectrometry in the study of oleuropein a ligstroside aglycon in virgin olive oil: aldehydic, dialdehydic forms and their oxidized products. *Riv. Ital. Sostanze Grasse* **2002**, *79*, 1−14.

(36) Kubo, I.; Matsumoto, A.; Takase, I. A multichemical defense mechanism of bitter olive *Olea europaea* (Oleaceae). Is oleuropein a phytoalexin precursor? *J. Chem. Ecol*. **1985**, *11*, 251−263.

(37) Furneri, P. M.; Piperno, A.; Saija, A.; Bisignano, G. Antimicoplasmal activity of hydroxytyrosol. *Antimicrob. Agents Chemother*. **2004**, *20*, 293−296.

(38) Paiva-Martins, F.; Gordon, M. H.; Gameiro, P. Activity and location of olive oil phenolic antioxidants in liposomes. *Chem. Phys. Lipids* **2003**, *124*, 23−36.

(39) Konno, K.; Hirayama, C.; Yasui, H.; Nakamura, M. Enzymatic activation of oleuropein: a protein cross-linker used as a chemical defense in the privet tree. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9159−9164.

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