



Avocado by-products as inhibitors of color deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage

J.G. Rodríguez-Carpena^a, D. Morcuende^b, M. Estévez^{b,*}

^a Food Science, Faculty of Veterinary, Autonomous University of Nayarit, 63190, Tepic, Nayarit, Mexico

^b Animal Production and Food Science, University of Extremadura, 10003, Cáceres, Spain

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ABSTRACT

Processing of avocados generates an important amount of by-products such as peels and seeds that are rich in bioactive substances with proven radical suppressing activities. The objective of this study was to evaluate the effectiveness of peel and seed extracts from two avocado varieties—'Hass' and 'Fuerte'—as inhibitors of lipid and protein oxidation and color deterioration of raw porcine patties during chilled storage (4 °C/15 days). Avocado extracts significantly ($p < 0.05$) reduced the loss of redness and the increase of lightness during storage of porcine patties. 'Fuerte' extracts were more efficient at inhibiting discoloration of chilled patties than 'Hass' extracts. Patties treated with avocado extracts had significantly lower amounts of TBA-RS than control ones throughout the storage. 'Hass' avocado extracts significantly inhibited the formation of protein carbonyls in chilled patties at day 15. The present results highlight the potential usage of extracts from avocado by-products as ingredients for the production of muscle foods with enhanced quality traits.

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1. Introduction

The oxidation of lipids and proteins is a major cause of meat deterioration (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Lipid oxidation promotes meat discoloration through the oxidation of myoglobin and leads to the formation of low molecular weight compounds which impart rancid odors and off-flavors (Mercier, Gatellier, & Renner, 2004; Shahidi, 1998). In relation to protein oxidation, the nature of the oxidation products formed is highly dependent on the amino acids involved and how the oxidation process is initiated (Lund, Heinonen, Baron, & Estévez, 2011). The side-chains of some particular amino acids such as arginine, lysine and proline, are oxidized through metal-catalyzed reactions into carbonyl residues (Davies & Dean, 2003; Garrison, 1987; Stadtman & Berlett, 1988). Lipid and protein oxidation have been reported to occur concurrently in meat systems, although relatively little is known about the repercussions of the latter on the quality of meat products (Decker, Xiong, Calvert, Crum, & Blanchard, 1993; Estévez, Ventanas, & Cava, 2006). The oxidation of myofibrillar proteins might play a role in the loss of enzyme activity, protein solubility and formation of protein complexes and non-enzymatic browning products and could

be linked to meat tenderness (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007a; Mercier et al., 2004). The occurrence of protein oxidation during chill storage of raw meat products and the interaction between oxidizing proteins with other food components such as lipids and myoglobin, require further research.

The use of antioxidant compounds is an effective way to minimize or prevent lipid oxidation and hence, retard the formation of toxic oxidation products, maintain nutritional and sensory quality and extend the shelf life of muscle foods (Pokorný, Yanishlieva, & Gordon, 2001). Antioxidants from natural resources are of increasing interest for consumers and meat technologists owing to their health implications and functionality. The benefits of plant phenolics and other natural antioxidants go beyond their efficacy against lipid oxidation as they are believed to enhance the quality and nutritional value of foods (Soong & Barlow, 2004; Wu et al., 2004). Crude extracts of fruits (De Oliveira et al., 2009; Ganhão, Estévez, Kylli, Heinonen, & Morcuende, 2010), herbs (Wojdylo, oszmianski, & Czemerys, 2007; Yoo, Lee, Lee, Moon, & Lee, 2008), vegetables (Ismail, Marjan & Foong, 2004), cereals (Ragaei, Abde-Aal, & Noaman, 2006), residual sources (Moure et al., 2001) and other phenolic-rich plant materials have been shown to display remarkable antioxidant potential. Little is known, however, about the effectiveness of these antioxidants against protein oxidation in meat products. Some authors have reported contradictory effects of natural antioxidants such as ascorbic acid, tocopherols and phenolic compounds on the oxidative stability of muscle proteins (Estévez & Cava, 2006; Estévez & Heinonen, 2010; Lund, Hviid, & Skibsted, 2007b).

* Corresponding author. Tel.: +34 9272571122; fax: +34 9272571110.
E-mail address: mariovet@unex.es (M. Estévez).

Avocado (*Persea americana* Mill.) is an oleaginous fruit native to tropical America (Mexico). Besides its pleasant sensory properties, the avocado consumption has caught considerable attention owing to its high nutritional value and reported health-benefits, including anti-cancer activity (Kritchevsky et al., 2003; Lu et al., 2005). Industrial processing of avocados generates a large amount of by-products such as peels and seeds. These materials are rich in bioactive substances such as polyphenols and chlorophylls which have been shown to have antioxidative and radical suppressing activities (Wang, Bostic, & Gu, 2010; Rodríguez-Carpena, Morcuende, Andrade, Kylli & Estévez, 2011). Exploiting the beneficial effects of these phytochemicals in muscle foods may lead to additional economical inputs to the avocado industry and would allow the development of novel and enhanced meat products. Nevertheless, the effect of extracts from avocado by-products on the oxidative stability of meat products is unknown.

The objective of the present study was to determine the effectiveness of peel and seed extracts from two avocado varieties as inhibitors of lipid and protein oxidation and color deterioration of raw porcine patties subjected to chilled storage.

2. Material and methods

2.1. Chemicals

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

2.2. Material

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 ripeness. In accordance to Gamble et al. (2010), full ripeness was identified by subjectively assessing the softening of the avocado pulp and by instrumentally measuring the darkening of his skin ('Hass' peel: L: 32.69; a*: -1.52; b*: 9.71; 'Fuerte' peel: L: 45.77; a*: -15.50; b*: 29.33). Full-ripened fruits were manually separated into seed, pulp and peel, and then frozen (-80 °C) until the manufacture of the corresponding extracts. For this purpose, 10 g of peel or seed samples were extracted with 30 mL of acetone/water (70:10 v/v). Sample and solvent were homogenized using an Omni-mixer homogenizer ("Omni", mod 5100, Omni International, INC, Waterbury, CT, USA). 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. These extracts were evaporated using rotary evaporator and redissolved using 50 g distilled water. Then, water solutions from each by-product were refrigerated until the manufacture of porcine patties (less than 24 h).

The meat (4 porcine *longissimus dorsi* muscles) and pork back-fat were randomly obtained from a homogeneous batch of Landrace × Large-White pigs (age at slaughter: 165 days, carcass weight: 83 kg) in a local slaughterhouse in Cáceres (Spain). The day after slaughter, the meat was freed from visible fat while the back-fat was cleaned and freed from the skin. Raw materials were manually chopped with a knife into pieces (~2 cm³), frozen (-18 °C, 24 h) and used as such for the manufacture of porcine patties.

2.3. Total phenolic content determination

The total phenolic content (TPC) of each extract was determined following the Folin-Ciocalteu method (Soong & Barlow, 2004) with minor modifications. An aliquot of 200 µL of diluted extract (1:250) 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 results expressed as mg gallic acid equivalents (GAE) per 100 g of fresh matter.

2.4. DPPH assay

The DPPH assay reported by Kähkönen and Heinonen (2003) was employed for the measurement of the antioxidant activity of extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. An aliquot of 33 µL of each diluted extract (1:20) was mixed with 2000 µL 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 mM) in 80% methanol. The absorbance of the reaction samples was compared to that of the Trolox standard and the results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC) and expressed as mM Trolox equivalents per gram fresh matter.

2.5. Manufacture of porcine patties

Five types of porcine patties were prepared depending on the addition of extracts from two avocado by-products [seed (S) and peel (P)] from two avocado varieties ['Hass' (H) and 'Fuerte' (F)] including a control (C) group of samples (no added extract). In the basic formulation, the ingredients per kg of patty were as follows: 700 g meat (porcine *longissimus dorsi* muscle), 180 g distilled water, 100 g pork back-fat and 20 g 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 by-product extracts. All ingredients were minced in a Stefan UMC 5 Electronic cutter at 10,000 rpm for 5 min until an apparent homogeneous raw batter was obtained. In total, sixteen patties per treatment were prepared in two independent manufacturing processes (eight patties per treatment each time). Patties (~100 g/patty) were formed using a conventional patty-maker (mod. MH3, J2, Barcelona, Spain) to give average dimensions of 10 cm diameter and 1 cm thickness. The raw patties were dispensed in polypropylene trays wrapped with PVC film (oxygen permeability: ~17 cm³/m² day atm; moisture permeability: <5 g/m² day; Tecnodur S.L., Valencia, Spain) and subsequently stored for 15 days at +5 °C in a refrigerator under white fluorescent light (1620 lx), simulating retail display conditions. At sampling times (days 0, 5, 10 and 15), four patties per group of patties were taken out of the refrigerator and analyzed for instrumental color parameters, TBARS and protein hydrazones. In addition, freshly made patties (day 0) were analyzed for chemical composition. After each refrigeration stage, patties were frozen (-80 °C) until analytical experiments were carried out (less than 2 weeks). Storage loss was calculated as the weight loss during refrigerated storage of raw patties as follows: storage loss = [(W₀ - W₁₅)/W₀] 100; where W₀ is the weight of the patty at day 0, and W₁₅ is the weight of the patty at day 15.

2.6. Chemical analysis

2.6.1. Proximate composition of porcine patties

Moisture and total protein contents were determined using official methods (A.O.A.C., 2000a,b). The method of Folch, Lees, and Sloane-Stanley (1957) was used for determining fat content in patties.

2.6.2. Color measurements

Surface color measurements of porcine patties were performed using a Minolta Chroma Meter 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). Before each measuring session the chromameter was calibrated on the CIE color space system using a white tile. The L^* value indicates lightness ($L^* = 0$ darkness, $L^* = 100$ lightness); a^* value indicates redness ($+60 = \text{red}$, $-60 = \text{green}$) and b^* value indicates yellowness ($+60 = \text{yellow}$, $-60 = \text{blue}$). Color measurements were made on the surface of each patty in triplicate at three randomly selected locations. Color measurements were made at room temperature ($\approx 22^\circ\text{C}$) with illuminant D65 and a 0° angle observer. A numerical total color difference (ΔE) between patties at day 1 and day 15 of storage was calculated as: $\Delta E_{1-15} = [(L_{15} - L_1)^2 + (a_{15} - a_1)^2 + (b_{15} - b_1)^2]^{1/2}$.

2.6.3. Determination of TBARS numbers

Malondialdehyde (MDA) and other TBARS were assessed using the method described by Salih, Smith, Price, and Dawson (1987) with some modifications. Briefly, 5 g of patty were dispensed in cone plastic tubes and homogenized with 15 mL perchloric acid (3.86%) and 0.5 mL BHT (4.2% in ethanol). In order to minimize the development of oxidative reactions during homogenisation, the plastic tubes were immersed in an ice bath. The slurry was filtered and centrifuged (3000 rpm for 4 min) and 2 mL aliquots were mixed with 2 mL 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 expressed as mg MDA per kg patty.

2.6.4. Determination of total protein carbonyls

Protein oxidation, as measured by the total carbonyl content, was evaluated by derivatisation with dinitrophenylhydrazine (DNPH) according to the method described by Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987) with slight modifications. Patties (1 g) were minced and then homogenized 1:10 (w/v) in 20 mM sodium phosphate buffer containing 0.6 M NaCl (pH 6.5) using an ultraturax 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 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. Afterwards, samples were precipitated by 10% TCA (1 mL) and washed twice with 1 mL ethanol:ethyl acetate (1:1, v/v) to remove excess of DNPH. The pellets were then dissolved in 1.5 mL of 20 mM 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 absorbance at 280 nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per milligram of protein using an absorption coefficient of $21.0 \text{ nM}^{-1} \times \text{cm}^{-1}$ at 370 nm for protein hydrazones.

Table 1
Total phenolic content and in vitro antioxidant activity of peel and seed of two avocado varieties.

	'Hass'		'Fuerte'		Significance level ^A		
	Peel	Seed	Peel	Seed	V ^B	M ^C	V*M
TPC ^D	8997 ^b ± 3103	6082 ^c ± 1446	17218 ^a ± 863	6912 ^{bc} ± 1699	<0.001	<0.001	<0.001
DPPH ^E	89 ^c ± 48	130 ^{bc} ± 36	200 ^a ± 33	168 ^{ab} ± 42	<0.001	0.721	0.007

Results are expressed as means ± standard deviations. Means with different superscripts (^{a-c}) within a row are significantly different ($p < 0.05$).

^A Statistical significance.

^B Variety ('Hass' vs. 'Fuerte').

^C Material (Peel vs. Seed).

^D TPC: total phenolic content (mg GAE/100 g dry matter).

^E Results are expressed as mM trolox/g fruit fresh matter.

2.6.5. Statistical analysis

Four patties per treatment and per sampling day were prepared and used as experimental units. All analytical experiments were made in duplicate in each experimental unit. Eight data per treatment and sampling day were analyzed by two-factor factorial analysis in the randomized design and Tukey tests by SPSS for Windows (v. 15.0) according to the following model:

$$Y_{ijk} = \mu + V_i + M_j + VM_{ij} + e_{ijk}$$

where: Y_{ijk} is the measured variable in the k th patties as affected by the V_i avocado variety and the M_j avocado material; μ is the overall mean; V_i is the effect of the i th avocado variety ('Hass' vs. 'Fuerte'); M_j is the effect of the j th avocado material (peel vs. seed); VM_{ij} is the interaction term; and e_{ijk} is the random error. In addition, Pearson correlation coefficients were calculated in order to establish relationships between instrumental and chemical measurements.

3. Results and discussion

3.1. In vitro antioxidant potential of avocado extracts

Table 1 shows the TPC and the in vitro antioxidant capacity of the avocado by-product extracts. Significant differences were found between the two varieties ('Hass' vs. 'Fuerte') and avocado materials (peel vs. seed). 'Fuerte' Peel (F-P) extracts had the highest polyphenol contents, followed by 'Hass' peel (H-P), 'Fuerte' Seed (F-S) and the lowest were observed in the extracts from 'Hass' seed (H-S). Consistently, F-P exhibited the most intense scavenging activity against the DPPH radical. The phenolics profile and further details about the antioxidant and microbial potential of these extracts have been published elsewhere (Rodríguez-Carpena et al., 2011).

3.2. Chemical composition and storage loss of porcine patties

The chemical composition of the raw patties manufactured using different avocado by-product extracts as well as their storage loss, are given in Table 2. As expected, the addition of avocado extracts did not affect the chemical composition of patties as no statistical differences were found amongst types of patties ($p > 0.05$). Moreover, the storage loss ranged from 4.02 g/100 g to 4.78 g/100 g and no statistical differences were found between treatments. The chemical composition of the patties from the present study is consistent with that described by other authors (López-López, Cofrades, Yakan, Solas, & Jiménez-Colmenero, 2010).

3.3. Color deterioration during chilled storage of porcine patties

The evolution of L^* , a^* and b^* (lightness, redness and yellowness) are shown in Figs. 1, 2 and 3, respectively. The addition of avocado extracts had a significant effect on the color displayed by freshly made patties (day 0). F-P extracts led to patties with significantly lower a^* -values while peel extracts from the two avocado varieties and F-S

Table 2
Chemical composition of raw patties and storage loss during chilled storage.

	C	H-P	H-S	F-P	F-S	Significance level ^A		
						V ^B	M ^C	V*M
Moisture%	70.80 ± 0.05	72.44 ± 0.45	71.83 ± 0.57	71.77 ± 1.28	72.09 ± 0.71	NS	NS	NS
Protein%	17.75 ± 1.20	17.42 ± 0.43	17.03 ± 0.24	17.02 ± 0.26	17.13 ± 0.35	NS	NS	NS
Fat%	9.05 ± 0.83	9.48 ± 1.17	9.62 ± 0.51	9.53 ± 0.60	9.93 ± 1.13	NS	NS	NS
Storage loss%	4.10 ± 1.62	4.17 ± 0.27	4.78 ± 0.09	4.02 ± 0.48	4.24 ± 0.09	NS	NS	NS

Results are expressed as means ± standard deviations.

C: Control patties; H-P: Patties treated with 'Hass' peel extract; H-S: Patties treated with 'Hass' seed extract, F-P: Patties treated with 'Fuerte' peel extract, F-S: Patties treated with 'Fuerte' peel extract.

NS: no significant.

^A Statistical significance.

^B Variety ('Hass' vs. 'Fuerte').

^C Material (Peel vs. Seed).

extracts caused a significant increase of *b**-values. The lightness of the patties was not affected by the addition of avocado extracts. Peel extracts had a distinctive greenish color as a result of the extraction of pigments (chlorophylls) from the avocado materials. Peel pigments were likely transferred to the patties during manufacture causing the modification of their color. In summary, at day 0 (at the beginning of the chilled storage) patties treated with peel extracts were less red and more yellow than control patties.

All color parameters showed differences (*p* < 0.05) between treatments and storage days. During chilled storage, *L**-values from the control and the treated patties presented different trends. *L**-value increased significantly with a positive trend (slope: 0.96) in C-patties whereas it decreased significantly with a negative trend (slope ranged from -0.21 to -0.71) over time in the treated patties. All types of patties suffered a significant decrease in redness during chilled storage. The loss of redness was considerably more intense in control patties (slope: -2.56) than in the treated counterparts (slopes ranged from -0.83 to -1.27). As aforementioned for the evolution of *L**, the progress of yellowness was different amongst treatments. Significant increases of yellowness were measured in control patties (slope: 0.48) while a slight decrease was detected in the treated ones (Slope ranged from -0.24 to -0.55).

The pleasant bright red color displayed by fresh meat cuts is caused by the presence of oxymyoglobin (OxyMb) on their surface (Ledward, 1991). Numerous authors have studied the color changes undergone by raw meat during chilled storage and reported similar trends to those described in the present study (Chen, Jo, Lee, & Ahn, 1999; Estévez, Morcuende, & Cava, 2003; Haak, Raes, & De Smet, 2009). These results confirm that patties suffered an intense decol-

ouration process which is mainly defined by the loss of redness. The discoloration occurred during chilled storage of red meat cuts is generally associated to the accumulation of metmyoglobin (MetMb) in the meat surface (reviewed by Faustman, Sun, Mancini, & Suman, 2010). During chilled storage, MetMb accumulation and meat discoloration largely depend on the presence of reducing systems in meat (Ledward, 1991) and on lipid oxidation (Estévez et al., 2003; Faustman et al., 2010; O'Grady, Monahan, & Brunton, 2001). Several authors have linked the loss of redness in raw meats subjected to chilled storage to the occurrence of oxidative reactions (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2009; Haak et al., 2009; Yu, Ahmedna, & Goktepe, 2010). Primary lipid oxidation products such as hydroperoxides and other reactive oxygen species (ROS) are known to oxidize the ferrous iron (Fe²⁺) from OxyMb into its ferric form (Fe³⁺) present in the MetMb (Faustman et al., 2010). Recent studies have also highlighted the role of secondary lipid oxidation products, such as unsaturated aldehydes, on the formation of MetMb in fresh porcine meat (Suman, Fastman, Stamer, & Liebler, 2006). On the other hand, yellowness increase may be due to the accumulation of Schiff pigments (lipofuscin) from lipid to protein complexes as a result of oxidative stress (Chelch, Gatellier, & Santé-Lhoutellier, 2007). According to the present results, the avocado extracts inhibited the discoloration of chilled patties by reducing the loss of redness and the increase of yellowness. It is reasonable to consider that the presence of antioxidant compounds in the avocado extracts retarded MetMb formation in patties. Interestingly, an updated revision on the factors influencing the formation of MetMb and the discoloration of meat, have reported that the consumption of oxygen resulting from the process of lipid oxidation could be sufficient to accelerate OxyMb

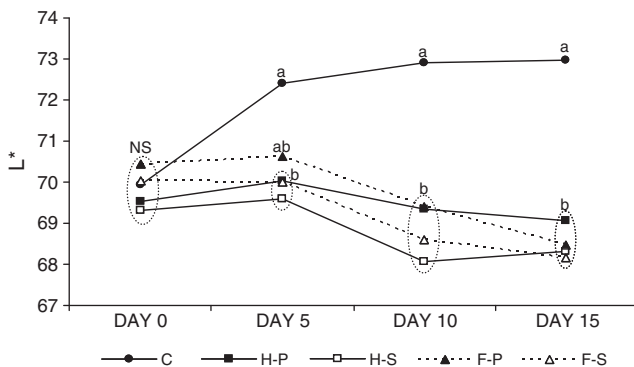


Fig. 1. Lightness (*L**) measured on the surface of raw patties during 15 days of chilled storage. Different letters (^{a-b}) within a day of storage denote significant differences between treatments (*p* < 0.05). NS: no significant. Values embraced by circles are similar (*p* > 0.05). C: Control patties; H-P: Patties treated with 'Hass' peel extract; H-S: Patties treated with 'Hass' seed extract, F-P: Patties treated with 'Fuerte' peel extract, F-S: Patties treated with 'Fuerte' peel extract.

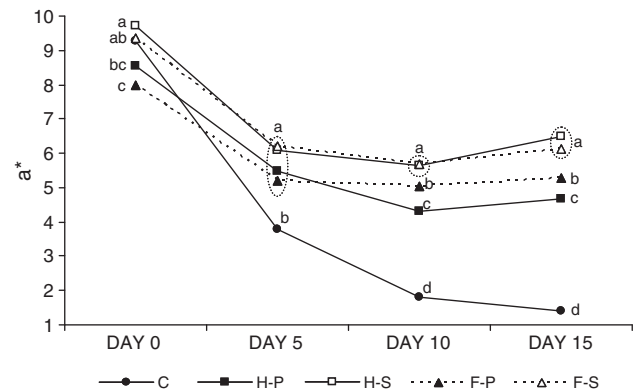


Fig. 2. Redness (*a**) measured on the surface of raw patties during 15 days of chilled storage. Different letters (^{a-d}) within a day of storage denote significant differences between batches (*p* < 0.05). Values embraced by circles are similar (*p* > 0.05). C: Control patties; H-P: Patties treated with 'Hass' peel extract; H-S: Patties treated with 'Hass' seed extract, F-P: Patties treated with 'Fuerte' peel extract, F-S: Patties treated with 'Fuerte' peel extract.

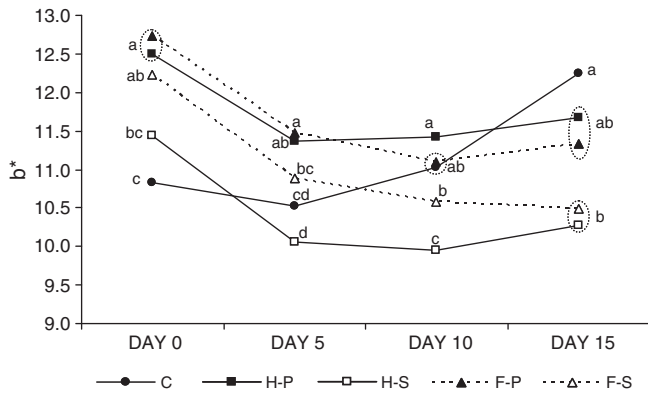


Fig. 3. Yellowness (b^*) measured on the surface of raw patties during 15 days of chilled storage. Different letters ($a-d$) within a day of storage denote significant differences between batches ($p < 0.05$). Values embraced by circles are similar ($p > 0.05$). C: Control patties; H-P: Patties treated with 'Hass' peel extract; H-S: Patties treated with 'Hass' seed extract, F-P: Patties treated with 'Fuerte' peel extract, F-S: Patties treated with 'Fuerte' peel extract.

oxidation through a localized lowering of partial oxygen pressure (Faustman et al., 2010). In this sense, Monahan, Skibsted, and Andersen (2005), hypothesized that α -tocopherol could inhibit MetMb formation by slowing oxygen consumption linked to lipid oxidation. This novel mechanism could also be attributed to the antioxidant constituents of the avocado extracts. Polyphenols and other pigments from avocado extracts have been proved to act as efficient radical scavengers and metal chelators in vitro (Wang et al., 2010; Rodríguez-Carpena et al., 2011). Other authors have reported the effectiveness of phenolic-rich extracts at minimizing the color changes occurred during chilled storage of porcine meat (McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001; Jo, Son, Son, & Byun, 2003; Haak et al., 2009; Shan, Cai, Brooks, & Corke, 2009). Unlike tocopherols, phenolic compounds are mostly water soluble compounds which would allow a straightforward interaction with myoglobin, a water-soluble protein known to reside in the cytoplasm.

The positive effect of avocado extracts on the color of chilled patties is supported by the calculated total color differences (ΔE_{0-15}) values (Table 3). According to the ΔE_{0-15} -values, C-patties suffered a more intense color deterioration than patties with added avocado extracts. According to Francis and Clydesdale (1975), the color modifications instrumentally measured can be considered as noticeable visual changes when the ΔE_{1-15} -values are higher than 2. Therefore, consumer's would perceive the color changes suffered by C-patties during the chilled storage. The intense color changes occurred during chilled storage of patties influenced the color displayed by the meat products at the end of the storage. C-patties were lighter and less red than patties treated with the avocado extracts. On the other hand, patties with added peel extracts were darker, redder and less yellow than control patties. It is worth noting

Table 3

Total color difference (ΔE_{0-15}) measured on the surface of raw patties between days 0 and 15 of chilled storage.

	ΔE_{0-15}	SD ^A
C	9.63 ^a	0.90
H-P	3.72 ^b	0.73
H-S	2.68 ^b	0.71
F-P	2.71 ^b	0.70
F-S	2.84 ^b	0.96

Means with a different letter ($a-b$) within a column are significantly different ($p < 0.05$). C: Control patties; H-P: Patties treated with 'Hass' peel extract; H-S: Patties treated with 'Hass' seed extract, F-P: Patties treated with 'Fuerte' peel extract, F-S: Patties treated with 'Fuerte' peel extract.

^A Standard deviation of the mean.

that L^* value (Zhu & Brewer, 1998) shows a higher correlation coefficient with visual perception of redness than a^* value and hence, might be considered the best instrumental indicator of visual redness (Joo, Kauffman, Kim, & Kim, 1995). The results obtained for lightness could worsen the sensorial perception of C-patties in contrast to the treated ones. The large differences between types of patties could influence the meat purchasing decision because consumers use discoloration as an indicator of freshness and wholesomeness (Mancini & Hunt, 2005). An intense red color is preferred by pork consumers (Brewer, Lan, & McKeith, 1998). Hence, the addition of avocado extracts on patties would contribute to preserve the color of freshly made patties for a longer time during chilled storage. This protective effect towards the desirable red color of raw patties may influence consumer's purchase decision.

3.4. Lipid oxidation during chilled storage of porcine patties

Lipid oxidation was quantified in raw patties subjected to chilled storage by using the TBA method (Fig. 4). As expected, TBARS numbers increased significantly in C-patties during the chilled storage (slope: 0.34) of patties as a likely result of the onset of lipid oxidative reactions. In contrast, the treated samples displayed slight changes over time. TBARS numbers increased in H-P patties the last sampling day (slope: 0.07), while remained almost unchanged for the other types of patties (slope ranged from 0.001 to -0.03). During the whole chilled storage, the amount of TBARS was significantly lower in patties with avocado extracts than in the control counterparts. Amongst treated patties, the samples with added F extracts had the lowest TBARS numbers by the end of the storage (day 15).

The results from the present study show that adding phenolic-rich avocado extracts protect patties against lipid oxidation. As aforementioned, phenolic compounds can inhibit free radical formation and the propagation of free radical reactions through the chelation of transition metal ions, particularly those of iron and copper (Shan et al., 2009; Ibrahim, Abou-Arab, & Abu, 2010). Like this, the intense antioxidant activity shown by the extracts in vitro had also a protective role in real meat products. Other authors have obtained similar results while testing other plant materials in raw meat patties (Chen et al., 1999; McCarthy et al., 2001; Jo et al., 2003; Bekhit et al., 2009; Ibrahim et al., 2010). In general, extracts from the F variety were more effective against lipid oxidation than the materials from the other variety which is in agreement with results from the in vitro extracts. Consistently, peel from the F-variety has higher amount of phenolic compounds and a more intense antioxidant activity than the H-variety.

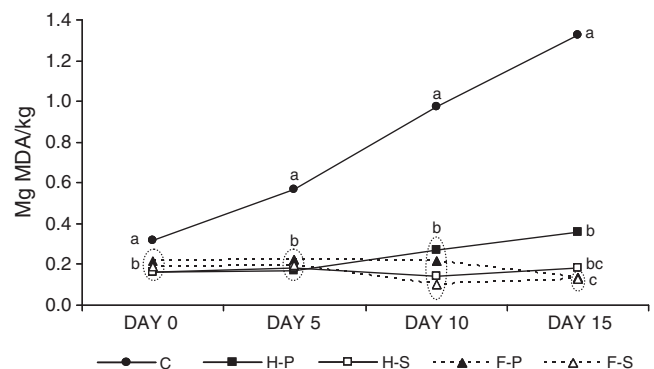


Fig. 4. TBARS in raw patties during 15 days of chilled storage. Different letters ($a-c$) within a day of storage denote significant differences between batches ($p < 0.05$). Values embraced by circles are similar ($p > 0.05$). C: Control patties; H-P: Patties treated with 'Hass' peel extract; H-S: Patties treated with 'Hass' seed extract, F-P: Patties treated with 'Fuerte' peel extract, F-S: Patties treated with 'Fuerte' peel extract.

According to these results, the usage of avocado extracts in meat patties may improve the quality of these products as lipid oxidation eventually leads to a net loss of sensory and nutritional quality. One of these unpleasant sensory changes would be linked to the color deterioration previously discussed. The timely coincidence of both color changes and lipid oxidation is supported by a negative and significant correlation ($r = -0.62$; $p < 0.01$) between redness and TBARS numbers. These data emphasize the likely interaction between lipid and myoglobin oxidation. In this sense, avocado extracts would have protected myoglobin against oxidative modifications by inhibiting lipid oxidation and hence, the formation of ROS. Other studies, however, support a lack of a clear tie between both oxidation processes as the addition of certain natural extracts and polyphenolic compounds inhibits lipid oxidation while showed no efficacy against color changes (McBride, Hogan, & Kerry, 2007; Hayes et al., 2009). This study confirms the effectiveness of the avocado extracts against both oxidation processes. Regarding the impact of lipid oxidation on other sensory attributes, Georgantelis, Blekas, Katikou, Ambrosiadis, and Dimitrios (2007) reported that rancid flavor is detected in meat products with TBARS values higher than 0.6 mg MDA/kg. TBARS numbers from treated patties remained below this threshold during the whole chilled storage while TBARS numbers in C-patties went beyond that line already after day 5. Hence, it is plausible to consider that avocado extracts would contribute to keep more desirable sensory features in patties after cooking and during consumption.

3.5. Protein oxidation during chilled storage of porcine patties

Raw patties with added avocado extracts were analyzed for the amount of total protein carbonyls during chilled storage using the DNPH method (Fig. 5). The amount of carbonyls derived from protein oxidation significantly ($p < 0.05$) increased during chilled storage in all types of patties, with the highest values being recorded at day 15. These results prove that muscle proteins underwent oxidative reactions during chilled storage of patties. Protein carbonyls are well-known oxidation products of certain amino acids such as proline, arginine and lysine and their detection using the DNPH method has been employed as an assessment of protein oxidation in muscle foods (Reviewed by Estévez, Ollilainen, & Heinonen, 2009). Specific protein carbonyls, α -amino adipic and γ -glutamic semialdehydes have been recently found to be present in patties (Ganhão, Morcuende, & Estévez, 2010a). These semialdehydes could have contributed to the carbonyl gain found as they account for around 70% of total protein carbonyls in oxidized proteins (Requena, Chao, Levine, & Stadtman, 2001). The level of protein carbonyls reported in the present study is

consistent with those described by Lund et al. (2007b) in chilled pork patties and noticeably lower than that found by Rowe, Maddock, Lonergan, and Huff-Lonergan (2004), Haak, Raes, Van Byck, and De Smet (2008), and Santé-Lhoutellier, Engel, Aubry, and Gatellier (2008) in chilled beef, pork and lamb meat, respectively. Compared to the results from the present study, Ganhão, Morcuende, and Estévez (2010b) recently reported considerably larger levels of protein carbonyls in pork patties subjected to cooking and a subsequent refrigeration procedure, which highlights the great impact of high temperatures on the oxidative stability of muscle proteins. Previous studies have reported contradictory results regarding the impact of chilled storage on the formation of protein carbonyls in raw meats. Some authors reported no increase of the amount of protein carbonyls during chilled storage of raw meats (Lund et al., 2007b; Smet et al., 2008) while the amount of TBARS increased steadily. It is known that the onset of lipid oxidation in meat systems would take place faster than the oxidative degradation of myofibrillar proteins (Vuorela et al., 2005; Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008; Estévez et al., 2009) and hence, it is plausible that lipid-derived radicals and hydroperoxides promote protein oxidation. The positive correlation found between protein carbonyls and TBARS in the present study ($r = 0.62$; $p < 0.01$) supports the belief that lipid and protein oxidation are timely coupled in food systems (reviewed by Lund et al., 2011).

In general, the addition of the avocado extracts inhibited to some extent the formation of protein carbonyls although the effects were variable throughout the chilled storage. At the point of the highest protein oxidation rate (day 15), patties with extracts from the Hass avocado variety had significantly lower protein carbonyls than C-patties while F-P and F-S showed intermediate values. In contrast to the TBARS results, differences between treatments were not so clear and this is in agreement with other authors (Lund et al., 2007b; Smet et al., 2008). However, the present results confirm the efficacy of avocado extracts against protein oxidation in raw meat patties. Extracts from F-avocados displayed the most intense antioxidant activity in vitro and against lipid oxidation in meat patties while extracts from H-avocados had the most intense protective effect towards proteins. It is noteworthy the lack of correspondence between the antioxidant activity of the extracts in the in vitro assays and the activity of such extracts against protein oxidation in meat patties. The aforementioned in vitro assays may provide a reliable prediction of the behavior of natural extracts against lipid oxidation in real meat systems whereas the eventual effect of the extracts on protein oxidation might not be related. These results reflect that the chemistry behind lipid and protein oxidation processes may differ considerably in meat systems which are in agreement with recent studies aimed to shed light on the precise mechanisms involved in the oxidation of myofibrillar proteins (Estévez et al., 2009; Estévez & Heinonen, 2010; Lund, Christensen, Fregil, Hviid, & Skibsted, 2008). In addition, these results reflect the different ability of extracts from different avocado varieties against the oxidation of each meat component which could respond to their different phenolics composition. The peel and the seed from avocados are rich in phenolic compounds with some of them being identified as procyanidins and chlorophylls (Wang et al., 2010). Besides the procyanidins, the avocado materials contain a large variety of catechins, hydroxybenzoic acids, hydroxycinnamic acids and flavonols (Rodríguez-Carpena et al., 2011). Some of the phenolic compounds found to be components of the avocado materials such as the gallic acid, the chlorogenic acid and the catechins have been described as inhibitors of the formation of carbonyl compounds from myofibrillar proteins (Estévez & Heinonen, 2010). The very few studies devoted to evaluate the impact of phenolic-rich materials on protein oxidation in chilled raw meats, report contradictory results. Smet et al. (2008) found no effect of dietary treatments with α -tocopherol and diverse phenolic-rich materials on protein oxidation in poultry meat while Lund et al.

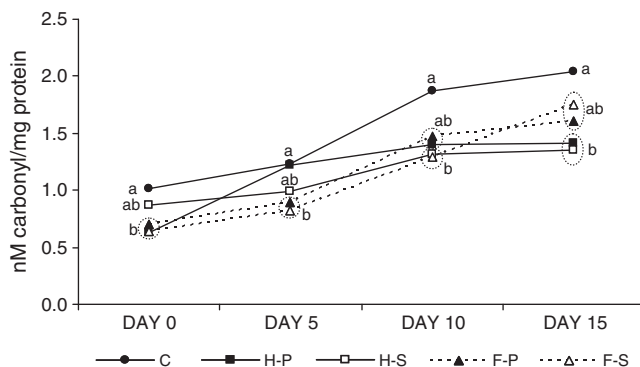


Fig. 5. Protein hydrazones in raw patties during 15 days of chilled storage. Different letters (^{a-b}) within a day of storage denote significant differences between batches ($p < 0.05$). Values embraced by circles are similar ($p > 0.05$). C: Control patties; H-P: Patties treated with 'Hass' peel extract; H-S: Patties treated with 'Hass' seed extract; F-P: Patties treated with 'Fuerte' peel extract; F-S: Patties treated with 'Fuerte' seed extract.

(2007b) reported pro-oxidant actions of added rosemary in chilled pork patties. Haak et al. (2008) also reported ambiguous results while studying the impact of α -tocopherol alone and in combination with phenolic compounds on the oxidation of proteins in raw and cooked pork. Recent studies aimed to shed light on the interactions between α -tocopherol, phenolic compounds and myofibrillar proteins, have reported the complex mechanisms involved during in vitro oxidation assays (Estévez et al., 2008; Estévez & Heinonen, 2010). As redox-active compounds, plant phenolics display a variety of actions which can lead to antioxidant and/or prooxidant actions towards food proteins. The overall effect depends on a variety of factors including the concentration of the active compounds, the presence of transition metals and other oxidation promoters and the composition and structure of the proteins (Estévez et al., 2008; Estévez & Heinonen, 2010). In the present study the antioxidant effect displayed by the avocado materials can be ascribed to the radical scavenging and metal chelating activities of their phenolic compounds. In agreement with the present results, Ganhão et al. (2010b) found a clear antioxidant effect of phenolic-rich fruit extracts against the formation of protein carbonyls in cooked patties. The impact of protein oxidation on the quality of meat products is largely ignored and hence, the likely beneficial effect of the avocado materials on chilled pork patties remains unknown. It is generally accepted, however, that the occurrence of protein oxidation in foods leads to a loss of nutritional value as carbonyl compounds derive from essential amino acids which are not available any more. Furthermore, previous studies have established reasonable casual relationship between the onset of protein oxidation and texture deterioration in fresh meat, cooked patties and frankfurters (Lund et al., 2008; Ganhão et al., 2010b; Estévez, Ventanas, & Cava, 2005). Hence, the protective role of 'HASS' avocado extracts towards muscle proteins could improve the nutritional value and sensory properties of patties.

4. Conclusions

The present results highlight the potential usage of extracts from avocado by-products as efficient inhibitors of oxidative reactions and color deterioration during chilled storage of raw porcine patties. Using these extracts as natural antioxidants could be an effective strategy to enhance the quality of muscle foods. The two avocado varieties evaluated—'Hass' and 'Fuerte'—display intense antioxidant effects whereas their efficacy against lipid and protein oxidation is different. Further research on the role played by specific phenolic compounds on the oxidative reactions affecting to muscle lipids and proteins would shed light on these complex mechanisms and would allow a more rational usage of phenolic-rich extracts as functional ingredients in muscle foods.

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References

- A.O.A.C. (2000a). Moisture content 950.46. *Official methods of analysis* (17th ed.). Association of Official Analytical Chemists Gaithersburgh, Maryland.
- A.O.A.C. (2000b). Protein content in meat 928.08. *Official methods of analysis* (17th ed.). Association of Official Analytical Chemists Gaithersburgh, Maryland.
- Bekhit, A. E. D., Geesink, G. H., Ilian, M. A., Morton, J. D., & Bickerstaffe, R. (2009). The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties. *Food Chemistry*, *81*, 175–187.
- Brewer, M. S., Lan, H. Y., & McKeith, F. K. (1998). Consumer evaluation of pork appearance with differing physiological and packaging conditions. *Journal of Muscle Foods*, *9*, 173–183.
- Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2007). Characterization of fluorescent Schiff bases formed during oxidation of pig myofibrils. *Meat Science*, *76*, 210–215.
- Chen, X., Jo, C., Lee, J. I., & Ahn, D. U. (1999). Lipid oxidation, volatiles and colour changes of irradiated pork patties as affected by antioxidants. *Journal of Food Science*, *64*, 16–19.
- Davies, M. J., & Dean, R. T. (2003). *Radical-mediated protein oxidation*. Oxford: Oxford Science Publications.
- De Oliveira, A. C., Valentim, I. B., Silva, C. A., Henriques, B. E. J., de Barros, M. P., Mano, C. M., et al. (2009). Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. *Food Chemistry*, *115*, 469–475.
- Decker, E. A., Xiong, Y. L., Calvert, J. T., Crum, A. D., & Blanchard, S. P. (1993). Chemical, physical, and functional properties of oxidized turkey white muscle myofibrillar proteins. *Journal of Agricultural and Food Chemistry*, *41*, 186–189.
- Estévez, M., Morcuende, D., & Cava, R. (2003). Oxidative and colour changes in meat from three lines of free-range reared Iberian pigs slaughtered at 90 kg live weight and from industrial pig during refrigerated storage. *Meat Science*, *65*, 1139–1146.
- Estévez, M., Ventanas, S., & Cava, R. (2005). Protein oxidation in frankfurters with increasing levels of added rosemary essential oil: Effect on color and texture deterioration. *Journal of Food Science*, *70*, 427–432.
- Estévez, M., & Cava, R. (2006). Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: Contradictory effects in different types of frankfurters. *Meat Science*, *72*, 348–355.
- Estévez, M., Ventanas, S., & Cava, R. (2006). Effect of natural and synthetic antioxidant on protein oxidation and colour and texture changes in refrigerated stored porcine liver pâté. *Meat Science*, *74*, 396–403.
- Estévez, M., Kylli, P., Puolanne, E., Kivikari, R., & Heinonen, M. (2008). Oxidation of skeletal muscle myofibrillar proteins in oil-in-water emulsions: Interaction with lipids and effect of selected phenolic compounds. *Journal of Agricultural and Food Chemistry*, *56*, 10933–10940.
- Estévez, M., Ollilainen, V., & Heinonen, M. (2009). Analysis of protein oxidation markers α -amino adipic and γ -glutamic semialdehydes in food proteins using liquid chromatography (LC)-electrospray ionization (ESI)-multistage tandem mass spectrometry (MS). *Journal of Agricultural and Food Chemistry*, *57*, 3901–3910.
- Estévez, M., & Heinonen, M. (2010). Effect of phenolic compounds on the formation of α -amino adipic and γ -glutamic semialdehydes from myofibrillar proteins oxidized by copper, iron, and myoglobin. *Journal of Agricultural and Food Chemistry*, *58*, 4448–4455.
- Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science*, *86*, 86–94.
- Folch, L., Lees, M., & Sloane-Stanley, C. H. (1957). A simple method for the isolation and purification of total lipid from animal tissues. *The Journal of Biological Chemistry*, *226*, 497–509.
- Francis, F. J., & Clydesdale, F. M. (1975). *Food colorimetry: Theory and applications*. Westport, CT: The AVI Publishing Co., Inc.
- Gamble, J., Harker, F. R., Jaeger, S. R., White, A., Bava, C., Beresford, M., et al. (2010). The impact of dry matter, ripeness and internal defects on consumer perceptions of avocado quality and intentions to purchase. *Postharvest Biology and Technology*, *57*, 35–43.
- Ganhão, R., Morcuende, D., & Estévez, M. (2010a). Tryptophan depletion and formation of α -amino adipic and γ -glutamic semialdehydes in porcine burger patties with added phenolic-rich fruit extracts. *Journal of Agricultural and Food Chemistry*, *58*, 3541–3548.
- Ganhão, R., Morcuende, D., & Estévez, M. (2010b). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. *Meat Science*, *85*, 402–409.
- Ganhão, R., Estévez, M., Kylli, P., Heinonen, M., & Morcuende, D. (2010c). Characterization of selected wild Mediterranean fruits and comparative efficacy as inhibitors of oxidative reactions in emulsified raw pork burger patties. *Journal of Agricultural and Food Chemistry*, *58*, 8854–8861.
- Garrison, W. M. (1987). Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chemical Reviews*, *87*, 381–398.
- Georgantelis, D., Blekas, G., Katikou, P., Ambrosiadis, I., & Dimitrios, J. F. (2007). Effect of rosemary extract, chitosan and α -tocopherol on lipid oxidation and color stability during frozen storage of beef burgers. *Meat Science*, *75*, 266–274.
- Haak, L., Raes, K., Van Byck, S., & De Smet, S. (2008). Effect of dietary rosemary and α -tocopherol acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. *Meat Science*, *78*, 239–247.
- Haak, L., Raes, K., & De Smet, S. (2009). Effect of plant phenolic, tocopherol and ascorbic acid on oxidative stability of pork patties. *Journal of the Science of Food and Agriculture*, *89*, 1360–1365.
- Hayes, J., O'Brien, N., Kerry, J., O'Grady, M., Stepanyan, V., & Allen, P. (2009). The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. *Meat Science*, *83*, 201–208.
- Ibrahim, H. M., Abou-Arab, A. A., & Abu, S. F. M. (2010). Addition of some natural plant extracts and their effects on Lamb patties quality. *Journal of Food Technology*, *8*(3), 134–142.
- Ismail, A., Marjan, Z. M., & Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, *87*, 581–586.
- Jo, C., Son, J. H., Son, C. B., & Byun, M. W. (2003). Functional properties of raw and cooked pork patties with added irradiated, freeze-dried green tea leaf extract powder during storage at 4 °C. *Meat Science*, *64*, 13–17.

- Joo, S. T., Kauffman, R. G., Kim, B. C., & Kim, C. J. (1995). The relationship between color and water holding capacity in postrigor porcine Longissimus muscle. *Journal of Muscle Foods*, 6, 211–226.
- Kähkönen, M. P., & Heinonen, M. (2003). Antioxidant activity of anthocyanins and their aglycons. *Journal of Agricultural and Food Chemistry*, 51, 628–633.
- Kritchevsky, D., Tepper, S. A., Wright, S., Czarnecki, S. K., Wilson, T. A., & Nicolosi, R. J. (2003). Cholesterol vehicle in experimental atherosclerosis 24: Avocado oil. *Journal of the American College of Nutrition*, 22, 52–55.
- Ledward, D. A. (1991). Letter to Editor: Meat color stability. *Journal of Food Science*, 56 vii.
- López-López, I., Cofrades, S., Yakan, A., Solas, M. T., & Jiménez-Colmenero, F. (2010). Frozen storage characteristics of low-salt and low-fat beef patties as affected by Wakame addition and replacing pork backfat with olive oil-in-water emulsion. *Food Research International*, 43, 1244–1254.
- Lu, Q. Y., Arteaga, J. R., Zhang, Q., Huerta, S., Go, V. L. W., & Heber, D. (2005). Inhibition of prostate cancer cell growth by an avocado extract: Role of lipid-soluble bioactive substance. *The Journal of Nutritional Biochemistry*, 16, 23–30.
- Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., & Skibsted, L. H. (2007a). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. *Meat Science*, 77, 295–303.
- Lund, M. N., Hviid, M. S., & Skibsted, L. H. (2007b). The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Science*, 76, 226–233.
- Lund, M. N., Christensen, M., Fregil, L., Hviid, M. S., & Skibsted, L. H. (2008). Effect of high-oxygen atmosphere packaging on mechanical properties of single muscle fibres from bovine and porcine longissimus dorsi. *European Food Research and Technology*, 227, 1323–1328.
- Lund, M. N., Heinonen, M., Baron, C. P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research*, 55, 83–95.
- Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science*, 71, 100–121.
- McBride, N. T. M., Hogan, S. A., & Kerry, J. P. (2007). Comparative addition of rosemary extract and additives on sensory and antioxidant properties of retail packaged beef. *International Journal of Food Science and Technology*, 42, 1201–1207.
- McCarthy, T. L., Kerry, J. P., Kerry, J. F., Lynch, P. B., & Buckley, D. J. (2001). Assessment of the antioxidant potential of natural food and plant extracts in fresh and previously frozen pork patties. *Meat Science*, 57, 177–184.
- Mercier, Y., Gatellier, P., & Renner, M. (2004). Lipid and protein oxidation in vitro, and antioxidant potential in meat from Charolais cows finished on pasture or mixed diet. *Meat Science*, 66, 467–473.
- Monahan, F. J., Skibsted, L. H., & Andersen, M. L. (2005). Mechanism of oxymyoglobin oxidation in the presence of oxidizing lipids in bovine muscle. *Journal of Agricultural and Food Chemistry*, 53, 5734–5738.
- Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, 49, 73–86.
- Moure, A., Cruz, J. M., Franco, D., Doínguez, J. M., Sineiro, J., Domínguez, H., et al. (2001). Natural antioxidant from residual sources. *Food Chemistry*, 72, 145–171.
- O'Grady, M. N., Monahan, F. J., & Brunton, N. P. (2001). Oxymyoglobin oxidation and lipid oxidation in bovine muscles—Mechanistic studies. *Journal of Food Science*, 66, 386–392.
- Oliver, C. N., Ahn, B., Moerman, E. J., Goldstein, S., & Stadtman, E. R. (1987). Age-related changes in oxidized proteins. *The Journal of Biological Chemistry*, 262(12), 5488–5491.
- Pokorný, J., Yanishlieva, N., & Gordon, M. (2001). *Antioxidants in food*. Cambridge: CRC Press.
- Ragaee, S., Abde-Aal, E. S., & Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, 98, 32–38.
- Requena, J. R., Chao, C. C., Levine, R. L., & Stadtman, E. R. (2001). Glutamic and amino adipic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins. *Proceeding of the National Academy of Sciences U. S. A.*, 98, 69–74.
- Rodríguez-Carpena, J. G., Morcuende, D., Andrade, M. J., Kylli, P., & Estévez, M. (2011). Avocado (*Persea americana* Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of Agricultural and Food Chemistry*, 59, 5625–5635.
- Rowe, L. J., Maddock, K. R., Lonergan, S. M., & Huff-Lonergan, E. (2004). Influence of early postmortem protein oxidation on beef quality. *Journal of Animal Science*, 82, 785–793.
- Salih, A. M., Smith, D. M., Price, J. F., & Dawson, L. E. (1987). Modified extraction of 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Science*, 66, 1483–1488.
- Santé-Lhoutellier, V., Engel, E., Aubry, L., & Gatellier, P. (2008). Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility. *Meat Science*, 79, 777–783.
- Shahidi, F. (1998). *Flavor of meat, meat products and sea foods* (2nd Edition). Blackie Academic and Professional.
- Shan, B., Cai, Y. Z., Brooks, J. D., & Corke, H. (2009). Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. *Journal of the Science of Food and Agriculture*, 89, 1879–1885.
- Smet, K., Raes, K., Huyghebaert, G., Haak, L., Arnouts, S., & De Smet, S. (2008). Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. *Poultry Science*, 87, 1682–1688.
- Soong, Y. Y., & Barlow, P. J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry*, 88, 411–417.
- Stadtman, E. R., & Berlett, B. S. (1988). Fenton chemistry revisited: Amino acid oxidation. *Basic Life Sciences*, 49, 131–136.
- Suman, S. P., Fastman, C., Stamer, S. L., & Liebler, D. C. (2006). Redox instability induced by 4-hydroxy-2-nonenal in porcine and bovine myoglobins, at pH 5.6, 4 °C. *Journal of Agricultural and Food Chemistry*, 54, 3402–3408.
- Vuorela, S., Salminen, H., Mäkelä, M., Kivikari, R., Karonen, M., & Heinonen, M. (2005). Effect of plant phenolics on protein and lipid oxidation in cooked pork meat patties. *Journal of Agricultural and Food Chemistry*, 53, 8492–8497.
- Wang, W., Bostic, T. R., & Gu, L. (2010). Antioxidant capacities, procyanidins and pigments in avocado of different strains and cultivars. *Food Chemistry*, 122, 1193–1198.
- Wojdylo, A., oszmianski, J., & Czemerz, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105, 940–949.
- Wu, X., Gu, L., Holden, J., Haytowitz, D. B., Gebhardt, S. E., Beecher, G., et al. (2004). Development of a database for total antioxidant capacity in foods: A preliminary study. *Journal of Food Composition and Analysis*, 17, 407–422.
- Yoo, K. M., Lee, C. H., Lee, H., Moon, B., & Lee, C. Y. (2008). Relative antioxidant and cytoprotective activities of common herbs. *Food Chemistry*, 106, 929–936.
- Yu, J., Ahmedna, M., & Goktepe, I. (2010). Potential of peanut skin phenolic extract as antioxidative and antibacterial agent in cooked and raw ground beef. *International Journal of Food Science and Technology*, 45, 1337–1344.
- Zhu, L. G., & Brewer, M. S. (1998). Discoloration of fresh pork as related to muscle and display conditions. *Journal of Food Science*, 63, 763–767.