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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.

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 discolouration through the oxidation of myoglobin and leads to the formation of low molecular weight compounds which impart rancid odors and off-flavors (Mercier, Gatellier, & Renerre, 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, Hvii, 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 (Ragaee, 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 927257122; fax: +34 927257110.
E-mail address: mariovet@unex.es (M. Estévez).

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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 (Merk, 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 cm^3), 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 cm^3/m^2 day atm; moisture permeability: <5 g/m^2 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 = $[(W_0 - W_{15})/W_0]$ 100; where W_0 is the weight of the patty at day 0, and W_{15} 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