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ORIGINAL ARTICLE

Application of the bacteriocinogenic *Lactobacillus sakei* CTC494 to prevent growth of Listeria in fresh and cooked meat products packed with different atmospheres

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The production of sakacin K by Lactobacillus sakei *CTC494 at different temperatures and pH was evaluated before its application as a bioprotective culture against* Listeria *in different meat-based food packaging systems, that is, oxygen-permeable film, under vacuum and under a modified atmosphere (20% CO_2: 80% O_2) and stored at 7°C. The* Lactobacillus *culture produced the bacteriocin at a range of temperatures from 4°C to 30°C and at initial pH from 5·5 to 6·5.* Listeria *inhibition in raw minced pork, poultry breasts and modelized cooked pork could not be achieved by the sole application of vacuum or a modified atmosphere. Inoculation of* Lb. sakei *CTC494 or sakacin K inhibited the growth of* Listeria *to different extents in all the products studied in each system; the greatest inhibition being observed in the vacuum packaged samples of poultry breasts and cooked pork, and in the modified atmosphere packaged samples of raw minced pork. Addition of sakacin K resulted in immediate bactericidal action against* Listeria *in every product and atmosphere studied.*

Introduction

In the last few years, there has been an increased interest in the use of modified-atmosphere packaging (MAP), to prolong the shelf-life of fresh refrigerated foods and cooked products. The microbiological safety of MAP products is dependent on the gas atmosphere and storage temperature. Carbon dioxide is capable of inhibiting the growth of aerobic spoilage micro-organisms and moulds, although lactic acid bacteria are still able to grow (Brody 1989). One of the risks associated with packaging meat under modified atmospheres is the differential survival and/or the stimulation of the growth of pathogens such as *Yersinia enterocolitica*, *Aeromonas hydrophyla*, *Campylobacter jejuni*, *Clostridium botulinum* and *Listeria monocytogenes*. *L. monocytogenes*, the causative agent of food-related listeriosis, has been isolated from different types of meat and meat products (Johnson et al. 1990). This organism is potentially pathogenic to the

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foetus, the newborn, elderly people, pregnant women and immunocompromised patients.

Control of *L. monocytogenes* in foods is difficult due to its ability to grow at refrigeration temperatures, and its tolerance to low pH and certain levels of sodium chloride and sodium nitrite, which are inhibitory to other food pathogens (Buchanan et al. 1989, Duffy et al. 1994, Farber et al. 1996, Thomas et al. 1996). Razavilar et al. (1992) and Farber et al. (1996) have found that the inhibitory effect of CO_2 on the growth of *Listeria* spp. was enhanced with decreasing storage temperature. Growth of *L. monocytogenes* in packaged meat under modified atmospheres has been the focus of numerous, although in some cases controversial, studies. The effect of CO_2 on the growth of *Listeria* is not clear. Hart et al. (1991) observed that *Listeria* does not grow on chicken breasts at 1°C and 6°C, held under 100% CO_2, although growth was detected in roast beef in this atmosphere at 3°C (Hudson et al. 1994). The effect of including O_2 in CO_2 atmospheres is also controversial (Wimpfhimer et al. 1990). Mano et al. (1995) have not detected the growth of *Listeria* spp. in pork at either 1°C or 7°C, or in turkey at 1°C packaged in CO_2–O_2 atmospheres (ratios of both 20:80) and 40:60), whereas growth was observed in turkey under these atmospheres at 7°C.

Biopreservation systems, such as bacteriocinogenic lactic acid bacteria cultures and/or their bacteriocins have received increasing attention and new approaches to control pathogenic and spoilage micro-organisms have been developed. Recent studies have demonstrated antagonism by bacteriocins towards *Listeria* species in meats and meat-related products, for example, raw meat (Nielsen et al. 1990, Schillinger et al. 1991. Skyttä et al. 1991, Mattila-Sandholm et al. 1991, Winkowski et al. 1993, Vignolo et al. 1996), German-style fresh sausages 'Mettwurst' (Schillinger et al. 1991), American-style fermented sausages and dry fermented sausages (Berry et al. 1990, Foegeding et al. 1992, Hugas et al. 1995, Hugas et al 1996), Italian salami (Campanini et al. 1993), frankfurter sausages (Berry et al. 1991), wiener sausages (Yousef et al. 1991, Degnan et al. 1992) and turkey summer sausages (Luchansky et al. 1992).

The inhibition of some food-emergent pathogens in food systems could be achieved through the application of technology (MAP and hydrostatic pressure) and bacteriocinogenic cultures of LAB (Fang et al. 1994a, 1994b, Kalchayanand et al. 1994).

In the current study *Lb. sakei* CTC494 (isolated from dry fermented sausages) which is active against *L. monocytogenes*, and sakacin K (a bacteriocin produced by the former strain), were investigated as bioprotection systems against listeria in minced raw pork, poultry breasts and cooked pork packaged under different atmospheres.

Materials and Methods

Bacterial strains and media

Bacteriocin-producing (Bac +) *Lb. sakei* CTC494 (Hugas et al. 1995) and bacteriocin negative (Bac −) *Lb. curvatus* CTC371 (used as a standard starter strain) isolated from dry fermented sausages and characterized by Hugas et al. (1993) were grown in MRS broth (Difco, Detroit, Michigan, USA) at 30°C. Both strains produced the same amount of lactic acid either *in vitro* or *in situ* (Hugas et al. 1995). *L. innocua* CTC1014 was used as the indicator strain; it was isolated from raw meat and identified using the API test for listeria (API, Baume-les-Grottes, France), and haemolysis, and grown in tryptic soy broth (TSB) (Oxoid Ltd., Basingstoke, UK) supplemented with 0·6% yeast extract (TSYE) at 30°C. Stock cultures were stored at −80°C with 20% glycerol. The solid medium was prepared by adding 1·5% agar (Difco) to the broth. The overlays were prepared with 0·75% agar.

Preparation of bacteriocin

Lb. sakei CTC494 was grown for 18 h at 30°C in MRS broth. The cells were removed by centrifugation at 5000 *g* for 10 min at 4°C. The supernatant fluid was collected and

the bacteriocin sakacin K was precipitated by adding 40% ammonium sulphate. After 30 min incubation at 0°C, the precipitated proteins were collected by centrifugation at 11 000 *g* at 4°C for 30 min. The precipitates were dissolved in 3 mmol l^{-1} sodium phosphate buffer pH 7·0 and heated at 80°C for 10 min. The concentrated bacteriocin was stored at 4°C. A modification of the critical dilution assay (Tagg et al. 1976) was used to determine the bacteriocin titre (Hugas et al. 1995).

Effect of temperature on bacteriocin production

In order to study the effect of the incubation temperature on growth and bacteriocin production, 50 ml of MRS broth were inoculated with 500 µl of an overnight culture of *Lb. sakei* CTC494, and incubated at 4°C, 10°C, 15°C and 20°C for 9 days, at 25°C for 6 days and at 30°C for 2 days. At selected sampling times, pH, number of colony forming units (cfu ml^{-1}), cell density (OD_{600}) in an Spectronic 20D (Milton Roy Co., UK) and sakacin K production (in AU ml^{-1}), as previously reported, were studied. All experiments were repeated three times.

Influence of pH on bacteriocin production

Fermentations were carried out using MRS medium supplemented by 0·5% tween and 2% yeast extract placed in a fermenter (Bioflo llc, New Brunswick Scientific Co., Inc.) with automatic pH control (model 465-35-90 k9 electrode; Ingold). The temperature was maintained at 30°C and the culture was stirred at 25 rpm.

To determine the influence of initial pH of the media, the MRS broth was initially adjusted to the desired pH with HCl or NaOH, and during the fermentation the pH was kept constant by automatically adding NaOH. The pH values studied were 5·0, 5·5, 6·0 and 6·5. After fermentation (24 h), cfu ml^{-1}, cell density (OD_{600}) and bacteriocin production (AU ml^{-1}) were measured. All experiements were repeated three times.

Inoculum preparation

To prepare inocula, the cultures were grown overnight in 5 ml of MRS (lactobacilli) or TSYE broth (listeria) at 30°C. Cells were harvested by centrifugation (5000 *g* for 10 min), resuspended in glycerol and MRS broth (at ratio of 1:1) or TSYE broth, and stored at −40°C prior to use. *L. innocua* was used for *in situ* inoculation experiments in order to avoid dissemination of *L. monocytogenes* in our pilot plant. Before inoculation, the cell suspension was diluted and viable counts were obtained on MRS agar or tryptic soy agar. When necessary, the cells were diluted to the appropriate concentration in 0·85% NaCl.

Meat inoculation

Fresh lean shoulder pork was ground through a 6 mm plate and mixed with the inocula. Four different samples (lots) of minced pork were prepared as shown in Table 1. Skinless and deboned chicken breasts were selected after slaughtering and four different samples (lots) were prepared as shown in Table 1. The bacterial cultures and the bacteriocin were spread over the surface of the chicken breasts with a glass spreader.

Table 1. Application scheme of inoccula in raw minced pork, poultry breasts and modelized cooked ham. In poultry breasts inoccula was cfu cm^{-2}

	Bacterial inoccula applied to different lots			
Lot	*L. innocua* CTC1014 (cfu g^{-1})	*L. curvatus* CTC371 (Bac −) (cfu g^{-1})	*L. sakei* CTC494 (Bac +) (cfu g^{-1})	Sakacin K (AU g^{-1})
A	2×10^2	—	—	—
B	2×10^2	1×10^6	—	—
C	2×10^2	—	1×10^6	—
D	2×10^2	—	—	400

Manufacture and inoculation of sliced cooked pork

Fresh shoulder pork was coarsely ground and brined for 48 h (150 g brine kg^{-1} of meat) at 3–5°C. The brine solution contained (in g kg^{-1}): NaCl, 60; $NaNO_2$, 0·45; sodium ascorbate, 0·15; dextrose, 15; lactose, 15; sodium pyrophosphate, 9; sodium glutamate, 1 and water 60·4. The mixture was then inserted into 7 cm diameter plastic casings. The pork was cooked until its internal temperature reached 67°C and after cooling it was cut into slices of 10 g each. Four different lots (Table 1) were prepared and the bacterial cultures spread over the surface of each slice.

Three different packaging atmospheres were used for storage; oxygen-permeable film, vacuum and modified atmosphere (80% O_2: 20% CO_2). For MAP, Cryovac barrier bags (O_2 transmission rate 2–5 ml m^{-2}, 24 h atm^{-1} at 4·4°C and 0% RH) were sealed with a heat seal packaging machine. A proportional gas mixer was used to give the desired proportion of CO_2 and O_2. All samples were stored at 7°C for up to 8 days.

Microbiological sampling

Triplicates from each treatment were sampled at selected times to determine *Listeria* and *Lactobacilli* populations. For the microbiological determinations, 10 g of each sample (raw minced and cooked pork) were mixed (1:10) with dilution medium (0·1% peptone; 0·85% NaCl or *Listeria* enrichment broth base (UVM, Oxoid)) and placed in a Stomacher bag (model 400; Cooke Laboratory Products, Alexandria, Virginia, USA) for 1 min. For the chicken breasts, an area of 39·3 cm^2 was sampled by the swab method.

Lactobacilli populations were enumerated, after serial 10 fold dilutions were plated on MRS agar and incubated anaerobically at 30°C for 48 h. *Lb. curvatus* CTC371 (Bac −) was identified from MRS plates by its plasmid profile (Garriga et al, 1996) and *Lb. sakei* CTC494 by both the plasmid profile and the inhibition of indicator strains by direct antagonism (Barefoot and Klaenhammer, 1982). The dominance of a given inoculated strain was ascertained by testing a certain number of colonies ($\alpha = 0{\cdot}05\%$ and $\beta = 0{\cdot}05\%$) according to a progressive colony sampling plan based on the accumulated binomial distribution (Malegeant, 1991). *L. innocua* CTC1014 was enumerated by the most probable number (MPN) technique in *Listeria* enrichment broth base (UVM, Oxoid) at 37°C: 10µl from turbid tubes were streaked in Palcam agar and incubated at 30°C for 48 h, when counts were lower than 10^2 cfu g^{-1}, or by the spread plate technique in Palcam agar (Merck) at 30°C for 48 h. In both cases, colonies with typical morphology were confirmed by testing its capability to be inhibited by sakacin K in a microtitre plate.

Results

Effect of temperature and pH on production of sakacin K in MRS

Lb. sakei CTC494 produced sakacin K at every temperature studied (4°C, 10°C, 15°C, 20°C, 25°C and 30°C). At 4°C, 10°C and 15°C, the maximum production was 6400 AU ml^{-1}. and at 20°C, 25°C and 30°C it was 3200 AU ml^{-1}. At all temperatures tested, the maximum production was found at the late exponential phase, when the bacterial population was around 10^9 cfu ml^{-1}. At 4°C the maximum production occurred after 7 days of incubation, at 10°C after 3 days, while at 15°C, 20°C and 25°C it was detected after 9 h of incubation. At every temperature a reduction of the antimicrobial activity was detected during the stationary phase. The rate of cell growth increased with temperature, reaching the maximum at 30°C. The same trend was observed with the optical density; the maximum OD at 4°C was 2·3 and at 30°C it was 4·0. the pH decreased from an initial value of 6·3 to 4·9 at 4°C, to 4·3 at 10°C, and to 4·0–4·1 at the other temperatures.

The effect of initial pH of MRS broth on cell growth and sakacin K production was determined at 30°C in a fermenter. Maximum cell growth was observed at pH 5·5 and 6·0. At pH 5·0, cell growth was slower but

reached high cell densities at the end of the incubation time. At initial pH of 5·0, 5·5 and 6·0, the maximum production of sakacin K was 6400 AU ml^{-1} after 9–11 h of incubation. At pH 5·0 the maximum sakacin K activity was maintained to the end of the incubation period, while at pH 5·5 and 6·0, sakacin K activity decreased during the stationary phase to 1600 AU ml^{-1} and 800 AU ml^{-1} respectively. At pH 6·5 a peak production of 3200 AU ml^{-1} after incubation for 11 h was observed, decreasing to 400 AU ml^{-1} at 24 h.

Inhibition of L. innocua *in minced raw pork*

By the end of the sampling time, the counts of *Lb. curvatus* CTC371 in lot B and *Lb. sakei* CTC494 in lot C in each atmosphere were similar (5×10^8 cfu g^{-1}), although during the first 2 days *Lb. curvatus* grew more slowly than *Lb. sakei* CTC494. Each strain constituted 100% of the population of the LAB count, as confirmed by plasmid analysis and bacteriocin production when indicated.

Under the oxygen permeable film packaging, *L. innocua* grew from 50 MPN g^{-1} 316 MPN g^{-1} in the control batch (lot A). When CTC371 (Bac −) was inoculated, listeria counts remained stable throughout the process, while in lot CTC494 (Bac +), a decrease was observed after 6 days of packaging (from 36 MPN g^{-1} to 2 MPN g^{-1}). In lot D, where sakacin K was added, listeria counts diminished within 10 min (from 50 MPN g^{-1} to <3 MPN g^{-1}), remaining at this range until the end of the sampling time.

The growth of *Listeria* under vacuum was similar to that of the oxygen permeable film

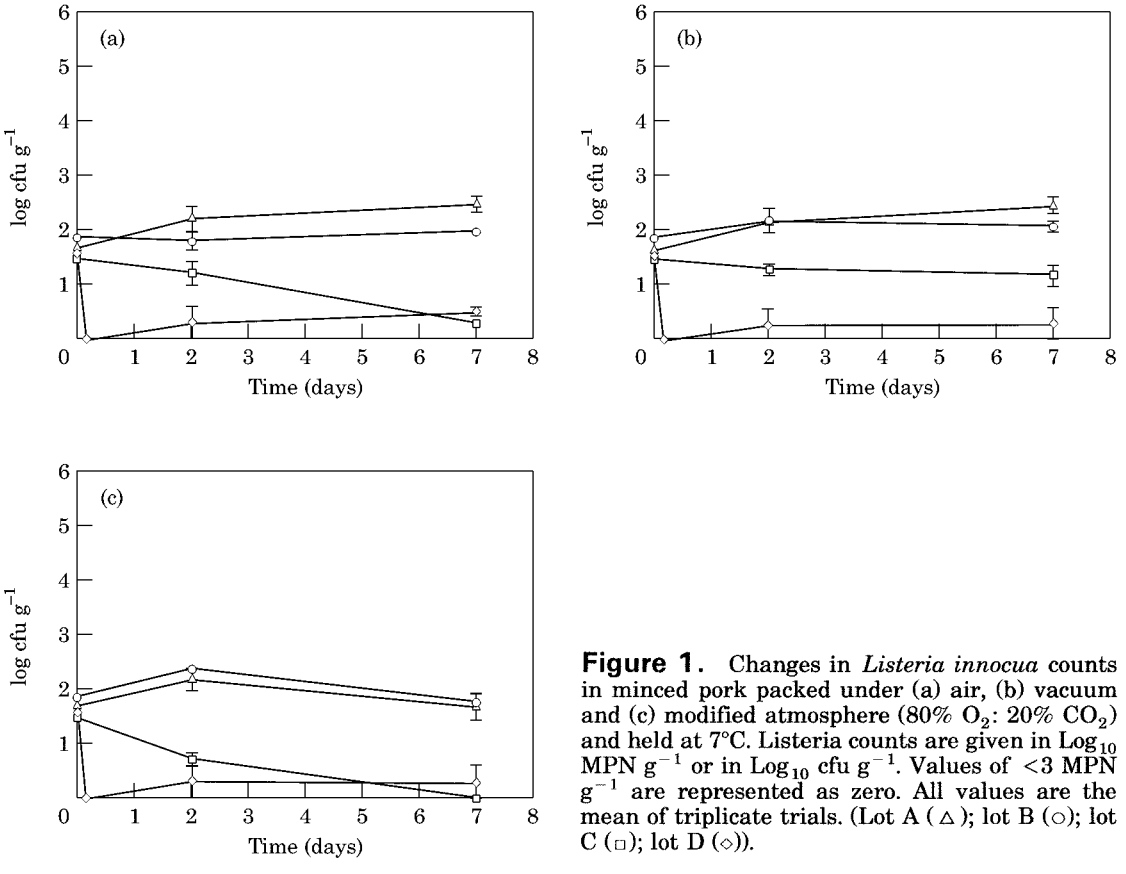

Figure 1. Changes in *Listeria innocua* counts in minced pork packed under (a) air, (b) vacuum and (c) modified atmosphere (80% O_2: 20% CO_2) and held at 7°C. Listeria counts are given in Log_{10} MPN g^{-1} or in Log_{10} cfu g^{-1}. Values of <3 MPN g^{-1} are represented as zero. All values are the mean of triplicate trials. (Lot A (△); lot B (○); lot C (□); lot D (◇)).

both in the control batch (lot A) and lot B (Bac −). When the samples were packed under vacuum, CTC494 (Bac +) was not as effective in reducing *L. innocua* as under air or MAP, except for lot D, with sakacin K, where the inhibition of *Listeria* was the same as under the oxygen-permeable film.

Under modified atmosphere packaging, *Listeria* evolution in lot A and B were identical. In lot C (Bac +), this atmosphere (80% O_2 : 20% CO_2) was the most effective in reducing *Listeria* at day 2. The inhibition of *Listeria* growth in lot D, with sakacin K, was very effective under each atmosphere.

Inhibition of L. innocua *in chicken breasts*

The kinetics of growth of the inoculated lactic acid bacteria CTC371 (Bac −) and CTC494 (Bac +) were similar in each atmosphere (Fig. 2).In the samples packaged under oxygen permeable film, *L. innocua* was able to grow to 10^5 cfu cm^{-2} in both the control (lot A) and in the non-bacteriocinogenic lot with CTC371 (Bac −) (lot B), while *Listeria* counts were reduced when CTC494 (Bac +) was inoculated (from 2×10^2 cfu cm^{-2} to 31 cfu cm^{-2} after 7 days) (lot C). In lot D, a decrease in *L. innocua* counts during the first minutes after inoculation (from 2×10^2 cfu cm^{-2}) was observed, although after 7 days *L. innocua* grew to 78 cfu cm^{-2}.

In the vacuum samples of poultry, *Listeria* grew slightly after 7 days in the non-bacteriocinogenic lots (A and B) while in lot C (Bac +), a growth inhibition was observed. With poultry, vacuum packaging was found to be the most effective atmosphere for inhibiting the growth of listeria in all treat-

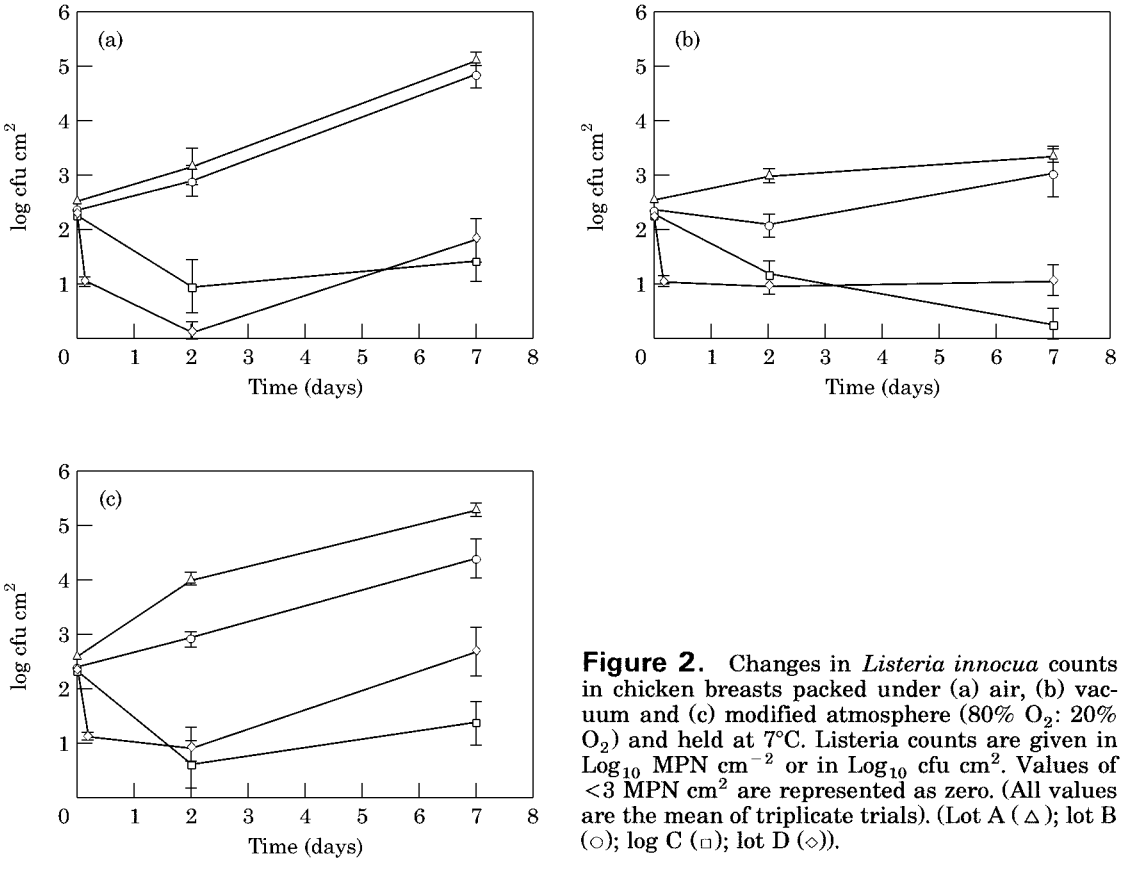

Figure 2. Changes in *Listeria innocua* counts in chicken breasts packed under (a) air, (b) vacuum and (c) modified atmosphere (80% O_2: 20% O_2) and held at 7°C. Listeria counts are given in Log_{10} MPN cm^{-2} or in Log_{10} cfu cm^2. Values of <3 MPN cm^2 are represented as zero. (All values are the mean of triplicate trials). (Lot A (△); lot B (○); log C (□); lot D (◇)).

ments evaluated.

No differences were observed in the growth of *L. innocua* in lot A when packed under oxygen-permeable film or in MAP. In lot B (Bac −) the growth of the indicator strain was slightly reduced compared to lot A. In lot C (Bac +) a 10 fold decrease of the indicator strain was observed. In lot D (sakacin K), *Listeria* counts diminished within 10 min, but by the end of the sampling an increase was observed ($5{\cdot}4 \times 10^2$ cfu cm^{-2}).

Inhibition of L. Innocua *in sliced cooked pork*

The strains of LAB inoculated in lots B and C reached similar counts throughout the sampling time in each packaging treatment studied (Fig. 3). Under the oxygen-permeable film packaging, *L. innocua* grew from 60 MPN g^{-1} to 1×10^5 cfu g^{-1} in the control batch (lot A) at the end of sampling and to 1×10^4 cfu g^{-1} in lot B (Bac −). In lot C (Bac +), *L. innocua* counts were maintained at the initial level for the first 3 days, reaching 8×10^2 cfu g^{-1} by the end of the sampling time. In lot D (sakacin K), *Listeria* counts showed a 10 fold decrease at the first stages of sampling but outgrowth occurred after 7 days of storage ($2{\cdot}5 \times 10^2$ cfu g^{-1}).

Under vacuum, *L. innocua* counts remained at the same level throughout the process in lot A, while in lot B it grew 0·6 log. Lot C under vacuum recorded the lowest *Listeria* counts (from 60 MPN g^{-1} to 2 MPN g^{-1} after 7 days). In lot D, an initial inhibition was observed, as in oxygen-permeable film packaging, which was maintained during the process.

The growth of *L. innocua* in lots A and B

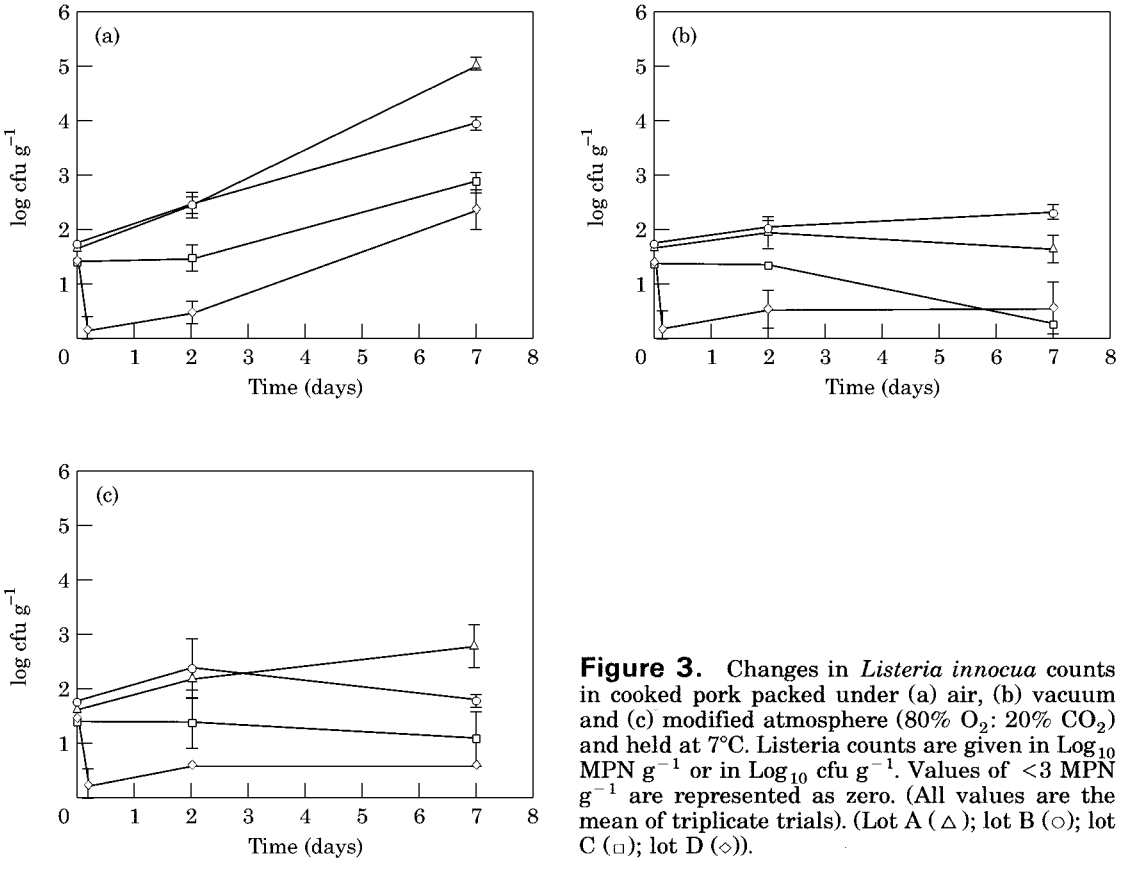

Figure 3. Changes in *Listeria innocua* counts in cooked pork packed under (a) air, (b) vacuum and (c) modified atmosphere (80% O_2: 20% CO_2) and held at 7°C. Listeria counts are given in Log_{10} MPN g^{-1} or in Log_{10} cfu g^{-1}. Values of <3 MPN g^{-1} are represented as zero. (All values are the mean of triplicate trials). (Lot A (△); lot B (○); lot C (□); lot D (◇)).

of sliced and cooked pork packaged under MA was the same until the third day, but then listeria counts diminished in lot B, while in lot A they increased to $6{\cdot}3 \times 10^2$ MPN g^{-1}. In lot C. *L. innocua* counts remained at the same low level throughout the process. In lot D, with the sakacin K, the inhibition of *L. innocua* was similar to the results obtained in the vacuum-packaging experiment.

In each treatment, LAB from MRS plates in lots B and C were identified as the inoculated strains (*L. curvatus* CTC371 and *L. sake* CTC494), by checking their plasmid profile and their ability to produce bacteriocin for CTC494. This confirmed their competitiveness in the meat environment.

Discussion

Effect of temperature and pH on production of sakacin K in MRS

Optimum conditions for bacteriocin biosynthesis differ even among closely related strains of lactic acid bacteria (Muriana and Luchansky 1993). The optimum temperature for bacteriocin production is sometimes different from that for cell growth (Larsen et al. 1993, Schillinger et al. 1993). *Lb. sakei* CTC494 showed a better production of sakacin K at low temperatures (4°C and 10°C) than at the optimum growth temperature (30°C). Due to the results obtained after the production of sakacin K at low temperatures, CTC494 was used in meat, poultry and cooked meat products to prevent *Listeria* outgrowth in three packaging atmospheres.

An initial pH of 6·0–6·5 has been reported as optimum for the production of bacteriocins in LAB, optimum pH for production of sakacin K was 5·0 (although production has also been observed at pH 6·0 and 6·5). The effect of pH control on cell growth and the production of various bacteriocins is variable in LAB. The use of pH control increased production of nisin at pH 6·8 (de Vuyst and Vandamme 1991), bavaricin MN at pH 6·0 (Kaiser and Montville 1993) and piscicolin at pH 6·5 (Schillinger et al. 1993), but not pediocin AcH at pH 4·0–5·0 (Biswas et al. 1991), plantaricins S and T at pH 4·0–7·0 (Jiménez-Díaz et al. 1993) or brevicin 286 (Coventry et al. 1996). As with nisin, bavaricin MN and piscicolin, the yield of sakacin K increased under pH control (5·0). From the results obtained it seems that optimum pH for the production of sakacin K is similar to that of fermented meat products.

Inhibition of L. innocua *in meat and meat products by* L. sakei *CTC494 and sakacin K*

Previous studies have shown that *L. monocytogenes* and *L. innocua* can grow in different conditions that would inhibit other pathogens, for example refrigeration temperatures, low pH and high levels of nitrite and sodium chloride (Hart et al. 1991, Hudson et al. 1994, Farber et al. 1996, Thomas et al. 1996). According to Franco-Abuín et al. (1997), there is no difference in the behaviour of *L. monocytogenes* and *L. innocua* in meat packed under modified atmospheres for 9 days. Besides this, sakacin K inhibits *L. monocytogenes* and *L. innocua* with the former being more sensitive to the bacteriocin than *L. innocua*, as shown by the minimum inhibitory concentration (MIC) and minimum killing concentration (MKC) results reported by Hugas et al. (1995).

In the last decade, the use of MAP to increase the shelf-life of different types of meat and meat products has been introduced in most distribution chains. Despite the interest in using this type of packaging, concern about the potential growth of some psycrotrophic pathogens has become a limiting factor in its acceptance (Mano et al. 1995). Numerous and contradictory studies about the growth of *L. monocytogenes* in MAP have been carried out. Some authors have observed growth of *Listeria* species in MAP (Ingham et al. 1990, Marshall et al. 1991, Zhao et al. 1992, Hudson et al. 1994), while other authors state that MAP inhibits listeria growth (Gill and Reichel 1989, Hart et al. 1991, Mano et al. 1995). Grau and Vanderlinde (1990) and Kaya and Schmidt (1991) suggested that the growth of *L. monocyto-*

genes could be affected by storage temperature, meat pH and competitive flora. Buchanan and Klawitter (1990) observed an unexpected inactivation of *L. monocytogenes* in minced beef. After an antagonistic strain against this pathogen was isolated from the minced meat, the authors suggested that the observed differences between laboratories could be due to the bacteriocin production by the endogenous meat flora.

In this work, *Listeria* inhibition in raw minced pork, poultry breasts and cooked pork could not be achieved by the sole application of vacuum or a modified atmosphere (20% CO_2, 80% O_2) although some influence of the type of atmosphere upon the growth of the pathogenic micro-organism could be observed. Nonetheless, application of the bacteriocinogenic strain *Lb. sakei* CTC494 or the bacteriocin sakacin K inhibited the growth of *L. innocua* to different extents compared to the control lots, in all the products studied either packed in air, vacuum or modified atmosphere.

In poultry breasts, vacuum packaging favoured the inhibition of *L. innocua*. Twenty percent CO_2 under MAP was not sufficient to inhibit the growth of *L. innocua*. Similar results were obtained by Harrison and Carpenter (1989). In cooked pork, the vacuum and modifed atmosphere packages maintained the *Listeria* counts at lower levels (10^3 cfu g^{-1}) than the oxygen-permeable film (10^5 cfu g^{-1}). In raw minced pork, the counts of *Listeria* in the control lot (lot A) packed in modified atmosphere did not grow during the sampling time, while *Listeria* outgrowth was observed in those samples packed in air or vacuum. Fang and Lin (1994b) observed a very slight reduction of *Listeria* growth in minced raw pork packed in 100% CO_2 compared to air.

Through the inocculation of the bacteriocinogenic *Lb. sakei* CTC494 or sakacin K, it has been possible to arrest the growth of *Listeria*. The greatest inhibition was observed in the vacuum packaged samples of poultry breasts and cooked pork, and in the modified atmosphere packaged samples of raw minced pork. Therefore, type of meat and levels of competitive flora seem to be very important in sustaining the growth of lactic acid bacteria. The total lactic acid bacteria count was higher in the cooked pork (10^9 cfu g^{-1}) compared to chicken breasts (5×10^7 cfu g^{-1}), although this did not imply a further inhibition of the indicator bacteria in the former product. The inhibition observed cannot be attributed solely to the lactic acid produced by the added culture, since lots inoculated with *Lb. curvatus* CTC371 achieved a final level of *L. innocua* 2–3 logs higher than lots with the bacteriocinogenic strain. Therefore, the observed inhibition in these lots can be attributed to bacteriocin production.

From the results obtained, it has been shown that the same kind of packaging atmosphere has a different effect on the indicator micro-organism depending on the type of meat; this could be due to the pH of the meat, water activity or endogenous flora. After addition of the bacteriocin, a bactericidal action of sakacin K could be observed within several minutes in every product and packaging atmosphere studied. Nielsen et al. (1990) and Motlagh et al. (1992) observed a similar inhibition pattern, although Vignolo et al. (1996) did not observe any initial inactivation after the inocculation of ground beef with 16800 AU ml^{-1} of lactocin 705. After the initial inactivation of *L. innocua* cells, the surviving bacteria grew to different levels depending on the packaging atmosphere and the product. Loss of the bacteriocin activity results from either the inactivation by meat proteases (Garriga et al. 1993) or the adsorption to certain meat particles such as fat (Schillinger et al. 1991).

In a previous study, raw minced pork was mixed with sakacin K and after the extraction of bacteriocin activity (Leisner et al. 1996), it was not possible to recover the same amount of bacteriocin added to the product (data not shown). Therefore, it has not been possible to assess the amount of bacteriocin produced *in situ* by *Lb. sakei* CTC494. Fang and Lin (1994a) and Chung et al. (1989) also found an inactivation of nisin after several days in pork. Thus, this could explain the outgrowth of *Listeria* after the initial inactivation produced by the addition of sakacin K.

In this study, temperature abuse has been applied to the products studied in order to simulate abusive storage conditions. In adequate refrigeration temperatures, inhibition of *Listeria* would probably have been higher. It has been shown by many authors that temperature is an important environmental factor greatly influencing listeria growth. Besides the anti *Listeria* activity of *Lb. sakei* CTC494 in fermented meat products (Hugas et al. 1995), this strain appears to be an effective anti *Listeria* bioprotective culture in raw and cooked products.

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