Ability of some natural antioxidants in combination with vitamin C to increase the oxidative stability of beefsteaks packaged in modified atmosphere.

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Background and objectives: The combined use of antioxidants and modified atmosphere packaging for retail display of meat represents a realistic and attractive strategy. Interest in the application of naturally occurring antioxidants in meat has increased, particularly since the use of synthetic antioxidants has become less acceptable. Chan and Decker, (1994) found that carnosine exert a multifunctional effect, acting as a hydrogen donor, a free radical scavenger and metal chelator. Ascorbic acid when used in combination with other antioxidants, functions as a synergist by promoting their antioxidative effects (Elliott, 1999). Rosemary extract exhibit a potent antioxidant activity (Chang *et al.*, 1977). In previous studies, Okayama et al, 1987 indicated that post-mortem dipping with Vitamin C and Vitamin E solutions was very suitable for the storage of beefsteaks. The aim of the present research was to determine the effect of various natural antioxidants in combination with vitamin C on the inhibition of both lipid and pigment oxidation, and therefore, on the extension of quality characteristics of fresh beefsteaks packaged in modified atmosphere.

MATERIALS & METHODS

Preparation of samples: Longissimus Dorsi muscles from beef carcasses was obtained. Steaks (1,5 cm thick) were cut. Samples beef steaks were sprayed with Carnosine (50mM), Taurine (50mM), Vitamin E (100ppm) and Rosemary (1000ppm) solutions all combined with 500 ppm of Vitamin C. The sprayed volume was the 2% of muscle's weight. Each steak was placed on a polystyrene tray. The tray with the steak was introduced in a pouch made of a polyethylene and polyamide laminate (PE/PA). The pouch was filled with a gas mixture of 70% O_2 +20% CO_2 + 10% N_2, thermosealed and stored at 1±1°C. **Colour Instrumental Measurement:** CIE L*.a*.b* parameters were measured at the surface of meat using a spectrophotometer. Each value of this parameters was the mean of 30 determinations. **Metmyoglobin Measurement:** Metmyoglobin (%) was estimated by measuring the surface reflectance at 525 and 572 nm according to Stewart et al. (1965). **Lipid oxidation analysis:** Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of Witte *et al.* (1970). TBARS values were expressed as mg malonaldehyde/kg meat. **Microbial analysis:** Microbial analysis were done by sprinkling an area of 10 cm^2 of steaks surface. Using conventional dilution procedures (in 0,1% peptone water), counts of aerobic psychrotrophic flora were determined from plates bearing 20-200 colonies in Plate Count Agar (PCA), incubated at 10°C for 7 days. **Sensory evaluation:** Scores for 'Fresh Meat Odour' were: 1 = excellent, not different from fresh meat; 2 = good, but slightly poorer than fresh meat; 3 = acceptable, but obviously poorer than fresh meat; 4 = hardly acceptable as fresh meat; 5 = non acceptable. Odour was examined by six member panel. **Statistical analyses:** 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 (SPSS, 1995). Differences were considered significant at the $p< 0.05$ level.

RESULTS & DISCUSSION

Metmyoglobin percentage: The formation of metmyoglobin is shown in Fig.1. The antioxidant ability of rosemary was more effective than of all other antioxidants tested after 12 days of storage ($P<0,05$), which resulted in 21,74%, 41,4%, 54,7% and 71% less metmyoglobin formation than in control samples after 12, 18, 22 and 29 days of storage. Carnosine and taurine seem to follow the same trends in the inhibition of metmyoglobin formation ($P>0,05$); treatment with both antioxidants in combination with vitamin C delayed metmyoglobin formation for about 13 days compared with control. Siqueira *et al.* (1997) found that taurine can act as an antioxidant by preventing or delaying oxidation. Vitamin E/Vitamin C (1/5), also inhibited pigment oxidation, which delayed metmyoglobin formation for about 10 days compared with control. In a previous study, Yin *et al.* (1993) tested the effect of vitamin E/Ascorbate (1/10) in MbO_2 liposome model and reported that MbO_2 oxidation were effectively inhibited test by this combination. **Colour measurement:** After 12 days of storage (Fig.2) the difference in CIE a* values was very significant between all samples treated with antioxidants and control ($P<0,05$). Samples treated with rosemary showed the highest final value of a* (16,15) during 29 days of storage. Hence, rosemary treated samples delayed red colour loss in beefsteaks for about 2 weeks compared with control. Treatments with carnosine and taurine delayed colour loss for about 10 days. Samples treated with vitamin E delayed colour loss for only about 7 days. These results confirmed those of MetMb (%), and reinforced the hypothesis that treatment with antioxidants was very useful for the oxidative stability of beefsteaks packaged in modified atmosphere. **TBA – Reactive Substances:** The greater antioxidant protection provided by carnosine compared to other antioxidants (Fig.3) was maintained even at the end of storage, which resulted in 45,8%, 55,02%, 72,16% and 64,72% less TBARS than in control after 12, 18, 22 and 29 days of storage, respectively. Luño *et al.* (1998) reported that beefsteaks injected with carnosine (25-50mM) had lower TBARS values. Variations in TBARS production were also observed in samples treated with alpha-tocopherol and samples treated with other antioxidants ($P<0,05$); inhibition of TBARS formation, which resulted in 11,24%, 27,83% and 32,51% less TBARS than in control samples after 18, 22 and 29 days of storage, respectively. Okayama *et al* .1987 also reported that beefsteaks dipped in vitamin E plus vitamin C showed very low TBARS during 13 days of storage. Rosemary treated samples also exhibited lower TBARS values, at 6 day to 18 days of storage. Rosemary had a higher antioxidant activity than taurine ($P<0,05$); from day 22 days to the end of storage the both showed a similar intermediate trend ($P>0.05$), which resulted in about 54,12% and 49,84% less TBARS than control after 22 and 29 days of storage, respectively. **Microbial analysis:** The psychrotrophic flora counts of all samples gradually increased during storage (Fig.4). Samples treated with rosemary exhibited relative reduction of total counts during the whole period of storage. It has been reported that microbial counts are not affected by ascorbic acid treatment (Shivas *et al.*, 1984). Treatment with vitamin E, also did not affect

microbial growth on fresh beefsteaks (Chan *et al.*, 1995). Microbial count of 7 Log_{10} cfu/cm^2 is considered the limit for acceptance of meat. In the present research conditions, none of the samples exceeded this limit, even at the end of storage. **Sensory analysis**: Results of sensory evaluation of odour are shown in Table.1. It appeared clearly evident that beefsteaks treated with antioxidants had the best scores throughout storage (P<0,05). In our study, the TBA value was in agreement with odour shelf life. Odour scores differed significantly (P<0,05), too, from day 12 of storage onwards between the samples treated with antioxidants and untreated samples. Odour of the control was acceptable for only 14 days, which corresponded to 1,6 TBARS values. Vit E treated samples exhibited odour scores hardly acceptable (about 4 in scale range), while all other antioxidants treated samples exhibited an odour scores of good (2, in scale range), and acceptable at the end of experiment (3, in scale range). These results were in good agreement with those of TBARS, which refers to the fact that odour spoilage of fresh meat is also probably due to the TBARS accumulation.

CONCLUSION: All natural compounds tested in this research are well-known antioxidants. The results indicate that rosemary and carnosine are superior to other antioxidants for inhibition of both lipid and pigment oxidation. Taurine and vitamin E inhibited both type of oxidation to a lesser extent. The combination of natural antioxidants with vitamin C may be very useful, since their synergistic effects contribute to maintaining sensory characteristics of beef steaks and, therefore, extending their shelf life in retail display.

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Table 1. Influence of some antioxidantants on sensory odour scores (mean±SD) of beefsteaks packaged in modified atmosphere at 1±1°C.

Attribute	Type of antioxidants	Days of storage 0	6	12	18	22	29
Odour quality*	Control	1,0±0,0^a	1,3±0,0^a	3,2±0,2^a	4,0±0,0^a	5,0±0,0 ^{a}	5,0±0,0^a
	Vitamin E+Vitamin C	1,0±0,0^a	1,0±0,0^a	1,2±0,1^b	3,2±0,2^b	3,4±0,3^b	3,9±0,3^b
	Taurine+Vitamin C	1,0±0,0^a	1,0±0,0^a	1,2±0,1^b	2,0±0,0^c	2,3±0,2^c	3,3±0,3^c
	Carnosine+Vitamin C	1,0±0,0^a	1,0±0,0^a	1,0±0,0^b	1,6±0,3^c	2,0±0,0^c	3,1±0,1^c
	Rosemary+Vitamin C	1,0±0,0^a	1,2±0,1^a	1,0±0,0^b	1,8±0,2^c	2,2±0,1^c	3,2±0,3^c

*1=Excellent, 2=Good, 3=Acceptable, 4=Hardly acceptable, 5=Not acceptable. ^{abc}Mean values in the same column and relating to attribute are significantly different when accompanied by different superscripts (P<0,05).

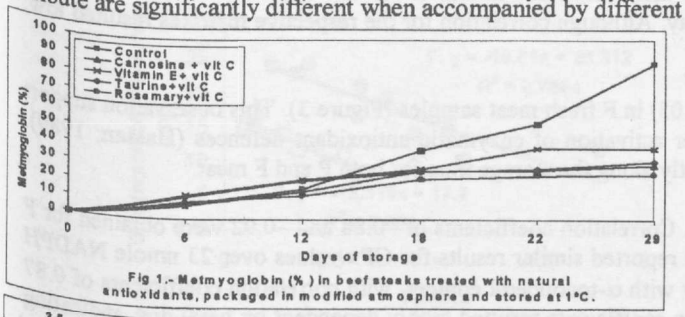

Fig 1.- Metmyoglobin (%) in beef steaks treated with natural antioxidants, packaged in modified atmosphere and stored at 1°C.

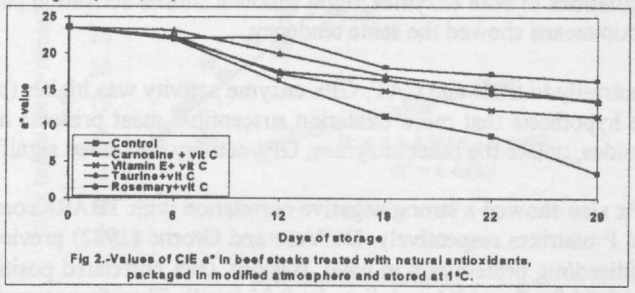

Fig 2.-Values of CIE a* in beef steaks treated with natural antioxidants, packaged in modified atmosphere and stored at 1°C.

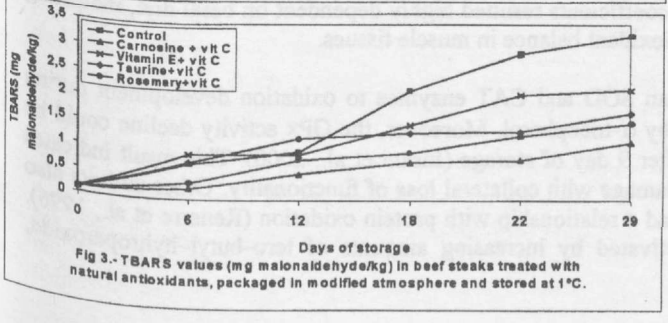

Fig 3.- TBARS values (mg malonaldehyde/kg) in beef steaks treated with natural antioxidants, packaged in modified atmosphere and stored at 1°C.

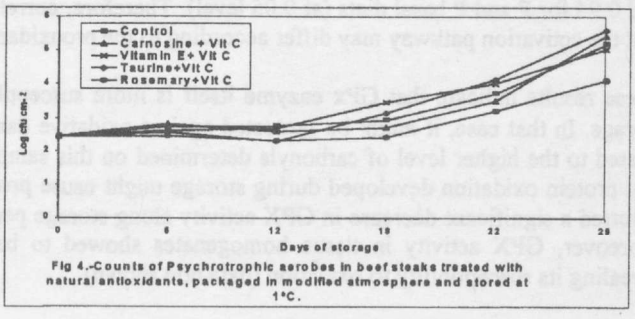

Fig 4.-Counts of Psychrotrophic aerobes in beef steaks treated with natural antioxidants, packaged in modified atmosphere and stored at 1°C.