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Avocado (*Persea americana* Mill.) Phenolics, In Vitro Antioxidant and Antimicrobial Activities, and Inhibition of Lipid and Protein Oxidation in Porcine Patties

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ABSTRACT: The first aim of the present work (study 1) was to analyze ethyl acetate, 70% acetone, and 70% methanol extracts of the peel, pulp, and seed from two avocado (*Persea americana* Mill.) varieties, namely, 'Hass' and 'Fuerte', for their phenolic composition and their in vitro antioxidant activity using the CUPRAC, DPPH, and ABTS assays. Their antimicrobial potential was also studied. Peels and seeds had higher amounts of phenolics and a more intense in vitro antioxidant potential than the pulp. Peels and seeds were rich in catechins, procyanidins, and hydroxycinnamic acids, whereas the pulp was particularly rich in hydroxybenzoic and hydroxycinnamic acids and procyanidins. The total phenolic content and antioxidant potential of avocado phenolics was affected by the extracting solvent and avocado variety. The avocado materials also displayed moderate antimicrobial effects against Gram-positive bacteria. Taking a step forward (study 2), extracts (70% acetone) from avocado peels and seeds were tested as inhibitors of oxidative reactions in meat patties. Avocado extracts protected meat lipids and proteins against oxidation with the effect on lipids being dependent on the avocado variety.

KEYWORDS: avocado, phenols, antioxidant activity, avocado extracts, antimicrobial activity

INTRODUCTION

Nowadays, there is a growing interest in finding phytochemicals as alternatives to the synthetic substances that are commonly used in the food, pharmaceutical, and cosmetic industries. This idea is supported by the consumer's concern about the safety of products containing synthetic chemicals because such molecules are suspected to cause or promote negative health effects. The principal function of antioxidants is delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and, therefore, reducing oxidative damage.[1] Antioxidants act in various ways, which include complexation of redox-catalytic metal ions, scavenging of free radicals, and decomposition of peroxides. Using multiple experimental approaches for the study of the antioxidant activity of food-related systems (e.g., extracts) allows a complete screening of the likely antioxidant mechanisms.[1] Crude extracts of herbs, fruits, spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of foods.[2,3] The phenolic compounds of plant origin act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers, and metal chelators.[1,3] Several natural antioxidants have already been isolated from different kinds of plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs.[4−7]

Efficient, inexpensive, and environmentally friendly use of agri-food industry waste is highly cost-effective and minimizes environmental impact. Many edible tropical fruits are processed into natural and concentrated juices, jellies, pulp, and extracts. In these processes, seeds, peels, and other parts are routinely discarded as useless, causing environmental problems. One of the most effective options is the recovery of bioactive plant food constituents, which could be used in the pharmaceutical, cosmetics, and food industries. In addition, economically advantageous alternatives for exploiting the antioxidant content of tropical fruit residues, from juice-processing industries, can provide the local food industries and impoverished population with low-cost nutritional supplements. Avocado (*Persea americana* Mill.) is a tropical and subtropical fruit, very rich in oil. Although avocados are native to southern Mexico, nowadays, they are grown in places as far from America as Australia, South Africa, or Spain.[8] Avocado production in 2008 was estimated at 3.2 million tonnes, with more than two-thirds produced in Latin America and the Caribbean and with the European Union being the major importer (around 290,000 tonnes). The avocado fruit consists of numerous varieties around the world, but the 'Fuerte' and 'Hass' varieties dominate the international market.[8] Due to the high economic importance of avocado fruit in Mexico, the food industry is showing a remarkable interest in processing and enhancing the value of this crop. Besides its pleasant sensory properties, the consumption of avocado-derived products has caught considerable attention owing to its high nutritional value and reported health benefits, including anticancer activity.[9]

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However, only a few studies have focused on the phytochemical composition of avocados.[10] There is scarce information available in the literature about the total phenolic content and antioxidant capacity of pulp[4] or residues from avocado fruit.[11] The impact of the addition of avocado extracts on the oxidative stability of real food systems has never been studied before. Exploiting the phytochemical content of avocado waste materials such as peel and seed may lead to new food products of enhanced quality, and that would have a significant impact on both the avocado and the processed-food industries.

The first aim of the present study was to determine the composition, the total phenolics content, phenolics profile, and antioxidant potential of different extracts (ethyl acetate, acetone, or methanol) from the peel, pulp, and seed of the two commonest avocado varieties, namely, 'Hass' and 'Fuerte' (study 1). In addition, the antimicrobial activity was investigated. The most efficient materials and extracting conditions from study 1 were selected to fulfill the second objective of the present paper: to evaluate the effectiveness of the selected avocado extracts as inhibitors of lipid and protein oxidation in meat patties (study 2).

MATERIALS AND METHODS

Chemicals. All chemicals and reagents used for the present work were purchased from Panreac (Panreac Química, S.A., Barcelona, Spain), Merck (Darmstadt, Germany), Extrasynthese (Genay, France), and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany). The extraction solvents were compatible for industrial food use.

Materials. Two avocado varieties ('Hass' and 'Fuerte') were purchased from a local supermarket in Madrid (Spain). The avocado varieties were maintained at room temperature until full maturity. Fully ripened fruits were manually separated into seed, pulp, and peel, measured for their length, width, and weight, and then frozen (−80 °C) until the remaining chemical analysis and extractions were carried out.

The meat (porcine longissimus dorsi muscle) and porcine back-fat belonged to industrial genotypes slaughtered in a local slaughterhouse in Cáceres (Spain). The day after slaughter, the meat was freed from visible fat, whereas the back-fat was cleaned and freed from the skin. Raw materials were immediately chopped into pieces (2 cm^3), frozen (−18 °C, 24 h), and used as such for the manufacture of the porcine patties.

Chemical Analysis. *Proximate Composition of Avocado Materials (Study 1).* Moisture, total protein, and ash contents were determined using official methods.[12] The method of Folch et al.[13] was used for determining fat content in the avocado materials.

Preparation of Avocado Extracts (Study 1). Three grams of peel or seed and 5 g of pulp were extracted in 15 mL of each solvent: ethyl acetate; acetone/water (70:30 v/v); or methanol/water (70:30 v/v). Samples and solvents were homogenized using an Omni-mixer homogenizer (Omni, model 5100). The homogenates were centrifuged at 2500 rpm for 3 min at 4 °C. The supernatants were collected with filter paper, and the residue was re-extracted once more following the procedure previously described. The two supernatants were combined.

For the antioxidant assays, these extracts were evaporated using a rotary evaporator, dispensed in 50 mL volumetric flasks, and brought to volume with distilled water. Then water solutions from each byproduct were stored in refrigeration until used (<24 h). In the case of the antimicrobial activity assays, the two supernatants from the acetone/water (70:30 v/v) extraction were combined and evaporated under vacuum at 40 °C with a rotary evaporator and taken to dryness under nitrogen. After determination of the yield, the extracts were dissolved in acetone to a final concentration of 100 mg/mL and sterilized by filtration by 0.45 μm Teflon membrane before storage under refrigeration until used (<24 h).

Total Phenolic Content (TPC) Determination (Study 1). The TPC of each extract was determined following the Folin−Ciocalteu method[14] with minor modifications. An aliquot of 200 μL of diluted extract (1:250 from peel or seed and 1:10 from pulp) was mixed with 1000 μL of 1:10 diluted Folin−Ciocalteu's phenol reagent, followed by 800 μL of 7.5% (w/v) sodium carbonate. The mixture was shaken and allowed to stand for 30 min at room temperature in the dark, after which the absorbance was measured at 765 nm using a spectrophotometer. Phenolic content was calculated from a standard curve of gallic acid, and the results were expressed as milligram gallic acid equivalents (GAE) per 100 g of fresh matter.

Extraction and UPLC Analysis of Phenolic Compounds (Study 1). An ASE 200 System (Dionex, Sunnyvale, CA) with 11 mL stainless steel ASE vessels was used for accelerated solvent extraction. About 1 g of freeze-dried powder was mixed homogeneously with 0.25 g of diatomaceous earth in triplicate and placed into an extraction cell. The solvent was acetone/water (70:30 v/v), and ASE settings were as follows: pressure, 1500 psi; temperature, 100 °C; heat time, 5 min; static time, 5 min; 1 static cycle. Each sample was extracted twice. After the extraction, supernatants were evaporated to dryness and redissolved in 10 mL of water. Waters Acquity UPLC was used for phenolic analyses (Waters, Milford, MA) in accordance with the method described by Kylli et al.[15] It consisted of a binary solvent manager, a sample manager, a column heater, a PDA eλ detector, and a FLD detector. Injection volume was 4 μL. Separation was achieved using a Waters HSS T3 C18, 1.8 μm, 2.1 × 150 mm column heated to 40 °C. The mobile phase consisted of a gradient performed with water/0.5% formic acid (solvent A) and acetonitrile/0.5% formic acid (solvent B) at a constant flow rate of 0.5 mL/min. Gradient (v/v) of B was as follows: 0−1 min 0%, B; 1−3.5 min, 0−6% B; 3.5−9.8 min, 6−10% B; 9.8−16 min, 10−16% B; 16−19 min, 16% B; 19−21 min, 16−24% B; 21−23 min, 24−32% B; 23−25 min, 32−64% B; 25−27 min, 64% B; 27−28 min, 64−0% B; next inject delay 3 min. Using the PDA detector, hydroxybenzoic acids (OH-B) were quantified as gallic acid equivalents at 280 nm, hydroxycinnamic acids (OH-C) as chlorogenic acid equivalents at 320 nm, and flavonols as rutin equivalents at 365 nm. Catechins and proanthocyanidins were detected by FLD by setting the excitation and emission wavelengths at 280 and 325 nm, respectively and quantified as (+)-catechin equivalents. An additional tentative identification of main compounds from each subgroup was made by using mass spectrometry according to Kylli et al.[15] All standard compounds were purchased from Extrasynthese (Lyon, France).

CUPRAC Assay (Study 1). The antioxidant capacity assay was carried out using the CUPRAC method as described by Apak et al.[16] with some modifications as follows. One milliliter of $CuCl_2$ 10^{-2} M, 1 mL of neocuproine solution 7.5 × 10^{-3} M in ethanol, and 1 mL of NH_4Ac buffer at pH 7.0 were added to 0.1 mL of diluted extract (prediluted as follows: extracts of peel or seed were diluted 1:150 and pulp extracts were diluted 1:5), so as to make the final volume 4.1 mL. The absorbance of the final solution at 450 nm was read against a reagent blank after 30 min of standing at room temperature in the dark. The calibration curves (absorbance vs concentration) of each antioxidant were constructed at various concentrations using Trolox standard solution (ranging from 0.25 to 2 mmol) in 80% ethanol under the described conditions. Results were calculated and expressed in terms of Trolox equivalent antioxidant capacity (TEAC) and expressed as millimole Trolox equivalents per gram of fresh matter.

ABTS Assay (Study 1). The antioxidant capacity assay was carried out using an improved ABTS method as described by Ganhão et al.[3] with some modifications as follows. The 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation (ABTS) solution was generated by the reaction of 7 mmol of ABTS and 2.45 mmol of potassium persulfate (in equal quantities) after incubation at room temperature in darkness for 15 h. The ABTS solution was then diluted with ethanol to obtain an absorbance of 0.700 ± 0.04 at 734 nm. Different dilutions of each extract

were prepared as follows: extracts from acetone and methanol of peel and seed were diluted 1:50; extracts from ethyl acetate of peel and seed were diluted 1:10; and pulp from all solvents was used pure. An aliquot of 10 μL of each diluted extract was added to 1000 μL of ABTS 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. The absorbance of the reaction samples was compared to that of the Trolox standard curve previously described, and the results were calculated as TEAC and expressed as millimole Trolox equivalents per gram of fresh matter.

DPPH Assay (Study 1). The DPPH assay reported by Turkmen et al.[17] was employed for the measurement of the antioxidant activity of extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Different dilutions of each extract were prepared as follows: extracts from acetone and methanol of peel and seed were diluted 1:20; extracts from ethyl acetate of peel and seed were diluted 1:10; and pulp from all solvents was used pure. An aliquot of 33 μL of each diluted extract was mixed with 2000 μL of DPPH solution (6×10^{-5} M) in methanol. The reaction mixture was stirred and allowed to stand at room temperature in the dark for 6 min, and the absorbance at 517 nm was immediately recorded. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0.25 to 2 mmol) in 80% methanol. The absorbance of the reaction samples was compared to that of the Trolox standard curve previously described, and the results were calculated as TEAC and expressed as millimoles Trolox equivalents per gram of fresh matter.

In Vitro Antimicrobial Test (Study 1). The antimicrobial activity assay was determined by agar disk diffusion method. The avocado extracts were prepared as described above and were individually tested against seven microorganisms including five bacterium strains (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* CECT 5192, *Listeria monocytogenes* CECT 934, *Escherichia coli* CECT 4267, and *Pseudomonas* spp.), one yeast strain (*Yarrowia lipolytica* CECT 1468), and one mold strain (*Aspergillus niger* CECT 2088). To prepare active cultures for inocula, bacteria were grown at 37 °C for 24 h in sterilized brain−heart infusion (BHI) broth except for *Pseudomonas* spp., which was cultured at 25 °C for 48 h in the same broth. Yeast and mold strains were incubated at 25 °C for 48−72 h in BHI broth and malt extract agar (MEA), respectively. A 100 μL aliquot of active bacteria and yeast cultures containing >10^6 cfu/mL was spread onto the surface of BHI agar and MEA to create a microbial lawn and then left to dry. Mold spores were obtained by washing the surface of the MEA plate with 1% Tween 20 in sterile water, and 100 μL of this suspension was used immediately as described before for the remaining microbial isolates. Sterile filter paper disks (5 mm in diameter) were impregnated with 20 μL of each extract and left to dry before being placed on each inoculated agar. Ten microliters of chloramphenicol (1 mg/mL) was used as positive control for bacteria and cycloheximide (1 mg/mL) for yeast and mold strains. Disks with 20 μL of acetone were employed as negative control. The assay was carried out in triplicate. The plates inoculated with bacteria were incubated at 37 °C for 24 h, except those inoculated with *Pseudomonas* spp., which were incubated at 25 °C for 48 h. Yeast and mold strains were incubated for 48−72 h at 25 °C. After incubation, the antimicrobial activity was determined by measuring the inhibition zone (clear zone) around each paper disk by means of a vernier calliper. All measures included the disk diameter.

Manufacture of the Porcine Patties (Study 2). Five types of porcine patties were prepared depending on the addition of extracts from two avocado byproducts (seed and peel) from the two avocado varieties ('Hass' and 'Fuerte') including a control batch (no added extract). In the basic formulation, the ingredients per kilogra, of patty were as follows: 700 g of meat (porcine longissimus dorsi muscle), 180 g of distilled water, 100 g of pork back-fat, and 20 g of sodium chloride. In the formulation of the patties treated with avocado extracts, 50 g of the distilled water was replaced by 50 g of a water solution containing the corresponding avocado extract. Avocado extracts were obtained by using acetone/water (70:30) as described for study 1 and employed in the formulation of the treated patties in the form of water solutions as aforementioned. The choice of the avocado materials and extracting solvent was based on results from study 1. All ingredients were minced in a cutter until a homogeneous raw batter was obtained. Burger patties were formed using a conventional patty-maker (∼100 g/patty), to give average dimensions of 10 cm diameter and 1 cm thickness. In total, four patties per batch were prepared in two independent manufacturing processes (two patties per batch each time).The raw burger patties were dispensed in polypropylene trays wrapped with PVC film and subsequently stored for 15 days at 5 °C in a refrigerator under white fluorescent light (1620 lx), simulating retail display conditions. At day 15, patties were taken out of the refrigerator and analyzed for thiobarbituric acid-reactive substances (TBARS) and protein carbonyls.

Determination of TBARS Numbers (Study 2). Malondialdehyde (MDA) and other TBARS were quantified using the method described by Ganhão et al.[18] with some modifications. Briefly, 5 g of patty was dispensed in cone plastic tubes and homogenized with 15 mL of perchloric acid (3.86%) and 0.5 mL of BHT (4.2% in ethanol). During homogenization, the plastic tubes were immersed in an ice bath to minimize the development of oxidative reactions during extraction of TBARS. The slurry was filtered and centrifuged (3000 rpm for 4 min), and 2 mL aliquots were mixed with 2 mL of thiobarbituric acid (0.02 M) in test tubes. The test tubes were placed in a boiling water bath (100 °C) for 45 min together with the tubes from the standard curve. After cooling, the absorbance was measured at 532 nm. The standard curve was prepared using a 1,1,3,3-tetraethoxypropane (TEP) solution (0.2268 g) in 3.86% perchloric acid. Results were calculated as milligram of MDA per kilogram of patty. The percent inhibition of avocado extracts against TBARS was calculated at day 15 as % inhibition = $[(C_{15} - T_{15})/C_{15}] \times 100$, where C_{15} is the relative amount of TBARS numbers in control patties at day 15 and T_{15} is the relative amount of TBARS numbers in the treated patties at day 15.

Determination of Total Protein Carbonyls (Study 2). Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatization with dinitrophenylhydrazine (DNPH) according to the method described by Ganhão et al.[3] with slight modifications. Patties (1 g) were minced and then homogenized 1:10 (w/v) in 20 mmol of sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an Ultraturrax homogenizer for 30 s. Two equal aliquots of 0.2 mL were taken from the homogenates and dispensed in 2 mL Eppendorf tubes. Proteins were precipitated by cold 10% TCA (1 mL) and subsequently centrifuged for 5 min at 5000 rpm. One pellet was treated with 1 mL of 2 M HCl (protein concentration measurement) and the other with an equal volume of 0.2% (w/v) DNPH in 2 M HCl (carbonyl concentration measurement). Both samples were incubated for 1 h at room temperature. Afterward, samples were precipitated by 10% TCA (1 mL) and washed twice with 1 mL of ethanol/ethyl acetate (1:1, v/v) to remove excess DNPH. The pellets were then dissolved in 1.5 mL of 20 mmol of sodium phosphate buffer containing 6 M guanidine−HCl (pH 6.5), stirred, and centrifuged for 2 min at 5000 rpm to remove insoluble fragments. Protein concentration was calculated from absorption at 280 nm using BSA as standard. The amount of carbonyls was calculated as nanomoles of carbonyl per milligram of protein using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones. The percent inhibition of avocado extracts against the total carbonyls content was calculated at day 15 as % inhibition = $[(C_{15} - T_{15})/C_{15}] \times 100$, where C_{15} is the relative amount of carbonyls in control patties at day 15 and T_{15} is the relative amount of carbonyls in the treated patties at day 15.

Statistical Analysis. All data were expressed as the mean ± standard deviation. Data collected for morphometric characteristics from avocado 'Hass' variety ($n = 24$) and 'Fuerte' variety ($n = 24$) were analyzed by one-way analyses of variance (ANOVA) and Tukey's tests, to study the effect of the avocado variety. Data ($n = 10$ for each batch) from the

Table 1. Morphometric Characteristics of the Whole Fruit, Peel, Pulp, and Seed of Two Avocado Varieties[a]

		whole fruit			peel		pulp		seed	
		length (cm)	width (cm)	weight (g)	thickness (mm)	weight (g)	weight (g)	length (cm)	width (cm)	weight (g)
'Hass'	mean	11.16 b	7.56 b	310.25 b	1.62 a	34.46 a	235.33 b	4.30 b	3.83 b	40.46 b
	SD[b]	0.77	0.22	17.44	0.37	3.73	15.87	0.19	0.23	6.52
'Fuerte'	mean	12.81 a	8.44 a	423.35 a	1.00 b	32.13 b	291.06 a	5.66 a	5.34 a	100.16 a
	SD	0.68	0.34	33.91	0.00	2.81	23.57	0.43	0.38	16.36

[a] Values with different letters (a, b) within a column are significantly different ($p < 0.05$). [b] Standard deviation of the mean.

chemical composition and phenolic profile were analyzed by two-factor (1 and 2) factorial analysis in the randomized design and Tukey's tests. Data ($n = 10$ for each batch) from total phenolic content and antioxidant activities were analyzed by a three-factor (1, 2, and 3) factorial analysis in the randomized design and Tukey's tests. The factors were (1) the three materials (peel, pulp, and seed); (2) the two avocado varieties ('Hass' and 'Fuerte'); and (3) the three extracting solvents (ethyl acetate, 70% acetone, and 70% methanol). The analysis of in vitro antimicrobial activity of avocado extracts and the analysis of significant differences between patties for the percent inhibitions against TBARS and protein carbonyls were accomplished through one-way ANOVA and Tukey's tests. For the assessment of the relationships between total phenolic content and the in vitro antioxidant assays, Pearson's correlation coefficients were calculated. Data were analyzed using the mixed procedure of SPSS for Windows (v. 15.0). Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Morphometric Characteristics of Avocado Fruits. Table 1 shows the morphometric measurements of the whole fruit, peel, pulp, and seed from two avocado varieties. All measurements were significantly different between avocado varieties, with these results indicating a clear differentiation between the two varieties. The 'Fuerte' variety was larger and displayed more weight and a bigger seed than the 'Hass' counterpart. However, the 'Hass' variety had a better ratio of edible portion (pulp) than the 'Fuerte' variety. The pulp percentage in the 'Fuerte' variety was lower compared to the 'Hass' counterpart due to the bigger seed in the former. The proportion of avocado byproduct (peel and seed) was higher in the 'Fuerte' than in the 'Hass' variety. According to the descriptors developed by Avilán et al.,[19] the 'Hass' variety is considered to have a high pulp percentage, whereas the 'Fuerte' variety is regarded as a variety with medium pulp percentage. The yields reported for peel, pulp, and seed in the 'Fuerte' variety (8.3, 72.9, and 18.8%, respectively)[19] and those reported for the seed and pulp in the 'Hass' variety (15 and 83%, respectively)[11,20] are in agreement with our results. This characterization could be used as a reference to replicate the experiments of this work with similar avocado varieties. However, besides the differences derived from the different avocado varieties, these results could be also explained by different growth conditions, environmental factors, state of maturation, and processing techniques among fruits.[19]

Chemical Composition of Avocado Fruits (Study 1). Table 2 shows the chemical composition of the peel, pulp, and seed from the two avocado varieties. The results indicated variability between the two avocado varieties. Moisture was the major component of the three materials. The peel and pulp from both varieties had high moisture values, whereas the seeds had considerably lower moisture content. As expected, the fat proportion in avocado pulps was considerably higher than in the peel and seed. The pulp from the 'Fuerte' variety had a higher content of moisture and a lower fat content than the 'Hass' variety. Schwartz et al.[20] and Rouse and Knight[21] described results similar to those from the present study, whereas Jiménez et al.[22] reported higher percentages of fat content in pulp from 'Fuerte' avocados.

Table 2. Chemical Composition of the Peel, Pulp, and Seed of Two Avocado Varieties[a]

		moisture (%)	fat (%)	protein (%)	ash (%)
peel	'Hass'	75.96 b ± 1.54	1.01 c ± 0.39	1.77 a ± 0.23	0.85 b ± 0.38
	'Fuerte'	76.76 b ± 1.16	1.91 c ± 0.37	1.33 b ± 0.12	0.32 c ± 0.10
pulp	'Hass'	77.38 b ± 2.03	15.80 a ± 1.76	1.83 a ± 0.47	1.01 a ± 0.16
	'Fuerte'	80.25 a ± 1.60	12.55 b ± 1.43	1.20 b ± 0.36	0.45 c ± 0.13
seed	'Hass'	55.76 c ± 4.34	1.39 c ± 0.54	2.19 a ± 0.38	0.70 b ± 0.14
	'Fuerte'	52.69 d ± 1.49	1.52 c ± 0.83	2.22 a ± 0.46	0.83 b ± 0.21

[a] Data are expressed as the mean ± standard deviation. Values with different letters (a–c) within a column are significantly different ($p < 0.05$).

Total Phenolic Content of Avocado Extracts (Study 1). The TPC of peel, pulp, and seed extracts from two avocado varieties were measured using Folin–Ciocalteu's colorimetric assay (Table 3). In general, the peel and seed from the two avocado varieties had considerably higher TPC values than the avocado pulp, which is in agreement with a previous study.[11] Consistently, Torres et al.[10] and Soong and Barlow[14] also found that 'Hass' and 'Fuerte' seeds had significantly higher phenolic concentrations than pulp.

The avocado byproduct from the present study generally showed higher TPC than other fresh fruits, vegetables, and plant extracts, described in the literature as good sources of polyphenols. For instance, the TPC of selected Mediterranean fruits and northern berries ranged from 69 to 4604 mg GAE/100 g[3] and from 1190 to 5080 mg GAE/100 g,[7] respectively, whereas common vegetables such as beetroot and carrots had between 40 and 740 mg GAE/100 g.[7] Even certain plant materials with exceptionally high TPC values such as red onion scale (10548 mg ferulic acid equivalents/100 g[6]), spruce needle (15530 mg GAE/100 g[7]), and mango seed (11700 mg GAE/100 g[14]) had slightly lower TPC than the acetone extracts from 'Fuerte' peel. The present results highlight the suitability of using the avocado byproduct, namely, peel and seed, as rich sources of phenolic compounds.

The extracting solvent had a significant impact on the TPC of peels and seeds from the two avocado varieties, whereas no effect was observed for pulp extracts. The TPC values reported by other authors for pulp extracts from different avocado varieties

Table 3. Total Phenolic Content of Ethyl Acetate, Acetone, and Methanolic Extracts from Peel, Pulp, and Seed of Two Avocado Varieties[a]

		total phenolic content (mg GAE/100 g dry mater)		
		'Hass'	'Fuerte'	p value[b]
peel	ethyl acetate	3293 b ± 925	4054 c ± 1008	NS
	acetone	8997 a ± 3103	17218 a ± 1446	***
	methanol	7841 a ± 2447	13770 b ± 2557	***
pulp	ethyl acetate	76 ± 23	116 ± 37	NS
	acetone	100 ± 21	175 ± 53	NS
	methanol	92 ± 22	145 ± 31	NS
seed	ethyl acetate	1699 b ± 408	2029 c ± 715	NS
	acetone	6082 a ± 863	6912 a ± 1699	NS
	methanol	3511 b ± 988	4164 b ± 1048	NS

[a] Data are expressed as the mean ± standard deviation. Means with different letters (a−c) from different extracting solvents within an avocado variety and material are significantly different ($p < 0.05$). [b] Between avocado varieties: ***, $p < 0.001$; NS, nonsignificant.

Table 4. Phenolic Profile of Peel, Pulp, and Seed of Two Avocado Varieties[a]

		phenolic profile (mg/100 g, dry wt)				
		catechins[b]	OH-B[c]	OH-C[d]	flavonols	procyanidins[e]
peel	'Hass'	228.5 b ± 13.5	1.3 c ± 0.2	328.1 b ± 5.8	129.0 b ± 11.0	4183.5 b ± 339.0
	'Fuerte'	751.9 a ± 24.8	22.5 b ± 0.6	599.4 a ± 13.4	361.1 a ± 15.6	13484.3 a ± 512.2
pulp	'Hass'	3.3 d ± 0.3	34.6 a ± 1.1	111.3 d ± 8.0	<LOD[f]	73.4 d ± 1.2
	'Fuerte'	0.4 d ± 0.1	31.9 a ± 1.9	315.7 b ± 15.8	<LOD	62.1 d ± 1.0
seed	'Hass'	237.8 b ± 4.2	<LOQ[g]	282.7 c ± 6.9	1.7 c ± 2.5	4592.0 b ± 129.4
	'Fuerte'	96.7 c ± 4.1	1.0 c ± 0.1	72.4 e ± 1.5	2.1 c ± 0.1	876.9 c ± 25.9

[a] Data are expressed as the mean ± standard deviation. Means with different letters (a−e) within the same column were significantly different ($p < 0.05$). [b] Catechins, sum of catechin and epicatechin. [c] Hydroxybenzoic acids. [d] Hydroxycinnamic acids. [e] Procyanidins, sum of dimers, oligomers and polymers. [f] LOD, limit of detection. [g] LOQ, limit of quantification.

are similar to those found in our experiment.[10,14] Compared to the present results, Wang et al.[14] reported slightly lower TPC values in extracts from 'Hass' seed and peel, using acetone/water/acetic acid (70:29.7:0.3). In general, acetone achieved the most efficient extraction of TPC in peel and seed from both varieties, followed by methanol and ethyl acetate. Between varieties, the acetone and methanol extracts of 'Fuerte' peel had higher TPC than the corresponding extracts from the 'Hass' variety. Several authors have emphasized the importance of the solvent used in the extraction efficiency.[5,17] Most phenolic acid derivatives present in the plant matrix are stored in vacuoles and are commonly extracted with alcoholic or organic solvents. Solvents, such as methanol, ethanol, acetone, propanol, ethyl acetate, and dimethyl formamide, have been commonly used at different concentrations for the extraction of phenolic compounds from fresh products.[3,5,17] The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Furthermore, solvent polarity plays a key role in increasing phenolic solubility.[5] Therefore, it is hard to develop a standard extraction procedure suitable for the extraction of all plant phenols. Usually, the least polar solvents are considered to be suitable for the extraction of lipophilic phenols. Chavan et al.[23] reported that aqueous acetone (70%) with or without acid was more efficient than absolute acetone for recovery of a maximum amount of condensed tannins from different peas. Zhou and Yu[24] reported that among several tested solvents, 50% acetone extracts contained the greatest level of total phenolics from wheat and that ethanol was the least effective solvent, which is in agreement with our results. In the study carried out by Turkmen et al.,[17] acetone was found to be more efficient than ethanol and methanol for extracting total phenolics from black tea, which is also consistent with the results from this study. The chemical structure and particularly the polarity of the phenolic compounds in each tissue largely determines their extractability and, hence, the efficiency of the extraction solvents.

Phenolic Profile of Avocado Fruits (Study 1). Analysis of the phenolic profile by UPLC of the peel, pulp, and seed from two avocado varieties resulted in a large variety of phenolic compounds, which were eventually classified into five subgroups (Table 4). Statistically significant differences were detected between avocado tissues and avocado varieties for all phenolic subgroups. The phenolics determined in the avocado tissues were catechins, hydroxybenzoic acids (OH-B), hydroxycinnamic

Table 5. In Vitro Antioxidant Activity of Ethyl Acetate, Acetone, and Methanolic Extracts from Peel, Pulp, and Seed of Two Avocado Varieties According to the CUPRAC Assay[a]

		in vitro antioxidant activity (mmol Trolox/g fresh matter)		
		'Hass'	'Fuerte'	p value[b]
peel	ethyl acetate	56.40 c ± 21.19	103.68 c ± 26.69	*
	acetone	218.04 a ± 42.42	456.24 a ± 77.07	***
	methanol	145.98 b ± 69.25	330.75 b ± 62.57	***
pulp	ethyl acetate	2.48 ± 0.33	2.44 ± 0.65	NS
	acetone	1.63 ± 0.39	2.04 ± 0.32	NS
	methanol	1.33 ± 0.43	1.64 ± 0.44	NS
seed	ethyl acetate	58.00 c ± 15.55	96.09 c ± 27.76	NS
	acetone	275.36 a ± 59.09	353.43 a ± 75.83	***
	methanol	141.67 b ± 41.24	184.42 b ± 66.05	*

[a] Data are expressed as the mean ± standard deviation. Means with different letters (a−c) from different extracting solvents within an avocado variety and material are significantly different ($p < 0.05$). [b] p value between avocado varieties: *, $p < 0.05$; ***, $p < 0.001$; NS, nonsignificant.

Table 6. In Vitro Antioxidant Activity against the ABTS Radical of Ethyl Acetate, Acetone, and Methanolic Extracts from Peel, Pulp, and Seed of Two Avocado Varieties[a]

		in vitro antioxidant activity (mmol Trolox/g fresh matter)		
		'Hass'	'Fuerte'	p value[b]
peel	ethyl acetate	16.12 c ± 6.98	34.82 c ± 12.61	NS
	acetone	103.75 a ± 44.49	242.26 a ± 28.31	***
	methanol	74.06 b ± 23.17	185.87 b ± 26.91	***
pulp	ethyl acetate	0.64 ± 0.10	0.56 ± 0.11	NS
	acetone	0.84 ± 0.24	0.91 ± 0.12	NS
	methanol	0.94 ± 0.23	0.78 ± 0.17	NS
seed	ethyl acetate	21.57 c ± 7.51	38.15 c ± 12.78	NS
	acetone	158.29 a ± 26.27	194.80 a ± 44.69	***
	methanol	78.93 b ± 26.73	121.61 b ± 31.87	***

[a] Data are expressed as the mean ± standard deviation. Means with different letters (a−c) from different extracting solvents within an avocado variety and material are significantly different ($p < 0.05$). [b] p value between avocado varieties: ***, $p < 0.001$; NS, nonsignificant.

acids (OH-C), flavonols, and procyanidins. In agreement with the TPC results, the avocado peels and seeds had, in general, considerably higher amounts and a larger variety of phenolic compounds than the avocado pulp. Whereas the avocado by-products were rich in catechins, procyanidins, and OH-C, the pulp was particularly rich in OH-B, OH-C, and procyanidins. Interestingly, the avocado pulps had significantly higher amounts of OH-B than the peels and the seeds. Flavonols were detected only in peels and seeds. A detailed analysis of the subclasses revealed further differences between avocado materials. For instance, epicatechins were the most abundant compounds (~98%) within the catechin subgroup in peels from both varieties, whereas the proportion between catechins and epicatechins was even (~50%) in the seed material. On the other hand, the chlorogenic acid and the neochlorogenic acids were the dominant OH-C in peels and seeds, whereas the pulp was particularly rich in *p*-coumaric acid derivatives. A previous study identified several OH-C and OH-B in pulp and seeds from several cultivated avocado varieties.[10] García-Alonso et al.[4] analyzed the global content of flavonols in avocado pulp. Recently, Wang et al.[11] reported the presence of procyanidins and certain chlorophylls in peel, pulp, and seed from several avocado cultivars. The present study contributes to original quantitative data on the detailed phenolic composition of avocado tissues. Among materials and avocado varieties, the peel from the 'Fuerte' variety had the largest amounts of procyanidins, catechins, OH-C, and flavonols. The amounts quantified in this avocado material are also higher than those reported previously for other fruit extracts.[3,25] It is worth noting that in contrast to the results obtained for the avocado peels, seeds from the 'Hass' variety had significantly higher amounts of catechins, procyanidins, and OH-C than the 'Fuerte' counterpart. The intense antioxidant activity of avocado phenolics and chlorophylls has been highlighted to be closely linked to the health-promoting effects of avocado fruit and oil.[26]

In Vitro Antioxidant Capacities of Avocado Extracts (Study 1). In agreement with the TPC analysis, the antioxidant activity of peel and seed extracts as assessed by the CUPRAC assay was

Table 7. In Vitro Antioxidant Activity against the DPPH Radical of Ethyl Acetate, Acetone and Methanolic Extracts from Peel, Pulp, and Seed of Two Avocado Varieties[a]

		in vitro antioxidant activity (mmol Trolox/g fresh matter)		
		'Hass'	'Fuerte'	p value[b]
peel	ethyl acetate	17.85 c ± 7.07	35.18 b ± 12.56	NS
	acetone	88.94 a ± 48.22	199.61 a ± 33.15	***
	methanol	71.92 b ± 28.93	174.71 a ± 29.80	***
pulp	ethyl acetate	0.37 ± 0.07	0.23 ± 0.07	NS
	acetone	0.33 ± 0.07	0.39 ± 0.10	NS
	methanol	0.32 ± 0.07	0.29 ± 0.09	NS
seed	ethyl acetate	17.78 c ± 4.34	27.80 c ± 10.16	NS
	acetone	130.26 a ± 36.80	167.50 a ± 42.08	***
	methanol	66.24 b ± 24.84	94.27 b ± 30.47	***

[a] Data are expressed as the mean ± standard deviation. Means with different letters (a−c) from different extracting solvents within an avocado variety and material are significantly different ($p < 0.05$). [b] p value between avocado varieties: ***, $p < 0.001$; NS, nonsignificant.

considerably more intense than that of pulp extracts (Table 5). Significant differences were found between solvents and varieties for peels and seeds. In the CUPRAC assay, acetone extracts of peels and seeds were the most efficient followed by the methanol and ethyl acetate counterparts. In addition, acetone and methanol extracts of 'Fuerte' peel and seed exhibited a higher antioxidant potential than the corresponding extracts from the 'Hass' variety. The present paper provides original data on the behavior of avocado extracts in the CUPRAC assay. The results obtained from the ABTS radical assay were comparable to those found in the CUPRAC assay (Table 6). Among avocado materials, the pulp extracts had the lowest antioxidant activity, and no significant differences were found between varieties and extracting solvents ($p > 0.05$). Significant differences were found between solvents and avocado varieties for peel and seed extracts ($p < 0.05$). Acetone used on peels and seeds had the greatest peroxyl radical scavenging ability in this method. Intermediate TEAC values were obtained for methanol extracts of the peels and seeds from the two avocado varieties, whereas the ethyl acetate extracts displayed the lowest TEAC values. Consistent with the CUPRAC assay, the 'Fuerte' peel and seed extracts had higher antioxidant activity compared to the 'Hass' extracts. In general, the free radical scavenging potentials of peel, pulp, and seed from the avocado varieties as assessed by the DPPH method (Table 7) showed the same trend as described above for the CUPRAC and ABTS assays. Therefore, the highest antioxidant activity against the DPPH radical was found in acetone and methanol extracts of the peel and the acetone extracts from the seed of the 'Fuerte' variety.

The consistency between the results from the different assays is in agreement with previous studies devoted to the evaluation of the antioxidant potential of plant and fruit extracts against several radicals in vitro.[3,25] In fact, significant and positive high correlations were found between the CUPRAC and ABTS ($r = 0.96$; $p < 0.01$) and the DPPH assays ($r = 0.94$; $p < 0.01$) and between the two latter ($r = 0.96$; $p < 0.01$). The DPPH approach seems to be a rapid and accurate method for assessing the antioxidant activity of fruit and vegetable extracts. The results are highly reproducible and comparable to those of other free radical scavenging methods such as ABTS.[25] However, as a distinct advantage over other electron-transfer-based assays (e.g., FRAP, ABTS, DPPH), CUPRAC is superior in regard to its realistic pH being close to the physiological pH, favorable redox potential, accessibility and stability of reagents, and applicability to lipophilic antioxidants as well as hydrophilic ones.[27] The ABTS method, on the other hand, has reagents (or chromophores) that are soluble in both aqueous and organic solvents and may therefore serve the need to simultaneously measure hydrophilic and lipophilic antioxidants.[16,27] Furthermore, the three in vitro assays were also significantly correlated with the TPC (CUPRAC, $r = 0.88$, $p < 0.01$; ABTS, $r = 0.87$, $p < 0.01$; DPPH, $r = 0.88$, $p < 0.01$), which supports phenolic compounds from avocado extracts playing a major role in their antioxidant effects against free radicals. Consistently, Wang et al.[11] described high correlations between the procyanidin and TPC in avocado tissues and between those and the antioxidant capacity of the corresponding extracts, which suggests that procyanidins were important contributors to their antioxidant capacities. Hence, the variations in the antioxidant capacity of different avocado materials observed in the present study may be attributed to differences in their TPC and phenolic compositions. The present work confirms the low antioxidant activity of the pulp in contrast to the other avocado materials. García-Alonso et al.[4] studied the antioxidant activity of extracts from 28 fruits in lipid and aqueous phases and reported that avocado fruit presented the lowest antioxidant activity among them. Soong and Barlow[14] evaluated the antioxidant potential of seeds and pulps extracts of some fruits including avocado and concluded that the seeds had higher TEAC values than the pulps. The DPPH assay has been employed by numerous authors to assess the antioxidant activity of different plant materials such as berries (3.9 mmol TEAC/100 g FW), cereals (1.2−3.5 mmol TEAC/100 g FW), common fruits (1.2 mmol TEAC/100 g FW), vegetables (0.40 mmol TEAC/100 g FW), and rice bran (24.3 mmol TEAC/100 g FW).[28] Compared with the aforementioned, the antioxidant activity displayed by the avocado byproduct from the present study is noticeably higher.

Whenever 70% acetone and 70% methanol were employed as extracting solvents, the peels and seeds from 'Fuerte' exhibited a more intense in vitro antioxidant activity than the 'Hass' tissues. The differences found between peels from the two avocado varieties could be ascribed to the significant differences in TPC. Surprisingly, no significant differences were found between seeds from the two varieties for the TPC and the concentration of particular phenolics such as catechins, procyanidins, and OH-C

Table 8. In Vitro Antimicrobial Activity of Peel, Pulp, and Seed of Two Avocado Varieties[a]

		in vitro antimicrobial activity (mm)						
		Bacillus cereus	*Staphylococcus aureus*	*Listeria monocytogenes*	*Escherichia coli*	*Pseudomonas* spp.	*Yarrowia lipolytica*	*Aspergillus niger*
peel	'Hass'	–[b]	5.80 d ± 1.06	5.07 d ± 0.12	5.73 b ± 1.10	5.73 ± 1.10	6.00 ± 0.20	–
	'Fuerte'	6.33 c ± 0.58	5.73 d ± 1.10	5.80 d ± 1.04	–	5.73 ± 1.10	5.53 ± 0.42	–
pulp	'Hass'	8.33 c ± 1.15	6.47 d ± 0.61	7.00 d ± 1.83	–	–	5.33 ± 0.12	–
	'Fuerte'	10.07 b ± 2.90	8.93 b ± 0.12	11.00 b ± 2.00	9.67 b ± 1.15	–	5.53 ± 0.42	–
seed	'Hass'	9.20 c ± 1.51	8.33 c ± 1.15	9.27 c ± 0.46	–	–	–	–
	'Fuerte'	7.87 c ± 1.94	6.80 d ± 0.72	7.33 d ± 1.53	7.67 b ± 2.31	–	5.20 ± 0.20	–
chloramphenicol		20.00 a ± 1.00	19.03 a ± 0.06	21.20 a ± 0.35	21.67 a ± 1.15	7.53 ± 0.92	–	–
cycloheximide		–	–	–	–	–	–	9.13 ± 0.23

[a] Values are the mean of three determinations ± standard deviation. Means with different letters (a–d) within the same column are significantly different ($p < 0.05$). [b] –, no inhibition.

was significantly higher in the 'Hass' seed than in the 'Fuerte' counterpart. These results suggest that the significant differences detected for the antioxidant potential of the seeds from the two avocado varieties may respond to differences in the phenolics profile. Like this, certain polyphenols in the seed from the 'Fuerte' variety would display a more intense antioxidant potential than those found in the seed from the 'Hass' variety. With the exception of the OH-B, the HPLC analysis performed in the present study did not allow the detection of significant differences in specific compounds between avocado varieties. The 'Fuerte' and 'Hass' avocado varieties have been reported to have considerably different genetic backgrounds[29] and, therefore, the differences between avocado varieties for their phenolic composition and antioxidant potential may respond to the genetic polymorphism between cultivars.

It is generally known that the behavior of plant and fruit extracts in the in vitro antioxidant assays is highly dependent on the extracting solvents employed. In the present study, 70% acetone allowed the highest yield and the most intense antioxidant activity followed by 70% methanol and ethyl acetate. It is reasonable to consider that the polarity of the solvents largely determines their ability to extract phenolic compounds with antioxidant potential. The results from the present study are in agreement with Pellegrini et al.,[30] who evaluated the antioxidant potential of vegetable foods using different sequences of extracting solvents. These authors concluded that using water and acetone was highly effective to obtain large extraction yields in foods rich in water-soluble antioxidants such as polyphenols and chlorophylls. Interestingly, these are the major antioxidant components of the avocado tissues analyzed in the present study.[11] Taking into consideration the overall results obtained from the antioxidant assays, 70% acetone extracts from the three avocado materials were also tested for their ability to inhibit the growth of microorganisms with potential to cause food spoilage and foodborne diseases. The extracts from the materials with the highest in vitro antioxidant activity (peel and seed) were analyzed for their effectiveness as inhibitors of the oxidative reactions affecting lipids and proteins in a real meat product (study 2).

In Vitro Antimicrobial Activity of Avocado Extracts (Study 1). To screen the antimicrobial activity of avocado extracts against several pathogen and spoilage microorganisms commonly found in meat products, the disk diffusion method was conducted. The inhibition zone diameters exerted by the extracts toward challenged microorganisms are given in Table 8. Acetone (negative control) was inactive against all tested microbial strains. Varied levels of antimicrobial effectiveness of avocado extracts were obtained and, in general, the extracts were more effective against bacteria. In the case of this microbial group, significant differences were observed between avocado tissues and avocado varieties except for *Pseudomonas* spp. Therefore, Gram-positive bacteria were generally found to be more sensitive than Gram-negative bacteria. These results agree with those reported by other authors, who observed a more intense effect of plant/fruit extracts against Gram-positive bacteria than against Gram-negative strains.[31,32] Due to the fact that Gram-negative bacteria have an extra protective outer membrane, they are usually considerably more resistant to antibacterial agents than their Gram-positive counterparts.[31,32] Among Gram-positive bacteria, the highest inhibitory effect was observed against *B. cereus* and *L. monocytogenes*, whereas *E. coli* was the most sensitive among Gram-negative bacteria. *Pseudomonas* spp. was found to be the most resistant bacteria. Kossah et al.[32] determined the antimicrobial activity of a *Rhus typhina* fruit extract against nine bacterium strains belonging to five different genera, and they also found that *B. cereus* and *L. monocytogenes* were the most sensitive isolates. Avocado extracts were active against the tested yeast isolate, but the effect was only moderate. A study on the antifungal effect of two avocado seed extracts also reported activity against yeasts of great importance for human and veterinary medicine.[33] Among all assayed microorganisms, *A. niger* was found to be the most resistant microorganism as all extracts were inactive against this mold strain. This result is in agreement with a study made on *Semenovia tragioides* extracts.[31] In the same line, Rauha et al.[34] assayed 28 tree, vegetable, and cereal extracts and none of them affected *A. niger* growth. Generally, extracts from the 'Fuerte' variety displayed higher antimicrobial activity than did extracts from the 'Hass' variety.

The differences in the antimicrobial activity of avocado extracts could be due to the nature of the antimicrobial substances present in the extracts and their mechanisms of action on the tested microorganisms. The antimicrobial activity of phenolic acids and flavonoids is well documented.[35] Surprisingly, pulp from avocado extracts showed, in general, the highest antimicrobial activity despite having lower TPC than peel and seed

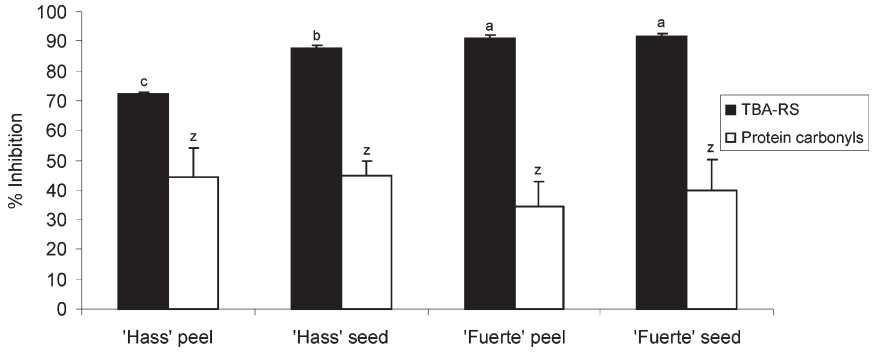

Figure 1. Percent inhibitions of avocado extracts against TBARS and protein carbonyls formation during chill storage of raw pork patties. Different letters (a−c) on top of columns denote significant differences between percent inhibitions against TBARS formation by different avocado extracts ($p < 0.05$). The same letter (z) on top of columns denotes the absence of significant differences between percent inhibitions against protein carbonyls formation by different avocado extracts ($p < 0.05$).

extracts. It is plausible to consider that avocado pulp has more active compounds with antimicrobial affects than the extracts from the other avocado tissues. Bystrom et al.[36] also found that pulp of *Melicoccus bijugatus* fruits had higher antimicrobial activity but less total phenolics than seed tissues. These authors ascribed the antimicrobial activity to *p*-coumaric acid derivatives. Interestingly, the pulp of both avocado varieties was found to be particularly rich in *p*-coumaric acid derivatives. On the other hand, the high fat content in avocado pulp could have contributed to the antimicrobial effect of this tissue against Gram-positive bacteria as long-chain unsaturated fatty acids are known to display such effects.[33] The findings from the present study highlight that avocado extracts are promising sources of potential antimicrobial activity and may be efficient natural additives for extending the shelf life of fresh food products.

Avocado Extracts against Lipid and Protein Oxidation in a Meat System (Study 2). To evaluate the effectiveness of the avocado extracts to act as antioxidants in a real food system, the oxidative stability of chilled porcine patties with added avocado extracts was compared to that of control patties. The oxidative reactions occurred during chill storage of porcine patties were assessed by monitoring the accumulation of lipid and protein oxidation products by means of TBARS and protein carbonyls, respectively. Figure 1 depicts the percent inhibitions displayed by the extracts of avocado byproduct against lipid and protein oxidation after 15 days of chill storage. The percent inhibition of avocado extracts against TBARS formation ranged from 72.36 to 91.54, with these percentages being considerably larger than those obtained against the formation of protein carbonyls (from 34.52 to 44.90). In agreement with the present results, previous studies have shown that the effectiveness of plant and fruit phenolics against lipid oxidation is higher than that against protein oxidation.[37] Estévez et al.[37] ascribed the limited antioxidant protection of plant phenolics on proteins to the partition behavior of phenolic compounds between the lipid and aqueous phases. In addition, the selective covalent binding of polyphenols to myofibrillar proteins was proposed to hinder the antioxidant action of plant phenolics. The major antioxidant action of phenolics derives from the radical scavenging via hydrogen atom donation. Other phenolic compounds prevent the oxidative reactions through the chelation of transition metals such as iron and copper.[37] Some of the phenolic components of the avocado extracts analyzed in the present study, such as catechins, OH-C (chlorogenic acid), and procyanidins, have been previously described as efficient inhibitors of lipid and protein oxidation in meat systems through the aforementioned antioxidant mechanisms.[37]

In agreement with the results from the in vitro antioxidant assays, 'Fuerte' extracts inhibited the oxidation of muscle lipids during chill storage of porcine patties to a larger extent than 'Hass' extracts. Between materials from the 'Hass' variety, seeds displayed a more intense antioxidant effect on lipids than the peel. Hence, the antioxidant potential exhibited by avocado extracts in vitro is confirmed in a real food product, and the antioxidant effect on lipids is dependent on the avocado variety. On the other hand, no significant differences were detected between avocado varieties and materials for the inhibition of protein oxidation. The accumulation of TBARS in muscle foods is commonly employed as a marker of quality deterioration. Lipid oxidation is responsible for the loss of nutritional value and the development of rancid flavors and odors in food products.[38] Whereas the impact of protein carbonylation on food quality has been scarcely studied, a recent paper has reviewed the negative effects of protein oxidation on muscle foods.[39] The formation of protein carbonyls involves the loss of essential amino acids, reduces the digestibility of myofibrillar proteins, and leads to the deterioration of particular quality traits such as texture.[39] Hence, the addition of avocado extracts on meat systems would enhance their nutritional and sensory properties through the effective inhibition of lipid and protein oxidation.

In conclusion, avocado tissues are interesting natural sources of rich-phenolic extracts with high antioxidant and antimicrobial potential. Interestingly, waste materials from the avocado processing industry (peels and seeds) displayed the most intense antioxidant effects. These effects are dependent on the avocado variety and can be improved by using 70% acetone as extracting solvent. To our knowledge, avocado extracts contain no potentially toxic or harmful components. Hence, results from the present study highlight remarkable technological applications of avocado extracts as natural food additives in the design of healthy meat products. The impact of the present extracts on the sensory properties of the treated foods should be elucidated in upcoming studies.

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