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The effects of varying gas concentrations and exposure times on colour stability and shelf-life of vacuum packaged beef steaks subjected to carbon monoxide pretreatment

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ABSTRACT

The aim of this study was to assess the effects of a range of carbon monoxide (CO) concentrations and exposure times on the colour stability and shelf-life of vacuum packaged *Longissimus thoracis et lumborum* (LTL) beef steaks. Steaks were exposed to five pretreatments: 0 (Control), 0.4, 1, 3 and 5% CO combined with 60% CO_2, (balance N_2) for 5, 7 or 24 h. They were then vacuum packed and stored at 2 °C for 28 days. The optimum pretreatment was 1% CO for 5 h as this enhanced initial desirable colour yet allowed discolouration to reach unacceptable levels (a* = 12, C* = 16) by the use-by-date (28 days). *K/S* ratios verified that the optimum CO pretreatment does not mask spoilage. All CO pretreatments had no effect on pH or purge loss ($P > 0.05$). Reducing the CO concentration and decreasing the exposure time achieved a desirable colour, without masking spoilage, thereby minimizing the processing time and improving the safety of workers.

1. Introduction

There is an emerging demand for more value-added meat packaging technologies which enhance meat quality. Overall, colour is the most important quality trait judged at point of purchase as sensory traits cannot be physically assessed prior to consumption (Carpenter, Cornforth, & Whittier, 2001; Van Rooyen, Allen, Crawley, & O'Connor, 2017). Consumers use colour as an indicator of freshness and wholesomeness and this influences perceived meat quality (Carpenter et al., 2001; Issanchou, 1996), while discoloration is associated with unwholesomeness (Faustman & Cassens, 1990) and leads to economic losses (Kropf, Hunt, & Piske, 1986) and food waste. Packaging directly affects the colour and quality of meat (Bernués, Olaizola, & Corcoran, 2003) but high oxygen MAP which is widely used to enhance meat colour negatively affects tenderness (Clausen, 2004; Tørngren, 2003). Innovations in meat packaging which enhance colour coupled with increased tenderness would greatly assist the meat industry. Vacuum packaging permits prolonged storage in an anoxic environment favouring tenderness but has a negative effect on meat colour. Carbon monoxide (CO) applied as a pretreatment prior to vacuum packaging would enhance the colour and tenderness (Van Rooyen, Allen, Gallagher, & O'Connor, 2018). CO binds to myoglobin to form carboxymyoglobin and produces a much more stable cherry red colour compared to oxygen (oxymyoglobin) (El-Badawi, Cain, Samuels, & Anglemeier, 1964). Legislation on the use of CO in meat packaging varies globally. The EU prohibited the use of CO in meat packaging systems due to concerns it might be used to mislead consumers by presenting microbiologically spoiled meat with an attractive colour so that consumers may falsely perceive the meat as "fresh" since the colour is retained (European Commission, 2001). This would be a major consumer safety concern as safety is considered a prerequisite by consumers (Van Wezemael, Verbeke, Kügler, de Barcellos, & Grunert, 2010).

Previous authors have demonstrated the benefits of applying 5% CO pre-treatments to enhance colour stability using a 24 h exposure period (Aspé, Roeckel, Martí, & Jiménez, 2008; Jayasingh, Cornforth, Carpenter, & Whittier, 2001). However, spoilage has been masked due the extended exposure period, raising concerns for consumer safety. Van Rooyen, Allen, Crawley et al. (2017), showed that the exposure time can be reduced to 5 h to enhance meat colour while allowing discoloration to occur by day 28 before spoilage occurred, without having adverse effects on microbiological safety or quality attributes. This study addressed consumer safety concerns that CO may be used to mask meat spoilage. Following this, a review (Van Rooyen, Allen, & O'Connor, 2017) concluded that the most important issues for the prohibition have been addressed including safety and consumer

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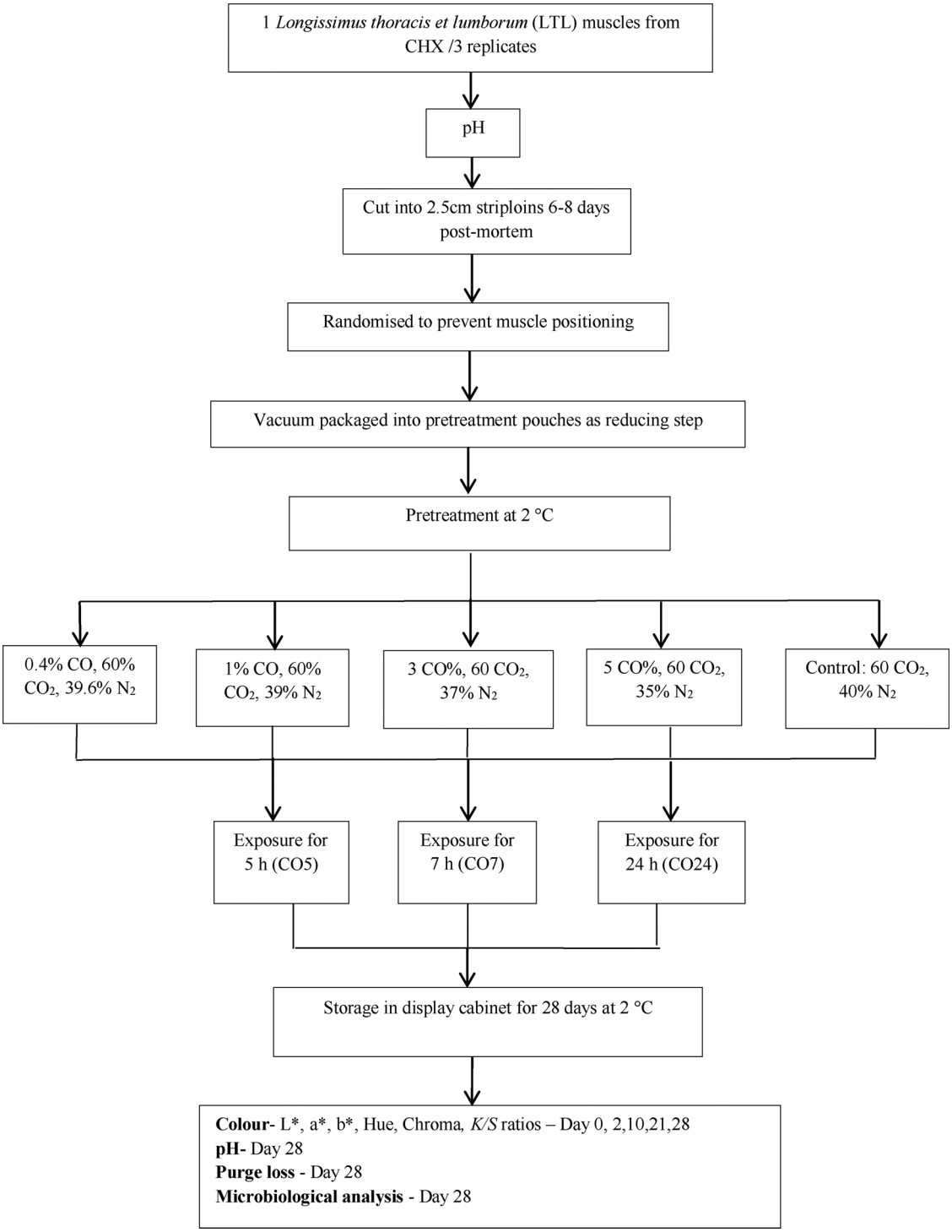

Fig. 1. Schematic of experimental process.

acceptance issues. The authors proposed that (Directive No 95/2/EC, 1995) has been satisfactorily addressed and therefore the use of CO in meat packaging systems should be reconsidered within the EU. In this context, if CO pretreatments were to be regulated further reducing CO pretreatment gas concentration may enable safer handling conditions for workers, while a reduced exposure time may minimise processing time.

The concentration of CO pretreatment and the effect of colour stability have been investigated (Sakowska, Guzek, Glabska, & Wierzbicka, 2016; Sorheim et al., 2006). Sorheim et al. (2006) applied 1% CO pretreatment for 5 days prior to vacuum packaging to pork and beef *Semimembranosus* muscles for fermented meat model systems (batter used to prepare salami sausages) and salami sausages or applied 1% CO pre-treatment for 1 day prior to vacuum packaging to pork and beef *Semimembranosus* muscles for cooked model meat systems (batter used to prepare hotdogs) and hotdogs. They concluded that CO could be applied as an alternative colourant to nitrite to enhance the colour of cooked or cured type sausages. Similarly, Sakowska, Guzek, Glabska et al. (2016) also applied very low concentrations of CO (0.1%, 0.3% or 0.5%) as a pretreatment for 48 h prior to vacuum packaging to raw and cooked beef striploin steaks. Results showed that pretreatment with a CO concentration of 0.5% for 48 h was optimum to enhance colour

stability while avoiding pinking (carboxymyoglobin layer being retained after cooking). The evidence provided by these authors suggests that it is possible to reduce the concentration of CO in the pretreatment gas mixture and still achieve the desirable colour stability.

Nevertheless, the CO concentration and exposure time is proportional to colour stability and prolonged exposure time may mask spoilage or result in pinking. Sorheim et al. (2006), proposed that varying the length of exposure to CO pretreatment may regulate the colour over storage. While, Suman, Hunt, Nair, and Rentfrow (2014) also reported that the duration of exposure time to CO can determine the extent of the colour stability and the depth of the carboxymyoglobin layer formed. In this context, more research is required to determine the appropriate exposure time tailored to a reduced concentration of CO, if CO pretreatments were to be implemented within the meat industry. Therefore, the objective of this study was to investigate a range of CO concentrations (Control), 0.4, 1, 3 and 5% CO and exposure times (5, 7 or 24 h) on the colour stability and shelf-life of vacuum packaged beef steaks.

2. Materials & methods

2.1. Sample preparation

Sample preparation was carried as described by Van Rooyen, Allen, O'Connor et al. (2017) with minor modifications. One bovine *Longissimus thoracis et lumborum* (LTL) muscle (n = 1) from a Charolais-cross (CHX) heifer (age between 21–29 months) was obtained from a commercial meat producer for each of the three replicates. Steaks were cut (n = 15) (25 mm thick, 285.2 g – 388.0 g) at 6–8 days post-mortem and randomized to account for muscle positioning effects. Steaks were vacuum packaged (New Diamond Vac J-V006 W, Heavy Duty Automatic Vacuum Machine, Jaw Feng Machinery Co., LTD, Taiwan; vacuum pressure < 0.01 Torr held for 32 s) in a pouch (5-layer coextruded film with PA/Tie/PE/Tie/PE (OTR: < -70 cm^3 O_2/m^2/24 h at 23 °C and 50% RH, Versatile Packaging, Ltd., Castleblayney, Co. Monaghan, Ireland). This step was a reducing step before the CO pretreatment to limit the amount of oxymyoglobin occurring. Steaks were assigned to one of five gas concentrations (5% CO, 60% CO_2 and 35% N; 3% CO, 60% CO_2 and 37% N; 1% CO, 60% CO_2 and 39% N; 0.4% CO, 60% CO_2 and 39.6% N; or control (60% CO_2 and 40% N_2)) for 5, 7 or 24 h respectively and stored at 2 °C. At the end of the designated pretreatment time, steaks were quickly removed from the pretreatment pouches and immediately individually vacuum packed (Product # S303, Synpac, PA/PE (OTR: < 38 cm^3 O_2/m^2/24 h at 23 °C and 0% RH, Synpac Ltd, Saxon way, Priory Park West, Hessle, East Yorkshire, UK). To simulate retail conditions, samples were placed randomly in an open front-display cabinet (Cronos fan-assisted cabinet, Criosbanc, Padova, Italy) for 28 d storage (2 °C–2.5 °C) under continuous fluorescent lighting (Meat - Fluorescent Touchcoat T5 F18 W T8 176 Foodstar Meat Toughcoat, Havells Sylvania Fixtures UK, Ltd) (2115 lx). The display cabinet temperature was recorded on all three shelves at the meat surface every five minutes using Dataloggers (Lascar EasyLog-USB, Lascar Electronics Ltd, Salisbury, SP5, UK). To limit temperature fluctuations throughout the storage period and particularly during the defrost cycles (4 x 35 min, maximum temperature of 8 °C for 1 min) an insulated blind was pulled down.

2.2. Experimental design

Fig. 1. Schematic of experimental process

2.3. Instrumental colour analysis

Surface colour measurements, reflectance and absorbance readings were carried out using a HunterLab UltraScan Pro (Hunter Associates Laboratory., Inc., Reston, VA) on vacuum packed pretreated samples. The 25 mm viewing port was used and illuminant D_{65}, 10° was selected to match daylight. Firstly, the UltraScan Pro was standardized using a light trap and white tile covered in the same vacuum packaging material to eliminate any packaging effect (AMSA, 2012). Triplicate measurements for both CIE L* (lightness), a* (redness) b* (yellowness) and reflectance spectra from 400 to 700 nm (5 nm interval) were recorded on each steak within the vacuum packages on three separate locations and averaged. Chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) values were calculated. The a* value of 12 was chosen as the threshold value to determine discolouration which is comparable to a C* value of 16 which was reported as the limit of acceptability by MacDougall, Down, and Taylor (1986). These authors also used a Hunterlab and an illuminant D.

Surface reflectance spectra values at 474, 525, 572 nm were calculated via linear interpolation. Surface reflectance data are converted to *K/S* ratios using the light absorbance (*K*) and scattering properties (*S*) and calculated following the Kubelka-Munk equation ($(1 - R)^2 \div 2R$) to estimate each myoglobin redox form (deoxymyoglobin (DMb) (K/S_{474})/(K/S_{525}), metmyoglobin (MMb) (K/S_{572})/(K/S_{525}) and carboxymyoglobin (COMb) (K/S_{610})/(K/S_{525})) and attain more linear data (AMSA, 2012). Additionally, 100% reference standards for 100% DMb, MMb and COMb were also prepared to estimate myoglobin redox forms (AMSA, 2012). Colour stability was analysed at 0, 2, 10, 21 and 28 days. A previous study carried out by this group (Van Rooyen et al., 2018) showed the benefits of using *K/S* ratios to estimate myoglobin redox forms and provide a greater understanding of surface colour stability.

2.4. pH

Determination of pH was carried out using a glass probe pH electrode (Thermo Scientific pH meter 420 A, Orion Research Inc.). Steaks were removed from their vacuum pouches and triplicate pH measurements were recorded on the surface of each pretreated steak.

2.5. Purge loss

Purge loss, a measurement of the water loss from meat, was carried out as described by (Krause, Sebranek, Rust, & Honeyman, 2003) and measured on samples after 28 days of display at 2 °C. Firstly, the package weights of the unopened pretreated steaks were recorded. The steaks were then removed from the packages, blotted dry and reweighed. The percentage of purge loss was calculated according to the following equation:

$$\%\,Purge\ loss = \frac{(Weight\ of\ package + steaks) - (Weight\ of\ steaks) \times 100}{(Weight\ of\ package + steaks)}$$

2.6. Microbiological analysis

To confirm that CO does not mask meat spoilage, microbiological analysis was determined at the end of the shelf-life (28 days storage at 2 °C). Microbiological analysis was performed as previously described by Van Rooyen, Allen, Crawley et al. (2017). Results were expressed as the log of colony forming units (CFU)/ per cm^2 of the steak surface area (log_{10} cfu/cm^2).

2.7. Statistical analysis

Three biological replicates were carried out on three separate occasions. Statistical analyses were performed using GenStat (Release 14.1 Copyright 2011) using two separate forms of analyses. The first was repeated measures ANOVA (rANOVA) with a 5 × 3 × 5 factorial split plot design was used to analyse colour variables including five CO concentrations (0.4%, 1%, 3%, 5% and 0% CO (control)) × three

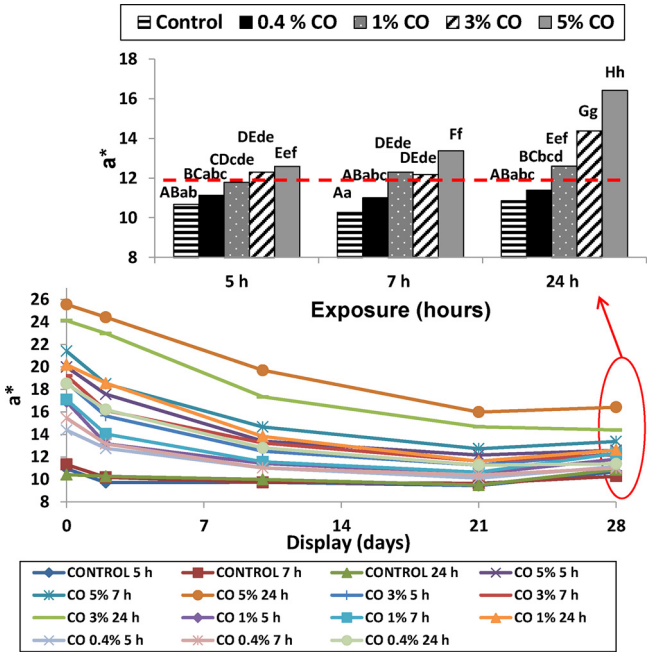

Fig. 2. Least square means showing CO pretreatments (0.4%–5% CO) and exposure times (5–24 h) for a* values over 28 days storage. Different uppercase (a–h) indicates significant differences by exposure*day. Different lowercase (a–h) indicates significant differences by gas concentration*day. Statistical significance: ($P < 0.05$). Least significant difference (L.S.D.). Exposure*day L.S.D. = 0.71. Gas concentration*day L.S.D = 0.91.

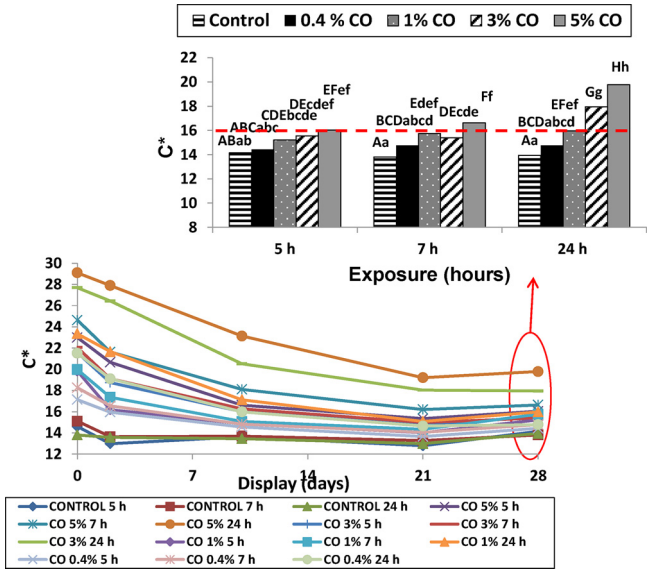

Fig. 3. Least square means showing CO pretreatments (0.4%–5% CO) and exposure times (5–24 h) for C* values over 28 days storage. Different uppercase (a–h) indicates significant differences by exposure*day. Different lowercase (a–h) indicates significant differences by gas concentration*day. Statistical significance: ($P < 0.05$). Least significant difference (L.S.D.). Exposure*day L.S.D. = 0.88. Gas concentration*day L.S.D = 1.32.

exposure times (5 h, 7 h and 24 h) × five display days (0, 2, 10, 21 and 28). Due to the large amount of interactions over display amongst the treatments, only the end of shelf life (day 28) interactions were presented for colour data (Figs. 2–6). In the second microbiological analysis, purge loss and pH were analysed separately using a 5 × 3 × 1 factorial split plot design including five CO concentrations (0.4%, 1%, 3%, 5% and 0% (control)) × three exposure times(5 h, 7 h & 24 h) ×

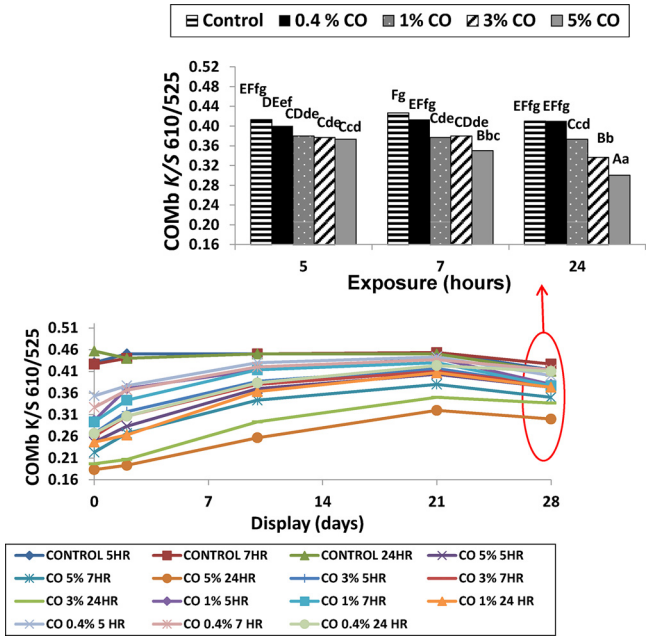

Fig. 4. Least square means showing the effect of CO pretreatments (0.4%–5% CO) and exposure times (5–24 h) for Carboxymyoglobin (COMb) *K/S* 610/525 ratios over 28 days storage. Different uppercase (a–h) indicates significant differences by exposure*day. Different lowercase (a–h) indicates significant differences by gas concentration*day. Statistical significance: ($P < 0.05$). Least significant difference (L.S. D.) Exposure*day L.S.D. = 0.02. Gas concentration*day L.S.D = 0.03.

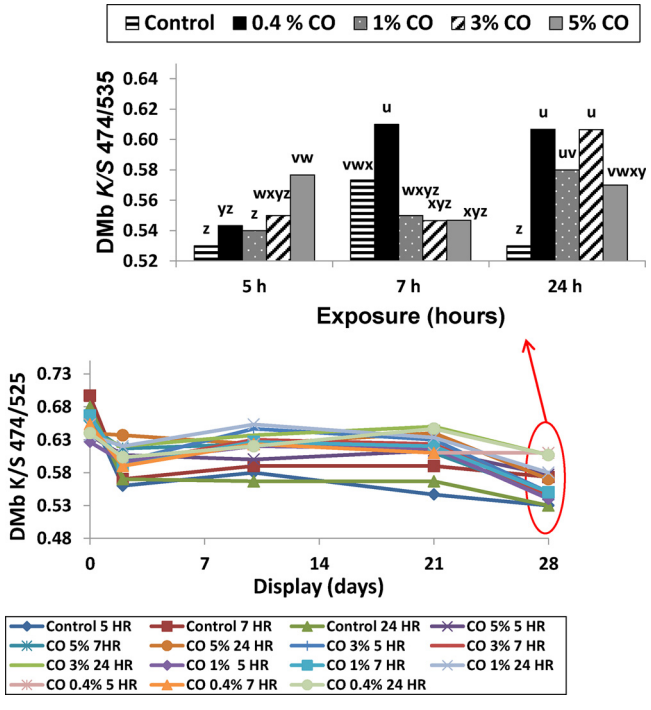

Fig. 5. Least square means showing the effect of CO pretreatments (0.4%–5% CO) and exposure times (5–24 h) for deoxymyoglobin (DMb) *K/S* 474/525 ratios over 28 days storage. Different lowercase (u–z) indicates significant differences by display day. Statistical significance: ($P < 0.05$). Least significant difference (L.S.D.) Exposure*day L.S.D. = 0.71. Display day L.S.D = 0.03.

one display day (28). Post-hoc analysis for both colour data and quality data using F-protected LSD was to test the significance of differences between means if factors were significant. Significance was defined at

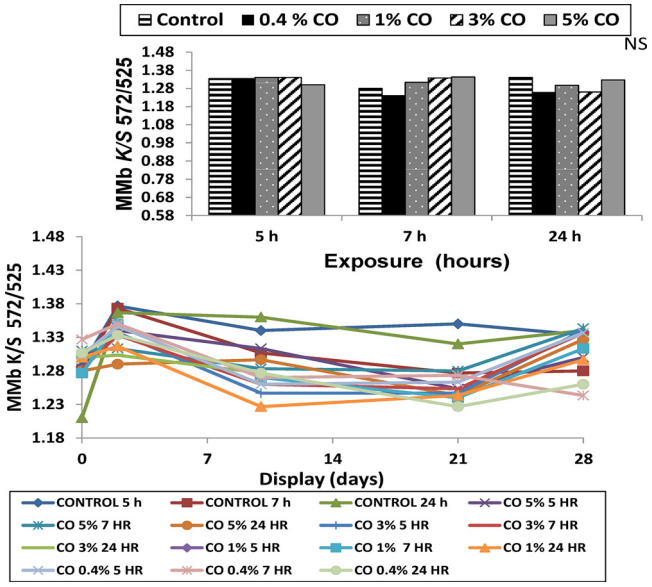

Fig. 6. Least square means showing the effect of CO pretreatments (0.4%–5% CO) and exposure times (5–24 h) for metmyoglobin (MMb) *K/S* 572/525 ratios over 28 days storage. Statistical significance: ($P < 0.05$). NS = not significant.

($P < 0.05$).

3. Results & discussion

3.1. Instrumental colour analysis

3.1.1. a* values

Immediately after pretreatment, surface redness (a*) increased with both CO concentration ($P < 0.05$) and exposure time ($P < 0.05$) (Fig. 2). For all combinations of CO concentrations and exposure times the a* value decreased during storage. However, there were significant CO concentration × display day ($P < 0.001$) and exposure time × display day ($P < 0.001$) interactions as the difference between gas concentrations and exposure times diminished with storage (Fig. 2).

The combinations of CO concentration and exposure time that achieved the desirable red colour while allowing discoloration to reach an unacceptable level by use-by-date (a* = 12) were 5% CO/5 h, 3% CO/5 h, 3% CO/7 h, 1% CO/5 h and 1% CO/7 h and 0.4% CO/ 24 h. This is consistent with previous work (Van Rooyen, Allen, & O'Connor, 2016; Van Rooyen, Allen, Crawley et al., 2017) who reported that 5% CO/5 h was the optimum pre-treatment. However, the results in the present study show that acceptable colour stability can be achieved by reducing the CO concentration to 1% with the same exposure time of 5 h as a* values fell to just below the discolouration threshold (a* = 12). Using a gas concentration of 0.4% CO for 5, 7 or 24 h resulted in the discoloration threshold (a* < 12) being reached after only 10 days. Therefore a pretreatment using a CO concentration lower than 1% would not be appropriate for exposure times of 24 h or less.

Others have investigated applying a range of CO pretreatments prior to vacuum packaging (Clark, Lentz, & Roth, 1976; Rozbeh, Kalchayanand, Field, & Johnson, 1993; Brewer & Wu, 1993; Jayasingh et al., 2001; Sakowska, Guzek, Glabska et al., 2016; Sakowska, Guzek, Sun, & Wierzbicka, 2016; Sakowska, Guzek, & Wierzbicka, 2016). However, in general it appears that reduced CO concentrations require extended exposure periods or vice versa. Recently, Sakowska, Guzek, Glabska et al. (2016) reported positive findings that pretreatment with 0.5% CO for 48 h can increase colour stability while avoiding persistent pinking. Although this study demonstrates positive results, an exposure time of 48 h may not be practically feasible for industry. Reducing the CO gas concentration to 1% for 5 h is may enable safer handling conditions for workers, while not reducing processing time compared to previous studies where CO pretreatments were applied for extended periods (24–48 h) (Aspé et al., 2008; Jayasingh et al., 2001; Sakowska, Guzek, Glabska et al., 2016).

3.1.2. Chroma values

Following a similar trend to a* values, immediately after pretreatment, surface chroma (C*) values increased with both CO concentration ($P < 0.001$) and exposure time ($P < 0.001$) (Fig. 3). For all combinations of CO concentration and exposure times the C* value decreased during storage. However, there were significant CO concentration × display day ($P < 0.001$) and exposure time × display day ($P < 0.001$) interactions as the difference between gas concentrations and exposure times diminished with storage (Fig. 3).

At the end of storage (28 days) all treatments except for the controls had C* values above 14, which is considered brown or discoloured (MacDougall et al., 1986). The 5% CO/5 h pretreatment had a C* value of (16.04) which is just above the limit of acceptability (C* = 16) (MacDougall et al., 1986) in line with previous work (Van Rooyen, Allen, Crawley et al., 2017, 2018). For 1% CO/5 h C* was 15.2 on day 28, just below the limit of acceptability, but were above C* = 14 which is considered as 40% metmyoglobin and causes consumer purchase rejection (Greene, Hsin, & Zipser, 1971). The results for C* values support those for a* values that a reduced CO concentration for CO-pretreatment can achieve an acceptable colour stability while allowing discolouration over storage.

3.2. K/S ratios

K/S ratios are a useful quantitative, non-destructive method used to assess the proportions of myoglobin redox forms on meat surfaces. The results for *K/S* ratios for COMb, DMb and MMb are presented in Figs. 4–6.

K/S ratios for COMb is an indicator of redness (AMSA, 2012; Jeong & Claus, 2010). The results for COMb *K/S* values were consistent with a* and C* values with values immediately after pretreatment increasing with CO concentration ($P < 0.001$) and exposure time ($P < 0.001$) (Fig. 4). COMb *K/S* values increased with storage up to 21 days and then declined slightly for all combinations of CO concentration and exposure time except the controls, which changed little during display. The increase in COMb values over storage indicates that discoloration occurred as lower values indicate more intense redness and higher values represent discoloration or in the case of the controls no carboxymyoglobin formation. There was a significant CO concentration × display day interaction ($P < 0.001$) for *K/S* COMb with the effect of gas concentration diminishing with display time. A significant exposure × display day interaction also occurred ($P < 0.001$) with the effect of exposure time decreasing with display time. Control samples had high COMb *K/S* values on day 0 (0.43- 0.46) as expected since these were not exposed to CO. The CO pretreatment combinations resulted in COMb *K/S* values ranging from 0.18 to 0.35 due to the binding of CO to the six co-ordination position of the haem group on the myoglobin molecule. CO is highly stable and has strong binding ability (Jeong & Claus, 2010; Sebranek, Hunt, Cornforth, & Brewer, 2006). However, over storage COMb *K/S* values for all CO pretreatments shifted towards and were near to the 0% COMb reference standard of 0.52 prepared according to AMSA (2012). This demonstrates that discolouration occurred in all CO pretreatments, verifying that CO disappears over time as it is lost from the six co-ordinate position of the iron-porphyrin ring so that very little COMb was present at the end of storage (28 days). This is in line a* and C* (Figs. 2 & 3) values and with previous work by this group and shows that the *K/S* ratio is a useful method to monitor meat discolouration (Van Rooyen et al., 2016). In contrast to this, Jeong and Claus (2010) reported that reflectance ratios were not definitive of colour changes in opened CO-MAP packages, however the conditions were different in this present study as colour

was measured in intact packages.

K/S ratios for DMb are shown in Fig. 5. Storage had a significant effect on DMb *K/S* ratios ($P < 0.001$) with the value increasing up to day 2 then decreasing up to day 21 towards the 100% DMb reference standard of 0.58 (O'Keefe & Hood, 1980) and increasing again at 28 days for all treatment combinations. This slight initial increase in deoxymyoglobin formation from day 0 to day 2 may be attributed to enzymatic activity within the muscle tissues utilising any residual oxygen within the vacuum packs, thus increasing reduction (deoxymyoglobin) (Bendall & Taylor, 1972). There was no gas concentration or exposure time effect observed for DMb values ($P > 0.05$). This result corresponds to previous work by this group where varying the CO exposure time had no effect on *K/S* ratios (Van Rooyen et al., 2016).

K/S ratios for MMb are a useful method for monitoring meat discolouration (Van Rooyen et al., 2018). The results for *K/S* ratios for MMb are presented in Fig. 6. There were no significant effects observed for CO concentration, exposure time or display in this present study ($P > 0.05$). In contrast, Lanier, Carpenter, Toledo, and Reagan, (1978), found that varying CO concentration (1–5%) increased MMb reduction. At 28 days, the ratios ranged from 1.24 to 1.34 for CO-pretreatments and from 1.28 to 1.34 for the controls indicating that very little MMb was present as these values were close to 1.4 which represent, 0% MMb according to O'Keefe and Hood (1980). This result indicates that this packaging system maintained an efficient anaerobic atmosphere throughout storage.

The authors conclude that carboxymyoglobin diminished over storage as confirmed by the a*, C* and *K/S* ratios for COMb values, therefore converting samples to the deoxymyoglobin redox form. However, the lack of MMb present from the *K/S* ratios MMb values suggests autoxidation of deoxymyoglobin to metmyoglobin had not occurred by 28 days even at low partial pressure. The results for *K/S* ratios MMb also suggests that a sufficient anaerobic environment was held below < 0.1% in this packaging system and very little residual oxygen was present which potentially could have assisted autoxidation.

3.3. pH

The pH is one of the main factors which can affect meat colour (AMSA, 2012). The pH values measured at the end of shelf-life are presented in Table 1. There were no significant differences reported amongst the treatment combinations or the controls ($P > 0.05$). Meat is considered to have a robust buffering system which may have contributed to this result (Ramanathan, Mancini, Naveena, & Konda, 2010). Similar findings were also evident in previous studies by this group and others where CO pretreatments before vacuum packaging beef steaks did not have a significant effect on the pH (Aspé et al., 2008; Sakowska, Guzek, Glabska et al., 2016; Van Rooyen et al., 2018).

3.4. Purge loss

Purge loss from fresh meat and can have a negative effect on the product yield and meat quality. Accumulation of purge is not only unappealing to consumers but can act as a substrate for bacterial growth and is particularly problematic in vacuum packaged meat products. However, applying CO pretreatments did not affect purge loss and there were no differences compared to the controls ($P > 0.05$) (Table 1). This result is in line with previous work by this group and others where CO pretreatments did not have a negative effect on purge loss (Aspé et al., 2008; Van Rooyen et al., 2018). Previous authors have shown that CO had no effect on purge loss when comparing CO-MAP and high-oxygen MAP (Krause et al., 2003; Stetzer et al., 2007).

3.5. Microbiological analysis

Gas concentration and exposure time had no effect on the microbial shelf-life as indicated by anaerobic mesophiles (TVCm), anaerobic psychrotrophiles (TVCp) (Table 2), Lactic acid bacteria (LAB) counts, and total *Enterobacteriaceae* counts (TEC) as values were similar for all treatments and the controls ($P > 0.05$) (Table 2). These results are in agreement with previous work by Van Rooyen, Allen, Crawley et al. (2017) where no bacteriostatic effect was evident due to the presence of CO. Initial bacterial counts were also enumerated on untreated steaks on day 0 and confirmed bacterial populations were < 2.0 log CFU/cm^2 (data not shown).

CO has previously been demonstrated to inhibit microbial populations at levels of above 5% CO (Gee & Duane Brown, 1981). Since the range of CO concentrations applied in this study were 5% CO or less, this may explain why no effect was observed in the present study. Although a CO concentration effect was observed for *Pseudomonas* spp. ($P < 0.01$) this is most likely a chance effect as no particular pattern was observed across treatments. All microbial values for all bacteria enumerated were within the acceptable threshold of 7–8 log CFU/cm^2 so the meat would not be considered spoiled (Ayres, 1960; FSAI (2014); James & James, 2000; Lavieri & Williams, 2014. Detection of meat spoilage is typically characterised by discolouration, the formation of slime and putrid off-odours which will occur when bacterial populations reach this upper limit of acceptability (7–8 log CFU/cm^2)(Egan, Eustace, & Shay, 1988; Reid, Fanning, Whyte, Kerry, & Bolton, 2017). Overall, this result confirms that applying CO pretreatments using various gas combinations and exposure times does not mask meat spoilage. More specifically, the optimum pretreatment of 1% CO for 5 h reached discolouration according to colour data (Figs. 2–4), and this seems to be proportional to bacterial populations nearing the upper the limit for acceptability.

Table 1
Effect of a range of carbon monoxide concentration pretreatment concentrations and exposure times for pH and purge loss (%) on LTL beef steaks after 28 day storage display.

Treatments	Exposure	pH	Purge Loss (%)
Control	24 h	5.29	4.39
Control	7 h	5.38	4.92
Control	5 h	5.32	5.46
CO 5%	24 h	5.37	5.10
CO 5%	7 h	5.38	5.26
CO 5%	5 h	5.36	5.11
CO 3%	24 h	5.37	6.07
CO 3%	7 h	5.37	3.76
CO 3%	5 h	5.37	4.90
CO 1%	24 h	5.32	4.47
CO 1%	7 h	5.35	6.16
CO 1%	5 h	5.38	5.46
CO 0.4%	24 h	5.45	4.97
CO 0.4%	7 h	5.40	5.26
CO 0.4%	5 h	5.33	4.97

	P value	L.S.D.	*P* value	L.S.D.
Gas Concentration	0.792	0.10	0.877	0.99
Exposure	0.828	0.08	0.890	0.79
GasConc*Exposure	0.931	0.18	0.152	1.72

Statistical significance: ($P < 0.05$).
Least significant difference (L.S.D).

4. Conclusion

While it is has been widely reported that CO acts as a colour enhancer for meat, there is limited knowledge on the effects of varying the pre-treatment CO concentration and exposure time on the colour stability of vacuum packaged beef steaks. We have shown that at least for the LTL muscle from prime Charolais X heifers, the concentration of CO can be reduced from 5% CO to 1% CO with the same exposure time of 5 h while achieving the objective of the colour deteriorating beyond acceptability before the meat is spoiled to avoid consumers being

Table 2
Effect of a range of carbon monoxide concentration pretreatment concentrations and exposure times for TVC, lactic acid bacteria, TEC and *Pseudomonas spp*. on LTL beef steaks after 28 day storage display (Means (log cfu/cm^2)).

Treatments	Exposure	TVCm	TVCp	LAB	TEC	*Pseudomonas spp.*
Control	24 h	6.66	8.12	5.32	2.97	4.00^{hi}
Control	7 h	6.55	7.69	5.72	2.78	3.95^{i}
Control	5 h	6.43	7.79	5.27	2.83	4.20^{gh}
CO 5%	24 h	6.40	7.92	5.52	3.27	4.76^{cde}
CO 5%	7 h	6.34	7.95	5.34	2.82	4.83^{cd}
CO 5%	5 h	6.63	7.83	5.53	3.11	4.54^{ef}
CO 3%	24 h	6.43	7.59	4.84	2.66	4.83^{cd}
CO 3%	7 h	6.66	7.87	5.40	2.80	5.16^{ab}
CO 3%	5 h	6.83	7.89	5.94	3.09	4.96^{bc}
CO 1%	24 h	6.44	7.90	5.53	2.66	5.11^{ab}
CO 1%	7 h	6.38	7.90	5.57	3.00	4.52^{f}
CO 1%	5 h	6.32	7.58	5.74	3.39	5.32^{a}
CO 0.4%	24 h	6.56	7.95	5.81	2.20	5.28^{a}
CO 0.4%	7 h	6.59	7.67	5.49	3.04	4.64^{def}
CO 0.4%	5 h	6.73	7.80	4.96	3.14	4.26^{g}

	P value	L.S.D.	*P* value	L.S.D.	*P* value	S.E.D.	*P* value	L.S.D.	*P* value	L.S.D.
Gas Concentration	0.947	0.76	0.884	0.29	0.989	0.91	0.981	1.08	0.002	0.47
Exposure	0.941	0.59	0.552	0.23	0.951	0.70	0.678	0.83	0.586	0.36
GasConc × Exposure	0.999	1.31	0.334	0.50	0.867	1.57	0.988	1.88	0.209	0.81

TVCm: Anaerobic mesophile.
TVCp: Anaerobic psychrotrophiles.
LAB: Lactic acid bacteria.
TEC:*Enterobacteriaceae*.
Statistical significance: ($P < 0.05$).
Least significant difference (L.S.D).

misled. Other combinations of CO concentration and exposure time gave the same result which indicates that there is sufficient flexibility in this technology to suit different meat processing regimes. *K/S* ratios were used to determine the reduction in COMB over storage and were in agreement with the instrumental colour analysis. All CO pretreatment combinations had no effect on pH or purge loss ($P > 0.05$).

The results from this present study show potential for the use of CO as a pretreatment to enhance the appearance of vacuum packaged beef LTL steaks. Further work on a broader range of animal types and muscles is required to determine whether these conclusions are broadly applicable. The use of CO in meat packaging systems in the EU should be re-evaluated as outlined in recent work by Van Rooyen, Allen, O'Connor et al. (2017) as CO pretreatments can be tailored to ensure that the meat colour becomes unacceptable prior to the meat becoming spoiled so that consumers are not misled about the safety of the meat on display.

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