APPLICATION OF TWO BACTERIOCINOGENIC LACTIC ACID BACTERIA TO THE PRESERVATION OF THE QUALITY OF BEEF STEAKS PACKAGED IN TWO DIFFERENT MODIFIED ATMOSPHERES; INHIBITORY EFFECT ON *LISTERIA MONOCYTOGENES*.

D. Djenane, A. Sánchez-Escalante, L. Martínez, D. Blanco, J. Yangüela, J.A. Beltrán and P. Roncalés.
Dept. of Animal Production and Food Science. Laboratory of Food Technology. Faculty of Veterinary Science. University of Zaragoza. C/ Miguel Servet, 177, 50013. Zaragoza, Spain.

INTRODUCTION: Modified atmospheres packaging (MAP) is becoming a common approach to extend the shelf life of fresh meat. Lactic acid bacteria (LAB) are also recognized for their benefits; they exert a strong antagonistic activity against many microorganisms, including spoilage and pathogenic bacteria (Aymerich et al., 1998). One of the concerns associated with packaging fresh meat under modified atmospheres is the risk of growth of psychrotrophic pathogens such as *Listeria monocytogenes* (Hugas et al., 1998), particularly following temperature abuse. In recent years, a number of new bacteriocins produced by bactriocinogenic LAB have been purified and characterized (Aymerich et al., 1996; Aymerich et al., 2000). This suggested that bacteriocin-producing LAB might be useful as natural preservatives to enhance meat shelf life and safety by reducing levels of spoilage and pathogenic bacteria.
OBJECTIVES: The aim of this report was to evaluate the effect of two bacteriocinogenic LAB strains (CTC 372 and CTC 711) on the preservation of meat quality characteristics of beef steaks packaged under two different modified atmospheres, as well as their inhibitory effect on *Listeria monocytogenes* in a broth system.

MATERIALS & METHODS

Bacterial strains and media: The strains of LAB used (*Lactobacillus sakei* CTC 372 and CTC 711 strains) were isolated from meat and meat products (Aymerich et al., 2000 and Hugas et al., 1993) and kindly provided by M. Hugas (Centro de Tecnología de la Carn, IRTA, Monells, Spain). *Listeria monocytogenes* (ATCC 15313; serotype 1 a) was used as the indicator strain and grown in tryptic soy broth (TSB) (Biolife, S.r.l. Milano) supplemented with 0.6% yeast extract (TSYE) at 30°C. Stock cultures were stored at –80°C with 20% (vol/vol) sterile glycerol. **Inoculum preparation:** To prepare inocula, the cultures were grown overnight in 10 ml of MRS (CTC 372 and CTC 711) or TSYE broth (*L.monocytogenes*) at 30°C. Before inoculation, the cell suspension was diluted at appropriate concentration. **Preparation and inoculation of meat:** *Longissimus dorsi* muscles were obtained from a beef carcass 24 h post-mortem (pH 5.6-5.7). Steaks (1.5 cm thick) were aseptically cut. The meat surface was inoculated with 2 ml of fresh culture (24 h) of CTC 372 or CTC 711, resulting in an inoculum level of 10^5 to 10^6/cm^2. 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 either 70% O_2 + 20% CO_2 + 10% N_2 (70/20/10) or 60% O_2 + 40% CO_2 (60/40), thermosealed and stored for 25 days at 1±1°C. **Inhibitory effect on *L. monocytogenes*.** A *L. monocytogenes* inoculum of 10^6 cfu/ml was suspended in TSYE broth, prepared with 50 mM sodium phosphate buffer (pH 5.6-5.8) to simulate pH meat conditions. The LAB strains were obtained from meat steaks by swabbing a surface of 10 cm^2. Swabs were stirred thoroughly in 10 ml of 0.1% peptone water. One ml (containing about 10^6 cfu/ml LAB strains) was added to the cell suspension. The broth was incubated up to 10 days at either 3, 8 or 25°C. **Microbial analysis:** Microbial analyses were done by swabbing 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. *L. monocytogenes* was enumerated by direct plating of 1ml of appropriate dilutions onto Palcam-Listeria-selective agar base (Merck. Germany) to which Listeria-selective supplement (Merck, Germany) was added. The plates were incubated at 30°C for 24 to 48 h. At time 0, and every one day, duplicate tubes for each treatment were sampled for microbial counts of *L. monocytogenes* on a selective medium. **Colour instrumental measurements:** CIE L* a* b* parameters were measured at the surface of meat using a Minolta reflectance spectrophotometer. Each value was the mean of 30 determinations. **Sensory evaluation:** 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. **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 (SPSS, 1995). Differences were considered significant at the $p < 0.05$ level.

RESULTS & DISCUSSION

Psychrotrophic flora. Counts of total psychrotrophic flora on meat surface are shown in Figure 1. First to point out is the fact that the presence of 40% of CO_2 (60/40) inhibited aerobic plate counts ($p<0.05$) by more than 1 log cfu/cm^2 compared to 20% CO_2 (70/20/10). With increasing levels of CO_2, the rate of bacterial multiplication decreases, and the length of the lag phase increase (Dixon, and Kell, 1989). In addition, beef steaks inoculated with LAB strains CTC 372 and CTC 711 in both atmospheres showed lower counts ($p<0.05$). These results were in agreement with those reported by Reddy et al. (1975). The biopreservation may be considered as an additional hurdle that could act cooperatively with CO_2 for inhibiting microorganisms growth. Treatment with bacteriocinogenic strains did not significantly alter the pH of meat steaks during storage (data not shown); therefore, population reductions of PCA may be attributed to the inhibitory activity of LAB strains and not to the effect of pH. **CIE a*.** The evolution of colour CIE a* (Fig. 2) had the same behaviour for all samples during the whole time of storage ($p>0.05$). Neither the variation in the CO_2 content of the atmosphere nor the inoculation with LAB strains exerted any effect on the colour of meat. **Sensory odour.** Table 1 shows the results of off odour scores for meat steaks during storage. Again, neither the variation in the CO_2 content of the atmosphere nor the inoculation with LAB strains exerted any effect on the odour of meat. Very little attention has been given to the effects of the bacteriocin-producing LAB on the sensory quality of meat during storage. In contrast with our results, Reddy et al. (1975) found that refrigerated beef steaks inoculated with LAB extended their sensory acceptance. **Effect on *Listeria monocytogenes*.** Figure 3 illustrates the inhibitory activity of meat surface microbial flora, containing a bacteriocinogenic LAB strain (either CTC 711 or CTC 372), on *L. monocytogenes* cultured in broth at different temperatures of incubation (3, 8 and 25°C). Both strains appeared to exert a clear inhibition of *L. monocytogenes* growth at any temperature. *Listeria* was most severely inhibited at 25°C ($p<0.05$) in the presence of both biopreservatives; an approximate 6 log reduction was reached from 5 hours of incubation onwards. Incubation at 3 or 8°C

gave rise to a lesser but significant ($p<0.05$) inhibition of *L. monocytogenes* (about 2-3 log reduction). Schillinger and Lücke (1990) already demonstrated that *Listeria* growth was dramatically inhibited at 8 and 15°C when it was cultured in minced meat and a nutrient solution, respectively, with bacteriocinogenic LAB strains. Regarding bacteriocinogenic LAB strains CTC 711 and CTC 372, Aymerich et al. (2000) showed that they are strong inhibitors of *Listeria monocytogenes* and *Staphylococcus aureus*. Despite 8 and 25°C are not appropriate temperatures for fresh meat storage, their use may be of interest in order to simulate abusive storage conditions. **CONCLUSION**: Our research demonstrated that MAP with increasing CO_2 concentration and bacteriocinogenic LAB strains CTC 372 and CTC 711 significantly reduced ($p<0.05$) aerobic plate counts of beef steaks. However, this inhibition did not result in an extension of the quality characteristics of meat. Additionally, the psychrotrophic pathogen *Listeria monocytogenes* was severely inhibited. Therefore, its use might provide a hurdle to the growth of *Listeria monocytogenes* at both chill and abuse temperatures. **Acknowledgements.** The authors wish to thank the Commisión Interministerial de Ciencia y Tecnología for research funding (grant No ALI 96-0587); Abelló Linde S.A. (Spain), which provided the packaging equipment and gas mixtures; Dr. M. Hugas, who kindly provided the strains of lactic acid bacteria; Dr. F.J. Sala, who kindly provided the strain of *L. monocytogenes*; the A.E.C.I. (fellowships of authors Djenane and Sánchez); the Diputación General de Aragón (fellowship of author L. Martínez); and Ms. A. Martínez for skilful technical assistance.

References

Aymerich M.T., Hugas M., & Monfort J.M. (1998). Food Sci. Technol. Int. 4, 141-158.
Aymerich T., Holo H., Havarstein L.S., Hugas M., Garriga M., & Nes I.F. (1996). Appl. and Environm. Microbiol. 62, 1676-1682.
Aymerich M.T., Garriga M., Monfort J.M., Nes I., & Hugas M. (2000). Food Microbiol. 17, 33-45.
Dixon N.M. & Kell D.B. (1989). J. Appl. Bacteriol. 67, 109-136.
Hugas M., Pagés,F., Garriga M., & Monfort J.M. (1998). Food Microbiol. 15, 639-650.
Hugas M., Garriga M., Aymerich T., & Monfort J.M. (1993). Int. J. Food Microbiol. 18, 107-113.
Reddy S.G., Chen M.L. & Patel P.J. (1975). J. Food Sci. 40, 314-318.
Schillinger U. & Lücke F. (1990). Fleischwirtschaft 70, 1296-1299.

Table 1. Sensory panel scores (mean±SD) for off odour of beef steaks inoculated with LAB.

	Treatments	Days of storage					
		0	5	10	15	20	25
Off odour*	60/40	1±0.0	1±0.0	1.2±0.3^a	2.3±0.6^a	3.2±0.4^a	4.2±0.6^a
	CTC 372+60/40	1±0.0	1±0.0	1±0.0^a	2.2±0.4^a	3.2±0.4^a	4.2±0.6^a
	CTC 711+60/40	1±0.0	1±0.0	1.2±0.3^a	2.4±0.5^a	3.3±0.4^a	4±0.0^a
	70/20/10	1±0.0	1±0.0	1.3±0.4^a	2.5±0.3^a	3.6±0.6^a	4.8±0.6^b
	CTC 372+70/20/10	1±0.0	1±0.0	1±0.0^a	2.3±0.6^a	3.3±0.4^a	4.3±0.4^a
	CTC 711+70/20/10	1±0.0	1±0.0	1±0.0^a	2.4±0.5^a	3.4±0.5^a	4.2±0.6^a

*1 = None, 2 = Slight, 3 = Small, 4 = Moderate, 5 = Extreme
^{a,b}Mean values in the same column are significantly different when accompanied by different superscripts ($p<0.05$)

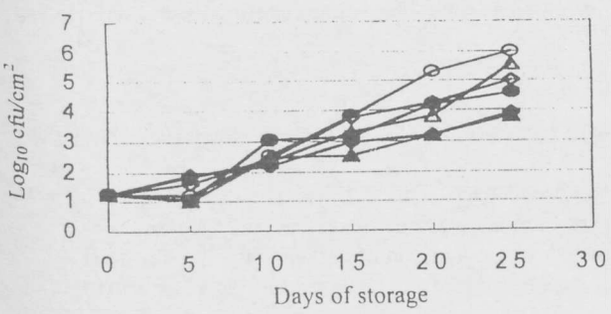

Fig.1. Plate Count Agar numbers of psychrotrophic aerobes in beef steaks inoculated with cultures of bacteriocinogenic LAB (either CTC372 or CTC 711), packaged in different modified atmospheres and stored at 1°C: (●) 60/40; (▲) 60/40+CTC372; (◆) 60/40+CTC711; (○) 70/20/10; (Δ) 70/20/10+CTC372; (◊) 70/20/10+CTC711.

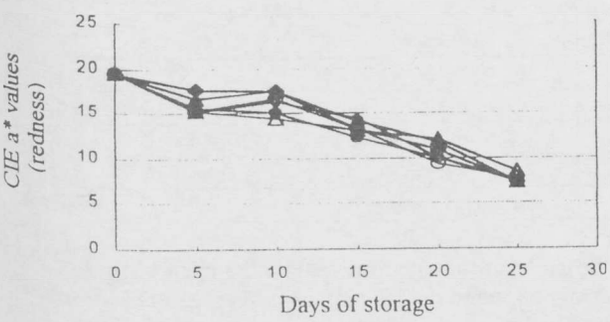

Fig.2. Values of CIE a* in beef steaks inoculated with cultures of bacteriocinogenic LAB (either CTC372 or CTC711), packaged in different modified atmospheres and stored at 1°C: (●) 60/40; (▲) 60/40+CTC372; (◆) 60/40+CTC711; (○) 70/20/10; (Δ) 70/20/10+CTC372; (◊) 70/20/10+CTC711.

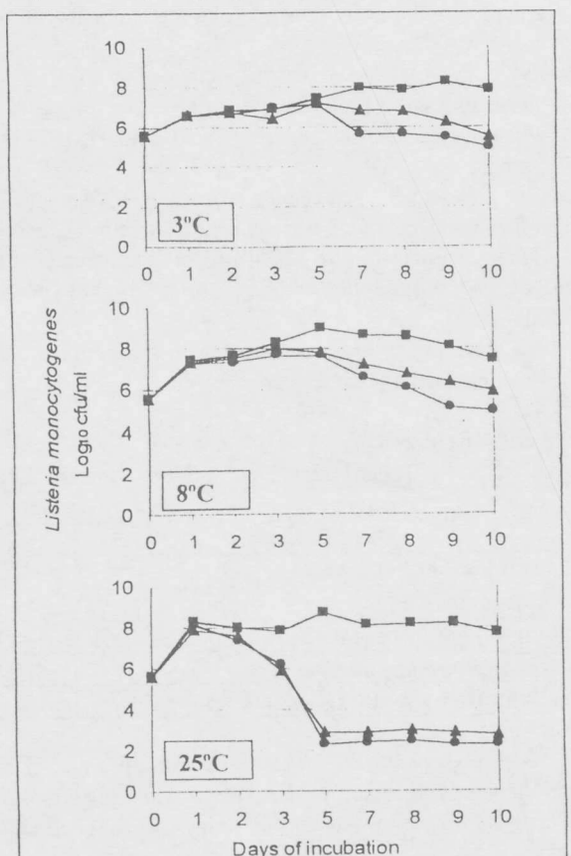

Fig.3. Effect of meat surface microbial flora containing a bacteriocinogenic LAB strains (either CTC 372 or CTC 711), on *L. Monocytogenes* cultured in broth at different temperatures of incubation: (■) *L. Monocytogenes*; (▲) *L. Monocytogenes* + CTC711; (●) *L. Monocytogenes* + CTC372.