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The shelf-life of beef steaks treated with DL-lactic acid and antioxidants and stored under modified atmospheres

Djamel Djenane, Armida Sánchez-Escalante[1], José A. Beltrán, Pedro Roncalés*

Laboratory of Food Technology, Department of Animal Production and Food Science, Faculty of Veterinary Sciences, University of Zaragoza, C/ Miguel Servet, 177, 50013 Zaragoza, Spain

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Abstract

Beef steaks were treated with 1.5% lactic acid alone or supplemented with antioxidants (0.1% rosemary extract and 0.05% ascorbic acid). The steaks were stored under modified atmospheres containing either 60% O_2/40% CO_2 or 70% O_2/20% CO_2/10% N_2. Both the 40% CO_2 atmosphere and the lactic acid treatment significantly ($P<0.05$) inhibited growth of lactic acid bacteria, *Brochothrix thermosphacta* and *Pseudomonas* spp. Neither CO_2 in the pack atmosphere, treatment with lactic acid, nor a combination of both, affected formation of thiobarbituric acid reactive substances, myoglobin oxidation, or CIE a^* values. However, treatment with antioxidants significantly ($P<0.05$) delayed oxidation of both myoglobin and lipids, and so extended the storage-life.

Keywords: DL-Lactic acid; Antioxidants; Beef steaks

1. Introduction

The storage-life of beef cuts can be extended by treating the meat with lactic acid, to reduce the initial numbers of spoilage bacteria (Zhang et al., 1996), and by packing the meat under modified atmosphere (MA) rich in carbon dioxide (CO_2), to inhibit growth of the spoilage flora (Zeitoun and Debevere, 1990). High concentrations of O_2 are also used in MA to maintain the meat pigment myoglobin in the oxygenated state. The oxygenated pigment, oxymyoglobin (MbO_2) has a desirable, bright red colour. However, high concentrations of O_2 also promote lipid oxidation, and retard but do not prevent the oxidation of red MbO_2 to brown MetMb (MetMb), which ultimately renders the meat unacceptable. Consequently, beef decontaminated with lactic acid and stored under an O_2 rich, MA will generally become unacceptable because of discolouration and/or rancidity rather than be spoiled by the activities of the microflora (Coventry et al., 1998).

The storage-life of decontaminated beef stored under a MA might then be extended if it were treated with antioxidants. As the use of synthetic antioxidants has become increasingly unacceptable to consumers, use of natural antioxidants would be preferable (Mielche and Bertelsen, 1994). Both ascorbic acid and rosemary extract have been reported to be effective for inhibiting oxidative deterioration of meat (Djenane et al., 2002a, b; Sánchez-Escalante et al., 2001). Therefore, the effects on the storage-life of beef stored under O_2 and CO_2 enriched MA of treatment of the meat with lactic acid, rosemary extract and ascorbic acid were examined.

2. Materials and methods

2.1. Meat samples

The Longissimus dorsi (LD) muscle from a single beef carcass was obtained at 48 h post-mortem (pH 5.6–5.7), and trimmed of external fat. Forty-five steaks, each 1.5 cm thick and weighing about 150 g, were aseptically cut and divided into halves. The steak portions were

*Corresponding author. Tel.: +34-976-761582; fax: +34-976-761-612.

E-mail address: roncales@posta.unizar.es (P. Roncalés).

[1] Affiliated with Centro de Investigación en Alimentación y Desarrollo A.C., Apartado postal 1735, Hermosillo, Sonora 83000, México.

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exposed to air for about 1 h at 1°C to allow for blooming.

2.2. Treatment with lactic acid and natural antioxidants

After blooming, 90 portions were divided into three groups of 30. One group was sprayed with a 1.5% lactic acid solution, pH 2.22, using 2 ml of solution to 100 g of meat. A second group was similarly sprayed with 1.5% lactic acid solution supplemented with 0.1% rosemary and 0.05% ascorbic acid. The final group of steaks was sprayed with sterile distilled water. Solutions of L-ascorbic acid and DL-lactic acid were freshly prepared in sterile distilled, deionized water. The DL-lactic acid (85%, wt/vol) was free of glucose and contained approximately equal amounts of D and L isomers (Sigma Chemical Co., St. Louis, Missouri, USA). The solution of rosemary extract (Flavorguard©, Chr. Hansen GmbH, Holdorf, Germany) was prepared using *n*-pentane (Panreac S.A., Barcelona, Spain).

2.3. Packaging and storage

With each group of steak portions, each portion was placed on a polystyrene tray ($15.5 \times 21.5 \times 2.5\,cm^3$). Each filled tray was placed into a polyethylene and polyamide (PE/PA, 80/20 μm) laminate pouch (Sidlaw Packaging-Soplaril, Barcelona, Spain) with a water vapour permeability of 5–7 $g\,m^{-2}\ 24\,h^{-1}$ at 23°C and oxygen permeability of 40–50 $ml\,m^{-2}\ 24\,h^{-1}\,atm^{-1}$ at 23°C. Forty-five (15 of each treatment) pouches were filled with 1.5 l of a mixture of 70% O_2/20% CO_2/10% N_2, and the other 45 (15 of each treatment) pouches were filled with 1.5 l of a mixture of 60% O_2/40% CO_2 (Abelló Linde S.A.; Barcelona, Spain). The pouches were thermosealed, and stored in the dark at 1 ± 1°C.

On days 6, 11, 16, 22 and 27 of storage, three packs containing each atmosphere from each group were open. One pouch from each of the set was used for micro-biological sampling, while the other two were used for sensory analysis, and for instrumental and chemical analyses.

2.4. pH measurements

The meat pH was measured using a CRISON mod. Micro-pH 2001 pH meter with an INGOLD-type U 402 electrode. Three readings were obtained from each steak portion.

2.5. Composition of pack atmospheres

Oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) concentrations in pack atmospheres were determined using a Hewlett Packard 4890 gas chromatograph equipped 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 the oven temperature 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. The temperature of the detector was 120°C. A calibration curve was prepared using a 70% O_2/20% CO_2/10% N_2 atmosphere (Abelló Linde S.A.). The total percentage of each gas was calculated from the average of three measurements of the appropriate peak on the chromatograph.

2.6. Micro-biological analysis

Two sterile cotton swabs moistened in 0.1% peptone-water were used to swab $10\,cm^2$ of meat surface delimited by a sterile, stainless-steel template. Swabs were stirred thoroughly in 10 ml of sterile 0.1% peptone-water. Serial 10 fold dilutions were prepared by diluting 1 ml in 9 ml of 0.1% peptone-water. Three plates were prepared from each dilution by pouring 1 ml into the fluid agar appropriate for each microbial species. Lactic acid bacteria (LAB) were enumerated in plates of deMan, Rogosa and Sharpe agar (Merck; Darmstadt, Germany), which were incubated anaerobically at 30°C for 48–72 h. *Brochothrix thermosphacta* were enumerated in plates of streptomycin thallous acetate actidione agar (Biolife s.r.l; Milano, Italy) which were incubated aerobically at 25°C for 72 h. *Pseudomonas* were enumerated in plates of cephaloridine fucidin cetrimide agar (Oxoid; Basingstoke, England) which were incubated at 25°C for 48–72 h (ICMSF, 1983. The logs of mean values for the counts from triplicate plates were recorded.

2.7. Colour determination

Meat surface colour was measured, using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan), 30 min after package opening, to allow for colour stabilization after exposure to air. CIE L^*, a^*, b^* parameters (CIE, 1978) were recorded. Hue-angle (h) and Chroma (C^*) were calculated using the formulae: $h = \tan^{-1}(b^*/a^*)$, and $C^* = (a^{*2} + b^{*2})^{1/2}$.

2.8. MetMb analysis

The MetMb percentage of the total myoglobin perceptible at the steak surface was estimated spectrophotometrically, according to Stewart et al. (1965), by measuring steak surface reflectance at 525 and 572 nm (Minolta CM-2002; Osaka, Japan). The maximum value of the ratios of $(K/S)_{572\,nm}$–$(K/S)_{525\,nm}$ 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 oxidizing 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.

2.9. Lipid oxidation

Lipid oxidation was assessed in triplicate by the 2-thiobarbituric acid (TBA) method of Pfalzgraf et al. (1995). Thiobarbituric acid reactive substances (TBARS) values were calculated from a standard curve of malonaldehyde and expressed as mg malonaldehyde kg^{-1} meat.

2.10. Statistical analysis

The significance of differences amongst treatments after each day of storage was determined by analysis of variance using the least-squares difference method of the General Linear Model procedure of Statistical Package for Social Sciences (SPSS, 1995) programme for Windows, version 6.1.2 (Chicago, Illinois, USA, 1995). Differences were considered significant at the $P<0.05$ level. The Pearson's correlation matrix was calculated using the same programme. Significance was defined as $P<0.01$.

3. Results

3.1. pH and atmosphere composition

The initial meat pH of 5.6–5.7 decreased to about 5.4 after treatment with lactic acid (data not shown). pH values did not differ significantly ($P>0.05$) within treatments throughout storage. Changes in pack atmospheres (O_2, CO_2, N_2) exhibited no significant differences ($P>0.05$) during the display period among samples (results not shown). Slight changes in CO_2 concentrations in the pack atmospheres were detected. CO_2 concentrations decreased by about 4% during the first week of storage in all pack atmospheres. O_2 concentrations also declined, by about 7%, during the first 7 days of storage in all pack atmospheres. After 7 days of storage, O_2 and CO_2 concentrations remained unchanged.

3.2. Lactic acid bacteria

Counts of presumptive LAB (Fig. 1) on beef steaks packaged in the 40% CO_2 atmosphere were significantly lower ($P<0.05$) than those on steaks packaged in an atmosphere with 20% CO_2, particularly after 16 days of storage. LAB numbers at the end of storage were <4 and >5 $\log_{10}$ cfu cm^{-2} in the 40% and 20% CO_2 atmospheres, respectively. The samples had similar initial microbial loads.

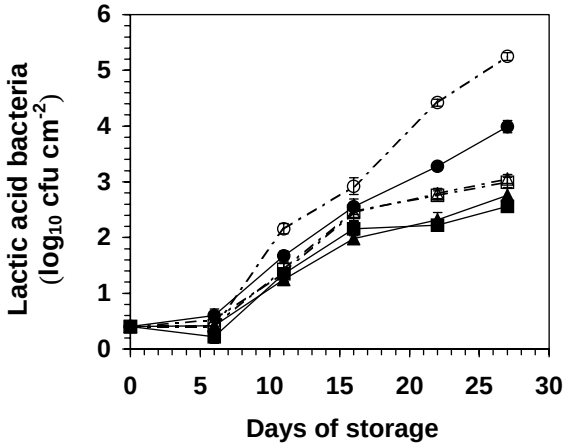

Fig. 1. Numbers (log cfu cm^{-2}) of LAB recovered from beef steaks stored at $1\pm1^{\circ}C$ under atmospheres of 70% O_2/20% CO_2/10% N_2 (open symbols) or 60% O_2/40% CO_2 (closed symbols) without being treated (circles) or after being treated with 1.5% lactic acid (triangles) or with 1.5% lactic acid/0.1% rosemary/0.05% L-ascorbic acid (squares).

Storage under either atmosphere after treatment with lactic acid led to inhibition of LAB growth by about 1 log and 2 log cfu cm^{-2} compared with 20% and 40% CO_2, respectively. Numbers recovered from samples treated with lactic acid and antioxidants were not significantly different ($P>0.05$) from the numbers recovered from steaks treated with lactic acid only.

3.3. B. thermosphacta

Presumptive *B. thermosphacta* was detected in all samples after 6 days of storage (Fig. 2). On day 11, counts of *B. thermosphacta* were <1 $\log_{10}$ cfu cm^{-2} for all samples, but reached maximum values of 5 $\log_{10}$ cfu cm^{-2} at the end of storage. Counts from beef steaks packaged in the 40% CO_2 atmosphere were significantly lower ($P<0.05$) than those from steaks packaged in 20% CO_2 after 27 days of storage. Treatment with lactic acid (1.5%) significantly ($P<0.05$) inhibited *B. thermosphacta* growth on steaks packaged in both atmospheres. After 3 weeks of storage, *B. thermosphacta* counts were about 1 $\log_{10}$ cfu cm^{-2} lower in samples treated with lactic acid. Samples treated with lactic acid and antioxidants also yielded fewer *B. thermosphacta* than samples treated with lactic acid only, although these differences appeared to be trivial.

3.4. Pseudomonas

Presumptive *Pseudomonas* were detected only after 6 or 11 days of storage of beef steaks not treated or

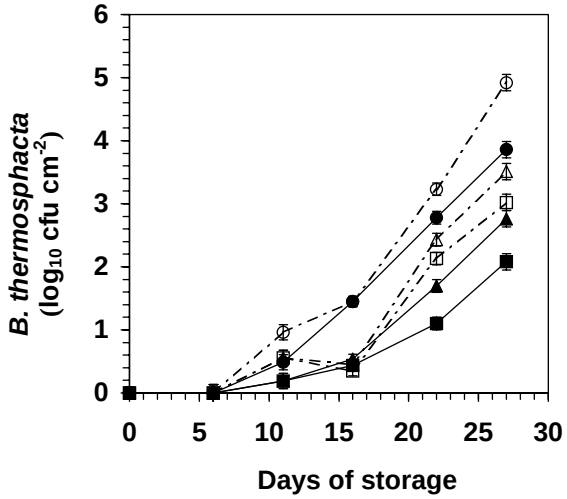

Fig. 2. Numbers (log cfu cm^{-2}) of *B. thermosphacta* recovered from beef steaks stored at $1 \pm 1°C$ under atmospheres of 70% O_2/20% CO_2/10% N_2 (open symbols) or 60% O_2/40% CO_2 (closed symbols) without being treated (circles) or after being treated with 1.5% lactic acid (triangles) or with 1.5% lactic acid/0.1% rosemary/0.05% L-ascorbic acid (squares).

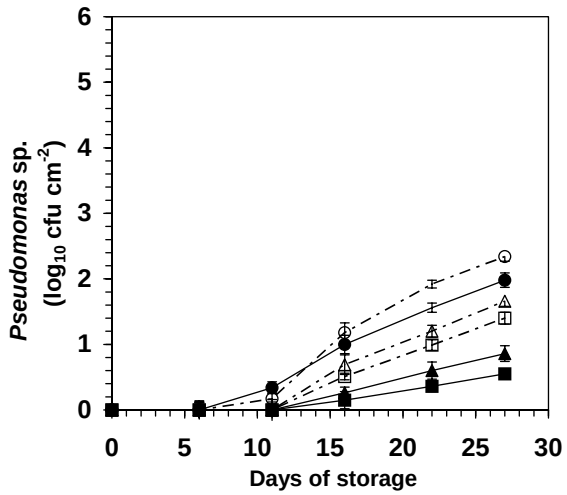

Fig. 3. Numbers (log cfu cm^{-2}) of *Pseudomonas* spp. recovered from beef steaks stored at $1 \pm 1°C$ under atmospheres of 70% O_2/20% CO_2/10% N_2 (open symbols) or 60% O_2/40% CO_2 (closed symbols) without being treated (circles) or after being treated with 1.5% lactic acid (triangles) or with 1.5% lactic acid/0.1% rosemary/0.05% L-ascorbic acid (squares).

treated with lactic acid, respectively (Fig. 3). Maximum *Pseudomonas* counts at the end of storage were <2.5 $\log_{10}$ cfu cm^{-2}. The presence of 40% CO_2 slightly inhibited *Pseudomonas* growth, but the effect was trivial after 15 days of storage. The presence of rosemary extract significantly reduced ($P<0.05$) *Pseudomonas* counts. At the end of storage, the numbers recovered from these steaks were <1 log cfu cm^{-2}.

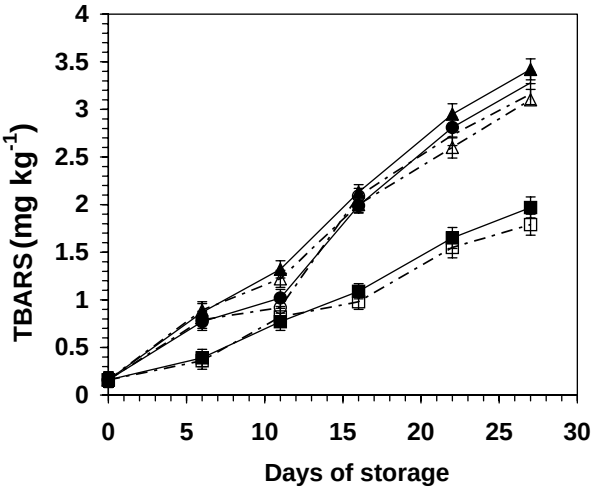

Fig. 4. TBARS (mg malonaldehyde kg^{-1} meat) in beef steaks stored at $1 \pm 1°C$ under atmospheres of 70% O_2/20% CO_2/10% N_2 (open symbols) or 60% O_2/40% CO_2 (closed symbols) without being treated (circles) or after being treated with 1.5% lactic acid (triangles) or with 1.5% lactic acid/0.1% rosemary/0.05% L-ascorbic acid (squares).

3.5. TBA reactive substances

Neither high CO_2 concentrations nor lactic acid significantly affected ($P > 0.05$) TBARS formation (Fig. 4), but treatment with antioxidants did significantly ($P<0.05$) inhibit TBARS formation.

3.6. MetMb percentage

Neither high CO_2 concentrations nor addition of lactic acid significantly affected ($P > 0.05$) MetMb formation (Fig. 5). However, treatment with antioxidants significantly ($P<0.05$) inhibited myoglobin oxidation and resulted in less MetMb than in untreated beef steaks, after 16, 22 and 27 days of storage.

3.7. Instrumental colour measurement

Neither high CO_2 concentrations nor lactic acid significantly affected ($P > 0.05$) surface meat colour, expressed in term of redness index (CIE a^*; Fig. 6). However, treatment with antioxidants significantly ($P<0.05$) delayed the decline in CIE a^* values.

4. Discussion

The fact that initial meat pH decreased slowly in the presence of lactic acid and that there were no significant differences ($P > 0.05$) within treatments may be explained by the buffering capacity of meat (Smulders, 1995). Results of the analysis of gas composition headspace are in agreement with those of Sørheim et al. (1999). Probably, solution of CO_2 in meat

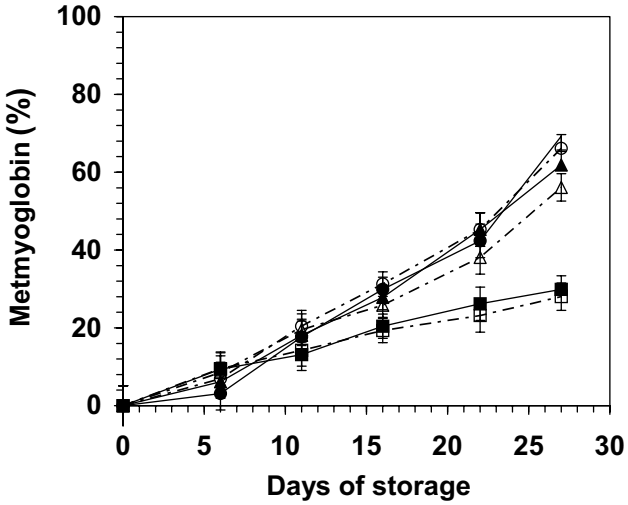

Fig. 5. MetMb percentages at the surfaces of beef steaks stored at $1\pm1^{\circ}C$ under atmospheres of 70% O_2/20% CO_2/10% N_2 (open symbols) or 60% O_2/40% CO_2 (closed symbols) without being treated (circles) or after being treated with 1.5% lactic acid (triangles) or with 1.5% lactic acid/0.1% rosemary/0.05% L-ascorbic acid (squares).

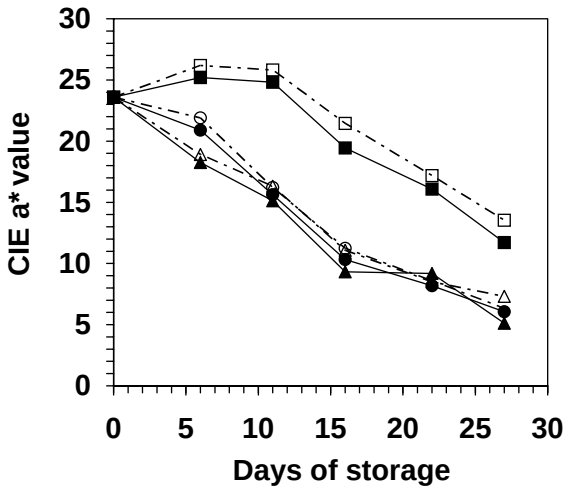

Fig. 6. Values of CIE a^* at the surfaces of beef steaks stored at $1\pm1^{\circ}C$ under atmospheres of 70% O_2/20% CO_2/10% N_2 (open symbols) or 60% O_2/40% CO_2 (closed symbols) without being treated (circles) or after being treated with 1.5% lactic acid (triangles) or with 1.5% lactic acid/0.1% rosemary/0.05% L-ascorbic acid (squares).

contributed to the early shift in gas composition (Gill, 1988). During the remainder of the storage period the gas composition was very stable, probably because of the high ratio of gas to product (Mano et al., 2000). O'Grady et al. (2000) found that changes of O_2 and CO_2 concentrations in pack atmospheres were high only when the initial oxygen concentrations were relatively low.

Numbers of LAB on steaks stored under 40% CO_2 were less than on steaks stored under 20% CO_2. This is in agreement with reports that LAB are only slightly inhibited by 20% CO_2 but are inhibited by higher concentrations (Rönner, 1994; Nissen et al., 1996). Treatment with lactic acid further inhibited LAB, as previously reported by Brink et al. (1985). *B. thermosphacta* were also inhibited by the higher concentrations of CO_2 and treatment with lactic acid, as would be expected (Nissen et al., 1996; Grau, 1980). The Gram-negative *Pseudomonas* spp. seemed to be the group most vulnerable to treatment with lactic acid, and to the lower as well as the higher concentrations of CO_2 (Lambert et al., 1991; Gould, 1996; Van Netten et al., 1994). Under MA conditions, LAB and *B. thermosphacta* predominated, reaching maximum levels of 5 $\log_{10}$ cfu cm^{-2}, while *Pseudomonas* spp. numbers increased only slowly during storage and so were always minor fractions of the flora. The inhibitory effects of rosemary extract were very low.

Accumulation of MetMb appeared to be highly correlated with oxidation of fatty acids, and the antioxidants inhibited both oxidative processes. A synergistic effect of ascorbic acid with rosemary extract could be expected (Elliott, 1999). In agreement with these results, Sánchez-Escalante et al. (2001) and Djenane et al. (2002a) reported beef patties and beef steaks, respectively, treated with rosemary exhibited lower TBARS values and MetMb percentage than untreated patties during storage. According to Greene and Cumuze (1981), TBA numbers of at least 2.0 are required for inexperienced panellists to detect oxidized flavours. The results of the present study showed that beef steaks treated with antioxidants would produce no perceptable off-odours during storage. With regard to MetMb, Greene et al. (1971) reported a consumer panel rejected samples of meat with a MetMb% greater than 40%. Untreated samples exceeded 40% MetMb by day 20 of storage, while treatment with lactic acid and antioxidants delayed MetMb formation until the end of the storage period.

Instrumental colour measurements paralleled the findings for MetMb formation. Neither 40% CO_2 nor addition of lactic acid accelerated colour deterioration, while antioxidants delayed red colour fading. Kotula and Thelappurate (1994) also failed to observe discolouration of retail cuts treated with lactic acid. In disagreement with present results, Arganosa and Marriott (1989) reported meat treated with organic acid had lower CIE a^* values, especially at lower pH values. pH values in the present study were unchanged throughout storage in all samples; therefore, an effect of pH on colour deterioration may be discounted. Bala et al. (1977) reported treatment of beef cuts with an organic acid in combination with a CO_2-rich atmosphere preserved fresh meat colour. Probably, the absence of effect of lactic acid on colour was due to its relative low concentration, and to the buffering capacity of meat. Brewer et al. (1991) reported addition of 2–3% sodium

lactate to fresh meat delayed microbial spoilage, pH decline, and off-odour development by 7–10 days at 4°C. In the present study, deterioration of beef colour was influenced more by the oxidative effect of high concentrations of O_2 than by pH values.

In summary, both high CO_2 (40%) concentrations and lactic acid treatment exerted significant inhibitory effects on the growth of spoilage bacteria on beef steaks. After 28 days, bacterial growth was delayed by about 1 week by the combined effect of both treatments, as compared with untreated samples packed under 20% CO_2. Neither CO_2 in the pack atmosphere, treatment with lactic acid, nor a combination of both, resulted in any modification of the physical and chemical characteristics of beef steaks. In addition, treatment with antioxidants delayed oxidation of both myoglobin and lipids, resulting in extension of meat shelf-life by about 10 days in terms of colour and, presumably, of odour. Therefore, results of the present study demonstrate modified atmosphere packaging in 40% CO_2 atmospheres of beef steaks previously treated with lactic acid and a mixture of natural antioxidants effectively extended the shelf-life of beef.

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