AN INVESTIGATION REGARDING THE USE OF CARBON MONOXIDE FOR COLOUR STABILITY AND INHIBITION OF LIPID AND PROTEIN OXIDATION IN MEAT

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Abstract – The use of carbon monoxide (CO) to enhance and stabilise the colour of red meat is well known. Its use on red meat has however raised concerns due to its ability to mask visible spoilage indicators (discolouration). Thus in an attempt to improve the consumer and scientific perception regarding its use, other suggested benefits such as the inhibition of lipid and protein oxidation were investigated. Yellowfin tuna was used as a model to investigate the effects of CO on the colour, lipid and protein oxidation in red meat. Samples were extracted from the dorsal loin of the tuna, vacuum packaged and frozen for 30 d before being subjected to a 32 d shelf-life trial at 4˚C. The surface colour, lipid and protein oxidation was measured every 4 d using the CIE Lab colour system, TBARS and DNPH methods, respectively. It was found that the CO did enhance and stabilise the colour of the tuna muscle, but had no effect on the lipid and protein oxidation. The findings did, however, reiterate the concerns regarding the ability of CO to mask visible spoilage indicators.

Key Words – CIE Lab, DNPH, TBARS

- INTRODUCTION

The use of CO to enhance the colour of meat has been around for centuries as the component in smoke which gives smoked products their distinctive colour [1]. Its use either as a pure gas (100% CO) or as a mixture of gasses in modified atmosphere packaging, is a relatively novel method. Its use has however raised concern as the resulting pigment formed, carboxymyoglobin (COMb), is highly resistant to autoxidation even under abusive conditions [2]. It thus has the potential to mask the visible indicators of spoilage and thermal abuse (discolouration) in red meat. For this reason it is currently not legal in many countries [3].
Despite these concerns, its potential economic importance should not be overlooked [1]. Consumer purchase intent of red meat is based on colour more than any other quality attribute [4]. Enhancement and stability of meat colour using CO could significantly decrease revenue lost due to discolouration [4].

Thus, in an attempt to influence the consumer and scientific perception regarding the use of CO on meat, other potential advantages of its used were investigated. These include its suggested potential to inhibit protein and lipid oxidation by inhibiting the pro-oxidant effect of myoglobin [5].

The objective of this study was to ascertain the effect of CO on red meat with regard to colour enhancement and stability and, lipid and protein oxidation using yellowfin tuna muscle as a model.
As an illustrative model of the potential effects of the CO treatment on meat, only two of the treatments from a broader study [6] will be discussed.

- MATERIALS AND METHODS

Harvesting, evisceration and freezing

The yellowfin tuna were harvested off the west coast of South Africa. The tuna were exsanguinated on board, directly after being caught. On that same day, the tuna were eviscerated and the head, gills, caudal fin (tail fin), second dorsal fin and anal fin were removed and discarded. The whole tuna was then frozen (-20°C) for seven days.

Sampling

The day prior to the commencement of the trial, the whole tuna were cut into 2.5 cm steaks. From these steaks, two samples, 10 cm in diameter, were extracted from the dorsal loins of the tuna (white muscle). The samples were then vacuum packed and defrosted overnight at 4°C.

CO treatment

The defrosted samples were removed from the vacuum packaging and treated with 100% CO (99.97% min, AFROX, Cape Town, South Africa) under 300 kPa pressure for 150 min. As a control, samples which had undergone the same sampling process (as above), were left untreated. The samples (both treated and untreated) were frozen (-20°C) for 30 d.

Shelf-life trial

The vacuum packaged treated (OI +CO) and untreated (OI -CO) samples were then defrosted the day prior to the commencement of the shelf-life trial. The samples were then subjected to a 32 d shelf-life trial during which colour measurements, and samples for lipid and protein oxidation were taken every 96 h. Thus nine time points (T_0-T_8) were established. The shelf-life trial was done according to the partially staggered design suggested by Gacula [7].

Surface colour measurements – CIE Lab

The surface colour of the tuna samples was measured according to the CIE Lab colour system using a colour-guide 45°/0° colorimeter (BYK-Gardner GmbH, Geretsried, Germany). Three L*, a* and b* measurements were taken with the colorimeter on different areas of the sample and the average of these colour ordinates, L*, a* and b* respectively, was used in the statistical analysis [8]. The hue angle ($h°_{ab}$) and the chroma (C*) were calculated using the a* and b* values.

Lipid oxidation – TBARS method

The level of lipid oxidation over time was assessed by the 2-thiobarbituric acid (TBARS) extraction method [9].

Protein oxidation – DNPH method

The amount of protein oxidation over time was determined using the protein carbonyl concentration with the derivation of DNPH [10].

Statistical analysis

The data was analysed with a two-way repeated measure analysis of variance (RMANOVA) using the general linear models (GLM) procedure.
The RMANOVA was performed on the assumption that the data had compound symmetry on

the time and treatment correlation. The data was also assumed to be normally distributed. The least significant interactions (LSD) were calculated at a 95% significance level to compare the treatment means i.e. results were defined as significant when $P \leq 0.05$ and not significant when $P > 0.05$. If the interaction between the main effects, time and treatment, was not significant ($P > 0.05$) then the main effects could be interpreted separately. In the case where the interaction is found to be significant, a Bonferroni pair wise comparison was done to identify the specific interactions. STATISTICA version 10 (StatSoft South Africa Pty (Ltd)) was used to analyse the data collected for each treatment.

- RESULTS AND DISCUSSION

Surface colour measurements

The b* (associated with metmyoglobin formation) and hue angle values indicate that both the treated and untreated samples brown to some extent over time (Fig. 1). The a* (associated with oxymyoglobin and carboxymyoglobin formation) and chroma values indicated that the untreated samples decrease in redness over time and become more saturated in colour (Fig. 1) i.e. a darker brown/tan colour. On the other hand, the treated samples displayed quite a dark (saturated) red colour initially, then became less red and lighter (less saturated) and yielded erratic colour changes near the end (Fig. 1), most probably due to microbial growth [11]. These results were expected since, in the absence of oxygen, the residual CO will bind to the unbound myoglobin resulting in a higher concentration of carboxymyoglobin [12], leading to a more saturated red colour. The low a* and chroma values of the untreated samples are ascribed to the increased myoglobin oxidation and loss in myoglobin in the tuna muscle as a result of the multiple freeze/thaw cycle [13](that was applied prior to vacuum packaging).

Figure 1. The a* values: () +CO and () -CO; combined b* values; chroma (C*): () +CO and () -CO; and combined hue angle (h_{ab}*).

Lipid oxidation

The overall increase in the TBARS values (Fig. 2) of the treatments are consistent with results found in vacuum packaged beef samples where an overall increase in TBARS values over time was also observed [14]. It is however expected that in the absence of oxygen, lipid oxidation would not occur. There are two possible reasons why, even though the samples were vacuum packed in oxygen impermeable packaging, lipid oxidation still occurred. The first is that it was found by Lynch et al. [14] that that vacuum packaging does not remove all the oxygen from the packaging. Thus any residual oxygen in the packaging could have led to the lipid oxidation which was seen in the results of this study. The second is that the TBARS method measures secondary lipid oxidation products and not primary lipid oxidation products [15]. These secondary reactions involve both oxidative and non-oxidative reactions [16]. Thus in the absence of oxygen, secondary lipid oxidation will still occur. There is some evidence that suggests primary lipid oxidation could occur during frozen storage (initial frozen storage of the whole tuna). This leads to accelerated secondary oxidation during thawing [17], the products of which are measured by the TBARS method [15].

Figure 2. (a) The mean TBARS values (with confidence intervals) for the () OI +CO and () OI -CO treatments; (b)TBARS values (with confidence intervals) for the combined treatments.

Protein oxidation

The results showed no significant interactions between the treatments. It can also be seen that little to no protein oxidation occurred over time. The apparent lack of protein oxidation is to

be expected since in the absence of oxygen, protein oxidation is not expected to occur [14]. However, in light of findings from the full study conducted [6], it was found that the more likely explanation for the lack of observed protein oxidation was due to the effect that freezing and thawing have on increasing protein oxidation, rather than due to a lack of oxygen. The protein oxidation reactions had thus proceeded further and the carbonyls (measured by the DNPH method) were no longer detectable.

Figure 3. (a) The mean carbonyl values (with confidence intervals) for the () OI +CO and () OI -CO treatments; (b) carbonyl values (with confidence intervals) for the combined treatments.

- CONCLUSION

It was found that the CO treatment did enhance and stabilise the colour of the yellowfin tuna meat but had no effect on the lipid and protein oxidation. The results did, however, reiterate the concerns regarding the use of CO on red meat and its ability to mask visible spoilage indicators. It would thus be recommended that when CO is used with red meat it should be done according to strict labelling and hygiene regulations and with strict control over the cold chain. The products which are to be treated, should also be regulated to prevent fraudulent practices.

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REFERENCES

- Otwell, W. S. (2006). Use of filtered smokes and carbon monoxide in fish processing. In W.S. Otwell, H.G. Kristinsson, & M.O. Balaban, Modified atmospheric processing and packaging of fish - filtered smokes, carbon monoxide and reduced oxygen packaging (pp 3-13). USA: Blackwell Publishing.
- Balaban, M. O., Kristinsson, H. G. & Otwell, W. S. (2005). Evaluation of color parameters in a machine vision analysis of carbon monoxide treated fresh tuna. Journal of Aquatic Food Product Technology 14: 5-24.
- European Parliament and Council Directive (1995). Food additives other than colours and sweeteners. Official Journal of the European Community 18: 1-40.
- Carpenter, C. E., Cornforth D. P. & Whittier, D. (2001). Consumer preferences for beef color and packaging did not affect eating satisfaction. Meat Science 57: 359-363.
- Kristinsson, H. G., Mony, S. S. & Petty, H. T. (2005). Properties of tilapia carboxy- and oxy hemoglobin at post-mortem pH. Journal of Agricultural and Food Chemistry 53: 3643-3649.
- Neethling, N. E. (2013). The effect of carbon monoxide on the colour stability and quality of yellowfin tuna (*Thunnus albacares*) muscle. Stellenbosch University, Stellenbosch, South Africa.
- Gacula, M. C. (1975). The design of experiments for shelf-life study. Journal of Food Science 40: 399-403.
- Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. Meat Science 49: 447-457.
- Lynch, S. M. & Frei, B. (1993). Mechanism of copper –and iron-dependent oxidative modification of human low density lipoprotein. Journal of Lipid Research 34: 1745-1753.
- Oliver, C. N., Ahn, B. W., Moerman, E. J., Goldstein, S. & Stadtman, E. R. (1987). Age-related changes in oxidised proteins. Journal of Biological Chemistry 262: 5488-5491.
- Mancini, R. A. & Hunt, M. C. (2005). Current research in meat color - a review. Meat Science 71: 100-121.
- Kristinsson, H. G., Balaban, M. O. & Otwell, W. S. (2006). Microbial and quality consequences of aquatic foods treated with carbon monoxide filtered wood smoke. In W. S. Otwell, H. G. Kristinsson & M. O. Balaban. Modified atmospheric processing and packaging of fish - filtered smokes, carbon monoxide and reduced oxygen packaging (pp 65-85). USA: Blackwell Publishing.
- Añón, M. C. & Cavelo, A. (1980). Freezing rate effects on the drip loss of frozen beef. Meat Science 4: 1-14.
- Lynch, M. P., Kerry, J. P., Buckley, D. J., Fraustman, C. & Morrisey, P. A. (1999). Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. Meat Science 52: 95-99.

- Draper, H. H. & Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. Methods in Enzymology 186: 421-431.
- Gray, J. I. & Monahan, F. J. (1992). Measurement of lipid oxidation in meat and meat products. Trends in food Science and Technology 3: 315-319.
- Owen, J. E., & Lawrie, R. A. (1975). The effect of an artificially induced high pH (hydrogen-ion concentration) on the susceptibility of minced porcine muscle to undergo oxidative rancidity under frozen storage. International Journal of Food Science and Technology 10: 169-180.