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Effect of long term dietary supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork ☆

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ABSTRACT

The effects of dietary supplementation in pigs with plant extract (PE) from Lippia spp., titrated in verbascoside (5 mg/kg feed), from weaning to slaughter (166 days), on carcass characteristics, meat quality, collagen characteristics, oxidative stability and sensory attributes of *Longissimus dorsi* (LD) muscle were examined. Ten pigs per treatment were slaughter at a live weight of 109.5 ± 1.4 kg. No influence on carcass characteristics, LD meat quality parameters and collagen characteristics were observed. Dietary PE increased ($P < 0.001$) α-tocopherol levels in LD muscle. Raw LD of pig fed PE showed lower ($P < 0.001$) lipid oxidation levels than controls. A reduction ($P = 0.05$) of fat odor and rancid flavor intensity in cooked LD muscle stored at 4 °C for 24 h was observed in the treated group. This study shows that PE is an effective antioxidant in pork meat, enhancing oxidative status and sensory attributes, without affecting other meat quality parameters.

1. Introduction

In recent years considerable attention has been given to the improvement of meat quality. Studies have shown that some feed additives (vitamins, mineral and antioxidant) improve pork sensory and nutritional characteristics (Nuernberg et al., 2002; Swigert, McKeith, Carr, Brewer, & Culbertson, 2004). In particular, additives for the control of lipid oxidation in meat and meat products have become increasingly important. Lipid oxidation is a major deteriorative phenomenon that negatively affects flavor, color and nutritional value of meat (Asghar, Gray, Buckley, Pearson, & Boeren, 1988) and is responsible to the potential formation of toxic compounds (Addis & Park, 1989). In addition to microbial spoilage, it causes loss of pork quality and thus determines the shelf life of pork products.

Many synthetic and natural substances have been investigated as potential antioxidants to prevent lipid oxidation. The trend is to decrease the use of synthetic antioxidants due to consumer concerns over safety and toxicity (Coronado, Trout, Dunsea, & Shah, 2002). Therefore, the search for natural additives, especially from plants, has increased in recent years (Meyer, Suhr, Nielsen, & Holm, 2002). Compounds from natural sources such as grains, oilseeds, spices, fruits and vegetables have been investigated (Que, Mao, & Pan, 2006; Sebranek, Sewalt, Robbins, & Houser, 2005).

Some plant extracts contain phenolic compounds that have anti-inflammatory, antimicrobic and antioxidant activities (Pereira, Valentão, Pereira, & Andrade, 2009).

Phenylpropanoid glycosides (PPG), like other phenolic compounds, are powerful antioxidants by direct scavenging of reactive oxygen and nitrogen species, or by acting as chain-breaking peroxyl radical scavengers (Afanasev, 2005). Beside phenolic compounds, verbascoside, shows the highest scavenger activity in the PPG (Wang et al., 1996) and has high antioxidant power in comparison with other phenolic compounds (Aleo, Ricci, Passi, & Cataudella, 2005).

Phenylpropanoids, particularly verbascoside, are the most abundant compounds in Verbenaceae extracts (Pascual, Slowing, Carretero, Sanchez Mata, & Villar, 2001). Previous studies showed that dietary supplementation with plant extracts of Verbenaceae (PE), improved the plasma oxidative status in weaning piglets (Pastorelli, Rossi, & Corino, 2012), in lacaune ewes (Casamassima, Palazzo, Martemucci, Vizzarri, & Corino, 2012) and Italian hares (*Lepus corsicanus*) (Palazzo, Vizzarri, Cinone, Corino, & Casamassima, 2011). Moreover, Rossi, Corino, Pastorelli, Durand, and Prost (2009) found that PE has greater antioxidant power compared to other phenolic compounds and compared to a water soluble analog of vitamin E (trolox).

On this basis the PE containing verbascoside could be used as a dietary supplement in association and/or partial replacement of

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synthetic vitamin E in animal feeding to enhance meat quality and consumer well-being. The literature contains no data on the effects of dietary supplementation of plant extracts of Verbenaceae, titrated in verbascoside, on meat quality.

The objective of the present study was to assess the effectiveness of long term supplementation of porcine diets with PE on carcass characteristics, meat quality parameters, oxidative stability and sensorial properties of *Longissimus dorsi* (LD) muscle. The influence of dietary PE on collagen characteristics and vitamin E content of LD muscle was also determined.

2. Material and methods

2.1. Animals and experimental design

Forty Dalland pigs, half barrows and half females, of average live weight (LW) 7 ± 0.4 kg, were assigned, on the basis of weight and sex, to two dietary treatments: control diet (C) and diet supplemented with plant extract (PE) to obtain 5 mg verbascoside/kg feed. The dose of plant extract in the feed was chosen on the basis of a previous study in piglets (Corino, Rossi, Musella, Cannata & Pastorelli, 2007).

Pigs were fed a corn-based diet with same amount of all-rac-α-tocopheryl acetate (30 mg/kg in the finishing phase; threefold the amount reported by NRC, 2012). The PE supplement contained a water-soluble extract of Verbenaceae (*Lippia* spp.) leaves, prepared on an industrial scale by a standardized procedure that included ultrasonic extraction with 60% aqueous ethyl alcohol followed by spray-drying with maltodextrins. The PE supplement did not contain vitamin E. The phenylpropanoid glycosides and benzoic acid content of the feed supplement, according to a certificate of analysis provided by the manufacturer, was: gallic acid, 1.75 ± 0.07; 3.4-dihydroxybenzoic acid, 0.45 ± 0.04; methyl gallate, 1.91 ± 0.09; isoverbascoside, 0.43 ± 0.04 and verbascoside, 4.47 ± 0.08 g/kg. The quantitative analysis of the phenolic compounds was performed by HPLC–UV–DAD (Rastrelli, personal communication). To avoid oxidation in the feed, the supplement was microencapsulated within a protective matrix of hydrogenated vegetable lipids using spray-cooling (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy). The experimental diets were formulated to meet the requirements for all nutrients for all the breeding phases (NRC, 2012), and were presented for ad libitum consumption. According to the gender, the animals were divided into 10 pens (4 pigs/pen) and reared in an environmentally controlled room.

The animals were cared for following the European Union guidelines (No. 86/609/EEC, 1986) approved by the Italian Ministry of Health (L. 116/92).

2.2. Carcass traits

Ten barrows per treatments were slaughtered at 109.5 ± 1.4 kg LW. After an on-farm fasting period of 8 h, the pigs were transported to the abattoir. The pigs were laired for 4 h with free access to water. Pigs were electrically stunned; following exsanguination, the carcasses were scalded, dehaired and eviscerated. Live weight at slaughter and hot carcass weight were recorded.

Dressing percentage was calculated, and midline backfat depth opposite the first rib, last rib, and last lumbar vertebrae was recorded. pH at 24 h postmortem was determined on LD muscle using a pH meter (HI 9023 microcomputer, Hanna Instruments, Vila do Conde, Portugal). The pH probe was calibrated using two buffers (pH 4.0 and 7.0), and calibration was monitored between samples. Color measurements were performed at 24 h postmortem on LD samples at the last lumbar vertebra, using a CR-300 Chroma Meter (Minolta Camera, Co., Osaka, Japan). The instrument was calibrated on the CIE LAB color space system using a white calibration plate (Calibration Plate CR-A43, Minolta Cameras). The colorimeter had an 8-mm measuring area and was illuminated with a pulsed Xenon arc lamp (illuminat C) at 0° viewing angle. Reflectance measurements were obtained at a viewing angle of 0° and the spectral component was included.

Longissimus dorsi muscle of all animals was removed from the carcass (after 24 h at 2–4 °C) at the last lumbar vertebra, vacuum-packed, and stored frozen (−20 °C) for chemical composition, vitamin E content, oxidative stability and intramuscular collagen (IMC) analyses. For the physical analyses fresh LD samples were employed.

2.3. Physical analyses

Drip and cooking losses were determined as described by Honikel (1998). A slice of fresh LD muscle (45 ± 2 g), was placed with a cut surface facing down on a grid in a closed plastic container. Drip loss was determined as percentage weight loss after 24 h of storage at 4 °C. For cooking loss determination, a fresh 25 mm thick slice from each sample was weighed (130 ± 5 g), placed in a plastic bag and cooked to an internal temperature of 70 °C in a 75 °C water bath. Internal temperature was monitored during cooking with a hand-held temperature probe. Cooked samples were allowed to cool for 30 min, blotted dry and weighed. The difference between pre- and post-cooking weights was used to calculate the percentage loss during cooking.

The Warner–Bratzler shear force (WBSF) was determined in samples cooled at 4 °C for 24 h after heat-treatment. For each sample, 6 cylindrical cores (Ø 1.25 cm), parallel to the longitudinal orientation of the muscle fibers, were taken. Each core was sheared with a WBSF device attached to an Instron Universal Testing Machine (model 4466; Instron Corp., Canton, MA) with a 50 kg tension/compression load cell using a crosshead speed of 50 mm/min. The maximum force (kg/cm^2) was recorded.

2.4. Chemical composition and cholesterol

Samples of LD of all animals were analyzed for dry matter, nitrogen, fat and ash according to Association of Analytical Chemists methods (AOAC, 2000).

Cholesterol was extracted using the method of Maraschiello, Diaz, and Garcia Regueiro (1996) and then quantified by HPLC. A Kontron HPLC (model 535, Kontron Instruments, Milan, Italy) equipped with a C18 reverse-phase column (250 mm × 4.6 mm × 5 μm; Phenomenex, Torrance, CA) was used. The mobile phase was acetonitrile 2 propanol (55:45 vol/vol) at a flow rate of 1.2 mL/min. The detection wavelength was 210 nm and retention time was 13.89 min.

2.5. Alpha tocopherol content

The levels of vitamin E in the LD muscle were determined and quantified as described by Zapel and Csallany (1983) and then quantified by HPLC (Kontron Instruments, Milan, Italy) model 535 equipped with a C18 reverse-phase column (250 cm × 4.6 mm × 5 μm) (Phenomenex, Torrance, CA). The mobile phase was 100% methanol at a flow rate of 1.5 mL/min. The detection wavelength was 292 nm and retention time was 4.1 min.

2.6. Collagen analyses

The IMC characteristics are responsible for the background toughness of meat (Maiorano, Kapelański, Bocian, Pizzuto, & Kapelańska, 2013). To study IMC characteristics, samples of LD from all pigs were thawed, trimmed of fat and epimysium, lyophilized for 48 h, weighed, and hydrolyzed in Duran tubes in 6 N HCl at 110 °C for 18–20 h (Etherington & Sims, 1981) for determination of hydroxyproline (Woessner, 1961) and crosslinking. IMC concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe & Leach, 1958) and expressed as micrograms of hydroxyproline per milligram of lyophilized tissue. Hydroxylysylpyridinoline (HLP) concentration, the principal nonreducible crosslink of muscle collagen (McCormick,

1999), highly correlated with the thermal stability of collagen (Horgan, 1991), was determined using a modification (Maiorano, Manchisi, Salvatori, Filetti, & Oriani, 1999) of the HPLC procedure developed by Eyre, Koob, and Van Ness (1984); it was expressed as moles of HLP per mole of collagen and also as micrograms of HLP per milligram of lyophilized tissue.

2.7. Measurement of oxidative stability

For oxidative stability evaluation the LD samples were analyzed as raw and cooked meat after storage at 4 °C for 0 and 24 h. Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) method as described by Monin, Hortos, Diaz, Rock, and Garcia-Regueiro (2003). Briefly, 4 g of minced meat was homogenized in 20 mL of distilled water using an Ultra Turrax T25 homogenizer (IKA, Cincinnati, USA). To the homogenate was added 5 mL of 25% trichloroacetic acid, that was then shaken at 4 °C for 30 min and centrifuged at 5000 *g* and 4 °C for 10 min. The supernatant was filtered with Whatman 52 filter paper. To 3.5 mL of supernatant 1.5 mL of 0.6% thiobarbituric acid was added and held at 70 °C for 30 min. The absorbance at 532 nm was measured immediately after cooling and compared with a standard curve of malonaldehyde prepared by hydrolysis of tetraethoxypropane. Determinations were made in duplicate. The results were expressed as micrograms of malondialdehyde (MDA) per gram of meat.

2.8. Sensory analysis

2.8.1. Sample preparation

The loin preparation for sensory analysis was performed using the method of Jonsäll (2000). After thawing for 24 h at 4 °C, the loins (total samples = 20) were prepared in one piece in an uncovered stainless steel dish in a conventional oven (REX, Italy) at 150 °C. A thermocouple (Pentronic AB, Gunnebobruk, Sweden) was inserted in the center of each piece of meat to register the core temperature. The core temperature was not allowed to exceed 68°, the loin was removed from the oven at 65 to 66.5 °C to allow for post-heating rise. After cooling the entire LD muscles were cut into 3 mm thick slices (Electrolux 50, 220–24, kW 0.2). The samples were evaluated for sensory characteristics after 0 (T1) and 24 h (T2) at 4 °C. The LD slices were warmed at 60 °C before the evaluation.

2.8.2. Descriptive analysis

A trained sensory panel, consisting of 8 members, familiar with pork and descriptive analysis procedures (EN ISO, 13299, 2010) was chosen. All assessments were carried out in a sensory laboratory equipped according to ISO 8598 (1989) recommendations. The list of descriptors, definitions, and standards is shown in Table 1 (Miller, 1998). The panel evaluated each sample in triplicate. During training and sampling, panelists had access to unlimited water and unsalted crackers. Within each session the design was balanced for order and carryover effects (MacFie, Bratchell, Greenhoff, & Vallis, 1989). Judges were requested to evaluate the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense).

2.9. Statistical analyses

Statistical analyses of the data were performed using SPSS (SPSS/PC Statistics 18.0 SPSS Inc., Chicago, IL, 2009). Data on carcass characteristics and LD parameters were analyzed by one-way analysis of variance (ANOVA) where diet was the main factor. Linear regression was used to evaluate correlations between muscle TBARS values and α-tocopherol content. One way ANOVA was used to test differences between dietary treatment on LD sensory characteristics for each storage time. Each pig was considered as the experimental unit. Data are presented as means ± SEM, and a value of $P < 0.05$ was used to indicate statistical significance.

Table 1
Descriptors, definitions and standards of sensory analysis.

Attribute	Definition	Standard
Odors		
Sulfury	Aromatics associated with boiled egg	Boiled egg
Fat	Aromatics associated with cooked pork fat	Cooked pork fat
Flavors		
Metallic	Flavor associated with blood or rare meat	Rare beef
Rancid	Flavor associated with rancid/oxidized fat	Oxidized flax seed oil
Roast peanuts	Flavor associated with roast peanuts	Roast peanuts
Cooked pork	Flavor associated with cooked pork loin	Cooked pork loin
Livery	Flavor associated with cooked liver	Cooked liver
Tastes		
Sweet	Taste associated with sucrose	Horse meat
Salty	Taste associated with sodium chloride	Pork loin boiled in salt water (15 g/of NaCl)
Sour	Taste associated with citric acid solutions	0.08% citric acid
Texture		
Juiciness	The degree of juice released while chewing the meat	Rare roast beef
Tenderness	The force needed to masticate the meat ready for swallowing (chewing 5 times)	Rare roast beef (extremely tender) Well done veal slice (extremely tough)
Stringy	Production of a large quantity of saliva for swallowing	Chicken breast boiled in no-salt water for 45 min
Fibrous	Presence of fibers during swallowing	Cattle meat boiled in no-salt water for 45 min

3. Results and discussion

Supplementing pig diets with α-tocopheryl acetate is a conventional way to decrease the susceptibility of pork to oxidation (Jensen, Lauridsen, & Bertelsen, 1998) and to improve meat quality (Corino, Oriani, Pantaleo, Pastorelli, & Salvatori, 1999).

Moreover, it has been shown that several plants or their phenolic extracts are efficient antioxidant when added in animal feeding (Lahucky, Nuernberg, Kovac, Bucko, & Nuernberg, 2010; O'Grady, Carpenter, Lynch, O'Brien, & Kerry, 2008) or in fresh meat (Kanatt, Chander, Radhakrishna, & Sharma, 2005; McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001).

In the recent years plant extracts have been widely used in animal nutrition for the prevention of some pathological conditions and as antioxidants (Frankič, Voljč, Salobir, & Rezaracta, 2009).

Several studies have been conducted in the growing-finishing phase to improve the oxidative stability of meat and meat products (Haak et al., 2006; Lahucky et al., 2010). No data have been reported on the effects of long-term supplementation from weaning to slaughter of plant extracts on carcass characteristics and meat quality parameters.

3.1. Carcass characteristics

In the present study, dietary supplementation with plant extract had no effects on carcass weight, dressing percentage and backfat thickness (Table 2), in agreement with Cullen, Monahan, Callan, and O'Doherty (2005) who reported no effect on carcass characteristics in pigs fed a diet supplemented with garlic and rosemary for 56 days. The same was also observed in pigs fed different levels of a plant extract mixture, containing thymol, carvacrol capsaicin, cinnamon aldehyde, eugenol,

Table 2
Carcass characteristics of pigs fed control (C) or plant extract (PE) supplemented diet from weaning to slaughter.[a]

Trait	C	PE	P
Final weight, kg	109.8 ± 1.9	109.4 ± 2.2	0.884
Carcass weight, kg	88.2 ± 2.1	87.7 ± 1.6	0.683
Dressing, %	80.2 ± 0.7	79.6 ± 0.7	0.569
Backfat thickness first rib, cm	3.39 ± 0.11	3.42 ± 0.06	0.812
Backfat thickness tenth rib, cm	2.58 ± 0.13	2.29 ± 0.09	0.095
Backfat thickness last rib, cm	2.52 ± 0.14	2.37 ± 0.08	0.390

[a] Data are reported as mean values ± SEM; n = 10; C, Control; PE, Plant extract.

flavonides and essential oils from 20 to 100 kg LW (Korniewicz et al., 2007). Hossain, Ko, and Yang (2012) observed no difference in carcass characteristics of finishing pigs fed different levels of green tea by-products for 6 weeks and other authors demonstrated no effect of plant extracts on these carcass traits (Grela, 2000; Paschma & Wawrzynski, 2003).

3.2. Physical parameters of LD muscle

The meat quality parameters, pH at 24 h, color indexes, drip and cooking losses, and shear force are presented in Table 3. Muscle pH values and color indexes were within the range expected for pork (Ryu & Kim, 2005). Dietary PE did not affect the pH at 24 h of LD muscle according to Peeters, Driessen, and Geers (2006) in pigs supplemented with dietary herbs (*Valeriana officinalis* L. and *Passiflora incarnata* L.). The same results were observed by Lahucky et al. (2010) in pigs fed an extract of Melissa, Origanum and Salvia for 30 days before slaughter. Also Grela (2000) reported no difference in pH at 45 min and 24 h post-mortem in meat from pigs fed a six-herb supplement. The long term supplementation with PE had no significant effect on the color indexes (L*, a*, and b*) of LD muscle, agreeing with Lahucky et al. (2010). The same result was also observed in pigs fed a diet containing different levels of green tea powder from 70 to 110 kg LW (Sarker et al., 2010). Haak, Raes, Van Dyck, and De Smet (2008) also found no difference on LD muscle color parameters after dietary administration of single antioxidants or antioxidant combinations (α-tocopheryl acetate and rosemary extract).

Neither cooking loss, nor shear force was affected by the dietary treatment, agreeing with Lahucky et al. (2010). Drip loss was lower in LD muscle of the treated pigs (−23.3% than control) although the difference was not significant (P = 0.101). No difference in cooking loss was reported in pigs fed green tea powder (Sarker et al., 2010). By contrast Kołodziej-Skalska et al. (2011) reported lower drip and cooking losses from the LD muscle of pigs fed plant extract compared with the control group. The same result was observed in pigs fed different levels of garlic (0.50, 1.00 and 1.50%) from 10 to 50 kg live weight (Omojola, Fagbuaro, & Ayeni, 2009). The present results indicate that long term supplementation with plant extract, titrated in verbascoside, has no detrimental effect on the physical parameters of pork quality.

Table 3
Physical characteristics of *Longissimus dorsi* muscle in pig fed control (C) or plant extract (PE) supplemented diet from weaning to slaughter.[a]

Trait	C	PE	P
pH, 24 h	5.83 ± 0.03	5.82 ± 0.04	0.861
Color indexes, 24 h postmortem:			
L*	52.07 ± 0.96	53.62 ± 0.71	0.215
a*	5.16 ± 0.27	5.25 ± 0.38	0.850
b*	1.46 ± 0.22	2.08 ± 0.26	0.097
Drip loss, %	3.00 ± 0.25	2.43 ± 0.23	0.101
Cooking loss, %	16.01 ± 0.52	15.18 ± 0.55	0.369
Warner–Bratzler shear force kg, cm^2	2.03 ± 0.10	1.83 ± 0.10	0.209

[a] Data are reported as mean values ± SEM; n = 10; C, Control; PE, Plant extract.

3.3. Chemical parameters of LD muscle

The intramuscular fat, protein, ash and moisture of the LD muscle of the pigs did not differ between the control and the supplemented groups (Table 4). The mean value of LD muscle intramuscular fat is higher than generally reported for light pigs (Corino, Rossi, Musella, Pastorelli, & Cannata, 2009). The value is near to that expected for heavy pigs (Corino et al., 2002; Corino et al., 2008) probably due to the ad libitum feed consumption (Candek-Potokar, Zlender, Lefaucheur, & Bonneau, 1998). No effect on pork LD muscle chemical composition due to dietary supplementation of plant extract has been observed (Kołodziej-Skalska et al., 2011; Lahucky et al., 2010). A difference in chemical composition of LD muscle was observed in pigs fed 1% of green tea powder, with a significant increase of crude protein and a decrease of crude fat (Sarker et al., 2010). This may be due to the inhibitory effect on lipid metabolism of green tea chatechin as observed in rats (Ikeda, Ismasato, & Sasaki, 1992).

The cholesterol content was not affected by dietary PE (Table 4), in accord with Kołodziej-Skalska et al. (2011). However, other authors reported a decrease of total cholesterol content in LD muscle with increasing levels of garlic supplementation in pig diets (Omojola et al., 2009). This cholesterol lowering effect is probably due to the garlic active principles that affect cholesterol biosynthetic pathways (Konjufca, Pesti, & Bakalli, 1997).

3.4. α-Tocopherol content and oxidative stability of LD muscle

Data on α-tocopherol content and TBARS in raw and cooked LD muscle are presented in Table 4. The LD muscle of pigs fed PE showed a higher α-tocopherol content than the controls (+34%; P < 0.001). This is in contrast to previous dietary studies where supplementation of porcine diets with plant antioxidant did not improve LD muscle α-tocopherol contents (Gonzalez & Tejeda, 2007; Haak et al., 2006).

Previous studies in different species showed that PE, titrated in verbascoside, improved the plasma oxidative status (Corino et al., 2007; Casamassima et al., 2012). This oxidative status effect is related to the increased serum levels of vitamins A and E (Palazzo et al., 2011).

Furthermore, a recent study demonstrated that long term supplementation with PE in pigs tended to improve total blood antioxidant status (Rossi, Pastorelli, & Corino, 2013). It was hypothesized that PE may improve vitamin E status in vivo by protecting α-tocopherol from oxidative decay, thus increasing muscle concentration. A similar result was observed by Iglesias, Pazos, Torres, and Medina (2012) who reported that the procyanidins could repair oxidized α-tocopherol in the medium-long term, and could delay ascorbic acid depletion in fish muscle.

Among the antioxidant components of muscle tissues, α-tocopherol plays a central role since it is the major endogenous lipid-soluble chain breaking antioxidant and is one of defensive barriers of muscle against

Table 4
Chemical composition and oxidative stability of *Longissimus dorsi* muscle in pig fed control (C) or plant extract (PE) supplemented diet from weaning to slaughter.[1]

Trait	C	PE	P
Dry matter, %	28.02 ± 0.17	28.72 ± 0.42	0.121
Crude Protein, %[2]	22.83 ± 0.30	23.29 ± 0.18	0.252
Ether extract, %[2]	2.80 ± 0.17	2.87 ± 0.40	0.876
Ash, %[2]	1.54 ± 0.11	1.77 ± 0.08	0.133
Cholesterol, mg/100 g	49.85 ± 2.61	51.63 ± 1.19	0.564
TBARS raw meat, μg/g	0.80 ± 0.09^A	0.30 ± 0.02^B	<0.001
TBARS cooked meat 0 h, μg/g	1.32 ± 0.09	1.16 ± 0.06	0.159
TBARS cooked meat 24 h, μg/g	1.43 ± 0.11	1.25 ± 0.07	0.334
α-tocopherol, μg/g	2.62 ± 0.09^A	3.52 ± 0.06^B	<0.001

[A,B]Values in the same row are different for P < 0.001.
[1] Data are reported as mean values ± SEM; n = 10; C, Control; PE, Plant extract.
[2] Data expressed as percentage of wet weight.

lipid oxidation (Monahan, Buckley, Morrisey, Lynch, & Gray, 1992). The useful effect on meat quality characteristics is related to decreased lipid oxidation, enhancing the products shelf life (Buckley, Morrissey, & Gray, 1995).

In the LD muscle, TBARS values were significantly lower ($P < 0.001$) in the raw meat of pigs fed PE than the controls. The TBARS value in PE group (0.30 μg/g muscle) was lower than the threshold level for raw meat, 0.5 μg MDA/g tissue (Lanari, Schaefer, & Scheller, 1995).

This is in part related to the higher α-tocopherol content in muscle of pigs fed PE, as demonstrated by the negative correlation between TBARS and muscle α-tocopherol level ($r = -0,72$; $P = 0.001$). The protective effects of phenolic compounds in the prevention of lipid oxidation have been investigated previous studies. Osada, Hoshina, Nakamura, and Sugano (2000) observed that in sausage, apple polyphenols inhibited linoleic acid and cholesterol oxidation through their radical scavenging abilities. Moreover phenolic compounds inhibit free radical development and the propagation of free radical reactions through the chelation of transition metal ions, principally copper and iron (Shan, Cai, Brooks, & Corkea, 2009). The TBARS values clearly indicated that pigs fed PE were best protected from lipid oxidation, in accord with previous studies in pigs fed plant antioxidant (Haak et al., 2006; Lahucky et al., 2010; Mairesse et al., 2010).

In contrast Gonzalez and Tejeda (2007) reported no difference in LD muscle TBARS values of pigs fed a diet supplemented with flavonoid and phenolic compounds. Also Augustin et al. (2008) observed that supplementation of pig diets with green tea catechins did not affect muscle TBARS values.

In cooked LD muscle, no difference was observed between control and PE supplemented pigs, in accord with Haak et al. (2006).

3.5. Collagen

It has been established that collagen, the major component of the intramuscular connective tissue (Light, Champion, Voyle, & Bailey, 1985), plays a key role in determining meat toughness (McCormick, 1999; Purslow, 2005). On the other hand, McCormick (1999) reported that mature crosslinks and collagen concentration have an additive effect on the toughening of meat. In the present study, the crosslink concentration is expressed as micrograms of HLP per milligram of muscle (Maiorano et al., 2001) in order to consider the influence of both these parameters. IMC amount and HLP crosslink concentration (μg/mg), as well as the degree of collagen maturation (moles of HLP/mole of collagen) were not influenced by dietary PE (Table 5). No data has been reported the influence of natural antioxidants on pork IMC characteristics. However, in agreement with McCormick (1999) and Maiorano et al. (2001), the results of HLP crosslink concentration (μg/mg) indicate that meat from C and PE pigs would be similar in tenderness.

3.6. Sensory evaluation

Mean sensory scores for odor, taste, flavor and textural attributes of cooked LD of control and treated pigs at two different storage times (0 and 24 h) are shown in Table 6. Many phenolic compounds are characterized by bitterness and astringency. Consumer studies indicate that taste is a determining factor in food choice (Drewnowski & Gomez-Carneros, 2000). The present results suggest that the use of dietary PE does not negatively affect the sensory quality of LD muscle.

At 0 h storage (T0) juiciness was lower ($P < 0.05$) in muscle of PE supplemented pigs. The differences related juiciness are difficult to interpret since no difference in LD muscle physical and chemical parameters were seen. At 24 h storage (T1) a higher ($P = 0.05$) fat odor and rancid flavor was observed in the LD muscle of control pigs. This agrees with a previous study that showed that rancid flavor and fat odors were reduced by the addition of grape seed extract in cooked and refrigerated beef and pork patties and in cooked and refrigerated ground chicken (Brannan, 2009; Rojas & Brewer, 2007). The present results suggest that PE was effective in reducing the intensity of attributes that are generally related with warmed over flavor in precooked and stored pork.

Table 5
Intramuscular collagen (IMC) characteristics of *Longissimus dorsi* muscle in pig fed control (C) or plant extract (PE) supplemented diet from weaning to slaughter.[1]

Trait	C	PE	P
IMC[a] lg/mg[c]	18.48 ± 0.58	17.48 ± 1.63	0.592
HLP[b] μg/mg[c]	3.59 ± 0.41	4.00 ± 0.35	0.466
HLP/IMC mol/mol	0.131 ± 0.01	0.170 ± 0.02	0.182

[1] Data are reported as mean values ± SEM; n = 10; C, Control; PE, Plant extract.
[a] IMC: intramuscular collagen.
[b] HLP: hydroxylysylpyridinoline.
[c] Of lyophilized muscle tissue.

Table 6
Sensory evaluation of *Longissimus dorsi* muscle in relation to dietary treatment and storage time.[1]

Attributes		C	PE	P Diet
Odors				
Sulfury	T0	1.35 ± 0.07	1.48 ± 0.07	0.217
	T1	1.15 ± 0.04	1.22 ± 0.05	0.237
Fat	T0	2.32 ± 0.18	2.21 ± 0.16	0.689
	T1	2.48 ± 0.14[a]	2.13 ± 0.11[b]	0.050
Flavors				
Metallic	T0	2.64 ± 0.17	2.59 ± 0.18	0.820
	T1	2.76 ± 0.19	2.45 ± 0.16	0.274
Peanuts	T0	1.78 ± 0.13	1.73 ± 0.12	0.752
	T1	1.65 ± 0.14	1.51 ± 0.10	0.340
Rancid	T0	1.41 ± 0.09	1.40 ± 0.09	0.915
	T1	1.80 ± 0.10[a]	1.52 ± 0.08[b]	0.050
Cooked meat	T0	4.88 ± 0.22	4.85 ± 0.23	0.930
	T1	4.63 ± 0.21	4.57 ± 0.22	0.845
Pork	T0	4.18 ± 0.24	3.90 ± 0.23	0.413
	T1	4.57 ± 0.25	4.45 ± 0.24	0.736
Livery	T0	1.17 ± 0.06	1.15 ± 0.07	0.810
	T1	1.11 ± 0.04	1.15 ± 0.05	0.574
Tastes				
Sweet	T0	3.41 ± 0.20	3.18 ± 0.18	0.398
	T1	3.04 ± 0.17	3.25 ± 0.17	0.402
Salty	T0	2.22 ± 0.16	2.31 ± 0.17	0.716
	T1	2.03 ± 0.15	1.81 ± 0.15	0.329
Sour	T0	1.81 ± 0.14	1.72 ± 0.13	0.629
	T1	2.07 ± 0.15	1.84 ± 0.12	0.261
Texture				
Juiciness	T0	4.48 ± 0.16[a]	4.01 ± 0.17[b]	0.033
	T1	4.65 ± 0.16	4.51 ± 0.18	0.965
Tenderness	T0	5.14 ± 0.15	4.64 ± 0.15	0.052
	T1	5.18 ± 0.18	5.17 ± 0.20	0.525
Stringy	T0	2.80 ± 0.15	2.77 ± 0.15	0.903
	T1	2.69 ± 0.17	2.59 ± 0.16	0.677
Fibrous	T0	2.51 ± 0.15	2.74 ± 0.15	0.292
	T1	2.31 ± 0.18	2.31 ± 0.20	0.972

[a,b]Values in the same row are different for $P \leq 0.05$.
[1] Data are reported as mean values ± SEM; C, Control; PE, Plant extract T0; cooked samples 0 h storage time, T1 cooked samples 24 h storage time. Intensity of attributes: score between 1 (absence of the sensation) and 9 (extremely intense).

4. Conclusion

The results suggest that long term supplementation with PE is effective as an antioxidant in LD muscle due to the reduced levels of lipid oxidation biomarkers and increased levels of α-tocopherol. Therefore, PE has the potential to improve LD sensory characteristic, reducing the intensity of warmed over flavor in refrigerated cooked pork meat.

In conclusion, this study shows that PE is an effective antioxidant in pork, enhancing oxidative status and shelf life, without affecting other meat quality parameters. Future studies are needed to clarify the

optimal length of dietary supplementation and to explore the efficacy of dietary PE in cooked and ripened products.

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