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ORIGINAL ARTICLE

Growth of *Listeria monocytogenes* on sliced cooked meat products

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During the shelf life (4–6 weeks) of artificially contaminated sliced cooked meat products such as luncheon meat, ham and chicken breast, the growth of Listeria monocytogenes *under vacuum was similar to the growth under modified atmosphere (30% CO_2/70% N_2) packaged products. The presence of competitors (lactobacilli), even in concentrations 100 times those of* L. monocytogenes*, only slightly inhibited growth of this pathogen. At the end of the shelf life levels were still 10^7 cfu g^{-1}. Due to the lower initial contamination, levels in naturally contaminated products were about 10^4 cfu g^{-1}. To prevent outgrowth of* L. monocytogenes *to such high levels it is necessary to prevent recontamination during slicing and packaging, and to shorten the rather long shelf life of these products. Due to the low pH of fermented sausage (saveloy) and (raw) Coburger ham the numbers of* L. monocytogenes *decreased below the detection level.*

Introduction

Listeria monocytogenes has been isolated from a wide variety of foodstuffs, including raw and cooked meat products. Conflicting results have been published about the growth of this pathogen on raw meat products. Nevertheless, growth appears to be highly dependent on pH, temperature, type of tissue and the competitive microflora (Farber and Peterkin 1991). In cooked meat products *L. monocytogenes* should be absent. The heating of these products for 2 min at 70°C, or to core temperatures of 71°C, is likely to inactivate any *L. monocytogenes* present (Gaze et al. 1989, Mackey et al. 1990, Zaika et al. 1990).

Although reports on survival of *L. monocytogenes* during processing have been published regularly, evidence for this is often obtained by heating meat products with unrealistic high levels (10^7–10^8 cfu g^{-1}) of added listeriae (Boyle et al. 1990, Michel et al. 1991, Hardin et al. 1993). The presence of heat-stressed cells is probably only of interest when cells have been exposed to sublethal temperatures for short periods of time, because this significantly increases the heat resistance (Linton et al. 1992). However, these results were also obtained in experiments using high initial levels of *Listeria*.

Because raw meats generally contain low numbers of *Listeria* (less than 100 cfu g^{-1}) (Sheridan et al. 1994), the decimal reductions obtained with the heating processes normally applied are sufficient to ensure safe products (Mackey et al. 1990). This may be supported by the findings of Wang and Muriana (1994). They found *Listeria* spp. in the liquid exudate only of retail frankfurters and not in the internal meat, indicating that the presence of *Listeria* was most likely due to post-process contamination. The incidence of *L. monocytogenes* on prepacked sliced cooked meat

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products has been reported by a number of workers (McLauchlin and Gilbert 1990, Grau and Vanderlinde 1992, Pinner et al. 1992, Sheridan et al. 1994). Because these products are supposed to have a refrigerated shelf life of several weeks, it is important to know the growth potential of this pathogen on cooked meat products. From previous work it is known that luncheon meat, ham and cooked chicken breast were the most frequently contaminated cooked meat products in the Netherlands (De Boer 1990). In this study, these products were artificially contaminated with low numbers of *L. monocytogenes*. The samples were packaged under modified atmosphere, stored at 7°C and within the shelf life of these products (4–6 weeks), the extent of growth of *L. monocytogenes* and competitors (lactobacilli) was determined.

Material and Methods

Bacterial strains and inoculum preparation

L. monocytogenes, isolated from sliced roast beef, *Lactobacillus curvatus*, isolated from luncheon meat, and *Lactococcus lactis* (a nisin producing strain from our collection) were used in this research. *L. monocytogenes* was grown in brain–heart infusion broth (BHIB, Difco 0037-01-6) and *L. curvatus* and *L. lactis* were grown in de Man, Rogosa and Sharpe broth (MRSB, Oxoid CM 359) at 30°C. After 20–24 h serial dilutions were made in peptone physiological saline solution (PPSS) (composition: NaCl 8·5 g l^{-1}, neutralized bacteriological peptone (Oxoid L 34) 1 g l^{-1}).

To check the inoculum level appropriate serial dilutions were plated onto tryptone soya agar (TSA, Oxoid CM 131) for *Listeria* and on MRS-agar (Oxoid CM 361) for lactobacilli. Plates were incubated 2–3 days at 30°C.

Meat products and inoculation

Sliced cooked meat products (luncheon meat, ham, chicken breast), fermented sausage (saveloy) and raw ham (Coburger) were transported 1–3 days after production in insulated cool boxes to the laboratory. The packages were opened aseptically and the contents was transferred, after removing the upper slice, into sterile vacuum bags (polyamide/polyethylene). The meat products were inoculated (on the surface or between two central slices) with 100 µl of the 10^4 inoculum of *L. monocytogenes*, resulting in an inoculum level of 10 cfu g^{-1}. In some experiments the surface of the meat products was also inoculated with 100 µl of 10^3–10^5 inocula of *L. curvatus* or *L. lactis*, to investigate inhibitory effects on the growth of *L. monocytogenes*. With a sterile Drigalsky the suspensions were spread over the surface. Directly after inoculation, the meat products were packaged in 30% CO_2/70% N_2 or vacuum, using a tabletop apparatus for vacuum and modified air packaging, type ALVAC I-90 (Stephan, Almelo, Netherlands). The inoculated products and control samples (uninoculated products in original packages and in vacuum bags) were stored at 7°C until the end of the shelf life indicated by the manufacturer.

Microbiological investigation

After 1, 4, 7, 11, 14, 28 and 37 days the levels of *L. monocytogenes* were determined on enhanced haemolysis agar. Composition of the medium (amount l^{-1}): tripticase soy blood agar base EH (TSA-EH, Difco 0028-17-9) 40 g; 4-methylumbelliferyl-β-D-glucoside (MUβG, Sigma M3633) 50 mg; lithium chloride 5 g; PALCAM supplement (Merck 12122) 2 vials; sphingomyelinase (Sigma S8633) 10 U; sheep blood (sterile, defribinated) 50 ml. TSA-EH and MUβG were weighed in distilled water (950 ml), after soaking the pH was adjusted to 7·3 and the medium was sterilized for 15 min at 121°C. After cooling the medium to 50°C the other ingredients were added in the following order. Lithium chloride was added first as a 50% (w/v) solution in distilled water, then the PALCAM supplement dissolved in 1 ml of distilled water, followed by sphingomyelinase dissolved in 1 ml PBS buffer (Composition (amount l^{-1}): $Na_2HPO_4 \cdot 2\ H_2O$ 1·44 g; NaCl 8 g; KCl 0·2 g). Finally, the sheep blood was added, the medium was shaken carefully and

thin plates 10–13 ml were poured. The shelf life of the plates was 1 month at 4–7°C.

All *Listeria* spp. appear to be fluorescent under UV light (366 nm), in addition, *L. monocytogenes* shows haemolysis. Presumptive *Listeria* colonies from control samples were purified by streaking on a non-selective medium (TSA) and incubated for 2 days at 30°C. With colonies demonstrating a characteristic blue colour under Henry illumination, confirmation was carried out testing for Gram reaction (Gram-positive bacilli or coccobacilli), motility (umbrella type), presence of catalase (+) and oxidase (–) and haemolytic activity (+ or –). Colonies complying these tests were transferred to TSA slants, incubated for two days at 30°C and further identified using the API *Listeria* (bioMérieux, Lyon, France).

For competitive micro-organisms (including lactobacilli) PCA^+-medium was used. Composition (g l^{-1}): plate count agar (PCA, Oxoid CM 325) 13 g; MRSB 13 g and Agar (Oxoid L 11) 5 g.

At each sampling three packages were opened aseptically and the whole contents transferred into a Stomacher bag. The meat product was diluted (1:2) with PPSS, divided into pieces by hand kneading (30 s) and subsequently homogenized in a Stomacher for 2 min. After 5 min, 15 ml of the homogenate was transferred into sterile tubes and placed in melting ice.

Within 1 h, this suspension and appropriate serial dilutions in PPSS, were plated onto EHA and PCA^+. Directly after plating, the pH of the samples was determined in the primary dilution. Plates were incubated at 37°C (2 days) for *Listeria* and at 30°C (3 days) for total counts. Growth curves were generated from the experimental data by using the Gompertz equation. The growth rate and lag time were calculated by using a growth-curve fitting program (Zwietering et al. 1990).

Determination of gas atmosphere

In all series concentrations of O_2, CO_2 and N_2 were determined at day 1, after 3 weeks and by the end of shelf life, using a gas chromatograph (Fisons, GC 8000).

Effect of added lactic acid on the pH of luncheon meat and ham

Lactic acid (CCA, Biochem) was added to 100 g meat products (not inoculated) to final concentrations of 0, 0·5. 1·0, 1·5, 2·0 and 2·5% (w/w). After mixing in a blender (Waring 8010, New Hartford, CT, USA) for 20 s, the product was placed at 7°C and pH was measured after 1·5, 3 and 24 h.

Production of lactic acid in meat products

After 3, 29 and 39 days of storage concentration of lactic acid was investigated using an enzyme kit (Boehringer lactic acid test combination, 1112821). After mixing 100 g of meat product in a blender, a 5 g sample was added to 20 ml of 1 M perchloric acid. Then the concentration of lactic acid was determined according to the manufacturer's instructions.

Results

Growth of *L. monocytogenes* and plate counts are presented in the graphs as mean values of three packages. The growth of *L. monocytogenes* on meat products in 30% CO_2/70% N_2 is presented in Fig. 1 and was similar to growth on vacuum packed products (Fig. 2). Within the producer's sell by date, the initial numbers increased up to 10^8 cfu g^{-1}.

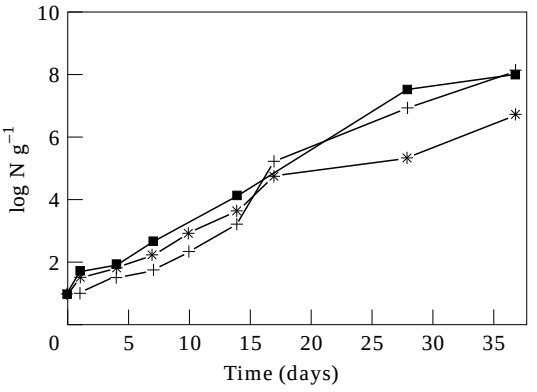

Figure 1. Growth of *Listeria monocytogenes* on MAP (30% CO_2/70% N_2) luncheon meat (+), cooked ham (*) and chicken breast (■) at 7°C.

The normal spoilage flora of cooked, vacuum or modified atmosphere packed, meat products mainly consists of lactic acid bacteria. *L. delbrueckii* subsp. *delbrueckii* and *L. curvatus* were the strains most frequently isolated from luncheon meat. Even high numbers of lactic acid bacteria did not affect the growth of *L. monocytogenes* in 30% CO_2/70% N_2 (Fig. 3) and the vacuum packed product (Fig. 4).

Leuconostoc mesenteroides subsp. *mesenteroides* and *L. lactis* subsp. *lactis* were the predominant spoilage organisms in ham, and *L. fermentum* and *L. mesenteroides* subsp. *mesenteroides* in chicken breast. Generation times for lactic acid bacteria in these products were comparable with those in luncheon meat or even shorter. When counts of lactic acid bacteria reached values of about 10^8 cfu g^{-1}, growth of *L. monocytogenes* decreased as is shown for vacuum packed ham in Fig. 4. In spite of the high numbers of lactobacilli in all products, only a slight decrease in pH (about 0·5 unit) was observed.

In control samples of ham and chicken breast, both in original packages and in repacked vacuum bags, *L. monocytogenes* was detected. Because this strain was not detected in all packages, growth curves were somewhat irregular. Fig. 5 shows that these listeriae reached numbers of 10^4 cfu g^{-1} at the end of the storage of chicken breast in the original packages. The growth rates on chicken breast for this isolate (μ=0·57 day^{-1}),

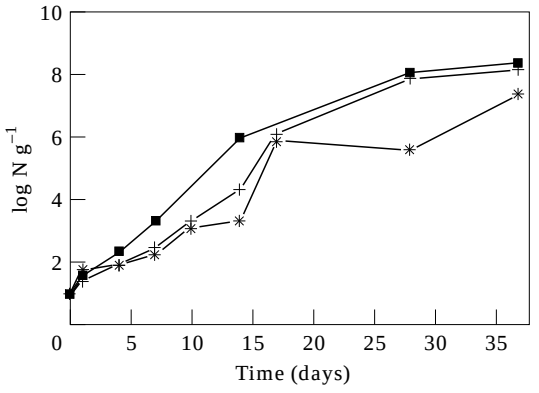

Figure 2. Growth of *Listeria monocytogenes* on vacuum packaged luncheon meat (+), cooked ham (*) and chicken breast (■) at 7°C.

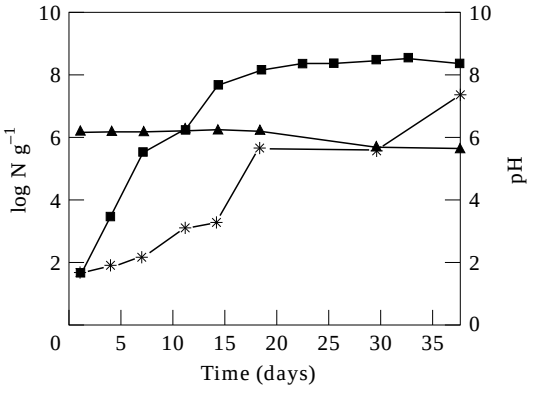

Figure 3. Growth of *Listeria monocytogenes* (*) and lactic acid bacteria (■) on MAP (30% CO_2/70% N_2) luncheon meat, and the effect on pH (▲) at 7°C.

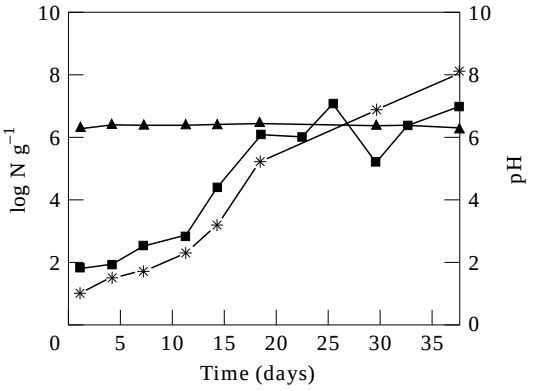

Figure 4. Growth of *Listeria monocytogenes* (*) and lactic acid bacteria (■) on vacuum packaged cooked ham, and the effect on pH (▲) at 7°C.

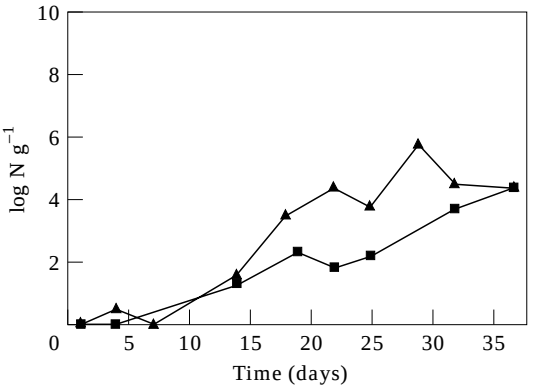

Figure 5. Growth of *Listeria monocytogenes* on chicken breast in controls (▲, repackaged in vacuum; ■, original package) at 7°C.

the isolate from vacuum-packaged chicken breast (μ=0·55 day^{-1}) and for the test strain (μ=0·60 day^{-1}) were comparable.

In Table 1 the growth parameters of *L. monocytogenes* in the presence of *L. curvatus* are shown. Increasing initial concentrations of lactobacilli slightly decrease the growth rate of *L. monocytogenes*. Because *L. curvatus* does not produce antibacterial substances such as bacteriocins, this experiment was repeated with *Lc. lactis*, a nisin producing strain. Growth rates and lag phases of *L. monocytogenes* were similar to those determined in presence of *Lb. curvatus* (data not shown).

No growth of *L. monocytogenes* was observed during the shelf life (6 weeks) of saveloy and (raw) Coburger ham. Initial concentrations of approximately 10^2 cfu g^{-1} decreased to below the detection level (< 3 cfu g^{-1}).

During storage, only slight differences in gas composition were observed. The O_2 concentration in the original packages decreased from 2 to less than 1%. In the other packages the O_2 concentration varied from 1–3%. Percentages of CO_2 increased from 12–28% in the original packages, and from 7–23% in repacked bags. In the original packages the N_2 concentration varied from 72–86%, which was similar to the concentrations determined in the other packages (76–90%).

Addition of lactic acid to meat products resulted in a decrease in pH, as is shown in Table 2. During the storage of the meat products no increase in lactic acid concentration was detected (data not shown).

Table 1. Growth parameters for *Listeria monocytogenes* in the presence of *Lactobacillus curvatus* in modified atmospheric packaged luncheon meat

Sample	Growth rate (day^{-1})	Lag phase (days)
Control, repacked	0·62	4·12
L. monocytogenes + *L. curvatus* 10^0 cfu g^{-1}	0·56	2·85
L. monocytogenes + *L. curvatus* 10^1 cfu g^{-1}	0·50	2·72
L. monocytogenes + *L. curvatus* 10^2 cfu g^{-1}	0·49	3·52

Table 2. Influence on pH by adding lactic acid to meat products

Lactic acid (%)	pH Luncheon meat	pH Ham
0	6·3	6·1
0·5	5·3	5·2
1·0	4·6	4·7
1·5	4·1	4·3
2·0	3·9	4·1

Discussion

In this study the growth of *L. monocytogenes* on cooked meat products has been investigated. In all experiments meat products were inoculated with diluted overnight cultures of *L. monocytogenes*, grown for 20–24 h at 30°C. In the production environment contamination with listeriae grown at lower temperatures is probably more relevant. However, the growth rates of test strains grown at 7°C and 30°C were similar, μ=0·64 and 0·62 respectively, the growth temperature only affected the lag phase. This is in agreement with the findings of Buchanan and Klawitter (1991). Neither the place of inoculation (on the surface or between slices) (data not shown) nor the composition of the gas atmosphere (30% CO_2/70% N_2 or vacuum) (Figs 1–4) influenced growth rate and lag phase. It has been shown previously that packaging of sliced roast beef in a saturated CO_2 atmosphere extended the shelf life compared with the identical product stored under vacuum (Penney et al. 1993). The CO_2 concentrations in the MAP products in this study varied from 7–28%. These concentrations are far too low to inhibit growth of *Listeria*, because growth of *L. monocytogenes* on sliced roast beef (pH 6·1) was even observed in a saturated CO_2 atmosphere at 3°C (Hudson et al. 1994). The effectiveness of MAP increases with decreasing temperatures and O_2 concentrations (Marshall et al. 1991). But it is unrealistic to maintain temperatures as low as 3°C in the chill-chain. The growth of *L. monocytogenes* at 7°C on precooked chicken nuggets in the absence of oxygen (80% CO^2/20% N_2) was similar to the growth we observed in our experiments.

It is well-known that reduced oxygen and

increased carbon dioxide concentrations in food packages can increase shelf life via the inhibition of aerobic spoilage bacteria. However, the growth of psychrotrophic *L. monocytogenes* and spoilage organisms under such environments will not be inhibited as is shown in Figs. 1–4. Within the shelf life of the investigated products low initial numbers of *L. monocytogenes* increased up to 10^8 cfu g^{-1}. This agrees with previous work on the fate of *L. monocytogenes* on processed meats (Glass and Doyle 1989, Johnson et al. 1990, McKellar et al. 1994). In our experiments the growth rate of the test strain on chicken breast and luncheon meat was slightly higher than that on ham. In other studies, levels of *L. monocytogenes* on ham were either incapable of multiplying or increased very slowly. This was attributed to differences in the composition of the products (Grau and Vanderlinde 1992, Farber and Daley 1994), but may also be explained by the use of lower storage temperatures and/or the presence of higher levels of background flora (lactic acid bacteria). It has often been reported that lactic acid bacteria might inhibit the growth of *Listeria* (McKellar et al. 1994, Schmidt 1995). As can be seen from Figs 3 and 4 development of *L. monocytogenes* is comparable with that of lactic acid bacteria. This can be explained by the fact that both the inoculation level of *Listeria* (about 10 cfu g^{-1}) and the contamination level of lactic acid bacteria (10–100 cfu g^{-1}) were low. Because of this, mutual interaction between the (micro)colonies formed was unlikely. Increasing the numbers of lactobacilli by a factor 100 resulted in a decrease of the growth of *L. monocytogenes*. However, it still reaches numbers of 10^7–10^8 cfu g^{-1} during storage. Other workers have observed similar effects (Hudson et al. 1994). To overcome this the use of bacteriocin-producing strains of lactic acid bacteria has been proposed (Berry et al. 1991, Foegeding et al. 1992). In our experiments we could not demonstrate a stronger inhibitory effect by the use of a nisin-producing *L. lactis*. Growth rates were similar to those of *L. curvatus* (Table 2). In all probability effective concentrations of nisin are only present when high levels of lactococci are reached (in the late log phase). In an experiment where nisin (Sigma N 5764) was added in a concentration of 25 mg kg^{-1} to luncheon meat, there was an initial decrease in numbers of *L. monocytogenes* in the product with nisin added. After storage for 1 week at 7°C, the levels were about 2 log units lower compared with the controls, but at the end of the storage time (after 6 weeks) counts in both products exceeded 10^7 cfu g^{-1} (data not shown). This phenomenon is due to the rapidly decreasing activity of nisin on meat surfaces (Chung et al. 1989, Fang and Lin 1994).

The decrease in the numbers of *L. monocytogenes* on saveloy and raw ham can be explained by the low pH of these products (saveloy, pH 4·3–4·5 and raw ham, pH 5·5–5·7). This agrees with results of other workers (Sabel et al. 1991, Farber et al. 1993).

Becuase spoilage may not be evident in MAP foods, consumers could judge such products as safe even in the presence of high numbers of pathogens. Processors should be aware of this and take measures to prevent recontamination of the products. Because such steps will never fully guarantee the absence of a single pathogen, the shelf life of products supporting the growth of *L. monocytogenes* and/or other pathogens should be restricted.

Because the recontamination of cooked meat products with *L. monocytogenes* is usually low (<10 cfu per package), only few (micro)colonies will be formed during shelf life. Therefore, it is recommended to investigate whole packages (as described in the section Material and Methods), and not to restrict the sampling to smaller portions and running the risk not to detect the presence of this pathogen.

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