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The pro-oxidant action of high-oxygen MAP on beef patties can be counterbalanced by antioxidant compounds from common hawthorn and rose hips

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ABSTRACT

The objective of this research was to evaluate the effectiveness of antioxidant-rich extracts from rose hip (*Rosa canina* L.; RC) and hawthorn (*Crataegus monogyna* Jacq.; CM) at minimizing the oxidative damage to proteins and lipids in beef patties subjected to a high-oxygen (HiOx-MAP) and vacuum (Vacuum) packaging atmosphere. The extracts of RC and CM were characterized by quantifying bioactive compounds, namely, phenolic compounds, tocopherols and vitamin C. Both fruits had high concentrations of bioactive compounds, with RC having the highest total phenolic and vitamin C content. Yet, CM was the most efficient in protecting beef patties against protein carbonylation, reducing, as a result, the instrumental toughness in cooked beef patties. The use of CM and RC extracts in beef patties significantly improved consumer purchase intention in HiOx-MAP packaging systems. The use of CM and RC extracts or their combination in future research would be an effective antioxidant means to decrease the pro-oxidative effects caused by HiOx-MAP in red meat.

1. Introduction

Burger meat remains as one of the most popular and consumed muscle food worldwide. Preservation of a desirable bright red colour in fresh beef burger patties during retail display is required owing to the impact of this quality trait on consumer's preference for red meat (Santos et al., 2021). To fulfil this objective, high-oxygen modified atmosphere packaging (HiOx-MAP) is commonly applied to red meat to guarantee the formation and stability of oxymyoglobin (OxyMb) during retail display (Holman, Kerry, & Hopkins, 2018). According to the scientific literature, percentages of oxygen between 50 and 80% in HiOx-MAP are effective for preserving OxyMb in fresh lamb (Morcuende et al., 2020) and beef (Shen et al., 2022) stored under chilled conditions. Yet, HiOx-MAP has been found to accelerate lipid and protein oxidation in beef (Bao, Puolanne, & Ertbjerg, 2016) lamb (Kim, Bodker, & Rosenvold, 2012) and pork meat cuts (Wang, Wang, Li, & Zhang, 2019). The occurrence of oxidative reactions under these packaging conditions is manifested in altered meat quality such as impaired water-holding capacity and decreased tenderness (Holman et al., 2018) Additionally, the accretion of lipid and protein oxidation products in meat is emphasized to display some safety concerns given the toxicity of such chemical species (Estévez & Xiong, 2019; Ge et al., 2021).

In this scenario, it seems reasonable to apply antioxidant strategies to guarantee the benefits of HiOx-MAP and counteract the negative impact of its noxious pro-oxidative environment. As recently reviewed by Estévez (2021), the application of bioactive compounds from plant kingdom to meat and meat products is an interesting option owing to their intense antioxidant properties, safety and consumer's preferences over conventional antioxidant additives. Common hawthorn (*Crataegus monogyna* Jacq.; CM) and rose hips (*Rosa canina* L., RC) are Mediterranean fruits with long recognized health properties owing to their high concentration in phenolic compounds and vitamin C (Ganhão, Morcuende, & Estévez, 2010). They have been previously characterized for their composition in bioactive compounds and tested for their effectiveness in controlling lipid and protein oxidation in processed meat products such as cooked pork hams (Armenteros, Morcuende, Ventanas, & Estévez, 2016), ready-to-eat pork patties (Ganhão et al., 2010), smoked beef sausages (Zheleuova, Uzakov, Shingisov, Alibekov, &

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Khamitova, 2021), and frankfurters (Vossen, Utrera, De Smet, Morcuende, & Estévez, 2012). Though these fruits are known for their antioxidant activities, it is ignored whether they can protect against lipid and protein oxidation and discoloration during retail display of ground beef packed under HiOx-MAP. Besides, it is of the utmost commercial interest to know whether the antioxidant protection of meat products is also manifested in improved sensory features and/or has an impact on consumers' preferences. In a recent study, we found limited effects of sprayed extracts of these fruits in intact lamb cutlets packed in 50% HiOx-MAP (Morcuende et al., 2020). We hypothesize whether the means of application was a limiting factor in their effectiveness, and we propose that adding extracts from CM and RC to ground beef meat could extend the shelf life and consumer perception of beef patties packed in HiOx-MAP. The assessment of proper oxidation markers together with a detailed characterization of the bioactive components of these fruits may also contribute to understanding the underlying action mechanisms.

In order to clarify all these issues, this study was designed to assess the effectiveness of extracts from CM and RC to inhibit oxidative reactions and discolouration in beef patties packed in HiOx-MAP and vacuum packaging and subjected to simulated retail display. The impact of such benefits on the consumer purchasing intention was also evaluated.

2. Material and methods

2.1. Materials

All chemical compounds used in the present work as reagents, solvents or standard compounds were purchased from Panreac (Panreac Química, S. A., Barcelona, Spain), Merck (Merk, Darmstadt, Germany) and Extrasynthese (Genay, France). The extraction solvents were compatible for industrial food use. Beef strip loins for the production of beef patties were donated by the European Protected Geographical Indication (PGI) "Ternera de Extremadura".

2.2. Extraction and characterization of bioactive compounds from fruits

Fruits, rose hips (*Rosa canina* L.; RC) and hawthorn (*Crataegus monogyna* Jacq.; CM) were harvested during late summer and autumn of 2021 from wild bushes nearby the city of Cáceres (GPS coordinates: 39°28′35.36″ N -6°22′20.06″ W). Upon hand harvesting and identification, fruits were cut into pieces, lyophilized and frozen at −80 °C. Extracts were prepared according to the procedure described by Morcuende et al. (2020). Bioactive compounds were extracted using 10 volumes of 60% food grade acetone. Supernatants were dried using rotary evaporator at 40 °C, dispensed in 25 mL volumetric flasks, brought to volume with distilled water and immediately transferred to the laboratory for characterization.

Tocopherols were quantified following the procedure described by Rodríguez-Carpena, Morcuende, and Estévez (2012). One millilitre of fruit extracts was diluted with isopropanol (1:10, *w/v*) prior to analysis by a Shimadzu "Prominence" HPLC equipped with a RF-10A XL fluorescence detector (Shimadzu Corporation, Kyoto, Japan). Samples were eluted on a reversed-phase C18 column (150 mm length, 4.6 mm i.d., 5 μm particle diameter) from Phenomenex (CA, USA) and an isocratic mobile phase consisting of acetonitrile (100%) (1.4 mL/min flow rate). Peaks were registered at 285 and 335 nm as excitation and emission wavelength, respectively. For quantification purposes, standard curves were prepared using standards of γ- and α-tocopherol supplied by Merck (Darmstadt, Germany).

Vitamin C content was determined according to AOAC official titrimetric method (method 967.21) (AOAC, 2006). The quantification was obtained by a calibration curve of suitable dilutions of standard ascorbic acid and the results are expressed as mg vitamin C/g of fruit (dry matter).

Analysis of monomeric phenolics was performed using an HPLC method described by Morcuende et al. (2020). Prior to analysis, fruit extracts were filtered using a PVDF 0.45 μm filter (Agilent, CA, USA) and injection volume was 2 μL. Analytical separation of phenolic compounds was carried out on a Shimadzu "Prominence" HPLC apparatus equipped with a RF-10A XL fluorescence detector and a SPD-M20A Diode Array Detector (DAD) (Shimadzu Corp., Kyoto, Japan). The column consisted of a reversed-phase Agilent Poroshell 120 SB-C18 column (150 × 4.6 mm, 2.7 μm particle size) and a guard column (10 × 4.6 mm) filled with the same material. Samples were eluted over a gradient from 7% (0.5% formic acid) to 65% acetonitrile (0.5% formic acid) over 55 min at a flow rate of 0.5 mL/min. Phenolics were quantified in five subclasses based on spectral identification, namely, hydroxybenzoic acids (as gallic acid equivalents, 280 nm), hydroxycinnamic acids (as caffeic acid equivalents, 320 nm), flavonols (as quercetin equivalents, 365 nm), and anthocyanins (as delphinidin chloride equivalents, 520 nm). Total procyanidin content was quantified by means of acid-catalysed cleavage reaction in the presence of excess phloroglucinol according to the procedure reported by Morcuende et al. (2020). Phloroglucinolysis products were characterized by their UV–vis spectra and retention time relative to external standards. A fluorescence detector (λex = 280 nm, λem = 322 nm) was used simultaneously to improve the identification procedure. Total procyanidins was determined as the sum of the quantified subunits.

2.3. Experimental design

Beef burger patties were elaborated using beef strip loin (80%), distilled water (18%), and NaCl (2%). Emulsified-type beef patties were shaped (10 cm diameter and 1 cm thickness) using a Mainca burger patty maker Mod. MH-55 (Equipamientos Carnicos S.L., Barcelona, Spain).

Depending on the addition of fruit extracts, three types of beef patties were produced, namely, CM (beef patties elaborated with *Crataegus monogyna* extract), RC (beef patties elaborated with *Rosa canina* extract) together with a Control group (C, no added extract). In the treated patties, the distilled water was replaced by the same amount of the fruit extract with the concentration of total phenolics set at 200 mg of gallic acid equivalent /kg. The election of this concentration was based on previous studies using the same fruits (Ganhão et al., 2010; Vossen et al., 2012) and on some preliminary studies to assure a positive antioxidant effect under the conditions of the present experiment. Patties from the three experimental groups were packed in polypropylene (PP) trays (Sarabia Plastics, Alicante, Spain) (130 mm × 160 mm × 50 mm) (0.90 mm thick) and polyester/PLPMC (Wipack, Hamburg, Germany) with an oxygen permeability of 114 $cm^3\ m^{-2}\ day^{-1}\ bar^{-1}$ at 25 °C and water transmission rate of 10 $g\ m^{-2}\ day^{-1}$ at 38 °C 90% RH, as a top film. Depending on the composition of the packaging atmosphere, two groups were considered: patties packed in vacuum (Vacuum) or in high-oxygen packaging atmosphere (HiOx-MAP) (70% O_2 + 30% CO_2). C, CM and RC patties were randomly packed in either one of these packaging systems totalling 6 experimental groups, namely: Vacuum-C (patties packed in a vacuum package and no added extract), Vacuum-CM (patties packed in a vacuum package and treated with 200 mg/kg CM extract); Vacuum-RC (patties packed in a vacuum package and treated with 200 mg/kg RC extract); HiOx-MAP-C (patties packed in a HiOx-MAP and no added extract), HiOx-MAP-CM (patties packed in a HiOx-MAP and treated with CM extract) and HiOx-MAP-RC (patties packed in a HiOx-MAP and treated with RC extract). Packaging was performed in a thermo-sealing equipment (Smart 500, ULMA, Spain) equipped with a gas mixer. The six types of samples were subjected to a retail display refrigerated storage for 10 days at 2 °C and 12 h cycles of fluorescent light (1620 lx) and darkness. Subsequent to this storage, samples were unpacked and grilled at 180 °C (external temperature) for 18 min (9 min each side). According to the aforementioned experimental design, samples from all groups were analysed at three processing stages: at day 0 (day of the

manufacture, FRESH), at day 10 of refrigerated storage (REFRIGERATED) and upon grilling (COOKED). Two burger patties per experimental group and processing stage were produced (technical replicates) and the whole experimental design was repeated twice (true replicates). Additionally, all experimental analysis were repeated twice and hence, means and standard deviations were calculated from 8 data (2 technical replicates x 2 true replicates x 2 analysis).

2.4. Analytical procedures on beef patties

2.4.1. Proximate composition of beef patties

Moisture and total protein content were determined using official methods (AOAC, 2000). The method of Folch, Lees, & Stanley (1957) was used for quantifying fat from beef patties.

2.4.2. Analysis of lipid oxidation: thiobarbituric acid reactive substances (TBARS)

TBARS were quantified following the procedure reported by Ganhão, Estévez, and Morcuende (2011). Concisely, five grams of beef patty were dispensed in cone plastic tubes and homogenized with 15 mL perchloric acid (3.86%). To lessen the onset of further oxidative reactions during extraction of TBARS, 0.5 mL of butylhydroxytoluene (BHT) (4.2% in ethanol) were added to plastic tubes that were immersed in an ice bath during homogenisation. Two mL of the filtered and centrifuged (600 *g* for 4 min) slurry were mixed with 2 mL of thiobarbituric acid (TBA) (0.02 M) in screw-capped test tubes. The tubes were placed in a boiling water bath (100 °C) for 45 min together with the tubes from the standard curve. After cooling, the absorbance was measured at 532 nm. The standard curve was prepared using a 1,1,3,3-tetraethoxypropane (TEP) solution in 3.86% perchloric acid. Results were calculated as mg MDA per kg of meat.

2.4.3. Analysis of lipid oxidation: hexanal counts

One gram of minced sample was transferred to a 4 mL SPME glass vial. Hexanal was analysed from the Headspace (HS) of the vials using a SPME fibre, coated with a divinylbenzene-carboxen-poly (dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm. Prior to extraction, the fibre was preconditioned for 2 h at 220 °C following the producers' recommendation. Once accomplished, the SPME fibre was exposed to the HS of the vial during 30 min while incubated in an oven at 37 °C and sequentially transferred to a HP5890GC series II gas chromatograph (Hewlett–Packard, USA) coupled to a mass-selective detector (Agilent model 5973). Volatiles were separated using a 5% phenyl-95% dimethyl polysiloxane column (Restek, USA) (30 m 0.25 mm id., 1.0 mm film thickness). GC–MS conditions were published elsewhere (Ganhão et al., 2010). Hexanal was positively identified by comparing its spectra and linear retention index (LRI) with that from the standard compound (Merck, Darmstadt, Germany). Chromatographic areas from MS are provided as units of arbitrary area (UAA 10^5) and used as a measurement of the relative abundance of hexanal.

2.4.4. Analysis of protein oxidation: depletion of tryptophan residues

The oxidation of tryptophan was assessed by the loss of the natural fluorescence of this amino acid as proposed by Utrera and Estevez (2013). One gram of sample was diluted (1:20) in 20 mL of 100 mM Na_3PO_4 buffer pH 6.0 with 8 M urea and then dispensed in a 4 mL quartz spectrofluorometer cell. Emission spectra of tryptophan were recorded from 300 to 400 nm with the excitation wavelength established at 283 nm (LS55 Perkin-Elmer luminescence spectrometer, MA, USA). Excitation and emission slit widths were set at 10 nm and data were collected at 500 nm per minute. These values were corrected according to the protein concentration of each sample by applying a correction factor (Cf = Pt/Pp) where Pt is the total average of the amount of protein from all samples and Pp is the content of protein in each type of sample. The fluorescence intensity recorded at 450 nm is used as a measurement of the relative abundance of tryptophan.

2.4.5. Analysis of protein oxidation: accretion of protein carbonyls

Specific protein carbonyls, namely, α-amino adipic and γ-glutamic semialdehydes (AAS and GGS respectively), were quantified following the procedure reported by Utrera, Morcuende, Rodríguez-Carpena, and Estévez (2011). One gram of patty was homogenized with 10 mL 100 mM Na_3PO_4 buffer pH 6.0. Upon precipitation of the protein using 20% cold trichloroacetic acid (TCA), protein carbonyls were derivatised with 0.5 mL p-aminobenzoic acid (ABA) (250 mM in MES buffer pH 6.0) in the presence of 0.25 mL sodium cyanoborohydride ($NaBH_3CN$) (100 mM in 250 mM MES buffer pH 6.0). Protein was then subjected to acid hydrolysis (6 M HCl) for 18 h at 110 °C and later dried at 45 °C using the vacuum concentrator. The dried hydrolysate was reconstituted in 0.2 mL HPLC grade water and filtered through hydrophilic polypropylene GH polypro (GHP) syringe filters (0.45 μm pore size, Pall Corporation, USA) for HPLC analysis. Two μL of the reconstituted derivatised protein hydrolysates were injected and analysed using a Shimadzu 'Prominence' HPLC apparatus (Shimadzu Corp., Kyoto, Japan) equipped with COSMOSIL 5C18-AR-II RP-HPLC column (150 × 4.6 mm) and a guard column (10 × 4.6 mm) containing similar packing material. The elution program was based on the low-pressure gradient method of Utrera et al. (2011) with Eluent A: 50 mM sodium acetate buffer pH 5.4 and Eluent B: Acetonitrile where the concentration of Eluent B varied from 0% (min 0) to 8% (min 20). The flow rate was kept at 1 mL/min, column temperature at 40 °C and a total run time of 30 min. The eluate was monitored with excitation and emission wavelengths of 283 nm and 350 nm, respectively. Identification of both AAS-ABA and GGS-ABA in the FLD chromatogram was done by comparing their retention times with those of the standards. Authentic AAS-ABA and GGS-ABA were respectively synthesized from Nα-acetyl-L-lysine and Nα-acetyl-L-ornithine following the procedure described by Utrera et al. (2011). Curves were manually integrated, and their resulting areas were plotted against a known concentration (0.01–0.05 mM) of ABA standard curve with $R^2 > 0.9997$. This follows the assumption that the fluorescence emitted by 1 mol of ABA is equivalent to that emitted by 1 mol of AAS-ABA or GGS-ABA. Results are displayed as the sum of the concentrations of both carbonyls and expressed as nmol carbonyl/mg protein.

2.4.6. Instrumental colour measurement

Surface colour measurements of beef patties were recorded on 15 min blooming time with a Minolta chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ) with 8 mm diameter aperture, illuminant D65 and 0° standard observer. Before each sampling day, the chromameter was calibrated on the CIE colour space system using a white tile. Only a^* values were recorded and used as indicators of the redness in beef patties. Colour measurements were made at room temperature on the surface of each sample in triplicate at three selected locations to cover the entire surface of the samples.

2.4.7. Instrumental hardness measurement

Hardness was measured in cooked beef patties using a TA.TXplus texturometer (Stable Micro Systems, Godalming, Surrey, UK). The samples cut into circular sections with dimensions of 20 × 10 mm (diameter × thickness, respectively) and compressed twice to 50% of their original height with a compression flat cylindrical aluminium probe (50 mm diameter) at a test speed of 50 mm min^{-1}. The results were expressed in newtons (N).

2.4.8. Analysis assessment of purchase intention

Consumer's willingness to purchase the beef patties under study was evaluated by 60 assessors ranging in age from 25 to 60 and who identified, themselves, as regular consumers of beef patties. They were recruited by using social networks and emails and all of them expressed their willingness to participate in the research study. Assessment was made on refrigerated beef patties (day 10) while displayed on trays. Assessors were asked to express their willingness to purchase the product according to their appearance. In addition, assessors were asked

to describe the main reasons behind their decision. Results are shown as percentage of consumers willing to purchase a particular sample.

2.5. Statistical analysis

The application of the fruit extracts (main variable under study) was repeated twice (true replicate) in two independent processing batches. Two beef patties per experimental group (Vacuum-C, Vacuum-CM, Vacuum-RC, HiOx-MAP-C, HiOx-MAP-CM, and HiOx-MAP-RC) and per sampling day (FRESH, REFRIGERATED and COOKED), were produced (technical replicates) in each batch totalling 72 beef patties. All experimental analyses were repeated twice and hence, means and standard errors were calculated from 8 data (2 technical replicates x 2 true replicates x 2 analysis). Data obtained from analyses at each processing stage were evaluated by two-way Analysis of Variance (ANOVA) where the addition of fruit extract (3 levels), the packaging system (2 levels) together with the interaction (fruits x packaging system) were regarded as fixed terms and the variance caused by the processing batch (2 replicates), was regarded as a random term. The General Linear Model (GLM) with repeated measures was used to analyse the data obtained from the analyses applied at different processing stages (FRESH, REFRIGERATED, COOKED). In the mixed statistical model, the replicate (processing batch) was considered as a random effect, while the addition of fruit extract, the packaging system and the processing stage were regarded as fixed terms. Tukey's test was performed when ANOVA revealed significant ($p < 0.05$) differences between treatments. The Tukey's test was used for multiple comparisons of the means. To evaluate the effect of treatments on consumers' purchase intention, the binary data obtained (Yes/No) was analysed using a chi-square test. The significance level was set at $P < 0.05$. SPSS (v. 18.0) software was used to carry out the statistic test.

3. Results and discussion

3.1. Characterization of fruit extracts from CM and RC

The extracts obtained from the two fruits under study had remarkable concentrations of total phenolic compounds (1575–2350 mg/100 g fruit dry matter) and within the range values reported by Kähkönen et al. (1999) in numerous fruits and berries (1200–5000 mg/100 g fruit dry matter). Results from Fig. 1 shows that RC presented a higher content of total polyphenols than CM (2350 vs 1575 mg/100 g fruit dry matter; $p < 0.05$) with procyanidins being the most abundant in both fruits (> 90% of TPC in both fruits). The concentration of procyanidins in RC from the present study (2322 mg/100 g fruit dry matter) is higher than that recently reported by Özdemir, Pashazadeh, Zannou, and Koca (2022) in a phenolic-rich extract made from the same fruit (*Rosa canina* L.) (~ 800 mg/100 g extract dry matter), which emphasizes the effectiveness of our extraction procedure. The effectiveness of this extraction may be explained by the combination of extraction solvents (40% water, 60% food-grade acetone), which has been proven to be highly efficient in extractive bioactive compounds from various plant materials (Morcuende et al., 2020). CM was more abundant in hydroxybenzoic acids (HBA) (0.8 vs 0.4 mg/100 g fruit dry matter; $p < 0.05$), hydroxycinnamic acids (HCAs) (38.9 vs 19.1 mg/100 g fruit dry matter; $p < 0.05$), flavonols (FV) (36.2 vs 9.0 mg/100 g fruit dry matter; $p < 0.05$), anthocyanins (AC) (1.9 vs 0.7 mg/100 g fruit dry matter; $p < 0.05$), and tocopherols (1.4 vs 0.8 mg/100 g fruit dry matter; $p < 0.05$), while RC had a higher content of vitamin C (4.8 vs 0.1 mg/ g fruit dry matter; $p < 0.05$) (Fig. 1). These results are similar to those presented by Morcuende et al. (2020) in their study of fruit extracts in lamb cutlets. Given the profusely documented bioactivities of phenolics, tocopherols and ascorbic acid (Shahidi & Ambigaipalan, 2015), the fruits selected as sources of antioxidants in the present study seem to be appropriate candidates to be applied to meat products according to the criteria recently reported by Estévez (2021).

3.2. Proximate composition of beef patties

FRESH (moisture, 75.7%; protein, 17.9%; fat, 3.2%) and REFRIGERATED patties (moisture, 74.9%; protein, 18.3%; fat, 3.2%) from all groups and treatments had similar proximate compositions. As expected, COOKED patties (moisture, 68.3%; protein, 22.7%; fat, 5.0%) had a lower content of moisture and higher percentages of protein and lipid compared to FRESH counterparts. Once more, the proximate composition of beef patties was not affected by either the addition of berry extract or the packaging system used.

3.3. Impact of fruit extracts and packaging system on lipid oxidation

The impact of CM and RC extracts on the oxidative damage to lipids from beef patties packed in vacuum or HiOx-MAP at different processing stages (FRESH, REFRIGERATED and COOKED) was determined by TBARS (Fig. 2A) and hexanal (Fig. 2B). Regardless of the packing strategy, the onset of oxidative reactions during refrigerated storage of patties led to an increase in TBARS and hexanal confirming profuse

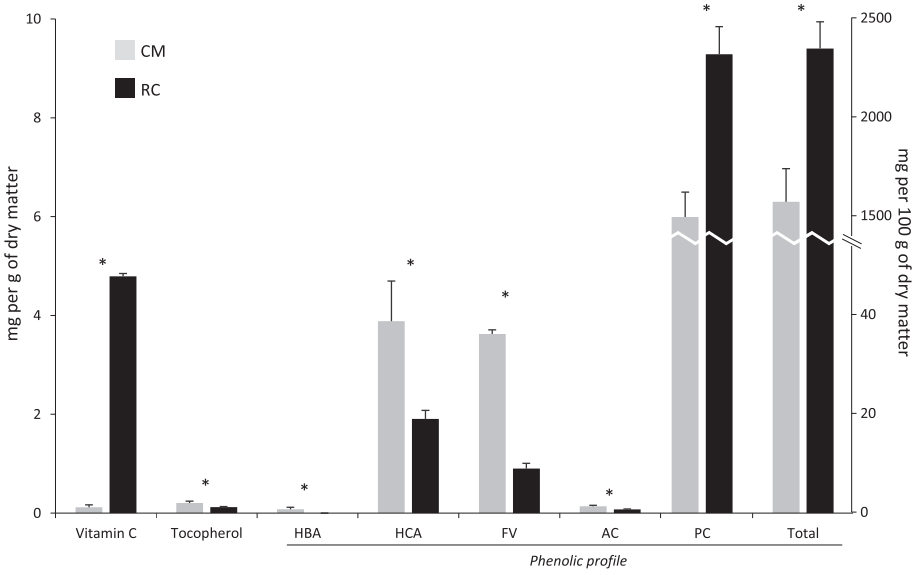


Fig. 1. Bioactive compound composition (means ± standard errors) of hawthorn (CM) and rose hip (RC). Vitamin C (left axis), tocopherol content and phenolic profile (right axis) of hawthorn (*Crataegus monogyna* Jacq.; CM) and rosehip (*Rosa canina* L.; RC) extracts. HBA: hydroxybenzoic acids; HCA: hydroxycinnamic acids; FV: flavonols; AC: anthocyanins; PC: procyanidins. Asterisks on top of 2 bars, indicate significant differences ($P < 0.05$) between the 2 fruits for a particular group of bioactive compounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

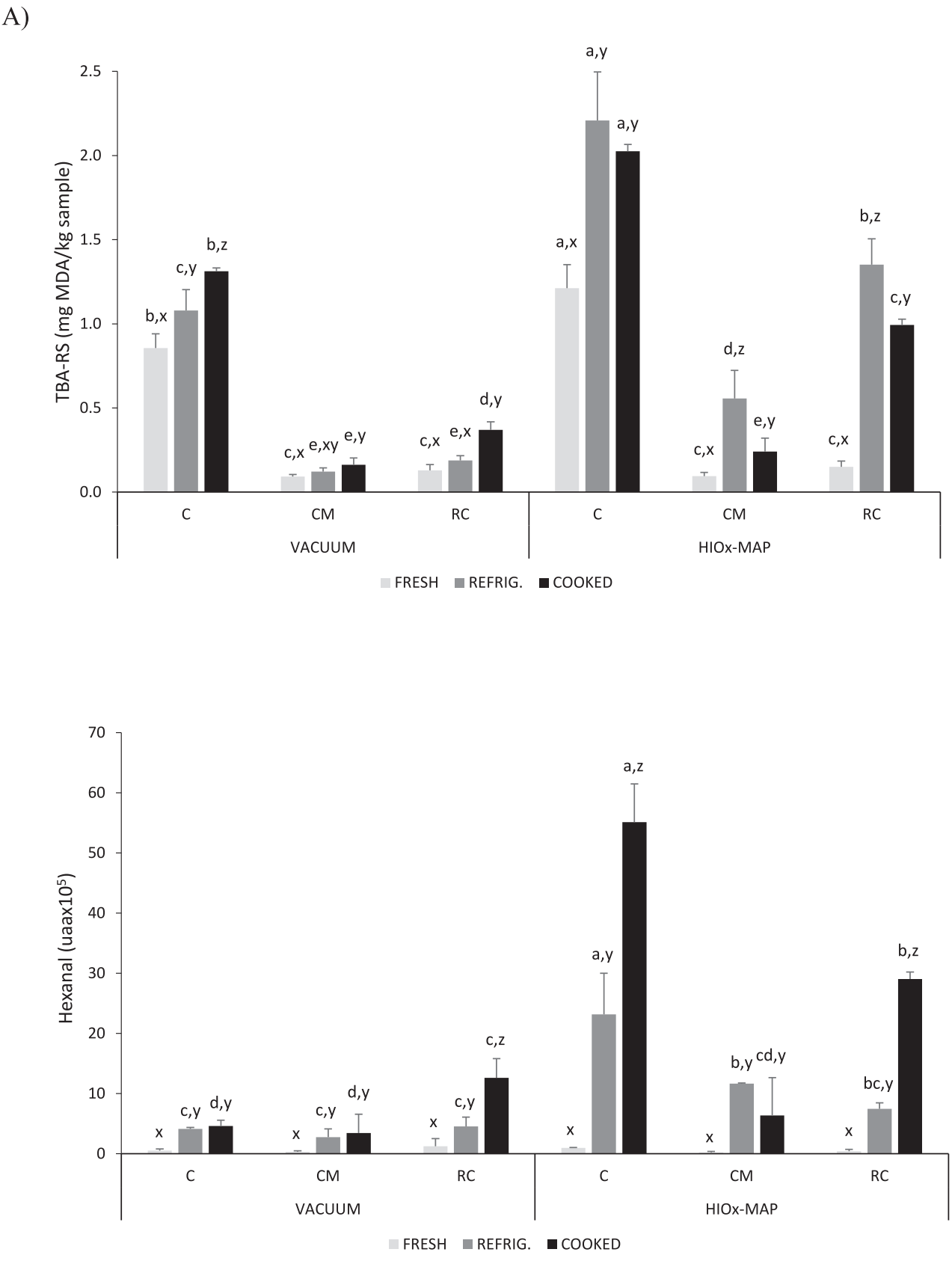


Fig. 2. Lipid oxidation: evolution of TBARS (A) and hexanal (B) (means ± standard errors) during simulated retail exposure (+2 °C/10 days) of beef patties treated with sea buckthorn (CM) and rose hip (RC) fruit extracts and vacuum-packed and HiOx-MAP-packed.
a-d Different letters at the top of the bars denote significant differences between treatments within a processing stage (fresh, refrigerated or cooked). x-z Different letters at the top of the bars denote significant differences between processing stages within a particular treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

existing evidence that refrigeration may slow oxidative reactions, yet they are not completely inhibited (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013; Domínguez et al., 2019). The onset of lipid oxidation begins in post-mortem meat due to loss of homeostasis and the action of pro-oxidant factors on unsaturated fatty acids (Bekhit et al., 2013). The processing of patties (mincing, chopping, oxygen exposure…) promotes oxidative reactions which is manifested as arise of lipid oxidation markers during storage (Domínguez et al., 2019). The onset of oxidative reactions during the subsequent cooking led to an additional increase of TBARS in Vacuum-C and of hexanal in HiOx-MAP-C which was expected, as it is known that heating accelerates chemical reactions, including lipid oxidation (Bekhit et al., 2013).

The extent of lipid oxidative damage during refrigerated storage was affected by the packing system. Although the use of HiOx-MAP packaging is commonly employed to ensure a bright red colour during retail display of red meat, it is known that lipid oxidation is promoted under these circumstances (Bao et al., 2016). This extent is also confirmed in the present study as the levels of both TBARS in refrigerated HiOx-MAP-C (2.2 mg/kg sample) were significantly higher ($p < 0.05$) than in Vacuum-C (1.1 mg/kg sample). Particularly remarkable were the differences in hexanal counts as those in HiOx-MAP-C were >4-fold higher than in Vacuum-C (23×10^5 area units vs. 4×10^5 area units; $p < 0.05$). The effects of packing were also reflected in samples upon cooking. This observation suggests that HiOx-MAP not only promotes lipid oxidation during storage, but it also makes burger patties more susceptible to oxidation during further processing such as cooking. The high concentration of molecular oxygen in HiOx-MAP is known to facilitate the occurrence of a pro-oxidative environment leading to increased lipid oxidation rates (Peng et al., 2019; Wang et al., 2019). Molecular oxygen is precursor of many radicals and ROS which would initiate oxidative reactions in meat systems (Estévez, 2011; Bekhit et al., 2013). The accumulation of lipid oxidation products has adverse effects from nutritional and/or sensorial points of view (Domínguez et al., 2019).

Compared to C samples, the formulation with fruit extracts caused a significant decrease in the TBARS index in the two packing systems (Vacuum and HiOx-MAP) and in the two processing stages (REFRIGERATION and COOKING). The protective effect of fruit extracts against the formation of hexanal was significant only in patties submitted to the HiOx-MAP, which were, in fact, the samples in which lipid oxidation occurred in a more severe manner. The antioxidant effects exerted by the fruit extracts can be attributed to the occurrence of several antioxidant phytochemicals already reported to be found in the extracts from the present study. Phenolics, ascorbic acid and tocopherols are known to display antioxidant properties in lipid systems, including meat and meat products (Estévez, 2021). Between fruits, CM seemed to have, overall, a more intense antioxidant protection on meat lipids than RC. Similar results have been reported in previous studies aimed to test the effectiveness of these fruits in other meat products including lamb chops (Morcuende et al., 2020), and ready-to-eat beef (Utrera, Morcuende, Ganhão, & Estévez, 2015) and pork meat (Papuc, Predescu, Tudoreanu, Nicorescu, & Gâjâilă, 2018). The present study confirms the efficacy of both fruits and, particularly of CM, in protecting beef lipids against oxidation. Unlike some previous studies, the detailed analysis of bioactive compounds in both fruits enables a more in-depth discussion of the potential underlying antioxidant mechanisms. Given that CM showed, in general, a more intense lipid antioxidant activity than RC and that the former had a significantly higher concentration of tocopherols, it is reasonable to ascribe to these phytochemicals the higher efficient protection of CM. This is consistent with the well-known polarity and hence, expected location of tocopherols in an emulsified complex meat system consisting of a water phase with lipid droplets. Unlike ascorbic acid and most phenolic compounds, tocopherol is a non-polar compound with a high affinity for muscle lipids, which partly explains its efficacy against the oxidation of this meat component (Estévez, 2021; Feng et al., 2020).

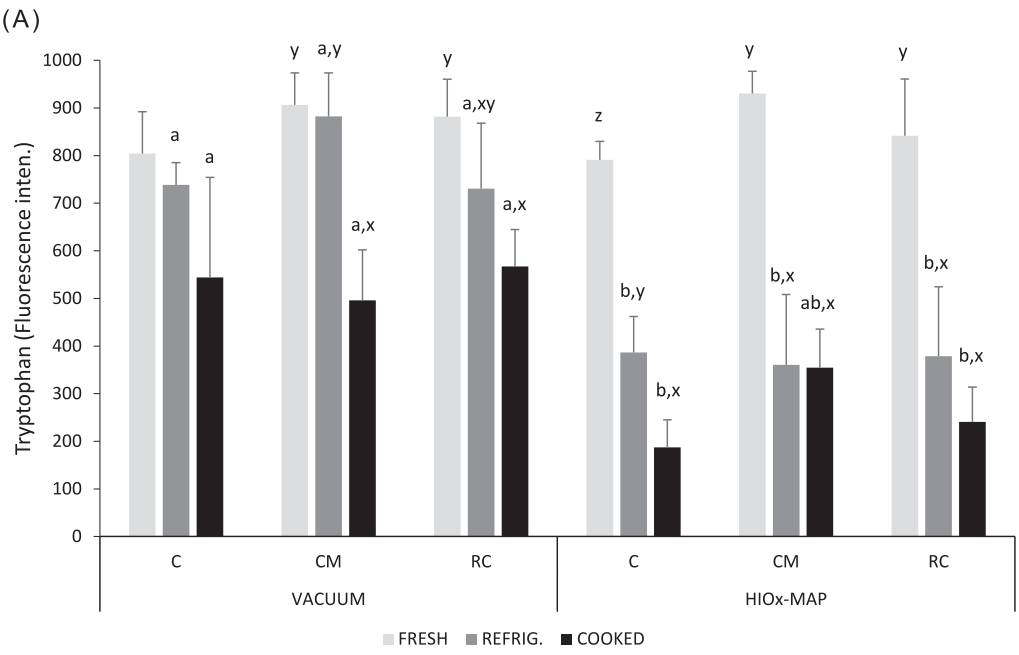
3.4. Impact of fruit extracts and packaging system on protein oxidation

Oxidative damage to proteins in beef patties was determined by oxidative depletion of tryptophan (Fig. 3A) and quantification of specific protein carbonyls, namely AAS and GGS (Fig. 3B).

Trp is, like sulfur-containing amino acids, sensitive to the pro-oxidant action of reactive oxygen species (ROS) and readily undergoes oxidative degradation (Ganhão et al., 2010; Utrera & Estevez, 2013). In fact, the loss of Trp in muscle foods has been recurrently used as indicator of the extent of protein oxidation (Hellwig, 2020). The depletion of Trp in C samples from the present study, was dependent on the packing system as the vacuum seemed to efficiently protect Trp against oxidation while HiOx-MAP promoted the loss of Trp during refrigerated storage. The description of the behavior of Trp in beef subjected to different packing systems is unprecedented in literature. It was known, however, that Trp is depleted in meat and meat products subjected to processing such as chilled storage (Wang, He, Zhang, Chen, & Li, 2021), cooking (Xia et al., 2021), and ripening (Li et al., 2022). The results from the present study originally shows the benefit of oxygen removal in terms of protection against Trp depletion in beef patties. Conversely, using HiOx-MAP promoted Trp oxidation, which would account for the loss of nutritional value of ground beef submitted to this packaging system. It is worth highlighting that the residual Trp content in HiOx-MAP-C patties upon refrigeration and cooking was approximately 25% of the initial Trp in fresh HiOx-MAP-C. The oxidation of Trp has been emphasized as a relevant negative consequence of the occurrence of protein oxidation in processed muscle foods (Ferreira, Morcuende, Madruga, Silva, & Estévez, 2018). Tryptophan (Trp) is considered an important essential amino acid given its multiple biological functions beyond its implication as building block in the de novo synthesis of proteins (Hellwig, 2020).

Neither CM nor RC extracts had a statistically significant effect on Trp depletion (Fig. 3A). The addition of the bioactive compounds from both tested fruits were unable to protect Trp against oxidative reactions as the levels of this essential amino acid were similar in patties from experimental groups within the same stage of processing. The information of the protective effect of phytochemicals against Trp oxidation in muscle foods in scarce. While phenolics from rosemary (Lara, Gutierrez, Timón, & Andrés, 2011) and white grape (Jongberg, Skov, Tørngren, Skibsted, & Lund, 2011) were found to protect Trp against oxidation in meat patties subjected to HiOx-MAP, other mechanistic studies revealed the complex chemistry behind the molecular interaction between Trp residues in proteins and phytochemicals (Salminen, Jaakkola, & Heinonen, 2008; Utrera & Estevez, 2013). According to these latter studies and recent review articles (Keppler, Schwarz, & van der Goot, 2020; Lund, 2021), certain phytochemicals display both antioxidant and pro-oxidant effects on Trp oxidation. This unpredictable outcome depends on various factors such as the protein conformation, the dose of the phenolics and the concentration of other redox species such as iron. Some of these differences could explain the different outcome from this study and that reported by Utrera et al. (2015) in which an RC extract was found to inhibit Trp oxidation in beef patties subjected to frozen storage and subsequent cooking. It seems obvious that additional studies should be specifically devoted to investigate further into the antioxidant protection of Trp in processed meat products.

In addition to Trp oxidation, protein oxidation was also assessed by the formation of AAS and GGS, which both account for around 70% of total protein carbonyls in meat systems (Estévez, 2011). Unlike lipid oxidation markers, protein carbonyls did not increase during the refrigerated storage of beef patties. In fact, a significant reduction was observed in refrigerated Vacuum-C patties as compared to the fresh counterparts. The variation in protein carbonyl concentration has been profusely described in meat systems and responds to their high reactivity, which is manifested in their oxidation into carboxylic acids and/or their involvement in the formation of protein cross-links (Estévez, 2011). However, the application of high temperatures during cooking



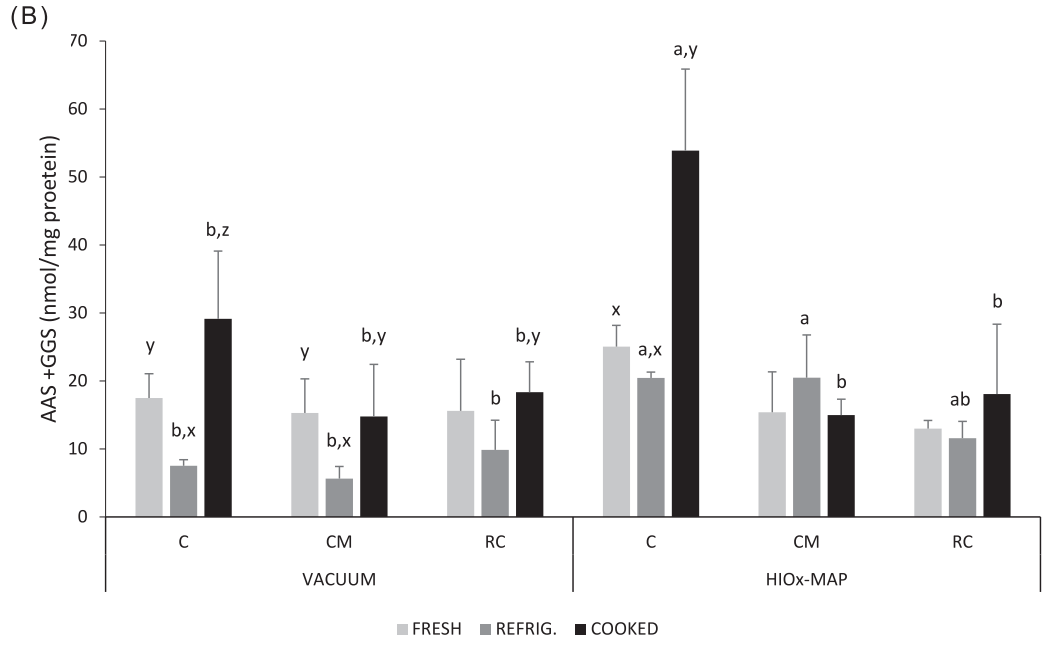


Fig. 3. Protein oxidation: evolution of trytophan (A) and protein carbonyls (B) (means ± standard errors) during simulated retail display (+2 °C/10 days) of beef patties treated with fruit extracts from common hawthorn (CM) and rose hips (RC) and packed in vacuum and HiOx-MAP.
a–d Different letters on top of bars denote significant differences between treatments within a processing stage (Fresh, refrigerated or cooked).
x–z Different letters on top of bars denote significant differences between processing stages within a particular treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

led to a significant accretion of protein carbonyls in beef patties, which is in agreement with many previous studies of similar nature (Morcuende et al., 2020; Shen et al., 2022; Utrera et al., 2015). Shen et al. (2022) recently found a connection between the severity of the cooking procedure, in terms of internal temperature, and the extent of protein carbonylation. The application of the HiOx-MAP promoted the formation of protein carbonyls as compared to the vacuum packaging. Similar results were reported, among others, by Morcuende et al. (2020) and Shen et al. (2022), confirming that enriching the packaging atmosphere with molecular oxygen has pro-oxidant effects on meat proteins. Protein carbonylation is regarded as a reaction of negative consequences as it is directly connected to loss of essential amino acids, impaired protein digestibility and toxicological effects (Estévez & Xiong, 2019).

CM and RC extracts significantly reduced the formation of AAS and GGS in beef patties during refrigeration and subsequent cooking in beef patties subjected to the HiOx-MAP (Fig. 3B). In this case, no differences were found between fruits as both seemed to have a similar antioxidant protection on meat proteins. The efficacy of CM and RC as inhibitors of protein carbonylation was previously reported by in pork (Papuc et al., 2018) as well as in frankfurters (Vossen et al., 2012) and cooked pork ham (Armenteros et al., 2016). While the antioxidant action on lipids requires a compatible chemical affinity with non-polar food components, the antioxidant action on proteins requires the molecular interaction with meat proteins located in the water phase of a given food system (Estévez, 2021). Therefore, the protection of meat proteins could be reasonably ascribed to small molecular weight polar phenolic compounds. The precise molecular mechanisms by which these phytochemicals could protect meat proteins from carbonylation have been reported in previous studies (Lund, 2021; Utrera & Estevez, 2013). These mechanisms involve radical scavenging activities and metal

chelation, among others (Estévez, 2021; Lund, 2021; Utrera & Estevez, 2013). In this study, we originally report the ability of these natural extracts to counteract the pro-oxidant action of HiOx-MAP on beef patties, which may have positive consequences on particular quality traits as explained below.

3.5. Impact of fruit extracts and packaging system on instrumental colour

The evolution of redness (a^*) during refrigerated storage and subsequent processing of red meat is of commercial importance due to the impact of this quality parameter on consumer preferences. In the present work, redness of fresh beef patties was higher in HiOx-MAP-C than in Vacuum-C (Fig. 4). During refrigeration, redness decreased as a likely consequence of oxymyoglobin depletion and unexpectedly, beef patties subjected to HiOx-MAP underwent more intense discoloration than those vacuum-packed. Although preservation of redness is indeed the presumed effect of a HiOx-MAP on red meat subjected to retail display, the conflicting outcome from the present study is not exceptional in literature. In fact, Muhlisin et al. (2010) and more recently Jaspal et al. (2022) reported similar results when studying the effect of packaging methods on the oxidative stability and colour displayed by beef patties subjected to chilled storage. In accordance with current results, those authors found that red meat patties packed in HiOx-MAP suffered a more severe discoloration than the control (VACUUM) counterparts. This result may be explained by the double role that oxygen may play in the conditions of the present experiment. In the one hand, molecular oxygen enables the formation of oxymyoglobin by combining with heme iron (~myoglobin oxygenation) (Jayawardana, Liyanage, Lalantha, Iddamalgoda, & Weththasinghe, 2015). On the other hand, oxygen is precursor of reactive oxygen species (ROS) which are known to be responsible for the oxidation of heme iron (Fe^{2+} to Fe^{3+}) leading to the formation of metmyoglobin, which is, in turn, responsible for the discolouration of the red meat (Li et al., 2022). For reasons that remain indefinite so far, under certain conditions, such as those of the present experiment, the pro-oxidative role of oxygen may be particularly relevant in HiOx-MAP, which leads to an unexpected, accelerated discoloration of red meat.

The use of CM and RC extracts significantly increased the redness of fresh beef patties. However, the redness of these groups of patties decreased over refrigerated storage and subsequent cooking to such extent that they eventually reached similar values than Vacuum-C patties. The effect of fruit extracts on fresh patties subjected to HiOx-MAP was similar as treated samples displayed a redder colour than CONTROL samples. After refrigerated storage and cooking treated samples remained with higher a^* values than those measured on CONTROL samples. It is, hence, reasonable to propose that the use of antioxidant-rich extracts from CM and RC diminished the discoloration of beef patties in the pro-oxidative environment created in HiOx-MAP. While it is highly speculative, we may attribute this antioxidant protection to water-soluble phytochemicals such as small phenolic compounds as Mb would be located in the water phase of the patties and tocopherols would mainly be located in the interphase. The lack of differences between fruit extracts on this regard would indicate that in fact, tocopherols an ascorbic acid, the most discriminating bioactive compounds, would have had little impact on Mb protection against oxidation.

3.6. Impact of fruit extracts and packaging system on instrumental hardness

In order to have a further insight into the impact of treatments on quality traits of burger patties, cooked samples from all experimental groups were subjected to assessment of instrumental hardness. According to the results presented in Fig. 5, HiOx-MAP-C patties presented a greater instrumental hardness than Vacuum-C counterparts. It has been reported that protein oxidation is directly responsible for the deterioration of the quality of meat products, and among these quality traits, texture is one of the most remarkable (Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015). The connection between the occurrence of protein oxidation in red meat subjected to HiOx-MAP and the increase in toughness was already described by Lund, Lametsch, Hviid, Jensen, and Skibsted (2007) and attributed to the formation of protein cross-links. This is, in fact, consistent with previous findings in which protein carbonyls was found to be related to the strengthening of meat and processed meat products measured by both, instrumental and sensory means (Armenteros et al., 2016; Ferreira et al., 2018; Shen et al., 2022). The underlying mechanism of such strengthening could be related to the implication of protein carbonyls, such as AAS, in the formation of protein aggregates and crosslinks (Estévez et al., 2019; Luna & Estévez, 2019) which is consistent with the reports made by Lund et al. (2007).

None of the two fruits under study was able to reduce significantly the hardness of the C samples. Fig. 5 shows how the addition of both CM and RC tended to counterbalance the increased hardness measured in HiOx-MAP-C, yet, this effect did not reach a significant extent. Therefore, the protection of the fruit extracts against protein oxidation may

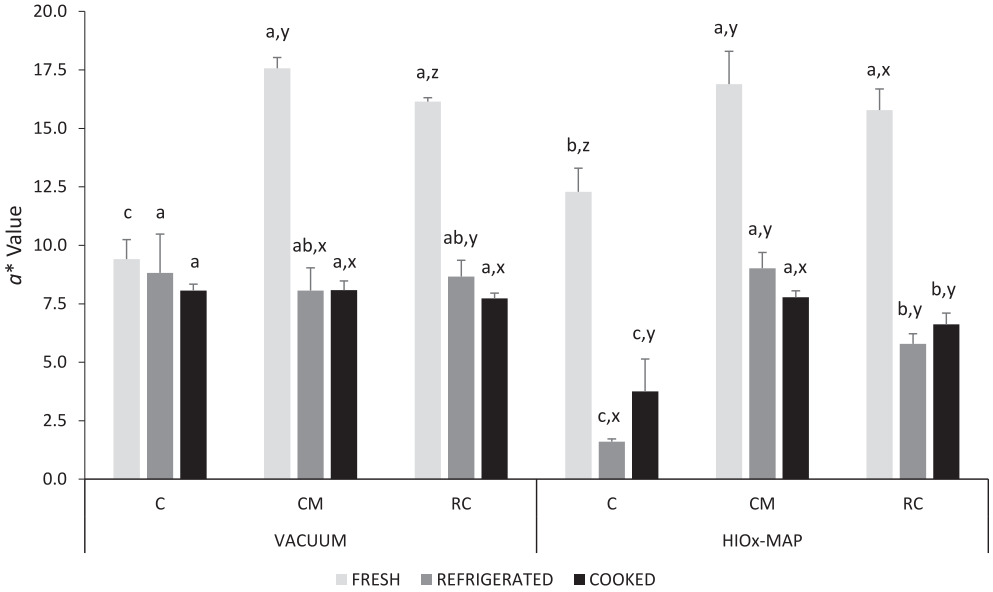


Fig. 4. Evolution of redness (means ± standard errors) during simulated retail display (+2 °C/10 days) of beef patties treated with fruit extracts from common hawthorn (CM) and rose hips (RC) and packed in vacuum and HiOx-MAP.
Different letters at the same sampling time denote significant differences between treatments.
a–d Different letters on top of bars denote significant differences between treatments within a processing stage (Fresh, refrigerated or cooked).
x–z Different letters on top of bars denote significant differences between processing stages within a particular treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

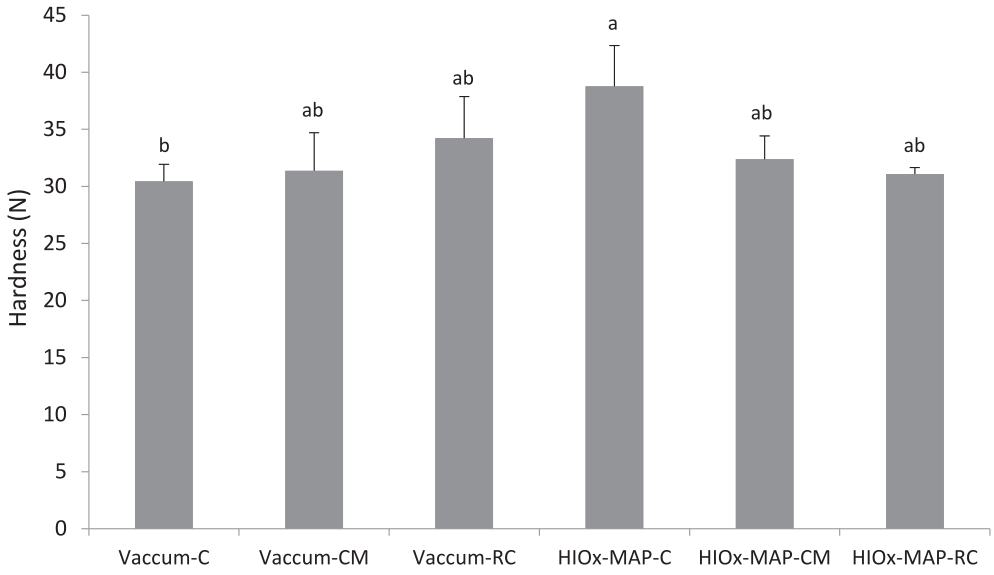


Fig. 5. Hardness (means ± standard errors) in grilled beef patties treated with fruit extracts from common hawthorn (CM) and rose hips (RC) after simulated retail display (+2 °C/10 days) packed in vacuum and HiOx-MAP. a–b Different letters on top of bars denote significant differences between treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

not have a direct reflection in significant texture benefits. Other authors such as Armenteros et al. (2016) or Morcuende et al. (2020) attributed the strengthening of oxidized meat products to the formation of protein carbonyl-mediated cross-links and reported how the addition of plant antioxidants (including RC) reduced both, the formation of such structures and the instrumental hardness of the products. In particular, the article from Morcuende et al. (2020) revealed that improving the texture of meat products by inhibiting the formation of oxidized protein aggregates in meat subjected to HiOx-MAP is feasible. We hypothesized whether an optimized concentration of RC and/or CM would have led to significant effects also in the present study.

3.7. Impact of fruit extracts and packaging system on consumers' purchase intention

To evaluate the effects of packaging systems and the addition of fruit extracts on consumer willingness to purchase the target beef patties, an assessment of purchase intent was conducted on refrigerated beef patties (day 10). During evaluation, samples were displayed on trays and assessors were asked to express their willingness to purchase the product based on its appearance. The results showed (Fig. 6) that Vacuum-C patties had 100% purchase intention versus HiOx-MAP-C, which had 5%. The deterioration of the appearance of HiOx-MAP-packed beef patties (discussed above) may explain these results, as vacuum samples (protected against aerobic bacteria and pro-oxidant oxygen action) were not affected by the discoloration factors aforementioned. The addition of fruit extracts led to a highly remarkable and statistically significant increase in the willingness to purchase HiOx-MAP beef patties as HiOx-MAP-CM and HiOx-MAP-RC patties received 98% and 80% purchase intention, respectively while HiOx-MAP-C had only a 5%. Therefore, it is considered that such improvement is due to the antioxidant effects of these extracts corroborating with what was reported by Kähkönen et al.

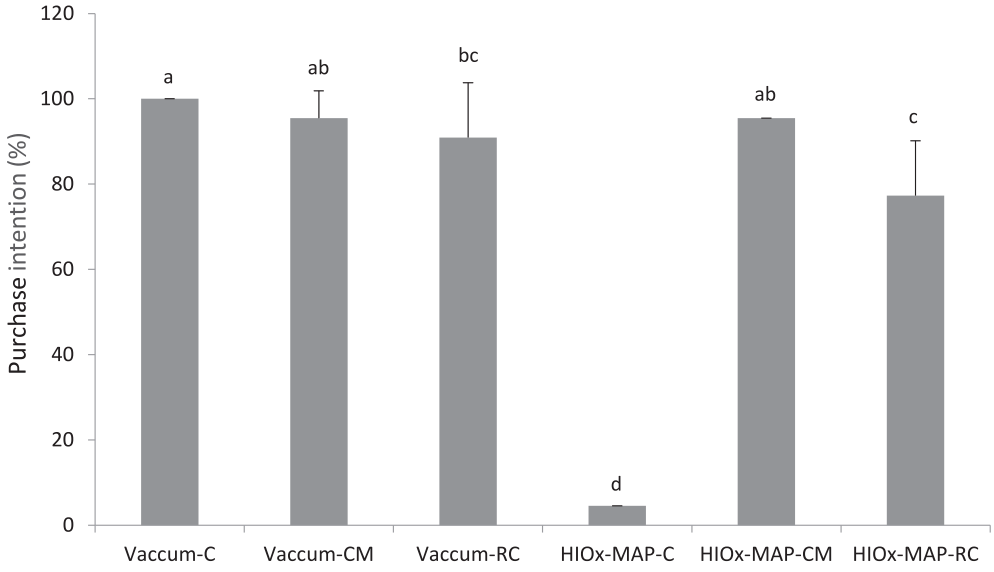


Fig. 6. Purchase intention (means ± standard errors) of beef patties treated with fruit extracts from common hawthorn (CM) and rose hips (RC) and packed in vacuum and HiOx-MAP, after a simulated retail display (+2 °C/10 days). a–b Different letters on top of bars denote significant differences between treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(1999) and Kalogianni, Lazou, Bossis, and Gelasakis (2020).

4. Conclusions

HiOx-MAP packaging systems are well-established packaging strategies for the marketing and sale of red meat, but the effects on proteins and lipids are notorious. The application of fruit extracts such as CM and to a lesser extent RC seems a feasible and efficient solution to reduce the pro-oxidative effects caused by HiOx-MAP. The protective effect of CM and RC on meat proteins and lipids is also reflected in decreased toughness and increased consumer purchasing choices. The application of these alternative preservatives may be a strategy of commercial interest owing to the growing concern of consumers towards additives and the preference for "clean-label" products.

CRediT authorship contribution statement

Christian Vallejo-Torres: Formal analysis, Writing – original draft. **Mario Estévez:** Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing. **Sonia Ventanas:** Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing. **Sandra L. Martínez:** Formal analysis, Writing – review & editing. **David Morcuende:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have 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.

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