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Microbial interaction in cooked cured meat products under vacuum or modified atmosphere at 4°C

J. Metaxopoulos, M. Mataragas and E.H. Drosinos
Agricultural University of Athens, Department of Food Science and Technology, Laboratory of Food Quality Control and Hygiene, Athens, Greece

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Aims: To investigate the antagonistic activity of two lactic acid strains against the spoilage microflora in cooked cured meat products, vacuum or modified atmosphere packed at 4°C and to determine the inhibitory capacity of their bacteriocins.
Methods and Results: Frankfurter-type sausages and sliced cooked cured pork shoulder were inoculated with *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442 or with their bacteriocins. The microbial, physico-chemical (pH, L- and D-lactate, acetate and ammonia) and colour changes were studied. Results under vacuum packaging showed that in the uninoculated samples of the pork product the spoilage microflora grew but in the inoculated ones the spoilage microorganisms (e.g. *Brochothrix thermosphacta* and enterococci) reduced during the storage. This observation was more pronounced in the samples with the addition of bacteriocins. In the frankfurter-type sausages the spoilage microflora did not grow in the uninoculated and inoculated samples. In the modified atmosphere enriched in CO_2 the population of spoilage microflora remained at low levels in both products, indicating that CO_2 has an effect on the spoilage microorganisms' growth. In the pork product the concentrations of acetate and D-lactate increased while L-lactate decreased, but in the frankfurter-type sausages increase of acetate and D-lactate was not observed.
Conclusions: Lactic acid strains had an effect on the spoilage microflora growth but did not affect, negatively, the organoleptic properties of the products. These strains may be used as biopreservative cultures or their bacteriocins could be an important contribution to microbiological quality of meat products.
Significance and Impact of Study: Establishment of biopreservation as a method for extension of shelf life of meat products.

INTRODUCTION

Cooked frankfurter-type sausages and sliced cooked cured pork shoulder (will be reported in the text also as pork product) are sensitive to the spoilage meat products. The low salt content (2·0% in water phase), a pH value above 6·0 and a water activity (a_w) higher than 0·95 are only small hurdles to inhibit the usual types of organisms associated with these products. After cooking, the normal flora of the product, consisting of lactic acid bacteria, is too low to protect the products against the growth of Gram-negative microorganisms (Kotzekidou and Bloukas 1996).

Cooked meat products are stored under vacuum or modified atmosphere packaging at chill temperatures. The bacterial flora is gradually selected towards a CO_2-tolerant but a slowly growing one (Borch *et al.* 1996), dominated mainly by lactic acid bacteria (von Holy *et al.* 1991). Data indicate that the shelf life of frankfurter-type sausages and sliced cooked cured pork shoulder under vacuum packaging is about 35–42 d and 18–20 d, respectively, at a storage temperature of 4°C (Blickstad and Molin 1983; Kotzekidou and Bloukas 1996).

Storage under modified atmosphere (CO_2 + N_2) (MA) is also used for cooked cured meat products. Comparisons of

Correspondence to: M. Mataragas, Agricultural University of Athens, Department of Food Science and Technology, Laboratory of Food Quality Control and Hygiene, Iera Odos 75, 118 55 Athens, Greece (e-mail: iqcf2mey@auadec.aua.gr).

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shelf life of these products in vacuum and in MA packs have resulted in different findings. Some investigations indicated no extension of the shelf life of MA packaged meat products but other studies reported an increase in the shelf life by MA (Blickstad and Molin 1983, 1984; Simard *et al.* 1983; Boerema *et al.* 1993; Borch *et al.* 1996). The microflora of the cooked cured meat products in vacuum or in MA consists mainly of *Lactobacillus* sp. (*Lact. sakei* and *Lact. curvatus*) (Dykes and von Holy 1994; McMullen and Stiles 1994) and *Leuconostoc* sp. (*Leuc. gelidum*, *Leuc. carnosum* and *Leuc. mesenteroides*) (Schillinger and Lücke 1987; Collins *et al.* 1993; Dykes *et al.* 1994). Other species that have been isolated are *Weissella viridescens*, *Carnobacterium divergens*, *C. piscicola*, *Brochothrix thermosphacta*, *Pseudomonas fragi*, *Ps. fluorescens* and *Ps. lundensis* and some other species (Egan *et al.* 1980; Gardner 1983; Collins *et al.* 1987; Hammes *et al.* 1992; Nychas 1994).

Antagonistic cultures that are only added to inhibit pathogens and/or to extend the shelf life, while changing the sensory properties of the product as little as possible, are termed as 'protective cultures'. Using them (or their metabolic products, namely bacteriocins or enzymes) is often designated as 'biopreservation' (Lücke 2000). Protective cultures are microorganisms, namely lactic acid bacteria, which can suppress the growth of food poisoning organisms in the product. Inhibition of the growth of undesirable microorganisms can take place in a number of ways, such as the production of bacteriocins, the competition for nutrients, etc. (Kotzekidou and Bloukas 1996).

The aim of this study was to investigate the effect of two lactic acid bacteria strains, *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442, on the spoilage microflora and on the organoleptic properties of the products, with a view to use them as protective cultures.

MATERIALS AND METHODS

Meat products, bacterial cultures, inoculation and storage

The two products studied were frankfurter-type sausages and sliced cooked cured pork shoulder. Table 1 shows the final composition of the products.

Table 1 Final composition of the products

	Frankfurter-type sausages (%)	Pork shoulder (%)
Moisture	61·5	68·0
Proteins	13·5	12·2
Fat	17·0	12·0
Salt (NaCl)	2·2	2·2
Nitrite (NO_2^-)	<0·03	<0·03
Ashes	3·2	3·5
Starch	4·0	4·5

The lactic acid strains *Leuc. mesenteroides* L124 and *Lact. curvatus* L442 were isolated from Greek dry fermented salami and kept frozen at −20°C in MRS broth (MERCK, Darmstadt, Germany) supplemented with 20% glycerol. The organisms were subcultured twice (24 h, 30°C) in 10 ml MRS broth (MERCK), 1% inoculum. The cells were harvested by centrifugation (11 000 × *g* for 30 min) (Heraeus Sepatech, Biofuge 22R, Osterode, Germany), washed twice and resuspended in 10 ml of 50 mmol l^{-1} sodium phosphate buffer pH 7. The bacterial suspension was diluted with sterile 1/4-strength Ringer's solution (OXOID, Basingstoke, UK), yielding an inoculum of about 10^4 cfu ml^{-1}. The frankfurter-type sausages and the pork product were immersed in 3 l of bacterial suspension and the surplus solution was allowed to drip off by placing the slices on a sterile grid. The inoculation took place in a laminar flow cabinet (Nuaire NU-425–400E, Plymouth, MN, USA).

Inoculated and uninoculated control slices were packaged in vacuum and MA (80% CO_2 + 20% N_2). The packaging material used was Cryovac-type bags with low oxygen permeability (35 cm^3 m^{-2} d^{-1} at 22°C, 65% r.h.). The packaging was carried out using a packaging machine (Henkovac 1900, Howden Food Equipment B.V. BA's – Hertogenbosch, The Netherlands). Each package contained two slices of each product. All samples were stored at 4°C for up to 28 d and one package from each occasion was examined at intervals of 0, 3, 7, 14, 21 and 28 d of storage for microbiological and physico-chemical analysis.

Partial purification of the bacteriocins, assay and inoculation

Supernatants (500 ml) (11 000 × *g* at 4°C for 15 min) from an overnight culture of the lactic acid bacteria in MRS broth (MERCK) were adjusted to pH 6·5 with NaOH (1 mol l^{-1}) and treated with catalase (300 U ml^{-1}) (C-3515, Sigma) (Schillinger and Lücke 1989). After that the supernatants were precipitated (60 and 50% saturation for the bacteriocins produced by *Leuc. mesenteroides* L124 and *Lact. curvatus* L442, respectively) with ammonium sulphate (SERVA, Heidelberg, Germany) at 4°C for 18 h (Schobitz *et al.* 1999). The pellicle was collected after the precipitation and the pellet by centrifugation (11 000 × *g* at 4°C for 30 min) and together resuspended in 20 ml of 50 mmol l^{-1} sodium phosphate buffer pH 7. The solutions with the bacteriocins were diluted with the same buffer to 1 : 2 and 1 : 4 for the bacteriocins of *Leuc. mesenteroides* L124 and *Lact. curvatus* L442, respectively, in order to give the same activity, 1280 AU ml^{-1} and sterilized

through a microbiological filter Acrodisc, 0·22 μm (Gelman, MI, USA).

To check bacteriocin activity, two-fold dilutions of the extracts were made in sterile 1/4-strength Ringer's solution. Then 50 μl of the diluted samples were spotted on MRS agar (MERCK) plates containing an indicator strain (*Lact. curvatus* L267). After incubation (30°C for 24 h) the arbitrary units of activity (AU) of the bacteriocin were determined as the reciprocal of the highest dilution showing inhibition of the indicator strain (Barefoot and Klaenhammer 1983). A 1-ml unit of bacteriocin solution was used on each surface of slice with pipette (Gilson, Villiers-le-Bel, France). After assuring good contact of the inoculum with the meat surface (manually massaging of the exterior of the bags), the samples were vacuum packaged (Henkovac 1900) and were stored at 4°C for 28 d.

Microbiological sampling and analysis

A 25-g unit of sample from each experimental case was weighed, aseptically, into a sterile stomacher bag (Seward stomacher 400 bags, London, UK). Then 225 ml of sterile 0·1% (w/v) peptone water (OXOID) were added and the sample was homogenized in a stomacher (Laboratory Blender, Seward, London, UK) for 2 min at normal speed at room temperature. Serial decimal dilutions in sterile 1/4-strength Ringer's solution (OXOID) were prepared from this 10^{-1} dilution and 1 or 0·1 ml samples of the appropriate dilutions were poured or spread in duplicate on total count and selective agar plates.

Total mesophilic counts were determined on Plate Count Agar (PCA, MERCK), incubated at 30°C for 72 h; lactic acid bacteria on de Man, Rogosa, Sharpe Agar (MRS, MERCK), incubated at 30°C for 72 h under anaerobic conditions (Gas-Pack, BBL); *Brochothrix thermosphacta* on Streptomycin Thallous Acetate Agar (STAA) (Gardner 1966), incubated at 25°C for 48 h; micrococci on Kranep Agar (KRA, MERCK), incubated at 30°C for 72 h; pseudomonads on Cetrimide-Fucidin-Cephaloridine (CFC) medium (OXOID), incubated at 25°C for 48 h; enterobacteria in Violet Red Bile Glucose Agar (VRBGA, MERCK), overlayed with 5 ml of the same medium and the plates were incubated at 37°C for 24 h; enterococci on Kanamycin Aesculin Azide Agar (MERCK), incubated at 37°C for 48 h; and staphylococci on Baird-Parker Agar (MERCK), incubated at 37°C for 48 h.

The selectivity of the growth media was checked by carrying out the following rapid tests on about 10% of the colonies grown on countable plates, according to Harrigan and McCance (1976).

The presence of *Listeria monocytogenes* was also determined on the day of packaging. For the detection of *Listeria*, enrichment was done by suspending 25 g unit of sample in 225 ml Listeria Enrichment Broth according to FDA (MERCK) followed by incubation at 30°C for 48 h. After the incubation, the culture was streaked on PALCAM Agar (MERCK) and the plates were incubated at 30°C for 48 h. Characteristic *Listeria* spp. colonies were further identified by the API Listeria system (BioMerieux, Marcy-l'Etoile, France).

pH, a_w assay and colour measurement

pH was measured immediately after the microbiological analysis, immersing the electrode into the Stomacher bag with the diluted sample. For the measurement a digital pH-meter was used (WTW, pH 526, Weilheim, Germany) (Nychas and Arkoudelos 1990). The water activity was measured with a calibrated electric hygrometer, Rotronic DT (Rotronic AG, Bassersdorf, Switzerland).

The surface colour (L [lightness], *a*, *b*) of the meat products was measured with a colorimeter Minolta (Chroma meter CR-200) after the microbiological analysis. The a and *b*-values are given as the average value of 10 determinations, five measurements on each surface.

Chemical analysis

A 10-g sample was homogenized with 60 ml perchloric acid (1 mol l^{-1}). The homogenate was transferred quantitatively with distilled water into a 100-ml volumetric flask, filled up to the mark with water. The homogenate was stored at −20°C until the day of analysis. After thawing, the deproteinized mixture was transferred to a centrifuge tube and was centrifuged at 11 000 *g* for 15 min at 4°C. After centrifugation, 50 ml of supernatant were transferred to a 100-ml beaker and the liquid was adjusted to the final pH 8 with KOH (5 mol l^{-1}). The contents were transferred to a 100-ml volumetric flask, filled up to the mark with distilled water and placed at 4°C for 60 min to precipitate the potassium perchlorate. After cooling in a refrigerator the mixture was filtered through a Whatman No. 1 filter paper. The clear supernatant after filtration was stored at 4°C and used as the sample solution in the assays. Sample concentration in the final sample solution was 50 g l^{-1} and the dilution factor, $F = 1:20$ (Drosinos and Board 1995). D-, L-lactic and acetic acid were assayed enzymatically by the methods described by Gawehn (1984), Noll (1984) and Beutler (1984), respectively. Ammonia was determined colorimetrically by the method of Cheney and Marbach (1962). Boehringer Mannheim GmbH, Mannheim, Germany supplied enzymes and coenzymes and the analytical method, as the calculations, were performed in accordance with the suppliers' instructions.

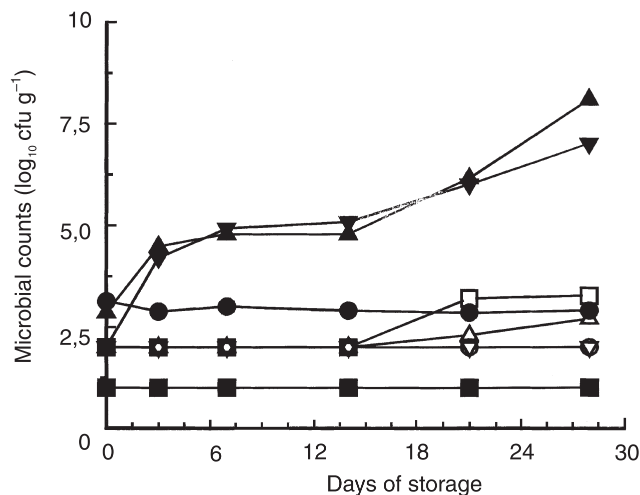

Fig. 1 Development of the spoilage microflora in the uninoculated frankfurter-type sausages, under vacuum at 4°C. □, pathogenic and nonpathogenic staphylococci; ○, enterococci; △, *Brochothrix thermosphacta*; ▽, pseudomonads; ■, enterobacteria; ●, micrococci; ▲, total viable count; ▼, lactic acid bacteria

RESULTS

Microbiological analysis

Figure 1 shows a representative growth of all microorganism types in the uninoculated samples of frankfurter-type sausages. The spoilage flora, apart from lactic acid bacteria, did not grow and regardless of the presence or not of the two bacteriocinogenic strains or their bacteriocins, the population at the end of storage was less than 3 log_{10} cfu g^{-1}, under vacuum or MA. Similar results in the uninoculated and inoculated pork product with the bacteriocinogenic strains or with their bacteriocins, packaged under MA, were obtained.

In the vacuum packaging of the uninoculated pork product growth of enterococci, *B. thermosphacta* and micrococci was observed, with final population *ca* 5–6 log_{10} cfu g^{-1}. The population of pseudomonads and staphylococci at the end of storage was *ca* 3 log_{10} cfu g^{-1} (Fig. 2a). In the inoculated pork product with the bacteriocin-producing strain *Leuc. mesenteroides* L124, after the initial increase of enterococci (0·5–1 log_{10} cfu g^{-1}) and *B. thermosphacta* (2 log_{10} cfu g^{-1}), followed a reduction of their population until the end of storage. The reduction of the two above-mentioned spoilage organisms was *ca* 1·5 log_{10} cfu g^{-1} (Fig. 2b). The results obtained from the other bac^{+} strain, *Lact. curvatus* L442, were similar. In the pork product samples, packed under vacuum inoculated with the bacteriocin produced by *Leuc. mesenteroides* L124, the microorganism *B. thermosphacta* did not grow, while the population of enterococci was *ca* 2 log_{10} cfu g^{-1} less compared to the uninoculated samples at the end of storage and also their growth was delayed by 21 d. The population of micrococci during the storage increased *ca* 4 log_{10} cfu g^{-1} in the uninoculated samples. But in the samples with the bacteriocins added the population increased only 1·8 log_{10} cfu g^{-1} and growth was delayed by 14 d, reflecting the antimicrobial activity of the two bacteriocins against Gram-positive microorganisms (Fig. 2c). The results obtained from the other bacteriocin were similar.

In the frankfurter-type sausages the lactic acid bacteria, endogenous or bacteriocinogenic, showed a delay in their

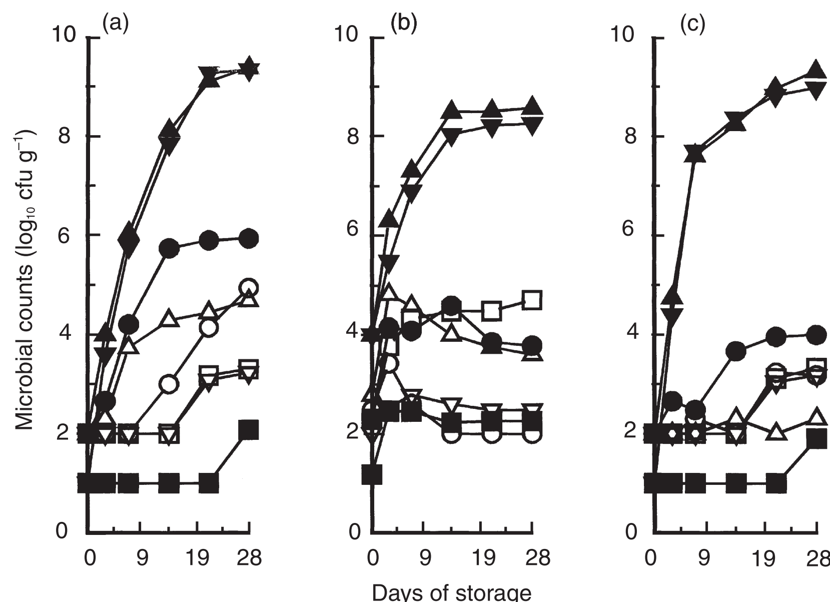

Fig. 2 Development of the spoilage microflora in the uninoculated (a) and inoculated samples of sliced cooked cured pork shoulder with *Leuconostoc mesenteroides* L124 (b) and the bacteriocin from *Leuconostoc mesenteroides* L124 (c), under vacuum at 4°C. □, pathogenic and nonpathogenic staphylococci; ○, enterococci; △, *Brochothrix thermosphacta*; ▽, pseudomonads; ■, enterobacteria; ●, micrococci; ▲, total viable count; ▼, lactic acid bacteria

growth compared to the pork product. The delay of the growth of lactic acid bacteria was more pronounced under MA in comparison with vacuum, showing the effect of MA on the microbial growth. The maximal population (7–8 $\log_{10}$ cfu g^{-1}) of lactic acid bacteria in the frankfurter-type sausages was attained at the end of storage (28th d) and in the pork product the same level of population was achieved earlier (14th d). The initial number of the lactic acid bacteria in the inoculated samples with the two strains was 1 $\log_{10}$ cfu g^{-1} more than the uninoculated samples, because of their inoculation with the bacteriocin-producing lactic acid bacteria. Also, in all samples the microflora was dominated by lactic acid bacteria. Therefore, the substrate and the packaging have an influence on the microbial growth.

pH, a_w assay and colour measurement

The a_w of the pork product remained constant (0·98) until the end of storage, whereas a_w of the frankfurter-type sausages showed a reduction from 0·99 to 0·97. The colour remained acceptable during the storage and did not show changes until the end of the experiment. The *a* and *b* values remained close to the initial values, in the uninoculated and inoculated samples with the bac^+ strains or with their bacteriocins, in both products (Table 2). In the frankfurter-type sausages the pH value remained constant (6·5–6·3) in both packages, except for the uninoculated and inoculated with *Lact. curvatus* L442 samples, stored under vacuum, in which the pH decreased (5·8) at the end of storage (28th d). In the pork product the reduction of pH, for the inoculated samples with the bac^+ strains and for those with their bacteriocins, under vacuum or MA packaging, was similar. The pH value decreased from 6·6 to 5·1–5·3 and to 5·5–5·6 for the vacuum and MA packaged samples, respectively.

Chemical analysis

In the frankfurter-type sausages, packed under vacuum or MA conditions, the concentration of L-lactate decreased during the storage in both uninoculated and inoculated samples (Fig. 3a). A production of D-lactate at the end of storage in the uninoculated (139 mg per 100 g or 15·4 mmol kg^{-1}) and inoculated (190·9 mg per 100 g or 21·2 mmol kg^{-1}) with *Lact. curvatus* under vacuum conditions coincided with a pH reduction. Under MA conditions the D-lactate concentration remained constant (14·1 mg per 100 g or 1·6 mmol kg^{-1}) for all samples (Fig. 3b). The levels of acetate and ammonia did not increase or decrease (Fig. 4a,b).

A reduction of the L-lactate concentration was observed for the inoculated samples of the pork product with *Leuc. mesenteroides* L124 and *Lact. curvatus* L442 under vacuum or MA (Fig. 5a). The D-lactate (Fig. 5b) and acetate (Fig. 6a) concentrations increased during the storage in the uninoculated and inoculated samples. Ammonia showed a slight increase at the end of storage, but it was not significant to assume that the proteolysis was extensive (Fig. 6b). The results obtained from the inoculated samples with the bacteriocins, under vacuum or MA, were the same as those mentioned before for the frankfurter-type sausages and for the pork product (results not shown).

Despite the high plate counts in the frankfurter-type sausages the overall aspect of the samples after 28 d of storage at 4°C was still better than that of the pork product. At the end of storage, when the spoilage was visible in the pork product (drip loss and slime formation) but not in the frankfurter-type sausages, the D-lactate levels were 37·8 mmol kg^{-1} (340·9 mg per 100 g) and 54 mmol kg^{-1} (486·9 mg per 100 g) in the vacuum, and in the MA were 13·3 mmol kg^{-1} (119·8 mg per 100 g) and 40·7 mmol kg^{-1} (366·5 mg per 100 g), in the presence of *Leuc. mesenteroides*

Table 2 Colour change in inoculated samples with the two strains

	Frankfurter-type sausage			Pork product		
	Initial numbers	Final numbers		Initial numbers	Final numbers	
Samples	VP/MAP	VP	MAP	VP/MAP	VP	MAP
Uninoculated	19·72*	19·76	19·53	15·69	16·32	15·98
	24·31†	24·61	24·05	9·77	10·05	9·83
Leuc. mesenteroides L124	19·15	19·56	18·85	13·01	13·68	13·08
	23·32	23·30	22·87	9·58	9·97	9·40
Lact. curvatus L442	18·82	18·99	18·61	14·62	14·12	14·69
	23·37	23·59	23·47	9·80	9·69	9·23

VP, vacuum packaging; MAP, modified atmosphere packaging.
*a-value.
†b-value.

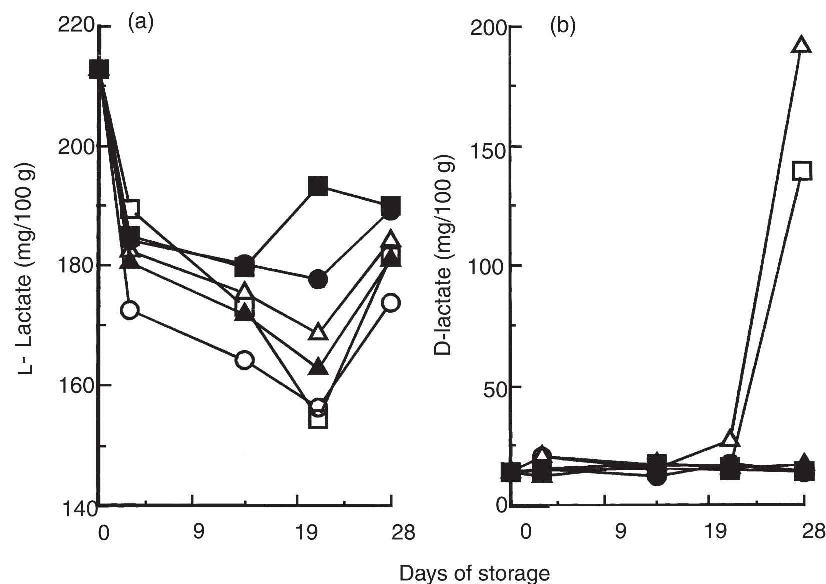

Fig. 3 Changes in (a) L-lactate and (b) D-lactate concentration in the frankfurter-type sausages, under vacuum (open symbols) and MA (80% CO_2 + 20% N_2) (solid symbols) at 4°C. □/■, uninoculated; ○/●, presence of *Leuconostoc mesenteroides* L124; △/▲, presence of *Lactobacillus curvatus* L442

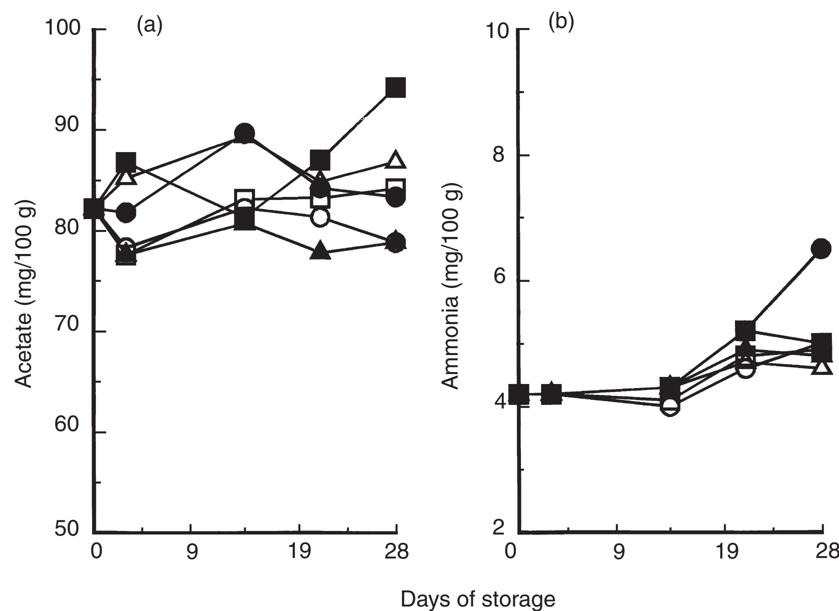

Fig. 4 Changes in (a) acetate and (b) ammonia concentration in the frankfurter-type sausages, under vacuum (open symbols) or MA (80% CO_2 + 20% N_2) (solid symbols) at 4°C. □/■, uninoculated; ○/●, presence of *Leuconostoc mesenteroides* L124; △/▲, presence of *Lactobacillus curvatus* L442

and *Lact. curvatus*, respectively. In the uninoculated samples the D-lactate concentration was 53·5 mmol kg^{-1} (482·4 mg per 100 g) and 30·9 mmol kg^{-1} (278·1 mg per 100 g) in vacuum and MA packaging, respectively. In the frankfurter-type sausages the D-lactate concentration was 1·5 mmol kg^{-1} (13·5 mg per 100 g) and 21·2 mmol kg^{-1} (190·9 mg per 100 g) in the vacuum, and in the MA was 1·6 mmol kg^{-1} (14·1 mg per 100 g) and 1·9 mmol kg^{-1} (16·7 mg per 100 g), in the presence of *Leuc. mesenteroides* L124 and *Lact. curvatus* L442, respectively. The results were similar for the uninoculated and inoculated with the bacteriocins samples of frankfurter-type sausages and pork product, under vacuum or MA packaging. The shelf life of the products was the same for both vacuum and MA, as between uninoculated and inoculated with the bac^+ strains or with their bacteriocins. The shelf life for the frankfurter-type sausages was more than four weeks, because there was no slime formation, drip loss and no, as can be seen from the results, D-lactic or acetic acid production. For the pork product the shelf life was three weeks because of the high concentration of D-lactic and acetic acid, but slime formation and drip loss was observed after four weeks.

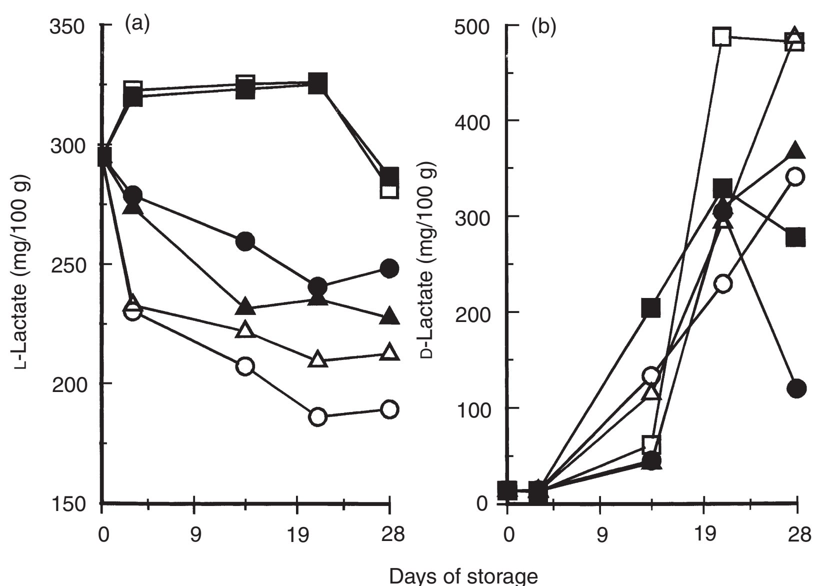

Fig. 5 Changes in (a) L-lactate and (b) D-lactate concentration in the sliced cooked cured pork shoulder, under vacuum (open symbols) and MA (80% CO_2 + 20% N_2) (solid symbols) at 4°C. □/■, uninoculated; ○/●, presence of *Leuconostoc mesenteroides* L124; △/▲, presence of *Lactobacillus curvatus* L442

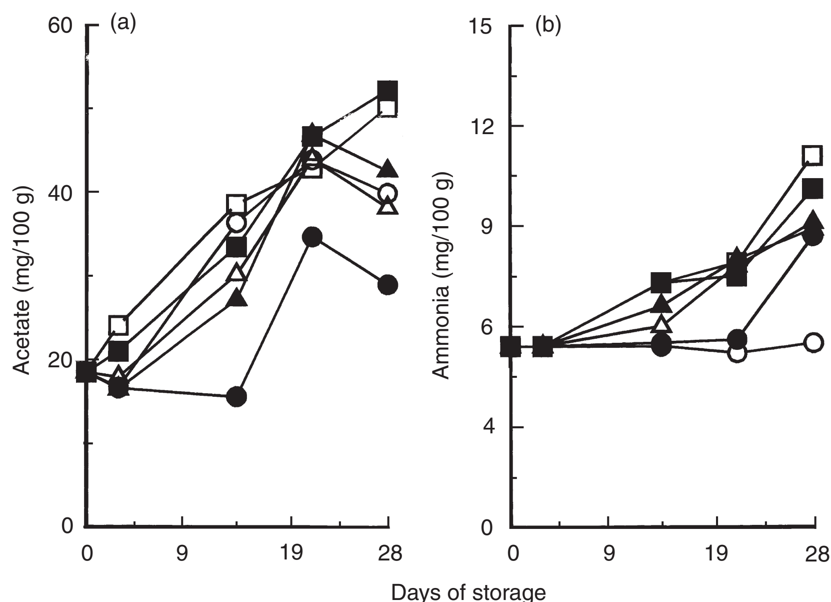

Fig. 6 Changes in (a) acetate and (b) ammonia concentration in the sliced cooked cured pork shoulder, under vacuum (open symbols) or MA (80% CO_2 + 20% N_2) (solid symbols) at 4°C. □/■, uninoculated; ○/●, presence of *Leuconostoc mesenteroides* L124; △/▲, presence of *Lactobacillus curvatus* L442

DISCUSSION

The substrate and the packaging are two factors which affect the spoilage flora growth. In respect of the microflora composition, there were differences between the two meat products. In the vacuum-packed pork product, growth of spoilage microorganisms, apart from lactic acid bacteria, such as pseudomonads, *B. thermosphacta*, enterococci and enterobacteria was observed, but the final population of pseudomonads and enterobacteria was not high. In the pork product inoculated with the two lactic acid bacteria, the population of *B. thermosphacta* and enterococci, after the initial increase, reduced and this indicates the production of antimicrobial substances by the lactic acid bacteria, probably bacteriocins. Inhibition of other species by the lactic acid bacteria has been ascribed to lactate or hydrogen peroxide production, though neither of these could be the cause in anaerobically stored meat because the amount of lactate produced is negligible compared with that already present in meat and the latter it is not formed in the absence of oxygen (Gill 1982). In addition, the results showed that the two bacteriocins had an effect on *B. thermosphacta* and enterococci growth because they were not able to grow compared to the uninoculated samples. In both products and in all

samples the predominant microorganisms were the lactic acid bacteria. As mentioned before *B. thermosphacta* and enterococci firstly increased and then reduced. This could have been explained by the fact that the bacteriocins from the two lactic acid strains are produced after 24–48 h and they reached the maximum activity after 7 d of incubation of lactic acid bacteria at 4°C (results from other experiment). Results from this study showed that the bacteriocins did not inhibit the endogenous lactic acid bacteria. In order to suppress the growth of the naturally growing lactic acid bacteria the inoculation of a lactic starter culture plus the bacteriocin could be an effective way to control undesirable organoleptic changes in vacuum or MA packaged meat products (Schobitz *et al.* 1999).

In the present study, maybe the reduction of a_w value from 0·99 to 0·97 was one of the reasons that spoilage flora did not grow in the frankfurter-type sausages. That did not apply to pork product. Also the lactic acid bacteria in the frankfurter-type sausages showed decrease in the growth rate because of the reduced a_w value, whereas in the pork product the a_w value remained constant until the end of storage (0·98).

In the vacuum packaging the combination of the micro-aerophilic conditions, the low temperature (4°C) and the presence of curing salts favours the growth of psychrotrophic lactic acid bacteria such as *Lactobacillus* and *Leuconostoc (*von Holy *et al.* 1991). The initial pH value of the frankfurter-type sausages and pork product were 6·5 and 6·6, respectively. The initial pH value does not affect the microbial growth of the spoilage flora because is high, between 6·0 and 6·5 (Dainty and Mackey 1992). The reduction of pH to the value 5·0–5·3, because of the lactic acid production by the lactic acid bacteria (Dykes *et al.* 1991), can inhibit the growth of *B. thermosphacta* because this microorganism does not grow at pH values less than 5·5, but this does not apply to genus of *Lactobacillus* (Blickstad 1983).

Storage under MA (CO_2 plus N_2) can increase the shelf life of the cooked cured meat products compared to vacuum packaging, because the growth rate of the lactic acid bacteria is reduced in CO_2-atmosphere (increase of the lag phase) compared to aerobic and vacuum storage (Blickstad and Molin 1983, 1984; Borch *et al.* 1996). But some studies indicated no extension of the shelf life of MA packaged meat products (Simard *et al.* 1983; Boerema *et al.* 1993). In our study, differences in the shelf life of the products between vacuum and MA packaging were not observed. The results showed that the bac^+ strains or their bacteriocins did not affect negatively the shelf life of the products. In addition, the final population of the spoilage microorganisms, namely lactic acid bacteria, cannot be the only parameter or the indicator for the evaluation of the shelf life of the meat products. Under MA the growth of the aerobic microorganisms, which are responsible for the spoilage of the meat products, packaged under aerobic conditions at chill temperatures is restricted because of the high concentration of CO_2 and the absence of O_2, thus the lactic acid bacteria become the dominant microflora (Ingram 1962). One of the reasons that the above spoilage microorganisms are the most common species detected, is their ability to catabolise the substracts present in meat and meat products. All the common psychrotrophic spoilage species under anaerobic conditions utilize the glucose as carbon source and one or two other substances to supply their energy needs (Nychas *et al.* 1988).

Lactic acid bacteria are the major group associated with the spoilage of refrigerated vacuum or MA packaged cooked cured meat products (Blickstad and Molin 1983; Shaw and Harding 1989; von Holy *et al.* 1991; Borch *et al.* 1996). Lactic acid bacteria spoil refrigerated meat products by causing defects such as sour off-flavours, discolouration, gas production, slime formation and decrease in pH (Borch *et al.* 1996). Also the spoilage of the products develops slowly and only after the attainment of the maximal population of lactic acid bacteria (Gill 1982). This observation explains the fact that, in the present study, at the end of storage the surface of the pork product was wet, because of the slime formation. That did not apply to frankfurter-type sausages. One explanation of this is that in the frankfurter-type sausages the lactic acid bacteria showed a time lag in their growth compared to the pork product. The slime formation associated with cooked cured meat products is caused by homofermentative *Lactobacillus* sp. and *Leuconostoc* sp. (Korkeala *et al.* 1988; von Holy *et al.* 1991; Dykes *et al.* 1994; Borch *et al.* 1996).

In this study, an increase in D-lactate and a decrease in L-lactate concentration were observed during the storage under vacuum or MA. Though racemase activity has been isolated from lactic acid bacteria (*Lactobacillus sakei*) (Garvie 1980), no such activity was observed with *Lactobacillus casei* subsp. *rhamnosus* (Hjorleifsdottir *et al.* 1990) and for one strain from the genus *Lactobacillus* (Borch and Agerhem 1992). Therefore, the reduction of the L-lactate concentration can be attributed to its metabolism by the microorganisms. These findings coincide with those of other researchers (de Pablo *et al.* 1989; Hjorleifsdottir *et al.* 1990; Ordoñez *et al.* 1991; Borch and Agerhem 1992; Kakouri and Nychas 1994; Nychas 1994; Drosinos and Board 1995; Lambropoulou *et al.* 1996). Additionally, it was observed that the D-lactate production is delayed in the frankfurter-type sausages packed under vacuum conditions compared to the pork product. This can be explained by the fact that the lactic acid bacteria delayed to attain the maximal population in comparison with the pork product. Generally, the production of D-lactate is more pronounced at the stationary phase (i.e. after the attainment of the maximal population), whereas the production of L-lactate usually occurs at the first stages of the microbial growth (Garvie 1980).

During the storage the concentration of acetic acid increased in the pork product, whereas in the frankfurter-type sausages the concentration of acetate remained constant. The increase in acetate found in the inoculated samples was most pronounced during the stationary growth phase. Therefore, a high level of acetate indicated a high bacterial number. The same results were found in other studies (de Pablo *et al*. 1989; Ordoñez *et al*. 1991; Borch and Agerhem 1992; Drosinos and Board 1994; Nychas 1994; Lambropoulou *et al*. 1996). The latter probably is explained by the fact that the lactic acid bacteria in the frankfurter-type sausages showed a lag time in their growth. Leuconostocs as heterofermentative bacteria produce acetic acid. Acetate is not an end product expected to be produced by a homofermentative *Lactobacillus* sp. under anaerobic conditions (Kandler 1983). However, it has been demonstrated for *Lactobacillus* sp. and for *Lactococcus lactis* that the production of acetate may be induced during conditions simulating semistarvation (Thomas *et al*. 1979; Borch *et al*. 1991; Hjorleifsdottir *et al*. 1991). Thus, the supply of glucose to each individual bacterial cell is likely to be insufficient to support a homofermentative metabolism when the bacterial numbers are high, for example on a meat surface during storage (Borch and Agerhem 1992). Also the anaerobic conditions can cause a shift in the metabolic pathways of the lactic acid bacteria (Thomas *et al*. 1979; Borch and Molin 1989; de Pablo *et al*. 1989; Ordoñez *et al*. 1991; Borch and Agerhem 1992; Kakouri and Nychas 1994; Nychas 1994).

Ammonia concentration in the frankfurter-type sausages remained constant, while in the pork product a slight increase was observed at the end of storage. Ammonia is a by-product of microbial metabolism and derived mainly from the deamination of amino acids, peptides or amines. The ammonia production is observed after the attainment of the maximal population and when the spoilage has already been started and this can lead to an increase of pH (Gill 1982; Kakouri and Nychas 1994; Nychas 1994; Lambropoulou *et al*. 1996).

D-Lactate has been suggested to be a parameter of the bacterial contamination of vacuum or MA packed meat and meat products (de Pablo *et al*. 1989; Borch and Agerhem 1992; Lambropoulou *et al*. 1996). Sinell and Luke (1979) concluded that meat products with a D-lactate level of less than 5·5 mmol kg^{-1} were sensorially acceptable, whereas levels above 11 mmol kg^{-1} were beginning to spoil and levels above 33 mmol kg^{-1} were completely spoiled. Borch and Agerhem (1992) also found that for meat samples that were beginning to spoil the concentrations of D-lactate were about 10 mmol kg^{-1} and 40 mmol kg^{-1} in the presence of *Leuconostoc* sp. and *Lactobacillus* sp., respectively. Schneider *et al*. (1983) found that, whereas D-lactate values above 100 mg per 100 g were invariably related to perceptible organoleptic changes, only when D-lactate reached values above 200–400 mg per 100 g were meat products overtly spoiled.

The two lactic acid strains (i) have the ability to produce antimicrobial agents (bacteriocins) during the cold storage, (ii) are well adapted to growth in the meat products, since they have been isolated from dry fermented salami, (iii) constitute the dominant flora, and finally (iv) have no undesirable influence on the product quality. These microorganisms could, therefore, be used as biopreservatives or protective cultures to inhibit other spoilage microorganisms or even pathogens, extending the shelf life of the products and increasing the meat safety. Additionally, their bacteriocins have an effect on the growth of spoilage microorganisms, such as enterococci and *B. thermosphacta*; therefore they could contribute to the increase of meat microbiological quality and safety.

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