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Beef shelf life in low O_2 and high CO_2 atmospheres containing different low CO concentrations

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Abstract

The use of atmospheres with low concentrations of CO (0.1 to 1%), in combination with O_2 (24%), high CO_2 (50%) and N_2 (25 to 25.9%), for preserving chilled beef steaks was investigated. The atmosphere used as reference contained 70% O_2 + 20% CO_2 + 10% N_2. Bacterial counts showed that all atmospheres containing CO greatly reduced total aerobic population numbers, including *Brochothrix thermosphacta*. Lactic acid bacteria, however, were not affected. CO concentrations of 0.5–0.75% were able to extend shelf life by 5–10 days at $1 \pm 1°C$, as demonstrated by delayed metmyoglobin formation (less than 40% of total myoglobin after 29 days of storage), stabilisation of red colour (no change of CIE a^* and hue angle after 23 days), maintenance of fresh meat odour (no variation of sensory score after 24 days) and significant ($P < 0.01$) slowing of oxidative reactions (TBARS).

Keywords: Beef; Meat; Carbon monoxide; Carbon dioxide; Modified atmosphere packaging; Psychrotrophic bacteria

1. Introduction

Modified atmosphere packaging (MAP) is well-known as a method for extending the shelf life of a variety of foods, including fresh meat. Atmospheres used combine oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) to maintain the quality of fresh red meat, both from a microbiological and an organoleptic point of view.

The inhibitory effect of CO_2 on microbial growth, and therefore on the extension of meat shelf life, is well documented (Clark & Lentz, 1972; Huffman, Davis, Marple & McGuire, 1975; Silliker & Wolfe, 1980; Taylor & McDougall, 1973). Nevertheless, several researchers have stressed that the use of high levels of CO_2, with a consequent low O_2 concentration, can cause meat discolouration (Ledward, 1970; Silliker, Woodruff, Lugg, Wolfe & Brown, 1977). Discolouration of the characteristic bright red meat colour is related to the conversion of oxymyoglobin to metmyoglobin (Ledward, 1984). This phenomenon may be counteracted by incorporation of CO to the atmosphere (Clark, Lentz & Roth, 1976; Luño, Beltrán & Roncalés, 1998; Silliker & Wolfe, 1980). CO combines with myoglobin to form carboxymyoglobin (MbCO), which is more stable to oxidation than oxymyoglobin and gives an attractive cherry-red colour to meat (El-Badawi, Cain, Samuels & Anglemeier, 1964). Because of the high stability of MbCO, only relatively low levels of CO are needed in order to maintain red colour of meat (Clark et al.; Luño et al.).

Carbon monoxide is a toxic gas; therefore, its use for food packaging is not allowed in most countries. However, the Norwegian meat industry, according to Sørheim, Nissen and Nesbakken (1997,1999), has been using a gas mixture containing 0.3–0.4% CO during the past 10 years; indeed, they estimated that 50–60% of the retail meat is packaged in this modified atmosphere. They reviewed the toxicological aspects of CO used in modified atmosphere packaging of meat, and concluded that gas mixtures with a low concentration of CO, up to about 0.5%, do not present any toxic threat to consumers. The calculation of the concentration of CO in a gas mixture necessary for reaching the toxicity limit is established in the International Standard for the Determination of Toxicity of Gases (ISO, 10298, 1995).

Colour is the most important sensory attribute for consumer decisions on the purchase of fresh meat. Hence, the effects of low concentrations of CO on the stabilisation of fresh meat colour is of interest. There

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has been little research on the effect of very low concentrations of CO on the stability of meat colour (Clark et al., 1976). Therefore, the effects of various very low CO concentrations on the shelf life of fresh red meat was examined to determine the critical level of CO necessary for colour stabilisation. Besides CO, gas mixtures contained high CO_2 and low O_2 (about atmospheric) concentrations, as used previously by Luño et al. (1998).

2. Material and methods

2.1. Samples and atmospheres

M. *Longissimus lumborum* was removed from three market-weight beef carcasses 48 h post mortem, and 1.5 cm thick steaks of about 100 g were cut from them. Each steak was placed on a polystyrene tray of size 15.5×21.5×2.5 cm. The tray with the steak was introduced in a pouch made of a polyethylene and polyamide laminate of water vapour permeability 5–7 g m^{-2} 24 h^{-1} at 23°C and oxygen permeability 40–50 ml m^{-2} 24 h^{-1} at 23°C (Sidlaw Packaging-Soplaril, Barcelona, Spain), which was filled with approximately 2.5 l of a selected gas mixture, sealed 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) or 24%O_2 + 50% CO_2 + 25% N_2 + 1% CO (LO-CO 1).

2.2. Microbial sampling and analysis

Two sterile cotton swabs moistened in 0.1% peptone water were used for swabbing 10 cm^2 of meat surface, delimited by a sterile stainless steel template. Swabs were stirred thoroughly in 10 ml of 0.1% peptone water. Serial 10-fold dilutions were prepared by diluting 1 ml in 9 ml of 0.1% peptone water. Two plates were prepared from each dilution by pouring 1 ml in fluid agar. Counts of aerobic psychrotrophic flora were determined from plates bearing 20–200 colonies in plate count agar (PCA; Merck, Darmstadt, Germany) incubated at 7°C for 10 days (Elliott, Clark & Lewis, 1983); lactic acid bacteria on DeMan-Rogosa-Sharpe (MRS) agar (Merck, Darmstadt, Germany), incubated anaerobically using an H_2 + CO_2 Gas-Pac generator (BBL), at 25°C for 4 days; and *Brochothrix thermosphacta* in streptomycin-thallous-acetate-actidione (STAA) agar (Oxoid, Basingstoke, UK) with streptomycin sulfate (500 mg l^{-1}), 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}.

2.3. Colour instrumental measurement

The colour (CIE L^*, a^*, b^*) of the surfaces of meat samples were measured objectively using a reflectance spectrophotometer (Minolta Chroma Meter CM-2002), 30 min after package opening. Hue angle (h) was calculated by the following formula: $h = \tan^{-1}(b^*/a^*)$. The average value of all parameters for each steak was the mean of 20–25 determinations.

2.4. Metmyoglobin percentage

The metmyoglobin percentage of the total myoglobin perceptable at the steak surface was estimated spectrophotometrically, according to Stewart, Zipser and Watts (1965), by measuring steak surface reflectance at 525 and 572 nm (Minolta Chroma Meter CM-2002). The average value of the ratios of K/S_{572} to K/S_{525} at the beginning of the experiment was fixed as 0% MetMb; K and S were the absorption and the scattering coefficients, respectively, and K/S ratios were calculated from reflectivity (R_∞) values using the Kubelka–Munk equation. The value of 100% MetMb was obtained following the same procedure after oxidising a sample in a 1% (w/v) solution of potassium ferricyanide (Ledward, 1970). The average value for each steak was the mean of 20–25 determinations.

2.5. Lipid oxidation

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of Witte, Krause and Bailey (1970). The amounts of substances reactive with TBA (TBARS) were expressed as mg malonaldehyde kg^{-1} sample.

2.6. Sensory analysis

Meat samples were evaluated by a six-member expert panel, trained according to the method of Cross, Moen and Stanfield (1978). Three open-discussion sessions were held to familiarise panellists with the attributes and the scale to be used. The attributes studied were: 'Red Colour', 'Discolouration' and 'Fresh Meat Odour'. All three attributes were scored using a 5-point scale. For 'Red colour', 5 denoted extremely high and 1 denoted extremely low. Scores for 'Discolouration' referred to percentage of discoloured surface, according to Sørheim, Kroff, Hunt, Karwoski and Warren (1996): 1 = none, 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, and 5 = 61–100%. Scores for 'Fresh Meat Odour' were: 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. Samples were scored by each panel member, and mean values were agreed thereof after discussion by all six panellists.

2.7. Determination of the proportion of gases in packaging atmospheres

Carbon monoxide concentrations in the headspace gases of meat packages were determined using a Hewlett Packard 4890 gas chomatograph equiped with a thermal conductivity detector. Samples of 50 μl were injected into a chrompack CP-carboplot P7 column of 0.53 mm inner diameter and 27.5 m length, with helium as the carrier gas at a flow rate of 12.6 ml min^{-1}. The initial temperature of the oven was set at 40°C. After 2.5 min it was raised at a rate of 45°C min^{-1} to a final temperature of 115°C. The temperature of the injector block was 59°C, and that of the detector was 120°C. A calibration curve was prepared using LO-CO atmospheres supplied by Abelló Linde S. A. (Barcelona, Spain). The total amount of gas (mmol) was calculated assuming an average package volume of 2.5 l.

2.8. Statistical analysis

The significance of differences among samples at each day of storage was determined by analysis of variance using the least square difference method of the general linear model procedure of SAS (1985).

3. Results and discussion

3.1. Development of microflora

As shown in Fig. 1, growth of the spoilage psychrotrophic flora was significantly inhibited ($P < 0.05$) when beef steaks were packaged in modified atmospheres with 50% CO_2; that is, in all LO-CO atmospheres irrespective of CO concentration. A difference of 1–1.5 log cfu cm^{-2} was found from 15 days of storage onwards with respect to samples packaged in CMA. Counts close to 6 log cfu cm^{-2} were reached only after 29 days of storage, about 10 days later than with control samples. Therefore, spoilage due to psychrotrophic bacteria would be delayed by about 10 days at 1°C by packaging in LO-CO atmospheres rather than in CMA. A similar finding was reported by Luño et al. (1998) for beef packaged in a low O_2 atmosphere containing 1% CO.

The growth on meat of the microorganisms which are selected on MRS agar is shown in Fig. 2. The growth of those lactic acid bacteria (LAB) was similar in all atmospheres. After 29 days of storage, counts reached values around 5 log cfu cm^{-2}. These results would be expected from the known insensitiveness of LAB to CO_2 (Farber, 1991; Parry, 1993).

B. thermosphacta grew rapidly in CMA-packaged samples after 15 days of storage, reaching maximum levels of 5 log cfu cm^{-2} at 29 days (Fig. 3). However, its growth was strongly delayed ($P < 0.05$) in samples packaged in atmospheres containing 50% CO_2, which grew at a lower rate throughout all the storage period to reach no more than 3 log cfu cm^{-2} after 29 days. This effect of CO_2 on *B. thermosphacta* growth is in good agreement with the results reported by Blickstad and Moulin (1983) and Gill and Harrison (1989).

3.2. Instrumental measurements of colour

Changes in CIE a^* values throughout the storage period of beef steaks packaged in CMA or LO-CO atmospheres are shown in Fig. 4. Values of a^*, which increase as red tones become more pronounced, demonstrated that redness was influenced by the

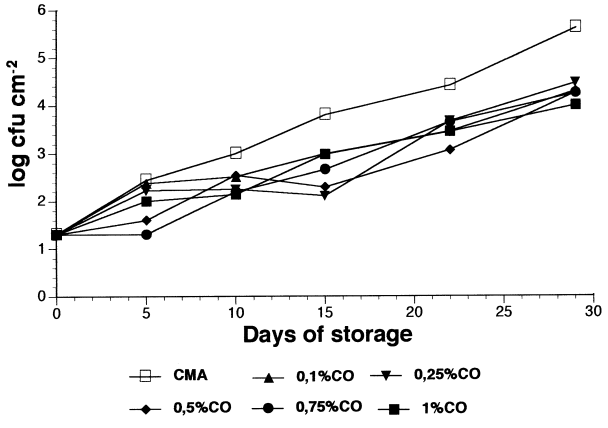

Fig. 1. Psychrotrophic flora counts from beef loin steaks stored at 1 ± 1°C in atmospheres: (□) CMA: 70% O_2 + 20% CO_2 + 10% N_2; all LO-CO atmospheres: 24% O_2 + 50% CO_2 + 25–26% N_2 + CO at the following concentrations: (▲) 0.1%; (▼) 0.25%; (◆) 0.5%; (●) 0.75%; and (■) 1%.

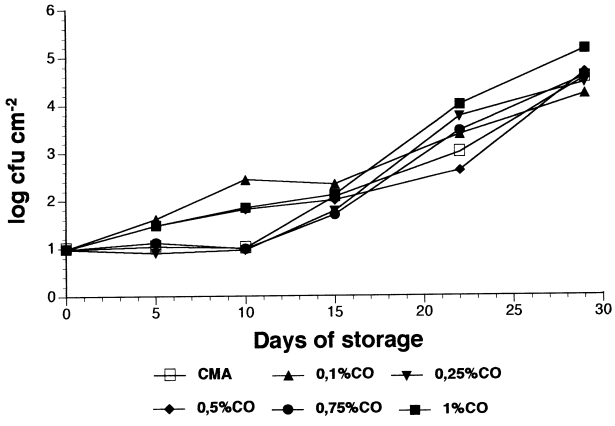

Fig. 2. Lactic acid bacteria counts from beef loin steaks stored at 1 ± 1°C in atmospheres: (□) CMA: 70% O_2 + 20% CO_2 + 10% N_2; all LO-CO atmospheres: 24% O_2 + 50% CO_2 + 25–26% N_2 + CO at the following concentrations: (▲) 0.1%; (▼) 0.25%; (◆) 0.5%; (●) 0.75%; and (■) 1%.

concentration of CO used. Thus, three types of atmosphere could be stablished according to their increasing ability for maintaining red colour of meat. These were: type 1 (LO-CO 0.1 and LO-CO 0.25), which did not increase red colour stability with respect to steaks stored in the CMA atmosphere; type 2 (LO-CO 0.5 alone) which increased stability of red colour; and type 3 (LO-CO 0.75 and LO-CO 1), which greatly increased its stability, in good agreement with Luño et al. (1998). Values of a^* for beef steaks did not differ ($P > 0.05$) within the same group, while differences were significant ($P < 0.01$) amongst groups.

Therefore, samples packaged in modified atmospheres with a minimum of 0.5% CO provided a significant ($P < 0.01$) stabilisation of red meat colour. Sørheim et al. (1997) obtained similar results for beef loin steaks packaged in a 0.4% CO + 60% CO_2 + 40% N_2 atmosphere. Our finding also demonstrated that colour stabilisation was extended by increasing CO concentration. Indeed, LO-CO 0.75 samples reached the maximum extension, of about 10 days at 1 ± 1°C, as that obtained with 1% CO in the atmosphere.

Changes in hue angle values during the storage of beef steaks, shown in Fig. 5, confirmed that surface colour could be grouped according to the three atmosphere types, as observed for redness a^* values. The maximum ($P < 0.01$) stabilising of meat colour was accomplished with the LO-CO 0.75 and LO-CO 1 atmospheres, while LO-CO 0.1 and LO-CO 0.25 were no more effective for maintaining meat colour than CMA.

3.3. Metmyoglobin percentages

Fig. 6 shows the changes in surface MetMb percentage of total myoglobin during storage of beef steaks packaged in CMA or LO-CO atmospheres. A

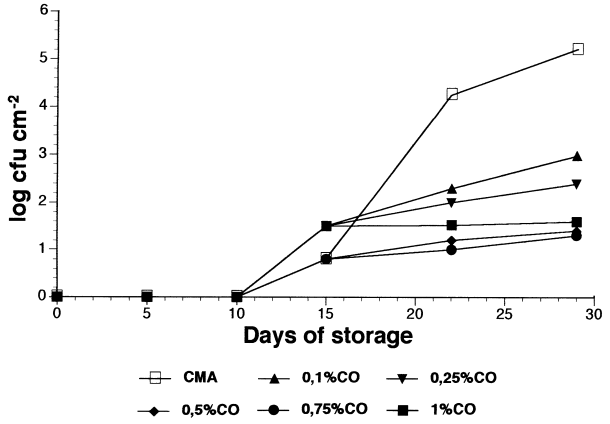

Fig. 3. *Brochothrix thermosphacta* counts from beef loin steaks stored at 1 ± 1°C in atmospheres: (□) CMA: 70% O_2 + 20% CO_2 + 10% N_2; all LO-CO atmospheres: 24% O_2 + 50% CO_2 + 25–26% N_2 + CO at the following concentrations: (▲) 0.1%; (▼) 0.25%; (◆) 0.5%; (●) 0.75%; and (■) 1%.

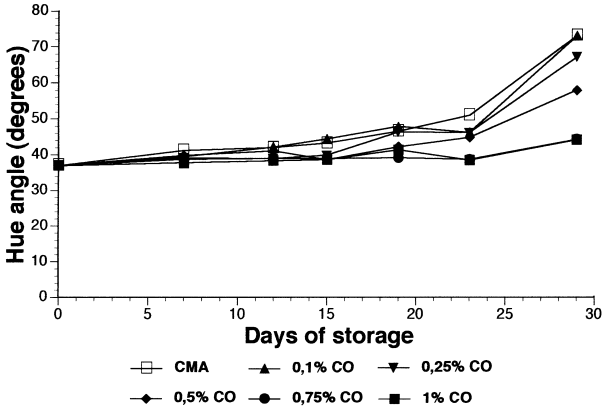

Fig. 5. Values of CIE hue angle in beef loin steaks stored at 1 ± 1°C in atmospheres: (□) CMA: 70% O_2 + 20% CO_2 + 10% N_2; all LO-CO atmospheres: 24% O_2 + 50% CO_2 + 25–26% N_2 + CO at the following concentrations: (▲) 0.1%; (▼) 0.25%; (◆) 0.5%; (●) 0.75%; and (■) 1%.

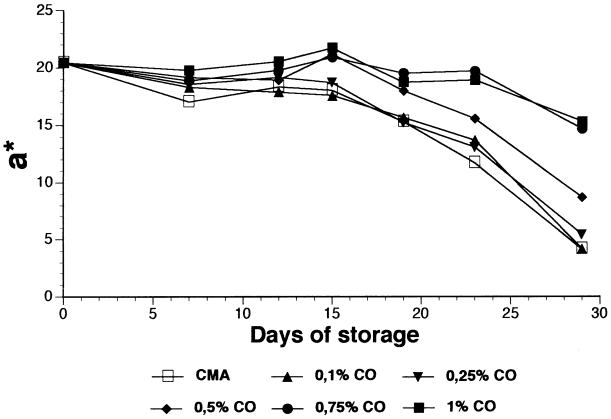

Fig. 4. Values of CIE a^* in beef loin steaks stored at 1 ± 1°C in atmospheres: (□) CMA: 70% O_2 + 20% CO_2 + 10% N_2; all LO-CO atmospheres: 24% O_2 + 50% CO_2 + 25–26% N_2 + CO at the following concentrations: (▲) 0.1%; (▼) 0.25%; (◆) 0.5%; (●) 0.75%; and (■) 1%.

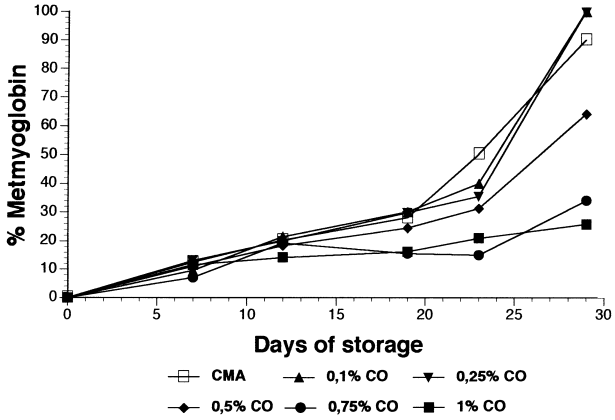

Fig. 6. Percentage of metmyoglobin at the surface of beef loin steaks stored at 1 ± 1°C in atmospheres: (□) CMA: 70% O_2 + 20% CO_2 + 10% N_2; all LO-CO atmospheres: 24% O_2 + 50% CO_2 + 25–26% N_2 + CO at the following concentrations: (▲) 0.1%; (▼) 0.25%; (◆) 0.5%; (●) 0.75%; and (■) 1%.

significant difference ($P < 0.01$) in the MetMb percentages was found between meat in CMA and meat in both LO-CO 0.75 and LO-CO 1 at day 19 and later times. After 29 days of storage, the MetMb fraction for meat in LO-CO 0.5 was also significantly different ($P < 0.01$) from that of meat in CMA, but the MetMb percentage of the former meat was 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 after 29 days of storage at $0 \pm 1°C$. In the present experiment, after the same storage time at $1 \pm 1°C$, only atmospheres containing 0.75% CO or more maintained MetMb below 40% for 29 days. This MetMb percentage might be considered as the limit of acceptance, since Greene, Hsin and Zipser (1971) reported that 40% MetMb caused meat rejection by consumers.

3.4. Formation of TBARS

Results of the TBARS analyses demonstrated that increasing concentration of CO led to increasing inhibition of oxidation (Fig. 7). Meat packaged in LO-CO atmospheres with 0.25% or more CO had significantly lower ($P < 0.01$) TBARS values than meat stored in CMA.

An antioxidant activity of CO was proposed by Besser and Kramer (1972), who demonstrated that CO is an enzyme inactivator. Indeed, they reported that CO delayed oxidation of myoglobin and haemoglobin. If it is considered that both pigments can promote other oxidative reactions, CO is likely to inhibit them. Silliker et al. (1977) corroborated the antioxidant action of CO on fat. The antioxidant effect of CO may therefore also play some part in the maintenance of desirable qualities in meat.

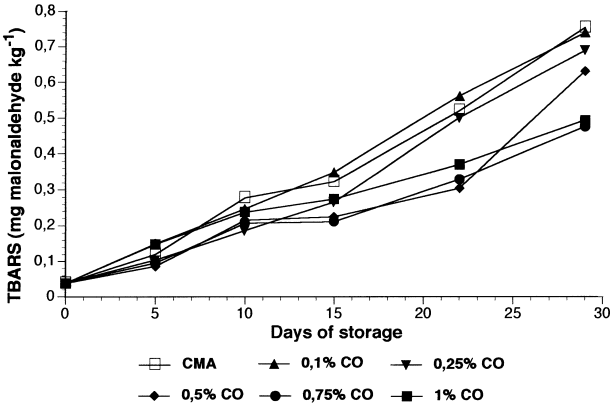

Fig. 7. Values of TBARS (reactive substances to TBA; expressed as mg malonaldehyde kg^{-1}) in beef loin steaks stored at $1 \pm 1°C$ in atmospheres: (□) CMA: 70% O_2 + 20% CO_2 + 10% N_2; all LO-CO atmospheres: 24% O_2 + 50% CO_2 + 25–26% N_2 + CO at the following concentrations: (▲) 0.1%; (▼) 0.25%; (◆) 0.5%; (●) 0.75%; and (■) 1%.

3.5. Sensory analysis

Results of sensory analysis of meat samples, including evaluation of red colour, discolouration and fresh meat odour, are summarised in Table 1.

Red colour increased simply as a consequence of the inclusion of CO in an atmosphere. The colour scores of meat packaged in CMA (4) were lower than those of meat in any of the CO atmospheres (5) throughout all the storage time (29 days). The literature indicates that 1% CO is able to produce and stabilise red colour (Garcıa-Matamoros & Moral, 1973; Gee & Brown, 1978; Luño et al., 1998). Clark et al. (1976) reported that 1% CO was the minimum concentration required for optimum colour, but 0.5% CO gave also good colour stability. Sørheim et al. (1997) obtained similar results with an atmosphere containing 0.4% CO. LO-CO 1, LO-CO 0.75 and LO-CO 0.5 maintained the

Table 1
Mean sensory values of beef loin steaks[a,b]

		Days of storage						
Parameter	Gas atmosphere	0	5	10	15	19	24	29
Red colour	CMA	4	4	4	4	3	3	1
	LO-CO 0.1	5	5	5	5	4	4	2
	LO-CO 0.25	5	5	5	5	5	4,5	2
	LO-CO 0.5	5	5	5	5	5	5	3
	LO-CO 0.75	5	5	5	5	5	5	3,5
	LO-CO 1	5	5	5	5	5	5	4
Discolouration	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	5	5	5	5	4	4	1
	LO-CO 0.1	5	5	5	5	5	4	2
	LO-CO 0.25	5	5	5	5	5	4	3
	LO-CO 0.5	5	5	5	5	5	5	4
	LO-CO 0.75	5	5	5	5	5	5	4
	LO-CO 1	5	5	5	5	5	5	4

[a] Packaged in an atmosphere of 70% O_2 + 20% CO_2 + 10% N_2 (CMA) or in an atmosphere of 24% O_2 + 50% CO_2 + 25 to 26% N_2 + 0.1 to 1% CO (LO-CO). CO concentrations in LO-CO atmospheres were 0.1% (LO-CO 0.1), 0.25% (LO-CO 0.25), 0.5% (LO-CO 0.5), 0.75% (LO-CO 0.75) or 1% (LO-CO 1).

[b] For 'Red colour', 5 denoted extremely intense red colour and 1 denoted extremely weak red colour. Values for 'Discolouration' referred to percentage of discoloured surface: 1 = none, 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, and 5 = 61–100%. Values for 'Fresh Meat Odour' were: 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. Samples were scored by each panel member, and mean values were agreed thereof after discussion by all six panellists.

improved colour for 24 days, LO-CO 0.25 for 19 days, while meat packaged in CMA and in LO-CO 0.1 lasted only 15 days with its original red colour.

Discolouration appeared after 15 days of storage in CMA packaged meat, after 19 days in meat in LO-CO 0.25 and LO-CO 0.1 and after 24 days in samples packaged in atmospheres containing 0.5% or more CO. If a score of 2 is regarded as the limit of acceptance, which corresponds to discoloured areas of $<10\%$, 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. Results regarding LO-CO 1 were in good agreement with those of Luno et al. (1998).

Fresh meat odour was largely preserved in meat steaks packaged in LO-CO atmospheres, probably because the presence of 50% CO_2 instead of 20% CO_2 in CMA inhibited microbial growth. After 19 days of storage at $1 \pm 1°C$ samples packaged in CMA still presented a fresh meat odour (4). In contrast, steaks packaged in LO-CO atmospheres maintained at this time an excellent fresh meat odour irrespective of the CO percentage. On day 29, a clear difference, related to CO content, was evident amongst the meat stored under the different atmospheres. CMA was scored 1, LO-CO 0.1 was scored 2, LO-CO 0.25 was scored 3 and atmospheres with 0.5% CO or more were scored 4. These results indicate that increasing concentrations of CO extended the odour shelf life of meat over that achieved with 50% CO_2. Previous results clearly showed that 1% CO improved odour shelf life (Luño et al., 1998). Clark et al. (1976) also reported that CO (above 0.5%) extended meat odour shelf life.

The explanation for this effect is not self-evident, but it could be related to both the inhibition of the growth of bacteria and the antioxidant activity of CO. The first hypothesis is supported by Clark et al. (1976), who found an inhibitory effect on microbial growth of an atmosphere with 0.5% CO at 5°C. Neither the results presented in this paper nor those from previous work (Luño et al., 1998) show any difference in psychrotrophic microbial counts of beef steaks as a function of CO content of the atmospheres, with the exception of small differences in the growth of *B. thermosphacta*. Luño et al. found a small difference in psychrotrophic flora in ground beef. This apparent inconsistency might be explained according to the report of Gee and Brown (1980–1981), who reported that CO had a selective action on microorganisms. The lowest CO concentration they used was 5% but, from the growth curves they obtained, it was suggested that 1% CO could also have an antimicrobial effect.

As the TBARS analyses showed that increasing concentration of CO led to increasing inhibition of oxidation, the antioxidant effect of CO could be involved in extending meat odour shelf life.

3.6. Residual carbon monoxide

A major concern regarding CO use in meat packaging is the amount of residual gas within the package, which will be released to the air when the pouch is opened. Fig. 8 shows the results of residual CO, expressed as the total amount of mmols within a package (2.5 l total volume), as a function of CO mmols in the original gas mixtures. Results showed that the amount of residual CO was a linear function of the concentration of gas present in the packaging mixtures, i.e. of their partial pressure within the package. Concomitantly, the amount of CO fixed by meat myoglobin decreased linearly with reducing CO concentration in the gas mixtures, as was previously noted by Sørheim et al. (1997).

3.7. General discussion

Clark et al. (1976) and Luño et al. (1998) had clearly demonstrated that 1% CO was an adequate concentration for assuring an optimum colour of beef steaks packaged in a modified atmosphere. The Norwegian meat industry is currently using gas mixtures with low concentrations (0.3–0.4%) of CO for beef packaging, with satisfactory colour stabilisation (Sørheim et al., 1997, 1999). Our results have shown that 0.5% CO stabilised red colour and odour, although results were improved by using 0.75% CO. Even a mixture containing only 0.1% CO improved and stabilised colour and odour at the early stages of storage.

The presence of 50% CO_2 in pack atmospheres also played a part in delaying odour changes as it considerably inhibited microbial growth. The presence of 24% O_2, besides presumably inhibiting anaerobes growth, allows the formation of oxyMb. The formation of a mixture of oxyMb and carboxyMb prevents an 'artificial' appearance of meat. In fact, panel members judged meat colour as a normal, attractive, beef bright-red colour.

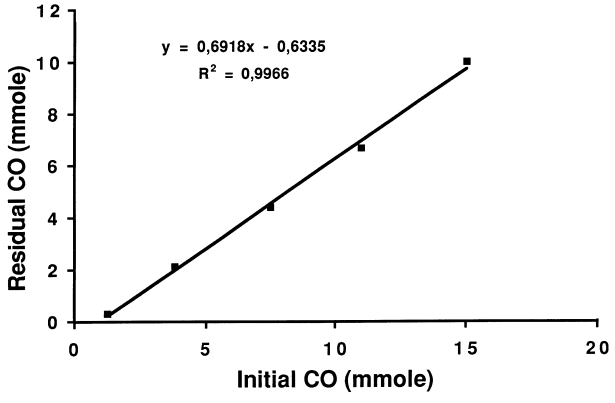

Fig. 8. Residual CO in meat packages after 3 weeks of storage at $1 \pm 1°C$ as a function of the initial amount of gas. The volume of atmosphere in each package was 2.5 l.

Regarding the intoxication risk arising from the use of low concentrations of carbon monoxide in meat packaging, it might be overestimated according to Sørheim et al. (1997) and ISO 10298 (1995). Besides this, in accordance with our results, residual CO in meat packages after storage is even lower, while concentration of carboxymyoglobin within meat steaks would result in a negligible formation of carboxyhaemoglobin within the organism (Sørheim et al., 1997).

We have shown that an atmosphere containing 50% CO_2 and 0.5 to 0.75% CO in the presence of a low concentration of O_2 (24%) is able to extend the shelf life of fresh beef steaks by 5 to 10 days, at $1 \pm 1°C$, when compared with the storage life in an atmosphere of 70% $O_2 + 20\%$ CO_2. The presence of CO and 50% CO_2 extends the shelf life by inhibition of spoilage bacteria growth, delayed metmyoglobin formation, stabilisation of red colour measured by instrumental and sensory techniques, maintenance of fresh meat odour and slow-down of oxidative reactions.

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