# ANTIOXIDANT ACTIVITY OF TOMATO, OREGANO AND ASCORBIC ACID AND THEIR MIXTURES ON BEEF PATTIES PACKAGED IN MODIFIED ATMOSPHERE

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**BACKGROUND AND OBJECTIVE**. Lipid oxidation is one of the major problems causing flavor deterioration and reduction in the shelf life of meat and its by-products. Unsaturated fatty acids become oxidised and produce undesirable organoleptic characteristics. Evaluation of lipid oxidation is essential to predicting the shelf life of muscle foods (Li *et al*., 2001). Surface discoloration is a common cause of consumer dissatisfaction with red meats and is costly to the meat industry (Faustman and Cassens, 1990). In certain meat products lipid oxidation is believed to be a particularly important contributor to oxymyoglobin oxidation. Minced or comminuted products are susceptible to lipid oxidation as a result of exposure of membrane lipids to atmospheric oxygen (Govindarajan *et al*., 1977) particularly if antioxidants are limiting (O'Grady *et al*., 1998). The use of antioxidants to increase the storage life of foods has made possible the marketing of many new products. Today antioxidants are widely used in processed foods and can be added directly to the meat or the meat products during processing (Mielche and Bertelsen, 1994). The incorporation of antioxidants protects lipids from oxidation and may indirectly stabilise oxymyoglobin from oxidation (O'Grady *et al*., 1996). A common approach to extending the colour shelf-life of fresh red meats is the use of modified atmosphere packaging (MAP) (Okayama, 1987). The objective of this study was to investigate the antioxidant effectiveness of tomato, oregano and ascorbic acid and their mixtures on the extension of shelf characteristics of fresh beef patties packaged in modified atmosphere.

## MATERIALS AND METHODS

**Samples and atmospheres**. Beef cuts (*Semimembranosus*) were obtained from 3 beef carcasses 48 hr *post mortem*, and ground using a conventional mincer through a plate with 4 mm holes. Portions of uniform weigh of the minced muscle (about 85 g) were formed, placed on polypropylene trays in gas impermeable bags (polyethilene and poliamide), and sealed after flushing with the gas mixture (70% $O_2$ + 20% $CO_2$ + 10% $N_2$). Twelve different formulations were prepared: 1) 500 ppm ascorbic acid, 2) 200 ppm oregano, 3) 500 ppm oregano, 4) 200 ppm oregano+500 ppm ascorbic acid, 5) 500 ppm oregano+500 ppm ascorbic acid, 6) 2.5% LRTP (lycopene rich tomato pulp), 7) 2.5% LRTP+500 ppm ascorbic acid, 8) 2.5% LRTP+200 ppm oregano, 9) 2.5% LRTP+500 ppm oregano, 10) 2.5% LRTP+200 ppm oregano+500 ppm ascorbic acid, 11) 2.5% LRTP+500 ppm oregano+500 ppm ascorbic acid, and 12) control (no antioxidant). All of the antioxidants were mixed with salt and then added to the mixtures. The patties were stored for 20 days at 2 $\pm$ 1°C in the dark. The evaluations were done on the 0, 4, 8, 12, 16 and 20 days of the display period. **Lipid oxidation.** Lipid oxidation was measured by the 2-thiobarbituric acid method of Pfalzgraf *et al.* (1995). TBA values were expressed as mg MA/kg sample. **Metmyoglobin**. Metmyoglobin percentage was estimated spectrophotometrically by measuring the reflectance at 525 and 572 nm according to Stewart *et al.* (1965). The maximum value of the quotient between $K/S_{572}$ and $K/S_{525}$ at the beginning of the experiment was fixed as 0% MetMb, while 100% MetMb was obtained after oxidising a sample in a 1% (w/v) solution of potassium ferricyanide (Ledward, 1970). **Colour measurement**. Objective measurement of colour (CIE L*, a*, b*) was performed at the surface of meat samples using a reflectance spectrophotometer (Minolta CM 2002, Japan). **Sensory analysis.** Samples of beef patties were evaluated by a six-member trained panel. The attribute Off-odour was evaluated using a 5-point scale. Statistical analysis: Data were analysed according to SPSS for Windows (1999).

## RESULTS AND DISCUSSION

**Lipid Oxidation**. Figure 1 shows the changes in TBA values. Lipid oxidation increased rapidly with increasing time ($p<0.05$) in all samples except those that included oregano (alone or in mixture with ascorbic acid and LRTP). Addition of oregano was concentration dependent since treatments with 500 ppm showed an important delay in TBARS formation, although when we added 200 ppm oregano+ascorbic acid lipid oxidation was delayed with same intensity that 500 ppm oregano. We considered that maximum acceptable limit of TBARS formation was 2.0 mg MA/kg (Greene and Cumuze, 1982). Antioxidant capacity of oregano has been proved (Martínez-Tomé *et al*., 2001). Addition of LRTP showed antioxidant activity when it was mixed with ascorbic acid and oregano, delaying the lipid oxidation up between 11 and 18 days. Martínez-Valverde *et al.* (2002) have reported antioxidant activity in tomato extracts and found that this capacity can related to lycopene and ferulic and caffeic acids. We also observed the synergistic capacity of ascorbic acid. This effect has been observed when it was mixed with rosemary and carnosine (Sánchez-Escalante *et al*., 2001).

**Metmyoglobin formation**. Changes in metmyoglobin percentage formation are shown in Figure 2. The amount of metmyoglobin was lower ($p<0.05$) during 12 days in all samples than in the control and those with ascorbic acid and LRTP alone. Beef patties containing oregano alone or in mixture with ascorbic acid maintained a highly acceptable level (below 40%) after 16 days of storage. Green *et al.* (1971) reported that 40% metmyoglobin caused meat rejection by consumers. Ascorbic acid only presented significant ($p<0.01$) inhibitory effect of myoglobin oxidation for 9 days. The efficiency of ascorbic acid to retard oxidation of meat pigments was reported by Mitsumoto *et al.* (1991). Sánchez-Escalante *et al.* (2001) reported that ascorbic acid was effective like antioxidant when was mixed with rosemary. The effectiveness of LRTP was evident until day 12 of storage when it was mixed with ascorbic acid and oregano (both 200 and 500 ppm).

**Colour Instrumental Measurement**. Figure 3 shows a* values. Beef patties containing LRTP+oregano+ascorbic acid presented a delay ($p<0.05$) in myoglobin oxidation (loss of redness) and had higher ($p<0.05$) a* values than those of control and samples with the other antioxidants. Also, addition of ascorbic showed a synergistic effect, since samples mixed with 500 ppm oregano were redder than those with 200 ppm of oregano. At the beginning of storage, red color of samples with LRTP was more intense due to the presence of pigments (carotenoids)(Nir and Hartal, 2000).

**Sensory analysis.** Odour evolution of beef patties is presented in Figure 4. Off-odour scores of all beef patties with oregano (alone or mixed with ascorbic acid) were lower ($p<0.05$), maintaining an acceptable meat odour until the day 16 of storage.

**CONCLUSION.** The presence of oregano, either alone or mixed with ascorbic acid or tomato pulp, was effective in inhibiting oxidation of both lipid and myoglobin. Oregano effectiveness was concentration-dependent; 500 ppm were most effective, inhibiting any oxidative process. LRTP alone was not effective in preventing lipid oxidation; however, when it was mixed with ascorbic acid and oregano it inhibited only partially lipid oxidation and metmyoglobin formation. Addition of these natural antioxidants contributed to extending the shelf life of beef patties until 16 days.

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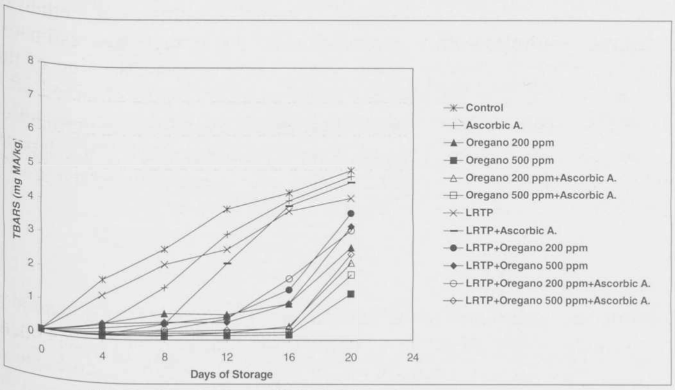


Fig. 1. Evolution of TBARS value.

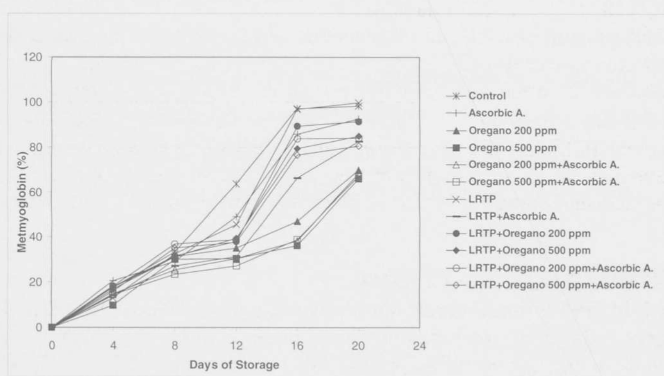


Fig. 2. Evolution of metmyoglobin percentage.

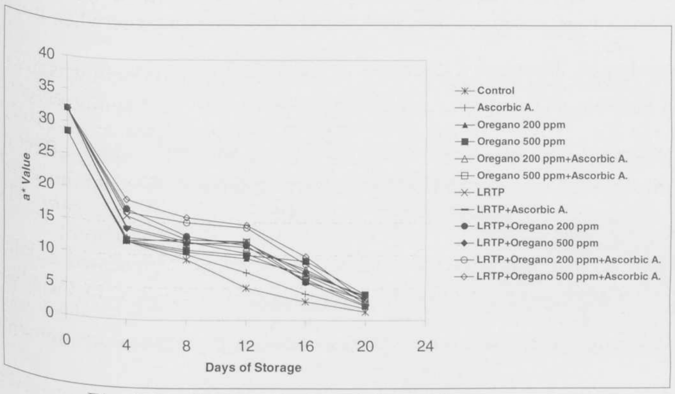


Fig. 3. Evolution of colour (a* value).

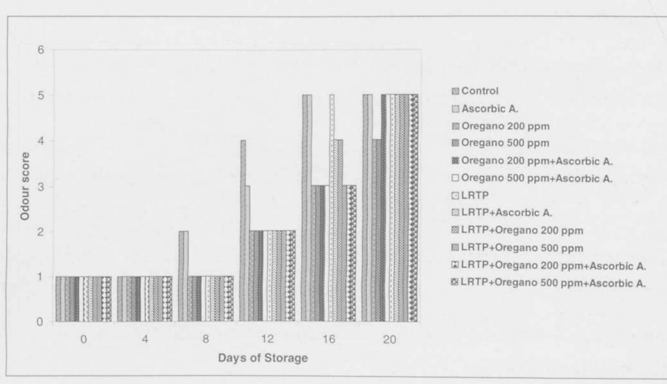


Fig. 4. Evolution of off-odour sensory scores.