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Antimicrobial and Antioxidant Activities of Turkish Extra Virgin Olive Oils

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Turkish extra virgin olive oils (EVOO) from different varieties/geographical origins and their phenolic compounds were investigated in terms of their antimicrobial and antioxidant properties in comparison to refined olive, hazelnut, and canola oils. Antimicrobial activity was tested against three foodborne pathogenic bacteria, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Enteritidis. Although all EVOOs showed a bactericidal effect, the individual phenolic compounds demonstrated only slight antimicrobial activity. Moreover, refined oil samples did not show any antimicrobial activity. Among the phenolic compounds, cinnamic acid (2 mg/kg of oil) had the highest percent inhibition value with 0.25 log reduction against *L. monocytogenes*. The synergistic interactions of tyrosol, vanillin, vanillic, and cinnamic acids were also observed against *Salmonella* Enteritidis. The antioxidant activities of oils were tested by β-carotene−linoleate model system and ABTS method. In both methods, EVOOs showed higher antioxidant activities, whereas refined oils had lower activity. The ABTS method provided a higher correlation (0.89) with total phenol content.

KEYWORDS: Olive oil; phenolic compounds; antimicrobial activity; antioxidant activity; *Escherichia coli* O157:H7; *Listeria monocytogenes*; *Salmonella* Enteritidis

INTRODUCTION

Olive oil is a major part of the diet of Mediterranean countries such as Spain, Italy, Greece, Tunisia, Turkey, Syria, and Portugal. In the past few years, olive oil has also become more popular among consumers in northern Europe, China, Japan, the United States, and Canada (*1*).

Virgin olive oil is the vegetable oil obtained from olive fruit by mechanical or other physical methods. If virgin olive oil has a free acidity of < 0.8 g/100 g in terms of oleic acid, it is designated extra virgin olive oil (EVOO). Refined olive oil is obtained from virgin olive oil by refining methods, which make olive oil with high acidity suitable for consumption (*2*).

Other edible oils that have a fatty acid composition similar to that of olive oil, such as sunflower, hazelnut, soybean, rapeseed, and canola oils, must be refined before consumption. This process changes their chemical compositions and causes loss of the most of the minor compounds. On the other hand, virgin olive oil is a natural juice of the olive fruit and has greater benefits for human health than those oils with similar fatty acid composition because of its high content of numerous micronutrients, particularly antioxidant molecules such as phenolic compounds, carotenes, and vitamin E (*3*).

Research on phenolic compounds showed that they play a role in the prevention of certain diseases such as cardiovascular heart diseases and cancers (*3*, *4*). Experiments carried out both in vitro and ex vivo have revealed that olive oil phenolics have greater antioxidant activity on oxidation of LDL and DNA compared to vitamin E (*5*, *6*). It has been indicated that short-term consumption of olive oils decreased the level of plasma oxidized LDL and increased the level of HDL cholesterol and glutathione peroxidase activity, in a dose-dependent manner with the phenolic content of the olive oil administered (*7*).

Phenolic compounds are also important in terms of virgin olive oil quality because of their contribution to oil flavor and aroma. They also protect the olive oil from oxidation through their antioxidant properties (*8*−*10*). Antioxidant activities of olives (*11*), olive oil (*12*, *13*), and olive mill waste extracts (*14*) were determined and associated with their phenolic content previously by other researchers.

In addition to their antioxidant properties, several studies showed that phenolic compounds also have antimicrobial properties by denaturing proteins and inactivating enzymes (*15*−*17*). It has been reported that phenolic compounds in olives and olive oil such as oleuropein, hydroxytyrosol, vanillin, and aliphatic aldehydes have the ability to inhibit or delay the growth of a range of bacteria (*18*, *19*) and fungi (*20*). Romero at al. reported that only the dialdehydic form of decarboxymethyl oleuropein aglycon and the dialdehydic form of decarboxymethyl ligstroside among the tested compounds showed antimicrobial activity against *Helicobacter pylori* (*21*). Moreover, antimicrobial activities of phenolic compounds found in olives (*22*) and wine (*23*, *24*) have been investigated in different studies, but phenolic concentrations were much higher than the levels determined in olive oil. Antimicrobial and antioxidant activities of olive oil have been studied, and these activities were correlated with the phenolic profiles of the oils (*12*, *13*, *25*). Although there are studies involving individual antimicrobial effects of olive oil phenolics as stated above, their synergistic and/or antagonistic interaction is not a well-researched area.

Turkey is one of the major olive oil producers, and there are several studies about the chemical and analytical properties of

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Turkish olive oils from Ayvalik and eastern Mediterranean cultivars (*26*−*29*). According to these reports variety and geographical origin determine both the major and minor component profiles of the olive oil. Our study evaluated the EVOOs that are economically the most important cultivars of Turkey. Furthermore, to the best of our knowledge, there is no report about antimicrobial activities of olive oils produced in Turkey.

The first purpose of this study was to evaluate the antimicrobial and antioxidant properties of olive oils obtained from various varieties of olives grown in different regions of Turkey along with the refined oils with similar fatty acid composition. In addition, the investigation of the antimicrobial activities of several individual olive oil phenolic compounds and their combinations was another aim of the study. In this paper, EVOOs from different regions (Altınoluk, Burhaniye, Dalaman, Gömeç, Koçarlı, and Ödemiş) and varieties (Erkence, Memecik, and Nizip) of Turkey and refined oil samples (refined olive oil and hazelnut and canola oils) were tested against three important foodborne pathogenic bacteria, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Enteritidis. Also, a group of phenolic compounds that were present in these EVOOs was evaluated for their antimicrobial activities against these bacteria as well. Total phenol content determination, β-carotene−linoleic acid model system, and ABTS radical scavenging assay were performed to evaluate antioxidant activities of all oil samples.

MATERIALS AND METHODS

Oil Samples. Nine EVOOs from different varieties and regions of Turkey were studied. Commercial EVOOs from the Aegean region of Turkey were provided by Tariş Olive and Olive Oil Cooperatives (Izmir, Turkey). These oils belong to Altınoluk, Burhaniye, Dalaman, Gömeç, Koçarlı, and Ödemiş districts.

The investigated Turkish EVOOs were produced from Erkence, Memecik, and Nizip olive varieties. Erkence and Memecik olives are native to the western coast of Turkey. On the other hand, Nizip is a high oil producing cultivar from southeastern Turkey. These oils were extracted using a small-scale olive oil mill (TEM Spermoliva, Italy). The olives used in extraction were hand-picked randomly at the same maturity level in the 2006 harvest year (*30*).

Refined oil samples, which are refined olive oil (Tariş, Izmir), hazelnut oil (Çotanak, Ordu), and canola oil (Olin, Edirne), were purchased from local markets. They were produced in 2008.

Oil samples in dark bottles were stored at 8 °C, and the headspaces were replaced by nitrogen after each use to prevent the deterioration of oils. About 20 min prior to analyses, the bottles were placed into a water bath at 20 °C to warm them to room temperature.

Microorganisms and Culture Conditions. All studied strains were purchased from the National Culture Type of Collection (NCTC, United Kingdom): *E. coli* O157:H7 NCTC 12900, *L. monocytogenes* NCTC 11994, and *S.* Enteritidis NCTC 12694. Luria broth (LB) and LB agar (Agar, Merck) for *E. coli*, brain heart infusion broth (BHI, Fluka) and BHI agar (Fluka) for *L. monocytogenes*, and tryptic soy broth (TSB, Fluka) and TSB agar (TSA, Merck) for *S.* Enteritidis were used as the growth media. LB was prepared with yeast extract (Fluka), tryptone (Fluka), and sodium chloride (Riedel-deHaen). A single colony of bacteria was inoculated in an appropriate medium. The overnight culture was transferred to fresh medium and incubated until the culture reached exponential phase. Spectrophotometric measurement and viable cell count methods were used to adjust bacterial cultures to the desired concentrations.

Analysis of Antimicrobial Activity. *Antimicrobial Activity of Oils.* The antimicrobial activity of oils was determined as previously reported (*25*) with minor modifications. Each EVOO sample was tested as follows: a mixture of 900 μL of sterilized phosphate-buffered saline with Tween 20 (PBST, pH 7.0) and 1 mL of oil sample was inoculated with 100 μL of bacterial culture to obtain an initial concentration of 5×10^3 cfu/mL. To examine the antimicrobial effect of buffer extract of oil, test tubes containing oil and PBST were shaken for 1 h at 200 rpm at 37 °C in an orbital shaker (GLF, Germany) and centrifuged at 2000 rpm (Sigma, Germany) for 1 min. Then, the aqueous phase, free of oil, was transferred into another test tube and inoculated. All tubes were shaken for 1 h at 200 rpm at 37 °C. After treatment, survivors were determined by viable cell count method. All controls were performed, and all tests were repeated in duplicate.

In addition, Burhaniye and Nizip EVOOs along with the refined olive oil and hazelnut and canola oils were tested with the increased initial bacterial concentration of 1×10^5 cfu/mL. Two EVOOs (Burhaniye and Nizip) were further tested for a shorter treatment time of 30 min.

Also, Burhaniye, Dalaman, and Nizip EVOOs were tested with the same procedure except with a 5×10^6 cfu/mlL initial bacterial concentration and a 5 min treatment time.

Antimicrobial Activities of Phenolic Compounds

(*a*) *Determination of Individual Antimicrobial Activity of Phenolics.* Antimicrobial activities of phenolic compounds were determined by a spectrophotometric microtiter plate method (*31*). The phenolic compound profiles of all of the EVOOs tested in this study were determined by reversed phase HPLC-DAD analysis (*32*) previously. Phenolic solutions of each compound were prepared in various concentration ranges according to this previous work (*32*). In addition, some phenols were tested in higher concentrations because generally their levels in olives are higher than those in oils. Each of these compounds was dissolved in ethanol and then diluted to the target concentration with the appropriate growth medium. The ethanol content of all solutions was decreased below 1% (v/v) during dilutions. All solutions were prepared fresh before the experiments.

One hundred microliters of each phenolic solution and 100 μL of 1×10^4 cfu/mL bacterial cultures in logarithmic growth phase were dispensed into a well of a flat-bottom 96-well microtiter plate (Bio-Grainer, Germany). Appropriate blanks and controls were also prepared. Then, absorbance measurements of each plate were taken in 3 h intervals by a Thermo Multiscan Spectra Reader (Finland) at 600 nm during incubation at 37 °C for 24 h.

(*b*) *Determination of Logarithmic Reduction of L. monocytogenes Exposed to Cinnamic Acid.* The microtiter plate was prepared as in the method for antimicrobial activities of phenolic compounds. The growth of *L. monocytogenes* was observed by measuring the absorbance at 4 h intervals for 24 h. At the same time, the solutions in the wells were plated after incubation times of 0, 8, 12, 16, and 24 h. Bacterial enumeration from each sampling time point was determined by viable cell count method. Then, the log reduction was evaluated by comparing the data obtained from the control sample.

(*c*) *Combinational Antimicrobial Activities.* The method for individual antimicrobial activities of phenolic compounds was modified to determine the synergistic interactions between four phenolic compounds ($k = 4$): tyrosol, vanillin, vanillic acid, and cinnamic acid. A two-level full-factorial design was applied with 9 center points (CP = 9) and 3 replications ($n = 3$) of each treatment for a total of 57 experiments ($N = n2^k + \mathrm{CP} = 3.2^4 + 9 = 57$).

A 25 μL sample of each solution and 100 μL of bacterial culture were added to each well of a microtiter plate to obtain a final volume of 200 μL as explained previously. All tests were performed with the appropriate controls. The absorbance measurements were taken at 600 nm at 3 h intervals during incubation at 37 °C for 24 h.

Analysis of Antioxidant Activity. *Methanolic Extraction of Olive Oil.* To prepare methanolic extracts of oils, 2 g of each oil sample, 10 mL of 80% v/v methanol, and 30 μL of Tween 20 (Sigma-Aldrich) were homogenized at 15000 rpm for 1 min (Heidolph Silent Crusher M Homogenizer, Germany). The homogenized mixture was centrifuged at 5000 rpm for 10 min at room temperature. Supernatant was collected in a graduated cylinder, and the oil phase was transferred to the beaker. Homogenization and centrifugation processes were repeated two more times with the same oil sample. At the end of three extraction cycles, about 30 mL of methanolic extract was collected in the graduated cylinder. Extracts were freshly prepared before each experiment.

β-Carotene−Linoleate Model System. The antioxidant activity of oil extracts was evaluated by the β-carotene−linoleate model system (*33*). After β-carotene (2.0 mg) had been dissolved in 10 mL of chloroform, 20 μL of linoleic acid and 200 mg of Tween 40 were added into 1 mL of the solution. Chloroform was removed at 45 °C under vacuum, then 50 mL of distilled water was added, and the mixture was vigorously shaken to form a stable emulsion. The emulsions were freshly prepared before each experiment.

Table 1. Antimicrobial Activity of Oils against Different Bacterial Concentrations and Treatment Time[a]

IC:	5×10^3 cfu/mL	1×10^5 cfu/mL					1×10^5 cfu/mL		5×10^6cfu/mL		
time:	1 h	1 h					30 min		5 min		
oil: MO	EVOOs	Burhaniye	Nizip	refined olive	canola	hazelnut	Burhaniye	Nizip	Burhaniye	Dalaman	Nizip
E. coli	NS	NS	NS	0.32[b]	0.16[b]	0.37[b]	NS	NS	5.99[b]	5.03[b]	0.22[b]
*S.*Enteritidis	NS	NS	NS	NLR	NLR	NLR	NS	NS	6.71[b]	2.70[b]	0.80[b]
L. monocytogenes	NS	NS	NS	0.16[b]	NLR	NLR	NS	NS	NS	NS	0.23[b]

[a] IC, initial bacterial concentration in test tube; MO, microorganism name; EVOOs, each of nine EVOO samples; NLR, no log reduction; NS, no survivors. [b] Log reduction: $\log(N_i/N_f)$, where N_i = cfu/mL initial bacteria concentration in test tube and N_f = cfu/mL final bacteria concentration.

An aliquot (250 μL) of β-carotene−linoleic acid emulsion and methanolic extracts (30 μL) of oils were dispensed into each of the 96 wells of the microtiter plate. Methanolic solution of butylated hydroxyanisole (BHA, 25−50 ppm) and methanol were also used as standard and control, respectively. As soon as the samples were added to the wells, the zero time absorbance was measured at 460 nm. The measurements were taken every 15 min for 180 min during incubation at 45 °C by a Thermo Multiscan Spectra Reader (Finland). Experiments were performed three times with three replicates for each sample.

The antioxidant activity (AA) of oil extracts was evaluated in terms of bleaching of the β-carotene using the formula

$$\mathrm{AA} = 100[1 - (A_0 - A_t)/(A'_0 - A'_t)]$$

where A_0 and A'_0 are the absorbance values at zero time of the incubation for sample and control, respectively, and A_t and A'_t are the absorbance values measured for sample and control, respectively, after incubation for 180 min.

ABTS Radical Scavenging Method. The determination of antioxidant activity by ABTS radical scavenging method was performed according to the procedure described by others (*13*, *34*) with minor modifications. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution (1.8 mM) with 0.63 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12−16 h before use. Then, the solution was diluted with ethanol to obtain an absorbance of 0.700 (±0.030) at 734 nm. Measurements were performed at ambient temperature. Oil extracts were diluted at a ratio of 1:10 with methanol (80%). Later, 190 μL of radical solution was mixed with 10 μL of diluted extracts in a microtiter plate. The absorbance at 734 nm was measured every minute for 13 min following initial mixing. Appropriate solvent blanks were run in each assay. BHA (2.5 ppm) and methanol (80%) were used as the standard antioxidant and the negative control, respectively. Experiments were performed three times with three replicates for each sample. The percent free radical scavenging activity (%FRSA) was calculated according to the formula

$$\%\mathrm{FRSA} = [(A_n - A_s) \times 100]/A_n$$

where A_n is the final absorbance value of negative control and A_s is the final absorbance value of sample.

Determination of Total Phenolic Contents of Refined Olive Oil, Hazelnut and Canola Oils. Total phenolic contents of oil samples were determined by Folin−Ciocalteu method, which is based on the reaction of a phosphowolframate−phosphomolybdate complex by phenolics to blue reaction products (*35*). The mixture was kept in the dark at ambient temperature, and then absorbance was measured at 765 nm and TPC values were calculated using gallic acid as the standard.

Statistical Analysis. Antimicrobial activity results of oil samples were calculated from the reduction in the numbers of bacteria that were transformed into $\log_{10}$. The experimental data of combinational antimicrobial activity test were analyzed with MODDE 8 (Umetrics, Umea, Sweden).

RESULTS AND DISCUSSION

Antimicrobial Activity of Oils. The antimicrobial properties of nine different types of EVOO samples, refined olive oil, and hazelnut and canola oils were tested against three foodborne pathogens, *E. coli* O157:H7, *L. monocytogenes*, and *S,* Enteritidis (**Table 1**). Hazelnut and canola oils and refined olive oil were tested because they have fatty acid compositions very similar to that of EVOO but their phenolics contents are different. All EVOO samples tested with a bacterial concentration of 5×10^3 cfu/mL for 1 h of treatment time showed strong antimicrobial activity against the three organisms. According to Folin−Ciocalteu analysis, Burhaniye and Nizip EVOOs have the highest and lowest TPC values, respectively (*32*). Therefore, these EVOOs were tested against a higher concentration of culture (1×10^5 cfu/mL) for 1 h. The same conditions were also applied to refined oils. Previously, the antimicrobial activity of fatty acids and their derivatives has been demonstrated (*36*, *37*). Although refined olive, hazelnut, and canola oils have fatty acid compositions similar to that of olive oil (*3*), they did not cause any significant

Table 2. Percent Inhibition in the Growth Rate of *E. coli* O157:H7 in the Presence of Phenolic Compounds

phenolic compound	concentration (mg/kg of oil)	% inhibition
cinnamic acid	0.05	2.05 ± 1.59
	0.10	4.62 ± 1.36
	1.00	5.39 ± 1.42
	1.50	6.00 ± 0.81
	2.00	5.35 ± 0.13
ferulic acid	0.55	2.62 ± 1.37
4-hydroxybenzoic acid	0.02	2.04 ± 1.18
	0.05	4.95 ± 0.01
	0.10	6.44 ± 0.14
	0.15	8.47 ± 3.47
luteolin	1.0	2.64 ± 0.29
	1.5	2.86 ± 0.16
	2.0	4.38 ± 0.32
syringic acid	0.1	3.74 ± 0.94
	0.2	7.81 ± 0.12
	0.8	9.88 ± 0.48
tyrosol	1.0	1.02 ± 0.07
	2.5	1.09 ± 0.68
	4.0	2.82 ± 2.43
	5.5	4.30 ± 0.79
	7.0	5.79 ± 0.10
	8.5	6.29 ± 1.55
vanillic acid	0.05	6.93 ± 0.12
	0.15	11.50 ± 0.02
	0.20	13.13 ± 2.52
vanillin	0.05	7.49 ± 3.08
	0.10	7.51 ± 2.74
	0.20	8.39 ± 1.74
	0.35	10.42 ± 0.95
	0.50	10.52 ± 0.60
	1386	80.16 ± 0.54

Table 3. Percent Inhibition in the Growth Rate of *L. monocytogenes* in the Presence of Phenolic Compounds

phenolic compound	concentration (mg/kg of oil)	% inhibition
cinnamic acid	0.05	6.93 ± 0.13
	0.10	14.29 ± 0.09
	0.50	17.34 ± 0.06
	1.00	19.36 ± 0.00
	1.50	23.23 ± 0.01
	2.00	27.68 ± 0.61
ferulic acid	0.10	2.12 ± 0.02
	0.25	8.14 ± 0.31
	0.40	9.68 ± 0.69
	0.55	9.99 ± 1.16
4-hydroxybenzoic acid	0.02	13.42 ± 0.00
	0.05	16.55 ± 1.76
	0.10	19.53 ± 0.07
	0.15	20.69 ± 0.07
luteolin	0.5	5.29 ± 0.12
	1.0	7.51 ± 0.47
	1.5	10.90 ± 0.50
	2.0	11.49 ± 0.09
	2.5	15.45 ± 0.67
	3.0	19.05 ± 0.20
syringic acid	0.1	9.14 ± 0.18
	0.2	9.92 ± 0.03
	0.3	12.12 ± 0.09
	0.4	13.38 ± 0.05
	0.8	15.28 ± 0.11
tyrosol	1.0	3.54 ± 0.26
	2.5	12.27 ± 1.57
	5.5	12.63 ± 0.03
	7.0	13.16 ± 0.64
	8.5	13.45 ± 0.55
vanillic acid	0.05	6.97 ± 0.20
	0.10	9.39 ± 0.09
	0.15	13.78 ± 0.17
	0.20	14.22 ± 1.18
	0.25	15.96 ± 0.01
vanillin	0.05	7.63 ± 0.10
	0.10	11.15 ± 0.40
	0.20	11.37 ± 0.20
	0.35	12.03 ± 0.19
	0.50	14.88 ± 0.04

decrease in the microbial population, whereas EVOOs showed bactericidal activity against all three microorganisms. This difference could be due to the fact that virgin olive oils contain high concentrations of phenolic compounds but refined oils do not (*38*). This result is consistent with a previous paper (*25*). When treatment time was decreased to 30 min, tests with Burhaniye and Nizip EVOOs still showed bactericidal activity.

To see the limits of the antimicrobial activity of EVOOs, treatment time was decreased to 5 min and the initial bacterial concentration was increased to 5×10^6 cfu/mL. Because there is a significant difference between the TPC of Burhaniye (342.93 mg of GA/kg of oil) and Nizip (125.29 mg of GA/kg of oil), Dalaman EVOO, which has an average TPC value of 277.99 mg of GA/kg of oil among nine EVOOs, was also tested. As a result, Burhaniye was still bactericidal against *L. monocytogenes*, but a few survivor colonies were observed after treatment against *E. coli* O157:H7 and *S.* Enteritidis. On the other hand, Nizip became ineffective in these conditions, as it caused < 1 log reduction. Dalaman EVOO was bactericidal against *L. monocytogenes*; however, it was not as effective as Burhaniye against *E. coli* O157:H7 and *S.* Enteritidis.

Table 4. Percent Inhibition in the Growth Rate of *S.* Enteritidis in the Presence of Phenolic Compounds

phenolic compound	concentration (mg/kg of oil)	% inhibition
cinnamic acid	0.05	8.27 ± 0.05
	0.10	9.70 ± 0.04
	0.50	11.14 ± 0.03
	1.50	11.02 ± 0.03
	2.00	11.13 ± 0.13
4-hydroxybenzoic acid	0.05	4.57 ± 2.19
	0.10	6.80 ± 0.28
	0.15	10.86 ± 0.92
ferulic acid	0.25	0.96 ± 1.21
	0.55	0.97 ± 0.77
luteolin	0.5	1.73 ± 0.07
	1.0	3.84 ± 0.31
	1.5	4.52 ± 0.84
	2.0	5.70 ± 0.71
syringic acid	0.1	3.24 ± 2.90
	0.2	5.65 ± 0.42
	0.3	5.72 ± 0.64
	0.4	6.80 ± 0.99
	0.8	7.94 ± 1.13
tyrosol	1.0	5.27 ± 0.30
	2.5	10.47 ± 0.06
	4.0	10.52 ± 0.14
	5.5	11.38 ± 0.07
	7.0	11.64 ± 0.22
	8.5	11.20 ± 0.03
vanillic acid	0.05	0.25 ± 3.39
	0.10	2.67 ± 0.85
	0.15	6.86 ± 0.35
	0.20	6.80 ± 1.91
vanillin	0.05	0.75 ± 0.06
	0.10	3.10 ± 0.06
	0.20	3.24 ± 0.25
	0.35	4.83 ± 0.07

Determination of Individual Antimicrobial Activity of Phenolics. The percent inhibition in growth rate at the end of 24 h of incubation was calculated for each phenolic compound existing in olive oil against *E. coli* O157:H7 (**Table 2**), *L. monocytogenes* (**Table 3**), and *S.* Enteritidis (**Table 4**), approximately in the same concentration range in which they are present in olive oil. These values were calculated by the reduction in the optical cell density of each sample with reference to the control that received no phenolic compounds.

Almost all tested phenolic compounds showed higher activity against *L. monocytogenes*, but a weaker effect against *E. coli* O157:H7 and *S.* Enteritidis. Therefore, it seemed that phenolic compounds were more active against Gram-positive organisms than against Gram-negative organisms. Our finding supports previous studies (*25*) that reported that Gram-positive bacteria are more prone to the action of oil extracts than Gram-negative bacteria. It was observed that some compounds were more effective against *E. coli* O157:H7, whereas others were more effective against *S.* Enteritidis. For example, 7 mg/kg oil of tyrosol caused 11.64% inhibition against *S.* Enteritidis and 5.79% inhibition against *E. coli* O157:H7. However, whereas 0.20 mg/kg oil of

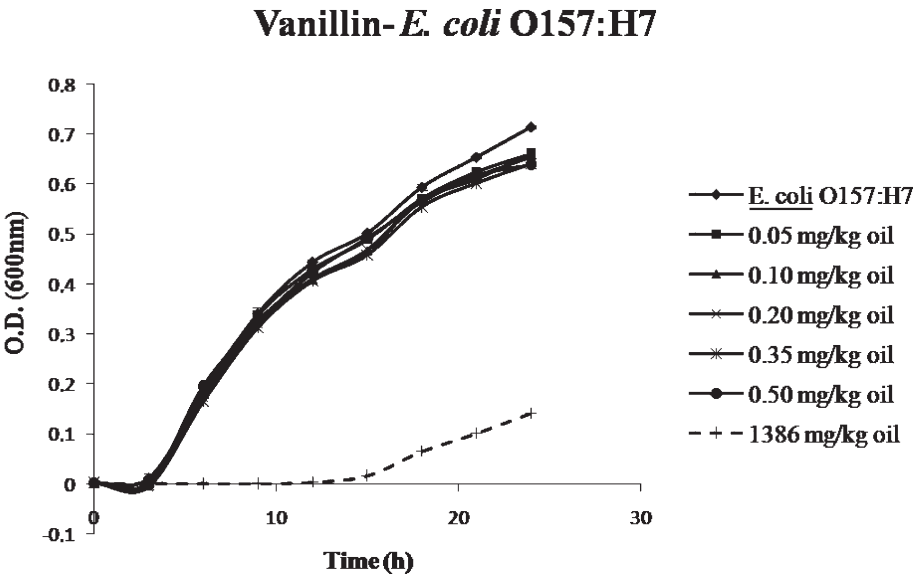

Figure 1. Growth curve of *E. coli* O157:H7 in the presence of vanillin. Standard deviation of measurements was found <0.03.

vanillic acid caused 13.13% inhibition in the growth of *E. coli* O157:H7, it resulted in 6.80% inhibition against *S.* Enteritidis.

The reported minimum concentrations of phenolic compounds that inhibit the bacterial growth are much higher than those found in olive oils. In the study by Tunçel and Nergis (*22*), minimum inhibitory concentrations of *o*-coumaric, ferulic, vanillic, and syringic acid and tyrosol against *E. coli* O157:H7 were determined as 450, 450, 550, 550, and 600 μg/mL, respectively, by the agar dilution method. These concentrations are about 1000 times higher than the levels of those in EVOOs. The antimicrobial activities of ferulic, vanillic, *p*-coumaric, and 4-hydroxybenzoic acids, which are also found in wine, were tested on *Campylobacter jejuni*. Whereas 4-hydroxybenzoic acid was found to be very effective at the lowest concentration (1 mg/L), vanillic, ferulic, and *p*-coumaric acid could show activity at concentrations of 10, 10, and 100 mg/L, respectively (*23*). In the paper of Rodriguez Vaquero et al. (*24*), vanillic acid was tested against *L. monocytogenes*. An inhibition of 10% in the final cell density after 18 h of incubation was observed by the addition of 50 mg/mL vanillic acid in 5% ethanol. An important point, in these studies, is that all tested solutions contained 5% ethanol, and this could be the reason for such high activity. In our study, vanillic acid at a concentration of 25 mg/kg oil (~23 mg/kg oil, with ethanol content lower than 1% (v/v)) was tested against *L. monocytogenes*. As a result, 4.95 and 23% inhibition was observed after 18 and 24 h of incubation, respectively. Our results are in agreement with these previous studies.

In the current study, it was found out that the effect of ferulic acid, in the concentration ranges determined in EVOOs, was not significant against *E. coli* O157:H7 (maximum 2.62% inhibition) and *S.* Enteritidis (maximum 0.97% inhibition). However, a stronger effect was observed against *L. monocytogenes* (**Table 3**). Also, higher concentrations (5.5 and 55.0 mg/kg oil) of ferulic acid caused only 3% inhibition in the growth of *E. coli* and *S.* Enteritidis, whereas they caused 12−16% inhibition on *L. monocytogenes*.

In general, our results indicate that the activities of all tested compounds were concentration dependent, but not directly proportional. Also, the activities of some compounds did not change with increased concentrations. For example, although the concentration of tyrosol was increased from 2.5 to 8.5 mg/kg oil, antimicrobial activity against *S.* Enteritidis and *L. monocytogenes* did not show any significant change. On the other hand, its activity against *E. coli* O157:H7 increased depending on concentration.

Cinnamic acid (0.50−2.00 mg/kg oil) was the most effective phenolic compound against *S.* Enteritidis, and tyrosol (5.5 and 7.0 mg/kg oil) also caused about 11% inhibition (**Table 4**).

Generally, all of the growth curves in the presence of phenolic compounds were similar to each other, and the lines were too close to discriminate them from one another. **Figure 1** is given as an example of the growth curve of *E. coli* O157:H7 in the presence of vanillin at the concentrations of 0, 0.05, 0.10, 0.20, 0.35, and 1386 mg/kg oil. The concentrations between 0.05 and 0.35 mg/kg oil are in the range of determined concentrations in EVOOs, and they yielded between 7.49 and 10.42% inhibition in the growth rate. Rupasinghe et al. (*19*) reported that the minimum inhibitory concentrations of vanillin against eight ATCC strains were between 6 mM (1040 mg/kg oil) and 12 mM (2079 mg/kg). In agreement with our results (**Figure 1**), it was demonstrated that 6 mM (1040 mg/kg oil) vanillin caused 73% inhibition in the growth rate of *E. coli*. In this study, 8 mM (~1386 mg/kg oil) vanillin caused 80% inhibition in the growth rate of *E. coli* O157:H7. In addition, such a high concentration of vanillin showed a bacteriostatic activity against *E. coli* O157:H7 during 15 h of incubation period. According to these results, the use of these phenolic compounds as antimicrobial agent in food could be considered. For example, vanillin is already used as an aromatic supplement in foods. Its potential use for the extension of the shelf life of foods could be suggested by means of its antimicrobial properties.

Determination of Logarithmic Reduction of *L. monocytogenes* Exposed to Cinnamic Acid. The level of cinnamic acid in Erkence EVOO was determined as 1.98 mg/kg of oil, which was the highest cinnamic acid concentration among all EVOO samples (*32*). Although 2.00 mg/kg oil cinnamic acid showed the highest percent inhibition (27%), it decreased the number of bacteria by 0.25 log at the end of 24 h of incubation. It can be concluded that the other phenolic compounds have very low log reduction effect, in comparison with cinnamic acid.

Combinational Antimicrobial Activities. To the best of our knowledge there is no published research about synergistic antimicrobial effect of phenolic compounds of olive oil.

A two-level factorial design was applied to reveal the main and interaction effects among the four phenolics. The experimental data revealed that the high inhibition rates were found at the

combinations where one phenolic compound was at low level, whereas the second one was at the high level (*39*). The result of ANOVA is given in **Table 5**. Factors and interactions with $p < 0.05$ were considered to be significant. In this study, the interactions between vanillic acid and cinnamic acid, between vanillic acid and tyrosol, and between cinnamic acid and tyrosol were found to be significant. Moreover, only vanillin was found to be significant individually.

It seems that because olive oil contains more than 30 different phenolic compounds in its composition, an increase of their overall effect due to the synergistic interaction or the sum of their individual antimicrobial effects is also very possible.

Total Phenolic Contents of Oils. TPC values of refined oil samples were as follows: refined olive oil is 91.67 ± 1.30 mg of GA/kg of oil, hazelnut oil is 41.67 ± 5.18 mg of GA/kg of oil, and canola oil is 58.88 ± 2.68 mg of GA/kg of oil. As expected, the phenolic content of refined oil samples was lower than those of EVOOs, because the refining process causes loss of phenolic compounds (*1*). TPC values of EVOO samples used in this study were determined to be between 125.29 and 353.36 mg of GA/kg of oil (*30*). In a study on the determination of TPC of EVOO, olive oil, and highly refined olive oil, it has been reported that TPC values for EVOO samples were between 73 and 265 mg of GA/kg of oil, whereas olive oil had TPC values of 14−30 mg of GA/kg of oil and the TPC of refined olive oil was 4 mg of GA/kg of oil (*40*).

Table 5. Results of ANOVA for the Effect of Phenolic Compounds

factors and interactions	*p* value
constant	0.000
vanillin	0.038[a]
vanillic acid	0.208
cinnamic acid	0.486
tyrosol	0.448
vanillin and vanillic acid	0.715
vanillin and cinnamic acid	0.404
vanillin and tyrosol	0.779
vanillic acid and cinnamic acid	0.000[a]
vanillic acid and tyrosol	0.000[a]
cinnamic acid and tyrosol	0.000[a]

[a] Significant parameters; *p* value <0.05.

Table 6. Antioxidant Activity (%AA) by β-Carotene Method and Percent Free Radical Scavenging Activity (%FRSA) Results of EVOOs, Refined Olive Oil, Hazelnut and Canola Oils, and Standard Solution (BHA)

oil extract	%AA	oil extract[a]	%FRSA
Erkence	64.54 ± 5.42	Erkence	21.97 ± 3.34
Burhaniye	60.58 ± 2.07	Burhaniye	16.68 ± 0.46
Koçarlı	57.93 ± 3.26	Koçarlı	13.95 ± 1.24
Ödemiş	42.91 ± 2.55	Ödemiş	13.20 ± 0.44
Dalaman	38.49 ± 5.61	Dalaman	11.79 ± 1.49
Gömeç	45.54 ± 2.75	Gömeç	10.70 ± 2.10
Altınoluk	53.38 ± 6.66	Altınoluk	9.93 ± 1.81
Memecik	29.83 ± 6.56	Memecik	5.34 ± 1.44
Nizip	21.22 ± 1.92	Nizip	5.60 ± 0.98
refined olive	23.50 ± 0.55	refined olive	3.95 ± 1.09
canola	22.52 ± 9.92	canola	1.55 ± 1.63
hazelnut	21.19 ± 1.14	hazelnut	1.31 ± 0.90
standard solution		standard solution	
BHA 50 ppm	85.43 ± 2.26	BHA 2.5 ppm	6.35 ± 0.20
BHA 25 ppm	74.76 ± 0.65		

[a] 1:10 diluted oil extracts.

Antioxidant Activity. In the β-carotene−linoleic acid method, the antioxidant activity level of a substance is determined by measuring oxidation products of linoleic acid that simultaneously attack β-carotene, resulting in bleaching of its characteristic yellow color (*12*, *41*). The results of this method are listed in **Table 6**. Activities of EVOO samples varied between 21.22 and 64.54%. These results are consistent with previous papers (*12*, *13*). In these studies, antioxidant activities were also found to be about 40% for EVOOs.

The $ABTS^{\bullet+}$ scavenging capacity method is a decolorization assay that measures the capacity of antioxidants directly reacting with $ABTS^{\bullet+}$ radicals generated by a chemical method (*42*). The results for ABTS scavenging capacity method were expressed as %FRSA values, which varied between 1.31 and 21.97% (**Table 6**).

As a result of both assays, Erkence EVOO, which has the highest TPC, showed the highest antioxidant activity. Refined olive, hazelnut, and canola oils have lower antioxidant activity values, which might be attributed to the low TPC content of these oils.

It has been reported that there is no single method that yields fully and reliably the antioxidant capacity of a complex mixture such as olive oil (*13*). In this study, when the results were ordered from high value to low, there was a difference in the sequence of the oils. This might be due to the difference in the working principals of the two methods. The ABTS method evaluates the activity directly, in contrast to the β-carotene assay.

In many studies, it was demonstrated that there is a correlation between total phenol content and antioxidant activity of oils (*12*, *13*). Although it has been reported that the β-carotene−linoleic acid model system was the best method for the determination of the antioxidant capacity of olive oils (*12*), this method provided lower correlation ($R^2 = 0.8075$) in this study (**Figure 2A**). The best correlation between TPC and antioxidant capacity was found by the $ABTS^{\bullet+}$ method ($R^2 = 0.9082$) (**Figure 2B**). This result is consistent with a previous paper determining the antioxidant activity of olive oil by four different methods, ABTS, DPPH, ORAC, and β-carotene methods (*13*). According to this study, the best correlation was obtained with the ABTS method ($R^2 = 0.8927$) and the worst was with the β-carotene method ($R^2 = 0.7258$).

In conclusion, the use of EVOOs in foods might be beneficial in terms of preventing foodborne diseases that arise from *E. coli* O157:H7, *L. monocytogenes*, and *S.* Enteritidis. As far as we know, this is the first study indicating antimicrobial and antioxidant

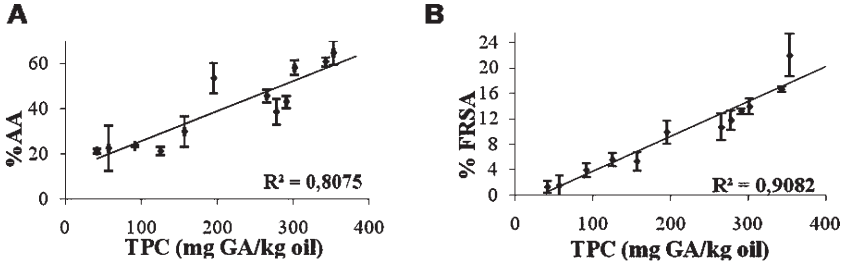

Figure 2. Correlation between total phenolic contents of oils and their antioxidant capacity using (**A**) the β-carotene−linoleic acid model system and (**B**) the ABTS method.

activities of Turkish EVOOs from different regions/varieties and directly correlating the antimicrobial activities with their phenolic content.

LITERATURE CITED

(1) Boskou, D. Olive oil. In *Vegetable Oils in Food Technology: Composition, Properties and Uses*; Gunstone, F. D., Ed.; Blackwell Publishing: Boca Raton, FL, 2002; pp 244−245.

(2) International Olive Oil Council. Designations and definitions of olive oils; http://www.internationaloliveoil.org/web/aa-ingles/oliveWorld/aceite.html, 2008 (accessed 2010).

(3) Waterman, E.; Lockwood, B. Active components and clinical applications of olive oil. *Altern. Med. Rev.* **2007**, *12*, 331–42.

(4) 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.

(5) Fito, M.; De la Torre, R.; Farre-Albaladejo, M.; Khymenetz, O.; Marrugat, J.; Covas, M. I. Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. *Ann. Ist. Super. Sanita* **2007**, *43*, 375–381.

(6) Masella, R.; Vari, R.; DArchivio, M.; Di Benedetto, R.; Matarrese, P.; Malorni, W.; Scazzochio, B.; Giovannini, C. Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. *J. Nutr.* **2004**, *134*, 785–791.

(7) Weinbrenner, T.; Fito, M.; de la Torre, R.; Saez, G. T.; Rijken, P.; Tormos, C.; Coolen, S.; Albaladejo, M. F.; Abanades, S.; Schroder, H.; Marrugat, J.; Covas, M. I. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J. Nutr.* **2004**, *134*, 2314–2321.

(8) Haddada, F. M.; Krichene, D.; Manai, H.; Oueslati, I.; Daoud, D.; Zarrouk, M. Analytical evaluation of six monovarietal virgin olive oils from northern Tunisia. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 905–913.

(9) Blekas, G.; Psomiadou, E.; Tsimidou, M.; Boskou, D. On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 340–346.

(10) Keceli, T.; Gordon, M. H. The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil. *J. Sci. Food Agric.* **2001**, *81*, 1391–1396.

(11) Pereira, J. A.; Pereira, A. P. G.; Ferreira, I. G. F. R.; Valentap, P.; Andrade, P. B.; Seabra, R.; Estevinho, L.; Bento, A. Table olives from Portugal; phenolic compounds, antioxidant potential and antimicrobial activity. *J. Agric. Food Chem.* **2006**, *54*, 8425–8431.

(12) Gorinstein, S.; Martin-Belloso, O.; Katrich, E.; Lojek, A.; Ciz, M.; Gligelmo-Miguel, N.; Haruenkit, R.; Park, Y. S.; Junk, S. T.; Trackhtenberg, S. Comparison of the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils as determined by four different radical scavenging tests. *J. Nutr. Biochem.* **2003**, *14*, 154–159.

(13) Sanchez, C. S.; Gonzalez, A. M. T.; Garcia-Parrilla, M. C.; Granados, J. J. Q.; de la Serrana, H. L. G.; Martinez, M. C. L. Different radical scavenging tests in virgin olive oil and their relation to the total phenol content. *Anal. Chim. Acta* **2007**, *593*, 103–107.

(14) Obied, H. K.; Bedgood, D. R., Jr.; Prenzler, P. D.; Robards, K. Bioscreening of Australian olive mill waste extracts: biophenol content, antioxidant, antimicrobial and molluscicidal activities. *Food Chem. Toxicol.* **2007**, *45*, 1238–48.

(15) Rawel, H. M.; Meidtner, K.; Kroll, J. Binding of selected phenolic compounds to proteins. *J. Agric. Food Chem.* **2005**, *53*, 4228–4235.

(16) Ortega-Garcia, F.; Blanco, S.; Angeles Peinado, M.; Peragon, J. Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. 'Picual' trees during fruit ripening. *Tree Physiol.* **2008**, *28*, 45–54.

(17) Cushnie, T. P. T.; Lamb, A. J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **2005**, *26*, 343–356.

(18) Bisignano, G.; Tomaino, A.; Cascio, R. L.; Crisafi, G.; Uccella, N.; Saija, A. On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. *J. Pharm. Pharmacol.* **1999**, *51*, 971–974.

(19) Rupasinhe, H. P. V.; Boulter-Bitzer, J.; Ahn, T.; Odumeru, J. A. Vanillin inhibits pathogenic and spoilage microorganisms in vitro and aerobic microbial growth in fresh-cut apples. *Food Res. Int.* **2006**, *39*, 575–580.

(20) Battinelli, L.; Daniele, C.; Cristiani, M.; Bisignano, G.; Saija, A.; Mazzanti, G. In vitro antifungal and anti-elastase activity of some aliphatic aldehydes from *Olea europaea* L. fruit. *Phytomedicine* **2006**, *13*, 558–563.

(21) Romero, C.; Medina, E.; Vargas, J.; Brenes, M.; De Castro, A. In vitro activity of olive oil polyphenols against *Helicobacter pylori*. *J. Agric. Food Chem.* **2007**, *55*, 680–686.

(22) Tunçel, G.; Nergiz, C. Antimicrobial effect of some olive phenols in a laboratory medium. *Lett. Appl. Microbiol.* **1993**, *17*, 300–302.

(23) Ganan, M.; Martinez-Rodriguez, A. J.; Carrascosa, A. V. Antimicrobial activity of phenolic compounds of wine against *Campylobacter jejuni*. *Food Control* **2009**, *20*, 739–742.

(24) Rodriguez Vaquero, M. J.; Alberto, M. R.; Manca de Nadra, M. C. Influence of phenolic compounds from wines on the growth of *Listeria monocytogenes*. *Food Control* **2007**, *18*, 587–593.

(25) Medina, E.; De Castro, E.; Romero, C.; Brenes, M. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. *J. Agric. Food Chem.* **2006**, *54*, 4954–4961.

(26) Kiralan, M.; Bayrak, A.; Ozkaya, M. T. Oxidation stability of virgin olive oils from some important cultivars in East Mediterranean area in Turkey. *J. Am. Oil Chem. Soc.* **2009**, *86*, 247–252.

(27) Ilyasoglu, H.; Ozcelik, B.; Van Hoed, V.; Verhe, R. Characterization of Aegean olive oils by their minor compounds. *J. Am. Oil Chem. Soc.* **2010**, *87*, 627–636.

(28) Andjelkovic, M.; Acun, S.; Van Hoed, V.; Verhé, R.; Van Camp, J. Chemical composition of Turkish olive oil − Ayvalik. *J. Am. Oil Chem. Soc.* **2009**, *86*, 135–140.

(29) Gurdeniz, G.; Ozen, B.; Tokatli, F. Classification of olive oils with respect to cultivar, geographic origin and harvest year, using fatty acid profile and mid-IR spectroscopy. *Eur. Food Res. Technol.* **2008**, *227*, 1275–1281.

(30) Ocakoglu, D.; Tokatli, F.; Ozen, B.; Korel, F. Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. *Food Chem.* **2009**, *113*, 401–410.

(31) Dufour, M.; Simmonds, R. S.; Bremer, P. J. Development of a method to quantify in vitro the synergistic activity of "natural" antimicrobials. *Int. J. Food Microbiol.* **2003**, *85*, 249–258.

(32) Ocakoglu, D. *Classification of Turkish Virgin Olive Oils Based on Their Phenolic Profiles*; Izmir Institute of Technology, Department of Food Engineering, 2008; pp 47−93

(33) Deba, F.; Xuan, T. D.; Yasuda, M.; Tawata, S. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. *Food Chem.* **2008**, *19*, 346–352.

(34) Ling, L. T.; Yap, S. A.; Radhakrishnan, A. K.; Subramaniam, T. Standardized *Mangifera indica* extract is an ideal antioxidant. *Food Chem.* **2009**, *113*, 1154–1159.

(35) Luther, M.; Parry, J.; Moore, J.; Meng, J.; Zhang, Y.; Cheng, Z.; Lucy Yu, L. Inhibitory effect of Chardonny and black raspberry seed extracts on lipid oxidation in fish oil and their redical scavenging and antimicrobial properties. *Food Chem.* **2007**, *104*, 1065–1073.

(36) Kabara, J. J.; Swieczkowski, D. M.; Conley, A. J.; Taurant, J. P. Fatty acids and derivatives as antimicrobial agents. *Antimicrob. Agents Chemother.* **1972**, *2*, 23–28.

(37) Ouattara, B.; Simard, R. E.; Holley, R. A.; Piette, G. J. P; Begin, A. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int. J. Food Microbiol.* **1997**, *37*, 155–162.

(38) Tripoli, E.; Giammanco, M.; Tabacchi, G.; Di Majo, D.; Giammanco, S.; La Guardia, M. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr. Res. Rev.* **2005**, *18*, 98–112.

(39) Karaosmanoglu, H. *Antimicrobial and Antioxidant Activities of Turkish Extra Virgin Olive Oil from Different Varieties*; Izmir Institute of Technology, Biotechnology and Bioengineering Program, 2009; pp 75−76
(40) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532–2538.
(41) Fukumoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597–3604.
(42) Moore, J.; Yu, L. Methods for antioxidant capacity estimation. In *Wheat Antioxidants*; Wiley-Interscience Press: Hoboken, NJ, 2008; pp 125−130.

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