Meat Science 213 (2024) 109519

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Protocatechuic acid as an inhibitor of lipid oxidation in meat

Teresa Deuchande *, Joana F. Fundo, Manuela E. Pintado, Ana L. Amaro

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo de Botelho 1327, Porto 4169-005, Portugal

ARTICLE INFO

Keywords:
Antioxidant capacity
Color
Lipid oxidation
Meat
Protocatechuic acid
Quality

ABSTRACT

Lipid oxidation is the principal driver of meat and meat product deterioration during shelf life, causing the loss of fresh meat color, flavor, and aroma. Currently, synthetic antioxidants are used to prevent oxidation, but increasing consumer demand for natural ones leaves the industry with few alternatives. In this study, protocatechuic acid (PCA), known to have high antioxidant activity, was evaluated as a potential inhibitor of meat lipid oxidation. For this purpose, the antioxidant capacity and lipoxygenase (LOX) inhibitory activity of PCA were evaluated *in vitro*, and a set of four experiments was conducted, treating minced meat with water (control), lactic acid (LA), rosmarinic acid (RA) and PCA, at different concentrations (1–12 mg mL^{-1}), depending on the experiment. The potential antioxidant effect of PCA when applied to meat cubes was also evaluated, as well as the potential of carboxymethyl cellulose (CMC) as a delivery system for PCA. The *in vitro* results showed that PCA is a potent antioxidant and an effective LOX inhibitor at 1 mg mL^{-1}. PCA effect on meat lipid oxidation prevention was dose-dependent, and at 2 mg mL^{-1}, it inhibited color change by 50% and lipid peroxidation by up to 70% when compared to water-treated samples, performing better than RA at 0.25 mg mL^{-1}. These results suggest that PCA is a promising molecule to the meat industry as a natural preservative for meat and meat products directly or in a formulation.

1. Introduction

Meat lipids content and composition impact the flavor and influence the tenderness and juiciness, determining meat quality and nutritional value. However, lipids are susceptible to degradation, and oxidation is the main non-microbial driver of meat and meat product deterioration during shelf life (Amaral, Da Solva, & Lannes, 2018). It is responsible for several undesirable reactions leading to the loss of color, flavor, and aroma. Lipids oxidize by i) autoxidation, ii) lipoxygenase action (LOX), or iii) photo-oxidation. In meat, the most impacting process is autoxidation, involving a continuous free-radical chain reaction (Domínguez et al., 2019). Lipid oxidation is triggered when atmospheric molecular oxygen reacts with fatty acids, mainly unsaturated fatty acids endowed with electron-deficient double bonds. During this primary oxidation, lipids form hydroperoxides. These molecules, although not causing a direct sensorial change to the product, are much more unstable, and can be further oxidized or react with other molecules. This results in secondary oxidation with the formation of several breakdown products, including hydrocarbons, aldehydes, ketones, alcohols, esters, and acids (Amaral et al., 2018). These compounds are the primary cause of off-odors and off-flavors, rancidity, and meat color change, being associated with meat quality decay, with aldehydes such as malondialdehyde (MDA) being considered the main contributors to aroma volatile flavors in meat (Al-Hijazeen & Al-Rawashdeh, 2019; Papuc, Goran, Predescu, & Nicorescu, 2017). Currently, the meat industry prevents lipid oxidation through the application of synthetic antioxidants such as propyl gallate or butylated hydroxytoluene (BHT) (Beya, Netzel, Sultanbawa, Smyth, & Hoffman, 2021; Oswell, Thippareddi, & Pegg, 2018). However, the increasing consumer demand for natural antioxidants due to concerns related to the safety of synthetic counterparts leaves the industry with few alternatives.

Many studies reported the beneficial effects of polyphenol-rich natural extracts in meat and meat product preservation (Beya et al., 2021; Kalogianni, Lazou, Bossis, & Gelasakis, 2020) due to their ability to inhibit oxidative processes in meat by scavenging reactive species, and inhibiting LOX activity or reducing metmyoglobin (Papuc, Goran, Predescu, Nicorescu and Stefan, 2017). The groups of naturally occurring phenolic compounds with potential applications in the food industry based on their antioxidant potential are (i) the phenolic acids, including hydroxybenzoic (*e.g.*, gallic and protocatechuic acids), and

* Corresponding author.
E-mail address: tdeuchande@ucp.pt (T. Deuchande).

https://doi.org/10.1016/j.meatsci.2024.109519
Received 30 November 2023; Received in revised form 17 April 2024; Accepted 17 April 2024
Available online 19 April 2024

hydroxycinnamic acids (*e.g.*, p-coumaric, caffeic, and ferulic acids); and ii) flavonoids, (*e.g.*, quercetin, kaempferol, luteolin, catechin, epicatechin), iii) phenolic diterpenes (carnosic acid and carnosol), iv) isoflavones (*e.g.*, genistein, daidzen) and v) volatile oils (*e.g.*, thymol, eugenol) (Beya et al., 2021; Kakkar & Bais, 2014; Singla et al., 2019). For instance, rosemary extract obtained from plant leaves has been shown to possess high antioxidant activity due to the high contents of phenolic diterpenes (carnosic acid, carnosol, and rosmanol) and phenolic acids such as rosmarinic acid (RA) (Al-Hijazeen & Al-Rawashdeh, 2019; Bianchin et al., 2017; Birtić, Dussort, Pierre, Bily, & Roller, 2015; Lešnik, Furlan, & Bren, 2021; Loussouarn et al., 2017; McBride, Hogan, & Kerry, 2007). Rosemary extract was regulated in the EU for application to meat products and permitted at a maximum concentration of 150 mg rosemary extract kg^{-1} of product (for meat with >10% fat) or 15 mg rosemary extract kg^{-1} of product (for meat with <10% fat) were established (European Commission, 2011, 2013a). Besides rosemary extract, other plant extracts, such as those from green tea, highly rich in flavan-3-ols such as catechins (Senanayake, 2013), or oregano leaves highly rich in RA (Khorsand et al., 2022), have been shown to reduce meat lipid oxidation during storage (Awad et al., 2021).

In this work, the potential of protocatechuic acid (PCA), chemically known as 3,4-dihydroxybenzoic acid was evaluated as an inhibitor of meat lipid oxidation. PCA is a phenolic acid naturally occurring in various fruits, vegetables, and traditional Chinese herbal medicines which has been shown to possess high antioxidant activity and anti-inflammatory, antibacterial, and antiviral activities (Kakkar & Bais, 2014; Liu et al., 2020; Mahfuz, Mun, Dilawar, Ampode, & Yang, 2022; Song et al., 2020; Zhang et al., 2021). As a phenolic acid, it has a high H^+-donating activity, trapping free radicals, and being an effective scavenger of H_2O_2 and superoxide radicals (Velasco & Williams, 2011). *In vitro* assays showed that PCA antioxidant activity was dose-dependent and that, at the adequate dose, it acts as i) a Fe^{3+} and Cu^{2+} reducing agent, ii) a scavenger of superoxide anion and hydroxyl radicals, and iii) a Fe^{2+} and Cu^{2+} chelating agent (Andjelković et al., 2006; Li, Wang, Chen, & Chen, 2011; Zhang et al., 2021). PCA contains two potential metal-binding sites, catechol, and carboxylic acid showing a high affinity for metal (Krishna & Muraleedharan, 2023). PCA has been also shown to be an effective antioxidant with 10 times the potency of α-tocopherol (Farombi et al., 2016; Song et al., 2020), playing an antioxidant role *in vitro* and *in vivo* by decreasing the levels of inflammatory markers, such as reactive oxygen species and MDA, upregulating activities of endogenous antioxidant enzymes, such as catalase and superoxide dismutase, regulating signaling pathways and decreasing oxidative damage (Song et al., 2020).

Despite the considerable promise of PCA as a natural antioxidant, supported by numerous studies demonstrating its robust antioxidant activity both *in vitro* and *in vivo* (Kakkar & Bais, 2014; Li et al., 2011; Song et al., 2020), there is a gap in research addressing its potential as a food preservative. While there is a wealth of literature investigating the impact of various antioxidant plant extracts on the preservation of meat and meat products (Awad et al., 2021; Beya et al., 2021; Kalogianni et al., 2020), the attention given to PCA in this context remains limited. Only a few studies have delved into the exploration of PCA's efficacy as a preservative in a food system (Stojković et al., 2013; Hernández-García, Vargas, & Chiralt, 2022; Yin & Chao, 2008; Zhong et al., 2021). It is noteworthy that the majority of existing research has predominantly focused on PCA's antibacterial properties (Chao & Yin, 2009; Stojković et al., 2013; Wu et al., 2022; Yin & Chao, 2008), rather than its potential to inhibit lipid oxidation and maintain the quality during shelf life. Consequently, there is a gap in the literature, requiring further investigation into PCA's role as a meat preservative with a focus on antioxidant aspects.

In the work presented herein, there is a sequence of experiments conducted to assess the PCA potential to inhibit meat lipid oxidation by evaluating its effect in minced meat, meat pieces, and during storage at room and cold temperatures. The effect of PCA combined with carboxymethyl cellulose (CMC) on meat quality preservation during storage was also evaluated. CMC is a water-soluble cellulose derivative widely used in the food and pharmaceutical industries (Riseh, Vazvani, Hassanisaadi, & Skorik, 2023). This biopolymer is *generally recognized as safe (GRAS)* and used to produce functional edible films incorporated with several additives such as nanoparticles, plant extracts, or essential oils, to increase the shelf life of packaged foods (Ezati & Rhim, 2021; Koushesh, Banin, Koushesh Saba, & Sogvar, 2016; Muppalla, Kanatt, Chawla, & Sharma, 2014; Priyadarshi, Kim, & Rhim, 2021; Riseh et al., 2023).

2. Materials and methods

2.1. In vitro evaluation of PCA antioxidant potential

2.1.1. PCA antioxidant capacity

The antioxidant capacity was determined using the Oxygen Radical Absorbance Capacity (ORAC) assay (Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, & Recio, 2011). The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and the final reaction volume was 200 μL. Fluorescein (120 μL and 116.66 nM), and the PCA or the standards (20 μL) were placed in the wells of a 96-well flat-bottom black microplate. A stock solution of 1 mg mL^{-1} of PCA was prepared by dissolving the molecule in phosphate-buffered saline (PBS) buffer (75 mM, pH 7.4). Serial dilutions were performed to attain optimum dilution. Standards solutions were prepared using a trolox solution at different sequential concentrations (10, 20, 30, 40, 50, 60, 70 and 80 μM). The mixtures were preincubated for 10 min at 37 °C before rapidly adding the 2,2′–Azobis (2-methylpropionamidine) dihydrochloride (AAPH) solution. The microplate was immediately placed in the reader (Synergy H11 multidetection microplate reader, Bio-Tek Instruments, Inc.) and shaken before each reading. Fluorescence values were recorded. The inhibition capacity was expressed as Trolox equivalents (mol TE kg^{-1}). All reaction mixtures were prepared in triplicate, and at least three independent assays were performed for each sample.

2.1.2. LOX inhibitory capacity of PCA

For the measurement of the PCA inhibitory effect on lipoxygenase the Lipoxygenase Inhibitor Screening Assay kit, Cayman Chemical (760700) was used. The kit detects and measures the hydroperoxides produced in the lipoxygenation reaction using a purified LOX (15-LO extracted from soybean). The analysis was conducted following the instructions of the kit. To evaluate the effect of PCA on the LOX activity, PCA solutions were prepared at different concentrations. Although PCA is soluble in water up to 12 mg mL^{-1}, highly concentrated solutions were required for the assay, considering the subsequent dilutions imposed by the method. Thus, to test the lower PCA concentrations (0.05, 0.15, and 0.3 mg mL^{-1}) PCA was dissolved in the assay buffer provided by the kit, but to test the higher concentrations (0.5 and 1 mg mL^{-1}), it was dissolved in DMSO. After subtracting the average absorbance of the blank from the absorbance of the inhibitor, the percentage of LOX inhibition was calculated using the following equation:

$$\%inhibition = \left[\frac{Initial\ activity - Inhibitor}{Initial\ activity}\right] \times 100 \quad (1)$$

where the 'Initial activity' corresponds to LOX activity in the presence of linoleic acid as substrate and without the addition of inhibitor, and the 'Inhibitor' corresponds to LOX activity in the presence of the inhibitor at a given concentration. For modeling, the absorbance of the blank was subtracted from the absorbance of the inhibitor, and the calculated values were used to fit the inhibitory response model.

2.2. Evaluation of PCA preservative effects in meat

2.2.1. Meat samples

For each trial conducted on different dates, minced meat samples were purchased fresh from a local supermarket. For all the trials the minced meat was purchased on the same weekday, considering the arrival of fresh meat pieces to the supermarket. In the market, the fresh beef meat was under refrigeration (0–2 °C) and normal atmosphere and was freshly minced locally. After mincing the meat was transported to the laboratory and immediately subjected to treatments.

The cubes of veal meat were also purchased in a local supermarket, where they were packaged in a normal atmosphere and stored under refrigeration conditions (between 0 and 2 °C). The meat was then transported to the laboratory and immediately treated.

2.2.2. Experiment 1 – Exploiting the PCA potential as a meat preservative compared with rosmarinic acid and lactic acid

To evaluate the potential of PCA as an inhibitor of meat oxidation, one trial (trial 1) was conducted. In this trial minced meat samples (three portions of about 30 g of meat, corresponding to approximately 100 g of meat) were placed in a perforated funnel and treated by spraying with 40 mL of the treatment solution including i) water (control), ii) RA at 0.25 mg mL^{-1} (100 mg kg^{-1} meat) and iii) lactic acid (LA) at 20 mg mL^{-1} (8000 mg kg^{-1} meat) and iii) PCA at 12 mg mL^{-1} (4800 mg kg^{-1} meat). RA is currently used as a natural meat preservative mainly due to its antioxidant activity (Al-Hijazeen & Al-Rawashdeh, 2019; Sánchez-Escalante et al., 2011; Velasco & Williams, 2011) whereas LA is also widely used in the industry at concentrations between 2% and 5% (European Commission, 2013), due to its antimicrobial activity (Carpenter, Smith, & Broadbent, 2011; Han et al., 2021; Heir et al., 2022; Manzoor et al., 2020; Rodríguez-Melcón, Alonso-Calleja, & Capita, 2017). After treatment, the samples were kept in the funnel for 1 min to drain the excess solution and then placed in polyethylene-terephthalate (PET) food-grade clamshell containers with each box containing about 30 g of treated minced meat (3 replicates per treatment) and stored at room temperature (20–23 °C and relative air humidity between 50 and 60%) under dim light for two days to accelerate the oxidation process. At the beginning and the end of the trial, meat color, lipid hydroperoxide concentrations, and lipid peroxidation were measured.

2.2.3. Experiment 2 – Determining the minimum PCA concentration to inhibit lipid oxidation

To develop the curve of lipid peroxidation inhibition as a function of PCA concentration, two trials were conducted. In the first trial (trial 2.1), minced meat samples (≈100 g) were treated with 40 mL of i) water, ii) RA at 0.25 mg mL^{-1} (100 mg kg^{-1} meat), and ii) PCA at 3, 6, and 9 mg L^{-1} (1200, 2400 and 3600 mg kg^{-1} meat). In the second trial (trial 2.2) the treatments were: i) water, ii) RA at 0.25 mg mL^{-1} (100 mg kg^{-1} meat), and iii) PCA at 0.5, 1, and 2 mg mL^{-1} (200, 400, and 800 mg kg^{-1} meat). In both trials, the samples were allowed to drain for 1 min before being placed in the clamshell boxes (≈ 30 g per box) and stored at room temperature under dim light for 2 days. At the beginning and the end of the trials, color and lipid peroxidation were determined.

To confirm the PCA minimum inhibitory concentration, an additional trial was conducted (trial 2.3). In this trial, samples of minced meat (≈ 100 g) were treated with 40 mL of i) water, ii) RA at 0.25 mg mL^{-1} (100 mg kg^{-1} meat), and iii) PCA at 1 mg mL^{-1} (400 mg kg^{-1} meat) and 2 mg mL^{-1} (800 mg kg^{-1} meat). The application and storage procedure were the same as conducted in the previous experiments. At the beginning and the end of the experiments, color and lipid peroxidation were determined.

2.2.4. Experiment 3 – Effect of storage temperature on PCA effectiveness to inhibit meat lipid oxidation

In this experiment (trial 3), about 200 g of minced meat samples were treated by spraying with 80 mL of i) water, ii) RA at 0.25 mg mL^{-1} (100 mg kg^{-1} meat), and iii) PCA at 2 mg mL^{-1} (800 mg kg^{-1} meat). After the treatments, 30 g of each sample was placed in 12 clamshell boxes, with six boxes being stored for two days at room temperature under dim light and the other six boxes under cold storage (4 °C) for seven days. The samples stored at room temperature were evaluated for color, the concentration of lipid hydroperoxides, and lipid peroxidation after 0, 1, and 2 days of storage, whereas the ones stored under cold conditions (4 °C) were evaluated for the same parameters but after 0, 2 and 7 days of storage.

2.2.5. Experiment 4– Exploring the potential of carboxymethyl cellulose (CMC) as a potential PCA delivery system

In this fourth experiment (trial 4), samples of nine meat cubes with 5–10 g were treated by immersion for 1 min in the following solutions: i) water, ii) 10 mg mL^{-1} CMC, iii) PCA at 2 mg mL^{-1}, and iv) solution containing 2 mg mL^{-1} PCA and 10 mg mL^{-1} CMC. After each treatment, the meat cube samples were placed in a perforated funnel and allowed to drain for 1 min before being placed in three clamshell boxes (3 meat cubes per box) and stored at room temperature for two days. At the beginning of the experiment and after the two days of storage, the samples were evaluated for color and then stored at −80 °C for lipid peroxidation determination.

2.3. Color measurements

The color of the sample surface was measured in the CIE L* a* b* color space with a Konica-Minolta CR-410 chroma Meter (Osaka, Japan) equipped with a D65 illuminant and the 2° observer for color interpretation aperture size of 50 mm. The three-dimensional color space is built up from three axes perpendicular to one another (L^*, a^*, b^*). L^* values represent lightness and range from 0 (black) to 100 (white). The chromatic colors are described by two axes in the horizontal plane, a^* and b^*. The a^* value indicates redness [green (negative values)-red (positive values)], and b*, yellowness [blue (negative values)-yellow (positive values)].

The hue angle ($h°$) indicates the tone, and it was calculated with the formula atan (b^*/a^*), with 0° corresponding to red color and 60° to yellow.

The total color difference (ΔE*) between the treated samples and fresh samples was calculated using the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

where ΔL* is the lightness difference, Δa* is the redness difference, and Δb* is the yellowness difference.

To evaluate the color stability of meat along the storage time, the values of a^* together with L*, h°, and ΔE* were the selected color parameters reported.

2.4. Meat hydroperoxides

Hydroperoxides were determined using the PeroxiDetect kit from Sigma Aldrich and following the manufacturer's instructions to quantify lipid hydroperoxides. Before hydroperoxide determination, lipids were extracted using the method of Bligh and Dyer (1959), as modified by Breil, Abert Vian, Zemb, Kunz, and Chemat (2017), with slight modifications. In brief, to 2.5 g of fresh minced meat samples, 3 mL of ethyl acetate: ethanol (2:1) were added. The mixture was thoroughly homogenized for 1 min in the vortex, and then 2.25 mL of ethyl acetate, 500 μL of ethanol, and 4.25 mL of distilled water were added. The mixture was homogenized in the vortex for 1 min and centrifuged at 1046*g* for 1 min, for phase separation. After complete separation and clarification, the organic phase (lipid extract) was transferred to another tube and used for hydroperoxides analysis.

2.5. Lipid peroxidation

Lipid peroxidation was determined by measuring MDA formation using the thiobarbituric acid reactive substances (TBARS) method described by Heath and Packer (1968), as modified by (Gheisari, Møller, Adamsen, & Skibsted, 2010), slightly adapted. For MDA extraction, 1.5 g of minced meat was homogenized with 6 mL 7.5% trichloroacetic acid (TCA) solution containing 0.1% propyl gallate and 0.1% ethylenediaminetetraacetic acid disodium salt (EDTA) using the vortex. The homogenate was centrifuged for 10 min at 4696*g*, and the supernatant was filtered. To aliquots of 750 μL of supernatant, 750 μL of 0.020 M TBA solution was added. The mixture was heated at 95 °C for 30 min and then cooled quickly in an ice bath. Afterward, the absorbance of the solution at 532 nm was measured in a microplate reader (Epoch 2, Biotek, USA). Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as mg kg^{-1} meat.

2.6. Statistical analysis

The LOX inhibiting curve fitted a sigmoidal inhibitor dose-response model.

All the experiments performed with meat samples followed a completely randomized experimental design with three replications. In experiments 1, 2, and 4, treatment was considered the single fixed effect. Significant differences among samples regarding color parameters (L^*, a^*, h°, and ΔE^*), hydroperoxide concentration, and MDA concentration, were determined by one-way ANOVA, followed by multiple comparisons of means using the Tukey test ($P < 0.05$). For the analysis of the results of experiment 3, treatment and storage time effects as well as the interaction of both effects were considered the fixed effects. Significant differences among samples regarding color parameters (L^*, a^*, h°, and ΔE^*), hydroperoxide concentration, and MDA concentration, among the samples of the different treatments during storage were determined by two-way ANOVA, followed by multiple comparisons of means using the Tukey test ($P < 0.05$).

The model of MDA as a function of PCA concentration was fitted using a sigmoidal dose response curve.

All the statistical and modeling analyses were performed on GraphPad Prism version 8.0.2 (San Diego, California, USA).

3. Results and discussion

3.1. PCA antioxidant capacity

The antioxidant capacity of PCA from Sigma Aldrich (≥ 97% purity), measured by the ORAC method, was 51.83 ± 2.95 mol TE kg^{-1}. PCA antioxidant capacity measured using the same method but with PCA dissolved in dimethyl sulfoxide (DMSO) was reported to be 20.76 mol TE kg^{-1} (Graton et al., 2022). Several extracts known to be rich in PCA have been also reported to have high antioxidant capacity. For instance, star anise spice, with 32.2 mg PCA 100 g^{-1} (Shan, Cai, Sun, & Corke, 2005), has been reported to have an antioxidant capacity of 0.113 ± 0.092 mol TE kg^{-1} using the ORAC method (Bi, Soong, Lim, & Henry, 2015) and red chicory, containing about 16.78 mg PCA 100 g^{-1} (Rossetto et al., 2005), showed an antioxidant capacity of 0.035 mol TE kg^{-1} (Ninfali, Mea, Giorgini, Rocchi, & Bacchiocca, 2005). According to this *in vitro* analysis, PCA is a potent antioxidant, with an antioxidant capacity comparable to that of rosemary extract already used as a meat preservative. Although the antioxidant capacity of rosemary extracts varies depending on several factors, including pre-harvest factors, harvest season, or extraction method, these extracts have high antioxidant capacity (Lešnik et al., 2021; Nieto, Ros, & Castillo, 2018). A rosemary extract prepared using acetone/perchloric acid has been reported to have an antioxidant capacity of 2.90 mol TE kg^{-1} (Ninfali et al., 2005), whereas another rosemary extracted with hexane followed by acetone/water/acetic acid has been reported to have an antioxidant capacity of 1.14 ± 0.06 mol TE kg^{-1} (Masuda et al., 2015). The high antioxidant capacity of rosemary extracts is mainly due to their phenolic diterpene content, mostly carnosic acid and carnosol, but other phenolic compounds such as RA are also known to confer antioxidant properties to these extracts (Lešnik et al., 2021) For instance, Ibarra et al. (2010) have shown that a rosemary extract standardized to contain 20% of RA had 1.5 fold higher antioxidant capacity than a rosemary extract standardized to have the same percentage of carnosic acid when measured using ORAC method. Considering this finding, for the *in vivo* studies, RA was used for application.

The high antioxidant capacity of PCA suggests that it may be a potential candidate as a meat preservative. However, *in vitro* assays, such as ORAC assay, do not measure bioavailability, *in vivo* stability, or interaction *in situ*. Therefore, an *in vivo* study is required to evaluate whether the application of PCA to meat products improves meat quality retention.

3.2. LOX inhibition capacity of PCA

LOXs are a class of non-heme iron enzymes that catalyze the oxidation of polyunsaturated fatty acids to generate hydroperoxides, which are further decomposed, forming several volatile compounds (Ivanov et al., 2010). Albeit the initial meat oxidation may favor meat flavor development, the generation of high levels of volatiles may produce detrimental effects on meat quality (Huang, Wu, Wang, & Li, 2015). Since LOX concentration determines the rate at which lipid oxidation develops, it plays a crucial role in meat oxidation, with high concentration favoring oxidative processes (Domínguez et al., 2019). Regarding PCA's potential to inhibit the activity of lipoxygenase (LOX), the results showed that PCA inhibition capacity was dose dependent. PCA at 0.3771 ± 0.0699 mg mL^{-1} inhibited LOX activity by 50% (IC_{50} value, Fig. 1) and at 1 mg mL^{-1} by 100% (Table 1, Fig. 1). LOXs comprise a family of non-heme iron-containing dioxygenases (Rackova, Oblozinsky, Kostalova, Kettmann, & Bezakova, 2007) and PCA may chelate metal ions that are essential co-factors for LOX activity. By binding to these metals, PCA can hinder the proper functioning of the enzyme, thereby reducing its activity (Sadik, Sies, & Schewe, 2003). Another possibility is that PCA may interact directly with the active sites of lipoxygenase, disrupting the enzyme's catalytic function (Borbulevych, Jankun, Selman, & Skrzypczak-Jankun, 2004). This interference can occur through binding to specific residues, altering the enzyme's conformation, or affecting substrate binding.

The results clearly show that PCA acts as a LOX inhibitor and that its effect on LOX inhibition is dose-dependent, hence, for applications aimed at preventing lipid oxidation, it should be applied at concentrations not lower than 1 mg mL^{-1} (Fig. 1).

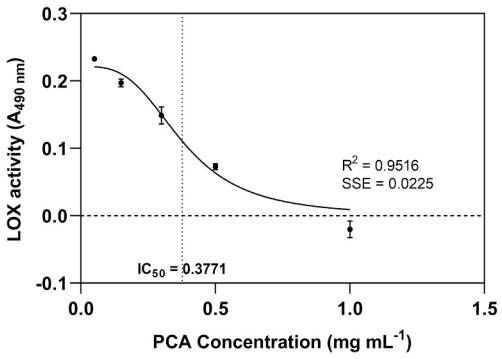

Fig. 1. Inhibition of soybean 15-lipoxygenase by protocatechuic acid (PCA) (IC_{50} = 0.3771 ± 0.0699 mg mL^{-1}). Linoleic acid was used as substrate in the analysis. The results represent the mean of three replicates ± S.E.

Table 1
PCA inhibitory effect on lipoxygenase (LOX) activity. The results represent the mean of three replicates ±S.E. Means with the same letter are not significantly different ($P < 0.05$).

PCA concentration (mg mL^{-1})	Solvent	LOX inhibition (%)
0.05	assay buffer	8.3 ± 0.8^{a}
0.15	assay buffer	22.6 ± 2.4^{b}
0.3	assay buffer	36.9 ± 3.4^{c}
0.5	DMSO	39.9 ± 3.7^{c}
1	DMSO	101.8 ± 1.5^{d}

3.3. Experiment 1: Initial assessment of PCA potential as a preservative for application in meat

The first experiment (trial 1) aimed to assess the potential of PCA as a meat preservative, comparing its antioxidant effects with those of LA at 20 mg mL^{-1} and RA at 0.25 mg mL^{-1}. The application of PCA at 12 mg mL^{-1} had a significantly higher positive effect in preventing color loss and oxidation than LA and RA. Color is an important quality parameter in meat. Changes in meat color occur when oxymyoglobin, a red-tone globin, is oxidized to metmyoglobin, a brown-tone globin, conferring a brown color to meat (Hunt & King, 2012). The meat samples of all treatments showed lightness loss after two days of storage (Table 2). The samples treated with LA and PCA showed the highest lightness loss, followed by water and RA-treated samples. This difference determined the major ΔE* in these samples. Although PCA-treated samples showed the highest total color difference and lightness loss, these samples did not show either redness or hue loss, while all the others showed significant changes in these parameters when compared to fresh meat (Table 2). Several studies showed that natural antioxidant extracts, including extracts containing PCA, contribute to preserving meat color (Awad et al., 2021; Sánchez-Escalante et al., 2011). Zhong et al. (2021) have recently shown that the application of gelatin-based films incorporating PCA at 0.1% to meat decreased discoloration. In contrast to the observed in PCA-treated samples, the samples treated with RA at 0.25 mg mL^{-1} showed a redness loss of 41% and an increase in the hue of 74%. The results suggest that the capacity of rosemary extract to preserve meat color may derive from the activity of these compounds together, and RA on its own may not be as effective in retaining meat color. Another possibility may be the need for a higher RA dose to reach the desired results. Concerning meat treated with LA at 20 mg mL^{-1}, the results showed that it slightly prevented redness loss (20% loss) in comparison with the observed in samples treated with water (46%) and RA (41%) (Table 2). However, the total color difference was higher in comparison with RA and water-treated meat, mainly due to lightness loss, as observed in the meat treated with PCA. LA, widely used for meat preservation due to its antimicrobial activity (Carpenter et al., 2011; Han et al., 2021; Rodríguez-Melcón et al., 2017), has been shown not to affect meat color. Remarkably, its application, at concentrations as high as 4%, may enhance color retention in beef (Rodríguez-Melcón et al., 2017). However, contradictory findings emerge, as buffalo meat treated with LA at concentrations up to 6% did not decrease redness loss compared to the control over a seven-day storage period (Manzoor et al., 2020). The contradictory results of these previous studies suggest that LA's ability to maintain meat color depends on the type of meat to which it is applied. Still, other factors may be involved, including raw meat quality (freshness, pH, pre-existing color changes, endogenous LA, and antioxidant concentrations) (Ma, Wang, Chen, Yu, & Han, 2021; Puolanne, Poso, Ruusunen, Sepponen, & Kyla-Puhju, 2002). LA, when used as a part of a preservation process, may contribute to maintaining the stability and activity of endogenous antioxidants naturally present, increasing the reducing activity (Kim, Keeton, Smith, Berghman, & Savell, 2009; Suman, Hunt, Nair, & Rentfrow, 2014). In addition, differences in processing conditions (temperature, and storage duration), and initial microbial load, may also impact color retention (Blandon et al., 2023). Our findings align with the notion that LA significantly prevents redness loss in minced meat.

The meat treatment with PCA contributed to the highest oxidation inhibition. The concentration of lipid hydroperoxides significantly increased in the samples of all treatments after two days of storage at room temperature. The lower increase was observed in the meat treated with RA (2.9-fold) followed by the treated with PCA (3.4-fold) (Table 2). This result is in accordance with the findings of several studies, which report that rosemary extract inhibits meat lipid oxidation (Al-Hijazeen & Al-Rawashdeh, 2019; Bianchin et al., 2017; Sebranek, Sewalt, Robbins, & Houser, 2005; Velasco & Williams, 2011). Despite the hydroperoxides results, lipid peroxidation, expressed as concentrations of MDA, was effectively inhibited in meat treated with PCA compared with RA, in which the levels of MDA increased by more than double the initial concentration (Table 2). This result suggests that PCA applied at 12 mg mL^{-1} (800 mg 100 g^{-1} meat) is more effective in preventing meat lipid oxidation than RA at 0.25 mg mL^{-1} (10 mg 100 g^{-1} meat). The application of 5 mg of PCA to 100 g of ground beef has been shown to reduce lipid peroxidation by 42% after six days of storage at 15 °C, compared to untreated meat (Yin & Chao, 2008) and more recently, Hernández-García et al. (2022) have also reported that meat packaged in bilayer films incorporating 2% PCA (*w*/w) reduced meat lipid peroxidation and peroxide value.

Regarding the LA effect, although it slightly inhibited meat lipid oxidation, the difference was only significant for MDA concentrations in comparison with water-treated samples, and the levels were still 3.5-fold higher than the initial concentrations (Table 2).

Considering the results, PCA at 12 mg mL^{-1} showed higher potential than RA and LA at preventing meat color loss and lipid oxidation.

3.4. Experiment 2: Minimal PCA concentration for meat oxidation inhibition

Considering the findings of experiment 1, in the second experiment three independent trials (trials 2.1, 2.2, and 2.3) were conducted. In trials 2.1 and 2.2, PCA solutions with decreasing concentrations were applied to minced meat samples and stored for two days at room temperature. The results allowed the development of a model (sigmoidal dose-response model) which showed that the application of a PCA

Table 2
Color measurements [lightness (L^*), redness (a^*) and hue angle (h°), and total color difference (ΔE*)] and lipid oxidation results [lipid hydroperoxides concentrations and concentrations of malondialdehyde (MDA)] in fresh meat (day 0) and meat treated with water, rosmarinic acid (RA) at 0.25 mg mL^{-1}, lactic acid (LA) at 20 mg mL^{-1}, and protocatechuic acid (PCA) at 12 mg mL^{-1}, after 2 days of storage at room temperature under dim light. The results represent the mean of three replicates ± S.E. Means within a column with the same letter are not significantly different ($P < 0.05$).

Experiment 1 (trial 1)	Color				Lipid oxidation	
	Lightness (L^*)	a^*	Hue (h°)	ΔE*	Hydroperoxides (μmol g^{-1} meat)	MDA (mg kg^{-1} meat)
Fresh meat	62.8 ± 0.5^{a}	15.3 ± 0.1^{a}	21.6 ± 0.2^{a}	–	0.563 ± 0.122^{a}	0.305 ± 0.006^{a}
Water	53.4 ± 0.6^{b}	8.3 ± 0.3^{b}	40.2 ± 1.0^{b}	16.0 ± 0.4^{a}	2.293 ± 0.137^{b}	1.530 ± 0.083^{b}
RA 0.25 mg mL^{-1}	55.8 ± 0.6^{c}	9.0 ± 0.2^{b}	37.7 ± 1.2^{b}	13.6 ± 0.4^{b}	1.657 ± 0.072^{c}	0.672 ± 0.045^{c}
LA 20 mg mL^{-1}	50.4 ± 0.9^{d}	12.2 ± 0.3^{c}	41.3 ± 2.0^{b}	19.2 ± 0.6^{c}	2.007 ± 0.069^{bc}	1.058 ± 0.076^{d}
PCA 12 mg mL^{-1}	50.2 ± 0.2^{d}	15.4 ± 0.6^{a}	21.0 ± 1.7^{a}	19.5 ± 0.2^{c}	1.940 ± 0.060^{bc}	0.205 ± 0.011^{e}

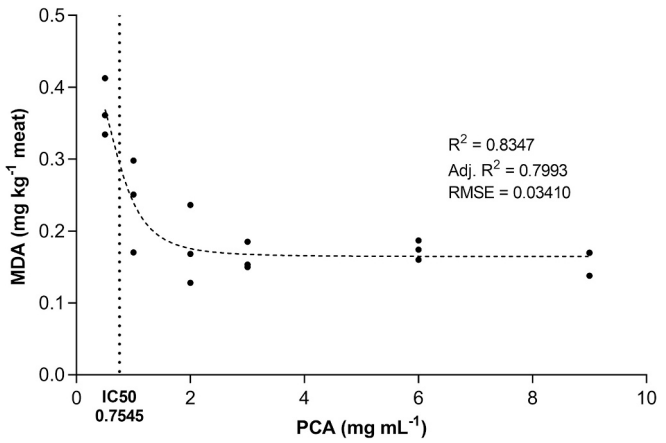

Fig. 2. Sigmoidal dose-response curve fitting of MDA formation as a function of PCA concentration applied to minced meat at different concentrations. Each data point represents the individual value of each replicate measured in triplicate. Results from experiment 2, trials 2.1 and 2.2.

solution at a concentration of 0.7545 mg mL^{-1} may be able to inhibit MDA formation by 50% and that PCA may be effective at inhibiting lipid oxidation when applied at a concentration of 2 mg mL^{-1} (Fig. 2).

Although there is no established MDA concentration limit in meat products, MDA concentrations above 0.5 mg kg^{-1} indicate oxidation and values above 1.0 mg kg^{-1} may be unacceptable (Reitznerová et al., 2017). In some previous studies, TBARS limits in beef have been suggested, and proposed limits varied from 1 up to >3 mg kg^{-1} (Campo et al., 2006; Hughes, McPhail, Kearney, Clarke, & Warner, 2015), having even been proposed a limit of 10 mg kg^{-1}, depending on the method used to determine TBARS concentrations (Zhang et al., 2019). It has been reported that the MDA concentrations measured using differently adjusted methods may lead to significantly different results (Reitznerová et al., 2017; Zhang et al., 2019). In the case of the samples analyzed in this study, after two days of storage at room temperature, though the measured MDA levels were below 0.5 mg kg^{-1} (Fig. 2), there was a significant decay in meat quality with the formation of off-odors. Hence, a lower limit of MDA may be proposed for minced meat, considering the method conducted herein to determine MDA levels.

To validate the developed model (Fig. 2), an independent trial was conducted (trial 2.3), with PCA applied to minced meat at 1 and 2 mg mL^{-1}. MDA concentrations were significantly lower in the meat samples treated with PCA at 2 mg mL^{-1} compared with the samples treated with 1 mg mL^{-1} ($P < 0.05$) (Table 3). The results showed that PCA applied at 1 mg mL^{-1} was not enough to prevent meat lipid oxidation, whereas, at 2 mg mL^{-1}, it was effective, confirming that the effective PCA inhibitory concentration is between 1 and 2 mg mL^{-1}.

Regarding color, the lightness results were in accordance with the results found in experiment 1, with PCA-treated samples showing the highest change (Table 2 and Table 3). The meat samples of all treatments showed similar redness loss, total color difference and the hue angle significantly increased (Table 3). The samples treated with PCA showed the lowest increase, followed by RA and water. These results suggest that PCA contributes to maintaining meat color. However, when applied to meat products at 1 and 2 mg mL^{-1} (Table 3), it is not as effective as at 12 mg mL^{-1} in preventing redness and hue loss (experiment 1, Table 2).

3.5. Experiment 3: PCA ability to inhibit lipid oxidation in meat stored at room temperature and 4 °C

The former experiments were conducted at room temperature to perform accelerated trials, rapidly assessing the potential of PCA for application in meat products. However, fresh meat and meat products are maintained in the cold chain and stored under cold conditions. Therefore, this trial (trial 3) aimed to validate the previous findings in meat stored under cold conditions. For this purpose, minced meat treated with water, RA at 0.25 mg mL^{-1}, and PCA at 2 mg mL^{-1} were stored for two days at room temperature and seven days at 4 °C. The meat samples treated with PCA after two days of storage at room temperature showed less color change when compared with water-treated. However, at each time-point of analysis, these differences were not significant among the samples of the different treatments (Fig. 3 a, c, e). This result is in accordance with the results found in experiments 1 and 2 (Tables 2 and 3). Similarly, when the samples were subjected to the same treatments and stored for seven days under cold conditions (4 °C), the meat samples treated with PCA showed significantly lower redness (a^*) and hue angle (h°) losses compared with water-treated (Fig. 4). Though in this case the differences were significant after seven days of storage ($P < 0.05$) (Fig. 5 b, d, f).

Regarding meat lipid oxidation, the results were in accordance with the results found in the former trials of the present study. The concentrations of hydroperoxides in the meat samples treated with PCA and RA were significantly lower than in the samples treated with water (Table 4). The concentration of hydroperoxides in PCA-treated samples was 39% lower after one day of storage and 20% lower after two days compared with the water-treated, in the respective time points (Fig. 5a). PCA also performed better than RA in inhibiting hydroperoxide formation after one day of storage at room temperature, with 20% lower accumulation of hydroperoxides (Fig. 5a). Despite the significant differences observed in the meat samples subjected to different treatments during storage at room temperature, for the stored at 4 °C, the concentrations of hydroperoxides at day two and day seven were not significantly different for any samples (Fig. 5b). The results of hydroperoxides obtained in the trial conducted at room temperature clearly showed that PCA inhibits meat lipid oxidation. Under cold storage, the kinetic of meat lipid oxidation differs from that occurring at room temperature (Huang et al., 2015), which may explain the non-significant differences found among the differently treated samples. In contrast with hydroperoxides, the concentrations of MDA, resulting from secondary oxidation, were significantly lower in the PCA-treated compared with the meat samples treated with water during storage under both

Table 3
Color measurements [lightness (L^*), redness (a^*), hue (h°) and total color difference (ΔE^*)], and lipid oxidation results [(lipid hydroperoxides and malondialdehyde (MDA) concentrations] in fresh meat (day 0) and meat treated with water, rosmarinic acid (RA) at 0.25 mg mL^{-1}, and protocatechuic acid (PCA) at 1 and 2 mg mL^{-1}, after 2 days of storage at room temperature under dim light. The results represent the mean of three replicates ± S.E. Means within a column with the same letter are not significantly different ($P < 0.05$).

	Color				Lipid peroxidation
Experiment 2 (trial 2.3)	Lightness (L^*)	a^*	Hue angle (h°)	ΔE^*	MDA concentration (mg kg^{-1} meat)
Fresh meat	51.3 ± 1.8^{a}	24.0 ± 0.2^{a}	29.4 ± 1.3^{a}	–	0.122 ± 0.035^{a}
Water	53.9 ± 0.4^{ab}	12.2 ± 0.7^{b}	43.7 ± 1.8^{b}	12.3 ± 0.6^{a}	1.151 ± 0.124^{b}
RA 0.25 mg mL^{-1}	51.5 ± 1.1^{a}	11.8 ± 0.3^{b}	42.3 ± 0.8^{bc}	12.5 ± 0.2^{a}	0.132 ± 0.009^{c}
PCA 1 mg mL^{-1}	54.9 ± 1.8^{ab}	12.3 ± 0.8^{b}	41.4 ± 1.3^{bc}	12.8 ± 0.9^{a}	0.255 ± 0.005^{d}
PCA 2 mg mL^{-1}	57.0 ± 1.2^{b}	12.2 ± 1.1^{b}	39.6 ± 1.6^{c}	13.6 ± 1.5^{a}	0.159 ± 0.018^{e}

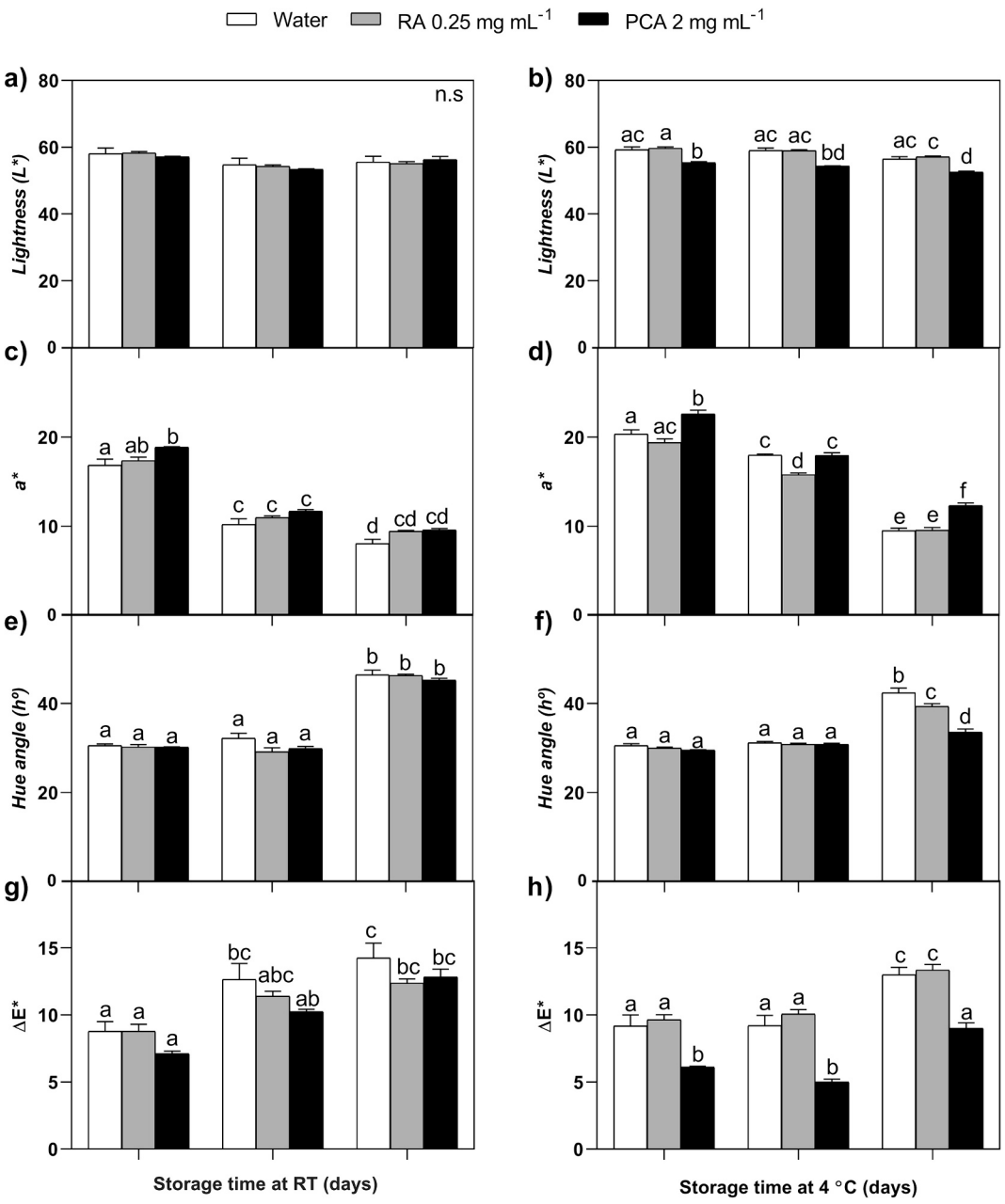

Fig. 3. Surface meat color changes in terms of lightness (L^*), redness (a^*), and hue (h°) and total color difference (ΔE^*) during storage at room temperature (a, c, e, and g) and under refrigeration (b, d, f and h) after treatment with water, rosmarinic acid (RA) solution at 0.25 mg mL^{-1} and PCA solution at 2 mg mL^{-1}. The results represent the mean of three replicates measured in triplicate ± S.E. Means with similar letters above the bars are not significantly different ($P < 0.05$). Results from experiment 3, trial 3.

conditions (Fig. 5 c, d). The concentration of MDA in the meat samples treated with 2 mg mL^{-1} of PCA and stored for two days at room temperature was significantly lower (47%) than in the samples treated with water. Similarly, after two and seven days under cold storage, the samples treated with PCA showed lower MDA concentrations (25% and 30%, respectively) than the samples treated with water, and the difference was significant after seven days of storage ($P < 0.05$). Hernández-García et al. (2022) recently reported similar results. The authors showed that meat packaged in bilayer films incorporating 2% PCA (*w/w*) reduced lipid peroxidation and peroxide value by about 30% after 15 days of storage at 5 °C, compared to meat packed using the same film without PCA. Despite the difference in the application mode, these results were similar to ours, indicating that PCA is promising as a food preservative on its own or as an ingredient in a preservative formulation or bioactive film.

The results found in this trial confirm that PCA applied at 2 mg mL^{-1} contributes to preventing lipid oxidation in minced meat during storage at room temperature and under refrigeration.

3.6. Experiment 4: The potential of CMC as a PCA delivery system

In experiment 4 (trial 4), PCA was applied to meat cubes, at 2 mg mL^{-1}, on its own and in combination with CMC to assess the potential of CMC as a delivery system to improve the effectiveness of PCA in inhibiting meat lipids oxidation.

In contrast to the observed in trial 2.3 (Table 3), in which PCA application at 2 mg mL^{-1} through spraying to minced meat did not inhibit redness loss, in this trial (trial 4), the immersion of meat cubes in

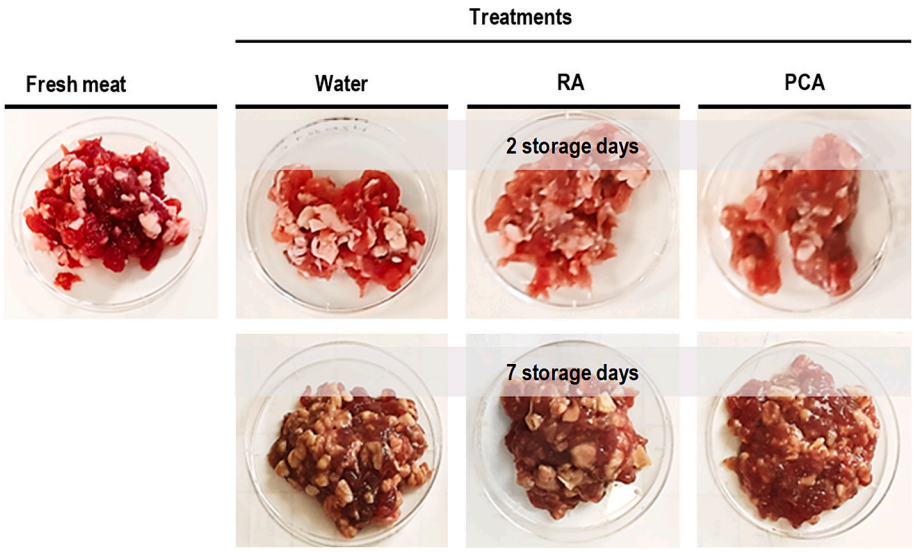

Fig. 4. Visual appearance of fresh minced meat and minced meat treated with water, rosmarinic acid at 0.25 mg $mL^{-1,}$ and protocatechuic acid at 2 mg mL^{-1} after 2 and 7 days of storage at 4 °C. Results from experiment 3, trial 3.

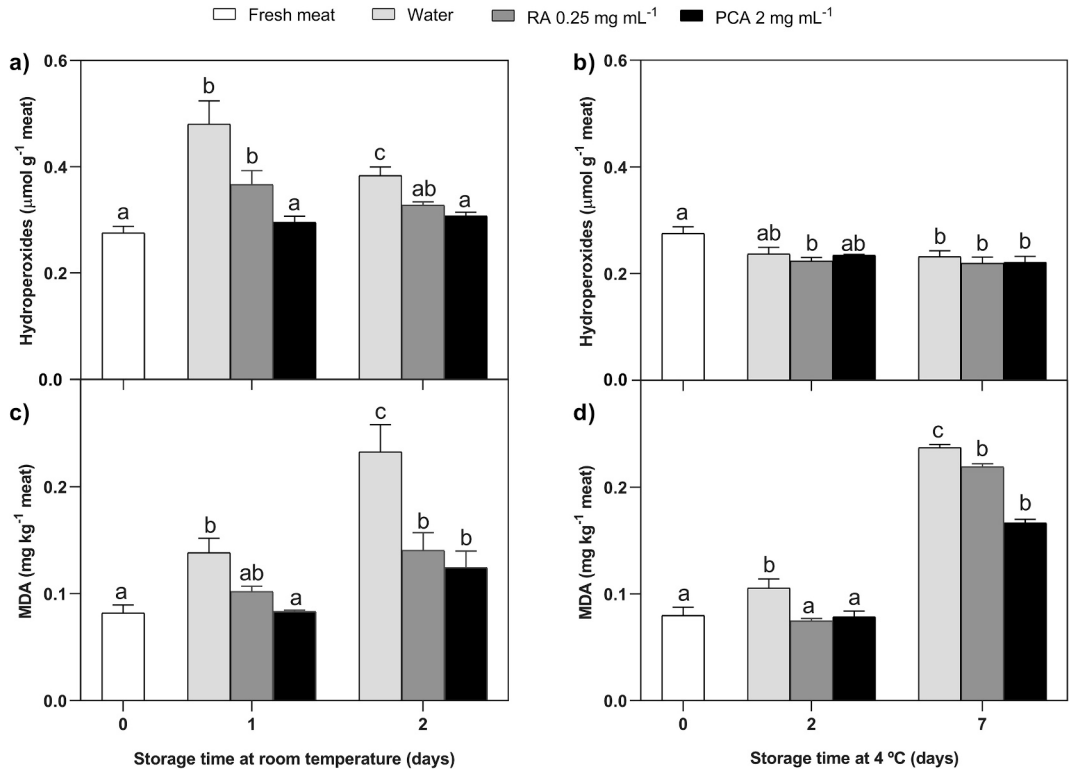

Fig. 5. Concentrations of lipid hydroperoxides and lipid hydroperoxides in fresh minced meat samples and samples treated with water, rosmarinic acid (RA) at 0.25 mg $mL^{-1,}$ and protocatechuic acid at 2 mg mL^{-1} during storage at room temperature for 2 days (a, c) and under refrigeration at 4 °C for 7 days (b, d). The results represent the mean of three replicates measured in triplicate ± S.E. Means with similar letters above the bars are not significantly different ($P < 0.05$). Results from experiment 3, trial 3.

a PCA solution at 2 mg mL^{-1} resulted in a redness (a^*) loss inhibition of 13%, compared with the redness (a^*) loss inhibition in the water treated samples (Table 4). This result suggests that PCA application by immersion may be more effective than spraying, or PCA at this concentration may be more effective when applied to meat cubes rather than to minced meat. The treatment with PCA combined with CMC resulted in a lower inhibition of redness loss (only 4%) compared to the application of PCA on its own. Similarly, meat samples treated with PCA had lower hue change, but the combination with CMC resulted in higher color change even when compared with the observed in water-treated meat (Table 4). CMC is commonly used to produce functional edible films incorporating biologically active molecules with antioxidant and antimicrobial activities to increase the shelf life of meat (El Sheikha et al., 2022; Ezati & Rhim, 2021; Razmjoo, Sadeghi, Alizadeh-Sani, Noroozi, & Azizi-Lalabadi, 2022). However, some studies reported that adding CMC at 2% to a model meat product does not confer resistance to oxidation but

Table 4
Color measurements [lightness (L^*), redness (a^*), hue (h°) and total color difference (ΔE^*)], and lipid peroxidation results expressed as malondialdehyde (MDA) concentrations in fresh meat (day 0) and meat treated with water, carboxymethyl cellulose (CMC) at 20 mg mL^{-1}, protocatechuic acid (PCA) at 2 mg mL^{-1} and PCA at 2 mg mL^{-1} plus 20 mg mL^{-1} CMC. after 2 days of storage at room temperature under dim light. The results represent the mean of three replicates ± S.E. Means within a column with the same letter are not significantly different ($P < 0.05$).

	Color				Lipid peroxidation
Experiment 4 (trial 4)	Lightness (L^*)	a^*	Hue angle (h°)	ΔE^*	MDA concentration (mg kg^{-1} meat)
Fresh meat	57.7 ± 2.8^{a}	19.1 ± 1.2^{a}	28.6 ± 0.9^{a}	–	0.070 ± 0.006^{a}
Water	67.9 ± 2.2^{b}	5.8 ± 0.6^{b}	49.3 ± 3.1^{bc}	15.6 ± 1.0^{a}	0.110 ± 0.003^{b}
CMC 20 mg mL^{-1}	60.2 ± 2.4^{ac}	7.2 ± 0.5^{bc}	48.5 ± 3.8^{bc}	12.7 ± 0.8^{b}	0.110 ± 0.004^{b}
PCA 2 mg mL^{-1}	65.1 ± 2.3^{bc}	8.0 ± 1.0^{c}	39.6 ± 7.4^{b}	17.0 ± 1.2^{a}	0.076 ± 0.007^{a}
PCA 2 mg mL^{-1} + CMC 20 mg mL^{-1}	64.4 ± 1.8^{ab}	6.5 ± 0.3^{bc}	56.5 ± 0.3^{c}	12.5 ± 1.2^{b}	0.088 ± 0.007^{ab}

improves the water-binding capacity and lowers meat hardness (Han et al., 2018). Our results support this hypothesis.

Concerning meat lipid oxidation, the results of lipid peroxidation showed that PCA applied at 2 mg mL^{-1} to meat cubes is effective in preventing oxidation since no significant differences were found between fresh meat cubes and PCA-treated cubes after storage for two days at room temperature (Table 4). Regarding meat samples treated with PCA combined with CMC, they showed higher MDA concentrations than PCA-treated, after storage, but they still were not significantly different from the concentrations found in fresh samples.

Considering the results, CMC as a delivery system for PCA did not contribute to increasing PCA effectiveness, but it had no significant detrimental effect and, for that reason, may be used to deliver PCA without constraints.

4. Conclusion

This work aimed to demonstrate the beneficial effect of PCA in preserving meat quality through the preservation of color and inhibition of lipid oxidation. The *in vitro* trials showed that PCA is a potent antioxidant and an effective LOX inhibitor at concentrations as low as 1 mg mL^{-1}.

The trials conducted *in vivo*, using minced meat bought in local markets, showed that PCA inhibits lipid peroxidation when applied at 12 mg mL^{-1} and that at this concentration, it is more effective at inhibiting meat lipid oxidation and meat discoloration than LA at 20 mg mL^{-1} and RA at 0.25 mg mL^{-1}. The results of subsequent trials conducted applying PCA solutions at different concentrations allowed the development of a model and the determination of the PCA concentration at which 50% of MDA formation was inhibited (IC_{50} = 0.7545 mg mL^{-1}). The model also allowed us to estimate the concentration at which PCA may be effective (2 mg mL^{-1}, applying 0.4 mL g^{-1} meat, corresponding to an application dose of 800 mg PCA kg^{-1}). In another trial, these results were confirmed, with the application of PCA at 1 mg mL^{-1} resulting in a significantly lower effect on meat oxidation than at 2 mg mL^{-1}. Having determined the concentration of PCA to apply to meat products, we evaluated the ability of this solution to prevent meat oxidation when meat samples were stored at room temperature and under cold conditions. The results showed that PCA at 2 mg mL^{-1} prevents meat lipid oxidation and discoloration when stored under both conditions. In addition, it was also of interest to understand if that concentration was also effective for application to meat products with other shapes. Therefore, PCA at 2 mg mL^{-1} on its own or combined with CMC, a biopolymer commonly used for bioactive compounds' delivery, was applied to meat cubes, which, after being treated, were stored at room temperature for two days. The results confirmed the ability of PCA to confer enhanced resistance to oxidation when applied to meat cubes, but CMC did not improve PCA performance.

Collectively, the results presented in this study clearly show that PCA activity is dose-dependent and that when applied to meat at concentrations above 2 mg mL^{-1}, it contributes to inhibiting meat lipid oxidation and meat discoloration in minced meat and meat pieces during storage under ambient and cold conditions and that PCA combination with CMC does not confer enhanced resistance to oxidation.

CRediT authorship contribution statement

Teresa Deuchande: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Joana F. Fundo**: Investigation, Methodology, Formal analysis. **Manuela E. Pintado**: Visualization, Project administration, Funding acquisition. **Ana L. Amaro**: Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: financial support and administrative support were provided by Amyris Bio Products Portugal. Teresa Deuchande, Joana F. Fundo, and Ana L. Amaro have a patent pending to Amyris Bio Products Portugal, Lda. and Universidade Católica Portuguesa. The other author declares that has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by Amyris Bio Products Portugal Unipessoal Lda. and Escola Superior de Biotecnologia—Universidade Católica Portuguesa through the Alchemy project—Capturing high value from industrial fermentation bioproducts (POCI-01-0247-FEDER-027578).

References

Al-Hijazeen, M., & Al-Rawashdeh, M. (2019). Preservative effects of rosemary extract (*Rosmarinus officinalis L.*) on quality and storage stability of chicken meat patties. *Food Science and Technology, 39*(1), 27–34. https://doi.org/10.1590/1678-457x.24817

Amaral, A. B., Da Solva, M. V., & Lannes, S. C. D. S. (2018). Lipid oxidation in meat: Mechanisms and protective factors - a review. *Food Science and Technology, 38*(1), 1–15. https://doi.org/10.1590/fst.32518

Andjelković, M., Van Camp, J., De Meulenaer, B., Depaemelaere, G., Socaciu, C., Verloo, M., & Verhe, R. (2006). Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chemistry, 98*(1), 23–31. https://doi.org/10.1016/j.foodchem.2005.05.044

Awad, A. M., Kumar, P., Ismail-Fitry, M. R., Jusoh, S., Ab Aziz, M. F., & Sazili, A. Q. (2021). Green extraction of bioactive compounds from plant biomass and their application in meat as natural antioxidant. *Antioxidants, 10*(9), 1–39. https://doi.org/10.3390/antiox10091465

Beya, M. M., Netzel, M. E., Sultanbawa, Y., Smyth, H., & Hoffman, L. C. (2021). Plant-based phenolic molecules as natural preservatives in comminuted meats: A review. *Antioxidants, 10*(2), 1–18. https://doi.org/10.3390/antiox10020263

Bi, X., Soong, Y. Y., Lim, S. W., & Henry, C. J. (2015). Evaluation of antioxidant capacity of Chinese five-spice ingredients. *International Journal of Food Sciences and Nutrition, 66*(3), 289–292. https://doi.org/10.3109/09637486.2015.1007452

Bianchin, M., Pereira, D., Reis, A. S., Almeida, J. F., Silva, L. D., Moura, C., & Carpes, S. T. (2017). Rosemary essential oil and lyophilized extract as natural antioxidant source to prevent lipid oxidation in pork sausage. *Advance Journal of Food Science and Technology, 13*(5), 210–217. https://doi.org/10.19026/ajfst.13.5070

Birtić, S., Dussort, P., Pierre, F. X., Bily, A. C., & Roller, M. (2015). Carnosic acid. *Phytochemistry, 115*(1), 9–19. https://doi.org/10.1016/j.phytochem.2014.12.026

Blandon, S. E., Vargas, D. A., Casas, D. E., Sarasty, O., Woerner, D. R., Echeverry, A., … Legako, J. F. (2023). Efficacy of common antimicrobial interventions at and above regulatory allowable pick-up levels on pathogen reduction. *Foods, 12*(4), 1–11. https://doi.org/10.3390/foods12040883

Bligh, E. G., & Dyer, W. J. (1959). Lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911–917. https://doi.org/10.1139/o59-099

Borbulevych, O. Y., Jankun, J., Selman, S. H., & Skrzypczak-Jankun, E. (2004). Lipoxygenase interactions with natural flavonoid, quercetin, reveal a complex with protocatechuic acid in its X-ray structure at 2.1 Å resolution. *Proteins: Structure, Function, and Bioinformatics, 54*(1), 13–19. https://doi.org/10.1002/prot.10579

Breil, C., Abert Vian, M., Zemb, T., Kunz, W., & Chemat, F. (2017). "Bligh and Dyer" and Folch methods for solid–liquid–liquid extraction of lipids from microorganisms. Comprehension of solvatation mechanisms and towards substitution with alternative solvents. *International Journal of Molecular Sciences, 18*(4), 1–21. https://doi.org/10.3390/ijms18040708

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*(2), 303–311. https://doi.org/10.1016/j.meatsci.2005.07.015

Carpenter, C. E., Smith, J. V., & Broadbent, J. R. (2011). Efficacy of washing meat surfaces with 2% levulinic, acetic, or lactic acid for pathogen decontamination and residual growth inhibition. *Meat Science, 88*(2), 256–260. https://doi.org/10.1016/j.meatsci.2010.12.032

Chao, C.-Y., & Yin, M.-C. (2009). Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. Foodborne Pathogens and Disease, 6(2), 201–206. doi: 10.1089=fpd.2008.0187.

Contreras, M., Hernández-Ledesma, B., Amigo, L., Martín-Álvarez, P. J., & Recio, I. (2011). Production of antioxidant hydrolyzates from a whey protein concentrate with thermolysin: Optimization by response surface methodology. *Lwt, 44*(1), 9–15. https://doi.org/10.1016/j.lwt.2010.06.017

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., Lorenzo, J. M., … Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants, 8*(429), 1–31. https://doi.org/10.3390/antiox8100429

El Sheikha, A. F., Allam, A. Y., Elobeid, T., Basiouny, E. A., Abdelaal, A. A., Amarowicz, R., … Oz, F. (2022). Impact of a carboxymethyl cellulose coating incorporated with an ethanolic propolis extract on the quality criteria of chicken breast meat. *Antioxidants, 11*(6), 1–17. https://doi.org/10.3390/antiox11061191

European Commission. (2011). Commission regulation (EU) no 1129/2011 of November 2011 amending annex II to regulation (EC) no 1333/2008 of the European Parliament and of the council by establishing a union list of food additives. *Official Journal of the European Union, 295*, 1–177. https://op.europa.eu/en/publication-detail/-/publication/28cb4a37-b40e-11e3-86f9-01aa75ed71a1/language-en.

European Commission. (2013a). Commission regulation (EU) no 723/2013 of 26 July 2013 amending annex II to regulation (EC) no 1333/2008 of the European Parliament and of the council as regards the use of extracts of rosemary (E 392) in certain low fat meat and fish products. *Official Journal of the European Union, 202*, 8–10. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32013R0723.

European Commission. (2013b). Regulation (EC) no 101/2013 of the European Parliament and of the council of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcasses. *Official Journal of the European Union, 9*(7), 2011–2013.

Ezati, P., & Rhim, J. W. (2021). Fabrication of quercetin-loaded biopolymer films as functional packaging materials. *ACS Applied Polymer Materials, 3*(4), 2131–2137. https://doi.org/10.1021/acsapm.1c00177

Farombi, E. O., Adedara, I. A., Awoyemi, O. V., Njoku, C. R., Micah, G. O., Esogwa, C. U., … Olopade, J. O. (2016). Dietary protocatechuic acid ameliorates dextran sulphate sodium-induced ulcerative colitis and hepatotoxicity in rats. *Food & Function, 7*(2), 913–921. https://doi.org/10.1039/C5FO01228G

Gheisari, H. R., Møller, J. K. S., Adamsen, C. E., & Skibsted, L. H. (2010). Sodium chloride or heme protein induced lipid oxidation in raw, minced chicken meat and beef. *Czech Journal of Food Sciences, 28*(5), 364–375. https://doi.org/10.17221/182/2009-CJFS

Graton, M. E., Ferreira, B. H. S. H., Troiano, J. A., Potje, S. R., Vale, G. T., Nakamune, A. C. M. S., … Antoniali, C. (2022). Comparative study between apocynin and protocatechuic acid regarding antioxidant capacity and vascular effects. *Frontiers in Physiology, 13*(1047916), 1–14. https://doi.org/10.3389/fphys.2022.1047916

Han, J., Liu, Y., Zhu, L., Liang, R., Dong, P., Niu, L., … Zhang, Y. (2021). Effects of spraying lactic acid and peroxyacetic acid on the quality and microbial community dynamics of vacuum skin-packaged chilled beef during storage. *Food Research International, 142*(110205), 1–11. https://doi.org/10.1016/j.foodres.2021.110205

Han, M., Clausen, M. P., Christensen, M., Vossen, E., Van Hecke, T., & Bertram, H. C. (2018). Enhancing the health potential of processed meat: the effect of chitosan or carboxymethyl cellulose enrichment on inherent microstructure, water mobility and oxidation in a meat-based food matrix. *Food & Function, 9*(7), 4017–4027. https://doi.org/10.1039/c8fo00835c

Heath, R., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts. *Archives of Biochemistry and Biophysics, 125*(1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Heir, E., Solberg, L. E., Jensen, M. R., Skaret, J., Grøvlen, M. S., & Holck, A. L. (2022). Improved microbial and sensory quality of chicken meat by treatment with lactic acid, organic acid salts and modified atmosphere packaging. *International Journal of Food Microbiology, 362*(109498), 1–13. https://doi.org/10.1016/j.ijfoodmicro.2021.109498

Hernández-García, E., Vargas, M., & Chiralt, A. (2022). Starch-polyester bilayer films with phenolic acids for pork meat preservation. *Food Chemistry, 385*(132650), 1–8. https://doi.org/10.1016/j.foodchem.2022.132650

Huang, Y., Wu, Z., Wang, Y., & Li, F. (2015). Examination of the effects of temperature and pressure on lipoxygenase activities in pork using response surface methodology. *Food Science and Biotechnology, 24*(4), 1257–1263. https://doi.org/10.1007/s10068-015-0161-5

Hughes, J. M., McPhail, N. G., Kearney, G., Clarke, F., & Warner, R. D. (2015). Beef *longissimus* eating quality increases up to 20 weeks of storage and is unrelated to meat colour at carcass grading. *Animal Production Science, 55*(2), 174–179. https://doi.org/10.1071/AN14304

Hunt, M. C., & King, D. A. (2012). *AMSA Meat Color Measurement Guidelines* (pp. 1–135). American Meat Science Association. https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012_12_meat_clr_guide.pdf?sfvrsn=d818b8b3_0.

Ibarra, A., Cases, J., Bily, A., He, K., Bai, N., Roller, M., Coussaert, A., & Ripoll, C. (2010). Importance of extract standardization and *in vitro/ex vivo* assay selection for the evaluation of antioxidant activity of botanicals: A case study on three *Rosmarinus officinalis* L. extracts. *Journal of Medicinal Food, 13*(5), 1167–1175. https://doi.org/10.1089/jmf.2009.0259

Ivanov, I., Heydeck, D., Hofheinz, K., Roffeis, J., O'Donnell, V. B., Kuhn, H., & Walther, M. (2010). Molecular enzymology of lipoxygenases. *Archives of Biochemistry and Biophysics, 503*(2), 161–174. https://doi.org/10.1016/j.abb.2010.08.016

Kakkar, S., & Bais, S. (2014). A review on protocatechuic acid and its pharamacological potential. *Journal of Acute Disease, 2014*(952943), 300–309. https://doi.org/10.1016/s2221-6189(13)60149-3

Kalogianni, A. I., Lazou, T., Bossis, I., & Gelasakis, A. I. (2020). Natural phenolic compounds for the control of oxidation, bacterial spoilage, and foodborne pathogens in meat. *Foods, 9*(6), 1–28. https://doi.org/10.3390/foods9060794

Khorsand, G. J., Morshedloo, M. R., Mumivand, H., Emami Bistgani, Z., Maggi, F., & Khademi, A. (2022). Natural diversity in phenolic components and antioxidant properties of oregano (*Origanum vulgare* L.) accessions, grown under the same conditions. *Scientific Reports, 12*(1), 1–9. https://doi.org/10.1038/s41598-022-09742-4

Kim, Y. H., Keeton, J. T., Smith, S. B., Berghman, L. R., & Savell, J. W. (2009). Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles. *Meat Science, 83*(3), 376–382. https://doi.org/10.1016/j.meatsci.2009.06.009

Koushesh, M., Banin, O., Koushesh Saba, M., & Sogvar, O. B. (2016). Combination of carboxymethyl cellulose-based coatings with calcium and ascorbic acid impacts in browning and quality of fresh-cut apples. *LWT - Food Science and Technology, 66*(1), 165–171. https://doi.org/10.1016/j.lwt.2015.10.022

Krishna, P. U. N., & Muraleedharan, K. (2023). Metal chelation ability of Protocatechuic acid anion with 210Po84; a theoretical insight. *Computational and Theoretical Chemistry, 1220*(December 2022), Article 113996. https://doi.org/10.1016/j.comptc.2022.113996

Lešnik, S., Furlan, V., & Bren, U. (2021). Rosemary (*Rosmarinus officinalis* L.): Extraction techniques, analytical methods and health-promoting biological effects. *Phytochemistry Reviews, 20*(6), 1273–1328. https://doi.org/10.1007/s11101-021-09745-5

Li, X., Wang, X., Chen, D., & Chen, S. (2011). Antioxidant activity and mechanism of protocatechuic acid in vitro. *Functional Foods in Health and Disease, 1*(7), 232–244. https://doi.org/10.31989/ffhd.v1i7.127

Liu, C., Chen, C., Jiang, A., Sun, X., Guan, Q., & Hu, W. (2020). Effects of plasma-activated water on microbial growth and storage quality of fresh-cut apple. *Innovative Food Science and Emerging Technologies, 59*(102256), 1–7. https://doi.org/10.1016/j.ifset.2019.102256

Loussouarn, M., Krieger-Liszkay, A., Svilar, L., Bily, A., Birtić, S., & Havaux, M. (2017). Carnosic acid and carnosol, two major antioxidants of rosemary, act through different mechanisms. *Plant Physiology, 175*(3), 1381–1394. https://doi.org/10.1104/pp.17.01183

Ma, G., Wang, Z., Chen, H., Yu, Q., & Han, L. (2021). Effect of low-dose sodium nitrite treatment on the endogenous antioxidant capacity of yak meat during wet curing: Pros and cons. *Lwt, 141*(110879), 1–8. https://doi.org/10.1016/j.lwt.2021.110879

Mahfuz, S., Mun, H. S., Dilawar, M. A., Ampode, K. M. B., & Yang, C. J. (2022). Potential role of Protocatechuic acid as natural feed additives in farm animal production. *Animals, 12*(6), 1–14. https://doi.org/10.3390/ani12060741

Manzoor, A., Jaspal, M. H., Yaqub, T., Haq, A. U., Nasir, J., Avais, M., … Yar, M. K. (2020). Effect of lactic acid spray on microbial and quality parameters of buffalo meat. *Meat Science, 159*(107923), 1–6. https://doi.org/10.1016/j.meatsci.2019.107923

Masuda, H., Hironaka, S., Matsui, Y., Hirooka, S., Hirai, M., Hirata, Y., Akao, M., & Kumagai, H. (2015). Comparative study of the antioxidative activity of culinary herbs and spices, and hepatoprotective effects of three selected lamiaceae plants on carbon tetrachloride-induced oxidative stress in rats. *Food Science and Technology Research, 21*(3), 407–418. https://doi.org/10.3136/fstr.21.407

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(10), 1201–1207. https://doi.org/10.1111/j.1365-2621.2006.01342.x

Muppalla, S. R., Kanatt, S. R., Chawla, S. P., & Sharma, A. (2014). Carboxymethyl cellulose-polyvinyl alcohol films with clove oil for active packaging of ground chicken meat. *Food Packaging and Shelf Life, 2*(2), 51–58. https://doi.org/10.1016/j.fpsl.2014.07.002

Nieto, G., Ros, G., & Castillo, J. (2018). Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis*, L.): A review. *Medicines, 5*(3), 98. https://doi.org/10.3390/medicines5030098

Ninfali, P., Mea, G., Giorgini, S., Rocchi, M., & Bacchiocca, M. (2005). Antioxidant capacity of vegetables, spices and dressings relevant to nutrition. *The British Journal of Nutrition, 93*(2), 257–266. https://doi.org/10.1079/bjn20041327

Oswell, N. J., Thippareddi, H., & Pegg, R. B. (2018). Practical use of natural antioxidants in meat products in the U.S.: A review. *Meat Science, 145*(1), 469–479. https://doi.org/10.1016/j.meatsci.2018.07.020

Papuc, C., Goran, G. V., Predescu, C. N., & Nicorescu, V. (2017). Mechanisms of oxidative processes in meat and toxicity induced by postprandial degradation products: A review. *Comprehensive Reviews in Food Science and Food Safety, 16*(1), 96–123. https://doi.org/10.1111/1541-4337.12241

Papuc, C., Goran, G. V., Predescu, C. N., Nicorescu, V., & Stefan, G. (2017). Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. *Comprehensive Reviews in Food Science and Food Safety, 16*(6), 1243–1268. https://doi.org/10.1111/1541-4337.12298

Priyadarshi, R., Kim, S. M., & Rhim, J. W. (2021). Carboxymethyl cellulose-based multifunctional film combined with zinc oxide nanoparticles and grape seed extract for the preservation of high-fat meat products. *Sustainable Materials and Technologies, 29*(e00325), 1–13. https://doi.org/10.1016/j.susmat.2021.e00325

Puolanne, E. J., Poso, A. R., Ruusunen, M. H., Sepponen, K. V., & Kyla-Puhju, M. S. (2002). Lactic acid in muscle and its effects on meat quality. In *Proceedings of the 55th Reciprocal Meat Conference. American Meat Science Association* (pp. 57–62). https://meatscience.org/docs/default-source/publications-resources/rmc/2002/lactic-acid-in-muscle-and-its-effects-on-meat-quality(3).pdf?sfvrsn=2.

Rackova, L., Oblozinsky, M., Kostalova, D., Kettmann, V., & Bezakova, L. (2007). Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *Journal of Inflammation, 4*(15), 1–7. https://doi.org/10.1186/1476-9255-4-15

Razmjoo, F., Sadeghi, E., Alizadeh-Sani, M., Noroozi, R., & Azizi-Lalabadi, M. (2022). Fabrication and application of functional active packaging material based on carbohydrate biopolymers formulated with *lemon verbena/Ferulago angulata* extracts for the preservation of raw chicken meat. *Journal of Food Processing and Preservation, 46*(e16830), 1–16. https://doi.org/10.1111/jfpp.16830

Reitznerová, A., Uleková, M., Nagy, J., Marcinčák, S., Semjon, B., Čertík, M., & Klempová, T. (2017). Lipid peroxidation process in meat and meat products: A comparison study of malondialdehyde determination between modified 2-thiobarbituric acid spectrophotometric method and reverse-phase high-performance liquid chromatography. *Molecules, 22*(1988), 1–12. https://doi.org/10.3390/molecules22111988

Riseh, S. R., Vazvani, G. M., Hassanisaadi, M., & Skorik, Y. A. (2023). Micro−/nano-carboxymethyl cellulose as a promising biopolymer with prospects in the agriculture sector: A review. *Polymers, 15*(440), 1–31. https://doi.org/10.3390/polym15020440

Rodríguez-Melcón, C., Alonso-Calleja, C., & Capita, R. (2017). Lactic acid concentrations that reduce microbial load yet minimally impact colour and sensory characteristics of beef. *Meat Science, 129*(1), 169–175. https://doi.org/10.1016/j.meatsci.2017.01.007

Rossetto, M., Lante, A., Vanzani, P., Spettoli, P., Scarpa, M., & Rigo, A. (2005). Red chicories as potent scavengers of highly reactive radicals: A study on their phenolic composition and peroxyl radical trapping capacity and efficiency. *Journal of Agricultural and Food Chemistry, 53*(21), 8169–8175. https://doi.org/10.1021/jf051116n

Sadik, C. D., Sies, H., & Schewe, T. (2003). Inhibition of 15-lipoxygenases by flavonoids: Structure-activity relations and mode of action. *Biochemical Pharmacology, 65*(5), 773–781. https://doi.org/10.1016/S0006-2952(02)01621-0

Sánchez-Escalante, A., Torrescano, G., Djenane, D., Beltrán, J. A., Giménez, B., & Roncalés, P. (2011). Effect of antioxidants and lighting conditions on color and lipid stability of beef patties packaged in high-oxygen modified atmosphere. *CyTA Journal of Food, 9*(1), 49–57. https://doi.org/10.1080/19476330903572945

Sebranek, J. G., Sewalt, V. J. H., Robbins, K. L., & Houser, T. A. (2005). Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Science, 69*(2), 289–296. https://doi.org/10.1016/j.meatsci.2004.07.010

Senanayake, N. S. P. J. (2013). Green tea extract: Chemistry, antioxidant properties and food applications - a review. *Journal of Functional Foods, 5*(4), 1529–1541. https://doi.org/10.1016/j.jff.2013.08.011

Shan, B., Cai, Y. Z., Sun, M., & Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry, 53*(20), 7749–7759. https://doi.org/10.1021/jf051513y

Singla, R. K., Dubey, A. K., Garg, A., Sharma, R. K., Fiorino, M., Ameen, S. M., … Al-Hiary, M. (2019). Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. *Journal of AOAC International, 102*(5), 1397–1400. https://doi.org/10.5740/jaoacint.19-0133

Song, J., He, Y., Luo, C., Feng, B., Ran, F., Xu, H., Ci, Z., Xu, R., Han, L., & Zhang, D. (2020). New progress in the pharmacology of protocatechuic acid: A compound ingested in daily foods and herbs frequently and heavily. *Pharmacological Research, 161*(105109), 1–25. https://doi.org/10.1016/j.phrs.2020.105109

Stojković, D. S., Živković, J., Soković, M., Glamočlija, J., Ferreira, I. C. F. R., Janković, T., & Maksimović, Z. (2013). Antibacterial activity of *Veronica montana*. *Food and Chemical Toxicology, 55*, 209–213. https://doi.org/10.1016/j.fct.2013.01.005

Suman, S. P., Hunt, M. C., Nair, M. N., & Rentfrow, G. (2014). Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Science, 98*(3), 490–504. https://doi.org/10.1016/j.meatsci.2014.06.032

Velasco, V., & Williams, P. (2011). Improving meat quality through natural antioxidants. *Chilean Journal of Agricultural Research, 71*(2), 313–322. https://doi.org/10.4067/s0718-58392011000200017

Wu, M., Tian, L., Fu, J., Liao, S., Li, H., Gai, Z., & Gong, G. (2022). Antibacterial mechanism of protocatechuic acid against *Yersinia enterocolitica* and its application in pork. *Food Control, 133*(108573), 1–12. https://doi.org/10.1016/j.foodcont.2021.108573

Yin, M.-C., & Chao, C.-Y. (2008). Anti-*Campylobacter*, anti-aerobic, and anti-oxidative effects of roselle calyx extract and protocatechuic acid in ground beef. *International Journal of Food Microbiology, 127*(1), 73–77. https://doi.org/10.1016/j.ijfoodmicro.2008.06.002

Zhang, S., Gai, Z., Gui, T., Chen, J., Chen, Q., & Li, Y. (2021). Antioxidant effects of protocatechuic acid and protocatechuic aldehyde: Old wine in a new bottle. *Evidence-based Complementary and Alternative Medicine, 2021*(6139308), 1–19. https://doi.org/10.1155/2021/6139308

Zhang, Y., Holman, B. W. B., Ponnampalam, E. N., Kerr, M. G., Bailes, K. L., Kilgannon, A. K., … Hopkins, D. L. (2019). Understanding beef flavour and overall liking traits using two different methods for determination of thiobarbituric acid reactive substance (TBARS). *Meat Science, 149*(1), 114–119. https://doi.org/10.1016/j.meatsci.2018.11.018

Zhong, C., Hou, P. F., Li, Y. X., Yang, W. Y., Shu, M., & Wu, G. P. (2021). Characterization, antioxidant and antibacterial activities of gelatin film incorporated with protocatechuic acid and its application on beef preservation. *LWT - Food Science and Technology, 151*(112154), 1–11. https://doi.org/10.1016/j.lwt.2021.112154