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Effect of Lactic Acid Bacteria on Beef Steak Microbial Flora Stored Under Modified Atmosphere and on *Listeria Monocytogenes* in Broth Cultures

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Beef steaks were inoculated with one or other of two protective strains of lactic acid bacteria, the bacteriocinogenic *Lactobacillus sakei* CTC 372 or the uncharacterised *Lactobacillus* CTC 711. They were stored under modified atmospheres (20–40% CO_2). Inoculation of meat with both strains inhibited the growth of the spoilage bacteria. Neither CO_2 in the pack atmosphere, inoculation with protective strains, nor a combination of both, affected formation of metmyoglobin or the development of off-odours. The formation of metmyoglobin in meat pigments and the sensory odour scores were compatible to those of fresh meat which had not undergone either oxidative deterioration or microbial spoilage. *Listeria monocytogenes* were inhibited in broth by meat surface microbiota containing either of the protective strains. With an initial population of 5.6 log cfu/mL, after 7 days incubation at 3 °C, *Listeria monocytogenes* were recovered at log mean population of 2.8 log cfu/mL when neither protective strain was present. At 8 °C, the population of *Listeria monocytogenes* recovered were reduced by about 2.5 or 1.5 log cfu/mL in the presence of *Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711, respectively. At 25 °C, the population of *Listeria monocytogenes* recovered from broth containing either protective strain were about 5 log cfu/mL less than the population recovered from broth containing *Listeria monocytogenes* only.

Key Words: beef meat, modified atmosphere, preservation, lactic acid bacteria, spoilage, *Listeria monocytogenes*

INTRODUCTION

Meat is a very perishable food, both oxidative and microbial processes are involved in meat spoilage. Oxygen (O_2) concentrations of 60–80% are used in modified atmosphere packaging (MAP) to maintain myoglobin (Mb) in its oxygenated form (MbO_2). High O_2 retards metmyoglobin (MetMb) formation at meat surfaces and does not accelerate growth of aerobic organisms (Djenane et al., 2003a; Martínez et al., 2005). The potential for psychrotrophic spoilage microorganisms to grow during the extended refrigerated storage period and decrease organoleptic quality or spoil the meat product is also a concern. With sufficient time at refrigeration temperatures, several types of psychrotrophic bacteria may grow to enough levels to cause meat spoilage (Djenane et al., 2006). These microorganisms include those of primary concern in extended shelf-life refrigerated meats: *Brochothrix thermosphacta*, lactic acid bacteria, and *Pseudomonas* spp. (Marth, 1998). *Brochothrix thermosphacta*, which is aerobic to facultatively anaerobic, has been recovered from packaged beef, pork and lamb. Spoilage may involve development of sliminess and production of off-odours and off-flavours conferred by short chain fatty acids (Jay, 1992).

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Pseudomonas spp. are among the most common aerobic spoilage agents of refrigerated meats. Growth of *Pseudomonas* spp., like other Gram-negative psychrotrophs, is affected by oxygen tension and other factors. During growth, pseudomonas produce proteases and lipases that catalyse reactions causing degradation of protein and fat. The consequence of these reactions is formation of peptides and fatty acids of undesirable flavour and odour. Sometimes these bacteria also produce unsightly green pigments (Marth, 1998).

If pathogens are present they may grow and the meat may become hazardous for consumers. A major concern associated with packaging fresh meat under modified atmospheres (MA) is the risk of growth of psychrotrophic pathogens such as *Listeria monocytogenes* (García de Fernando et al., 1995; Hugas et al., 1998). *Listeria monocytogenes* is a mesophilic foodborne pathogen, which shows psychrotrophic behaviour. It is widely distributed in nature and domestic animals, including sheep, cattle, pigs and fowl, which can carry *L. monocytogenes* without exhibiting signs of disease and whose control in food is difficult by its relatively high tolerance to inhibitory conditions compared to other food borne pathogens (Farber and Peterkin, 1991). Growth of *Listeria monocytogenes* in meat packaged under modified atmospheres has been the focus of many studies, but the effect of CO_2 on the growth of *Listeria monocytogenes* is not clear, while the effect of including O_2 in the atmosphere is also uncertain (Wimpfheimer et al., 1990). Hugas et al. (1998) found that inhibition of *Listeria monocytogenes* in meats cannot be achieved by modified atmospheres alone.

Some strains of lactic acid bacteria (LAB) are antagonistic against many microorganisms, including spoilage and pathogenic bacteria, because of their production of bacteriocins (Aymerich et al., 1998; Jamuna et al., 2005). It has been suggested that bacteriocin-producing lactic acid bacteria might be useful as natural preservatives to enhance meat shelf life and safety by inhibiting spoilage and pathogenic bacteria (Bredholt et al., 2001). The bacteriocinogenic LAB strains examined in this study grew and produced antimicrobial substances at refrigeration temperatures. Thus, *Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711, originally isolated from meat products could be useful for the control of microorganisms in refrigerated meats. Inhibition of spoilage by application to fresh meat of bacteriocinogenic *Lactobacillus sakei* CTC 494 (sakacin K-producing) together with modified atmosphere packaging has been reported (Hugas et al., 1995). Aymerich et al. (2000) characterised *Lactobacillus sakei* CTC 372 as a sakacin T-producing strain; they also demonstrated that it can strongly inhibit *Listeria monocytogenes* and *Staphylococcus aureus*. *Lactobacillus* CTC 711 has not been characterised for bacteriocin production. However, according to Hugas (personal communication) it inhibited not only *Listeria monocytogenes* and *Staphylococcus aureus* but also Gram-negative bacteria, such as *Salmonella* and *Escherichia coli*. The effects of bacteriocin-producing LAB on pathogens in meat may be difficult to evaluate because intrinsic qualities of the product can influence the activities of bacteriocins (Hugas et al., 2002). The inhibitory effects of LAB on *Listeria monocytogenes* might then be more realistically discussed in broth cultures than with meat (Castellano et al., 2004).

The aims of this study were to examine the effects of two protective lactic acid bacteria inoculated onto meat on the preservation of the quality characteristics of beef steaks stored under CO_2-rich atmospheres, as well as to test the effects of the lactic acid bacteria against *Listeria monocytogenes* in broth at various temperatures.

MATERIALS AND METHODS

Material

Meat Samples

The *Longissimus dorsi* (LD) muscle from a single beef carcass was obtained at 48 h *post-mortem* (pH 5.6–5.7), and trimmed of external fat. Forty-five steaks, each 1.5 cm thick and weighting about 150 g, were aseptically cut and divided into halves. The steak portions were exposed to air for about 1 h at 1 °C to allow for blooming.

Bacterial Strains

The strains of lactic acid bacteria used, bacteriocinogenic *Lactobacillus sakei* CTC 372 and the uncharacterised *Lactobacillus* CTC 711, were isolated from meat or meat products (Aymerich et al., 2000) and kindly provided by Dr M. Hugas (Centro de Tecnología de la Carne, IRTA, Monells, Spain). They were grown on De Man, Rogosa and Sharpe (MRS) agar (Merck; Darmstadt, Germany) at 30 °C.

The indicator strain of *Listeria monocytogenes* used in this study was from the Spanish Type Culture Collection (STCC 4031, corresponding to ATCC 15313; Valencia, Spain) and was kindly provided by Prof. Dr Sala (University of Zaragoza, Spain). *Listeria monocytogenes* was grown in Triptic Soy Broth (TSB; Biolife, S.r.l. Milano, Italy) supplemented with 0.6% yeast extract (YE; Biolife) and maintained on slants of Tryptic Soy Agar (TSA, Biolife) supplemented with 0.6% of yeast extract. The inoculum was prepared by transferring one colony of *Listeria monocytogenes* from a plate to a test tube containing 5 mL of sterile supplemented Triptic Soy Broth yeast extract (TSBYE). After inoculation, the tube was incubated at 36 ± 1 °C for 24 h. Erlenmeyer flasks (250 mL) containing 50 mL

of triptic soy broth yeast extract were inoculated with this culture to obtain approximately 10^6 cfu/mL.

To prepare inocula of LAB, an isolated colony of each strain was transferred from De Man, Rogosa and Sharpe agar into a test tube containing 10 mL of sterile De Man, Rogosa and Sharpe broth (Merck; Darmstadt, Germany) and incubated overnight at 30 °C to obtain a culture containing approximately 10^6 cfu/mL. When necessary, the culture was diluted with De Man, Rogosa and Sharpe broth to obtain the required cell population. Stock cultures of lactic acid bacteria strains were maintained as frozen stocks at −80 °C in 20% (v/v) sterile glycerol (Panreac, Barcelona, Spain).

Methods

Inoculation of Meat with LAB Strains

After blooming, the 90 portions of meat were divided into three groups of 30 units. One group was sprayed with a culture of *Lactobacillus sakei* CTC 372 to inoculate the surface of beef steaks with 10^4–10^5 *Lactobacillus sakei*/cm^2. The second group was similarly sprayed with a culture of *Lactobacillus* CTC 711. The final group of steaks was sprayed with sterile 0.1% peptone water. Uniform spraying of the surface of beef steaks was achieved using a spray gun. To ensure that the inoculum was evenly distributed on meat surfaces, steaks were selected at random for determination of population of LAB.

Packaging and Storage

Each meat portion was placed on an expanded polystyrene tray (15.5 × 21.5 × 2.5 cm). Each tray was placed into a polyethylene and polyamide (PE/PA, 80/20 μm thickness) laminate pouch (Sidlaw Packaging-Soplaril, Barcelona, Spain) with a water vapour permeability of 5–7 g/m^2·24 h at 23 °C and oxygen permeability of 40–50 mL/m^2·24 h·atm at 23 °C. For pouches containing steaks subjected to the same treatment, 15 were filled with 1.5 L of 70% O_2/20% CO_2/10% N_2 (Abelló Linde S.A.; Barcelona, Spain), and the other 15 with 1.5 L of 60% O_2/40% CO_2 (Abelló Linde S.A.). The pouches were heat sealed, and stored in the dark at 1 ± 1 °C.

On days 7, 12, 17, 22 and 28 of storage, three packs from each treatment group with each atmosphere were opened. One steak from each set of three was used for microbial sampling, while the other two were used for sensory analysis and for instrumental and chemical analyses.

Inhibitory Effect on Listeria monocytogenes

An overnight culture of *Listeria monocytogenes* was suspended in triptic soy broth yeast extract, prepared with 50 mM sodium phosphate buffer, pH 5.6–5.7, to simulate the normal pH of meat. Cultures of *Lactobacillus sakei* CTC 372 and *Lactobacillus* CTC 711 were obtained from inoculated meat on day 7 of storage by swabbing 10 cm^2 of the meat surface with a sterile cottonwool swab. Swabs were stirred in 10 mL of 0.1% peptone water. One millilitre of each suspension was added to each of four test tubes containing 10 mL of the suspension of *Listeria monocytogenes*. The broth containing LAB and *Listeria monocytogenes* was incubated for up to 10 days at 3, 8 or 25 °C. To prepare the control samples, uninoculated beef steaks were swabbed on day 7 of storage and cultures containing lactic acid bacteria and *Listeria monocytogenes* were prepared and incubated as before.

pH Measurements

The meat pH was measured after homogenisation of 5 g of meat in distilled water, using a micro pH 2001 meter (CRISON mod.) with an INGOLD type U 402 electrode. Three readings were obtained for each steak portion.

Microbiological Analysis

Two sterile cottonwool swabs moistened with 0.1% peptone water were used to swab 10 cm^2 of the meat surface delimited by a sterile, stainless steel template. Swabs were stirred 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. Three plates were prepared from each dilution by pouring 1 mL into the fluid agar appropriate for each microbial species. LAB were enumerated in plates of MRS agar, which were incubated anaerobically at 30 °C for 48–72 h. *Brochothrix thermosphacta* were enumerated in plates of streptomycin thallous acetate actidione (STAA) agar (Biolife s.r.l.; Milano, Italy), which were incubated aerobically at 25 °C for 72 h. *Pseudomonas* spp. were enumerated in plates of cephaloridine fucidin cetrimide (CFC) agar (Oxoid; Basingstoke, England) which were incubated at 25 °C for 48–72 h (International Commission of Microbiological Specifications: ICMSF, 1983). *Listeria monocytogenes* were enumerated in plates of PALCAM agar (Merck; Darmstadt, Germany). The plates were incubated aerobically at 36 ± 1 °C for 24 to 48 h. The logs of mean values for the counts from plates were recorded.

Colour Determination

Meat surface colour was measured using a reflectance spectrophotometer (CM-2002, Minolta, Osaka, Japan) equipped with a D65 standard illuminant, at 20 °C, 30 min after package opening, to allow for colour stabilisation after exposure to air. The

Commision International de l'Eclairage (CIE) L*, a*, b* values (CIE 1978) were recorded. Hue-angle (h) and Chroma (C*) were calculated using the formulae: $h = \tan^{-1}(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{\frac{1}{2}}$, respectively.

Metmyoglobin Analysis

The metmyoglobin percentage of the total myoglobin perceptible at the steak surface was estimated spectrophotometrically by the method of Stewart et al. (1965), with measurement of 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 at 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 $[K/S = (1 - R_\infty)^2/2R_\infty]$. The value of 100% MetMb was obtained following the same procedure after oxidising a sample of meat in a 1% (w/v) solution of potassium ferricyanide (Ledward, 1970). The average value for each steak was the mean of 20–25 determinations.

Lipid Oxidation

Lipid oxidation was assessed in triplicate by the 2-thiobarbituric acid (TBA) method of Pfalzgraf et al. (1995). Thiobarbituric acid reactive substance (TBARS) values were calculated from a standard curve of malonaldehyde (MA) and expressed as mg malonaldehyde/kg meat.

Sensory Evaluation

Meat samples were evaluated for off-odour by a six-member panel, trained according to the method of Cross et al. (1978). For rating odour, meat samples presenting different off-odour characteristics within the range of the evaluation scale were used. Samples used for rating included packaged beef steaks that were either fresh or stored at 4 °C for times up to 3 weeks.

In all assessments, beef steaks were evaluated 20 min after pack opening. Two samples for each treatment and time were taken as needed from a cold room, identified with three-digit random population and placed in polystyrene trays of 15.5 cm × 21.5 cm. Each panellist received two half steak portions from each treatment, randomly numbered and served. The samples for evaluation were presented at room temperature of about 25 °C.

The attribute off-odour was rated using a 5-point descriptive scale: 1 = no off-odour, 2 = slight off-odour, 3 = little off-odour, 4 = moderate off-odour, and 5 = strong off-odour (Djenane et al., 2001).

Analysis of Data

The significance of differences among treatments after each day of storage was determined by analysis of variance using the least square difference (LSD) method of the General Linear Model procedure of Statistical Package for Social Sciences (SPSS) program for Windows, version 6.1.2 (SPSS 1995). All other calculations were performed using Microsoft Excel, version 5, statistical functions (Microsoft Corp., Redmond, WA, USA). Differences were considered significant at the $p < 0.05$ level.

RESULTS

Few presumptive *Pseudomonas* spp. were recovered from steaks after 12 days of storage (Figure 1). The population of *Pseudomonas* spp. recovered from uninoculated steaks packaged in the 40% CO_2 atmosphere were 0.8 log units less than the population recovered from uninoculated steaks packaged in 20% CO_2 atmosphere after 17 days of storage. However, after 22 days of storage, the population recovered from uninoculated steaks packaged under either atmosphere were not significantly different ($p > 0.05$).

Initial population of lactic acid bacteria were less than 10 cfu/cm^2 on uninoculated steaks (result not shown). The inoculated steaks all carried lactic acid bacteria at 4 to 5 log cfu/cm^2. After inoculation with *Lactobacillus sakei* CTC 372, the population of *Pseudomonas* spp. recovered from steaks were about one log unit less than the population recovered from uninoculated steaks after 28 days of storage under both

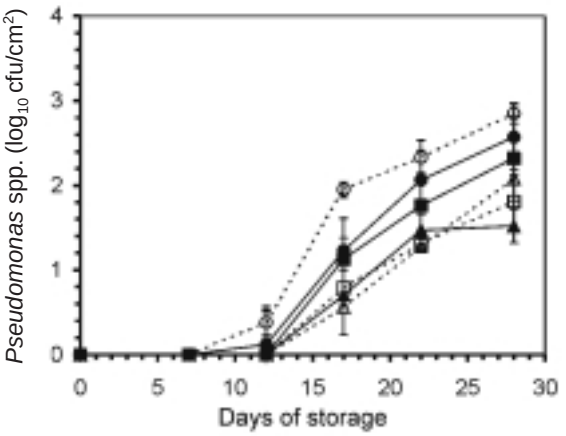

Figure 1. Population of *Pseudomonas* spp. recovered from beef steaks stored at 1 ± 1 °C under atmospheres of 70% O_2/20% CO_2/10% N_2 (○△□) or 60% O_2/40% CO_2 (●▲■) without being inoculated (○●) or after being inoculated with *Lactobacillus sakei* CTC 372 (△▲) or *Lactobacillus* CTC 711 (□■).

CO_2 atmospheres. However, the population recovered from steaks inoculated with *Lactobacillus* CTC 711 were about one log unit less than the population recovered from uninoculated steaks throughout storage, when steaks were packaged under 20% CO_2. Presumptive *Brochothrix thermosphacta* reached maximum values of about 5 log cfu/cm^2 at the end of storage (Figure 2). The log population recovered from uninoculated steaks were similar during 22 days of storage; but the population recovered from steaks packaged under 40% CO_2 were about one log unit less than the population recovered from steaks packaged under 20% CO_2 after 28 days of storage. The population of *Brochothrix thermosphacta* recovered from steaks inoculated with *Lactobacillus sakei* CTC 372 were about 2 log units less than the population recovered from uninoculated steaks after 28 days of storage under either CO_2 atmosphere. The population recovered from steaks inoculated with *Lactobacillus* CTC 711 were about one log unit less than the population recovered from uninoculated steaks throughout storage when steaks were packaged under 20% CO_2.

Neither high CO_2 concentrations nor inoculation with lactic acid bacteria protective strains appeared to affect metmyoglobin formation (Figure 3). The results of the Commision International de l'Eclairage indices of redness (CIE a*, hue and Chroma; data not shown) agreed with those for metmyoglobin formation. The pH values of steaks did not differ significantly ($p > 0.05$) during storage (data not shown).

Neither variation in CO_2 concentration, nor inoculation with the protective LAB strains affected the odour of the meat (Table 1).

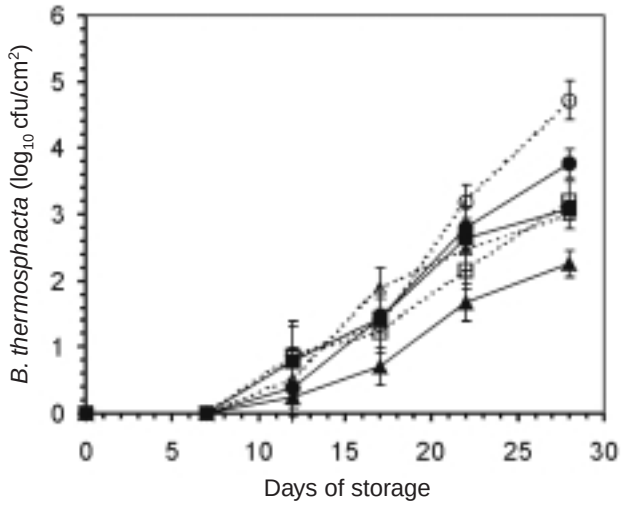

Figure 2. Population of *Brochothrix thermosphacta* recovered from beef steaks stored at 1 ± 1 °C under atmospheres of 70% O_2/20% CO_2/10% N_2 (○△□) or 60% O_2/40% CO_2 (●▲■) without being inoculated (○●) or after being inoculated with *Lactobacillus sakei* CTC 372 (△▲) or *Lactobacillus* CTC 711 (□■).

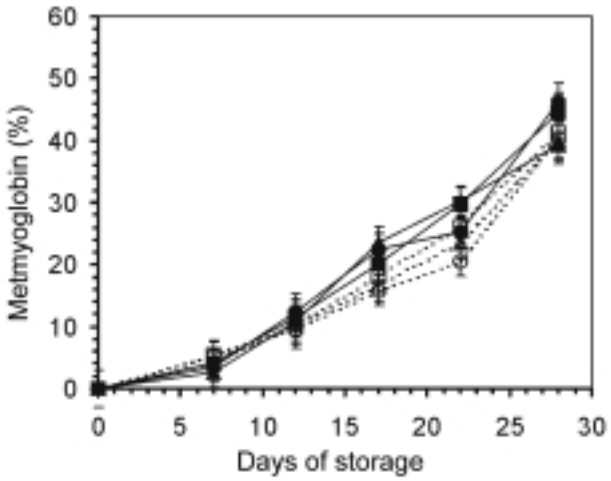

Figure 3. Metmyoglobin percentage on the surface of beef steaks stored at 1 ± 1 °C under atmospheres of 70% O_2/20% CO_2/10% N_2 (○△□) or 60% O_2/40% CO_2 (●▲■) without being inoculated (○●) or after being inoculated with *Lactobacillus sakei* CTC 372 (△▲) or *Lactobacillus* CTC 711 (□■).

Table 1. Sensory panel scores for off-odours of beef steaks packaged under atmospheres containing 20 or 40% CO_2 without or after being inoculated with lactic acid bacteria, after storage at 1 ± 1 °C at several times.

Treatments		Sensory scores* (mean ± SD)					
		Days of storage					
CO_2 (%)	Inoculum	0	7	12	17	22	28
20	None	1 ± 0.0	1 ± 0.0	1.3 ± 0.4^x	2.5 ± 0.3^x	3.6 ± 0.2^x	4.8 ± 0.3^y
20	*Lactobacillus sakei* CTC 372	1 ± 0.0	1 ± 0.0	1.0 ± 0.0^x	2.3 ± 0.6^x	3.3 ± 0.4^x	4.3 ± 0.2^x
20	*Lactobacillus* CTC 711	1 ± 0.0	1 ± 0.0	1.0 ± 0.0^x	2.4 ± 0.5^x	3.4 ± 0.5^x	4.2 ± 0.3^x
40	None	1 ± 0.0	1 ± 0.0	1.2 ± 0.3	2.3 ± 0.6^x	3.2 ± 0.4^x	4.2 ± 0.4^x
40	*Lactobacillus sakei* CTC 372	1 ± 0.0	1 ± 0.0	1.0 ± 0.0^x	2.2 ± 0.4^x	3.2 ± 0.4^x	4.2 ± 0.3^x
40	*Lactobacillus* CTC 711	1 ± 0.0	1 ± 0.0	1.2 ± 0.3^x	2.4 ± 0.5^x	3.3 ± 0.4^x	4.2 ± 0.2^x

*1 = No off-odour, 2 = Slight off-odour, 3 = Little off-odour, 4 = Moderate off-odour, 5 = Strong off-odour.
^{xy}Mean values in the same column are significantly different when accompanied by different superscripts ($p < 0.05$).

After 5 or 7 days incubation at 3 or 8 °C, respectively, and at latter times, *Listeria monocytogenes* recovered from broths containing *Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711 were substantially less than the population recovered from broth containing *Listeria monocytogenes* only (Figure 4). The presence of either protective strains resulted in a severe inactivation of *Listeria monocytogenes* after 5 days at 25 °C. The pH values of broths did not differ significantly ($p > 0.05$) during incubation (data not shown).

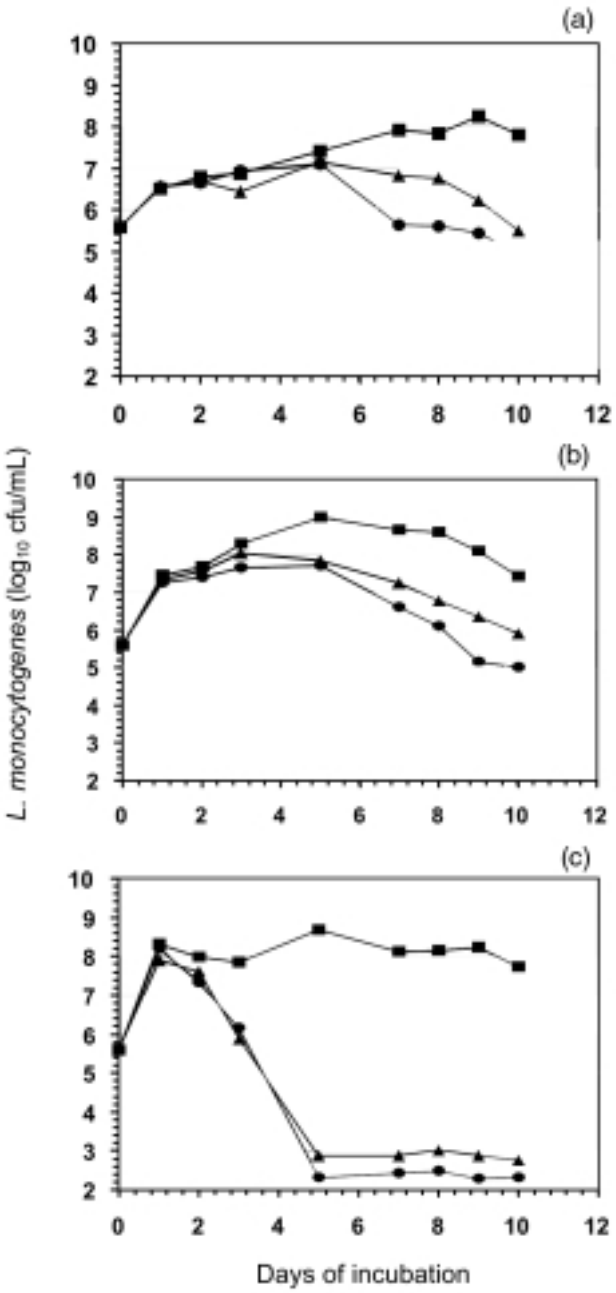

Figure 4. Effect of meat surface microbial flora without (■) or with *Lactobacillus sakei* CTC 372 (●) or *Lactobacillus* CTC 711 (▲) on the population of *Listeria monocytogenes* in broths incubated at (a) 3 °C, (b) 8 °C or (c) 25 °C.

DISCUSSION

Due to consumer demands and convenience at retail stores, there is great interest in developing modified atmosphere packaging of consumer-ready cuts of meat. Spoilage bacteria tolerate high concentrations of O_2 and their growth rate can be reduced by including CO_2 in the gas mixture. As to the microbiological effects of CO_2 atmospheres, these findings indicated that population of bacteria recovered from steaks packaged under 40% CO_2 atmosphere were less than those packaged under 20% CO_2. Similar results were observed in packed steaks for 28 days of storage at 1 °C (Djenane et al., 2003b). An increase of shelf-life of meat packaged in high CO_2 atmospheres was expected and previously reported (Asensio et al., 1988), as was the synergistic effect between CO_2 and low temperature (Gill, 1988).

Studies of bacteriocin-producing strains have mainly focused on the inhibition of food pathogens, but the effect of these bacteria on specific spoilage organisms is unknown. The inoculation of steaks with *Lactobacillus sakei* CTC 372 reduced the population of *Pseudomonas* spp. and *Brochothrix thermosphacta* on steaks packed under every CO_2 atmosphere. After 28 days of storage, *Lactobacillus sakei* CTC 372 delayed microbial growth by about 10 days for steaks packed under either CO_2 atmosphere, as compared with uninoculated steaks. However, the population of bacteria were apparently reduced by inoculation of steaks with *Lactobacillus* CTC 711 only when steaks were packaged under 20% CO_2, with bacterial growth being delayed approximately 9 days in this case. Further studies are required to explain the ineffectivity of *Lactobacillus* CTC 711 to inhibit spoilage bacteria in steaks packed under 40% CO_2. Inhibition of spoilage bacteria on inoculated steaks can be attributed to the production of bacteriocins by either lactic acid bacteria strains. The combination of meat packaging under modified atmosphere and inoculation with bacteriocins has been shown to reduce the population of *Brochothrix thermosphacta*, which reached undetectable levels after 25 days of storage (Tu and Mustapha, 2002).

In this study, *Pseudomonas* spp. seemed to be the group most vulnerable to either protective strains of lactic acid bacteria, and to the lower as well as the higher concentration of CO_2 consequently, *Pseudomonas* spp were always minor fractions of the flora, while *Brochothrix thermosphacta* was predominant. If it is accepted that a number of 7 log cfu/cm^2 is the approximate point at which spoilage becomes apparent (Hunt et al., 2004), the population of the spoilage flora recovered from uninoculated and inoculated steaks were always less than the population required for spoilage.

With respect to meat quality characteristics, the

meat would be acceptable for about 22 days of storage. The CIE a* values, metmyoglobin percentage and sensory odour scores during this time of storage might be considered similar to those of fresh meat. It has been suggested that ≤40% metmyoglobin on the meat surface might be considered as satisfactory (Greene et al., 1971). According to this, all the steaks should be considered satisfactory during the 22 days of storage. With respect to off-odours, Greene and Cumuze (1981) found that a TBA reactive substances value of at least 2.0 mg malonaldehyde/kg is required for perception of rancid odours. Results not shown of the present study showed that TBA reactive substances values were <2 mg malonaldehyde/kg in steaks inoculated by either protective strain; therefore, they would not present perceptible off-odours during 3 weeks of storage, what is in accordance with sensory results.

Steaks inoculated with either protective strains had lower populations of total psychrotrophic aerobes throughout storage (results not shown). However, the reduction in population of total psychrotrophic aerobes did not result in any extension of the shelf life of steaks. Those findings suggest that changes in the colour and odour of steaks packed in modified atmospheres were due to oxidative processes rather than to the growth of spoilage microorganisms.

The ubiquity of *Listeria monocytogenes* is well known. In food and in the environment, as *in vivo* infection, *Listeria monocytogenes* are exposed to many stress signals that can alter its virulence. According to Kathariou (2002), stresses caused by heating, freezing, dehydration, refrigeration, acids and salts, as well the exposure to disinfectants and other antimicrobial substances are of special relevance for the physiological status and virulence of this pathogen in foods. *Listeria monocytogenes* was detected in many types of processed meat products: cooked meats, raw cured meats, mayonnaise-salads and prepared meals (Uyttendaele et al., 1999).

Several studies have been conducted using lactic acid bacteria strains to inhibit *Listeria monocytogenes* in dairy, fish and meat products (Hugas et al., 1998; Ammor et al., 2006). Unfortunately, individual foods may also be inhibitory towards bacteriocins and thus reduce or eliminate their efficiency (Ganzle et al., 1999). It has been shown that the use of lactic acid bacteria protective strains was less efficient in foods compared to broths (Budde et al., 2003). Thus, other studies have examined their effect only in broth cultures and extrapolated results to a wider range of uses. This may be in part explained by a more thorough contact between *Listeria monocytogenes* and bacteriocin in broth than in meat, which seems to be crucial for the efficacy of the protective culture. An even distribution of lactic acid bacteria on the surface of the product was also found to be essential for the antilisterial activity. Tsigarida et al. (2000) found that volatile compounds of oregano essential oil are capable of affecting growth of microbial association of meat stored at modified atmospheres; however, such inhibition is not as strong as that due to the contact of pure essential oil with microorganisms when this is added directly on the surface of meat.

In the present study, the authors used a high inoculum in broth of *Listeria monocytogenes* in order to assure that the presence of a high population of other bacteria would not give rise to inhibition because of microbial competition. The effect of the inoculated lactic acid bacteria on *Listeria monocytogenes* was by far higher at 25 °C than at the lower temperatures. *Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711 inhibited *Listeria monocytogenes* in broth by 70.24 and 64.30%, respectively after 10 days of incubation. Inactivation of *Listeria monocytogenes* at 25 °C can be attributed to the rapid growth and early production of bacteriocins by the bacteriocinogenic strains of lactic acid bacteria at this temperature. But inhibition of *Listeria monocytogenes* was substantial at refrigeration temperatures also. At 8 °C, inhibitions by *Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711 were 32.70 and 20.72%, respectively, after 10 days incubation. But at 3 °C, *Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711 inhibited *Listeria monocytogenes* by 36.5 or 29.5%, respectively. Mataragas et al. (2003) found that the bacteriocins were able to inhibit strains of different *Listeria* species. Also, the bacteriocins or the LAB as protective strains were able to inhibit *Listeria* spp. in contaminated meat products, with complete inhibition of the pathogen after two weeks of storage. The enhancement of the antilisterial efficacy of bacteriocinogenic protective culture had already been shown in broth in previous reports (Schillinger et al., 1998). Inhibition of pathogenic microorganisms by lactic acid bacteria may be due to the effect of one or synergism between several mechanisms, such as lowering of pH, competition for nutrients, production of organic acid, hydrogen peroxide, gas composition of atmosphere or antimicrobial substances such as bacteriocins (Drosinos and Board, 1994).

In the samples inoculated with the lactic acid bacteria, the population of *Listeria monocytogenes* decreased, probably due to the production of bacteriocin. *Listeria monocytogenes* population, in the presence of the lactic acid bacteria strains, firstly increased and then decreased. This may be explained by the fact that the bacteriocins are produced after 24–48 h and they reach the maximum activity after 7 days of incubation at all temperatures.

Experiments were conducted at higher temperatures in order to simulate possible temperature abuse throughout the meat commercial chain. Inhibition of *Listeria monocytogenes* by lactic acid bacteria at low temperatures has been reported in broth or on meat or salmon (Katla et al., 2001; Castellano et al., 2004). That

contrast with reports of the higher production of bacteriocin by LAB at low temperature than at temperatures optimal for growth (Hugas et al., 1998). In accordance with the findings of this study, Luchansky et al. (1992) reported that *Listeria monocytogenes* was inhibited at 25°C but not at 4°C on meat inoculated with *Listeria monocytogenes* and lactic acid bacteria. Inoculation with protective strains did not significantly alter the pH of either steaks or broths (results not shown); so pH was not a factor in the inhibition of bacteria growth.

CONCLUSION

This study demonstrated that 40% CO_2 atmosphere exerted a significant inhibitory effect on the growth of spoilage bacteria on steaks. Lactic acid bacteria protective strains *Lactobacillus sakei* CTC 372 or *Lactobacillus* CTC 711 might be useful as natural preservatives for further controlling spoilage bacteria, as well as providing a hurdle to the growth of *Listeria monocytogenes* at either refrigeration or abusive temperatures. Neither the increase of CO_2 in the packaging atmosphere, inoculation with lactic acid bacteria protective strains, nor a combination of both, resulted in any modification of meat quality characteristics.

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