USE OF CARBON MONOXIDE IN BEEF PACKAGING ATMOSPHERES

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***Abstract* — Carbon monoxide (CO) has recently been allowed in the US for meat modified atmosphere packaging (MAP). It's still forbidden in the EU: according to European Commission, stabilizing meat colour could mask spoilage and therefore be potentially dangerous for the consumer. That's why this study wanted to explore the specific problem of the evolution of both commercial appearance and microbiological status of beef meat packaged under modified atmosphere (MA) including traces of CO. Two kinds of case-ready MAP including 0.4% CO were studied: with (high-O_2) or without oxygen (low-O_2). For each of them, two temperature storage conditions were tested: decent and poor. Commercial and microbiological evaluations were made at different times up to 12 and 31 days respectively in high- and low-O_2 atmospheres. The use of CO at 0.4% level in a gas mixture without oxygen (30%CO_2/70%N_2) in beef retail-ready packages gave an extend shelf life to the product regarding the meat colour. On a microbial point of view, our results showed a very low health risk even this one could not be totally excluded. On the other hand, 0.4% of carbon monoxide seemed to be of little interest when meat was packaged under high- O_2 (70%O_2/30%CO_2). It did not delay the early colour deterioration of case-ready meat: the commercial alteration took place still earlier than the microbiological one. These results raise questions about bacteriostatic effect of the carbon monoxide, since this gas effect on bacterial growth seems to be complex according to the scientific literature.**

***Index Terms* — beef, carbon monoxide, MAP, microbiological growth**

I. INTRODUCTION

In many countries, case-ready packaging has an increasing share of the domestic retail red meat market (Sørheim, Nissen and Nesbakken, 1999). It is the same situation in France. The most common retail packaging is still the foam tray wrapped with an air permeable film leading to a short shelf life. The High-O_2 MAP is now widely used to prolong the microbiological shelf life and to maintain an attractive red colour of the product. Unfortunately the colour stability is limited, so the shelf life does not exceed 8-9 days and early discoloration causes meat wastes. Vacuum packaging allows more than 20 days of shelf life for retail sale, but the non-attractive purple colour (deoxymyglobin) of the meat is a disadvantage. One interesting solution could be the use up to 0.4% CO in MAP for meat, in order to benefit from a red and stable meat colour. Adding 0.3-0.5% CO in a gas mixture is estimated not to constitute any health risk by the European Commission (2001), but it is presently not on the list of accepted gas combinations as it is in the USA (Sørheim, 2003, FDA, 2004). The major objection raised against using CO as a small component of a MA for retail-ready meat, is the colour stability could possibly exceed the microbiological shelf life, with the risk of masking meat spoilage.

The aim of the present study was to work on this objection by investigating if there were some signs which could warn the consumer on a possible microbiological spoilage of the meat under a gas mixture containing a small amount of CO. This study has also been a good opportunity to achieve, from an official source, data on performances of a packaging method which interests many researchers and could avoid waste of money for meat plants and retailers.

II. MATERIALS AND METHODS

Two experiments were conducted using small amount of CO in the case-ready packaging gas mixture, in French conditions: beef loin steaks (*m. longissimus lumborum et thoracis*) were packaged in 0.4% CO/69.6% N_2/30%CO_2 (low O2 with O_2 scavenger) or 0.4% CO/69.6% O_2/30%CO_2 (high O_2).

Loins were deboned from 22 French Prim' Holstein reformed dairy cows: 12 of them where allocated to the low-O_2 mixture, the 10 others to the high-O_2 one.

For each experiment, two storage temperature conditions were studied. In the decent (acceptable) one, the meat was stored in a dark chilling room at 2°C during the two first thirds of the official expiration date and then alternately 12h in light/12h in darkness at 8°C until the last evaluation. In the poor condition, the packaging steaks were stored in darkness at 2°C only during the first third of the retail life. The decent condition was used as control, the cold chain break was supposed to reflect bad refrigeration in the consumer's fridge. According to the French habits, the official expiration date was 21 days for low-O_2, as for vacuum packaged meats, and 8 days for high-O_2, as for standard high-O_2 MAP

(without CO).

The loins of each animal were cut into 14 steaks, which were randomly assigned to one of the 4 evaluation times: 0 (just before packaging), 16, 23 and 31 days for low-O_2 and 0, 6, 9 and 12 days for high-O_2, with 2 steaks at 0 day and also 2 at each time for each temperature condition.

The low temperature condition and the belated evaluations (after the end of the official expiration date) were chosen in order to take no chances to obtain microbiological altered meats.

Except for day 0, evaluations included a commercial appreciation of the case-ready meat products. This was done by a sensory trained panel (5 members), by a few consumers and by instrumental color measurements (Minolta Chroma Meter CR-300 and spectrocolorimeter). Some trained sensory investigations were also made on cooked grilled meats in order to detect odor and/or flavor deteriorations. Each sensory parameter studied was graded using a 5 points scale, from 5 (perfect meat) to 1 (total degradation), through 3 (commercial deadline for the evaluated parameter). Microbiological status was evaluated through following microorganisms using appropriate media: total viable counts, *lactic acid bacteria*, Enterobacteria, *Pseudomonas* spp., *Brochotrix thermosfacta*, *E. coli*.

Some additional data were obtained concerning the low-O_2 mixture, on meat from 4 other animals. Those meats were partly artificially contaminated, either with 10^3 *Pseudomonas* spp. or with 10^3 Enterobacteria. This was done in order to be sure to have a bad but reasonable meat microbiological status just before the packaging. The storage conditions were the same as they were in previous experiments: decent or bad.

III. RESULTS AND DISCUSSION

In low-O_2 gas mixture, 0.4% CO was very efficient in preservation of meat red color for a very long time, exceeding the official limit of 21 days, since this gas has 20 times higher affinity to bind to myoglobine compared to oxygen (Turubatovic, Milijasevic, Jovanovic and velebit, 2008). This cherry-red color is a bit unusual, but seemed to be commercially acceptable for some French consumers. The commercial evaluations and microbiological results were surprisingly disparate, but rarely dangerous for an eventual consumer.

Two kinds of results were mainly obtained:

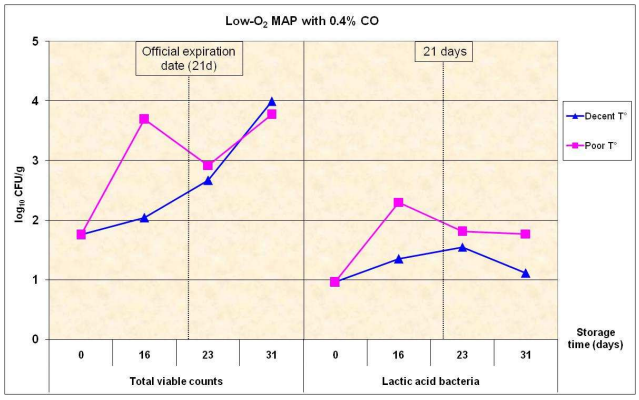
- in the main experiment, steaks showed very small microbial evolutions, even under bad temperature conditions (figure 1 & 2, with no *Pseudomonas* evolution whatever the temperature conditions : graphics confused),
- on the contrary, in the small added experiment with artificial contaminations, case-ready packs had very big contaminations in bad conditions, notably in enterobacteria, which came with a blowing up of the packs, these packs becoming unsealable (figure 3).

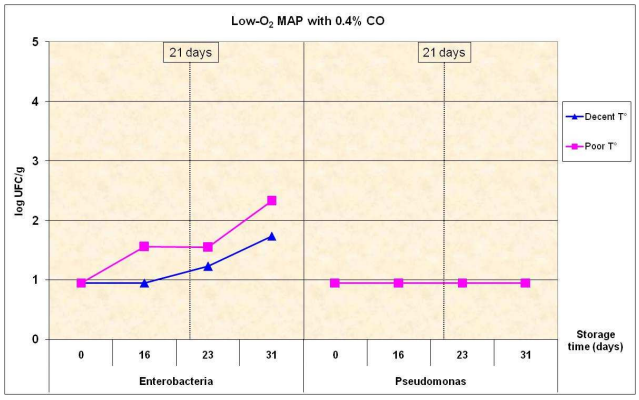
The real problem could be linked to those few packs which presented a notable microbial evolution inside a non-evaluating group (the first mentioned). Neither the raw meat sensory evaluation, before or after the packaging opening, nor the cooked odor evaluation, could systematically spot those steaks (n = 18). However, the contamination levels achieved were mostly not excessive, notably before or near the expiration date: the highest total viable count was 6.48 with 5.62 enterobacteria (log_{10} CFU/g). Moreover, in theory, pathogens were not concerned.

Because of those few cases, the sanitary risk could not be ruled out, even it seemed difficult to concluded categorically on this point.

Figure 4 shows the steaks sensory commercial evolution, which is rather slow before the expiration date and with a small temperature effect. Afterwards differences between temperature conditions increased, the meat showing a more important degradation. Although a good colour stability, the trained panel observed commercial degradations: odours and sticky coating spoilages developed and were perceptible. This fact is opposite to the sanitary risk mentioned by the European Commission.

Figures 1 & 2. Main experiment without O_2 . Mean values for microbiological counts for beef steaks stored in 0.4%CO/69,6%N_2/30%CO_2 at 2°C during 14 days and then at 8°C until 31 days (Δ decent temperature conditions), or at 2°C during 7 days and then at 8°C until 31 days (□ poor temperature conditions)





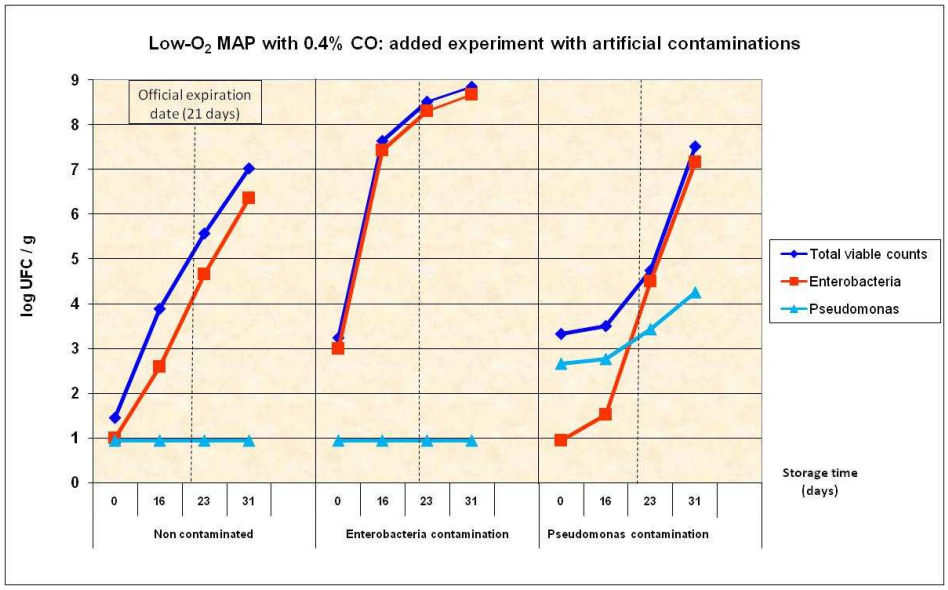


Figure 3. Small added experiment without O_2 - Mean values for microbiological counts for 3 kinds of beef steaks stored in 0.4%CO/69,6%N_2/30%CO_2 at 2°C during 7 days and then at 8°C (poor temperature conditions) until 31 days: without artificial contamination (on the left), with enterobacteria artificial contamination (center) and with *Pseudomonas spp.* artificial contamination (on the right)

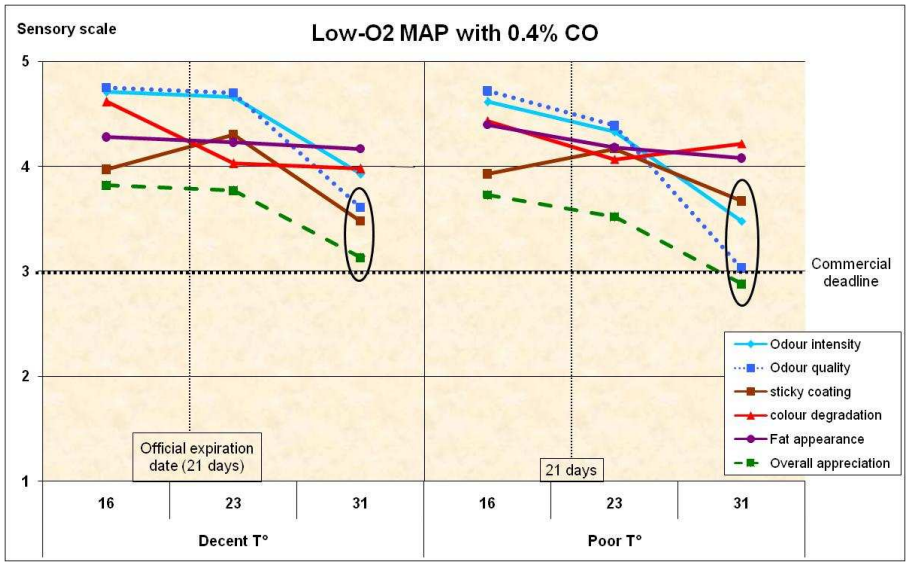


Figure 4. Main experiment without O_2 - Mean sensory commercial evaluation values for beef steaks stored in 0.4%CO/69,6%N_2/30%CO_2 in decent or poor temperature conditions until 31 days, using 5 points scales from 5 = perfect meat for the studied parameter to 1 = totally degraded, with a commercial deadline at score 3

The use of **high-O_2 MAP** near those currently used in the retail stores in France, but including 0.4% of carbon monoxide, was apparently on little interest. High level of oxygen allowed keeping the usual red color of the meat, but without avoiding his quick deterioration. Beef packaged 0.4% CO-atmosphere had color stability similar to that packaged in usual high-O_2-atmosphere, not greater. So the first limit of the shelf life was always the meat discoloration not the microbiological status. In this experiment the commercial deadline was reached 9 days after the packaging, whatever the temperature conditions. Color and fat degradations were mainly responsible for this situation whereas there were very few odors and spoilage (sticky coating) development, suggesting low microbiological loads. The total viable counts were indeed very satisfactory as never exceeded 3 $\log_{10}$ CFU/g, even after 12 days of storage in bad temperature conditions (table 1). So the sanitary risk seemed to be non-existent. On the other hand the early meat discoloration was not solved. This result was disappointing.

Our results raises questions about microbiological effects of carbon monoxide in meat: in the 2 main experiments the very good mean bacteriological status could be linked to a bacteriostatic effect of CO. This was not the case for the added experiment which showed big spoilage evolutions. However this small experiment was conducted under the poor temperature conditions that are 8°C during the main part of the storage duration. One question still remained: why didn't we see such big microbiological growths in the part of the main experiments under the same temperature conditions? According to the scientific literature, the microbial influence of CO seems to be complex. Using CO in MAP could potentially extend the shelf life of fresh meat because it is selectively bacteriostatic for selected microbial populations (Stetzer, Wicklund, Paulson, Rucker, Macfarlane & Brewer, 2007). Luño, Roncalés, Djenane & Beltrán (2000) reported that CO-MAP greatly reduced aerobic plate counts in beef, although lactic acid bacteria appeared unaffected. Fraqueza, Ferreira & Barreto (2006) showed that the introduction of 0.5% CO in gas mixtures without oxygen promoted a significant inhibition of *Brochotrix thermosphacta* and did not affect Pseudomonas spp. counts.

Sorheim et al. (1999) suggested that 0.4% CO probably had little or no direct effect on the growth of bacteria.

Zee, Bouchard, Simard, Richard & Holley (1984) indicated that CO influence on bacteria could be linked to the gas concentration, but not necessarily proportionally. In the present study, the gas mixtures were separately bought for each experiment. These ready mixtures had a confidence level at 95% (5% relative blend tolerance). Could this uncertainty explain part of our disparate results?

Table 1. Experiment with O_2 - Mean sensory, instrumental and microbiological values of steaks packaged under 0.4%CO/69,6%O_2/30%CO_2

		D0	Decent T°			Poor T°		
			D0 + 6 d	D0 + 9 d	D0 + 12 d	D0 + 6d	D0 + 9 d	D0 + 12 d
Trained panel sensory evaluation after packaging opening with a 5 points scale (5 = perfect, 3 = commercial deadline, 1= totaly deteriorated) N = 50/cell and 100/cell for odours (5 judges)	Odour intensity	-	4.72	4.38	3.98	4,65	4.37	3.78
	Odour quality	-	4.55	4.37	3.82	4.54	4.39	3.53
	Colour degradation	-	4.50	3.14	**2.68**	4.16	3.02	**2.56**
	Fat appearance	-	4.18	3.50	**2.86**	4.10	3.50	**2.80**
	Sticky coating	-	4.48	4.38	3.80	4.36	4.30	3.68
	Overall appreciation	-	4.36	3.22	**2.76**	4.02	3.04	**2.56**
Microbiological counts (log_{10} CFU/g) - N = 10/cell	Total viable counts	1.22	1.12	1.60	2.50	1.30	2.44	3.83
	Lactic acide bacteria	0.95	0.95	0.95	1.31	0.95	1.52	1.41
	Enterobacteria	0.95	0.95	1.02	0.95	0.95	0.96	1.67
	E. coli	0.95	0.95	0.95	0.95	0.95	0.95	0.95
	Pseudomonas spp.	0.95	0.95	1.03	1.06	0.95	1.37	2.56
	Brochotrix thermosph.	0.95	0.95	0.95	0.95	0.95	1.10	2.07

IV. CONCLUSION

Regarding these results, it could be of interest for the French beef industry to use 0.4% CO in a low-O_2 gas mixture for case-ready packs, combined with a short expiration date. The aim would not be to reach a long retail shelf life as for vacuum case-ready meats, but to close the one of the high-O_2 MAP used in French retail stores at present, which is around 8-10 days. By stabilizing meat color, this solution could reduce the meat waste as meat plants and/or retailers would like to, without a major risk for consumer health.

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