HOW MUCH CARBON MONOXIDE IS NECESSARY FOR COLOUR STABILISATION AND SHELF-LIFE EXTENSION OF BEEF PACKAGED IN MODIFIED ATMOSPHERES?.

Manuel Luño, Pedro Roncalés and José Antonio Beltrán

Dept. of Animal Production and Food Science. Laboratory of Food Technology. Faculty of Veterinary Science. University of Zaragoza. C. Miguel Servet 177. 50013 ZARAGOZA. SPAIN.

INTRODUCTION

Modified atmosphere packaging (MAP) is a usual system of meat retail sale. The atmospheres used combine oxygen (O_2), carbon dioxide (CO_2) and eventually nitrogen (N_2) to maintain the quality of fresh red meat, both from a microbiological and an organoleptic point of view. The incorporation of low levels of CO stabilises red colour of meat (Clark *et al.*, 1976). CO combines with myoglobin to form carboxymyoglobin (MbCO), which is more stable than oxymyoglobin and gives an attractive cherry-red colour to meat (El-Badawi *et al.*, 1964). Carbon monoxide is considered a toxic gas; therefore, its use for food packaging is not allowed in most countries. However, according to the International Standard for the determination of toxicity of gases (ISO 10298, 1995), the concentration of CO in a gas mixture necessary for reaching the toxicity limit is much higher than generally thought. The Norwegian meat industry, according to Sorheim *et al.* (1997a), has been using a gas mixture containing 0.3-0.4% CO during the past 10 years, and they concluded that gas mixtures with a low concentration of CO, up to about 0.5%, do not present any toxic threat to consumers. The aim of the present research was to investigate the effect of various low CO concentrations on shelf-life extension of fresh red meat, as well as to determine the critical level of CO necessary for colour stabilisation.

MATERIALS AND METHODS

Samples and atmospheres M. *Longissimus lumborum* was removed from 3 beef carcasses 48 hr *post mortem* and 2 cm thick steaks were cut. Steaks were individually placed on polystyrene trays of 15.5 x 21.5 cm and sealed (after flushing with the selected gas mixtures) within a laminated pouch of polyethylene and polyamide (water vapour permeability of 5-7 g m^{-2} 24 hr^{-1} at 23°C and oxygen permeability of 40-50 ml m^{-2} 24 hr^{-1} at 23°C), supplied by Sidlaw Packaging-Soplaril (Barcelona, Spain), and stored at 1±1°C. Gas mixtures were supplied by Abelló Linde S. A. (Barcelona, Spain) and consisted of 70% O_2 + 20% CO_2 + 10% N_2 (control modified atmosphere: CMA), 24 %O_2 + 50% CO_2 + 25.9% N_2 + 0.1% CO (LO-CO 0.1), 24 %O_2 + 50% CO_2 + 25.75% N_2 + 0.25% CO (LO-CO 0.25), 24 %O_2 + 50% CO_2 + 25.5% N_2 + 0.5% CO (LO-CO 0.5), 24 %O_2 + 50% CO_2 + 25.25% N_2 + 0.75% CO (LO-CO 0.75) and 24 %O_2 + 50% CO_2 + 25% N_2 + 1% CO (LO-CO 1). Steaks from each animal were assigned to all treatments. **Microbial analysis.** Counts of aerobic psychrotrophic flora were determined in Plate Count Agar (Merck; Darmstadt, Germany) incubated at 7°C for 10 days (Elliott *et al.*, 1983); lactic acid bacteria on MRS agar (Merck; Darmstadt, Germany), incubated anaerobically at 25°C for 4 days; and *Brochothrix thermosphacta* in STAA agar (Oxoid; Basingstoke, UK) with streptomycin sulfate (500 mg/l), thallous acetate (50 mg l^{-1}) and cycloheximide (50 mg l^{-1}) incubated aerobically at 25°C for 3 days. Counts were expressed as log cfu cm^{-2}. **Colour instrumental measurement.** Objective measurement of colour (CIE L*, a*, b*) was performed at the surface of meat samples using a reflectance spectrophotometer (Minolta Chroma Meter CM-2002), 30 min after package opening. Each value was the mean of 20-25 determinations. **Metmyoglobin percentage.** Metmyoglobin percentage was estimated spectrophotometrically by measuring the reflectance at 525 and 572 nm according to Stewart *et al.* (1965). The maximum value of the quotient between K/S_{572} and K/S_{525} at the beginning of the experiment was fixed as 0% MetMb, while 100% MetMb was obtained after oxidising a sample in a 1% (w/v) solution of potassium ferricyanide (Ledward, 1970). Each value was the mean of 20-25 determinations. **Sensory analysis.** Meat samples were evaluated by a six-member trained panel. Three open-discussion sessions were held to familiarise the individual with the attributes and the scale to use. The attributes studied were: 'Red Colour', 'Discoloration' and 'Fresh Meat Odour'. 'Red Colour' was scored using a 9-point scale; 9 denoted extremely high and 1 denoted extremely low. 'Discolouration' (% of discoloured surface) was assessed using a 5-point scale, according to Sorheim *et al.* (1996); 1 = none, 2 = 0-10%, 3 = 11-20%, 4 = 21-60%, and 5 = 61-100%. 'Fresh Meat Odour' was evaluated using a 5-point scale; 1 = Excellent, not different from fresh meat ; 2 = Good, but slightly poorer than fresh meat; 3 = Acceptable, but obviously poorer than fresh meat; 4 = Hardly acceptable as fresh meat; and 5 = Non acceptable.

RESULTS AND DISCUSSION

Microbial analysis. Psychrotrophic flora counts (Fig. 1) showed a similar pattern to that reported by Luño et al. (1998). Bacterial growth was significantly inhibited ($p < 0.05$) when beef steaks were packaged in modified atmospheres with 50% CO_2, that is, all LO-CO atmospheres independently of CO concentration. A reduction of 1-1.5 log cfu cm^{-2} was found from 15 days of storage onwards. Spoilage due to psychrotrophic bacteria growth was then delayed by about 10 days by effect of packaging in LO-CO atmospheres. The evolution of counts on MRS agar is shown in Fig. 2. Growth of **lactic acid bacteria (LAB)** was quite similar under the different atmospheres. After 29 days of storage, counts reached values around 5 log cfu cm^{-2} for all atmospheres. These results agreed with the well known fact that LAB are relatively insensitive to CO_2 (Farber, 1991; Parry, 1993). Counts of ***Brochothrix thermosphacta*** (*B. th.*; Fig. 3) grew quite rapidly in CMA-packaged samples after 15 days of storage, reaching maximum levels of 5 log cfu cm^{-2} at 29 days. Samples packaged in atmospheres containing 50% CO_2 showed a strong delay ($p<0.05$) in *B. th.* growth, which remained at a lower rate throughout all the storage period (≤3 log cfu cm^{-2} at 29 days). This effect of CO_2 on *B. th.* growth is in good agreement with the results reported by Blickstad and Molin (1983) and Gill and Harrison (1989).

Colour instrumental measurement. Figure 4 shows that samples packaged in LO-CO 0.75 and LO-CO 1 had much higher (significantly different; $p<0.01$) a* values, than those of samples packaged in CMA (in good agreement with Luño et al., 1998), after 29 days of storage. Difference was also significant ($p<0.01$) with all other LO-CO atmospheres. There were significant differences ($p<0.01$), too, between LO-CO 0.5 and those of group 1 (LO-CO 0.1 and LO-CO 0.25) and CMA. Therefore, samples packaged in modified atmospheres with a minimum of 0.50% CO provided a significant ($p<0.01$) stabilisation of red meat colour and, consequently, shelf life extension. Sorheim et al. (1997b) obtained similar results for beef loin steaks packaged in a 0.4% CO + 60% CO_2 + 40% N_2 atmosphere.

Metmyoglobin percentage. Fig. 5 shows a significant difference ($p < 0.01$) in the MetMb percentage, from day 19 to the end of the experiment, between CMA and both LO-CO 0.75 and LO-CO 1. After 29 days of storage, LO-CO 0.5 was also significantly different ($p < 0.01$) from CMA, although MetMb percentage was in this case much higher than that of steaks packaged in atmospheres containing over 0.75% CO.

In a previous work (Luño et al., 1998), beef loin steaks packaged in an atmosphere containing 1% CO had less than 30% MetMb at 29 days of storage (0±1°C). In the present experiment, for the same storage time and temperature, only atmospheres containing over 0.75% CO evidentiated its ability for maintaining MetMb below 40% for 29 days. This MetMb percentage might be considered as the limit of acceptance, since Greene *et al.* (1971) reported that 40% MetMb caused meat rejection by consumers. LO-CO 0.5 showed only a partial ability to inhibit MetMb formation, limited to a 4-5 days period.

Sensory analysis. The results of sensory analysis are summarised in Table 1, and include evaluation of red colour, discolouration and fresh meat odour. Regarding **red colour**, it increased simply as a consequence of the inclusion of CO into the atmosphere. Colour score of meat packaged in CMA (7.5) was lower than in any CO atmosphere (9) at the same storage time. Literature review (García-Matamoros and Moral-Rama, 1973; Gee and Brown, 1978; Luño *et al.*, 1998) revealed that 1%CO is the most used concentration in order to stabilise red colour and increase colour shelf-life of meat. Clark *et al.* (1976) established that 1%CO was the minimum concentration required for optimum colour, but 0.5% CO gave good colour stability, too. Sorheim *et al.* (1997a) obtained similar results with an atmosphere containing 0.4% CO. In our experiment, meat packaged in all atmospheres containing CO had a better red colour than CMA throughout all the storage time (29 days). LO-CO 1 maintained the improved colour during 24 days, LO-CO 0.75 and LO-CO 0.5 for 19 days, LO-CO 0.25 and LO-CO 0.1 for 15 days, while meat packaged in CMA lasted only 10 with its original red colour. **Discolouration** appeared after 15 days of storage in CMA packaged meat, 19 days for LO-CO 0.25 and LO-CO 0.1 samples and 24 days for samples packaged in atmospheres containing 0.5% CO or more. If we consider a value of 2 as the

limit of acceptance, which correspond to 0-10% discoloured area, beef steaks packaged in CMA would be refused after 15 days of storage, while samples packaged in atmospheres containing 0.5% CO or higher would not be refused until 24 days of storage. These results were in good agreement with previous work (Luño et al., 1998). **Fresh meat odour** was better maintained in meat steaks packaged in LO-CO atmospheres, probably due to the presence of 50% CO_2 instead of 20 % CO_2 of CMA, and related to microbial growth inhibition. After 19 days of storage samples packaged in CMA evidentiated still good odour, but slightly poorer than fresh meat. In contrast; steaks packaged in LO-CO atmospheres maintained an excellent fresh meat odour independently of CO percentage. After 24 days, CMA, LO-CO 0.25 and LO-CO 0.1 were scored with a 2, while atmospheres with 0.5% CO or more were scored with a 1. On day 29th, a clear difference, related to CO content, was evident amongst the atmospheres; CMA was scored with a 5, LO-CO 0.1 with a 4, LO-CO 0.25 with a 3 and atmospheres with 0.5% CO or more were scored with a 2. These results proved that increasing concentrations of CO were able to extend the odour shelf-life of meat, independently of the presence of 50% CO_2. Previous results (Luño et al., 1998) clearly showed that 1 % CO improved odour shelf life. Clark *et al.* (1976) already reported that CO (above 0.5%) extended meat odour shelf life.

CONCLUSION

A modified atmosphere containing 0.5 to 0.75% CO, 50% CO_2, 24% O_2 and about 25% N_2 combines a number of worthwhile effects for meat storage: very good and stable colour and odour, extended microbiological shelf-life with minimum pathogen hazard and no fire or explosion risk. This mixture is nontoxic according to ISO 10298.

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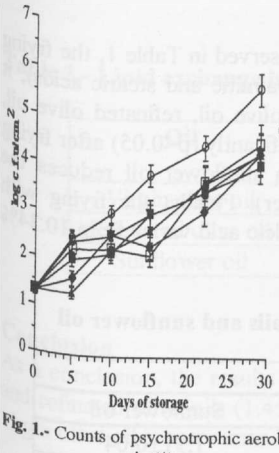

Fig. 1.- Counts of psychrotrophic aerobes in (*).

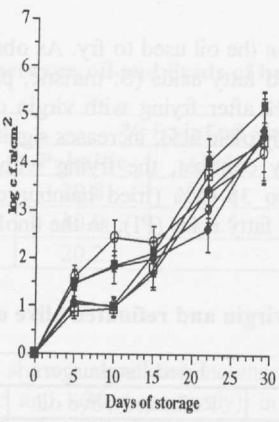

Fig. 2.- Counts of acid lactic flora in (*).

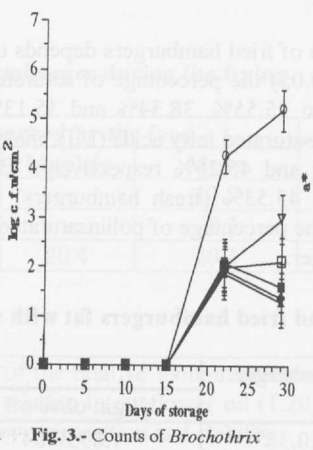

Fig. 3.- Counts of *Brochothrix termosphacta* in (*).

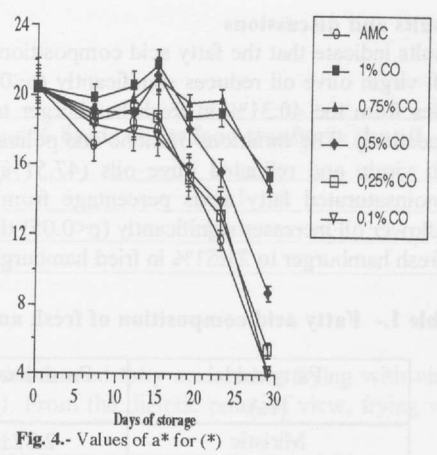

Fig. 4.- Values of a* for (*)

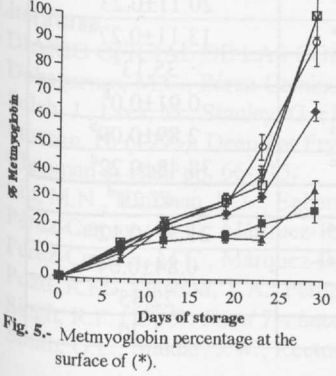

Fig. 5.- Metmyoglobin percentage at the surface of (*).

Table 1.- Mean Sensory Values [†] of (*) using a 9-point scale: 9 denoted extremely high and 1 denoted extremely low.

Parameter	Gas atmosphere	Days of storage						
		0	*5*	*10*	*15*	*19*	*24*	*29*
Red Colour	CMA	7.5	7.5	7.5	6.5	5.9	4	1
	LO-CO 0.1	9	9	9	9	7	6	2.5
	LO-CO 0.25	9	9	9	9	8.3	7.5	2.5
	LO-CO 0.5	9	9	9	9	9	8.7	4
	LO-CO 0.75	9	9	9	9	9	8.7	5.3
	LO-CO 1	9	9	9	9	9	9	7
Discoloration	CMA	1	1	1	2	3	4	5
	LO-CO 0.1	1	1	1	1	2	3	5
	LO-CO 0.25	1	1	1	1	2	3	5
	LO-CO 0.5	1	1	1	1	1	2	3
	LO-CO 0.75	1	1	1	1	1	2	2
	LO-CO 1	1	1	1	1	1	2	2
Fresh meat odour	CMA	1	1	1	1	2	2	5
	LO-CO 0.1	1	1	1	1	1	2	4
	LO-CO 0.25	1	1	1	1	1	2	3
	LO-CO 0.5	1	1	1	1	1	1	2
	LO-CO 0.75	1	1	1	1	1	1	2
	LO-CO 1	1	1	1	1	1	1	2

[†] Data are means of 3 samples from different carcasses.

(*) Beef Loin Steaks Packaged in the Following Gas Atmospheres: CMA (70% O_2 + 20% CO_2 + 10% N_2); LO-CO 0.1 (24% O_2 + 50% CO_2 + 25.9% N_2 + 0.1% CO), LO-CO 0.25 (24% O_2 + 50% CO_2 + 25.75% N_2 + 0.25% CO), LO-CO 0.5 (24% O_2 + 50% CO_2 + 25.5% N_2 + 0.5% CO), LO-CO 0.75 (24% O_2 + 50% CO_2 + 25.25% N_2 + 0.75% CO) and LO-CO 1 (24% O_2 + 50% CO_2 + 25% N_2 + 1% CO).