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ORIGINAL ARTICLE

Investigations on the growth of *Listeria monocytogenes* on lamb packaged under modified atmospheres

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The growth of Listeria monocytogenes *in air and modified atmosphere packaged lamb pieces and mince, stored at 5 or 0°C was investigated at 42 days. The modified atmospheres included: (i) vacuum pack; (ii) 80% O_2/20% CO_2; (iii) 50% CO_2/50% N_2; and (iv) 100% CO_2. On lamb pieces at 5°C, growth of* L. monocytogenes *occurred in air and all the modified atmospheres, except 100% CO_2.* L. monocytogenes *growth on minced lamb at 5°C was reduced compared with lamb pieces. Growth did not occur in vacuum packaged mince or in an atmosphere containing 100% CO_2. At 0°C, growth of the organism was completely inhibited on pieces and mince in all the modified atmospheres tested and in air. It was noted that pH did not increase or decrease in a regular manner throughout the storage period. Thus pH was eliminated as a factor controlling the growth of* L. monocytogenes *on modified atmosphere packaged lamb.*

Introduction

Listeria monocytogenes is ubiquitous in the environment and occurs widely in various fresh foods such as milk (Lovett et al. 1987), cheese (McLauchlin et al. 1990), turkey (Genigeorgis et al. 1990), meat and fish products (Sheridan et al. 1994). Due to the psychrotrophic nature of this organism, refrigeration alone cannot be relied upon to control its growth.

Modified atmosphere packaging (MAP) can extend the shelf-life of many perishable products including meat, fish and poultry (Hotchkiss 1988). The use of reduced oxygen (O_2) and increased carbon dioxide (CO_2) concentrations can increase the shelf-life by inhibiting the growth of aerobic spoilage bacteria. Under such conditions, the growth of psychrotrophic pathogenic bacteria, including such species as *L. monocytogenes*, may not be inhibited. *L. monocytogenes* can, under certain circumstances, outgrow spoilage bacteria on cooked chicken (Marshall et al. 1991). Thus, concerns have been expressed regarding the microbiological safety of MAP food (Farber 1991).

Individuals at greatest risk from listeriosis are pregnant women and their fetuses, the elderly, and immunosuppressed patients. The clinical symptoms of listeriosis mainly include central nervous system infections and primary bacteraemia, but can also include endocarditis (Farber and Peterkin 1991). The Food and Drug Administration has determined that there is zero tolerance (<one organism per 25 g of sample) for *Listeria* species in food.

The relative growth rates of spoilage organisms and pathogenic organisms such as *L. monocytogenes* in MAP foods is a critical factor

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in determining the safety of MAP. If the normal aerobic spoilage flora is suppressed, while pathogen growth continues, foods could become unsafe before being rejected due to the development of overt spoilage. The objective of the present work was to determine if MAP would inhibit the normal spoilage flora while allowing the growth of *L. monocytogenes* on lamb pieces and mince, packaged under four modified atmospheres and air, and stored at 5 or 0°C. The modified atmospheres investigated were vacuum pack, 80% O_2/20% CO_2, 50% CO_2/50% N_2 and 100% CO_2.

Materials and Methods

Animals

Lambs purchased in local markets were rested overnight on straw, given water *ad libitum* and slaughtered the next day in the pilot scale abattoir at The National Food Centre. After dressing, carcasses were not washed and were chilled for 16 h at 4°C at an air speed of $<0{\cdot}2$ m s^{-1} and at a relative humidity (RH) of 85–90% until the temperature of the deep round reached 4°C. The pH of the striploin was measured after chilling. Muscles were selected from carcasses in the pH range 5·4–5·8, dissected into small pieces and all visible fat was removed. The meat was stored in sterile plastic bags at 0°C before use. A sample was removed from this bulk stored material and tested for the presence of *Listeria* spp. as described below.

Organism

L. monocytogenes serotype 4b (NCTC 11994) was obtained from the National Collection of Type Cultures at the Central Public Health Laboratory, Colindale, London. It was maintained on tryptone soya agar (TSA; Oxoid, Unipath Ltd, Basingstoke, UK) at 0°C.

Inoculation

Before inoculation, the lamb pieces were examined for the presence of *Listeria* spp. A random 25 g sample of lamb pieces was homogenized in 225 ml UVM I broth (Oxoid) in a Colworth Stomacher (Model No. BA6021, A. J. Seward & Company Ltd, London, UK) for 1 min and incubated at 30°C overnight. The following day a 0·1 ml aliquot of the homogenate was aseptically transferred into 9·9 ml of UVM II (Oxoid) and incubated at 30°C overnight. After incubation, a loopful of UVM II was streaked on two *Listeria* selective agar plates (LA; Oxoid) containing 200 mg of cycloheximide, 10 mg of colisitin sulphate, 2·5 mg of acriflavine, 1·0 mg of cefotetan and 5·0 mg of fosfomycin in 500 ml of agar. The LA plates were incubated at 30°C for 48 h.

Approximately 14 kg of lamb pieces per replicate were inoculated by immersion for 5 s in maximal recovery diluent (MRD, BBL Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) containing 1000–1500 *L. monocytogenes* organisms ml^{-1}. The inoculum was prepared from an overnight culture of *L. monocytogenes* grown in brain heart infusion broth (Oxoid) at 30°C. Excess liquid was allowed to drain off the meat. Minced lamb was prepared by double mincing approximately 7 kg of inoculated lamb pieces per replicate through a 10 mm plate and then through a 5 mm plate using a Crypto-Peerless mincing machine (Model EB12F, Crypto-Peerless Ltd, London, UK), sterilized by autoclaving at 121°C for 15 min.

Packaging

The inoculated lamb pieces or mince were weighed out in 100 g lots into Dynopack (Dynopack AS, Kristiansand, Norway) high density polyethylene trays with an oxygen transmission rate (OTR) of 3·5 cm^3 m^{-2} 24 h^{-1} atm at 25°C and a RH of 50%. The meat was modified atmosphere packaged with a Transoplan (A&R, Flexible, Lund, Sweden) top web film with an OTR of 8·0 cm^3 m^{-2} 24 h^{-1} atm at 25°C and a RH of 50%. The packs were filled with a modified gas atmosphere or air and sealed using a Mecapac 500 semi-automatic packaging machine (rue Diderot, 93170 Bagnolet, France). A KM 100-3M (Witt Gasetechnik, D-5810 Witten 1, Postfach 2550, Germany) gas mixer was used to mix food grade CO_2, O_2 and N_2 (Air Products PLC, Walton-on-Thames, UK) to obtain pack atmospheres of: 80% O_2/20% CO_2; 50% CO_2/50% N_2 and 100% CO_2. The final pack volume was 475 ml giving a gas : meat ratio in excess of 3 : 1.

Vacuum packaging bags with a capacity of approximately 200 g were made from Cryovac BB6 bags (W.R. Grace Ltd, Co. Dublin, Ireland) with an OTR of 48 cm^3 m^{-2} 24 h^{-1} atm at 25°C and a RH of 50%. The bags were filled with 100 g of inoculated lamb pieces or mince and heat sealed using a Swissvac vacuum packaging machine (Model 380, Swissvac (GB) Ltd, Langley, UK). After packing, the

vacuum packs were dipped in water at 90°C for 5 s to shrink the bags.

Storage

All packs were stored at 5 or 0°C for up to 42 days. At 7 day intervals, packs (n = 20) were removed and examined. The inoculated meat pieces or mince were randomly distributed over each atmosphere/temperature/time combination. Four replicates were set up for each atmosphere/temperature/time combination.

Gas analysis

A Gow-Mac gas chromatograph (Spectra 250, Gow-Mac Instrument Co. Ltd, Co. Clare, Ireland) fitted with an Alltech Speciality CTRI column, was used to confirm the individual gas ratios in the gas mixtures before packaging and to analyse head space contents when packs were opened for microbiological examination. A Cl-4100 Integrator (Milton Roy, Co. Clare, Ireland) was used to plot the chromatographs and calculate the gas percentages from the areas under the curves using the normalization method (Anon 1987).

Microbiology

A 10 g sample of lamb pieces or mince was removed from the pack and homogenized in a Colworth Stomacher for 1 min with 20 ml of MRD. Counts for *L. monocytogenes* were obtained by surface inoculating duplicate LA plates with 1 ml or 0·1 ml of the homogenate or successive 10-fold dilutions in MRD. The inoculum was spread on the surface using a sterile glass rod. Before incubation, the 1 ml plates were placed in a laminar air flow cabinet (Nuaire, Class II, type A, MN, USA) for approximately 30 min to dry excess liquid from the surface of the agar plate. All plates were incubated at 30°C for 48 h.

A presumptive *L. monocytogenes* colony from each LA plate was checked to ensure it was a Gram-positive rod, catalase positive and displayed umbrella motility at 25°C in SIM medium (Oxoid).

Total counts were obtained on all purpose tween (APT; BBL) agar. Duplicate plates were inoculated with the homogenate or successive 10-fold dilutions in MRD using a spiral plate maker (Don Whitley Scientific Ltd, Shipley, UK) and incubated at 25°C for 3 days.

pH measurement

When packs were opened, a 2 g sample was removed and added to 10 ml of a solution of sodium idoacetate (5 mM; BDH Chemicals Ltd, Poole, UK) and potassium chloride (150 mM; AnalaR, BDH) (Bendall 1973). The sample was homogenized (Silverson Machines Ltd, Chesham, Bucks, UK) for two 15 s intervals with a 5 s interval between treatments. A combination electrode (Model 91-06, ATI Orion Europe, Cambridge, UK) was inserted into the homogenate and the pH recorded using an Orion pH meter (Model 221). Before each determination the meter was calibrated using standard phosphate buffers of pH 4·01 and 7 (Radiometer Analytical A/S, Bagsvaerd, Denmark).

Statistical analysis

The data were analysed as a split-plot design in which meat type was the main plot effect and pack type and storage temperature were the sub-plot effects. Differences between means were determined using one or two tailed t-tests as appropriate. The standard deviation for the pH data was calculated.

Results

When the meat was examined before inoculation, it was found to be *Listeria* free.

At 5°C *L. monocytogenes* grew on lamb pieces stored under all atmospheres except 100% CO_2 (Table 1). There were no significant differences between the counts at 42 days achieved in all

Table 1. Mean *Listeria monocytogenes* counts (log_{10} cfu g^{-1}) at 42 days on lamb pieces and mince packaged in different gas atmospheres at 5°C

Meat type	Pieces	Mince
Gas atmosphere		
Air	6·07	5·37
Vacuum pack	3·81	1·74
80% O_2/20% CO_2	4·91	2·68
50% Co_2/50% N_2	4·22	3·68
100% CO_2	1·35	0·58

Standard error of difference between means = 1·16, except when comparing pieces vs mince for the same gas atmosphere = 1·18. Degrees of freedom = 24.

atmospheres at this temperature with the exception of 100% CO_2 which was significantly lower than the others ($P < 0{\cdot}05$).

On minced lamb at 5°C, growth of *L. monocytogenes* occurred in air. Although there appeared to be small amounts of growth in 80% O_2/20% CO_2($\log_{10}$ 2·68 cfu g^{-1}) and 50% CO_2/50% N_2 ($\log_{10}$ 3·68 cfu g^{-1}), the significance of these counts could not be established statistically. Growth of *L. monocytogenes* was inhibited on vacuum packaged minced lamb. In 100% CO_2, numbers were reduced below the initial inoculum level ($\log_{10}$ 1·45 cfu g^{-1}). The counts obtained from minced lamb stored at 5°C in air were significantly different from all the others except 50% CO_2/50% N_2 ($P < 0{\cdot}05$). There was also a significant difference between 50% CO_2/50% N_2 and 100% CO_2 ($P < 0{\cdot}05$).

In all atmospheres growth on minced lamb was less than on pieces but the differences were not significant after 42 days storage, for any of the atmospheres.

At 0°C, growth of *L. monocytogenes* on pieces and mince was completely inhibited under all the atmospheres examined. A comparison of *L. monocytogenes* counts at 5 and 0°C for lamb pieces packaged in 80% O_2/20% CO_2 is presented (Fig. 1). Similar inhibition patterns at 0°C were observed in all other atmospheres for pieces and mince.

The total aerobic counts at 25°C on lamb pieces packaged in different gas atmospheres and held at 5 or 0°C are shown in Table 2. There were no significant differences between any of the counts at 5°C. At 0°C, significant differences were observed between air and all other atmospheres except 80% O_2/20% CO_2 ($P < 0{\cdot}05$). There was also a significant difference between 80% O_2/20% CO_2 and 100% CO_2 ($P < 0{\cdot}05$). The total aerobic count after 42 days storage at 5°C was higher than at 0°C in 50% CO_2/50% N_2 and 100% CO_2 ($P < 0{\cdot}05$).

No significant differences were noted between counts for minced lamb stored at 5°C

Table 2. Mean total aerobic counts ($\log_{10}$ cfu g^{-1}) at 42 days on lamb pieces and mince packaged in different gas atmospheres at 5 or 0°C

Meat type	Pieces		Mince	
Storage temperature (°C)	5	0	5	0
Gas atmosphere				
Air	8·86	8·81	9·20	8·35
Vacuum pack	7·53	6·75	8·65	6·35
80% O_2/20% Co_2	8·91	7·50	8·97	7·24
50% Co_2/50% N_2	8·15	6·33	7·75	6·51
100% CO_2	7·55	5·41	8·07	5·66

Standard error of difference between means = 0·87, except when comparing pieces vs mince for the same gas atmosphere = 0·86. Degrees of freedom = 54.

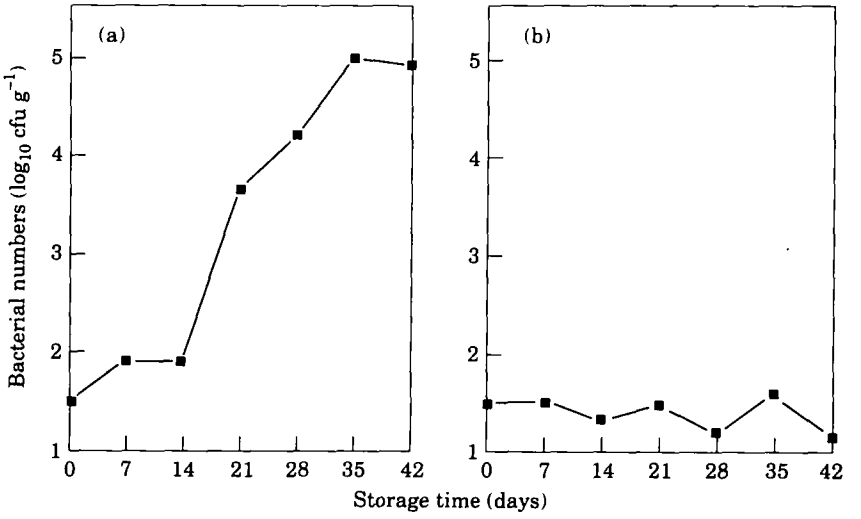

Figure 1. Growth of *Listeria monocytogenes* on lamb pieces packaged in an atmosphere containing 80% O_2/20% CO_2 and stored at (a) 5°C or (b) 0°C.

under different atmospheres. The count obtained after storage at 0°C in air was significantly different from the counts obtained under all other atmospheres except 80% O_2/20% CO_2 ($P < 0.05$). There were significant differences between the counts for minced lamb stored in vacuum packs, 80% O_2/20% CO_2 and 100% CO_2 at 5 and 0°C ($P < 0.05$).

There were no significant differences in total counts between pieces and mince at either 5 or 0°C.

The mean pH values and standard deviations over the 42 days of storage, for lamb pieces and mince, packaged in different gas atmospheres and stored at 5 or 0°C are shown in Table 3. In general, pH did not increase or decrease in a regular manner over 42 days for pieces or mince in any of the atmospheres at either storage temperature. An example of the variation in pH observed is shown for lamb pieces packaged in 100% CO_2 and stored at 5 or 0°C (Fig. 2). The higher mean pH values were recorded from pieces and mince stored in air and in an atmosphere containing 80% O_2/20% CO_2 at 5 and 0°C. Lower mean pH values were recorded from vacuum packaged lamb pieces and mince at 5°C. It was noted that there appeared to be greater variation in mean pH values between atmospheres for lamb pieces and mince at 5°C than at 0°C.

Discussion

The literature contains conflicting reports on the ability of *L. monocytogenes* to grow in air on refrigerated meat. In the present study, this pathogen grew on lamb pieces and mince stored

Table 3. Mean pH and standard deviation values over 42 days on lamb pieces and mince packaged in different gas atmospheres at 5 or 0°C

Meat type	Pieces		Pieces		Mince		Mince	
Storage temperature (°C)	5	5	0	0	5	5	0	0
Gas atmosphere								
Air	5·80	0·14*	5·68	0·08*	5·71	0·13*	5·75	0·12*
Vacuum pack	5·41	0·15	5·62	0·04	5·39	0·17	5·62	0·06
80% O_2/20% CO_2	5·81	0·18	5·67	0·07	5·70	0·09	5·70	0·18
50% CO_2/50% N_2	5·52	0·07	5·62	0·05	5·50	0·11	5·61	0·10
100% CO_2	5·58	0·08	5·63	0·05	5·50	0·12	5·61	0·11

*Standard deviations.

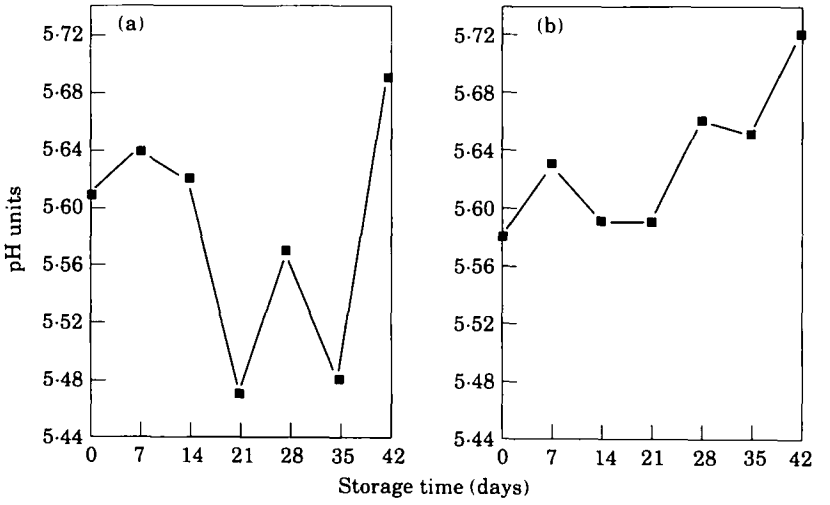

Figure 2. Mean pH values over 42 days on lamb pieces packaged in an atmosphere containing 100% CO_2 and stored at (a) 5°C or (b) 0°C.

in air at 5°C. Growth has been previously reported on lamb stored at 8°C (Khan et al. 1973), the sarcoplasmic protein ('drip') of lamb, beef and pork stored at 4°C (Khan et al. 1975) and beef stored at 5°C (Gibbs et al. 1993). However, Buchanan et al. (1987) reported no growth of *L. monocytogenes* in hamburger stored at 4°C for 7 days, and Johnson et al. (1988) reported no detectable growth of *L. monocytogenes* in beef mince packaged in O_2-permeable bags held at 4°C for 14 days. Shelef (1989) reported that *L. monocytogenes* counts remained relatively unchanged on inoculated ground beef and liver during storage at 4 or 25°C.

There is a similar lack of agreement among previous reports on the ability of this organism to grow under vacuum storage. Our results indicated that there was no growth on vacuum packaged minced lamb at 5°C and that very limited growth occurred at this temperature on vacuum packaged lamb pieces. Other workers have reported very small increases in *L. monocytogenes* numbers (1·0 $\log_{10}$ cfu cm^{-2} in 9 days) on pork stored at 1°C (van Laack et al. 1993). Other studies note the absence of growth of *L. monocytogenes* on vacuum packaged beef (pH 5·6) stored at 2, 4, 5 or 7°C (Kaya and Schmidt 1991, Gibbs et al. 1993). On the other hand, there are reports of substantial growth of *L. monocytogenes* on vacuum packaged beef striploins stored at 5 and 10°C (Grau and Vanderlinde 1990, Avery et al. 1994). Grau and Vanderlinde (1990) and Kaya and Schmidt (1991) have suggested that growth of *L. monocytogenes* on vacuum packaged beef is affected by the temperature of storage, the type of tissue, i.e. lean or fat, the pH of the lean and the competing flora. In other studies, Buchanan and Klawitter (1990) noted an unexpected inactivation of *L. monocytogenes* in a raw ground beef sample. On further investigation, a lactic acid bacterium was isolated which was antagonistic to this pathogen. This observation led them to suggest that the production of bacteriocin or bacteriocin-like agents against *L. monocytogenes* may be responsible for the variations observed between laboratories (Buchanan and Klawitter 1992).

In the present study, an inhibitory effect of 100% CO_2 on the growth of *L. monocytogenes* was recorded. This inhibitory effect of CO_2 on *L. monocytogenes* has been reported by Khan et al. (1973). These authors noted that more listeria survived on inoculated lamb meat packaged in O_2-permeable bags than on inoculated lamb meat packaged in O_2-impermeable bags. They suggested that increased concentrations of CO_2 in impermeable bags may have inactivated the organism. The results of the present study show that growth of *L. monocytogenes* was significantly inhibited on lamb pieces and mince packaged in 100% CO_2 and stored at 5 or 0°C. This is in agreement with the results of Avery et al. (1994) who reported inhibition of the growth of *L. monocytogenes* on beef striploin steaks packaged under a saturated CO_2 controlled atmosphere at 5 and 10°C. In contrast to these results, Hudson et al. (1994) observed growth of *L. monocytogenes* at 3°C on sliced roast beef packaged in a saturated CO_2 controlled atmosphere. Although growth of *L. monocytogenes* was inhibited in 100% CO_2, our results showed that growth of the pathogen occurred on lamb pieces and mince packaged in atmospheres containing 20 or 50% CO_2 at 5°C. This is at variance with the results of Gibbs et al. (1993) who found that *L. monocytogenes* did not grow on beef steaks packaged in atmospheres containing 20, 50 or 100% CO_2 at 5 or 0°C (Gibbs et al. 1993).

It has been suggested that the growth of *L. monocytogenes* is influenced by the bacterial flora. Gouet et al. (1978) noted that growth of the lactobacilli increased on minced beef, while growth of *L. monocytogenes* decreased. Kaya and Schmidt (1991) suggested that the domination of the lactic acid bacteria on vacuum packaged beef of pH 5·6 was responsible for suppressing the growth of *L. monocytogenes*. On beef of pH > 6·0, growth of this organism occurred where the dominant flora consisted of *Brocothrix thermosphacta* and Enterobacteriaceae. A sterile minced beef medium with inhibitory activity for *L. monocytogenes* has been demonstrated by Mattila-Sandholm and Skyttä (1991). It was not possible to determine if the inhibitory activity was microbial in origin or due to the meat itself since the sterile beef medium was prepared from beef which had a natural microflora.

In this study growth of *L. monocytogenes* occurred on meat in the pH range 5·4–5·8.

These results differ from previous reports, e.g. Johnson et al. (1988) found that *L. monocytogenes* did not grow on beef mince at pH values similar to those observed in the present study (pH 5·6–5·9). Grau and Vanderlinde (1990) noted that growth of this organism on vacuum packaged beef was higher at pH > 6·0 than at pH 5·6. In addition, Kaya and Schmidt (1991) only obtained growth of the pathogen on vacuum packaged beef of pH > 6·0.

Although *L. monocytogenes* has been reported as being psychrotrophic (Farber 1991), we found that this organism did not grow at 0°C on lamb pieces and mince in all the atmospheres tested, including air. Inhibition at 0°C has previously been reported for lamb (Khan et al. 1973, 1975) and beef (Gibbs et al. 1993). On vacuum packaged beef (pH 5·6) *L. monocytogenes* did not grow on the lean tissue after 9 weeks storage at 0°C (Grau and Vanderlinde 1990). After 11 weeks, the viable count was only 10-fold greater than the initial count.

Such differences in psychrotrophic ability may be due to the difference in the metabolic state of the test organism inoculum. Walker et al. (1990) and Hart et al. (1991) have reported that low temperature pre-incubation of *L. monocytogenes* reduces the length of the lag phase during subsequent growth under chill conditions. Gibbs et al. (1993) observed that *Listeria* inoculum preconditioned at 5°C survived better in certain atmospheres than a similar inoculum preconditioned at 30°C. As most contamination with *Listeria* is thought to occur during chilling, chill holding, and further processing (van Laack et al. 1993), it is very likely that such contaminants are fully low temperature conditioned and, therefore, well adjusted to survival and growth on meats. Such phenotypic variation may underlie the observations made in this investigation. The *L. monocytogenes* inoculum used in this study, pre-incubated at 30°C grew on lamb pieces and mince stored at 5°C in some storage atmospheres but did not grow under the same atmospheres at 0°C.

Conclusions

The above discussion emphasizes the confused state of the literature in relation to the growth of *L. monocytogenes* on lamb, beef and pork packaged under different atmosphere/temperature combinations. Differences in the microflora of each meat type may be responsible for controlling the growth of this pathogen on meat. In particular, the production of bacteriocins by the lactic acid bacteria, towards *L. monocytogenes*, may be a significant factor influencing growth of the organism on each meat type. Further examination of the microflora is necessary to confirm this conclusion.

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