ELSEVIER

Food Chemistry 85 (2004) 453–459

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidant effect of carnosine and carnitine in fresh beef steaks stored under modified atmosphere

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Received 30 May 2003; received in revised form 24 July 2003; accepted 7 August 2003

Abstract

The exogenous aplication of carnosine (*β-alanine-L-histidine*) (50 mM), carnitine (50 mM) and L-ascorbic acid (500 ppm) solutions on the shelf life of fresh beef steaks packaged in modified atmosphere (70% O_2 + 20% CO_2 + 10% N_2) was studied. Beef steaks were sprayed with natural antioxidant solutions at a ratio of 2 ml solution to 100 g meat. Lipid oxidation (TBARS formation), colour changes (CIE *a** value and metmyoglobin formation), antimicrobial properties (psychrotrophic flora), and sensory (off odour and discolouration) changes were examined throughout 28 days of storage. Results showed that the combination of carnosine with ascorbic acid provided the best antioxidative protection with regard to meat deterioration. Surface aplication of carnosine or ascorbic acid alone resulted in an effective delay of oxidation of meat. However, carnitine was significantly ($P < 0.05$) less effective than any other antioxidant in delaying meat oxidation, while the combination of carnitine and ascorbic acid exerted no antioxidant effect.

Keywords: Beef; Modified atmosphere; Natural antioxidants; Carnosine; Carnitine

1. Introduction

In order to minimise metmyoglobin (MetMb) formation in fresh beef, oxygen (O_2) must be either totally excluded from the packaging environment or injected at saturating levels (Faustman & Cassens, 1990). High O_2 atmospheres are used to maintain the red pigment in its oxygenated form (MbO_2) (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2003b), but high O_2 levels can also promote lipid oxidation and, ultimately, MetMb accumulation. Many studies have indicated that lipid oxidation in meat can be effectively controlled or, at least, minimised by adding antioxidants (Gray, Gomaa, & Buckley, 1996). Lipid oxidation in muscle systems is initiated at the membrane level in the intracellular phospholipid fractions. Lipid oxidation and MetMb accumulation show a close similarity in their progression (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2001). An approach involving the use of both high O_2 atmospheres and natural antioxidants seems therefore convenient to keep myoglobin (Mb) in its oxygenated state and inhibit lipid oxidation, consequently extending meat shelf or retail life (O'Grady, Monahan, Burke, & Allen, 2000).

Carnosine (*β-alanine L-histidine*) is an endogenous buffering agent and antioxidant found in skeletal muscle (Zhou & Decker, 1999). The antioxidant activity of carnosine has been re-evaluated recently in a model system (Decker, Livisay, & Zhou, 2000) and in beef patties (Sánchez-Escalante, Djenane, Torrescano, Gimenez, Beltrán, & Roncalés, 2003), due to the presence of contaminating hydrazine in commercial carnosine preparations. Those authors demonstrated that hydrazine-free purified carnosine possessed the same

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doi:10.1016/j.foodchem.2003.08.007

antioxidant activity as commercial carnosine both in a model system and in meat.

Carnitine and some of its acyl esters might have a beneficial effect on pathological conditions in which it is possible to recognize free radicals as potential mediators of cellular damage (Arduini, 1992). Recent studies have shown that L-carnitine suppresses oxidative damage during aging (Arockiarani & Panneerselvam, 2001). Vanella et al. (2000) reported that L-propionylcarnitine showed a dose-dependent free radical scavenging activity; it was shown to scavenge superoxide anion, to inhibit lipoperoxidation of linoleic acid, and to protect against damage induced by H_2O_2 in the presence of ultraviolet radiation (UV). The use of carnitine as an antioxidant in meat has been, as far as we are aware, not addressed.

Wheeler, Koohmaraie, and Shackelford (1996) indicated that ascorbic acid can be very effective in stabilising colour when added to steak surfaces, thus extending retail display life, even at high temperature (9 °C). Ascorbic acid has been used as a steak surface treatment alone or in combination with other antioxidants to stabilise meat colour during display (Mitsumoto, Cassens, Schaefer, Arnold, & Scheller, 1991). Ascorbic acid, when used in combination with other antioxidants, functions as a synergist by promoting their antioxidative effects (Elliot, 1999). Sánchez-Escalante, Djenane, Torrescano, Beltrán, and Roncalés (2001) demonstrated that ascorbic acid showed an additive antioxidant effect with carnosine when added in combination to beef patties.

The objective of this study was to investigate the effects of treatment of fresh beef steaks with carnosine and carnitine, alone or in combination with ascorbic acid, prior to packaging in modified atmosphere, with the aim of extending their shelf life by delaying oxidative reactions.

2. Materials and methods

2.1. Preparation of samples

Beef loins (*M. longissimus dorsi*) from three beef carcasses were obtained at 48 h post-mortem (pH 5.6–5.7), and trimmed of external fat. Sixty-three steaks (1.5 cm thick, and weighting about 150 g) were aseptically cut and divided into halves, using sterile cutting boards and knives to reduce contamination, and exposed to air during about 1 h at 1 °C to allow blooming.

2.2. Treatment with natural antioxidants and gas packaging

After blooming, samples were randomly divided into six groups of 21 steaks. One group was sprayed on meat surface with a carnosine (50 mM) solution, according to a ratio of 2 ml solution to 100 g meat, in a fine mist from a distance of approximately 10 cm from the surface. The second group was sprayed with the same amount of a carnitine (50 mM) solution. The third group was treated with a L-ascorbic acid (500 ppm) solution. The fourth group was treated with carnosine (50 mM) and ascorbic acid (500 ppm) solutions. The fifth group was treated with carnitine (50 mM) and ascorbic acid (500 ppm) solutions. The last group (control samples) was sprayed with the same volume of sterile distilled water. Solutions of ascorbic acid ($\geqslant$99%), carnosine ($\geqslant$99%), and carnitine ($\geqslant$98%), (all from Sigma Chemical Co) were freshly prepared in sterile distilled, deionized water.

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/polyamide laminate (PE/PA, 80/20 μm of thikness) (Sidlaw Packaging-Soplaril, Barcelona, Spain) 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} atm^{-1} at 23 °C. The pouch was filled with a gas mixture of 70% O_2 + 20% CO_2 + 10% N_2, supplied by Abelló Linde S. A. (Barcelona, Spain), at a pression of 2 bar, reaching a headspace gas volume of 1.5 l, thermosealed and stored in the absence of illumination at 1 ± 1 °C.

On days 0, 7, 13, 16, 20, 24 and 28 of storage, three packs for each treatment were open. One steak was used for only microbial sampling, while the other two were used first for sensory analysis and then for instrumental and chemical analyses.

2.3. Headspace gas analyses

O_2, CO_2 and N_2 concentrations in the headspace gases of meat packages were determined using a Hewlett Packard 4890 gas chromatograph 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 70% O_2 + 20% CO_2 + 10% N_2 atmosphere supplied by Abelló Linde S.A. (Barcelona, Spain). Total percentage of the different gases was calculated assuming an average of three measurements of total areas.

2.4. Meat colour

Meat colour was measured at the surface of beef steaks, using a reflectance spectrophotometer (Minolta

CM-2002; Osaka, Japan), 30 min after packaging opening, in order to allow colour stabilisation on air exposure. CIE L^*, a^*, b^* (lightness, redness and yellowness indices, respectively (CIE, 1978) parameters were recorded.

2.5. Metmyoglobin analysis

The MetMb 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 CM-2002; Osaka, Japan). The maximum value of the ratios of $K/S_{(572\ nm)}$ to $K/S_{(525\ nm)}$ 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 determinations.

2.6. pH measurements

The meat pH was measured using a CRISON mod. Micro- pH 2001 pH meter with an INGOLD-type U402 electrode. Three readings were obtained from each steak portion.

2.7. Lipid oxidation analysis

Lipid oxidation was assessed in triplicate, using 10 g of each sample, by the 2-thiobarbituric acid (TBA) method of Pfalzgraf, Frigg, and Steinhart (1995). TBARS values were calculated from a standard curve of malonaldehyde and expressed as mg malonaldehyde/kg meat.

2.8. 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 ten-fold dilutions were prepared by diluting 1 ml in 9 ml of 0.1% peptone water. Two duplicate plates were prepared from each dilution by pouring 1 ml in fluid plate count agar (PCA; Merck; Darmstadt, Germany); plates were incubated at 7 °C for 10 days (ICMSF, 1983). Counts of aerobic psychrotrophic flora were determined from plates bearing 20–200 colonies. After enumeration, bacterial populations from duplicate plates were averaged and converted to $\log_{10}$ of colony forming units (cfu) /cm^2.

2.9. Sensory evaluation

Meat samples were evaluated by a trained six-member panel. Training consisted of four sessions of approximately 1 h, in which panelists were served beef steak samples for evaluation of selected attributes, followed by an open discussion. For rating surface discolouration, samples with about 0, 10, 20, 60 and 100% discolouration were presented. For rating odour, meat samples presenting different "off odour" characteristics within the range of the evaluation scale were used. Samples included packaged beef steaks stored at 4 °C for different times up to 3 weeks to allow off odour formation related to meat spoilage; absence of off-odour was rated as 1 and maximum off-odour was rated as 5. Panelists were male ($n=4$) and female ($n=2$) laboratory coworkers, and ranged in age from 26 to 50 years.

In all assessments, the surface of beef steaks was evaluated 20 min after pack opening. The samples were taken as needed from the cold room, identified with 3-digit random numbers and placed in polystyrene trays of 15.5 cm × 21.5 cm. Each panelist received one sample (half steak) of each treatment randomly numbered and served. All samples were evaluated under cool white fluorescent lighting, positioned so that it provided about 800 lux at the counter surface. The samples for evaluation were presented at room temperature (about 25 °C).

The attributes "discolouration" and "off odour" were rated using a 5-point descriptive scale, according to Djenane et al. (2001), using a paper scorecard. Scores for "discolouration" referred to percentage of discolored surface: 1 = none, 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, and 5 = 61–100%. Scores for "off odour" referred to the intensity of odours associated to meat spoilage: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme.

2.10. Statistical analysis

The significance of differences amongst treatments at each day of storage was determined by analysis of variance (ANOVA) using the least square difference (LSD) method of the general linear model procedure of SPSS (1995). Differences were considered significant at the $P<0.05$ level.

3. Results and discussion

3.1. Headspace gas analysis and pH

Changes in gaseous environment are shown in Table 1. There were no significant differences ($P>0.05$) during the display period among samples. These results were in

Table 1
Changes in gas composition in packages headspaces during storage of beef steaks at 1±1 °C

Days of storage	Gas composition (%)[a]					
	Spray treatment with antioxidants + 70%O_2 + 20%CO_2 + 10%N_2			Control + 70%O_2 + 20%CO_2 + 10%N_2		
	O_2	CO_2	N_2	O_2	CO_2	N_2
0	68.6±1.4	18.45±0.6	13.15±0.9	68.2±1.3	19.23±2.1	12.60±1.4
7	62.31±0.9	15.59±1.6	13.45±09	63.40±1.2	16.09±1.3	13.12±1.2
15	61.31±1.3	19.2±0.7	13.4±1.5	63.51±0.7	19.62±0.9	11.25±0.4
29	61.56±1.7	19.52±1.2	11.42±1.7	64.01±1.2	19.25±1.2	11.13±0.7

[a] Mean (n = 3)±standard deviation.

agreement with those reported by O'Grady et al. (2000), who found that relative changes of O_2 and CO_2 in the gaseous environment pack were larger at the lowest oxygen level, while only small differences were enregistered at the highest level of O_2 (80%). The same results were obtained by Djenane, Sánchez-Escalante, Beltrán, and Roncalés (2003a). It is very probable that static head space may be related to conditions of saturation. Mano (1997) did not find significant changes in the gaseous atmosphere of pork loin packaged in 40/60 CO_2/O_2 at 7 °C. In contrast, Zhao, Wells, and McMillin (1994) found that the gas headspace within the modified atmosphere pack was not static, since microbial metabolism and gas mobilisation continually act to modify headspace composition. pH values (results not shown) did not differ significantely ($P > 0.05$) within treatments throughout storage. Probably due to the buffering capacity of meat.

3.2. Metmyoglobin accumulation

The results shown in Fig. 1 demonstrated that the combination of carnosine with ascorbic acid was the most effective in delaying metmyoglobin formation. This effect was evident from day 16 of storage, showing significant ($P < 0.05$) differences with any other treatment. In fact, after 28 days of storage these samples reached only about 30% of surface metmyoglobin, while metmyoglobin of control steaks and those with carnitine alone and carnitine + ascorbic acid reached 72.09, 62.09 and 74.29%, respectively, at the end of the storage period. Treatment with either carnitine alone or carnitine plus ascorbic acid showed no significant differences with the control during the whole period of storage ($P > 0.05$).

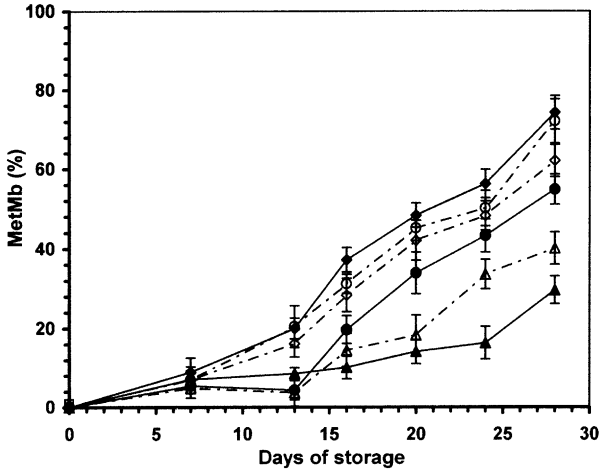

Fig. 1. Metmyoglobin percentage (MetMb%) (±SD; n = 25) in beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (○) Control; (●) Vitamin C; (△) Carnosine; (▲) Carnosine + Vitamin C; (◇) Carnitine; (◆) Carnitine + Vitamin C.

In agreement with present results, previous reports demonstrated that the addition of carnosine inhibited myoglobin oxidation in ground beef (Lee, Hendricks, & Cornforth, 1999). Several authors found that carnosine combined with ascorbic acid was very useful for preventing myoglobin oxidation in meat (Lee et al., 1999). The synergistic effect of ascorbic acid when used in combination with other antioxidants has been already proposed (Elliot, 1999). However, our results suggested that carnosine and ascorbic acid exerted an additive effect. Regarding carnitine, no reports on its antioxidant ability in relation to meat colour protection have been presented thus far.

3.3. Colour instrumental measurement

Changes in CIE a^* values throughout storage of beef steaks packaged in modified atmosphere and treated with antioxidants are shown in Fig. 2. Results essentially agreed with those of metmyoglobin formation. Treatment with carnosine + ascorbic acid led to significant differences ($P < 0.05$) with any other treatment from day 13 of storage. At the end of the storage period (28 days), control samples and those with carnitine and carnitine + ascorbic acid had very low CIE a^* values, below 4, while samples treated with the combination of carnosine + ascorbic acid possessed CIE a^* values above 11, corresponding to a red colour. This value of redness was reached only at day 17, 18 and 19 of storage in samples treated with carnitine + ascorbic acid, carnitine alone and control, respectively. Consequently,

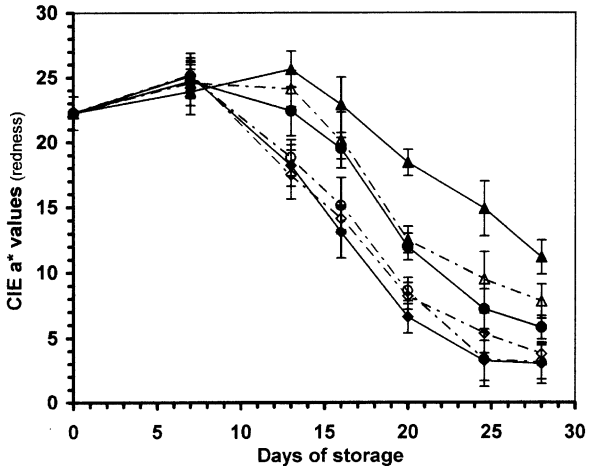

Fig. 2. Values of CIE a^* (±SD; $n=25$) in beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (○) Control; (●) Vitamin C; (△) Carnosine; (▲) Carnosine + Vitamin C; (◇) Carnitine; (◆) Carnitine + Vitamin C.

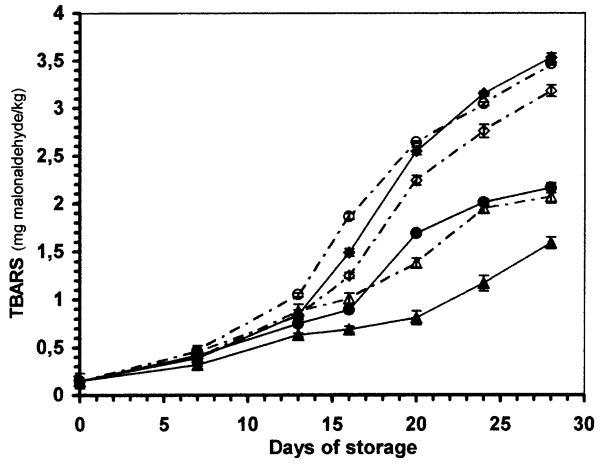

Fig. 3. TBARS (mg malonaldehyde/kg meat) (±SD; $n=6$) in beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (○) Control; (●) Vitamin C; (△) Carnosine; (▲) Carnosine + Vitamin C; (◇) Carnitine; (◆) Carnitine + Vitamin C.

treatment with carnosine + ascorbic acid retarded colour loss by about 11, 10 and 9 days, respectively, in comparison with the control.

3.4. TBA-Reactive substances

Values of TBARS formation are shown in Fig. 3. Carnosine exerted a significant ($P<0.05$) inhibition of TBARS formation. The considerable antioxidant protection provided by carnosine was in agreement with the results of Decker and Crum (1991), Zhou and Decker (1999) and Sánchez-Escalante et al. (2001, 2003). The largest antioxidant protection was provided by carnosine combined with ascorbic acid, which resulted in 62.90, 69.31, 61.51 and 54.04% less TBARS than in control samples after 16, 20, 24 and 28 days of storage, respectively.

A small inhibition ($P<0.05$) of TBARS production was observed in samples treated with carnitine with respect to the control. However, the combination of carnitine and ascorbic acid led to an absence of inhibition of TBARS formation. Hu, Chen, and Lin (1995) suggested that a stimulation of lipid oxidation by carnitine could be due to the use of large amounts of the compounds. Halcak, Rendekova, Pechan, and Kubaska (1998) found that carnitine at 1 mmol l^{-1} concentration decreased the value of lipid oxidation (TBARS) in a homogenate of brain cortex, but its effect was lower than the effect of other antioxidants. Table 2 shows that, at 20 days of storage, the percentage inhibition of lipid oxidation of fresh beef treated with various natural antioxidants was 35.98, 47.72, 15.5, 69.31 and 3.4% for ascorbic acid, carnosine, carnitine, carnosine + ascorbic acid, and carnitine + ascorbic acid, respectively. The inhibition percentage of pigment oxidation at the same day of storage was respectively, 24.97, 59.44, 6.81, 68.62 and −7.08 for the same treatments.

3.5. Microbial analysis

Psychrotrophic aerobe counts in all samples gradually increased along storage (results not shown). They reached values around 7 $\log_{10}$ cfu/cm^2 in all samples after 28 days of storage. The differences in Plate Count Agar amongst samples were not significant. ($P>0.05$). In our results a critical value of 7 $\log_{10}$ cfu/cm^2 was only reached after 29 days of storage in all samples. Few studies on the antimicrobial activity of natural antioxidants have been reported. Regarding ascorbic acid, Shivas, Kropf, Hunt, Kastner, Kendall, and Dayton (1984) and Djenane, Sánchez-Escalante, Beltrán, and Roncalés (2002) reported that microbial populations were not affected by treatment with ascorbic acid.

3.6. Sensory analysis

Results of sensory analysis of beef steaks are summarised in Table 3. Results clearly demonstrated that beef steaks treated with a combination of carnosine and ascorbic acid were given lower scores ($P<0.05$) than any other steaks throughout the whole storage period, both for "off odour" and discolouration, followed by carnosine and ascorbic acid alone, which also differed significantly from the control ($P<0.05$). This intensity order of the protective ability on meat quality of the antioxidant combinations, as measured by sensory evaluation, consistently agreed with their effectiveness in preventing both myoglobin and lipid oxidation.

Discolouration scores given to control and steaks treated with carnitine + ascorbic acid showed that broad discoloured areas were evident at the 16th day of storage, while only small discoloured areas were present in samples treated with carnosine alone and carnosine +

Table 2
Inhibition (%) of pigment and lipid oxidation at day 20 of storage of fresh beef steaks treated with carnosine, carnitine and vitamin C, packaged in MAP[a] at 1±1 °C

	Vitamin C	Carnosine	Carnitine	Carnosine + vitamin C	Carnitine + vitamin C
TBARS[b]	35.98±2.30a	47.72±2.31b	15.5±3.65c	69.31±2.82d	3.40±1.39e
Metmyoglobin[c]	24.97±6.67a	59.44±4.23b	6.81±5.32c	68.62±4.31d	−7.08±3.70e

Data represent 6 and 25–30 determinations±standard deviation, respectively for TBARS and Metmyoglobin (%). Mean values in the same line and relating to each attribute are significantly different when accompanied by different letters ($P<0.05$).

[a] 70%O_2 + 20%CO_2 + 10%N_2.

[b] Inhibition (%) = (TBARS (control) – TBARS (antioxidant)/ TBARS (control))*100.

[c] Inhibition (%) = (MetMb% (control) – MetMb% (antioxidant)/ MetMb% (control))*100.

Table 3
Effect of antioxidant treatments on sensory panel scores (mean±SD) for discolouration and off odour of beef steaks packaged in modified atmosphere (70% O_2 + 20% CO_2 + 10% N_2) at 1±1 °C

Attribute	Antioxidants treatment	*Days of storage*					
		7	13	16	20	24	28
Discolouration[a]	Control	1.0±0.0	1.2±0.2a	1.7±0.6a	4.0±0.0a	4.6±0.9a	5.0±0.0a
	Vitamin C (Vit. C)	1.0±0.0	1.0±0.0a	1.5±0.5a	2.8±0.6b	3.6±0.7b	4.0±0.0b
	Carnosine	1.0±0.0	1.2±0.2a	1.2±0.4b	2.2±0.5b	3.2±0.8b	3.4±0.7c
	Carnosine + Vit. C	1.0±0.0	1.0±0.0a	1.1±0.2b	1.7±0.4c	2.4±0.8c	2.6±0.6d
	Carnitine	1.0±0.0	1.2±0.3a	1.6±0.7a	2.8±0.8b	3.8±0.3b	4.4±0.2b
	Carnitine + Vit. C	1.0±0.0	1.0±0.0a	1.9±0.7a	4.0±0.0a	4.7±0.9a	5.0±0.0a
Off-odour[b]	Control	1.0±0.0	1.3±0.6a	3.2±0.9a	4.0±0.0a	4.7±0.0a	5.0±0.0a
	Vitamin C (Vit. C)	1.0±0.0	1.2±0.2a	1.4±0.6b	3.3±0.6b	3.6±0.5b	3.8±0.4b
	Carnosine	1.0±0.0	1.0±0.0a	1.2±0.7b	2.8±0.6b	3.0±0.0c	3.4±0.5b
	Carnosine + Vit. C	1.0±0.0	1.0±0.0a	1.2±0.7b	2.0±0.0c	2.3±0.3d	3.2±0.8b
	Carnitine	1.0±0.0	1.2±0.4a	3.1±0.5a	3.2±0.3b	3.9±0.4b	4.1±0.2c
	Carnitine + Vit. C	1.0±0.0	1.3±0.3a	3.3±0.2a	4.2±0.4a	4.8±0.7a	5.0±0.0a

Mean values in the same column and relating to each attribute are significantly different when accompanied by different letters ($P<0.05$).

[a] 1 = None (0%), 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, 5 = 61–100%.

[b] 1 = None, 2 = Slight, 3 = Small, 4 = Moderate, 5 = extreme.

ascorbic acid until the end of storage period. Odour of the control and carnitine + ascorbic acid samples was acceptable for about only 2 weeks, which corresponded to a value of 1.86 TBARS. Greene and Cumuze (1981), in a study to determine the relationship between TBARS values and inexperienced taste panel assessments of oxidised lipid flavour, found that a maximum TBA value of 2.0 was sufficient for inexperienced taste panelists to detect oxidised flavours. Samples treated with carnosine and carnosine + ascorbic acid exhibited an "off odour" score of slight (about 3 in the scale range), and small at the end of experiment (2 in the scale range), respectively. That is to say that the shelf life of treated steaks with the combination of carnosine and ascorbic acid would be extended, on the basis of the perceived odour, for about 2 weeks. Treatment with ascorbic acid and carnosine alone also extended odour shelf life. These results were in good agreement with those of TBA reactive substances, which may be referred to the fact that the characteristic odour deterioration of beef steaks is mainly due to accumulation of volatile carbonyl compounds by oxidation of unsaturated fatty acids and meat phospholipids, and not to microbial spoilage.

4. Conclusions

It is concluded that the combination of carnosine with ascorbic acid provided the best antioxidant protection against meat deterioration during 28 days of storage. Results also demonstrated that surface aplication of carnosine or ascorbic acid alone resulted in a an effective delay of oxidative deterioration of meat. However, carnitine was significantly ($P<0.05$) less effective than carnosine in delaying meat oxidation, while the combination of carnitine and ascorbic acid exerted no antioxidant effect.

Acknowledgements

This research was supported by the Comisión Interministerial de Ciencia y Tecnología (grants ALI

96-0587 and AGL 2000-1407), Abelló Linde S.A. (Barcelona, Spain), which provided the packaging equipment and gas mixtures and the Agencia Española de Cooperación Internacional (fellowships of authors D. Djenane and A. Sánchez-Escalante, the ANUIES Mexico; SUPERA program; fellowship of A. Sánchez-Escalante) and the Diputación General de Aragón (fellowship of L. Martínez). The technical assistance of Mrs. A. Martínez is greatly acknowledged.

References

Arduini, M. D. (1992). Carnitine and its acyl esters as secondary antioxidants? *American Heart Journal*, *123*, 1726–1727.

Arockiarani, P., & Panneerselvam, C. (2001). Carnitine as a free radical scavenger in aging. *Experimental Gerontology*, *36*, 1713–1726.

CIE (1978). *Recommendatios on uniform color spaces—color difference equations psychometric color terms*. Commision International de l'Eclairage, Paris. Supplement N°.2 to CIE Publication N°15 (E-1.3.1) 1971/(TC-1.3.).

Decker, E. A., & Crum, A. D. (1991). Inhibition of oxidative rancidity in salted ground pork by carnosine. *Journal of Food Science*, *56*, 1179–1181.

Decker, E. A., Livisay, S. A., & Zhou, S. (2000). A re-evaluation of the antioxidant activity of purified carnosine. *Biochemistry (Moscow)*, *65*, 766–770.

Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2001). Extension of the retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. *Journal of Food Science*, *66*, 181–186.

Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2002). Ability of a-tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. *Food Chemistry*, *76*, 407–415.

Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2003a). The shelf-life of beef steaks treated with DL-lactic acid and antioxidants and stored under modified atmospheres. *Food Microbiology*, *20*, 1–7.

Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2003b). Extension of the shelf life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. *Meat Science*, *64*, 417–426.

Elliot, J. G. (1999). Application of antioxidant vitamins in foods and beverages. *Food Technology*, *53*(2), 46–48.

Faustman, C., & Cassens, R. G. (1990). The biochemical basis for discoloration in fresh meat: a review. *Journal of Muscle Foods*, *1*, 217–243.

Gray, J. I., Gomaa, E. A., & Buckley, D. J. (1996). Oxidative quality and shelf-life of meats. *Meat Science*, *43*, S111–S123.

Greene, B. E., & Cumuze, T. H. (1981). Relationship between TBA-numbers and inexperienced panelists assessments of oxidized flavor in cooked beef. *Journal of Food Science*, *47*, 52–54 58.

Halcak, L., Rendekova, V., Pechan, I., & Kubaska, M. (1998). The effect of uric acid, creatine phosphate and carnitine on lipid peroxidation in cerebral cortex and myocardium homogenates. *Bratislavské Lekárske Listy*, *99*, 343–346.

Hu, M. L., Chen, Y. K., & Lin, Y. F. (1995). The antioxidant and prooxidant activity of some B vitamins and vitamin-like compounds. *Chemistry & Biology Interact*, *30*, 63–73.

ICMSF. In R. P. Elliott, D. S. Clark, K. H. Lewis, H. Lundbeck, J. C. Olson Jr., & B. Simonsen (Eds.), *Microorganisms in foods. Their significance and methods of enumeration (Vol. 1)*. Toronto: University of Toronto Press.

Ledward, D. A. (1970). Metmyoglobin formation in beef stored in carbon dioxiiide enriched and oxygen depleted atmospheres. *Journal of Food Science*, *35*, 33–37.

Lee, B. J., Hendricks, D. G., & Cornforth, D. P. (1999). A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef pattie model system. *Meat Science*, *51*, 245–253.

Mano, S. B. (1997). *Comportamiento de la microbiota natural y* Listeria monocytogenes, Aeromonas hydrophila *y* Yersinia enterocolitica *en carne envasada en atmósferas modificadas*. Tesis Doctoral. Universidad Complutense de Madrid, Spain.

Mitsumoto, M., Cassens, R. G., Schaefer, D. M., Arnold, R. N., & Scheller, K. K. (1991). Improvement of colour and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. *Journal of Food Science*, *56*, 1489–1492.

O'Grady, M. N., Monahan, F. J., Burke, R. M., & Allen, P. (2000). The effect of oxygen level and exogenous alpha-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. *Meat Science*, *55*, 39–45.

Pfalzgraf, A., Frigg, M., & Steinhart, H. (1995). Alpha Tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage. *Journal of Agricultural and Food Chemistry*, *43*, 1339–1342.

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Beltrán, J. A., & Roncalés, P. (2001). The effects of ascorbic acid, taurine, carnosine and rosemary powder on color and lipid stability of beef patties packaged in modified atmosphere. *Meat Science*, *58*, 421–429.

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Gimenez, B., Beltrán, J. A., & Roncalés, P. (2003). Evaluation of the antioxidant ability of hydrazine-purified and untreated commercial carnosine in beef patties. *Meat Science*, *64*, 59–67.

Shivas, S. D., Kropf, D. H., Hunt, M. C., Kastner, C. L., Kendall, J. L. A., & Dayton, A. D. (1984). Effects of ascorbic acid on display life pf groung beef. *Journal of Food Protection*, *47*, 11–15 -19.

SPSS. (1995). *SPSS for Windows, 6.1.2*. Chicago, IL: SPSS Inc.

Stewart, M. R., Zipser, M. W., & Watts, B. M. (1965). The use of reflectance spectrophotometry for the assay of raw meat pigments. *Journal of Food Science*, *30*, 464–469.

Vanella, A., Russo, A., Acquaviva, R., Campisi, A., Di Giacomo, C., Sorren, V., & Barcellona, M. L. (2000). L-propionyl-carnitine as superoxide scavenger, antioxidant, and DNA cleavage protector. *Cell Biology and Toxicology*, *16*, 99–104.

Wheeler, T. L., Koohmaraie, M., & Shackelford, S. D. (1996). Effect of vitamin C concentration and Co-Injection with calcium chloride on beef retail display colour. *Journal of Animal Science*, *74*, 1846–1853.

Zhao, Y., Wells, J. H., & McMillin, K. W. (1994). Applications of dynamic modified atmosphere packaging systems for fresh red meats: review. *Journal of muscle Foods*, *5*, 299–328.

Zhou, S., & Decker, A. A. (1999). Ability of carnosine and other skeletal muscle components to quench unsaturated aldehydic lipid oxidation products. *Journal of Agricultural and Food Chemistry*, *47*, 51–55.