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Rinse and chill®, frozen storage and retail packaging influence the quality of lamb loins

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ABSTRACT

This study evaluated effects of Rinse&Chill® vs. control, storage condition (fresh, frozen-thawed) and retail packaging (high oxygen modified atmosphere packaging, vacuum skin packaging) on quality of lamb loins. Thirty-two lambs were slaughtered, and carcasses were allocated to Rinse&Chill® or control ($n = 16$ for each). Loins were aged for seven-days (fresh), and then allocated to fresh or freezing-thawing (frozen-thawed). After storage completion loins were cut into steaks then packaged in high oxygen modified atmosphere packaging (HiOxMAP) or vacuum skin packaging (VSP) and into 8-days simulated retail display. Rinse&Chill® samples had lower heme protein content and lipid oxidation ($p < 0.05$ for both) compared to control samples. In frozen-thawed lamb samples, VSP resulted in lower lipid oxidation, higher red values (a^*), and lower Warner-Bratzler shear force compared to those in HiOxMAP ($p < 0.05$ for all). This study showed that Rinse&Chill® reduced lipid oxidation in lamb loins, and VSP can be used to improve the quality of frozen-thawed lamb in retail.

1. Introduction

Ensuring consistent eating quality of lamb is an important but challenging goal for the sheep meat industry. The quality characteristics of lamb include texture, water-holding capacity, flavour and colour stability (Lawrie & Ledward, 2014). These quality characteristics are influenced by various supply chain factors. Frozen storage is widely used in the meat industry to extend shelf life, as the quality deterioration of meat, especially oxidation is largely retarded under frozen storage conditions (generally −18 °C) (Muela, Monge, Sañudo, Campo, & Beltrán, 2015). However, several chemical and physical changes, including the formation of ice crystals and accumulation of prooxidant substances, are known to occur at a slow but steady rate during frozen storage (Leygonie, Britz, & Hoffman, 2012; Soyer, Özalp, Dalmış, & Bilgin, 2010; Vieira, Diaz, Martínez, & García-Cachán, 2009). In addition, these factors have been shown to impair the oxidative stability, texture, and water-holding capacity of lamb upon thawing, thus accelerating the quality deterioration of the frozen-thawed lamb in retail display (Bellés et al., 2018; Muela et al., 2015; Muela, Sañudo, Campo, Medel, & Beltrán, 2010). Aside from frozen storage, the use of high oxygen modified atmosphere packaging (HiOxMAP; 70–80% O_2 and 20–30% CO_2) in retail display is another main cause of quality deterioration in lamb (Frank et al., 2017). HiOxMAP is used in the meat industry to enhance the stability of the bright red colour on the meat surface and retard the growth of microorganisms (Lawrie & Ledward, 2014). However, the high oxygen content in this packaging is also known to induce lipid and protein oxidation, resulting in toughening and off-flavour in lamb (Frank et al., 2017; Kim, Bødker, & Rosenvold, 2012). For these reasons, overcoming the adverse effects of frozen storage and HiOxMAP are of significant interest to the sheep meat industry.

To address the quality deterioration of lamb caused by the above factors, several novel technologies are recently used in the supply chain. The Rinse & Chill® (RC) is a technology designed to accelerate the chilling of animal carcasses and reduce the weight loss. The technology involves vascular rinsing of the carcasses post-exsanguination and early postmortem using a patented chilled isotonic solution (98.5% water; balance is glucose, polyphosphates, and maltose, MPSC. Inc.; Fowler, Claus, & Hopkins, 2017). RC was reported to tenderise and improve the colour stability of lamb, although the underlying mechanisms remain unclear (Fowler et al., 2017). In addition, Kethavath et al. (2021) reported that RC reduced lipid oxidation in pork, likely through the removal of the residual blood, thus reducing heme-meditated lipid oxidation. Although the positive effects of RC on lamb quality was

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reported in previous studies (Fowler et al., 2017; Mickelson, Warner, Seman, Crump, & Claus, 2018), its interaction with other factors in the supply chain remains unknown. We expected that RC could counteract some of the quality deterioration caused by frozen storage or HiOxMAP and result in a better quality of lamb in retail. In addition to RC, vacuum skin packaging (VSP) is a recommended alternative to HiOxMAP for use in the retail display of lamb (Frank et al., 2017). VSP refers to the shrink-wrapping of a flexible polymer with low oxygen transmission rate on meat, and is reported to provide better appearance than the conventional vacuum packaging by reducing air pockets and wrinkles (Aaslyng, Tørngren, & Madsen, 2010; Frank et al., 2017). VSP enhances the quality of lamb by providing a low-oxygen environment that limits lipid oxidation and the toughening of lamb seen in HiOxMAP (Frank et al., 2017). However, the effect of VSP on frozen-thawed lamb has not been studied. Therefore, we hypothesised that the use of RC and VSP can improve the quality of fresh and frozen-thawed lamb under retail display conditions. To test this hypothesis, we examined the effects of RC vs. control, storage condition (frozen-thawed vs. fresh), and retail packaging (HiOxMAP vs. VSP) on lipid oxidation, texture, water-holding capacity, and colour of lamb loin.

2. Materials & methods

2.1. Carcass collection and experimental design

The experimental design is summarised in Fig. 1. Thirty-two lambs (from within one group of sheep, unknown live animal data) were slaughtered at a commercial meat processing plant and were randomly allocated to Rinse & Chill® treatment ($n = 16$) or control (no Rinse & Chill®, n = 16). The hot carcass weight was 24 ± 3.0 kg and the fat depth at the GR site (110 mm from the midline over the 12th rib) was 18 ± 4.5 mm. Rinse & Chill® was performed by inserting a catheter intracardially, through the thoracic cavity, into the heart of the carcass within five minutes of slaughter. The vascular system of the carcass was rinsed with a chilled (8 °C) isotonic solution (98.5% water; balance: glucose, polyphosphates, and maltose, MPSC. Inc.; Fowler et al., 2017) to 10% of the carcass weight. The formula of this solution is a trade secret. At one-day postmortem, loins (*Longissimus lumborum*) from both the left and right sides of the lamb carcasses were boned and vacuum packed. The loins were transported to the University of Melbourne under refrigerated conditions. The pH was measured in each loin and then the loins were aged in vacuum packaging for seven days at 0.5 °C. Thirty-two loins from 16 randomly selected carcasses (8 RC and 8 control) were frozen in vacuum at −20 °C for three months. The remainder of the loins were cut into steaks immediately after the seven-day ageing (fresh) and subjected to packaging as described below. The frozen primals were thawed at 0.5 °C, in the dark, for 16 h. Upon thawing, the frozen-thawed loins were cut into steaks and subjected to the same packaging treatments as the fresh loins. For each carcass, the loins from left and right sides were randomly allocated to HiOxMAP or VSP. The loins were cut into 6 cm-long steaks before packaging and four steaks per carcass (two steaks from each side) were used. For samples allocated to HiOxMAP, two steaks were placed on 130 × 90 mm absorbent pads (Complete Butcher Supplies, Carrums Down, Victoria, Australia) in a polypropylene MAP tray measuring 90 mm long × 70 mm wide × 80 mm deep (Sealed Air Corporation, Fawkner, Victoria, Australia). The trays were sealed with an oxygen impermeable film (high barrier laminated lidding film, 45 μm; Sealed Air Corporation, Fawkner, Victoria, Australia) in 80% O_2 and 20% CO_2 using a Multivac T200 (Multivac Pty Ltd., Keilor Park, Victoria, Australia). The gases were mixed using a Gas Mixer KM100-200_2M (WittGas, Epping, Victoria, Australia) and the mixture was validated using an Oxybaby M+ headspace analyzer (Witt Gas, Epping, Victoria, Australia). Vacuum skin packaging was conducted with CRYOVAC® DARFRESH® film and trays (Sealed Air Corporation, Fawkner, Victoria, Australia) using a Multivac T200 (Multivac Pty Ltd., Keilor Park, Victoria, Australia). All packaged samples were labelled and stored at 4 °C in a Bromic upright display fridge flat glass door 976 L LED GD1000LF (Bromic Refrigeration, New South Wales, Australia), fitted with two 18 W bulbs with approximately 310 lx, measured at the surface of the meat, to simulate the supermarket retail display conditions. The samples were rotated daily to minimise light and temperature exposure bias. The instrumental colour of the fresh meat was measured before the start of retail display. After 8 days of retail display, for both fresh and frozen-thawed, steaks were removed from packaging. One steak in each pack was collected for pH, colour and texture analysis, and cooked immediately after retail display. At the same time, the other steak in the same package was immediately frozen at −20 °C for TBARS, total carbonyl content, and total heme protein.

2.2. pH, instrumental colour, purge loss, cooking loss, and texture measurement

The pH of the interior of the loin was measured by insertion of a spear-head Ionode IJ44 pH probe attached to WP-80 pH-mV-tempera-

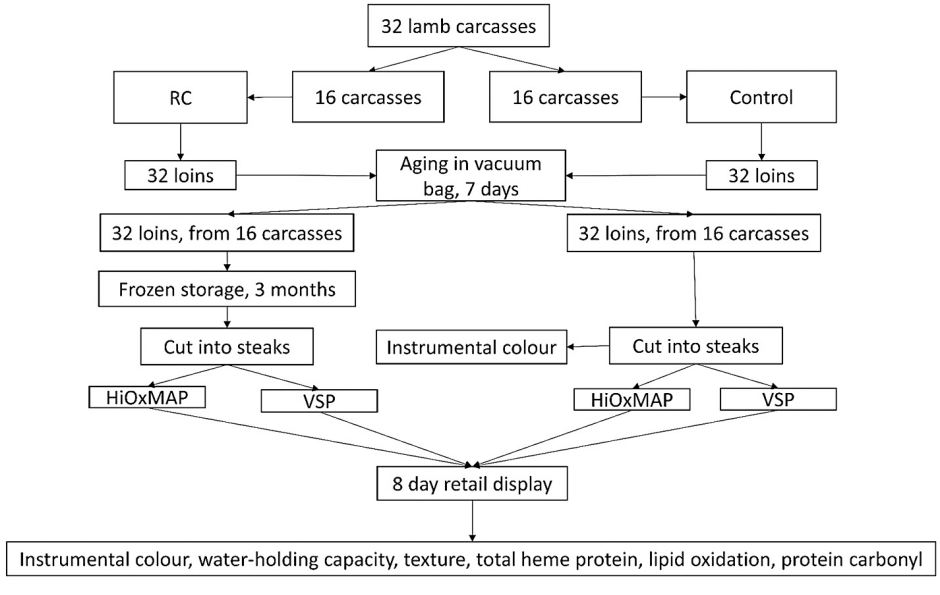

Fig. 1. Illustrated experimental design. RC - Rinse and chill®, HiOxMAP – high oxygen modified atmosphere packaging, VSP – vacuum skin packaging.

ture meter (both TPS Pty Ltd., Brisbane, QLD). Temperature compensation was allowed using a TPS temperature probe supplied with the meter. Instrumental colour of each sample was measured three times at the surface of each lamb steak using a HunterLab MiniScan™ EZ 4500 L Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA). The HunterLab was calibrated using a white tile provided by the supplier (Serial No: MSEZ1535; X: 79.19, Y: 84.27, Z: 90.06). The colour of all fresh (non-frozen) loin steaks were measured after ageing and before packaging, and all fresh and frozen-thawed samples were measured after retail display. Meat in VSP was measured after blooming at 6 °C for 30 min. Meat in HiOxMAP was measured without blooming. The samples were measured with a 2.54 cm diameter aperture, 10° standard observer, D65 illuminant setting. The CIE L^* (lightness), a^* (redness, scale from green to red), b^* (yellowness, scale from blue to yellow) colour scale were recorded. The L^*, a^*, b^* values for each steak were averages of three measurements. The chroma and hue angle were calculated as Chroma $= \sqrt{a^2 + b^2}$; Hue angle $= tan^{-1}\left(\frac{b^*}{a^*}\right)$ and averaged for each steak.

The lamb steaks were weighed before and after retail display. Purge loss was calculated as:

$$\text{Purge loss}\,(\%) = \left(\text{weight}_{\text{before packaging}} - \text{weight}_{\text{after packaging}}\right) / \left(\text{Weight}_{\text{before packaging}}\right) \times 100$$

The lamb steaks for texture measurement were cooked according to the method described by Hopkins, Toohey, Warner, Kerr, and Van De Ven (2010) with modifications. The fresh and frozen steaks were cooked in two different batches. In both fresh and frozen-thawed samples, the steaks were cooked in complete treatment sets (RC vs control, HiOxMAP vs VSP, $n = 32$). The lamb steaks were individually placed in plastic bags and the bags were secured to a metal rack with paper clips. The steaks were then submerged in two temperature equilibrated Julabo F38-ME water baths set at 75 °C (JULABO GmbH, Seelbach, Germany). The samples were cooked to an internal temperature of 71 °C. The temperature was monitored using a Grant data logger 2020 series fitted with T-type thermocouples (Temperature Controls, Victoria, Australia). Once the internal temperature was reached, the steaks were cooled in ice-water for 30 mins. After refrigeration overnight at 4 °C, water on the surface of the steak was patted dry with paper towel before weighing to measure the cooking loss. The cooking loss was calculated as:

$$\text{Cooking loss}\,(\%) = \left(\text{weight}_{\text{before cooking}} - \text{weight}_{\text{after cooking}}\right) / \left(\text{Weight}_{\text{before cooking}}\right) \times 100$$

The cooked samples were subjected to texture measurement. Warner-Bratzler shear force (WBSF) and texture profile analysis (TPA) hardness were conducted at room temperature according to the procedures of De Huidobro, Miguel, Blázquez, and Onega (2005). Each steak was first cut parallel to the direction of muscle fibres into four to five 1-cm thick slices. For WBSF measurement, 6 strips measuring 1 cm^2 × 4 cm long, were then cut from the steaks, keeping the cuts parallel longitudinally to the muscle fibres. These strips were sheared individually using a Lloyd TA1 texture analyzer (Lloyd Instruments Ltd., Hampshire, UK) with a Warner Bratzler V-shaped shear blade attachment. Shearing was perpendicular to the direction of the muscle fibre with a 60^o triangular aperture, 300 mm/min crosshead speed and 500 N load cell. Results were expressed as peak shear force (N). For the TPA double bite compression test, one 1 cm-thick slice was compressed at five different positions across the surface of the steak, using the same Lloyd instrument, with a blunt cylindrical metal rod (diameter = 0.63 mm) attachment. The samples were compressed to 80% of their height with a test speed of 50 mm/min. Compression occurred twice at each of the five positions for each sample and perpendicular to the muscle fibre direction. TPA hardness (N) was reported.

2.3. Total heme protein assay

Total heme protein was measured on all 64 samples from 32 carcasses, after retail display, following the method of Wadhwani, Murdia, and Cornforth (2010) with modifications. Meat samples (3 g) were homogenised in 25 mL ice-cold 40 mM sodium phosphate buffer pH 6.8 using an IKA T25 digital Ultraturrax homogeniser (IKA® Works Asia Sdn Bhd, 48,000 Rawang, Selangor Malaysia) at 15,000 rpm for 50 s. The homogenates were kept on ice for 1 h and centrifuged at 21,500 *g* at 2 °C for 30 min. The supernatant was collected, and the absorbance measured at 525 nm and 700 nm against the homogenisation buffer using a Thermo Scientific Multiskan Spectrum (Thermo Fisher Scientific Australia Pty Ltd., Scoresby, Victoria, Australia). The total heme protein content (myoglobin + hemoglobin) was calculated as:

$$\text{Total heme protein} = (A_{525} - A_{700}) \times 2.303 \times \text{dilution factor}$$

A_{525} is the isosbestic point for myoglobin, where the three forms of myoglobin (deoxymyoglobin, oxymyoglobin, and metmyoglobin) have equal absorbance. Similarly, hemoglobin has the same extinction coefficient at A_{525} on a mass basis (Kethavath et al., 2021). The absorbance at 700 nm was subtracted to correct for turbidity.

2.4. Thiobarbituric acid reactive substances assay

The TBARS values of lamb samples were determined using the method described in the study of Witte, Krause, and Bailey (1970) with modifications. In brief, lamb samples (2.0 ± 0.02 g) were minced and homogenised in 5 mL 20% (*w/v*) trichloroacetic acid (TCA) and 2 M phosphoric acid for 50 s using an IKA T25 digital Ultraturrax homogeniser (IKA® Works Asia Sdn Bhd, 48,000 Rawang, Selangor Malaysia) at 15,000 rpm. The homogenate was filtered through Whatman #1 filter paper. The filtrate (0.8 mL) was then mixed with equal volume of 5 mM thiobarbituric acid and incubated at 90 °C for 40 min. The solution was read at 532 nm using a Thermo Scientific Multiskan Spectrum (Thermo Fisher Scientific Australia Pty Ltd., Scoresby Victoria, Australia). A standard curve with malondialdehyde (MDA) was constructed. The TBARS results are expressed as mg MDA/kg lamb.

2.5. Protein carbonyl content assay

The protein carbonyl content in lamb loins were measured following the method of Fagan, Sleczka, and Sohar (1999) with modifications. Lamb samples (4.0 g ± 0.01 g) were minced and homogenised in 5 mL pyrophosphate buffer (pH 7.4). The homogenates were split into 2 × 1 mL aliquots, which were then washed with HCl-acetone and 10% (*w/v*) TCA solution. From two aliquots, one was incubated with 0.5 mL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl. The other aliquot was incubated with 0.5 mL of 2 M HCl for 30 min in the dark. After the incubation, the two aliquots were then precipitated with 1 mL 20% (w/v) TCA and the pellets were extensively washed with ethanol/ethyl-acetate. The washed pellets were resuspended in 1 mL of 6 M guanidine chloride solution (pH 2.3) and incubated overnight. The absorbance of the DNPH aliquot and the HCl aliquot were measured at 370 nm and 280 nm, respectively. Finally, the protein concentration of the HCl tube was measured against bovine serum albumin (BSA) standards. The concentration of protein carbonyl in the DNPH aliquot was calculated using an absorption coefficient of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$ and the final results were expressed as nmol carbonyl/mg protein.

2.6. Statistical analysis

Statistical analysis was performed with Restricted Maximum Likelihood (REML) analysis using GenStat (16th Edition, VSN International, Hemel Hempstead, UK). The TBARS and WBSF data were transformed with natural logarithm due to inhomogeneity of variance. The main effects used in the fixed model were carcass treatment (RC, control),

storage condition (fresh, frozen-thawed), and retail packaging (HiOx-MAP, VSP). Carcass ID and side (left, right) (all nested within each other) were used in the random model. The average standard error of difference (SED) was calculated for main effects and their interactions. Differences between treatments larger than 2 × SED were considered significant ($p < 0.05$).

3. Results

3.1. Heme protein content, lipid oxidation and protein carbonyl

The effects of RC on the total heme protein content and lipid oxidation (TBARS) are shown in Table 1. RC reduced the total heme protein content in lamb ($p = 0.007$). Storage condition and retail packaging had no effect on the total heme protein content of lamb ($p > 0.05$ for both). Likewise, RC reduced the TBARS in lamb loins at the end of the 8-day retail display ($p = 0.016$), and the effect of RC on TBARS had no interaction with the effects of storage condition or retail packaging ($p > 0.05$ for both).

Fig. 2 shows the effect of storage condition and retail packaging on lipid oxidation of lamb. The frozen-thawed lamb had higher TBARS values than the fresh lamb ($p < 0.001$). The effect of storage condition on TBARS had an interaction with the effect of retail packaging ($p = 0.01$). In the fresh lamb, VSP resulted in lower TBARS compared to HiOxMAP ($p < 0.05$), although the difference between these two packaging treatments was relatively small. Similarly, the TBARS value of the frozen-thawed lamb in VSP was five times lower than that in HiOxMAP ($p < 0.05$). Therefore, VSP resulted in lower lipid oxidation in both fresh and frozen-thawed lamb compared to HiOxMAP, but the extent of reduction was much greater in frozen-thawed lamb compared to the fresh lamb.

The protein carbonyl content of lamb in HiOxMAP (3.19 nmol/mg protein) was higher ($p < 0.001$) than that of lamb in VSP (2.29 nmol/mg protein). Whereas storage condition and RC had no effect on the protein carbonyl content ($p > 0.05$ for both). Treatments had no interaction for the protein carbonyl content in lamb ($p > 0.05$ for all).

3.2. pH and instrumental colour

The average pH in the loin before application of ageing, packaging and freezing was 5.90 ± 0.134 and there was no effect of RC ($p > 0.05$).

Before the retail display, RC increased the $L^*$of fresh lamb ($p = 0.046$) loins but had no effect on other instrumental colour parameters ($p > 0.05$ for both a^* and b^*) (Fig. 3). In contrast, RC had no effect on the instrumental colour of fresh lamb loins at the end of the 8-day retail display ($p > 0.05$; data not shown). The effects of storage condition and retail packaging on instrumental colour of lamb are shown in Table 2. Frozen-thawed lamb had higher L^*, lower a^* and lower b^* compared to the fresh lamb ($p < 0.05$ for all). The effect of retail packaging on a^*, b^*, hue and chroma instrumental colour, interacted with storage condition ($p < 0.05$ for all). In fresh lamb, retail packaging had no effect on the a^*, b^*, chroma, and hue angle ($p > 0.05$ for all). However, in the frozen-thawed lamb, VSP resulted in higher a^* and chroma, but lower b^* and hue angle ($p < 0.05$ for all).

Table 1
Effect of Rinse & Chill® on total heme protein content and lipid oxidation (TBARS) of lamb loins after 8 days of retail display, values in the table are least-square means and standard error of difference (SED).

	Rinse & Chill®	Control	SED	*p*-value
Total heme protein content (mg heme protein/g meat)	2.18	2.57	0.13	0.007
TBARS[1] (mg malonaldehyde/kg meat)	0.19 (1.21)	0.56 (1.75)	0.15	0.016

[1] TBARS values were transformed with natural logarithm for data analysis. Values in parentheses are back transformed data and the SED and *p*-value relate to the transformed data.

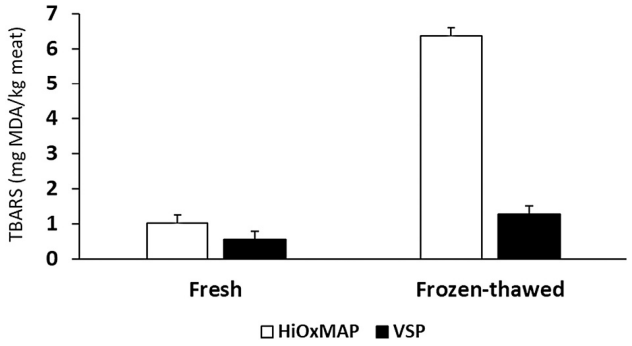

Fig. 2. TBARS values of lamb loins, after 8 days of retail display, under different storage condition (fresh vs. frozen-thawed) and packaging type (HiOxMAP vs. VSP). $p < 0.001$ for storage condition × packaging type interaction. HiOxMAP – High oxygen modified atmosphere packaging, VSP – vacuum skin packaging. Least-square means are shown, and error bars are standard error of difference (SED) for interaction, MDA – malondialdehyde.

3.3. Texture

RC had no effect on the WBSF (17.67 N for control, and 15.17 N for RC) and TPA-hardness (26.56 N for control, and 26.95 N for RC) of lamb loin ($p > 0.05$ for both). Table 3 shows the effect of storage condition and retail packaging on the WBSF and TPA hardness of lamb loin. The WBSF of lamb loins was affected by the interaction between these two treatments ($p = 0.011$). In fresh lamb, retail packaging had no effect on the WBSF ($p > 0.05$). However, VSP led to lower WBSF compared to HiOxMAP for the frozen-thawed lamb ($p < 0.05$). Similarly, VSP led to lower TPA hardness of lamb loins compared to HiOxMAP ($p = 0.009$), but storage condition or their interaction had no effect ($p > 0.05$).

3.4. Water-holding capacity

The water-holding capacity of lamb loins, measured by purge loss and cooking loss, is shown in Table 3. The purge loss and cooking loss of lamb loins were only influenced by the interaction between the storage condition and the retail packaging ($p < 0.05$ for both), whereas RC had no effect on the purge loss (8.02% for control, and 7.57% for RC; SED = 0.75%) and cooking loss (15.79% for control, and 16.03% for RC; SED = 0.86%) ($p > 0.05$ for both). In fresh lamb, VSP resulted in higher purge loss compared to HiOxMAP ($p < 0.05$). By contrast, the frozen-thawed lamb in VSP had lower purge loss than in HiOxMAP ($p < 0.05$). In addition, the VSP resulted in higher purge loss in fresh lamb than that of frozen-thawed lamb ($p < 0.05$). For the cooking loss, the retail packaging had no effect on the fresh lamb ($p > 0.05$), but the VSP resulted in higher cooking loss in the frozen-thawed lamb compared to HiOxMAP ($p < 0.05$).

4. Discussion

RC reduced the total heme protein content in the lamb loins in the present study. This result is supported by the study of Bekhit et al. (2005) who used water infusion of lamb carcasses. They postulated that the reduction in total heme protein content was caused by the extraction of myoglobin from the muscle by the infused water. Erazo-Castrejón et al. (2019) reported that RC reduced the concentration of hemoglobin in pork but had no effect on myoglobin and total heme protein content, indicating that RC contributed to blood removal. Similarly, Kethavath et al. (2021) found no difference in total heme protein content between RC pork and that of control. However, they suggested that that their measurement of total heme protein content was inadequate to

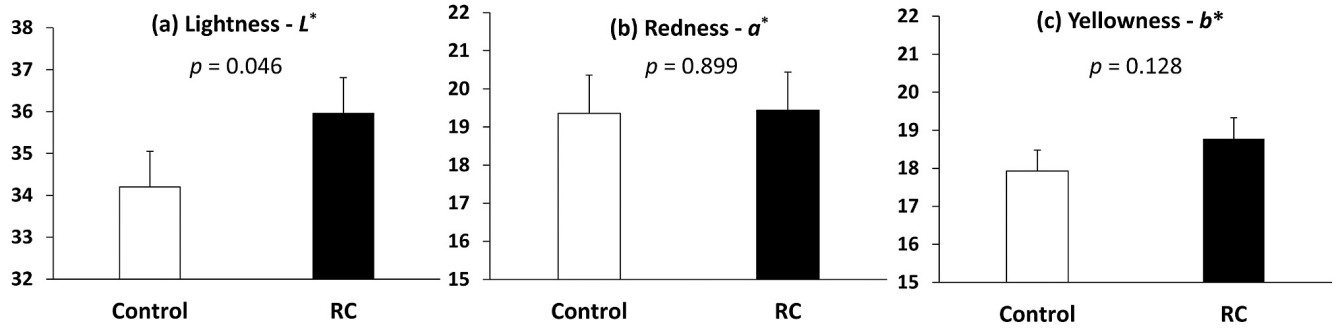

Fig. 3. Effect of Rinse&Chill® (RC) on instrumental colour measurements of fresh lamb loins before packaging and retail display. RC = Rinse&Chill®; control = no RC. Least-square means are shown, and error bars are standard error of difference (SED).

Table 2
Effects of storage condition (SC; fresh vs. frozen-thawed) and packaging type (PT; HiOxMAP vs. VSP) on instrumental colour measurements of lamb loins at the end of 8-day retail display, values in the table are least-square means and the SED of the interaction is shown.

	Fresh		Frozen-thawed		SED	*p*-values		
	HiOxMAP[1]	VSP[1]	HiOxMAP	VSP		SC	PT	SC × PT
*L**	38.03	35.28	42.73	38.43	1.17	0.006	<0.001	0.139
*a**	17.29	18.11	8.24	17.37	1.34	<0.001	<0.001	<0.001
*b**	16.69	16.66	12.26	10.00	0.57	<0.001	<0.001	0.004
Chroma	24.09	24.63	15.18	20.22	1.02	<0.001	<0.001	0.009
Hue angle (degrees)	44.25	42.69	57.37	30.63	3.35	0.812	0.001	<0.001

[1] HiOxMAP – High oxygen modified atmosphere packaging (80% O_2, 20% CO_2), VSP – Vacuum skin packaging. SED – standard error of differences for two-way interactions. There were no three-way interactions ($p > 0.05$).

Table 3
Effect of storage condition (SC; fresh vs. frozen-thawed) and packaging type (PT; HiOxMAP vs.VSP) on water-holding capacity and texture of lamb loins at the end of 8-day retail display, values in the table are least-square means and the SED of the interaction is shown.

	Fresh		Frozen-thawed		SED	*p*-values		
	HiOxMAP[1]	VSP[1]	HiOxMAP	VSP		SC	PT	SC × PT
WBSF[2] (N)	2.78 (16.10)	2.80 (16.33)	3.00 (20.03)	2.68 (14.63)	0.10	0.522	0.007	0.011
TPA Hardness (N)	28.34	27.31	27.21	24.18	2.55	0.201	0.009	0.21
Purge Loss (%)	5.74	9.19	10.83	5.45	1.22	0.375	0.091	<0.001
Cooking Loss (%)	15.47	14.68	14.98	18.5	1.21	0.064	0.06	0.019

[1] HiOxMAP – High oxygen modified atmosphere packaging (80% O_2, 20% CO_2); VSP – Vacuum skin packaging; [2]WBSF – Warner-Bratzler shear force values were transformed with natural logarithm for data analysis. Values in parentheses are back transformed data; TPA – Texture profile analysis; SED – Standard error of differences for two-way interactions. No effect of carcass treatment (Rinse&Chill® vs. control) on any parameters ($p > 0.05$). There were no three-way interactions ($p > 0.05$).

distinguish the difference in hemoglobin concentration between treatments. Compared to the study of Kethavath et al. (2021) and Erazo-Castrejón et al. (2019), we postulate that RC removed more residual hemoglobin in lamb in the present study, hence causing a detectable reduction in total heme protein content. It is also possible that RC removed some of the myoglobin from the lamb, similar to the effect of water infusion found by Bekhit et al. (2005). The reduction in total heme protein content coincided with an increase in *L** in the present study, in agreement with previous studies on infused lamb (Farouk & Price, 1994; Fowler et al., 2017; Kethavath et al., 2021).

RC reduced lipid oxidation in lamb loins compared to control at the end of the 8-day retail display. This is supported by the results of Kethavath et al. (2021) who found that RC reduced TBARS and hexanal in cooked pork, which are indicators of lipid oxidation. Bekhit et al. (2005) also found that water infusion reduced the TBARS values in over-wrapped lamb after 6 days of retail display. However, water infusion had no effect on the TBARS of over-wrapped lamb after 3-day retail display under the same conditions (Bekhit et al., 2005). Similarly, Fowler et al. (2017) found no difference in TBARS between RC and control lamb after 3 days of retail display in overwrap. Based on the results in the present and previous studies, it appears that RC only exhibits its antioxidant effect in a challenged meat system, such as cooked meat or meat in overwrap for longer than 3 days.

The effect of RC on lipid oxidation could possibly be explained by the reduction of heme protein in the RC lamb. Bekhit et al. (2005) attributed the reduced lipid oxidation in water-infused lamb to the reduction in total heme protein content. Kethavath et al. (2021) also suggested that RC reduced lipid oxidation in their study by reducing the hemoglobin content. It is well established that the heme proteins can induce lipid oxidation through multiple pathways (Faustman, Sun, Mancini, & Suman, 2010). Hence, we speculated that RC reduced lipid oxidation in lamb as a result of a lower total heme protein content.

Regardless of the RC or control treatment applied to the carcasses, HiOxMAP increased lipid oxidation, especially in the frozen-thawed lamb. Similar effects of HiOxMAP on lipid oxidation of the frozen-thawed lamb have been reported in previous studies (Bellés et al., 2018; Muela et al., 2015). Frozen-thawed meat is known to be more susceptible to oxidation due to the accumulation of prooxidant substances during frozen storage (Bellés et al., 2018; Muela et al., 2010; Muela et al., 2015). It is well-known that HiOxMAP results in higher oxidation in lamb (Frank et al., 2017; Kim et al., 2012), thus explaining the exacerbated lipid oxidation in frozen-thawed lamb packed in

HiOxMAP in our study. In the present study, the TBARS in the frozen-thawed lamb (6.33 mg MDA/kg meat) in HiOxMAP largely exceeded the recommended 2 mg MDA/kg meat threshold for TBARS (Campo et al., 2006), thus it is likely that the oxidised flavour would lead to a lower consumer acceptance. In contrast, the TBARS value in the frozen-thawed lamb in VSP was maintained at a relatively low level (1.29 mg MDA/kg meat), which was below the acceptability threshold. Therefore, VSP is preferred to HiOxMAP for the retail display of the frozen-thawed lamb to enhance its oxidative stability and to ensure a consistent eating quality.

Similarly, HiOxMAP overall led to higher protein carbonyl content than VSP. Protein carbonyl content is a commonly used indicator for protein oxidation in animal muscle (Fagan et al., 1999). HiOxMAP has been reported to facilitate protein oxidation and formation of protein carbonyls in meat (Estévez, 2011). Surprisingly, RC and storage condition had no effect on the protein carbonyls in lamb. Myoglobin (heme protein) and oxidised lipids are considered promotants in the formation of protein carbonyls (Estévez, 2011). However, the reduction in heme protein concentration did not influence protein carbonyl, and the HiOxMAP did not result in higher formation of protein carbonyl in frozen-thawed lamb compared to that of fresh lamb. This phenomenon could be explained by the limitations of the DNPH method, including the relatively low selectivity and sensitivity, in measuring protein carbonyl content in meat (Estévez, 2011). Estévez (2011) suggested that the DNPH method is more suitable for descriptive or qualitative purpose. Therefore, there is not enough evidence for us to conclude on the effect of RC and frozen storage on protein oxidation in lamb.

Retail packaging not only influenced lipid and protein oxidation but also influenced the instrumental colour of the frozen-thawed lamb. In the frozen-thawed lamb, the lower a^* and chroma and higher b^* and hue angle indicate that discolouration of the samples occurred during retail display in HiOxMAP compared to VSP. Specifically, a higher b^* and hue angle but lower a^* usually indicate browning of meat and loss of the red colour, while the decrease in chroma is associated with the loss of vivid and attractive colour (Mancini & Hunt, 2005; Miltenburg, Wensing, Smulders, & Breukink, 1992). A main cause for the discolouration of red meat is autooxidation of myoglobin to brown metmyoglobin (Mancini & Hunt, 2005), which can be facilitated by lipid oxidation in meat (Faustman et al., 2010). Therefore, the discolouration of the frozen-thawed lamb in HiOxMAP agreed with the prooxidative effect of HiOxMAP on lipid oxidation observed in this study. In addition, frozen storage has been shown to induce several changes, including the denaturation of myoglobin, cell disruption, and the loss of metmyoglobin reducing activity which impairs the redox stability of myoglobin (Faruk & Swan, 1998; Leygonie et al., 2012). All these factors could interact with the high oxygen content in HiOxMAP and accelerate the autooxidation of myoglobin. In contrast, the discoloration of the frozen-thawed lamb was less in VSP. The colour could, to a large extent, affect consumer's meat purchasing decision, and the discolouration of meat negatively affects consumer acceptance (Polkinghorne & Thompson, 2010). Hence, retail display of lamb in VSP is expected to add value to the frozen-thawed lamb by providing a better lamb colour stability.

HiOxMAP led to higher WBSF in the frozen-thawed lamb compared to VSP. The toughening effect of HiOxMAP on meat was also reported in previous studies (Kim et al., 2012; Lagerstedt, Ahnström, & Lundström, 2011). The toughening of lamb in HiOxMAP is most likely caused by the crosslinking of the myofibril proteins induced by the protein oxidation under the high oxygen environment (Kim et al., 2012). Whereas VSP effectively prevented these changes in the retail display of the frozen-thawed lamb. This was supported by the results of the protein carbonyl assay in our study. This could also explain the effect of packaging on the TPA hardness of the lamb samples. Unlike the frozen-thawed lamb, the retail packaging had no effect on the WBSF of the fresh lamb, hence we postulated that the fresh lamb is less susceptible to protein crosslinking. This difference between the fresh and the frozen-thawed lamb may, again, be attributed to the loss of oxidative stability during frozen storage (Leygonie et al., 2012). In summary, VSP is recommended to replace HiOxMAP for the purpose of retail packaging of frozen-thawed lamb to prevent meat toughening.

RC had no effect on the WBSF or pH of lamb in our study. Thess results are supported by the study of Mickelson et al. (2018) on lamb and the study of Yancey, Dikeman, Addis, Katsanidis, and Pullen (2002) on beef. Fowler et al. (2017) reported that RC reduced the WBSF of lamb, but did not change the pH. There is no consensus in literature on whether RC can tenderise meat, and it is likely that multiple factors, such as animal, muscle type, and ageing time, could mask any potential tenderizing effect of RC.

The purge loss of both the fresh and the frozen-thawed lamb was affected by the retail packaging. In the fresh lamb, the higher purge loss in VSP can be simply explained by the negative pressure applied to meat during the packaging process (Doherty, Sheridan, Allen, McDowell, & Blair, 1996; Gariepy, Amiot, Simard, Boudereau, & Raymond, 1986). In the frozen-thawed lamb, HiOxMAP resulted in a higher purge loss compared to VSP. The effects of HiOxMAP and VSP on the purge loss of the frozen-thawed lamb has not been reported previously. We hypothesise that the higher purge loss in HiOxMAP could be caused by the crosslinking of myofibril proteins mentioned previously, as the oxidation and crosslinking of the myofibril proteins are known to negatively impact the water-holding capacity of meat (Lund, Heinonen, Baron, & Estévez, 2011). However, the underlying mechanism needs further investigation.

It is worth noting that VSP led to higher purge loss in the retail display of fresh lamb compared to that of frozen-thawed lamb. We postulate that a considerable amount of free water in lamb loins was already lost during thawing and thus cannot be lost again in retail display. Muela et al. (2015) reported that around 1–4% of water in lamb was lost in the thawing process in vacuum packaging. Since the thawing loss was not measured in our study, there is not enough evidence for us to compare the purge loss of fresh and frozen-thawed lamb. Therefore, the water loss of lamb during thawing followed by retail display in VSP needs to be further investigated in future studies to help retailers improve the water-holding capacity of lamb.

VSP led to higher cooking loss in the frozen-thawed lamb, but not in fresh lamb. The high cooking loss in VSP could be explained by the continued ageing of meat during the display period of VSP lamb. The display period for VSP lamb is known to be similar to the ageing process in traditional vacuum environment, which allows effective proteolysis of the meat proteins (Frank et al., 2017). Long-term ageing has been shown to increase cooking loss in beef, pork, and lamb (Peng et al., 2019; Purslow, Oiseth, Hughes, & Warner, 2016; Vaskoska, Ha, Naqvi, White, & Warner, 2020). Purslow et al. (2016) postulated that degraded proteins in the aged meat are less capable of entrapping water during cooking relative to unaged meat. In addition to the VSP, the freezing process and the frozen storage are known to impair the water-holding capacity of meat due to the disruption of cellular and myofibril structures by the ice crystals (Leygonie et al., 2012). The combination of these two factors could lead to an increase in the cooking loss, although their individual effect might be minor. Therefore, the high cooking loss was possibly caused by the synergistic effects of frozen storage and VSP in the present study.

5. Conclusion

RC led to lower lipid oxidation in the lamb loins, likely through reducing the concentration of heme proteins. Also, RC increased the lightness of lamb before retail display but had no effect on other quality characteristics at the end of the retail display period. The effects of retail packaging on quality of lamb were dependent on its storage condition. In the frozen-thawed lamb, VSP led to lower lipid oxidation, redder instrumental colour, and more tender meat compared to HiOxMAP. Therefore, it is recommended that RC and VSP be used in the lamb industry, especially in the supply chain of the frozen-thawed lamb, to

deliver a more consistent quality at the retail point.

CRediT authorship contribution statement

Zhenzhao Li: Investigation, Methodology, Data curation, Formal analysis, Writing – original draft, Visualization. **Robyn D. Warner:** Resources, Writing – review & editing, Visualization, Supervision. **Minh Ha:** Resources, Writing – review & editing, Visualization, Supervision.

Declaration of Competing Interest

Robyn D Warner and Minh Ha declare a relationship with MPSC Incorporation that includes consulting and advisory. MPSC Inc. had no influence on the experimental design, data collection, analysis or manuscript preparation of this study.

Data availability

The authors do not have permission to share data.

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