CO PRE-TREATMENT OF VACUUM PACKAGED BEEF STEAKS

Lauren A. Van Rooyen[1, 2,*], Paul Allen[1], Eimear I. Gallagher[2] and David. I. O'Connor[2]

[1] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

[2]Dublin Institute of Technology, School of Food Science and Environmental Health, Cathal Brugha Street, Dublin 1, Ireland

[*]Corresponding author email: Lauren.VanRooyen@teagasc.ie

Abstract –Carbon monoxide (CO) exposure prior to vacuum packaging gives the meat a stable bright red colour while avoiding the problems associated with oxidation. The objective of this study was to assess the effect of display temperature and novel CO pre-treatments on the colour stability of vacuum-packed beef steaks *Longissimus thoracis et lumborum* (LTL). Steaks were exposed to a gas mixture of 5% CO, 60% CO_2 and 35% N_2 for 3, 5 & 7 h, followed by 28 days display at 2 °C (good industry practice) or 6 °C (mild abuse). Steaks were assessed for colour stability (a*, chroma values and *K/S* reflectance ratios) at days 0, 2, 10, 21, 28. A 5 h CO pre-treatment induced the desirable red colour, while discolouration reached unacceptable levels (a* > 12, C*>16) by day 28 so that meat spoilage was not masked by acceptable colour. Temperature had no effect on a* and chroma values (P>0.05), but did affect *K/S* values (P<0.05).

Key Words – Carbon monoxide, colour stability, packaging.

I. INTRODUCTION

Consumer perception of meat quality is based on an attractive appearance and good eating quality. High oxygen MAP packaging enhances the colour but eating quality can be impaired by oxidation. Carbon monoxide (CO) enhances appearance by producing a stable cherry red colour in the vacuum-pack and allows ageing to occur within the package increasing tenderness while prolonging shelf-life. Previous research has focused on using 5% CO-pretreatments for 24 h [6, 2]. Van Rooyen *et al* [10] 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, addressing concerns that CO may be used to mask meat spoilage. However, further research is necessary to determine the stability of CO-pretreatments, in the case of mild temperature (6 ˚C) abuse, which may occur due to mishandling during storage, as temperature has a direct influence on colour stability [9]. There are no reports on the effect of 5% CO-pretreatments prior to vacuum packaging beef steaks on the reflectance and absorbance properties of meat surfaces. The objective of this study was to investigate the effect of temperature and CO exposure time on the colour stability of beef striploin (LTL) steaks during storage.

II. MATERIALS AND METHODS

Bovine LTL (n = 4) were obtained from a commercial meat producer for each of the three replicates. Steaks were cut (25 mm thick, 285.2g – 388.0g) at 6-8 days post-mortem and randomized within each loin (block) to account for muscle positioning. Steaks were vacuum packaged (IIpra Foodpack VG 400 packaging machine, IIpra, Vigevano, Italy) as a reducing step prior to pre-treatment. Samples were exposed to a gas mixture of either CO-pre-treatment (5% CO, 60% CO_2 and 35% N_2) or control (60% CO_2 and 40% N_2) for 3, 5 & 7 h, and stored at 2 °C. They were then 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) and placed under retail display at 2 °C (good industry practice) or 6 °C (mild abuse) for 28 days under continuous fluorescent lighting (2115 lux) to simulate retail conditions. Temperature was recorded every five minutes using Dataloggers (Lascar EasyLog-USB, Lascar Electronics Ltd, Salisbury, SP5, UK). Surface colour measurements, reflectance and absorbance readings were performed using a HunterLab UltraScan Pro (Hunter Associates Laboratory., Inc., Reston, VA) with a viewing port of 25 mm and illuminant (D_{65}, 10°). Calibration was carried out using a white standard tile (L=100) and a light trap (L=0). The white tile was covered with the vacuum packaging film to eliminate any effect on the colour readings. Triplicate measurements were

recorded on vacuum packaged samples in three separate locations for each of the three replicates. Chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) values were calculated using CIE a* (redness) and b* (yellowness) measurements. Surface reflectance and absorbance measurements (n=3) were also measured from 400 to 700 nm (5 nm interval). Surface reflectance data for (474, 525, 572 nm) was calculated via linear interpolation. *K/S* ratios were determined using the Kubelka-Munk equation $((1-R)^2 \div 2R)$ to obtain each myoglobin redox form to obtain more linear data AMSA [1]. Deoxymyoglobin (DMb) $(K/S_{474})/(K/S_{525})$, Metmyoglobin (MMb) $(K/S_{572})/(K/S_{525})$ and Carboxymyoglobin (COMb) $(K/S_{610})/(K/S_{525})$ were estimated. Reference standards for 100% MMb, DMb, COMb were prepared (AMSA, 2012). Surface colour analysis was measured at 0, 2, 10, 21 and 28 days.

Data were analysed using a complete randomized block design with the loin being the block (SAS ver. 9.3, SAS Institute Inc., Cary, NC, USA). ANOVA (PROC GLIMMIX) was used to carry out a 3×2×5 split plot factorial design with three exposure times (3h, 5h, 7h), two display temperatures (2 °C, 6 °C) and five storage times (0d, 2d, 10d, 21d, 28d) as fixed effects and the rep as a random effect. Where factors were significant, differences between means were determined using Tukey's multiple comparisons test with $P<0.05$. The entire experiment was repeated three times.

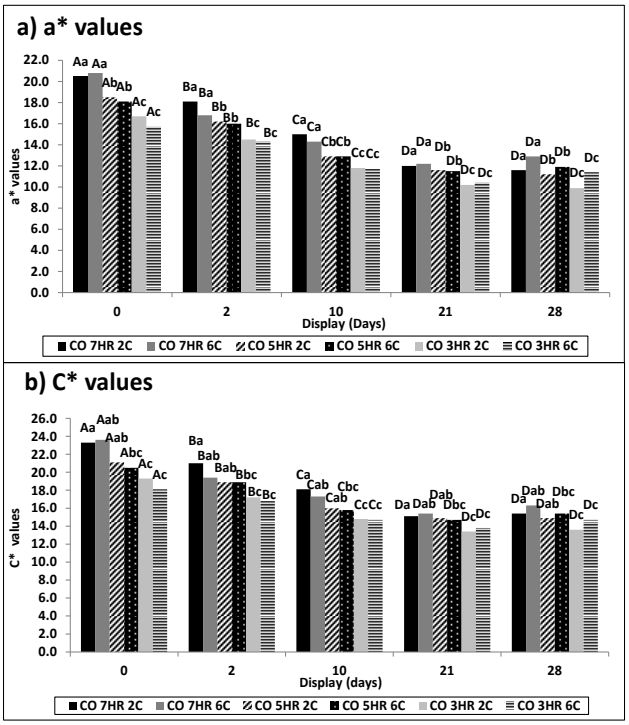
III. RESULTS AND DISCUSSION

An exposure time × display day interaction was observed for a* values ($P<0.05$) with the difference between exposure times diminishing with storage time (Fig.1a). Increased exposure time increased redness ($P<0.05$). No temperature interaction occurred for a* values ($P>0.05$). CIE a* values decreased over the display period, with the exposure time of 5 h (CO5) being the optimum to induce redness, while allowing discolouration by the use-by date, in agreement with Van Rooyen et al, [10]. The threshold used to detect discoloration is a*<12 [7]. Mean a* values for CO5 at 2 ˚C were 11.2 and 11.9 for CO5 at 6 ˚C, i.e. just below the colour threshold. This result means that the colour of CO-pretreated steaks could continue to be used as a reliable quality cue of product freshness by consumers, even after mild temperature abuse (6 ˚C), as this did not affect colour stability.

Chroma is a measure of the colour intensity of meat. MacDougall [8], reported that chroma values >16 represent the limit of acceptability and values below 14 are discoloured and considered brown. Additionally, consumers may reject meat products which contain 40% metmyoglobin (C*>14) [4]. Chroma values increased with exposure time to CO ($P<0.05$) with mean values on d 0 ranging from 18.1-23.6 for CO3-CO7, and decreased over the storage period ($P<0.05$) (Fig.1b). Temperature had no effect on chroma values ($P>0.05$), suggesting that the colour stability of CO pre-treated steaks is unaffected by mild temperature abuse (6 ˚C). All treatments were above the threshold for discolouration (C*<14) on day 28, except for CO3 at 2 ˚C (C*~13.6). Mean C* values on day 28 for CO5 (C*14.9, 15.4) at 2 ˚C and 6 ˚C respectively, were just below the limit.

K/S ratios are useful for estimating myoglobin redox forms, and give a more detailed understanding of the colour stability of meat surfaces. Varying the exposure time to CO did not affect reflectance ratios for DMb ($P>0.05$) (Table 1), however, there was a significant temperature × display day interaction ($P<0.05$). This was possibly due to oxidation of DMb and formation of MMb during storage. K/S ratios of 0.58 represent 100% DMb [9]. K/S ratios for MMb were affected by CO exposure time ($P<0.05$) and there was a temperature × display day interaction ($P<0.05$). K/S ratios of 0.58 and 1.4 represent 100% and 0% for MMb [9]. Reflectance standards prepared according to AMSA [1] corresponded to these values (data not shown). CO exposure time affected K/S ratios for MMb ($P<0.05$) (Table. 1) and there was a temperature × display day interaction ($P>0.05$). MMb values decreased over the display period with the lowest values being for CO3 (1.07). CO exposure time had a significant effect on COMb K/S values ($P<0.05$) (Table 1), in agreement with a* and chroma values. A temperature × display day interaction occurred for COMb K/S values ($P<0.05$). Reference standards prepared according to AMSA [1] showed a K/S $(K/S_{610})/(K/S_{525})$ value of 0.16 for 100% COMb and 0.52 for 0% COMb.

Figure 1.Mean values of 5% CO pre-treatments over 28 days storage (2 °C, 6 ˚C) for a) a* and b) C*values



Different uppercase (A-D) indicates significant differences by display day (P<0.05). Different lowercase (a-f) indicates significant differences by exposure time to CO-pretreatment (P<0.05). Different lowercase letters (x, y) indicates significant differences by temperature (P<0.05).

The mean COMb K/S values on day 0 ranged from 0.25-0.32 for CO7 – CO3 at 2 ˚C & 6 ˚C, respectively. COMb K/S values increased over storage and on day 28 ranged from 0.38-0.47 for CO7 – CO3 for 2 ˚C & 6 ˚C, respectively. The increased K/S COMb values over storage indicate discoloration occurred as K/S COMb values shifted towards the 0% COMb reference standard of 0.52 representing 0% COMb. These results are in agreement with the discolouration trend observed for a* and chroma values (Fig.1 a & b). This result demonstrates that discolouration occurred and very little CO was present for all treatments at the end of storage so as to not mask meat spoilage and address consumers' concerns. Gee and Brown [3] reported that an increased formation of MMb in CO treated meat over storage is equalised with a decreased concentration of COMb. Jeong *et al.* [6] reported that the COMb reflectance ratio showed similar colour discolouration patterns in comparison to a* values, however reported reflectance ratios are not definitive of the colour changes with CO exposure time. This could be a possible explanation for the effect that temperature had on all K/S ratio values (P<0.05), while it had no effect on a* and chroma values (P>0.05). While, the CO-pretreated steaks in this present study were stored under anaerobic conditions, discolouration may also have occurred due to oxygen permeating through the vacuum packaging material slowly over the storage period. Thus, CO-pretreated meat may be subjected to oxidation due to the loss of the CO ligand over the storage period and reoxygenation, which then oxidises and the formation of MMb occurs [5].

Table 1. Effect of CO-pretreatment and temperature (2 °C, 6 °C) on *K/S* values

	Display (days)				
	0	**2**	**10**	**21**	**28**
K/S 474/525					
CO 3 h					
2℃	0.69^{BAx}	0.70^{BAx}	0.69^{Bx}	0.76^{Ax}	0.74^{BAx}
6℃	0.67^{BAy}	0.65^{BAy}	0.61^{By}	0.67^{Ay}	0.64^{BAy}
CO 5 h					
2℃	0.68^{BAx}	0.69^{BAx}	0.67^{Bx}	0.74^{Ax}	0.72^{BAx}
6℃	0.67^{BAy}	0.65^{BAy}	0.61^{By}	0.60^{Ay}	0.61^{BAy}
CO 7 h					
2℃	0.68^{BAx}	0.69^{BAx}	0.67^{Bx}	0.77^{Ax}	0.69^{BAx}
6℃	0.68^{BAy}	0.64^{BAy}	0.62^{By}	0.65^{Ay}	0.62^{BAy}
K/S 572/525					
CO 3 h					
2℃	1.22^{Abx}	1.20^{Abx}	1.17^{Abx}	1.03^{Bbx}	1.07^{Bbx}
6℃	1.25^{Aby}	1.25^{Aby}	1.29^{Aby}	1.19^{Bby}	1.19^{Bby}
CO 5 h					
2℃	1.25^{Aax}	1.22^{Aax}	1.24^{Aax}	1.09^{Bax}	1.13^{Bax}
6℃	1.25^{Aay}	1.26^{Aay}	1.30^{Aay}	1.30^{Bay}	1.29^{Bay}
CO 7 h					
2℃	1.27^{Abax}	1.23^{Abax}	1.24^{Abax}	1.03^{Bbax}	1.08^{Bbax}
6℃	1.26^{Abay}	1.25^{Abay}	1.27^{Abay}	1.22^{Bbay}	1.24^{Bbay}
K/S 610/525					
CO 3 h					
2℃	0.32^{Dax}	0.36^{Cax}	0.42^{Bax}	0.47^{Aax}	0.47^{Aax}
6℃	0.34^{Day}	0.37^{Cay}	0.42^{Bay}	0.45^{Aay}	0.43^{Aay}
CO 5 h					
2℃	0.29^{Dbx}	0.32^{Cbx}	0.39^{Bbx}	0.43^{Abx}	0.43^{Abx}
6℃	0.29^{Dby}	0.32^{Cby}	0.39^{Bby}	0.41^{Aby}	0.40^{Aby}
CO 7 h					
2 ℃	0.25^{Dcx}	0.29^{Ccx}	0.34^{Bcx}	0.43^{Acx}	0.42^{Acx}
6 ℃	0.25^{Dcy}	0.31^{Ccy}	0.35^{Bcy}	0.40^{Acy}	0.38^{Acy}

Statistical significance: (P<0.05). Different uppercase (A-D) per column indicates significant differences by display day. Different lowercase (a-f) per row indicates significant differences by exposure time to CO-pretreatment. Different lowercase letters (x, y) per row indicates significant differences by temperature.

IV. CONCLUSION

In summary, increasing the CO pre-treatment exposure time of LTL steaks enhanced colour stability. All treatments discoloured over storage irrespective of display temperature, and therefore meat spoilage would not be masked, thus addressing consumer concerns. A CO-pre-treatment of 5 hours is the optimum exposure time to induce colour stability while allowing discolouration to occur by a use by date of 28 days. Surface reflectance ratios may give a more detailed understanding of CO pre-treated meat colour stability, as similar trends between these and a* and chroma values were observed but, while temperature had no effect on a* and chroma values, it did affect the K/S values. This merits further research for the application of 5% CO pre-treatment prior to vacuum packaging, a value-added technology which improves meat quality through enhanced colour stability.

ACKNOWLEDGEMENTS

Lauren-Anne Van Rooyen is in receipt of a Teagasc Walsh Fellowship. The authors would like to acknowledge the Department of Agriculture, Food and Marine (DAFM) for their financial support through the Food Research Institutional Measure (FIRM) (project 11/F/060). The authors would also like to thank Paula Reid for her statistical assistance.

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