MICROBIOLOGICAL AND SENSORY PROPERTIES OF FRESH BEEF STEAKS UNDER LOW CARBON MONOXIDE WITH OR WITHOUT OXYGEN

A.C.Venturini [1], J.A.F.Faria[1], C.J. Contreras-Castillo*[2] and C.R.Gallo[2]

[1]*Department of Food Technology, State University of Campinas, São Paulo State, SP, PO Box 6121, Brazil*
[2]*Department of Agroindustry, Food and Nutrition, University of São Paulo State, SP, PO Box 11, Brazil*
*****ccastill@esalq.usp.br

Key Words: Beef steaks, case-ready, modified atmosphere packaging, carbon monoxide

Introduction

The addition of low concentrations of carbon monoxide (CO) can avoid discoloration of beef stored in virtually oxygen-free atmospheres for lengthened periods and restore the bloomed color through the interaction of CO with myoglobin (Hunt *et al*., 2004). A possible disadvantage of using CO in beef packaging is the masking of the microbiological conditions, since the red bloom color of carboxymyoglobin keeps stable despite the poor microbiological quality of the beef (Jayasingh *et al*., 2001, Sϕrheim *et al*., 1999). Oxygen is injected, not only to inhibit anaerobic growth, but also to obtain carboxymyoglobin and oxymyoglobin mixtures in an attempt to maintain the natural color of the beef (Luño *et al*., 2000).

Luño *et al*. (2000) showed that in atmospheres containing 24% oxygen and 50% CO_2, at least 0.5% CO was needed to associate color optimization and the microbiological safety of fresh steaks using modified atmosphere packaging. On the other hand, Seyfert *et al.* (2007) showed that addition of 0.4% CO to package atmospheres with 20 or 80% oxygen had no impact on color stability of fresh steaks at 0.2±3ºC. The objective of this work was to investigate color, microbiology and odor interrelations in order to increase the stability of fresh beef steaks by 21 days at 2±2ºC.

Materials and Methods

Gluteus medium (GM) and *Longissimus dorsi* (LD) with normal pH (<5.8) were randomly removed from twenty market-weight carcass of Nelore-cross bulls. The muscles were obtained 72h post-slaughter from castrated males raised in open *Brachiaria brizantha* pasture at the age of 24 month old (with 2 incisive teeth and 1-3 mm fat layer). Two steaks of about 1.5cm thickness were placed into thermoformed trays and randomly divided into four experimental lots. In each lot, a gas mixture of 30% CO_2 and variable concentrations of CO (0.2 or 0.4%, O_2 (0 or 21.0%) and N_2 (balance) was studied. The codified trays were sealed with a laminated film with a ethylene-vinyl alcohol (EVOH) barrier layer using a thermosealing machine. A self-activated oxygen-absorbing sachet was placed in the trays of anoxic treatments. Two trays of each lot remained exposed to air for initial analyses (0 d). The gas mixtures of the cylinders was made and certificated by AGA (Linde Group). The sealed trays were stored at 2±2ºC and analyzed for color, microbiology and odor after 7, 14 and 21 days of storage in MAP at 2ºC.

All plates were incubated according to the methods described by Downes & Ito (1992), except for the plates incubated for the *Brochothrix thermosphacta* tests, which were carried out according to the methods described by Gardner (1966) and *Listeria monocytogenes* (Downes & Ito, 2001). The sensorial color, discoloration (%) and immediate off-odor was evaluated by twelve panelists trained using the scale suggested by AMSA (1991).

The experiment was a 4 x 4 x 2 x 2 factorial design with four packaging treatments (T_1: 69.8 %N_2 / 30%CO_2 / 0.2%CO; T_2: 69.6 %N_2 / 30%CO_2 / 0.4%CO; T_3: 48.8 %N_2 / 30%CO_2 / 0.2%CO / 21%O_2; T_4: 48.6 %N_2 / 30%CO_2 / 0.4%CO / 21%O_2), four display times at 2ºC (0, 7, 14 and 21 days), two muscles (M. *Gluteus medium* and M. *Longissimus dorsi*) and two replications of entire experiment. Means of the treatments were calculated by the analysis of variance (ANOVA) using Statistica™ software (Statsoft Inc., Tulsa, OK, USA). Significant differences among the means were determined by calculation of Tukey Test, when appropriate. The significance level used for all statistic analyses was 5%.

Results and Discussion

The discoloration rate (%) and off-odor of the GM and LD steaks were affected (p<0.05) by oxygen concentration (0 or 21%), display time (0, 7, 14 and 21 days) and the interaction between the oxygen concentration and the display time (Table 1). Panel scores indicated increased browning, discoloration % and off-odor with increase in display time at higher oxygen concentration. The GM steaks packaged in CO without oxygen presented a bloomed color that was similar to or higher than the initial color of the fresh beef (0 d) and practically did not present a visible discoloration or perceptive bad odor along the display time. The visual color of the GM or LD steaks in oxygen-free atmosphere was statistically affected by the increase of the CO concentration. The LD steaks displayed in 0.4% CO without oxygen presented a stable pinkish tonality, after 21 days of storage, which was regarded as 'artificial' by some panelists. The LD fat was infiltrated by exsudate of steaks that positively influenced the acceptance of the product. These data suggest that an increase in the CO concentration can change significantly the natural color of fresh beef with inherent color stability, such as LD.

Thus, there must be an optimal concentration of CO for each cut, that is, there must be a concentration from which the maximum stability of the typical color of fresh beef is achieved. In the samples packed in CO with 21%O_2 the change from red to dark-red generally coincided with the beginning of the formation of visible brown spots. In the GM steak samples of aerobic treatments, the formation of brown spots depended on the CO concentration. In the steaks packed in 0.2%CO, an area around 11-20% became visible after 14 days of storage and the number reached ≥60% of the steaks after 21 days. As to the samples displayed in 0.4%CO, discoloration became apparent (≤20%) only after 21 days of storage in MAP. The formation of perceptive brown spots in the LD steaks displayed in 21% O_2 occurred after 21 days of storage, regardless the CO concentration. The perception of a moderately off-odor (≤2.0) only occurred in the samples displayed with oxygen, after the 21st day of storage at 2ºC, when aerobic and anaerobic psychrotrophic counts reached about 7 log CFU/g, the threshold for spoilage. However oxidative processes more then microbial must be involved in such unpleased odor since the different levels O_2 (0 or 21%) in the atmosphere did not affect significantly the aerobic and anaerobic psychrotrophic counts in the samples. *Salmonella*, *Listeria monocytogenes* and sulphite-reducing *Clostridia* remained absent in all samples along the whole experiment.

Table 1. Means and standard error for O_2 concentration x display time interaction for sensory analysis (n=12).

Muscle	Trait	without O_2				with O_2				SE
		0	7	14	21	0	7	14	21	
GM	Visual score	6.5 ^{b}	7.3 ^{ab}	8.1 ^{a}	8.5 ^{a}	8.2 ^{a}	4.1 ^{c}	4.1 ^{c}	2.5 ^{d}	3.4
	% discoloration	4.5 ^{ab}	4.7 ^{a}	4.9 ^{a}	4.9 ^{a}	4.5 ^{ab}	4.8 ^{a}	4.1 ^{b}	2.6 ^{c}	0.5
	Off-odor	4.8 ^{a}	5.0 ^{a}	4.8 ^{a}	4.8 ^{a}	4.8 ^{a}	4.8 ^{a}	4.6 ^{a}	2.2 ^{b}	0.3
LD	Visual score	6.9 ^{a}	8.0 ^{ab}	8.8 ^{b}	8.7 ^{b}	4.8 ^{c}	3.2 ^{d}	3.2 ^{d}	3.3 ^{d}	3.0
	% discoloration	4.8 ^{a}	4.8 ^{a}	4.9 ^{a}	4.7 ^{a}	4.5 ^{a}	4.6 ^{a}	4.7 ^{a}	2.6 ^{b}	0.3
	Off-odor	4.9 ^{ab}	5.0 ^{a}	5.0 ^{a}	4.9 ^{ab}	4.8 ^{ab}	4.9 ^{ab}	4.6 ^{b}	2.8 ^{c}	0.2

Means in a row with a different letter differ (p<0.05)
Visual score: 0 = brown; 5.0 = dark red; 9.0 = bright red; % discoloration: 1 = 61-100%, 2 = 21-60% discoloration, 3 = 11-20% discoloration, 4 = 1-10% discoloration and 5 = 0% discoloration; Off-odor: 1 = extreme off-odor; 2 = moderate off-odor; 3 = small off-odor; 4 = slight off-odor and 5 = no off-odor.

Conclusions

The extension of color and odor of GM and LD steaks packaged under low carbon monoxide was best achieved using atmospheres virtually without oxygen. Anoxic atmospheres with 0.4%CO produced no additional color effect over 0.2% CO, which is a clear advantage from the toxicological point of view as a suitable additive for extending the display life of fresh beef steaks using low-CO packaging technology. The packaging of the fresh beef in atmosphere containing 69.8% N_2 / 30% CO_2 / 0.2% CO showed to be the most suitable technique for the maintenance of the sensory and microbiological properties at 2ºC.

Acknowledgements

The authors would like to thank the financial support provided by the State of São Paulo Research Foundation - FAPESP (Project nº 2004/14644-8).

References

1. AMSA. (1991). Guidelines for meat color evaluation. Proceedings Reciprocal Meat Conference.
2. Downes, F. P. & ITO, K. (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D. C.
3. Gardner, G.A. (1966). A selective medium for enumeration of microbacterium thermosphactum in meat and meat products. Journal of Applied Bacteriology, 29(3), 455-460.
4. Hunt, M.C., Mancini, R.A., Hachmeister, K.A., Kropf, D.H., Merriman, M., DelDuca, G. & Milliken, G. (2004). Carbon monoxide in modified atmosphere packaging affects color, shelf life, and microorganisms of beef steaks and ground beef. Journal of Food Science, 69(1), 45-52.
5. Luño, M., Roncales P., Djenane D., Beltran J.A. (2000). Beef shelf life in low O_2 and high CO_2 atmospheres containing different low CO concentrations, Meat Science 55 (4): 413–19.
6. Seyfert, M., Mancini, R.A., Hunt, M.C., Tang, J. & Faustman, C. (2007). Influence of carbon monoxide in package atmospheres containing oxygen on colour, reducing activity, and oxygen consumption of five bovine muscles. Meat Science, 75(3), 432–42.
7. Sϕrheim, O., Nissen, H. & Nesbakken, T. (1999). The storage life of beef and pork packaged in atmosphere with low carbon monoxide and high carbon dioxide. Meat Science, 52(2), 157-164.