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Ability of α-tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere

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Abstract

Fresh beef steaks were sprayed on the surface with vitamin C (500 ppm), taurine (50 mM), rosemary (1000 ppm) and vitamin E (100 ppm), the three latter in combination with 500 ppm of Vitamin C, packaged in modified atmosphere (70% O_2 + 20% CO_2 + 10% N_2) and stored at 1 ± 1 °C for 29 days. Metmyoglobin formation, lipid oxidation (TBARS), instrumental colour (CIE a^*), psychrotrophic bacterial counts (PCA) and sensory discolouration and odour were determined. Results demonstrated that surface application of antioxidant combinations resulted in an effective delay of oxidative deterioration of fresh beef steaks. Shelf life was extended beyond that of control, according to evaluation of sensory attributes. Both combinations of vitamin C with either rosemary or taurine significantly ($P < 0.01$) extended the shelf life of fresh beef steaks by about 10 days. Rosemary was the most effective in delaying oxidation processes. The combination of vitamins E and C was significantly ($P < 0.01$) less effective than those combinations in delaying meat oxidation.

Keywords: Beef; Modified atmosphere; Antioxidants; Vitamin C; Rosemary; Taurine, α-Tocopherol; Meat colour

1. Introduction

A bright red colour for meat is perceived by consumers as indicative of freshness (Faustman, Cassens, Schaefer, Buege, Williams, & Scheller, 1989). In fact, colour is the most important single characteristic on which consumers decide meat purchase (Jeremiah, Carpenter, & Smith, 1972). With prolonged storage oxymyoglobin oxidises to metmyoglobin and gives meat an unattractive brown colour. A common approach to extending the shelf life of fresh red meats is the use of modified atmosphere packaging. However, besides its positive effect on meat colour, O_2 also favours oxidation reactions and therefore, discolouration (Faustman & Cassens, 1990; O'Grady, Monahan, Bailey, Allen, Buckley, & Keane, 1998). Oxidation of membrane phospholipids leads to the formation of unpleasant flavours (Gandemer, 1997). Oxymyoglobin and lipid oxidation appear to be interrelated in meat (Anton, Salgues, Gatellier, & Renerre, 1993). The balance between pro-oxidative factors and antioxidative capacity favours oxidation in stored meat (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998).

The combined use of antioxidants and modified atmosphere packaging for meat represents a realistic and attractive strategy to increase the shelf life of fresh meat (Giese, 1996). The interest in the application of naturally occuring antioxidants has increased over recent years, particularly since the use of synthetic antioxidants has become less acceptable (Mielche & Bertelsen, 1994).

A few studies have been conducted on direct meat treatment with α-tocopherol (vitamin E), although much research has focused on supplementation of animal feed with α-tocopherol for improving myoglobin and lipid stability of fresh beef along storage or retail

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display (Arnold, Scheller, Arp, Williams, & Schaefer, 1993; Eikelenboom, Hoving, Kluitman, Houben, & Klont, 2000; Morrissey et al., 1998; O'Grady, Monahan, Burke, & Allen, 2000; Okayama, Imai, & Yamanoue, 1987). Mitsumoto, Arnold, Schaefer, and Cassens (1993) compared dietary versus postmortem meat supplementation with vitamin E (solved in mineral oil), and concluded that endogenous vitamin E improved pigment and lipid stability much better than exogenous vitamin E.

Regarding taurine, Siqueira, Oetterer, and Regitano d'Arce (1997) reported that it acts as an antioxidant by preventing or delaying oxidations. Keys and Zimmermann (1999) also studied the antioxidant activity of taurine; they found that taurine, combined with other compounds, showed greatest protection against lipid oxidation.

Rosemary extracts exhibit a potent antioxidant activity, and are widely used in the food industry. A number of authors have reported the effectiveness of rosemary for reaching higher sensory scores and lowering lipid oxidation in various foods: Chang, Matijasevic, Hsieh, and Huang (1977) used 200 ppm of rosemary, together with 500 ppm of ascorbic acid, in lard; Barbut, Josephson, and Maurer (1985) used 20 ppm in turkey meat; Stoick, Gray, Booren, and Buckley (1991) used 500–1000 ppm in beef steaks; Shahidi and Wanasundara (1992) recommended concentrations ranging between 200 and 1000 ppm in various foods; Huisman, Madsen, Skibsted, and Bertelsen (1994) used 0.05% in cooked minced pork; Sánchez-Escalante, Djenane, Torrescano, Giménez, Beltrán, and Roncalés (2001) used 1000 ppm of rosemary, combined with 500 ppm of vitamin C, in beef patties. It has been demonstrated that rosemary extracts effectively inhibited hydroperoxide formation (Frankel, Huang, Aeschbach, & Prior, 1996). The antioxidant activity of rosemary extracts has been associated with the presence of phenolic compounds, which break free radical chain reactions by hydrogen atom donation (Basaga, Tekkaya, & Acikel, 1997).

Ascorbic acid (vitamin C) has been also considered for extending the retail display life of meat (Wheeler, Koohmaraie, & Shackelford, 1996). Decker and Xu (1998) found that ascorbic acid either promoted or inhibited lipid oxidation reactions in muscle foods, depending on its concentration. Vitamin C acts as singlet oxygen quencher (Elliott, 1999). According to this author, ascorbic acid functions as a synergist, when used in combination with other antioxidants, by promoting their antioxidant effects. In fact, both vitamin E and rosemary extracts have been used together with ascorbic acid for preventing food oxidation. It has been found that vitamin C regenerates vitamin E for protection against free radical attacks in vitro (Kinsella, Frankel, German, & Kanner, 1993). Okayama et al. (1987) indicated that postmortem dipping with vitamin C and vitamin E solutions before modified atmosphere packaging was very suitable for the storage of beef steaks. Mitsumoto, Faustman, Cassens, Arnold, Schaefer, and Scheller (1991) concluded that vitamin E (6 ppm) + vitamin C (500 ppm) resulted in a lower pigment and lipid oxidation than with any of the antioxidants alone, showing a synergistic effect. Chang et al. (1977) and Sánchez-Escalante et al. (2001) also demonstrated that vitamin C enhanced the antioxidant effect of rosemary extracts, though they afforded no data to support an additive or synergistic effect.

The objective of the present research was to determine the effect of natural antioxidants vitamin E, taurine and rosemary extract, in combination with vitamin C, on the inhibition of both lipid and pigment oxidations and, consequently, on the extension of quality characteristics of fresh beef steaks packaged in modified atmosphere.

2. Materials and methods

2.1. Preparation of samples

Longissimus dorsi muscles from three beef carcasses (right sides) were obtained from the abattoir 48 h post-slaughter, and trimmed of external fat. Steaks of about 100 g weight (1.5 cm thick and about 75 cm^2 surface) were aseptically cut, using sterile cutting boards and knives, and exposed to air during 1 h at 1 °C to allow blooming.

After blooming, samples were randomly divided into six groups. One group was sprayed on meat surface with vitamin C (500 ppm), according to a ratio of 2 ml solution to 100 g meat. The second group was sprayed with the same amount of a solution of taurine (50 mM) and vitamin C (500 ppm). The third group was treated with α-tocopherol (100 ppm) and vitamin C (500 ppm) solutions. The fourth group was treated with rosemary (500 ppm) and vitamin C (500 ppm) solutions. The fifth group (water control samples) was sprayed with 2 ml of sterile distilled water. The sixth group (water + n-pentane control samples) was sprayed with 2 ml of n-pentane, which readily evaporated, and 2 ml of sterile distilled water. Solutions of taurine and L-ascorbic acid (both from Sigma Chemical Co) were freshly prepared in sterile distilled, deionised water. Alpha-tocopherol (Sigma Chemical Co) and rosemary extract (Flavorguard©, Chr. Hansen GmbH) were prepared using n-pentane.

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 (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} 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), thermosealed and stored in the dark at 1 ± 1 °C.

The packs were stored for either 6, 12, 18, 22 or 29 days. Thirty-six samples (six for each treatment) were removed from the cabinet at each selected time for subsequent analysis. Half of them were used for microbial sampling alone, whereas the other half were used firstly for sensory analysis, secondly for colour instrumental analysis and thereafter for the determination of TBARS.

2.2. Meat colour and metmyoglobin analysis

Meat colour was measured at the surface of beef steaks using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan), 30 min after pack opening, in order to allow colour stabilisation on air exposure. CIE L^* (lightness), a^* (redness) and b^* (yellowness) parameters were recorded (CIE, 1978). The average value for each steak was the mean of 20–25 determinations.

The metmyoglobin (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 $(K/S)_{572}$ to $(K/S)_{525}$ at the beginning of the experiment was fixed as 0% metmyoglobin; 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 ferricyanid (Ledward, 1970). The average value for each steak was the mean of 20–25 determinations.

2.3. Lipid oxidation analysis

Lipid oxidation was assessed in duplicate by the 2-thiobarbituric acid (TBA) method of Pfalzgraf, Frigg, and Steinhart (1995), using 10 g of meat samples. TBARS values were calculated from a standard curve of malondialdehyde and expressed as mg malondialdehyde kg^{-1} meat.

2.4. 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 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. Counts were expressed as the $\log_{10}$ of colony forming units/cm^2.

2.5. Sensory evaluation

Meat samples were evaluated for discolouration and odour by a six-member panel, trained according to the method of Cross, Moen, and Stanfield (1978). Training consisted of four sessions of approximately 1 h, in which panelists were served beef steak samples for evaluation of selected attributes. Training sessions were conducted to acquaint panelists with the products and attributes to be evaluated, and were 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 non-packaged beef steaks either fresh or stored at 2 °C for different times up to 3 weeks to allow off odour formation related to meat spoilage. Panelists were male ($n = 4$) and female ($n = 2$) laboratory coworkers, and ranged in age from 24 to 50 years. They were familiar with meat and taste panels, and consisted of graduate students and faculty of the Food Technology laboratory.

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 15.5×21.5 cm. Each panelist received one sample (half steak) of each treatment randomly numbered and served. Panelists were instructed to wash hands and cleanse with distilled water to minimise extraneous odours. All samples were evaluated under cool white fluorescent lighting, positioned so that it provided about 800 lux (74.4 foot candles) 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 Sørheim, Kropf, Hunt, and Warren (1996), and Djenane, Sánchez-Escalante, Beltrán, and Roncalés (2001) using a paper scorecard. Scores for 'discolouration' referred to percentage of discoloured 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 off odours associated to meat spoilage: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme.

2.6. 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 SPSS (1995). Differences were considered significant at the $P<0.05$ level. The Pearson's correlation matrix was calculated according to SPSS (1995); correlations were considered significant at the $P<0.01$ level. Data were also analyzed using a multivariate statistical method. The principal component loading and rotated factor matrix were determined by using SPSS for Windows, release 7.5.2.S (SPSS, 1995). Double cross-validation was performed in order to define the number of significant components in Principal Component Analysis. This analysis included a large data matrix integrated by all chemical, microbial and instrumental parametres, measured in beef patties subjected to all treatments on the day 29 of storage.

3. Results and discussion

3.1. Metmyoglobin percentage

Fig. 1 depicts the results of metmyoglobin formation, expressed as percentage of metmyoglobin of total surface myoglobin. In order to avoid misunderstanding, control data shown were the mean of all samples sprayed with either water or n-pentane + water, since no significant ($P>0.05$) differences were found among them. Results demonstrated that all of the antioxidant combinations effectively delayed metmyoglobin formation. This effect was evident from day 18 of storage onwards, showing significant ($P<0.05$) differences with untreated control samples. In fact, after 29 days of storage, none of the treated steaks reached 40% of surface metmyoglobin, with the exception of vitamin C alone, while myoglobin of control steaks was oxidised to nearly 100%. Treatment with vitamin E + vitamin C was significantly ($P<0.05$) less effective than any other combination. Greene, Hsin, and Zipser (1971) reported that a consumer panel rejected samples of fresh beef with a percentage of MetMb greater than 40%. Therefore, according to them, the presence of any of the antioxidant combinations would contribute to meat acceptance by consumers along the whole storage time. However, Hood and Riordan (1973) stated that consumers discriminated already at 20% MetMb formation.

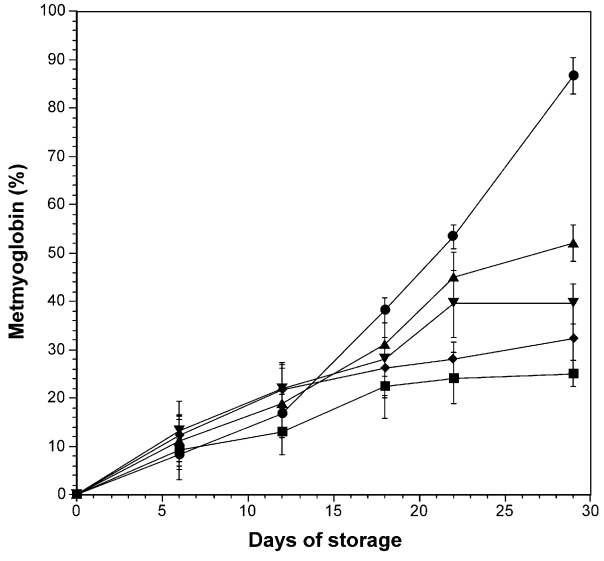

Fig. 1. Metmyoglobin percentage (±S.D.; $n=3$) in beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (●) control; (▲) vitamin C; (▼) vitamin E + vitamin C; (◆) taurine + vitamin C; (■) rosemary + vitamin C.

Regarding vitamin E, Okayama et al. (1987) reported that treatment with vitamin E + vitamin C was very useful for delaying metmyoglobin accumulation. Yin, Faustman, Riesen, and Williams (1993) also reported that myoglobin oxidation in a liposome model was greatly reduced by its combination with ascorbate. Mitsumoto et al. (1991) demonstrated that exogenous treatment with vitamin E + vitamin C maintained a very low surface metmyoglobin percentage in ground meat. However, the same authors reported a very low effect of exogenous vitamin E when compared to dietary supplementation (Mitsumoto et al., 1993).

With regard to taurine effect, Siqueira et al. (1997) already reported that taurine exerted an antioxidant effect by preventing or delaying oxidative reactions. However, no assessment of its effect on delaying myoglobin oxidation had been thus far reported. The effect of rosemary extract agreed with the results of Sánchez-Escalante et al. (2001), who found that this extract combined with vitamin C was very useful for preventing myoglobin oxidation in ground beef patties. The synergistic effect of vitamin C when used in combination with other antioxidants was already proposed by Elliott (1999).

3.2. Instrumental colour

Values of CIE a^* (redness) are depicted in Fig. 2. Control data were as explained for Fig. 1. Treatment with antioxidant combinations led to significant differences ($P<0.05$) with the controls from day 18 of storage onwards. At the end of the storage period (29 days), untreated samples had very low a^* values, below 5, while all other possessed a^* values above 10, representative of a manifest bright red colour, which reflected a sharp difference in redness index among them.

Treatment with rosemary combined with vitamin C resulted in the most intense red colour at the end of storage, showing the highest ($P<0.05$) a^* values, of about 16. This value of a^* was reached at day 12 of storage of the control samples; therefore, rosemary + vitamin C retarded colour loss by about 2 weeks. Treatment with taurine + vitamin C had a lower protective effect of

redness than rosemary, while the combination of vitamin E and vitamin C was less effective in maintaining red colour. However, even the latter retarded colour loss by about 10 days with respect to untreated samples.

The overall effect of antioxidant combinations on a^* values agreed fairly with the results of metmyoglobin formation discussed earlier. They were in agreement, too, with previous reports regarding treatment with antioxidants before modified atmosphere packaging; indeed, Okayama (1987) indicated that this treatment was very suitable for the storage of beef steaks.

3.3. TBA reactive substances

Fig. 3 shows the results of TBA reactive substances (TBARS) throughout the storage of treated and untreated steaks. Control data were as explained for Fig. 1. All of the antioxidant combinations exerted a significant ($P<0.05$) inhibitory effect on the formation of TBARS, although not with the same intensity. The most effective combinations were those of taurine and rosemary extract with vitamin C, while the effect of the combination of vitamins E + C was significantly lower ($P<0.05$) than those. Differences were significant ($P<0.05$) from day 18 of storage onwards, except for treatment with vitamin C alone, which were significant ($P<0.05$) from day 22 onwards.

Rosemary and taurine exerted considerable inhibition of oxidative processes, which resulted in about 50% less TBARS than in the control after 29 days of storage. The antioxidant effect of rosemary extracts has been well documented (Frankel et al., 1996), and was used in a variety of meat preparations (Huisman et al., 1994; Sánchez-Escalante et al., 2001). Regarding taurine, its antioxidant effect has been demonstrated in some human organs (Keys & Zimmerman, 1999), whereas it had been thus far not tested as an antioxidant in foods.

Vitamins E + C were effective in inhibiting lipid oxidation, although their effect was the lowest of all combinations, in agreement with their relatively low protective effect on myoglobin oxidation. Exogenous vitamin E alone was shown to reduce lipid oxidation in minced meat stored in high O_2 atmospheres (O'Grady et al., 2000). Though it has been reported that α-tocopherol with vitamin C had a higher effect than α-tocopherol alone. In fact, Mitsumoto et al. (1991) found that the exogenous treatment with vitamin E + vitamin C maintained low TBARS values in ground meat during 7 days of storage. Okayama et al. (1987) also reported that beef steaks dipped in a vitamin E + vitamin C solution had low TBARS values throughout 13 days of storage.

3.4. Microbial analysis

Counts of psychrotrophic aerobic flora throughout storage of beef steaks are shown in Fig. 4. Control data were as explained for Fig. 1; no significant ($P>0.05$) differences were found between both control samples, which were sprayed on the surface with either water or n-pentane + water (data not shown). Therefore, an antimicrobial effect of n-pentane must be discarded. Microbial counts of all samples gradually increased along storage, and reached final relatively low values of 5–6

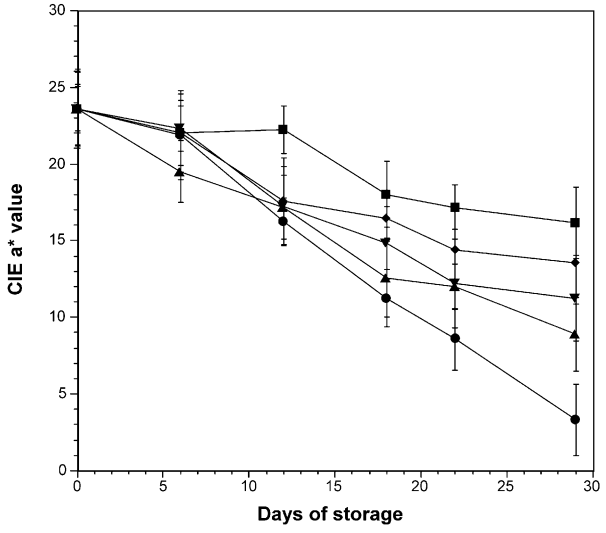

Fig. 2. Values of CIE a^* (±S.D.; $n=3$) in beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (●) control; (▲) vitamin C; (▼) vitamin E + vitamin C; (◆) taurine + vitamin C; (■) rosemary + vitamin C.

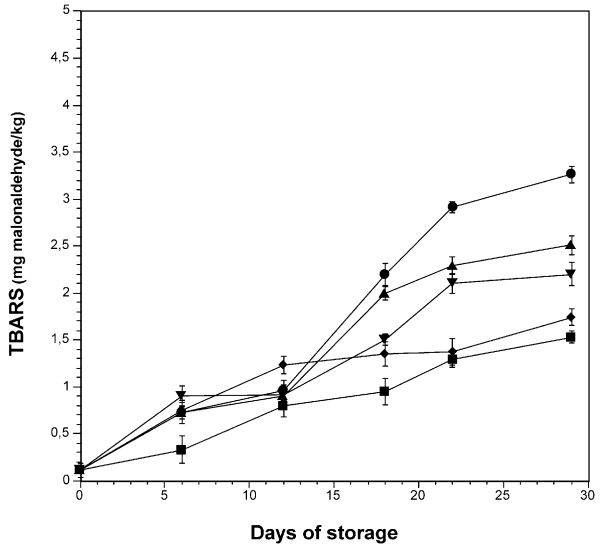

Fig. 3. TBARS (mg malonaldehyde/kg meat) (±S.D.; $n=9$) in beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (●) control; (▲) vitamin C; (▼) vitamin E + vitamin C; (◆) taurine + vitamin C; (■) rosemary + vitamin C.

$\log_{10}$ cfu cm^{-2}. Samples treated with rosemary extract + vitamin C exhibited lower counts during the whole period of storage, but the difference was only significant ($P < 0.05$) from day 22 of storage onwards. Several researchers indicated the antibacterial effect of many herbs and spices. Regarding rosemary extracts, Pandit and Shelef (1994) showed that *Listeria monocytogenes* was strongly inhibited by addition (0.3%) of a rosemary extract; however, its inhibitory effect on meat psychrothrophic bacteria growth had been, as far as we are aware, reported only by Ouattara, Simard, Holley, Piette, and Begin (1997) using pure rosemary essential oils. It has been also reported that microbial counts were not affected by treatment with ascorbic acid (Shivas, Kropf, Hunt, Kastner, Kendall, & Dayton, 1984).

3.5. Sensory analysis

Results of the sensory evaluation of discolouration and odour are shown in Table 1. Control data were as explained for Fig. 1. These results clearly demonstrated that beef steaks treated with any of the antioxidant combinations were given lower scores ($P < 0.05$) than untreated steaks from day 18 of storage onwards for discolouration and from day 12 for off-odour. However, the various antioxidants differed significantly ($P < 0.05$) in their protective effect. Rosemary extract + vitamin C were the most effective, followed by taurine with vitamin C, while vitamins E and C together were the least effective of the combinations. 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.

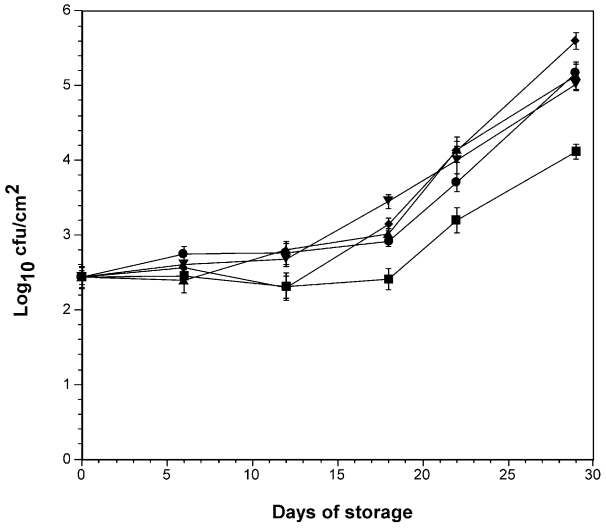

Fig. 4. Plate count agar numbers (±S.D.; $n = 6$) of psychrotrophic aerobes in beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (●) control; (▲) vitamin C; (▼) vitamin E + vitamin C; (◆) taurine + vitamin C; (■) rosemary + vitamin C.

Discolouration scores given to untreated steaks showed that broad discoloured areas were evident at the 18th day of storage, while only small discoloured areas were present in treated samples until the end of the storage period. Only those containing vitamins E + C showed significant discolouration from day 18 onwards. Therefore, steaks treated with either taurine and rosemary extract, both with vitamin C, extended significantly ($P < 0.05$) the shelf life of packaged steaks.

Our results demonstrated similar patterns for the rates of discolouration, a^* values and metmyoglobin percentage changes. The storage time at which discolouration scores reached a value of three was assigned to samples with a proportion of MetMb of about 30–40%, which coincided with previous reports of Greene et al. (1971) and Renerre and Mazuel (1985) for causing beef steak rejection by purchasers in retail display.

Off-odour was absent or slight on the untreated samples for 12 days; this corresponded to a TBARS value of about 1.0, which is in good agreement with values previously reported by Tarladgis, Watts, Younathan, and Dugan (1960). Off-odour of steaks treated with either taurine or rosemary extract, together with vitamin C, was scored as absent, slight or small until the day 22 of storage, in correspondence to TBARS values of about 1.0. That is to say that the shelf life of treated steaks would be extended, on the basis of perceived off-odour, for over 10 days. Treatment with vitamin E extended shelf life for about 6 days.

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 TBA range of 0.6–2.0 was required for inexperienced taste panelists to detect oxidised flavours; Tarladgis et al. (1960) reported a range of 0.5 to 1.0 for experienced panelists.

3.6. Principal components analysis

Fig. 5 depicts the results of principal components analysis (PCA) of all data corresponding to the 36 samples of day 29 of storage. The first component, which explained 72.9% of the differences among treatments, was able to neatly discriminate control samples from any antioxidant treatment. The second component, which explained 21.4% of the differences, discriminated mainly treatments among them. Therefore, PCA provided an unquestionable demonstration of the effectiveness and significance of all antioxidant treatments. As discussed previously, PCA confirmed that vitamin C alone and the combination of vitamins E + C exerted a significant but moderate effect, while taurine and rosemary extract, both with vitamin C, exerted a significant and remarkable antioxidant effect.

Table 1
Effect of antioxidant treatments on sensory panel scores (mean ± S.D.) 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[a]

Attribute	Antioxidant treatment	Days of storage					
		0	6	12	18	22	29
Discolouration[b]	Untreated samples (control)	1.0±0.0a	1.0±0.0a	1.7±0.6a	4.0±0.0a	4.8±0.9a	5.0±0.0a
	Vitamin C	1.0±0.0a	1.0±0.0a	1.6±0.5a	2.8±0.6b	3.7±0.7b	4.0±0.0b
	Vitamin E + vitamin C	1.0±0.0a	1.0±0.0a	1.9±0.7a	2.5±0.7b	3.2±0.8b	3.6±0.7b
	Taurine + vitamin C	1.0±0.0a	1.0±0.0a	1.8±0.8a	2.1±0.8b	2.4±0.8c	2.6±0.6c
	Rosemary + vitamin C	1.0±0.0a	1.0±0.0a	1.6±0.7a	1.8±0.8c	2.0±0.0c	2.0±0.0d
Off Odour[c]	Untreated samples (control)	1.0±0.0a	1.3±0.6a	3.2±0.9a	4.0±0.0a	5.0± 0.0a	5.0±0.0a
	Vitamin C	1.0±0.0a	1.0±0.0a	1.4±0.6b	3.3±0.6b	3.6±0.5b	4.0±0.0b
	Vitamin E + vitamin C	1.0±0.0a	1.0±0.0a	1.2±0.7b	3.2±0.8b	3.4±0.7b	3.9±0.8b
	Taurine + vitamin C	1.0±0.0a	1.0±0.0a	1.2±0.7b	2.0±0.0c	2.3±0.8c	3.3±0.6c
	Rosemary + vitamin C	1.0±0.0a	1.2±0.4a	1.0±0.0b	1.8±0.8c	2.2±0.7c	3.2±0.7c

[a] Mean values in the same column and relating to each attribute are significantly different when accompanied by different letters ($P < 0.05$).
[b] **1** = None (0%), **2** = 0–10%, **3** = 11–20%, **4** = 21–60%, **5** = 61–100%.
[c] **1** = None, **2** = Slight, **3** = Small, **4** = Moderate, **5** = extreme.

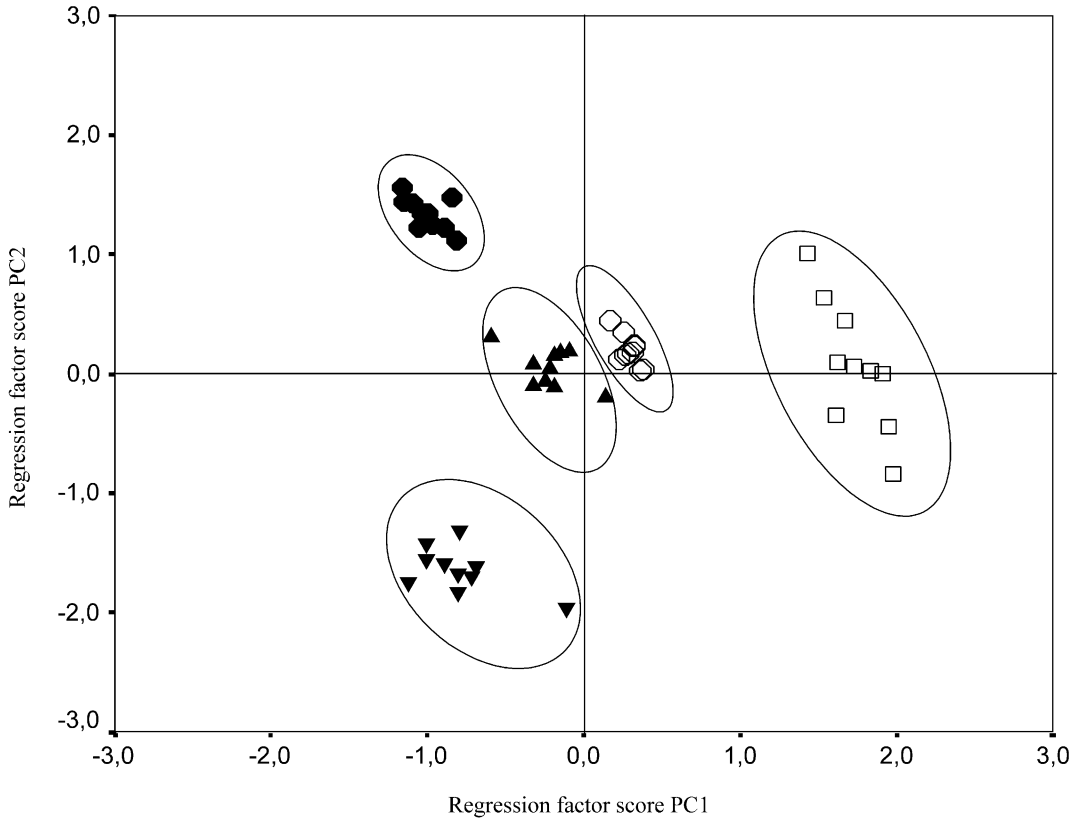

Fig. 5. Regression factor scores of principal components analysis of all measured parameters (±S.D.; $n = 18$) at the day 29 of storage of beef steaks treated with different antioxidants, packaged in modified atmosphere and stored at 1 °C: (□) control; (○) vitamin C; (▲) vitamin E + vitamin C; (▼) rosemary + vitamin C; (●) taurine + vitamin C.

4. Conclusions

Our results demonstrated that surface application of natural antioxidants prior to modified atmosphere packaging resulted in an effective delay of oxidative deterioration of fresh beef steaks. The results of PCA of all data corresponding to day 29 of storage were conclusive of the effect brought about by antioxidant treatments. Consequently, shelf life was extended and, therefore, treated meat maintained its quality characteristics for a longer

period than the control, according to the evaluation of its sensory attributes. Both combinations of vitamin C with either rosemary extract or taurine extended the shelf life of fresh beef steaks by about 10 days. Rosemary combination with vitamin C was the most effective in delaying myoglobin oxidation and lipid oxidation. The combination of vitamins E and C was significantly less effective than any other in delaying meat oxidation, though its effect was more intense than that of vitamin C alone. Those data strongly suggest that all the combinations tested have a valuable potential for their use as natural antioxidants in fresh beef steaks packaged in modified atmosphere.

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References

Anton, M., Salgues, C., Gatellier, P., & Renerre, M. (1993). Etude des relations oxydatives entre les lipides membranaires et la myoglobine in vitro. *Science des aliments*, *13*, 261–274.

Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N., & Schaefer, D. M. (1993). Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Science*, *71*, 105–118.

Barbut, S., Josephson, D. B., & Maurer, A. J. (1985). Antioxidant properties of rosemary oleoresin in turkey sausage. *Journal of Food Science*, *50*, 1356–1359 1363.

Basaga, H., Tekkaya, C., & Acikel, F. (1997). Antioxidative and free radical scavenging properties of rosemary extract. *Lebensmittel Wissenschaft und Technologie*, *30*, 105–108.

Chang, S. S., Matijasevic, B. O., Hsieh, O. L., & Huang, C. L. (1977). Natural antioxidants from rosemary and sage. *Journal of Food Science*, *42*, 1102–1106.

CIE (1978). *Recommendations on uniform color spaces–color difference equations psychometric color terms*. Commision International de l'Eclairage, Paris. Supplement No 2 to CIE Publication No 15 (E-1.3.1) 1971/(TC-1.3.).

Cross, H. R., Moen, R., & Stanfield, M. S. (1978). Training and testing of judges for sensory analysis of meat quality. *Food Technology*, *32*(7), 48–54.

Decker, E. A., & Xu, Z. (1998). Minimizing rancidity in muscle foods. *Food Technology*, *52*, 54–59.

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.

Eikelenboom, G., Hoving, B. A. H., Kluitman, I., Houben, J. H., & Klont, R. E. (2000). Effect of dietary vitamin E supplementation on beef colour stability. *Meat Science*, *54*, 17–22.

Elliott, 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.

Faustman, C., Cassens, R. G., Schaefer, D. M., Beuge, D. R., Williams, S. N., & Scheller, K. K. (1989). Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *Journal of Food Science*, *54*, 858–862.

Frankel, E. N., Huang, S. W., Aeschbach, R., & Prior, E. (1996). Antioxidant activity of rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil—in water emulsion. *Journal of Agricultural and Food Chemistry*, *44*, 131–135.

Gandemer, G. (1997). Lipides du muscle et qualité de la viande. Phospholipides et flaveur. *Oleagineux, Corps Gras et Lipides*, *4*, 1–7.

Giese, J. (1996). Antioxidants: tools for preventing lipid oxidation. *Food Technology*, *50*(11), 73–80.

Greene, B. E., Hsin, I. M., & Zipser, M. W. (1971). Retardation of oxidative color changes in raw ground beef. *Journal of Food Science*, *36*, 940–942.

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.

Hood, D. E., & Riordan, E. B. (1973). Discoloration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. *Journal of Food Technology*, *8*, 333–343.

Huisman, M., Madsen, H. L., Skibsted, L. H., & Bertelsen, G. (1994). The combined effect of rosemary (*Rosmarinus officinalis* L.) and modified atmosphere packaging as protection against warmed-over flavour in cooked minced pork meat. *Zeitschrift für Lebensmittel Untersuchung und Forschung*, *198*, 57–59.

ICMSF (1983). Microorganisms in foods, Vol. 1. Their significance and methods of enumeration. In R. P. Elliott, D. S. Clark, K. H. Lewis, H. Lundbeck, J. C. Olson Jr., & B. Simonsen. Toronto: University of Toronto Press.

Jeremiah, L. E., Carpenter, Z. L., & Smith, G. C. (1972). Beef color as related to consumer acceptance and palatability. *Journal of Food Science*, *37*, 476–479.

Keys, S. A., & Zimmerman, W. F. (1999). Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. *Experimental Eye Research*, *68*, 693–702.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology*, *47*(4), 85–89.

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

Mielche, M. M., & Bertelsen, G. (1994). Approaches to the prevention of warmed-over flavour. *Trends in Food Science & Technology*, *5*, 322–327.

Mitsumoto, M., Arnold, R. N., Schaefer, D. M., & Cassens, R. (1993). G, Dietary versus postmortem supplementation of vitamin E on pigment and lipid stability in ground beef. *Journal of Animal Science*, *71*, 1812–1816.

Mitsumoto, M., Faustman, C., Cassens, R. G., Arnold, R. N., Schaefer, D. M., & Scheller, K. K. (1991). Vitamins E and C improve pigment and lipid stability in ground beef. *Journal of Food Science*, *56*, 194–197.

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability in meat and meat products. *Meat Science*, *49*, 73–86.

O'Grady, M. N., Monahan, F. J., Bailey, J., Allen, P., Buckley, D. J., & Keane, M. G. (1998). Colour-stabilising effect of muscle vitamin E in minced beef stored in high oxygen packs. *Meat Science*, *50*, 73–80.

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.

Okayama, T. (1987). Effect of modified gas atmosphere after dip treatment on myoglobin and lipid oxidation of beef steaks. *Meat Science*, *19*, 179–185.

Okayama, T., Imai, T., & Yamanoue, M. (1987). Effect of ascorbic acid and α-tocopherol on storage stability of beef steaks. *Meat Science*, *21*, 267–273.

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J. P., & Begin, A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *International Journal of Food Microbiology*, *37*, 155–162.

Pandit, V. A., & Shelef, L. A. (1994). Sensitivity of *Listeria monocytogenes* to rosemary (*Rosmarinus officinalis*-L). *Food Microbiology*, *11*, 57–63.

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.

Renerre, M., & Mazuel, J. P. (1985). Relation entre méthodes de mesure instrumentales et sensorielles de la couleur de la viande. *Science des Aliments*, *5*, 541–557.

Sánchez-Escalante, A., Djenane, D., Torrescano, G., Giménez, B., Beltrán, J. A., & Roncalés, P. (2001). The effects of natural antioxidants on the quality characteristics of beef patties packaged in modified atmosphere. *Meat Science* (in press).

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, *32*, 67–103.

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 of ground beef. *Journal of Food Protection*, *47*, 11–15 -19.

Siqueira, F. M., Oetterer, M., & Regitano d'Arce, M. A. B. (1997). Antioxidant nutrients. *Boletim da Sociedade Brasileira de Ciencia e Technologia Alimentaria*, *31*, 192–199.

Sørheim, O., Kropf, D. H., Hunt, M. T., & Warren, K. E. (1996). Effects of modified gas atmosphere packaging on pork loin colour, display life and drip loss. *Meat Science*, *43*, 203–212.

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

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.

Stoick, S. M., Gray, J. I., Booren, A. M., & Buckley, D. J. (1991). Oxidative stability of restructured beef steaks processed with oleoresin rosemary, tertiary butylhydroquinone, and sodium tripolyphosphate. *Journal of Food Science*, *56*, 597–600.

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L. (1960). A distillation method for the quantitative determination of malonaldehyde in foods. *Journal of the American Oil Chemistry Society*, *37*, 44–48.

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

Yin, M. C., Faustman, C., Riesen, J. W., & Williams, S. N. (1993). Alpha-tocopherol and ascorbate delay oxymyoglobin and phospholipid oxidation in vitro. *Journal of Food Science*, *58*, 1273–1281.