Conference papers

2015-8

The Effect of Carbon Monoxide pre-Treatments on the Colour Stability of Vacuum Packaged Beef Steaks

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Recommended Citation

The effect of carbon monoxide pre-treatments on the colour stability of vacuum packaged beef steaks

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Abstract – Carbon monoxide (CO) as a component of wood smoke has a long history of applications in meat, fish, vegetable and fruit processing. More recently it has been used in meat packaging to enhance colour stability. Concerns have been raised by regulatory authorities that CO may mask meat spoilage and meat might be sold beyond its sell-by-date due to the bright red colour being retained. This study investigated the use of 5% CO as a pre-treatment prior to vacuum packaging beef striploin steaks (*Longissimus thoracis et lumborum*, LTL) to induce the desirable cherry red colour, while determining the optimum pre-treatment time to allow discoloration by 28 days of storage (2°C). A range of pre-treatment exposure times (1, 3, 5, 7, 9, 15 and 24 h) were applied to steaks using a gas mixture of 5% CO, 60% CO_2 and 35% N_2. Colour analysis was measured over 28 days of storage and microbiological analysis was analysed at the end of storage. The CO5 treatment appears to be the most appropriate as the bright cherry red colour desirable to consumers was achieved, and discolouration reached unacceptable levels (a* = 12, C* = 16)by the use-date of 28 days, thus ensuring the consumer of a reliable visual indication of freshness and addressing concerns about consumer safety. The 5% CO pre-treatment had no negative effect on the microbiological safety of steaks ($P > 0.05$).

Key Words – Modified Atmosphere Packaging, Colour, Carboxymyoglobin

I. INTRODUCTION

Meat colour is the primary quality attribute used by consumers to assess meat quality at point of sale. However, for eating experience tenderness is considered the most important palatability attribute (Miller et al., 1995, Grobbel et al., 2008). Consumers perceive meat colour as a strong indication of freshness or wholesomeness (Kropf, 1980). The most common form of packaging for fresh red meat is high-oxygen modified atmosphere packaging (MAP) which induces the cherry red colour (oxymyoglobin) of beef which is desirable to consumers. Unfortunately, disadvantages of this packaging technology include limited shelf-life, reduced tenderness and off-odours due to myoglobin and lipid oxidation. Vacuum packaging (VP) is an anoxic technology that prevents lipid oxidation, prolongs shelf-life, reduces microbial spoilage and is the most commonly applied ageing method (wet ageing) for tenderisation of primals. Wet ageing is also more cost effective than dry ageing. The major drawback of VP is the dark purple colour of the beef due to deoxymyoglobin, which many consumers perceive as poor quality or old meat thus giving rise to consumer purchase rejection. Carbon monoxide (CO) induces a stable cherry red colour (carboxymyoglobin) similar to oxygen. In the USA, low concentrations of 0.4% CO have been GRAS (Generally recognised as safe) approved by the FDA and CO is permitted as a primary packaging gas in case-ready packaging systems (FDA, 2004). However in the EU, CO has not yet been approved as a packaging gas even though the application of low concentrations of CO to meat packaging systems have been reported to be consumer friendly and have no toxic effect (Sorheim et al., 1997). Concerns have also been raised by regulatory authorities that CO may mask meat spoilage so that meat might be sold beyond its sell-by-date due to the bright red colour being retained. Previous researchers have investigated applying 5% CO pre-treatments prior to vacuum packaging (Aspé et al., 2008, Jayasingh et al., 2001, O'Connor and Allen, 2011). However a pre-treatment exposure time which discolours by a use-by date of 28 days (2°C), using colour as reliable visual indication, has not yet been determined. The objective of this study was to determine the pre-treatment exposure time of 5% CO prior to vacuum packaging striploin steaks that would give a colour stability of 28 days storage (2°C). Microbiological analysis was carried out at 28

days storage to determine if the pre-treatment had any effect on the microbiological safety of LTL steaks.

II. MATERIALS AND METHODS

Sample Preparation:

Bovine *Longissimus thoracis et lumborum* (LTL) muscles from Charolais-cross (CHX) heifers (aged between 21-29 months) were obtained from a commercial meat producer. Two muscles from the same heifer were obtained for each of three replicates. Striploin steaks were cut (2.5cm in thickness) at 6-8 days post-slaughter and randomized to account for muscle positioning and animal side effects. Steaks were vacuum packaged (IIpra Foodpack VG 400 packaging machine, IIpra, Vigevano, Italy) as a reducing step prior to pre-treatment. Pre-treatments involved exposure of steaks to 5% CO, 60% CO_2 and 35% N_2 (Air Products and Chemicals, Inc.,) for a range of exposure times (1, 3, 5, 7, 9, 15 and 24 h). Steaks were then vacuum packaged after pre-treatments and placed in an open front-display cabinet (Cronos fan-assisted cabinet, Criosbanc, Padova, Italy) at 2°C with lighting to simulate retail conditions. The display cabinet temperature was monitored every five minutes using Dataloggers (Lascar EasyLog-USB).

Instrumental Colour Analysis:

Instrumental Colour analysis using a HunterLab UltraScan Pro (Hunter Associates Laboratory., Inc., Reston, VA) with a viewing port of 25.54 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 (AMSA, 2012). Steaks were measured in the vacuum packages and triplicate measurements were taken in three separate locations avoiding intramuscular fat. CIE a* (redness) and b* (yellowness) values were used to calculate Chroma ($C* = (a*^2 + b*^2)^{1/2}$) values. . Surface colour analysis was carried over 28 days of storage (2°C) at 0, 7, 14, 21 and 28 days.

Microbiological Analysis:

Sterile carcass swabs were used to swab the surface area of the LTL steaks at 28 days storage (2°C). The total viable counts (TVC) for anaerobic psychrotrophic bacteria were enumerated following ISO 4833:2003/ ISO 17410:2001 using standard Plate Count Agar (SPCA, Oxoid Ltd, England, CM0463). Plates were incubated (6.5°C, 10 d) in anaerobic jars. Lactic acid Bacteria were obtained on de Man Rogosa sharpe agar (MRS, Oxoid, CM 0361) at (30°C, 72 h) following ISO 15214:1998. Total *Enterobacteriaceae* counts (TEC) were determined following ISO 21528:2004 on Violet Red Bile Glucose Agar (VRBGA, Oxoid Ltd, England, CM0485) and incubated at (37°C, 24 h). Results were expressed as the log of colony forming units (CFU) per cm^2 of the surface area of the steak (log_{10} cfu/cm^2).

Statistical Analysis:

Data was analysed by one-way ANOVA, General Linear Model (PROC GLM) with Tukey's multiple comparisons test to determine if the pre-treatment had any effect on a* values, chroma and microbiological analysis of LTL vacuum packaged steaks using SAS ver. 9.3 (SAS Institute Inc., Cary, NC, USA). Significance was defined at $P < 0.05$.

III. RESULTS AND DISCUSSION

Instrumental Colour Analysis:

CIE a*, and Chroma values are shown for the control and each CO pre-treatment over the 28 day storage period in Figures 1 and 2. In agreement with preliminary work carried out by O'Connor & Allen (2011), the 5% CO pre-treatment significantly increased redness (P<0.001) in LTL steaks on day 0 in comparison to the control (untreated vacuum packaged) . Mean a* values of pretreatments at Day 0 ranged from 15.4 – 23.9 for CO1-CO24 in comparison to the control which had an a* value of 9.4. Redness decreased over the storage time of 28 days, as expected from previous studies investigating 5% CO pre-treatment (Aspé

et al., 2008, Jayasingh et al., 2001). The threshold which was used to detect discoloration from the instrumental colour analysis was CIE a* >12. Jeong and Claus, (2010) reported a* values >12 to be considered the limit of acceptability and values below 12 to be considered brown or discoloured. A pretreatment exposure time of 5 hours (CO5) is of particular interest in this study as a* values decreased over storage and had a mean value of 12.2 by day 28 (P<0.001) as seen in Figure 1. Thus, following CO pre-treatment colour may continue to be used as reliable indication of freshness.

Chroma determines the colour intensity and higher values represent a more vivid colour. Chroma values >16 are considered the limit of acceptability (MacDougall et al., 1986). Meat surface that has been affected by 20% of metmyoglobin can affect the purchase decisions of consumers and discrimination may occur (MacDougall, 1982). Meat with metmyoglobin levels above 40% can lead to purchase rejection at point of sale (Greene et al., 1971). The CO pre-treatments had an effect on chroma values with mean chroma values on day 0 ranging from 18.5-27.4 for CO1-CO24 (P<0.001) as shown in Figure 2. Chroma values decreased over storage as expected (28 days). The mean chroma values for pre-treatments ranged from 14.7- 19.3 for CO1-CO24. The mean chroma value for CO5 was 16.3 on day 28 which was just above the limit of acceptability according to MacDougall et al, (1986).
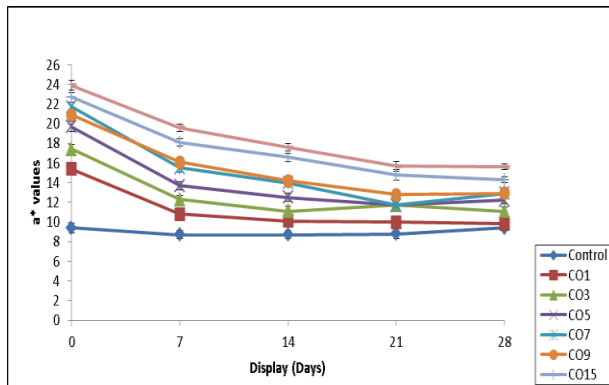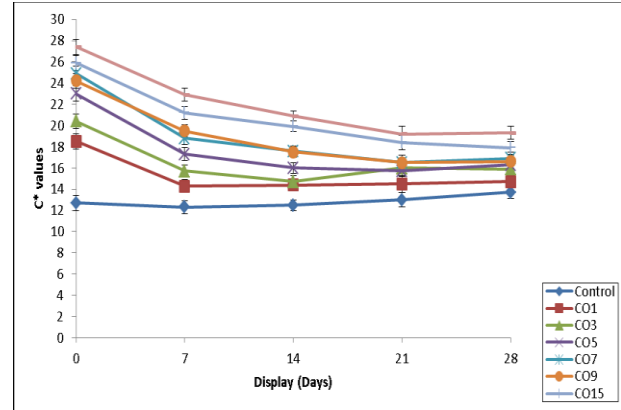

Figure 2 Chroma values of 5% CO pre-treatments and untreated control over 28 days storage (2°C).

Microbiological Analysis

Mean total viable counts (TVC) for anaerobic psychrotrophic bacteria, Lactic acid bacteria counts and total *Enterobacteriaceae* counts (TEC) are shown Table 1. The CO pre-treatments had no effect on microbial shelf-life (P>0.05). The highest mean total viable counts (TVC) for anaerobic psychrotrophic bacteria was 7.9 log cfu/cm^2 which was below the upper limit of detection (8.0 log cfu/cm^2) for microbial spoilage in vacuum packaged meat products (FSAI, 2014).The highest mean Lactic acid bacteria count of 6.1 log cfu/cm^2 was considerably lower than upper microbiological limit for Lactic acid bacteria of 9 log cfu/cm^2 (FSAI, 2014). The highest total *Enterobacteriaceae* counts (TEC) for pre-treated LTL was 3.5 log which was below the upper microbiological limit for *Enterobacteriaceae* (4.0 log) to be considered unsatisfactory, in comparison to the untreated control which was 4.5 log cfu/cm^2 (FSAI, 2014). Lower TEC in pre-treated LTL may be due to the bacteriostatic effect of CO_2 in the pre-treatment gas mixture. In summary, these results show that pre-treatment with 5% CO had no negative effect on the microbiological safety of LTL steaks (P>0.05).

Figure 1 a* values of 5% CO pre-treatments and untreated control over 28 days (2°C).

Table 1 Mean TVC, Lactic Acid Bacteria and TEC levels ($\log_{10}$ cfu/cm^2) of 5% CO pre-treated and untreated vacuum packaged LTL steaks at 28 days storage (2°C).

Treatments	Anaerobic psychrophiles		Lactic Acid Bacteria		*Enterobacteriaceae*	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control	7.6	0.27	6.0	0.50	4.5	0.74
CO1	7.9	0.33	6.0	0.61	3.5	0.91
CO3	7.9	0.33	5.8	0.61	3.2	0.91
CO5	7.8	0.28	5.6	0.50	3.3	0.91
CO7	7.3	0.28	5.7	0.50	3.1	0.74
CO9	7.6	0.28	6.0	0.50	3.1	0.74
CO15	7.8	0.28	6.1	0.50	3.1	0.91
CO24	7.8	0.28	5.9	0.50	2.9	0.91
Significance	NS		NS		NS	

IV.   CONCLUSION

In summary, all the CO pre-treatments improved the colour stability of beef steaks without any adverse effects on the microbiological status after 28 days storage. The CO5 pre-treatment appears to be the most appropriate as the discoloration reached an unacceptable level by the use-date of 28 days ensuring the consumer of a reliable visual indication of freshness and addressing concerns about consumer safety. In addition, this anoxic packaging technology should prevent any negative quality issues related to high oxygen MAP packaging. Applying 5% CO pre-treatments may be a potential innovative solution to current packaging issues within the meat sector adding value, safety and enhancing meat quality while facilitating exports to distant markets.

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