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ARTICLE

Characterization of Selected Wild Mediterranean Fruits and Comparative Efficacy as Inhibitors of Oxidative Reactions in Emulsified Raw Pork Burger Patties

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In the present study, water, ethanolic, and methanolic extracts from seven selected wild fruits originally from the Mediterranean area, namely, strawberry tree (*Arbutus unedo* L., AU), azarole (*Crataegus azarolus* L., CA), common hawthorn (*Crataegus monogyna* L., CM), blackthorn (*Prunus spinosa* L., PS), dog rose (*Rosa canina* L., RC), elm-leaf blackberry (*Rubus ulmifolius* Schott, RU), and rowan (*Sorbus aucuparia* L., SA), were analyzed for the total amount and profile of phenolic compounds and for the in vitro antioxidant activity against the DPPH and ABTS radicals (study 1). The seven fruits showed different chemical compositions, which consequently led to different antioxidant potentials. Among the seven fruits initially analyzed, AU, CM, RC, and RU had the highest amount of phenolic compounds and displayed the greatest antioxidant activity in vitro. Extracts from these four fruits were tested as inhibitors of lipid oxidation in raw pork burger patties subjected to refrigerated storage at 2 °C for 12 days (study 2). The quantitative measurements of thiobarbituric acid reactive substances (TBA-RS), hexanal content, and color stability were used as indicators of oxidative reactions. The four selected fruits displayed intense antioxidant activity against lipid oxidation, which highlights the potential usage of these fruits as ingredients for the manufacture of healthy meat products. Among them, RC and AU were particularly efficient as their protective effect against lipid oxidation was more intense than that displayed by quercetin (230 mg/kg of burger patty).

KEYWORDS: Phenolics; antioxidant activity; wild Mediterranean fruits; meat; lipid oxidation

INTRODUCTION

The serious consequences of oxidative reactions in muscle foods challenge scientists and the meat industry to develop effective strategies against oxidation to diminish quality changes and avoid the consumer's rejection. The oxidative degradation of lipids involves the loss of fatty acids and causes a decline of nutritional and sensory quality of meat and meat products (*1*). Color changes and lipid oxidation play a major role in meat quality and consequently in the consumer's acceptance of fresh meat. During storage, lipid oxidation occurs due to an autoxidative mechanism involving free radical formation (*2*). Primary lipid peroxidation products are unstable and decompose to generate various secondary products, such as aldehydes, that can contribute to food rancidity. Secondary and final oxidation products, namely, malondialdehyde and hexanal, are reliable indicators of oxidative deterioration in meat products (*3*).

The introduction of antioxidants derived from plant materials in the food industry is becoming popular. Phenolic metabolites are common constituents of fruits and vegetables, and the interest of plant phenolic derives from the evidence of their potent antioxidant activity and their wide range of pharmacologic properties including anticancer, antioxidant, and platelet aggregation inhibition activities (*4*, *5*). The antioxidant properties of phenolic acids and flavonoids depend on their redox properties and chemical structures, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (*4*). In addition, some of them display a metal chelation activity, which hinders transition metals from acting as oxidation promoters (*4*). In vitro antioxidant assays such as those based on hydrogen atom transfer and electron transfer reactions are commonly applied to evaluate the antioxidant capacity of fruits and vegetables (*6*). The extrapolation of conclusions based on the results from model systems or antioxidant assays to real complex food systems should generally be done with great care and should ideally be based on results from more than one model system or assay (*7*). A large variety of fruits and phenolic extracts have been reported to be effective enhancers of the oxidative stability of muscle foods (*8*), with berries being among the best sources of phenolic compounds (*9*). The Mediterranean forest is an ecosystem generally composed of broadleaf evergreen trees that provide multiple wild fruits. Some particular fruits from Mediterranean forest

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such as *Rosa canina*, *Crataegus monogyna*, *Arbutus unedo*, or *Prunus spinosa* have been used for ages in rural southwestern Spain as alternative sources of food or in folk medicine. Nevertheless, the phenolic content and composition as well as the antioxidant capacity of wild fruits from the Mediterranean area are mostly unknown.

The primary objective of the present work was to investigate the composition and antioxidant potential of different extracts (water, methanolic, and ethanolic) of selected fruits harvested from the Mediterranean forest, strawberry tree (*A. unedo* L., AU), azarole (*Crataegus azarolus* L., CA), common hawthorn (*C. monogyna* L., CM), blackthorn (*P. spinosa* L., PS), dog rose (*R. canina* L., RC), elm-leaf blackberry (*Rubus ulmifolius* Schott, RU), and rowan (*Sorbus aucuparia* L., SA), using ABTS and DPPH assays (study 1). The fruits showing the most intense in vitro antioxidant activity in study 1 were selected and subsequently analyzed for their ability to inhibit the lipid oxidation and color deterioration occurring during refrigerated storage of raw pork burger patties (study 2). In the latter study, the effectiveness of the selected fruit extracts in inhibiting oxidative reactions in the meat system was compared to that exhibited by a pure phenolic compound, namely, quercetin.

MATERIALS AND METHODS

Chemicals. All chemicals and reagents used for the present work were of AAS grade and purchased from Panreac (Panreac Química, S.A., Barcelona, Spain), Merck (Merk, Darmstadt, Germany), Extrasynthese (Genay, France), and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

Fruits. Samples of strawberry tree (*A. unedo* L., AU), azarole (*C. azarolus* L., CA), common hawthorn (*C. monogyna* L., CM), blackthorn (*P. spinosa* L., PS), dog rose (*R. canina* L., RC), elm-leaf blackberry (*R. ulmifolius* Schott, RU), and rowan (*S. aucuparia* L., SA) cultivars were collected at the stage of full ripeness in the Cáceres region, Spain (altitude = 450 m) during the summer and autumn of 2007. After hand-harvest, the samples were immediately transferred to the laboratory, cleaned and sorted to eliminate damaged and shriveled fruits, and then frozen at −80 °C.

Physicochemical Composition of Wild Fruits (Study 1). The fruits were cleaned, air-dried at temperature room, and ground through a 1 mm screen in preparation for chemical analysis. Seeds were not included in the analysis. The proximate composition (moisture, crude protein, ashes) as well as pH and acidity of the selected fruits was analyzed according to AOAC methods (*10*). The fat content was determined by using the Bligh and Dyer procedure (*11*).

Extraction of Wild Fruit Phenolics (Studies 1 and 2). Fruits (30 g), including peel and pulp, were cut into pieces while the seeds were carefully removed. Each fruit was finely ground, dispensed in a falcon tube, and homogenized with 10 volumes (w/v) of each solvent (water, ethanol, or methanol) using an Omni-mixer homogenizer (model 5100). The homogenates were centrifuged at 4000 rpm for 10 min at 6 °C using an Eppendorf centrifuge 5810 R. The supernatants were collected, and the residue was re-extracted once more following the procedure previously described. The two supernatants were combined and stored under refrigeration until analyzed (< 24 h). These extracts were used for determination of total phenol content and the in vitro antioxidant assays (study 1).

With the results of study 1 taken into consideration, ethanolic extracts from four particular fruits were selected for study 2. These ethanolic extracts were evaporated using a rotary evaporator and redissolved using 250 g of distilled water. Then water solutions from each fruit were prepared and stored under refrigeration until used for the manufacture of pork burgers (< 24 h) as described below (study 2). No insoluble fragments or residues were observed in the water solutions.

Total Phenolic Content (TPC) (Study 1). TPC of fruit extracts was determined following to the Folin−Ciocalteu method (*12*). TPC was estimated from a standard curve of gallic acid, and results are expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh fruit.

Determination of Phenolic Profiles by HPLC (Study 1). Phenolic compound extraction was performed on freeze-dried fruit samples with accelerated solvent extraction (ASE) using a Dionex ASE 200 Accelerated Solvent Extractor (Salt Lake City, UT). Homogenized fruit was weighed (3−4 g) in a 5 mL steel extraction cell previously prepared with a cellulose filter. The solvent was acetone/water (7:3, v/v), and the extraction program was carried out as follows: temperature, 100 °C; pressure of nitrogen, 1500 psi; two cycles of 5 min; purge, 60% with 22 mL collector tube; final time to fill the tube containing extract time, 90 s. Total solvent collected was 30−35 mL. The extract was removed in a rotary evaporator, and the solid residues were dissolved in water and refrigerated until the HPLC analysis was performed (the following day).

Phenolic profiles were determined using an analytical HPLC method described by Lamuela-Raventós and Waterhouse (*13*) and modified by Kähkönen et al. (*9*). Analytical separation of phenolic compounds was carried out on a Nova-Pak C18 column (150 mm × 3.9 mm, 4 μm; Waters) equipped with a C18 quard column. The mobile phase consisted of 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid (solvent A), 20% A with 80% acetonitrile (solvent B), and 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5 (solvent C). The temperature of the column oven was set at 40 °C. The elution conditions were as follows: isocratic elution 100% A, 0−5 min; linear gradient from 100% A to 96% A/4% B, 5−15 min; to 92% A/8% B, 15−25 min; stepwise to 8% B/92% C, 25−25.01 min; linear gradient to 20% B/80% C, 25.01−45 min; to 40% B/60% C, 45−55 min; to 80% B/20% C, 55−65 min; isocratic elution 80% B/20% C, 65−70 min; linear gradient to 100% A, 70−75 min; post-time 15 min before next injection; flow rate, 0.5 mL/min. On the basis of spectral identification, phenolics were quantified in seven subclasses: catechin (expressed as (+)-catechin equivalents; detection wavelength, 280 nm), hydroxybenzoic acids (as gallic acid equivalents, 280 nm), ellagitannins (as ellagic acid equivalents, 280 nm), ellagic acid (as ellagic acid equivalents, 365 nm), hydroxycinnamic acids (as chlorogenic acid equivalents, 320 nm), flavonols (as rutin equivalents, 365 nm), and anthocyanins (as cyanidin 3-glucoside equivalents, 520 nm), expressed as milligrams per 100 g of dry material. Total procyanidin content was determined using an analytical HPLC method described by Hellström and Mattila (*14*). Normal-phase HPLC with a 250 × 4.6 mm i.d., 5 μm, Silica Luna column (Phenomenex Inc., Darmstadt, Germany) was used with the column temperature set at 35 °C. The injection volume was 10 μL. The mobile phase consisted of A, dichloromethane/methanol/water/acetic acid (41:7:1:1, v/v/v), and B, dichloromethane/methanol/water/acetic acid (5:43:1:1, v/v/v). Elution was started with 100% A, followed by 0−13.5% B, 0−20 min; 13.5−29.2% B, 20−50 min; 29.2−100% B, 50−55 min; 100% B, 55−60 min. Separation was monitored using both UV (λ = 280 nm) and fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 323 nm). Catechin was used as standard for the quantification of total procyanidin content.

Antioxidant Assays (Study 1). The antioxidant activity of fruit extracts was measured by using the DPPH and ABTS assays. The procedure reported by Turkmen et al. (*15*) was employed for the measurement of the antioxidant activity of fruits extracts using the DPPH radical. The antioxidant capacity of each sample was expressed as the amount of sample necessary to decrease the initial DPPH concentration by 50% (EC_{50}).

The antioxidant capacity assay was also carried out using an improved ABTS method as described by Re et al. (*16*), with some modifications as follows. The ABTS radical cation ($ABTS^{\bullet+}$) solution was generated by the reaction of 7 mM ABTS and 2.45 mM potassium persulfate (in equal quantities), after incubation at room temperature in the dark for 15 h. The $ABTS^{\bullet+}$ solution was then diluted with ethanol to obtain an absorbance of 0.70 ± 0.04 at 734 nm. Fresh $ABTS^{\bullet+}$ solution was prepared daily. An aliquot of 10 μL of the diluted fruit extracts was added to 1 mL of $ABTS^{\bullet+}$ solution and mixed thoroughly. The reaction mixture was allowed to stand at room temperature in the dark for 6 min, and the absorbance at 734 nm was immediately recorded. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 2.0 mM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard, and the results are expressed in terms of Trolox equivalent antioxidant capacity (TEAC), expressed as micromolar Trolox equivalents per gram of fruit fresh matter.

Manufacture of Burger Patties (Study 2). The experimental burgers were prepared in a pilot plant. Six types of pork burger patties were prepared depending on the addition of different fruit extracts (AU, CM, RC, and RU) including negative (no added extract, CT) and positive control (added quercetin; 230 mg/kg of burger patty, Q) groups. The

Table 1. Physicochemical Properties of Seven Wild Mediterranean Fruits[a]

	crude fat (%)	crude protein (%)	moisture (%)	ash (%)	pH	acidity[b]
Arbutus unedo (AU)	0.31 ± 0.08	0.77 ± 0.12	71.43 ± 0.76	0.33 ± 0.08	3.74 ± 0.06	0.62 ± 0.06
Crataegus azarolus (CA)	0.43 ± 0.01	0.89 ± 0.07	70.04 ± 1.07	0.83 ± 0.09	3.62 ± 0.05	1.55 ± 0.10
Crataegus monogyna (CM)	0.52 ± 0.14	1.09 ± 0.03	71.16 ± 0.27	1.19 ± 0.12	4.23 ± 0.08	0.71 ± 0.06
Prunus spinosa (PS)	0.37 ± 0.07	1.58 ± 1.50	48.64 ± 0.73	2.07 ± 0.16	3.92 ± 0.03	2.24 ± 0.09
Rosa canina (RC)	0.35 ± 0.02	1.28 ± 0.04	57.60 ± 0.47	1.73 ± 0.05	4.00 ± 0.07	2.00 ± 0.07
Rubus ulmifolius (RU)	0.70 ± 0.07	2.13 ± 0.19	73.05 ± 0.90	0.73 ± 0.06	4.57 ± 0.09	0.46 ± 0.03
Sorbus aucuparia (SA)	0.74 ± 0.05	1.37 ± 0.09	72.03 ± 0.91	0.60 ± 0.05	3.68 ± 0.02	1.32 ± 0.05

[a] Data are expressed as mean ± SD of triplicate experiments and are given as fresh matter. [b] Data are expressed as percent of malic acid.

choice of fruit extracts was based on results from study 1. The amount of quercetin added is equivalent to the average amount of phenolic compounds added to the burger patties through the addition of the fruit extracts. In the basic formulation, the ingredients per kilogram of patty were as follows: 725 g of meat (porcine longissimus dorsi muscle), 250 g of distilled water, and 25 g of sodium chloride. In the formulation of the treated patties, the 250 g of distilled water was replaced by 250 g of a water solution containing the corresponding fruit extracts or the quercetin. All ingredients were minced in cutter (Stephan UMC 5 Electronic) until a homogeneous raw batter was obtained. Sixteen burger patties per batch were prepared in two independent manufacturing processes (eight patties per batch each time). Burger patties were formed using a conventional burger-maker (100 g/patty) to give average dimensions of 10 cm diameter and 1 cm thickness. The raw burger patties were dispensed in polypropylene trays wrapped with PVC film and subsequently stored for 12 days at 2 °C in a refrigerator under white fluorescent light (620 lx), simulating retail display conditions. At sampling times (days 1, 4, 8, and 12), four burger patties per batch were taken out of the refrigerator and analyzed for color parameters and TBA-RS and hexanal content with day 1 being the day after that of manufacture. After each refrigeration stage, burger patties were analyzed for instrumental color and then frozen (−80 °C) until the other analytical experiments were carried out (< 2 weeks).

Color Measurement (Study 2). Surface color measurements of raw burgers were accomplished using a Minolta Chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ), which consisted of a measuring head (CR-300) with an 8 mm diameter measuring area and a data processor (DP-301). Color measurements were made at room temperature with illuminant D_{65} and a 0° angle observed at days 1, 4, 8, and 12. A numerical total color difference (ΔE^*) between burgers at days 1 and 12 of refrigerated storage was calculated: $\Delta E_{1-12} = [(L_{12} - L_1)^2 + (a_{12} - a_1)^2 + (b_{12} - b_1)^2)]^{0.5}$.

TBA-RS Numbers (Study 2). Lipid oxidation in raw burgers was determined by the thiobarbituric acid (TBA) assay using the distillation method reported by Tarladgis et al. (*17*) with some modifications. Briefly, 12 g of each burger meat was homogenized with 35 mL of 3.86% perchloric acid. The homogenate blended was centrifuged (3000 rpm for 3 min) and filtered through Whatman no. 54 filter paper into a 100 mL Erlenmeyer flask and washed with perchloric acid. The filtrate was adjusted to 50 mL by adding perchloric acid (3.86%); then the samples were distilled, and the first 50 mL of distillate was collected. Next, a 2 mL aliquot of the distillate was mixed with 2 mL of 0.02 M TBA in perchloric acid (3.86%) in test tubes (duplicate). The test tubes were vigorously vortexed, and these together with the tubes from the standard curve were incubated at room temperature (24 °C) in the dark for 20 h to develop the color reaction. After this period, all test tubes were centrifuged at 3000 rpm for 2 min. The absorbance was measured at 532 nm using a Hitachi U-2000 spectrophotometer against a blank containing 2 mL of distillate water and 2 mL of TBA reagent. The results from the samples were plotted against a standard curve prepared with known concentrations of tetraethoxypropane (TEP). The results were expressed as milligrams of malonodialdehyde (MDA) per kilogram of meat. The percent inhibition against TBA-RS was calculated at day 12 as $[(C_{12} - T_{12})/C_{12})] \times 100$, where T_{12} is the TBA-RS value in the treated burger at day 12 and C_{12} is the TBA-RS value in control burger at day 12.

Hexanal Content (Study 2). Hexanal was assessed in the headspace from raw pork burgers by solid-phase microextraction (SPME) and gas chromatography−mass spectrometry (GC-MS) using a Hewlett-Packard 5890 series gas chromatograph coupled to a Hewlett-Packard HP-5793 mass selective detector. The method developed by Estévez et al. (*18*) was employed with minor modifications as follows: The SPME fiber, coated with divinylbenzene−carboxenpoly (dimethylsiloxane) (DVB/CAR/PDMS) 50/30 μm, was preconditioned prior to analysis at 220 °C during 45 min. One gram of minced sample was placed in a 4 mL SPME vial and sealed with a silicone septum. The sample was allowed to equilibrate during 30 min while immersed in water at 37 °C. During the extraction, the SPME fiber was inserted through the septum and exposed to the headspace of the vial. After extraction, the SPME fiber was immediately transferred to the injector of the chromatograph, which was in splitless mode at 220 °C. Hexanal was separated using a 5% phenyl−95% dimethyl polysiloxane column (Restek, Bellefonte, PA) (30 m × 0.25 mm id., 1.05 μm film thickness). The GC-MS conditions were as follows: the carrier gas was helium at 18.5 psi, resulting in a flow of 1.6 mL min^{-1} at 40 °C. The SPME fiber was desorbed and maintained in the injection port at 220 °C during the whole chromatography run. The temperature program was isothermal for 10 min at 40 °C and then raised at the rate of 7 °C min^{-1} to 250 °C and held for 5 min. Transfer line to the mass spectrometer was maintained at 270 °C. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV and a multiplier voltage of 1650 V, collecting data at a rate of 1 scan s^{-1} over a range of m/z 40−300. Hexanal was positively identified by comparing its mass spectra and retention time with those displayed by the standard compound. The area of each peak was integrated using ChemStation software, and the total peak area was used as an indicator of hexanal generated from the samples. Results from the hexanal analysis were provided in arbitrary area units (AAU). The percent inhibition of fruit extracts against formation of hexanal was calculated at day 12 as $[(C_{12} - T_{12})/C_{12})] \times 100$, where T_{12} is the relative amount of hexanal in the treated burger at day 12 and C_{12} is the relative amount of hexanal in control burger at day 12.

Data Analysis. In study 1, experiments were conducted four times and analyses were made in duplicate. All data were expressed as mean ± standard deviation of eight measurements. In study 2, four burger patties per batch and per storage day were produced and used as experimental units. All analyses were performed in triplicate in each burger patty (4 burger patties × 3 analysis; $n = 12$ per batch and storage day). In studies 1 and 2, analyses of variance (ANOVA) and Tukey tests by SPSS for Windows (v. 15.0) were carried out to study significant differences on the measured parameters. Differences were considered to be significant at $p < 0.05$. Relationships among measured parameters were calculated using Pearson's correlation coefficients.

RESULTS AND DISCUSSION

General Composition of Wild Fruits (Study 1). The chemical compositions of seven wild Mediterranean fruits (AU, CA, CM, PS, RC, RU, and SA) as well as their pH and acidity are given in **Table 1**. Quantitative results indicated differences among fruits for their chemical composition. Besides the differences derived from the different varieties of fruits, these results could be also explained by different growth conditions, environmental factors, state of maturation, and processing techniques among fruits (*5*, *19*). However, the chemical composition of the present fruits can be considered to be within the expected range for wild fruits and is in accordance with several authors (*19*−*22*).

Total Phenolic Content of Wild Fruit Extracts (Study 1). TPC of water, ethanolic, and methanolic extracts of seven wild

Mediterranean fruits are shown in **Table 2**. TPC among the tested fruits ranged from 69 to 4604 mg of GAE/100 g of fruit, from 100 to 2377 mg of GAE/100 g of fruit. and from 134 to 2068 mg of GAE/100 g of fruit in water, methanolic, and ethanolic extracts, respectively. The highest TPC was found in the water extract of RC (4604 mg of GAE/100 g of fruit) followed by methanolic and ethanolic extracts of RC (2377 and 1175 mg of GAE/100 g of fruit) and by ethanolic extract of CM (2068 mg of GAE/100 g of fruit). The lowest TPC was observed in the extracts from CA. In general, we can consider that our results are coherent with those reported by others authors (*21*, *24*, *26*), although we must emphasize that environmental factors (*5*) greatly influence the chemical characteristics of fruits and consequently their antioxidant potential (*23*). Similar results were found in previous studies for RU (*24*), AU (*25*), SA (*26*), and RC (*21*). The scientific literature available for the remaining fruits is scarce. In fact, this work reports, for the first time, considerably high levels of phenolic compounds in RC compared to other Mediterranean fruits, regardless of the extraction solvent employed. According to our results, rose hips contained a higher TPC than black currants (3−4 mg/g), blueberries (2.70−3.50 mg/g), strawberries (1.6−2.9 mg/g), and raspberries (2.7−3.0 mg/g) (*27*). In previous studies, TPC of rose species were found to range from 55 to 122 mg of GAE/g of DW (*28*, *29*), which is in accordance with our findings. Halvorsen et al. (*30*) evaluated TPC in different types of berries harvested in Nordic countries. Among the 19 fruits analyzed in that study, RC showed significantly higher levels of phenolic compounds, and those levels were between 5- and 30-fold higher than in the other evaluated fruits. Besides the presence of phenolic compounds, this fruit is known to contain high amounts of vitamin C (*28*) that could have contributed to increase TPC as these compounds are also oxidized by the Folin−Ciocalteu reagent, enhancing the development of bluish color.

The extraction yield is dependent on the solvent, the method of extraction, and the raw material used (*23*). The results obtained in this study show that the extractable fraction of TPC was strongly dependent on the solvents. In general, polar fractions contain more phenolics than nonpolar fractions (*31*). Consistently, our results showed that increasing the polarity of the solvent resulted in higher content of phenolic compounds as water and methanol gave the highest levels of phenolic compounds. However, these solvents could have extracted water-soluble substances that count as TPC but do not display antioxidant potential (i.e., sugars, organic acids, and proteins/amino acids). Some exceptions were observed, particularly in CM, because its ethanolic extract showed the highest extractable fraction of TPC. The effectiveness of different solvents for extracting phenolic compounds from various fruits can be explained by the different compositions of the chemical species that comprise the set of phenolic compounds in each fruit. The chemical structure and especially the polarity of the phenolic compounds in each fruit mainly determine their extractability and, hence, the efficiency of the extraction solvents.

Table 2. TPC (Mean ± Standard Deviation) in Water, Methanolic, and Ethanolic Extracts from Seven Wild Mediterranean Fruits According to the Folin−Ciocalteu Method[a]

	water extract	methanolic extract	ethanolic extract
Arbutus unedo (AU)	472 by ± 65	586 bcx ± 53	428 cy ± 61
Crataegus azarolus (CA)	69 by ± 16	100 dxy ± 21	150 cx ± 44
Crataegus monogyna (CM)	450 by ± 138	600 bcy ± 105	2068 ax ± 457
Prunus spinosa (PS)	473 bx ± 80	326 cdy ± 29	134 cz ± 35
Rosa canina (RC)	4604 ax ± 877	2377 ay ± 492	1175 bz ± 222
Rubus ulmifolius (RU)	575 by ± 132	871 bx ± 80	493 cy ± 73
Sorbus aucuparia (SA)	142 by ± 21	396 cdx ± 51	360 cx ± 123

[a] Results are expressed as mg of GAE/100 g of fruit fresh matter. Different letters a−d within a column of the same extract denote a statistical difference between means from different fruit ($p < 0.05$). Different letters x−z within a row of the same fruit denote statistical differences between means from different extract ($p < 0.05$).

Phenolic Profiles of Wild Fruits (Study 1). Phenolic compounds have been frequently examined in European berry varieties (*5*). However, no previous study has compared AU, CA, CM, PS, RC, RU, and SA with respect to their phenolic profile. **Table 3** shows the amount of particular phenolic compounds subgroups (catechins, hydroxybenzoic acids (HBA), hydroxycinnamic acids (HCA), flavonols, ellagitannins, ellagic acid, anthocyanins, and procyanidins) in these wild fruits. The phenolic profile among wild Mediterranean fruit extracts varied considerably, but some consistencies were observed within families and/or genera. Procyanidins were the most abundant phenolics in AU (52%, of the phenolics analyzed), CA (73%), CM (59%), PS (75%), and RC (78%), whereas ellagitannins and ellagic acid were predominant in RU (70%). On the other hand, HCA were the most abundant in SA (68%). In addition, catechins were the second largest group after procyanidins in the same fruits, with the exception of SA, which had flavonols and catechins as second most abundant groups of phenolic compounds. In general, our results are in agreement with other studies in berries (*9*, *32*). In AU, procyanidins, catechins, and HBA (**Table 3**) were the predominant subgroups (474.14, 313.35, and 112.15 mg/100 g of dry weight (dw), respectively). The phenolic profile of AU found in this work is in agreement with a previous study (*33*). According to these authors, procyanidins are the most abundant group detected of the total flavonoid contents in AU. However, anthocyanins are also present as glycosides of cyanidin and delphinidin, with cyanidin-3-galactoside being the most abundant. Alarcão-E-Silva et al. (*25*) reported that the relative content of tannins was higher than that of anthocyanins, which is in agreement with the results of this study. In accordance also with Pallauf et al. (*33*), ellagic acid was detected in AU. Qualitatively, a similar phenolic pattern was found for the fruits belonging to the *Crataegus* genus (*34*, *35*). However, large quantitative differences were found between CA and CM as the amounts of procyanidins and catechins in CM were 4 and 11 times higher than in CA, respectively. RC showed the highest content of procyanidins (4624.92 mg/100 g of dw) and

Table 3. Catechins, Hydroxybenzoic Acids, Hydroxycinnamic Acids, Flavonols, Ellagitannin, Ellagic Acid, Anthocyanins, and Procyanidins of Wild Mediterranean Fruit Extracts[a]

	catechins	hydroxybenzoic acids	hydroxycinnamic acids	flavonols	ellagitannin	ellagic acid	anthocyanins	procyanidins
Arbutus unedo (AU)	313.4 ± 26.2	112.2 ± 9.4	1.0 ± 0.1	3.6 ± 0.4	nd	6.9 ± 0.6	5.8 ± 0.5	474.1 ± 72.2
Crataegus azarolus (CA)	131.0 ± 20.3	3.0 ± 0.9	16.7 ± 1.4	33.3 ± 5.5	nd	nd	nd	505.3 ± 77.8
Crataegus monogyna (CM)	1438.4 ± 84.8	1.9 ± 1.0	81.0 ± 16.9	89.7 ± 2.1	nd	nd	2.9 ± 0.5	2307.7 ± 229.2
Prunus spinosa (PS)	55.6 ± 5.9	4.1 ± 0.4	87.3 ± 7.3	42.1 ± 4.8	nd	nd	3.5 ± 0.6	588.2 ± 91.8
Rosa canina (RC)	1178.9 ± 140.3	9.3 ± 0.8	47.7 ± 4.2	22.5 ± 2.7	nd	17.1 ± 1.5	1.6 ± 0.2	4624.9 ± 209.2
Rubus ulmifolius (RU)	27.5 ± 22.3	7.6 ± 0.9	39.2 ± 6.7	28.2 ± 12.6	994.7 ± 233.1	100.9 ± 5.3	373.1 ± 80.0	nd
Sorbus aucuparia (SA)	92.7 ± 7.6	1.5 ± 0.1	403.7 ± 37.3	92.5 ± 7.5	nd	nd	1.8 ± 0.2	nd

[a] Data are expressed as mg of/100 g of fruit dw (mean and ± standard deviation). nd, not detected.

Table 4. Scavenging Activity against DPPH Radical (Mean ± Standard Deviation) of Water, Methanolic, and Ethanolic Extracts from Seven Wild Mediterranean Fruits[a]

	water extract	methanolic extract	ethanolic extract
Arbutus unedo (AU)	0.70 de ± 0.37	0.59 cd ± 0.18	0.83 c ± 0.16
Crataegus azarolus (CA)	4.70 a ± 0.85	3.83 a ± 0.64	5.22 a ± 1.50
Crataegus monogyna (CM)	1.21 cdx ± 0.36	0.70 cdy ± 0.16	0.42 cy ± 0.16
Prunus spinosa (PS)	2.70 by ± 0.79	1.98 by ± 0.32	5.26 ax ± 0.73
Rosa canina (RC)	0.08 ey ± 0.02	0.18 dy ± 0.05	0.63 cx ± 0.11
Rubus ulmifolius (RU)	0.53 dey ± 0.09	0.41 cdy ± 0.05	0.64 cx ± 0.07
Sorbus aucuparia (SA)	1.97 bcy ± 0.27	0.81 cz ± 0.30	2.36 bx ± 0.15

[a] Results are expressed as EC_{50}. Different letters a−d within a column of the same extract denote a statistical difference between means from different fruit ($p < 0.05$). Different letters x−z within a row of the same fruit denote statistical differences between means from different extract ($p < 0.05$).

Table 5. Antioxidant Activity against ABTS Radical (Mean ± Standard Deviation) of Water, Methanolic, and Ethanolic Extracts from Seven Wild Mediterranean Fruits[a]

	water extract	methanolic extract	ethanolic extract
Arbutus unedo (AU)	65.7 bc ± 16.6	51.1 d ± 6.3	50.3 cd ± 11.4
Crataegus azarolus (CA)	22.9 d ± 2.3	32.3 d ± 5.0	34.3 d ± 15.7
Crataegus monogyna (CM)	56.8 cz ± 18.7	196.6 bx ± 17.2	134.7 ay ± 10.7
Prunus spinosa (PS)	55.1 cx ± 12.2	35.9 dx ± 9.9	7.1 ey ± 2.6
Rosa canina (RC)	393. 3 ax ± 21.2	224.1 ay ± 40.0	94.1 bz ± 14.2
Rubus ulmifolius (RU)	82.2 by ± 19.2	122.1 cx ± 10.2	44.3 cdz ± 15.9
Sorbus aucuparia (SA)	21.6 dy ± 7.1	59.9 dx ± 11.9	61.5 cx ± 28.4

[a] Results are expressed as μM TEAC/g of fruit fresh matter. Different letters a−e within a column of the same extract denote a statistical difference between means from different fruit ($p < 0.05$). Different letters x−z within a row of the same fruit denote statistical differences between means from different extract ($p < 0.05$).

the second highest content of catechins (1178.87 mg/100 g of dw) when compared with to the wild fruits counterparts. However, procyanidins were not detected in RU or SA. Results of the present study are in accordance with previous works in which procyanidins were highlighted as the main phenolic compounds in dog rose species (*36*, *37*). In RU, ellagitannins predominated (994.66 mg/100 g of dw) followed by anthocyanins (373.12 mg/100 g of dw). These results are in agreement with other authors (*38*−*40*). The *Rubus* ellagitannins comprise a complex mixture of monomeric and oligomeric tannins. *Rubus* oligomeric ellagitannins contain the well-known ellagic acid (100.91 mg/100 g of dw), and it has received much attention for its nutritional and pharmacological potential (*38*). Total anthocyanins followed the expected pattern as considerable amounts are mainly found in the dark red fruit (RU) (*39*). The commonest anthocyanins in red or purple colored fruits are cyanidin glycosides followed by delphinidin glycosides (*9*). In a recent study, Elisia et al. (*41*) showed that the presence of cyanidin-3-glucoside in RU contributes a major part of the antioxidant ability in this fruit. However, the presence of anthocyanins and their antioxidant properties can vary considerably among species and fruit cultivars (, *42*, *43*). According to the present study and some previous works, SA contains high concentrations of HCA, catechins, and flavonols (*9*, *26*, *32*). On the other hand, comparison of the phenolic contents of different fruits from the literature should be carefully done because of the variety of analytical methods employed, including different extraction solvents, among studies (*9*).

In Vitro Antioxidant Capacity of Wild Fruit Extracts (Study 1). In the present study, the in vitro antioxidant activity of water, ethanolic, and methanolic extracts of seven selected wild Mediterranean fruits was determined using the DPPH and ABTS methods. In all cases, significant differences were found among fruits and extracts (**Tables 4** and **5**).

The radical scavenging activities of the different fruit extracts against the DPPH radical are shown in **Table 4**. The values range from 0.08 to 4.70 EC_{50}, from 0.18 to 3.83 EC_{50}, and from 0.42 to 5.26 EC_{50} for water, methanolic, and ethanolic extracts, respectively. The smallest EC_{50} value, which corresponds to the highest antioxidant activity, was found for the water extract of RC, followed by the three extracts of AU and RU, the methanolic and ethanolic extracts of RC and CM, and the methanolic extract of SA. The highest value of EC_{50}, corresponding to the lowest antioxidant activity, was displayed by CA. In agreement with results described above for TPC, the antioxidant potential of RC was clearly superior to that of the other fruits. These results are consistent with those found recently by Su et al. (*44*), who described an intense antioxidant activity of extracts of RC against DPPH radical. In direct relation with the antioxidant activity, Olsson and Gustavsson (*29*) described RC as a fruit with a high content of phenolic compounds and an interesting antitumor activity. In general, fruits with the highest TPC (AU, CM, RC, and RU) showed the highest antioxidant activity against the DPPH radical. However, the correspondence between TPC and antioxidant activity against DPPH is not so obvious for water extracts from CM and PS or for the ethanolic extract of SA.

The antioxidant activity of the fruit extracts against the radical ABTS is shown in **Table 5**. The total antioxidant activity of water, methanolic, and ethanolic extracts of the investigated fruits ranged from 21.6 to 393.3 μM TEAC/g of fruit, from 32.3 to 224.1 μM TEAC/g of fruit, and from 7.1 to 134.7 μM TEAC/g of fruit, respectively. In agreement with the results obtained using the DPPH assay, the water and methanolic extracts from RC displayed the highest TEAC values, followed by the ethanolic and methanolic extracts from CM, as well as by the methanolic extract from RU. The lowest TEAC values, which correspond to the lowest antioxidant activity, were found for CA and PS. Deighton et al. (*45*) analyzed the antioxidant activity of different extracts from RU and SA against the ABTS radical, and the results are coherent with those obtained in the present study. Similar results of antioxidant activity were described by other authors (*7*, *28*) in berries. In another study, Su et al. (*44*) reported an even higher antioxidant activity of RC than that described in the present study. The solvents used by Su et al. (*44*) (50% acetone and 80% ethanol) probably provided a greater efficiency of extraction of compounds with antioxidant potential. In the present study, the efficiency of the antioxidant capacity of these extracts was in the order water > methanol > ethanol. It can be concluded that the extracts obtained using high-polarity solvents were considerably more effective radical scavengers than were those obtained using low-polarity solvents.

In general, the results obtained from both antioxidant assays (ABTS and DPPH) gave comparable results for the antioxidant activity. These two assays are well correlated with TPC ($R^2 = 0.87$, $p < 0.01$ and $R^2 = -0.62$, $p < 0.01$, respectively), confirming that phenolic compounds play a main role in the antioxidant activity of the fruits. These results are also in good agreement with the findings of many other authors who reported such positive correlation between TPC and antioxidant activity in different fruits and vegetables (*45*−*47*). Gil et al. (*48*) found a high correlation ($R^2 > 0.9$; $p < 0.05$) between antioxidant activities as determined by DPPH and ABTS assays and TPC in nectarines, peaches, and plums. The Folin−Ciocalteu assay gives a crude estimate of the total phenolic compounds present in an extract. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentration (*49*). Moreover, different phenolic compounds

respond differently to this assay, depending on the number of phenolic groups they contain (*12*). Thus, TPC does not incorporate necessarily all of the antioxidants that may be present in an extract. Hence, this may explain the lack of correlation between TPC and the antioxidant activity for certain fruits and extracts. According to the results obtained, fruits RC, CM, RU, and AU had the highest amount of phenolic compounds and displayed particularly intense in vitro antioxidant activity. Therefore, these four wild fruits were selected (study 2) to investigate their efficacy as enhancers of the oxidative stability in pork burger patties.

Effect of Fruit Extracts in Refrigerator-Stored Burger Patties (Study 2). The quantitative measurements of TBA-RS, hexanal, and color changes were used as indicators of oxidative deterioration occurring during refrigerated storage (2 °C for 12 days) of raw pork burger patties.

The effect of extracts of AU, CM, RC, RU, and Q against lipid oxidation is shown as percent inhibition against TBA-RS and hexanal formation at day 12, when the highest oxidation values were recorded (**Figure 1**). All fruit extracts significantly ($p < 0.05$) reduced TBA-RS numbers by the end of the refrigerated storage (day 12) compared to the control samples, which indicates effective protection of meat against lipid oxidation. The percent inhibition against TBA-RS formation was highest ($p < 0.05$) in RC and AU compared to Q, whereas CM and RU displayed intermediate values. The ability of these fruits to inhibit the oxidative deterioration of meat products can be attributed to the antioxidant activity of phenolic compounds naturally present in AU, RC, CM, and RU, which is in accordance with the results previously reported. In fact, fruits containing higher amounts of phenolic compounds and displaying the most intense antioxidant potential in vitro against DPPH and ABTS radicals displayed as well the most effective antioxidant activity in burger patties. The radical scavenging activity displayed by fruits in the in vitro oxidation assays could have been greatly helpful in protecting muscle lipids from oxidative reactions. It is plausible to consider that phenolic compounds from the tested fruits inhibited the formation of TBA-RS through the protection of polyunsaturated fatty acids against reactive oxygen species (ROS). Results from the present study agree with those obtained by other authors (*50*, *51*), who evaluated the antioxidant potential of diverse plant extracts on muscle lipid oxidation. The same authors highlighted the possibility of replacing synthetic antioxidants such as BHT by natural plant extracts. TBA-RS comprise a heterogeneous collection of carbonyl compounds that contribute to deteriorate the overall quality of muscle foods in terms of meat odor, flavor, and color (*1*). Beyond the influence of lipid oxidation products on the sensory quality of meat products, MDA and other TBA-RS have been highlighted as mutagenic compounds with carcinogenic potential (*52*). By inhibiting the formation of TBA-RS in burger patties, added fruit extracts might improve the overall quality of these products and increase the nutritional value from a health perspective.

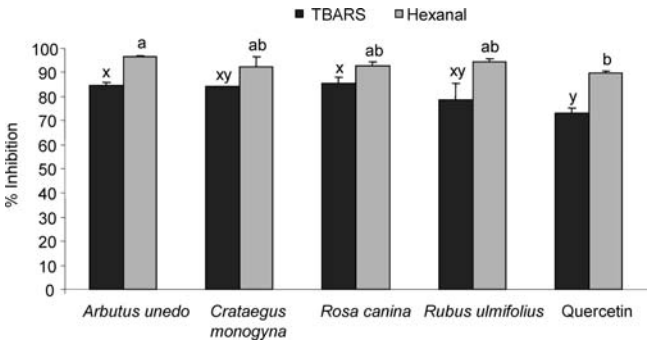

Figure 1. Percent inhibitions of selected fruit extracts and quercetin against TBARS and hexanal formation during refrigerated storage of pork burgers. Different letters (a, b; x−y) above columns denote statistical differences between means from different fruit extracts ($p < 0.05$).

The formation of volatile carbonyl compounds in muscle foods is also attributed to the oxidation of unsaturated lipids. Hexanal is the main volatile compound, formed from omega 6-fatty acids in an oxidizing meat system (*2*). Hexanal has been traditionally used to follow the course of lipid oxidation and off-flavor development in meat products and employed as an indicator of the oxidative deterioration of muscle foods (*53*). All fruits were highly effective at inhibiting hexanal formation ($p < 0.05$) at day 12. The percent inhibitions displayed by fruits and Q against the formation of hexanal in raw burgers patties was in general higher than those found against TBA-RS (**Figure 1**). Among the five treated samples, the greatest antioxidant efficacy was displayed by AU, followed by CM, RC, and RU ($p > 0.05$). Taking into consideration that the antioxidant effect of fruits can be ascribed to the ability of their phenolic compounds at inhibiting oxidative reactions, the differences between fruits for the antioxidant effect could be attributed to their different TPC and phenolic profile. Q displayed significantly lower percent inhibitions against hexanal formation than AU. These results are in agreement with other authors, who reported a higher efficacy of plant extracts against lipid oxidation than pure phenolic compounds (*18*, *50*, *51*). The oxidation of linoleic acid and further oxidation of preformed volatiles are considered to be responsible for the abundant occurrence of hexanal in food systems (*2*). Therefore, the inhibition of hexanal formation by fruit extracts reflects the protective role of fruit phenolics toward unsaturated fatty acids from muscle lipids. Hexanal as well as other lipid-derived volatiles contribute to muscle foods with rancid and other off-odors, which worsen the overall quality of the commodity (*2*, *53*). Therefore, the addition of fruit extracts in burger patties would enhance the sensory quality of the product by successfully reducing the generation of volatiles responsible for off-odors during chilled storage.

At day 1, all types of burgers showed similar a^* values with the exception of RU (**Table 6**). The addition of this fruit extract had a

Table 6. Evolution of Redness (a^* Value) and Total Color Difference (ΔE^*) during Refrigerated Storage of Pork Burgers with Added Selected Fruit Extracts and Quercetin[a]

	day 1	day 4	day 8	day 12	ΔE^*
control (CT)	7.98 bx ± 1.49	7.02 bcx ± 1.57	6.30 bx ± 1.67	2.46 cy ± 0.48	37.99 a ± 5.35
Arbutus unedo (AU)	7.63 bx ± 0.29	6.90 cxy ± 0.54	6.27 by ± 0.43	3.94 bz ± 0.39	16.51 b ± 1.60
Crataegus monogyna (CM)	8.25 bx ± 0.96	7.74 bcx ± 1.32	7.41 bx ± 1.11	4.34 by ± 0.45	17.86 b ± 6.19
Rosa canina (RC)	8.44 bx ± 0.38	7.84 bcxy ± 0.84	7.02 by ± 0.64	4.92 bz ± 0.55	16.22 b ± 1.62
Rubus ulmifolius (RU)	15.92 ax ± 1.03	16.06 ax ± 0.91	15.09 axy ± 1.12	14.42 ay ± 0.59	3.60 c ± 1.60
quercetin (Q)	8.65 bx ± 0.51	8.25 bxy ± 0.84	7.62 by ± 0.63	4.29 bz ± 0.35	22.01 b ± 2.34

[a] Different letters a−c within a column denote a statistical difference between means from different fruit extracts ($p < 0.05$). Different letters x−z within a row of the same fruit extract denote statistical differences between means from different storage days ($p < 0.05$).

significant influence on the color displayed by raw burgers. Anthocyanins, which are major pigment constituents of RU, literally dyed burger patties with a distinct purplish color. The impact of RU pigments on burger patties led to an intense increase of redness values compared to burger patties from the other batches. The color displayed by raw pork burgers patties changed significantly during refrigerated storage as shown by the evolution of redness (a^* value) and the total color difference (ΔE^*) (**Table 6**). All samples showed similar trends over time, with the redness decreasing ($p < 0.05$) throughout refrigerated storage. However, the decrease of redness was significantly affected by the addition of the fruit extracts. Burger patties with added RU extracts displayed significantly higher a^* values at all days of storage than burgers from the other batches. CT samples, in contrast, displayed the lowest a^* values at day 12. Burgers with added fruits extracts and Q displayed, at day 12, higher a^* values than CT. According to the ΔE^* values, RU displayed the most intense color stability followed by AU, CM, and RC. CT samples suffered the most intense color changes, whereas burger patties with added Q displayed intermediate values. The color changes described in this study are consistent with those previously reported for meat products subjected to refrigerated and frozen storage (*50*, *54*). The decrease of redness is generally known to happen during refrigerated storage of meat cuts, and it is a sign of quality loss or deterioration of fresh meat (*1*, *54*). The decrease in a^* values has frequently been associated with the formation of metmyoglobin and thus with meat discoloration induced by lipid oxidation products (*54*). The free radicals produced by lipid oxidation can initiate the reaction of oxidizing oxymyoglobin to metmyoglobin. Similarly, hydrogen peroxide activates metmyoglobin to form ferryl-myoglobin radicals which are catalysts of lipid oxidation in muscle foods (*1*, *55*). Oxygen concentrations at storage conditions could be responsible for the formation of ROS that eventually causes the discoloration of meat. It is reasonable that the color changes in burger patties were caused by oxidative reactions as the addition of substances with proven antioxidant activity inhibits to some extent the discoloration of meat products. The addition of fruit extracts significantly reduced the discoloration of fresh burger patties, with this effect being more intense than that displayed by the pure phenolic quercetin. Fruit extracts would have protected myoglobin against oxidative modification by inhibiting lipid oxidation and, hence, the formation of ROS. According to Francis and Clydesdale (*56*), the color modifications instrumentally measured can be considered as noticeable visual changes when the ΔE^* values are >2. Therefore, the intense color changes recorded in the present study would have been noted by consumers and plausibly interpreted as color deterioration. The addition of fruit extracts could have contributed to diminish this unpleasant change and, therefore, improve the appearance of burger patties during refrigerated storage.

In conclusion, wild Mediterranean fruits contain phenolic compounds, namely, flavonoids and phenolic acids, which compose two large and heterogeneous groups of biologically active non-nutrients. The polarity of the extracting solvent influences the amount of phenolic compounds in the extracts and the extent of their antioxidant activity. According to the results obtained, RC had the highest antioxidant potential in vitro followed by AU, CM, and RU. The fruits tested in the present study display intense antioxidant potential and could play an important role as functional ingredients in meat products, improving their oxidative stability and quality. The results from the present study highlight remarkable technological applications of these wild Mediterranean fruits as natural food additives in the design of healthy meat products.

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