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Influence of high-pressure processing and varying concentrations of curing salts on the color, heme pigments and oxidation of lipids and proteins of Iberian dry-cured loins during refrigerated storage

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ABSTRACT

The effect of high hydrostatic pressure (HHP) on reduced ingoing amounts of both nitrate and nitrite (150, 75, 37.5, and 0 mg/kg of each curing salt) dry-cured loins during the refrigerated storage was studied. Changes in instrumental color, nitrosylmyoglobin (NOMb) and Zinc protoporphyrin (ZnPP) contents, and lipid and protein oxidation were analyzed. The reduction of ingoing amounts of nitrate/nitrite in loins decreased the redness (CIE a*) and NOMb content in the dry-cured products while increased the ZnPP content and the lipid oxidation measured as TBA-RS. In contrast, the diminution of ingoing curing agents up to 37.5 mg/kg NO_3^-/NO_2^- did not significantly affect color, lipid, and protein oxidation over refrigerated storage. In comparison to control samples, HHP increased lightness (CIE L*) and the extent of lipid oxidation, meanwhile reduced protein carbonyl and thiol contents over storage. Neither NOMb nor ZnPP contents were affected by HHP treatment over storage time. Therefore, the reduction of ingoing amounts up to 37.5 ppm NOx together with a pressurization treatment at 600 MPa 7 min controlled lipid and protein oxidation.

1. Introduction

Iberian dry-cured meat products are highly appreciated due to their exclusive sensory characteristics. These are related to a characteristic cured red color, high juiciness, and intense flavor consequence of the genetic background of the Iberian pigs, the rearing system, and the long ripening period. The use of curing salts is common in the manufacture of this type of dry-cured product with a long drying process. Nitrite participates in the development of the sensory characteristics, giving them their characteristic color, modulate the oxidative processes that generate aromatic compounds and contribute to food safety (Cava, Ladero, González, Carrasco, & Ramírez, 2009; Higuero, Moreno, Lavado, Vidal-Aragón, & Cava, 2020; Stadnik & Stasiak, 2016).

In cured meat products, nitrite reacts with myoglobin resulting in nitrosylmyoglobin (NOFe(II)Mb), providing a typical cured red color. Whereas, Zn-protoporphyrin, a red heme pigment, is present in uncured dry meat products (Huang et al., 2020; Wakamatsu, Ito, Nishimura, & Hattori, 2007). Additionally, nitrite imparts antioxidant activity by the formation of NOFe(II)Mb that stabilizes the Fe^{2+} in the myoglobin molecule, which inhibits the release of Fe^{2+} and consequently less Fe^{2+} is available to catalyze the Fenton reaction resulting in the control of lipid oxidation (Chen, Pearson, Gray, Fooladi, & Ku, 1984). Other antioxidant effects are associated with the chelation of metal ions, elimination of free radicals, and formation of nitroso- and nitrosyl-compounds (Sebranek, 2009). Compared to lipid oxidation, the impact on protein oxidation is more complex and nitrite can act as both antioxidant and pro-oxidant on proteins (Vossen & De Smet, 2015).

Despite the beneficial role of nitrite on the sensory and safety of meat products, there is a growing concern about the safety of nitrite as precursors of nitrosamines. These nitrosamines could be formed in meat and meat products during and after curing leading to potential carcinogenic products (Alahakoon, Jayasena, Ramachandra, & Jo, 2015). This concern is reflected in Regulation (EC) No 1333/2008 amended by Regulation (EU) No 2015/647 on food additives (European Commission, 2008, 2015). This trend of European authorities of bringing down the levels of nitrate and nitrite additives together with public concerns

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about their safety and the increasing demand for healthier products triggered both the academic and industrial research towards the manufacture of nitrite-free meat products (Alahakoon et al., 2015).

The utilization of high hydrostatic pressure processing (HHP) as a non-thermal technology has been widely used for shelf-life extension and safety assurance in ready-to-eat meat products, especially relevant in those with heat-sensitive attributes and characteristics. The ability of HHP to destroy spoilage and pathogenic microorganisms, such as *L. monocytogenes,* in meat products is well documented (Cava, García--Parra, & Ladero, 2020; Cava, Higuero, & Ladero, 2021) and the effectiveness is related to the intensity (pressure and holding time) of the treatment. Furthermore, this technology satisfies consumer interest for the development of products minimally processed with clean labels (without/with fewer chemicals preservative) with comparable nutritional value and an extended shelf-life that assure microbial safety (Bajovic, Bolumar, & Heinz, 2012; Bolumar et al., 2021; Giménez, Graiver, Califano, & Zaritzky, 2015).

However, pressurization could cause changes in the color and induce oxidative changes of lipids and proteins immediately after treatment and/or storage that could lead to modifications of quality parameters (Guyon, Meynier, & de Lamballerie, 2016). High pressure-induced changes are dependent on the intensity of the process as well as the characteristics of the meat product (Bak, Bolumar, Karlsson, Lindahl, & Orlien, 2019; Cava et al., 2020, 2021). In general, cured meat color is particularly resistant to pressurization, though some color changes in lightness had been reported. Literature provides conflicting results on the impact of HHP on lipid and protein oxidation in meat and meat products. However, several studies reported a negative influence of HHP on lipid and protein oxidation in dry-cured products (Bajovic et al., 2012; Bak et al., 2019; Guyon et al., 2016).

The present study aims to evaluate the effect of a high-pressure treatment at 600 MPa/7 min on the instrumental color, the content of nitrosylmyoglobin and Zn–protoporphyrin, and lipid and protein oxidation of Iberian dry-cured loins formulated with reduced ingoing amounts of nitrate and nitrite during 240 days of refrigerated storage.

2. Materials and methods

2.1. Iberian dry-cured loin samples

Iberian dry-cured loins from Iberian x Duroc pigs free-range reared and fed on concentrate were manufactured under commercial conditions. Four different formulations of dry-cured loins were produced with decreasing ingoing amounts of both sodium nitrite and potassium nitrate: 1) 150 mg/kg $NaNO_2$ + 150 mg/kg KNO_3 (150NOx), 2) 75 mg/kg $NaNO_2$ + 75 mg/kg KNO_3 (75NOx) −50% reduction-, 3) 37.5 mg/kg $NaNO_2$ + 37.5 mg/kg KNO_3 (37.5NOx) −75% reduction- and 4) 0 mg/kg $NaNO_2$/KNO_3 (0NOx) −100% reduction-. A detailed description of the process of production is described in Higuero et al. (2020).

Each dry-cured loin (n = 5/batch) from the experimental batches was portioned into 5 cm thick slices and randomly divided into two experimental groups: 1. Non-pressurized (Control) and 2. High Hydrostatic Pressure (HHP) treated. Samples were vacuum packed in polyamide/polyethylene bags (70 μm, OTR 50.7 cm^3/m^2/day 0.1 MPa at 23 ± 2 °C and 75% RH) and stored at 4 °C for 24 h until the pressure treatment.

The HHP samples were pressurized at 600 MPa for 7 min at 18 °C in a semi-industrial hydrostatic pressure unit Wave 6000/55 (Hiperbaric, Burgos, Spain). The initial water temperature inside the vessel and samples were 10 °C and 4 °C, respectively. Operating pressure (600 MPa) was reached in 230 s and decompression of the vessel was instantaneous (<3s). The water in the vessel increased at 18 °C due to the adiabatic compression heating. Immediately after treatment, the samples were stored at 4 °C in the dark and sampled at days 0, 30, 60, 120, and 240 of storage. Five packages per treatment and day of storage were analyzed.

2.2. Instrumental color

CIE L*a*b* instrumental color coordinates measurements were performed immediately after opening the package following AMSA guidelines (AMSA, 2012). A Minolta CM-600d spectrophotometer (Minolta Camera, Osaka, Japan) with a 0.8 cm port/viewing area, a 10° viewing angle, and a D65 illuminant were used. Before readings, a white calibration plate CR-A43 and a zero-calibration tube CM-A182 were used. The measurement was repeated on nine randomly selected locations on the surface of each portion, and mean values were calculated.

2.3. Reflectance spectrum

Reflectance spectra were measured using a Minolta CM-600d spectrophotometer operating in the 360–740 nm spectral range following AMSA recommendations (AMSA, 2012). The cured index (CI) was obtained by the reflectance ratio λ650 nm/λ570 nm.

2.4. Determination of nitrosylmyoglobin (NOMb) content

The NOMb content determination was conducted using the spectrophotometric method described by Hornsey (1956) with slight modifications. Samples (1g) were homogenized (6.0 m/s; 60 s, 2 cycles) in 10 ml (acetone: water 80:20 v/v) in a 15 ml tube that contained a lysing matrix type A (MP Biomedicals Inc., Santa Ana, California, USA) using a Fast Prep-24TM 5G (MP Biomedicals Inc., Santa Ana, California, USA). The homogenate was stirred using a tube roller at 4 °C for 5 min in the dark and subsequently centrifuged (4700 g, 4 °C, 5 min). The absorbance of the supernatant was read at 540 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The NOMb concentration was calculated as NOMb (mg/kg acid hematin) = Abs_{540nm} x 290.

2.5. Determination of Zn-protoporphyrin (ZnPP) content

The ZnPP content was determined by fluorescence spectroscopy based on the method described by Bou, Llauger, Arnau, and Fulladosa (2018) with some modifications. Samples (2 g) were homogenized twice (4.0 m/s; 30 s) in a 15 ml tube with 10 ml of ethyl acetate/acetic acid solvent mixture (4:1, v/v) and a lysing matrix type A using a Fast Prep-24TM 5G. Both extracts and the sample residue were mixed using a tube roller in the dark (60 rpm, 4 °C, 20 min), subsequently centrifuged (1100 g, 4 °C, 14 min) and the supernatants filtered through a filter paper. The ZnPP was detected by fluorescence in a Varioskan LUX Multimode Microplate Reader (Thermo Fisher Scientific, Vanta, Finland) with excitation and emission wavelengths set at 415 and 590 nm, respectively. The ZnPP content was calculated from a standard curve of ZnPP (4.00–0.0079 mg/l), and the contents were expressed as mg ZnPP/kg. Readings were made in duplicate.

2.6. UV–vis absorption and fluorescence spectra analysis

At days 0 and 240, the UV–vis absorption spectra of the extracts (obtained as described in section 2.4) were measured from 350 to 650 nm with 1 nm intervals using a Shimadzu UV-1800 spectrophotometer. The fluorescence spectra of the extracts (obtained as described in section 2.5) were recorded from 470 to 700 nm with 1 nm intervals at 415 nm for excitation using a microplate reader.

2.7. Lipid oxidation

The extent of lipid oxidation was estimated as thiobarbituric acid reactive substances (TBA-RS) according to the method of Salih, Smith, Price, and Dawson (1987). Absorbance readings were measured at 532 nm with a Multiskan Go spectrophotometer microplate reader. The TBA-RS values were calculated from a standard curve of 1,1,3,3-tetramethoxypropane (TEP) (0.16–0.001 mM) and expressed as mg

malondialdehyde (MDA)/kg. Measurements were assessed in duplicate.

2.8. Total protein carbonyls

Protein oxidation was estimated by measuring the total protein carbonyl groups as described in Oliver, Ahn, Moermans, Goldstein, and Stadtman (1987). Protein carbonyl groups were quantified by treatment with 2,4-dinitrophenylhydrazine (DNPH) to form protein hydrazones. Absorbances were measured using a Multiskan Go spectrophotometer microplate reader. Carbonyl concentration was determined using an absorption coefficient of 21.0 mM^{-1} cm^{-1} at 370 nm. Protein concentration was measured spectrophotometrically at 280 nm using a standard curve of bovine serum albumin (BSA) (2.00–0.125 mg/ml). Results were expressed as nmol carbonyls/mg protein. Assays were done in duplicate.

2.9. Protein thiol analysis

The thiol groups concentration was determined after derivatization with Ellman's reagent, 5-5′-Dithiobis(2-nitrobenzoic acid) (DTNB) (Ellman, 1959). The thiol and protein concentrations were estimated according to the method described by Martínez, Jongberg, Ros, Skibsted, and Nieto (2020). The thiol concentration was quantified using a standard curve of L-cysteine in 5% SDS/TRIS buffer (pH 8.0, 0.10 M) (1000–32.25 μM). The protein concentration was determined using the Thermo Scientific Pierce Rapid Gold BCA Protein Assay Kit, measured spectrophotometrically at 480 nm, and quantified using a standard curve of BSA in 5% SDS/TRIS buffer (2.5–0.3125 g/l). Results were expressed as nmol thiol/mg protein. Duplicate assays were performed.

2.10. Statistical analysis

The effect of curing agent reduction and the high-pressure treatment in Iberian dry-cured loins and their interaction on the parameters studied at each sampling day were analyzed by the Two-way Analysis of Variance (ANOVA) procedure of SPSS, version 22.0 (IBM. IBM Corp. Released, 2013). A one-way ANOVA was used to determine differences in studied parameters throughout storage time. Tukey's test was applied to compare differences among mean values of the treatments. Means and standard errors of the means (SEM) values were reported. Partial least squares (PLS) modeling was used to relate the effect of curing salts and HHP with each of the variables under study. PLS modeling was performed using the statistical add-on XLStat (Addinsoft, 2014) for Microsoft Excel.

3. Results and discussion

3.1. Instrumental color parameters

The ingoing amounts of NOx and HHP treatment differently affected the instrumental color parameters throughout 240 days of refrigerated storage (Table 1).

While the CIE L*-values were not affected by the ingoing amounts of nitrate and nitrite introduced, HHP caused a significant increase in lightness from day 30 to the end of storage. Results are in line with findings previously reported in pressurized dry-cured loin (Campus, Flores, Martinez, & Toldrá, 2008), dry-cured ham (de Alba, Montiel, Bravo, Gaya, & Medina, 2012), and dry-cured Iberian loin (Cava et al., 2021), but contrast with those described by Amaro-Blanco, Delgado-Adámez, Martín, and Ramírez (2018) in Iberian dry-cured shoulder. The CIE L* increases are associated with HHP-induced denaturalization of the myofibrillar component, which leads to the formation of larger insoluble aggregates resulting in an increased light reflectance (Bak et al., 2019).

Discrepancies in the effect of HHP could be explained by the interaction between the water content and the pressurization conditions (intensity/holding time). Thus, higher water contents result in increased lightness while no color changes are found at low water contents after

Table 1
Effect of nitrate and nitrite ingoing amounts (0, 37.5, 75 and 150 mg/kg) and HHP treatment (Control, HHP treated at 600 MPa/7 min) on instrumental color parameters (CIE L*a*b*) of Iberian dry-cured loins over 240 days of refrigerated storage.

	Day	Nitrate/Nitrite (mg/kg)				HHP			Sig.		
		0	37.5	75	150	Control	Treated	SEM	1	2	3
CIE L*	0	42.0	43.5	41.0	40.2	41.4 ^{x}	41.9 ^{z}	0.56	n.s.	n.s.	n.s.
	30	39.9	40.2	40.8	40.4	38.2 ^{x,y,z}	42.5 ^{y,z}	0.64	n.s.	**	n.s.
	60	40.2	42.0	40.9	41.3	37.6 ^{y,z}	44.6 ^{x,y}	0.76	n.s.	***	n.s.
	120	38.1	40.8	37.8	38.8	37.2 ^{z}	40.6 ^{z}	0.61	n.s.	**	n.s.
	240	42.4	44.6	43.4	41.7	41.0 ^{x,y}	45.1 ^{x}	0.63	n.s.	***	n.s.
	SEM	0.65	0.57	0.70	0.45	0.33	0.43				
	Sig.	n.s.	n.s.	n.s.	n.s.	**	***				
CIE a*	0	8.2 $^{b\ y}$	9.3 $^{a,b\ y}$	9.8 $^{a,\ y}$	9.6 $^{a\ y}$	9.0 ^{y}	9.4 ^{y}	0.19	*	n.s.	n.s.
	30	8.8 $^{b\ x,y}$	10.2 $^{a\ x,y}$	9.9 $^{a,b\ y}$	9.9 $^{a\ x,y}$	9.9 ^{x,y}	9.5 ^{y}	0.16	**	n.s.	n.s.
	60	9.1 $^{b\ x,y}$	10.2 $^{a\ x,y}$	10.1 $^{a,b\ x,y}$	9.8 $^{a,b\ x,y}$	10.2 ^{x}	9.4 ^{y}	0.15	*	**	n.s.
	120	9.5 $^{b\ x}$	10.4 $^{a,b\ x}$	11.4 $^{a\ x}$	11.2 $^{a\ x}$	10.5 ^{x}	10.8 ^{x}	0.23	*	n.s.	n.s.
	240	9.4 $^{b\ x}$	9.9 $^{a,b\ x,y}$	10.1 $^{a,b\ x,y}$	10.5 $^{a\ x,y}$	10.0 ^{x,y}	9.9 ^{x,y}	0.13	*	n.s.	n.s.
	SEM	0.13	0.13	0.18	0.18	0.11	0.13				
	Sig.	**	*	*	*	**	***				
CIE b*	0	6.5	5.4 ^{z}	5.8 ^{y}	5.0 ^{y}	4.0 ^{z}	7.3 ^{x,y}	0.39	n.s.	***	*
	30	9.2	8.0 ^{x,y}	8.4 ^{x}	8.0 ^{x}	8.2 ^{x}	8.6 ^{x}	0.21	n.s.	n.s.	n.s.
	60	9.4 ^{a}	8.7 $^{a,b\ x}$	8.2 $^{a,b\ x}$	7.5 $^{b\ x}$	8.7 ^{x}	8.3 ^{x}	0.23	*	n.s.	n.s.
	120	8.6	6.7 ^{y,z}	8.0 ^{x,y}	7.2 ^{x}	7.3 ^{x,y}	7.9 ^{x,y}	0.34	n.s.	n.s.	n.s.
	240	7.8 ^{a}	6.0 $^{b\ y,z}$	5.9 $^{b\ y}$	7.0 $^{a,b\ x}$	6.6 ^{y}	6.8 ^{y}	0.24	**	n.s.	n.s.
	SEM	0.35	0.28	0.28	0.25	0.17	0.24				
	Sig.	*	***	**	***	***	**				

n.s.: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$; ***: $P < 0.001$.
a, b: Means within the same row with different superscripts differ significantly (Tukey's test, $P < 0.05$).
x, y, z: Means within the same column with different superscript differ significantly (Tukey's test, $P < 0.05$).
[1] Significance. 1: Effect nitrate/nitrite. 2: Effect high-pressure treatment. 3: Interaction.

pressurization (Bak et al., 2019). Pressurization could probably also increase the sensitivity of the product to changes during storage, increasing paleness over time.

The nitrate/nitrite ingoing amounts did not affect the evolution of CIE L*, but the application or not of high hydrostatic pressure did. The CIE L*-values, in non-treated dry-cured loins, followed a decreasing trend during the first half of storage to significantly increase at day 240 while, in HHP-treated loins, there was a constant increase throughout storage, except for day 120 that decreased. Conflicting results on the evolution of CIE L*-values in different dry-cured meat products have been reported, which is likely due to the type of meat product studied, the HHP parameters, and the duration of storage (Cava et al., 2021; de Alba et al., 2012; Stadnik & Stasiak, 2016).

Contrastingly, CIE a* values and CIE b* values were significantly affected by the ingoing amounts of curing salts. Nitrate/nitrite removal resulted in significantly lower CIE a* values than in 75NOx loins (except at day 240) and 150NOx (except at day 60) during 240 days of storage. At days 0, 120, and 240, CIE a* showed no significant differences between the 37.5NOx and 0NOx samples. Regarding CIE b* values, uncured loins (0NOx) showed significantly higher values than 150NOx loins and 37.5NOx and 75NOx loins, at days 60 and 240, respectively. No differences were found within batches with different ingoing levels of curing salts in both parameters.

These findings agree with those described by Stadnik and Stasiak (2016) that reported a substantial reduction in CIE a* values in dry-cured loins in which curing salts (99.5% NaCl, 140 mg/kg sodium nitrite) were replaced by sea salt (99.74% NaCl). Changes in CIE a*-value could be associated with the formation of NOMb in cured loin samples and ZnPP in the uncured counterparts (Wakamatsu et al., 2007). Nonetheless, the pigmentation due to added paprika in this kind of meat products could interfere with the color parameters, affecting mainly a*-values (Aquilani et al., 2018). Moreover, Flores, Salafia, López-Diez, and Belloch (2019) reported that paprika could be also an external source of nitrate apart from curing agents in dry-cured loin processing, contributing to 17–20 ppm sodium nitrate. This fact could affect the residual nitrate concentration affecting CIE a*-value. Indeed, a minimum amount of nitrite in a meat product (2–14 ppm) is enough to develop a desirable cured color, while higher concentrations were necessary to avoid non-uniform curing and preserve the cured color throughout storage (Alahakoon et al., 2015). In addition to this effect, nitrite contributes to the inhibitory activity against *C. botulinum* in cured meat products, and 50–150 mg/kg nitrite is necessary to inhibit the growth of *C. botulinum* in prolonged shelf life meat products. Removal or reduction of nitrite could seriously compromise the food safety of this kind of product (EFSA, 2004). Recently, Hospital, Hierro, Stringer, and Fernández (2016) reported that decreasing or even removing nitrate/-nitrite (112.5 and 75 mg/kg of both nitrate/nitrite) from the formula of fermented dry-sausages did not compromise safety regarding *C. botulinum* in the conditions tested in their study. These authors stated that the antimicrobial role of nitrite should not be underestimated in the case that other hurdles could fail (i.e. pH, Aw, temperature) or another ripening or storage conditions were used, as well as the inhibitory effect of nitrite on other foodborne pathogens, such as *Listeria monocytogenes* and *Salmonella* spp should be considered.

On the contrary, HHP affected a lesser extent CIE a* and b*-values, being redness only significantly higher for control loins at day 60 and yellowness significantly lower for control loins just after treatment. The lack of effect of pressurization on redness over the storage is in agreement with previous studies in treated Iberian dry-cured loins at lower pressure levels (200–300 MPa) (Cava et al., 2009) meanwhile in opposition to our results, other authors observed a decrease in redness in pressurized dry-cured loins treated at 350–400 MPa and 600 MPa (Campus et al., 2008; Cava et al., 2021). In addition, these authors described that decreases in CIE a* and b* values could be associated with high water contents in the samples. During storage, slight reductions in CIE a* were found until day 120, followed by a slight increase at the end of storage. These results differ from the stable values observed in control dry-cured loins over 90 days of storage (Stadnik & Stasiak, 2016) and the decreasing trend observed in non-treated dry-cured ham during 60 days of storage (de Alba et al., 2012).

3.2. NOMb and ZnPP contents and cured index

The nitrosylmyoglobin contents were significantly affected by the amount of incoming nitrate and nitrite (Table 2). At days 0 and 30, NOMb contents were significantly lower in 0NOx samples than in the 75NOx and 150NOx groups and the 150NOx samples at day 60. On days 120 and 240, cured samples had higher contents, but not to a significant extent, of NOMb than the uncured counterparts. In contrast, Zn-protoporphyrin was found only in the uncured samples. In the uncured (0NOx) and non-pressurized samples, the NOMb content increased significantly during storage while the ZnPP content remained unchanged.

These findings are in accordance with other studies in uncured Parma and Iberian hams (Adamsen, Møller, Laursen, Olsen, & Skibsted, 2006; Møller, Adamsen, Catharino, Skibsted, & Eberlin, 2007; Wakamatsu et al., 2007). Hence, these results support ZnPP, instead of NOMb, as the main heme pigment in uncured Iberian dry meat products. Previous studies have attributed an important role of red dried paprika, used in the formulation of dry-loins in the present study, as an external source of nitrate on the formation of NOMb in dry-cured meat products, and also it could interfere in the measurement of color (Flores et al., 2019). This fact could explain the formation, although at low levels of NOMb, in the loins manufactured without nitrate/nitrite added. Thus, the residual amount of nitrate obtained from paprika may be reduced to nitrite and result in a higher NOMb formation throughout the storage. Therefore, more studies are needed to know in depth the role played by the added paprika in the formation of NOMb.

On the contrary, HHP treatment affected neither NOMb nor ZnPP contents. Bruun-Jensen and Skibsted (1996) demonstrated in NOMb solutions that HHP protects NOMb against oxidation, observing that the rate of oxidation decreased with increasing pressure intensity, although the maximum pressure intensity was 300 MPa, much lower than 600 MPa used in the present study. The stabilizing effect of HHP on NOMb could be because of the formation of intermolecular hydrogens bonds with water (Bak et al., 2019).

Removal of nitrate/nitrite from loin formulation significantly affected CI values being lower than in 75NOx and 150NOx loins, at day 0, and lower than in 150NOx, at day 240 (Table 2). No significant effect was found in CI values by the NOx dose for the rest of the sampling days. Moreover, the processing conditions only affected significantly CI values at day 0, with significantly higher values for pressurized loins, and at day 60, with significantly higher values for control loins. Similarly to CIE a*, CI values generally showed a significant increase in all the experimental batches until day 120 of storage followed by a decrease up to day 240. This ratio tracks cured meat color fade and ratio values of 2.2–2.6 represent an excellent cured color (AMSA, 2012). Only the reflectance ratio value of the batch formulated without NOx at day 0 had a noticeable cured color according to this scale (1.5–2.1).

3.3. UV–vis absorption and fluorescence spectra analysis

The UV–vis absorption spectra of extracts from loins with added nitrate/nitrite showed maximal absorptions at 393, 472, 539, and 560 nm, while those from loins with no nitrates/nitrites added showed three absorption peaks at 415, 545, and 583 nm from the beginning of the assay and a new absorption peak at 393 nm gradually increased over storage (Fig. 1).

The acetone-extracted myoglobin pigment from cured loins matched with NOMb (Morita, Yoshikawa, Sakata, Nagata, & Tanaka, 1997). However, the pattern of peaks observed in the absorption spectra of uncured loins was similar to those reported in dry-cured ham without

Table 2
Effect of nitrate and nitrite ingoing amounts (0, 37.5, 75 and 150 mg/kg) and HHP treatment (Control, HHP treated at 600 MPa/7 min) on NOMb content (mg/kg), ZnPP content (mg/kg) and on cured index (Reflectance λ_{650nm}/Reflectance $\lambda_{570\ nm}$) of Iberian dry-cured loins over 240 days of refrigerated storage.

	Day	Nitrate/Nitrite (mg/kg)				HHP			Sig.		
		0	37.5	75	150	Control	Treated	SEM	1	2	3
NOMb (mg kg^{-1})	0	17.4 $^{b\ y}$	24.1 ^{a,b}	25.4 ^{a}	27.8 ^{a}	21.8 ^{y}	25.5	1.10	**	*	n.s.
	30	18.9 $^{b\ y}$	24.3 ^{a,b}	25.3 ^{a}	27.5 ^{a}	23.0 ^{y}	25.0	0.87	**	n.s.	n.s.
	60	21.3 $^{b\ x,y}$	25.1 ^{a,b}	25.5 ^{a,b}	29.6 ^{a}	25.4 ^{x,y}	25.4	0.87	**	n.s.	n.s.
	120	24.3 ^{x,y}	26.4	27.0	29.9	26.1 ^{x,y}	27.7	0.84	n.s.	n.s.	n.s.
	240	28.1 ^{x}	27.7	28.4	30.7	27.7 ^{x}	29.7	0.88	n.s.	n.s.	n.s.
	SEM	0.93	0.85	0.52	0.77	0.64	0.56				
	Sig.	**	n.s.	n.s.	n.s.	**	n.s.				
ZnPP (mg kg^{-1})	0	17.0 ^{a}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	4.4	4.1	1.37	***	n.s.	n.s.
	30	16.4 ^{a}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	4.3	3.8	1.35	***	n.s.	n.s.
	60	17.3 ^{a}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	4.1	4.5	1.42	***	n.s.	n.s.
	120	15.6 ^{a}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	4.0	3.7	1.31	***	n.s.	n.s.
	240	16.2 ^{a}	0.0 ^{b}	0.0 ^{b}	0.0 ^{b}	4.3	3.8	1.37	***	n.s.	n.s.
	SEM	1.33	–	–	–	0.85	0.87				
	Sig.	n.s.	–	–	–	n.s.	n.s.				
Cured index	0	2.1 $^{b\ y}$	2.3 $^{a,b\ y}$	2.4 $^{a\ y}$	2.4 $^{a\ x,y}$	2.2 ^{z}	2.4 ^{y}	0.04	**	**	n.s.
	30	2.3 ^{x,y}	2.5 ^{x}	2.5 ^{y}	2.5 ^{x,y}	2.5 ^{x,y}	2.4 ^{y}	0.04	n.s.	n.s.	n.s.
	60	2.3 ^{x,y}	2.5 ^{x,y}	2.4 ^{y}	2.4 ^{y}	2.5 ^{x,y}	2.3 ^{y}	0.03	n.s.	***	n.s.
	120	2.6 ^{x}	2.6 ^{x}	2.9 ^{x}	2.8 ^{x}	2.7 ^{x}	2.7 ^{x}	0.06	n.s.	n.s.	n.s.
	240	2.2 $^{b\ y}$	2.2 $^{a,b\ y}$	2.3 $^{a,b\ y}$	2.4 $^{a\ y}$	2.3 ^{y,z}	2.2 ^{y}	0.03	**	n.s.	**
	SEM	0.04	0.03	0.05	0.04	0.03	0.03				
	Sig.	***	***	**	*	***	***				

n.s.: P > 0.05, *: P < 0.05, **: P < 0.01; ***: P < 0.001.
a, b: Means within the same row with different superscripts differ significantly (Tukey's test, P < 0.05).
x, y, z: Means within the same column with different superscript differ significantly (Tukey's test, P < 0.05).
[1] Significance. 1: Effect nitrate/nitrite. 2: Effect high-pressure treatment. 3: Interaction.

nitrite added (Møller, Adamsen, & Skibsted, 2003), which was not the characteristic absorption spectrum for NOMb. These results suggested that more NOMb was formed in 150NOx loins than 75NOx and 37.5NOx and that just after HHP treatment there was a higher content of NOMb in HHP-treated loins with added nitrate/nitrite than in control ones. Furthermore, among those loins formulated without curing salts, the formation of NOMb seemed to increase over storage. The formation of NOMb, even though nitrate/nitrite were not added in the formulation, could be explained by other ingredients added like common salt, paprika, etc.

The fluorescent spectra showed two fluorescence emission peaks approximately at 588 and 641 nm in uncured loins samples meanwhile negligible peaks were detected in cured counterparts (Fig. 2). This pattern of peaks was previously described in Parma ham and coincided with ZnPP (Møller et al., 2007; Wakamatsu, Nishimura, & Hattori, 2004). Therefore, these results confirmed the presence of two different pigments in uncured (ZnPP) and cured (NOMb) loins.

3.4. Lipid and protein oxidation

From day 30 until the end of the storage period, TBA-RS values were significantly higher in 0NOx loins than in cured loins (Table 3). The reduction of incoming amounts of nitrite or its removal leads to an increase in TBARS in different fermented meat products; although in fermented sausages with high ingoing amounts of both curing salts, peroxynitrite formation might increase oxidation phenomena including lipid oxidation (Flores, Perea-Sanz, & Belloch, 2021). This antioxidant effect is attributed to nitric oxide generated from nitrite that can be readily oxidized in presence of oxygen, which results in a rapidly sequestering of oxygen and other reactive oxygen species. Furthermore, nitric oxide, as a free radical, binds free irons and stabilizes heme iron limiting its prooxidant activity (Alahakoon et al., 2015; Honikel, 2008). At days 120 and 240, HHP had a significant effect on lipid oxidation, increasing TBA-RS values. Slight and not relevant changes were found in TBA-RS values during storage that ranged from 0.7 to 0.8 mg MDA/kg. The well-known antioxidant activity of nitrite could explain the changes of TBA-RS when levels of nitrate/nitrite were reduced.

Considering previous works on the effect of HHP on dry-cured meat products, Campus et al. (2008) did not observe significant differences in HHP-treated (300–400 MPa/10 min) dry-cured loins after 45 days of storage. In contrast, Cava et al. (2021) reported only an increase of lipid oxidation in pressurized (600 MPa/8 min) dry-cured loins during short-term storage (30 days) without differences until 120 days of storage. Also, Cava et al. (2009) observed an increase in TBA-RS values in treated Iberian dry-cured loins (200–300 MPa/15–30 min) after 90 days of storage as the pressure level and holding time increased, although a slight reduction was noticed after pressurization treatment. According to Guyon et al. (2016), pressurization above 350 MPa have a prooxidant effect for all types of meat, yet aspects such as holding time, treatment temperature, the initial packaging, and mostly the type of meat have a relevant influence on the extent of lipid oxidation. Dry-cured meat products could be more sensitive to the development of lipid oxidation reactions induced by HHP due to their long shelf-life.

At day 0, uncured samples (0NOx) showed significantly higher protein carbonyl contents than their cured counterparts and, among them, an increased protein carbonyl formation was found in 37.5NOx and 75NOx loins compared to 150NOx (Table 3). At days 30 and 240, a significant interaction between both main effects on carbonyls was found. At day 30, 0NOx untreated loins had significantly higher carbonyl contents than their counterparts with 75NOx and 150NOx and pressurized loins formulated with 0, 37.5, and 150 mg/kg NOx. At the end of storage, untreated 75NOx loins had significantly higher carbonyls than their HHP-treated counterparts (Table 4). Vossen and De Smet (2015) also reported a negligible effect of sodium nitrite (0, 18, or 180 mg/kg) on carbonyl contents in raw porcine patties after 7 days of illuminated chilled display.

These authors suggested that $NaNO_2$ could act as an antioxidant since the mechanism behind the protein oxidation could proceed via free

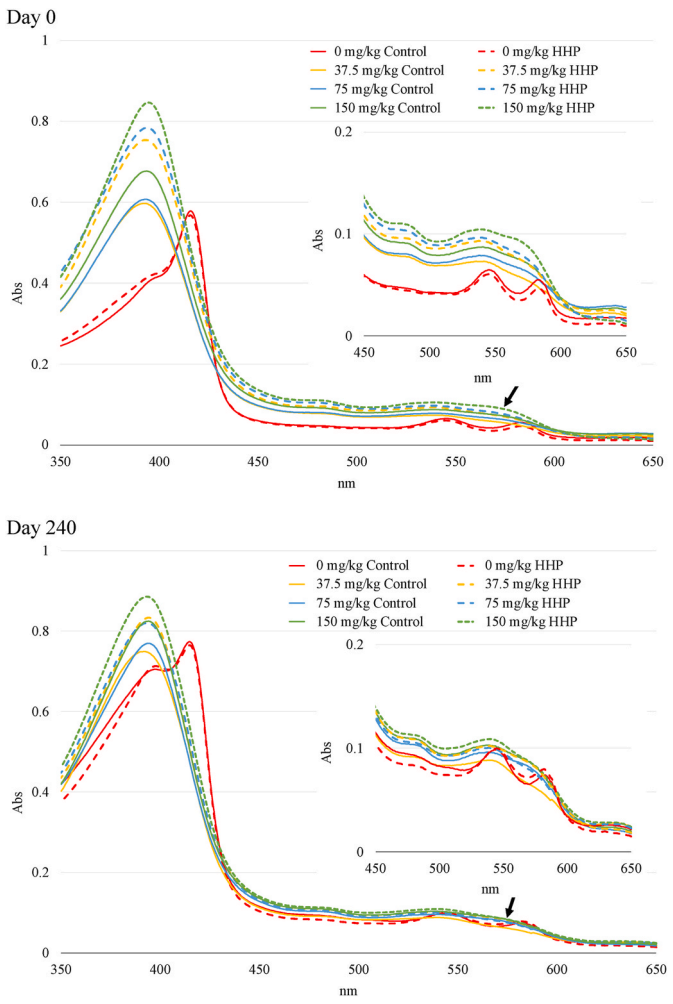

Fig. 1. UV–Vis absorption spectra (λ 350–650 nm) of water: acetone extracts obtained from control and HHP treated dry-cured loins with different nitrate and nitrite ingoing amounts at day 0 and 240 of refrigerated storage.

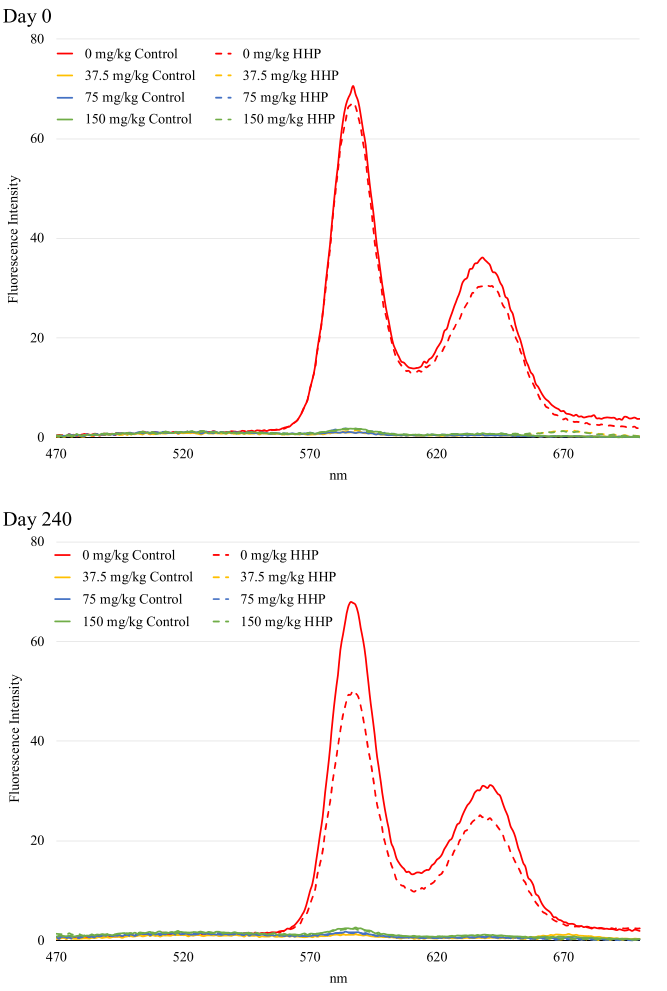

Fig. 2. Fluorescence spectra (λ ex: 415 nm λ em: 470–700 nm) of ethyl acetate: acetic acid extracts obtained from control and HHP treated dry-cured loins with different ingoing amounts of nitrate and nitrite at day 0 and 240 of refrigerated storage.

radical chain processes as those of lipid oxidation. Moreover, myofibrillar proteins from cooked sausages formulated with nitrite doses of 200 and 400 mg/kg showed lower carbonyl values than those with lower nitrite doses (0, 50, 100 mg/kg) after 21 days of cooled storage, which could indicate the importance of sodium nitrite in carbonyl generation prevention (Feng et al., 2016). Meanwhile, pressurization decreased significantly carbonyl contents from day 60 up to the end of the refrigerated storage. Meanwhile, higher carbonyl values were found in HHP-treated dry-cured loins (Cava et al., 2021) and no significant differences were found among both treatments in carbonyl contents neither in dry-cured Iberian hams after 150 days of chilled storage nor in dry-cured loins after 90 days of storage (Amaro-Blanco et al., 2018; Cava et al., 2009).

Conversely to lipid oxidation, the effect of HHP on protein oxidation is a very recent topic and has been scarcely studied. Thus, there are conflicting results that could be due to the different conditioning and HHP treatment applied, the storage conditions, and the type of meat studied. In contrast to our results, most researchers agree about the prooxidant impact of pressurization on protein oxidation. In this sense, several hypotheses about the mechanism followed were provided, such as the release of the metal iron from heme and the conformational modifications of hemoproteins under pressure, which leads to greater exposure to radical attack (Guyon et al., 2016).

Carbonyls slightly increased throughout storage in all batches, although changes were only statistically significant in 37.5NOx and 150NOx samples and when data were grouped as control/HHP-treated. Our results differ from those observed in sliced Iberian dry-cured shoulder (Amaro-Blanco et al., 2018) and in cured cooked sausages (Feng et al., 2016) in which carbonyl contents remained stable after shorter storage periods than those of the present work (150 and 21 days of refrigerated storage, respectively). In dried meat products, the low water content and water activity limit protein oxidation, the prolonged periods of conservation can trigger protein carbonylation induced by myoglobin, oxidizing lipids, or metal catalysts (Guyon et al., 2016).

A decrease in the content of free SH groups is often linked to protein oxidation in meat and meat products (Guyon, Le Vessel, Meynier, & de Lamballerie, 2018; Rakotondramavo et al., 2019). The reduction or removal of ingoing amounts of curing salts did not produce significant changes in the thiol contents of the loins during the refrigeration period (Table 3). Our results contrast with lower SH groups described in meat model systems formulated with 180 mg/kg of nitrite than in those with lower contents (18 and 0 mg/kg) after refrigerated display (Vossen & De Smet, 2015). Moreover, Sullivan and Sebranek (2012) observed that high sodium nitrite amounts (500 mg/kg) resulted in decreased thiol groups in cysteine and myoglobin model systems, which could be explained by the formation of S-nitrosothiol groups. Differences in nitrite levels added and characteristics of meat models/meat products could be the cause for the lack of agreement with previous works.

However, the HHP treatment significantly decreased thiol contents in loins from day 30 up to the end of the storage period. Finally, at day

Table 3
Effect of nitrate and nitrite ingoing amounts (0, 37.5, 75 and 150 mg/kg) and HHP treatment (Control, HHP treated at 600 MPa/7 min) on lipid oxidation measured as TBA-RS and protein oxidation as protein carbonyl and protein thiols contents of Iberian dry-cured loins over 240 days of refrigerated storage.

	Day	Nitrate/Nitrite (mg/kg)				HHP			Sig.		
		0	37.5	75	150	Control	Treated	SEM	1	2	3
TBA-RS (mg MDA kg^{-1})	0	0.9	0.8	0.8 ^{x}	0.8 ^{x}	0.8 ^{x}	0.8	0.01	n.s.	n.s.	n.s.
	30	0.9 ^{a}	0.8 ^{b}	0.7 $^{b\ y}$	0.7 $^{b\ y}$	0.8 ^{x,y}	0.8	0.02	***	n.s.	***
	60	0.9 ^{a}	0.7 ^{b}	0.8 $^{b\ x,y}$	0.7 $^{b\ x,y}$	0.8 ^{x}	0.8	0.02	***	n.s.	**
	120	0.9 ^{a}	0.7 ^{b}	0.7 $^{b\ y}$	0.7 $^{b\ y}$	0.7 ^{y}	0.8	0.02	***	***	**
	240	0.9 ^{a}	0.7 ^{b}	0.8 $^{b\ x,y}$	0.7 $^{b\ y}$	0.7 ^{x,y}	0.8	0.02	***	**	n.s.
	SEM	0.02	0.01	0.01	0.01	0.01	0.01				
	Sig.	n.s.	n.s.	**	**	**	n.s.				
Carbonyls (nmol mg $protein^{-1}$)	0	1.1 ^{a}	0.9 $^{b\ y}$	0.8 ^{b}	0.6 $^{c\ y}$	0.8 ^{z}	0.8 ^{y}	0.03	***	n.s.	n.s.
	30	1.0	0.9 ^{y}	0.8	0.8 ^{x,y}	1.0 ^{y,z}	0.9 ^{y}	0.04	n.s.	n.s.	***
	60	1.1	1.1 ^{x,y}	1.0	0.9 ^{x,y}	1.1 ^{x,y}	0.9 ^{y}	0.04	n.s.	**	n.s.
	120	1.1	1.2 ^{x}	0.9	1.0 ^{x,y}	1.2 ^{x,y}	1.0 ^{x,y}	0.06	n.s.	*	n.s.
	240	1.3	1.2 ^{x}	1.1	1.2 ^{x}	1.3 ^{x}	1.1 ^{x}	0.05	n.s.	*	*
	SEM	0.04	0.04	0.04	0.05	0.03	0.03				
	Sig.	n.s.	**	n.s.	**	***	**				
Thiols (nmol mg $protein^{-1}$)	0	33.6 ^{x}	34.6	34.4	34.3	34.5 ^{x,y}	34.0 ^{x}	0.55	n.s.	n.s.	n.s.
	30	30.1 ^{x,y}	33.1	32.7	32.2	36.3 ^{x}	27.7 ^{z}	0.81	n.s.	***	n.s.
	60	31.1 ^{x,y}	33.1	31.8	31.8	33.1 ^{y,z}	30.8 ^{y}	0.34	n.s.	***	n.s.
	120	30.8 ^{x,y}	33.1	33.2	33.3	34.7 ^{x,y}	30.5 ^{y}	0.51	*	***	n.s.
	240	29.6 ^{y}	31.5	30.1	29.8	31.6 ^{z}	28.9 ^{y,z}	0.44	n.s.	**	*
	SEM	0.44	0.47	0.62	0.51	0.36	0.28				
	Sig.	*	n.s.	n.s.	n.s.	***	***				

[1] Significance. 1: Effect nitrate/nitrite. 2: Effect high-pressure treatment. 3: Interaction.
n.s.: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$; ***: $P < 0.001$.
a, b, c: Means within the same row with different superscript differ significantly (Tukey's test, $P < 0.05$).
x, y, z: Means within the same column with different superscript differ significantly (Tukey's test, $P < 0.05$).

Table 4
Means for the interaction of nitrate and nitrite ingoing amounts (0, 37.5, 75 and 150 mg/kg) and HHP treatment (Control, HHP treated at 600 MPa/7 min) on CIE b*-value, cured index (CI), TBA-RS (mg MDA kg^{-1}), carbonyls (nmol mg $protein^{-1}$) and thiols (nmol mg $protein^{-1}$) contents.

	Day	Control				HHP					
		0	37.5	75	150	0	37.5	75	150	SEM	Sig.
CIE b*	0	3.4 ^{c}	4.2 ^{b,c}	4.7 ^{b,c}	3.8 ^{b,c}	9.6 ^{a}	6.6 ^{a,b,c}	7.0 ^{a,b}	6.1 ^{b,c}	0.04	***
CI	240	2.1 ^{c}	2.3 ^{a,b,c}	2.4 ^{a,b}	2.3 ^{a,b,c}	2.2 ^{c}	2.2 ^{b,c}	2.1 c	2.5 ^{a}	0.03	***
TBA-RS	30	0.8 ^{b}	0.7 ^{c,d}	0.7 ^{c,d}	0.7 ^{c,d}	1.0 ^{a}	0.8 ^{b,c}	0.7 ^{d}	0.7 ^{d}	0.02	***
	60	1.0 ^{a}	0.8 ^{b,c}	0.7 ^{c}	0.8 ^{b,c}	0.9 ^{a,b}	0.7 ^{c}	0.8 ^{b,c}	0.7 ^{c}	0.02	***
	120	0.7 ^{b}	0.7 ^{b}	0.7 ^{b}	0.7 ^{b}	1.0 ^{a}	0.8 ^{b}	0.8 ^{b}	0.7 ^{b}	0.02	***
Carbonyls	30	1.3 ^{a}	1.0 ^{a,b}	0.7 ^{b}	0.8 ^{b}	0.7 ^{b}	0.9 ^{b}	1.0 ^{a,b}	0.9 ^{b}	0.04	***
	240	1.1 ^{a,b}	1.3 ^{a,b}	1.4 ^{a}	1.3 ^{a,b}	1.4 ^{a}	1.2 ^{a,b}	0.8 ^{b}	1.1 ^{a,b}	0.05	*
Thiols	240	30.2 ^{a,b}	33.2 ^{a}	3.0 ^{a}	29.9 ^{a,b}	29.0 ^{a,b}	29.7 ^{a,b}	27.3^{b}	29.8 ^{a,b}	0.44	**

*: $P < 0.05$, **: $P < 0.01$; ***: $P < 0.001$.
a, b, c, d: Means within the same parameter and day of storage with different superscript differ significantly (Tukey's test, $p < 0.05$).

240, a significant interaction was detected on thiol contents. In this case, 37.5NOx and 75NOx HHP-treated samples had significantly lower thiol contents than their non-pressurized counterparts (Table 4).

In accordance with our results, Guyon et al. (2018) reported a decrease in the free thiol amounts in HHP treated minced beef (200–500 MPa/5 min) just after treatment, which modifications of the thiol groups were irreversible at 500 MPa. Rakotondramavo et al. (2019) assessed both the accessible and free thiol content in HHP treated cooked ham (500 MPa/5 min) and described lower free thiol contents and slightly higher accessible thiol contents in HHP treated samples after 21 days of refrigerated storage. These authors suggested that the conformational changes in the proteins due to the pressure increased the accessibility of the SH groups and therefore the exposure to free radicals that promoted thiols oxidation (Rakotondramavo et al., 2019). Additionally, high pressure also induces the formation of new disulfide bonds that could contribute to a diminution in the content of thiols. All these studies (Guyon et al., 2018; Rakotondramavo et al., 2019), reflect the great complexity of factors that influence the decrease in the amounts of thiols in dry-cured products treated with high pressure. Beyond the aspects related to oxidative changes, the formation of new disulfide bonds

induced by high pressure can stabilize the denatured proteins and/or produce protein aggregation that could impair the texture of the Iberian dry-cured loin during storage.

The thiol content decreased gradually with the storage time in all batches, although changes were only statistically significant in uncured samples. Both control and HHP treated dry-cured loins reduced thiols content throughout storage. In this sense, besides that the oxidation of the thiol groups in meat could lead to the formation of new disulfide bonds and/or sulfenic, sulfinic, or sulfonic acid, the myofibrillar proteins could be altered by different reactions between protein side chains with secondary lipid oxidation products. Thus, changes induced in sulfur amino acids by long storage times storage could lead not only to modifications in the textural properties of the stored dry-cured loins but also the free radical generation could be increased with an impact on food safety (Martínez et al., 2020).

3.5. Correlations among ingoing amounts of nitrate and nitrite, post-process treatment, and analyzed parameters

A partial least squares regression was performed to obtain a simultaneous map of ingoing amounts of nitrate and nitrite, post-process treatment, and instrumental color, cured index, heme pigments contents and protein and lipid oxidation markers. Fig. 3 shows the correlation circle among dependent and explanatory variables in the first two components (t1, t2) obtained from the PLS analysis.

Nitrosylmyoglobin, CIE a*-value, and cured index show proximity in t1 axis indicating positive correlations among them and with qualitative explanatory variables ingoing amounts of nitrate and nitrite (37.5, 75, and 150 mg/kg NOx). On the other hand, ZnPP shows a positive correlation to 0 mg/kg NOx and is opposed to NOMb, redness, and cured index. This distribution demonstrates that these variables are associated with each other and with the effect of NO_2^- on the formation of NOMb and ZnPP, and their relationships with the development of the characteristic cured color and redness in packed dry-cured loins. Oxidation of lipids and proteins show different associations and thus correlations with explanatory variables (NOx and HHP). The TBA-RS value is negatively correlated with NOx contents in loins and therefore located close to 0NOx, indicating the antioxidant effect of NO_2^- on lipid oxidation. For protein oxidation, thiol and carbonyl contents show a negative correlation and are far from HHP treatment, indicating a negative correlation with the pressurization treatment and suggesting that the decrease in thiol content is not only associated with oxidative phenomena but also with the formation of new disulfide bonds generated by the action of pressure.

4. Conclusions

Changes induced by the reduction/removal of curing salts in color and oxidation of lipids and proteins were maintained in vacuum-packed sliced products during storage. A significant reduction of ingoing amounts of both nitrate/nitrite to 75–37.5 mg/kg could be possible without affecting instrumental color, lipid, and protein oxidation. Regardless of the level of nitrate/nitrite added, high pressure processing (600 MPa, 7 min) does not induce changes beyond increased lightness and reduced thiol contents in Iberian dry loin slices. Given that a high-pressure treatment (600 MPa, 7 min) minimally affected the color and oxidation of lipids and proteins, future studies focused on the influence of the combined effect of nitrate/nitrite reduction/removal and HPP on the food safety of cured and nitrate/nitrite-reduced Iberian dry loins during storage are required.

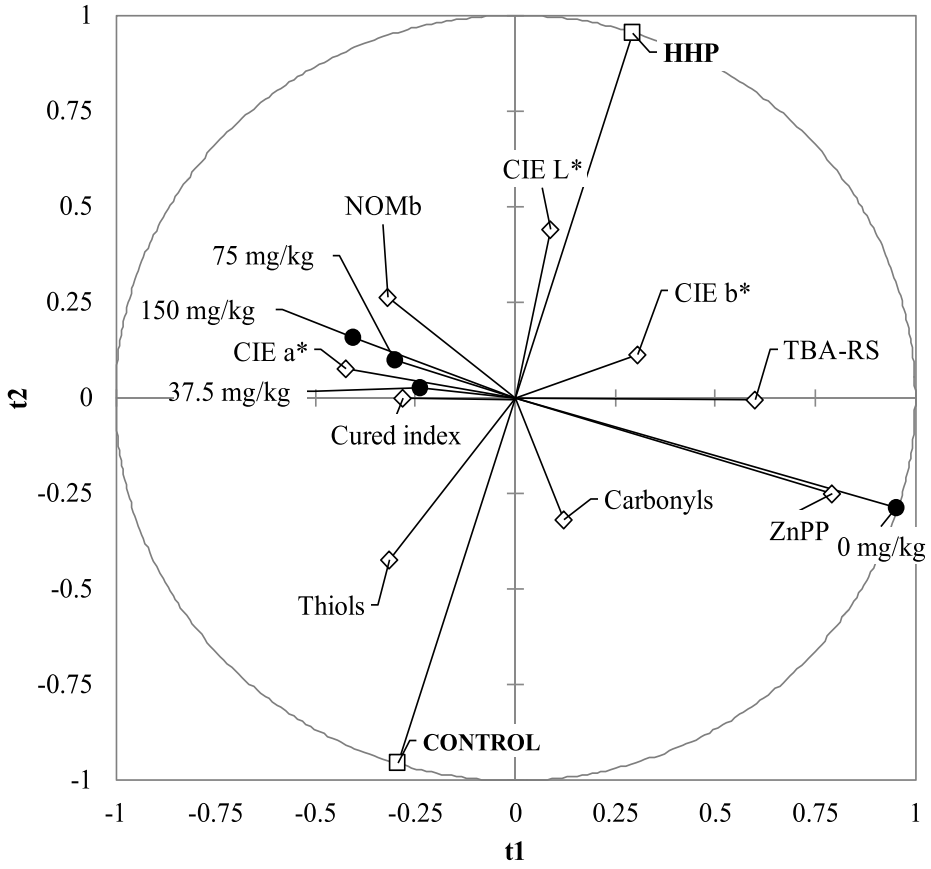

Fig. 3. Correlation circle on t1 and t2 between different ingoing amounts of nitrate and nitrite (0, 37.5, 75 and 150 mg/kg nitrate/nitrite -close circle-), post-process treatment (HHP, control -open square-) and analyzed parameters (CIE L* a*, b*, cured index, TBA-RS, carbonyls, thiols, NOMb and ZnPP) open diamonds) and the first two components (t1, t2).

CRediT authorship contribution statement

Nieves Higuero: Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **María Rosario Ramírez:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **María del Carmen Vidal-Aragón:** Writing – original draft, Writing – review & editing, Supervision. **Ramón Cava:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

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Glossary

BSA: bovine serum albumin
CI: cured index
DNPH: 2,4-dinitrophenylhydrazine
DTNB: 5-5′-Dithiobis(2-nitrobenzoic acid)
HHP: high hydrostatic pressure
MDA: malondialdehyde
NOMb: nitrosylmyoglobin
SDS: sodium dodecyl sulfate
TBA-RS: thiobarbituric acid reactive substances
TRIS: tris(hydroxymethyl)- aminomethane
SPSS: Statistical Package for the Social Science
ZnPP: Zinc protoporphyrin