

Combinations of Lyophilized Cell-adsorbed Bacteriocin of *Lactobacillus curvatus* CWBI-B28 with Organic Acids or Salts to Control *Listeria monocytogenes* on Smoked Salmon Stored at 4°C in Vacuum Packages

Running head: Extension of Anti-Listerial Activity of Bacteriocin by Organic Acids or Salts

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Abstract. This study evaluated dipping solutions of lyophilized cell-adsorbed bacteriocin (LCaB) of *Lactobacillus curvatus* CWBI-B28 enriched with or without organic acids or salts to control *Listeria monocytogenes* introduced artificially on sliced smoked salmon and stored at 4°C for six weeks. Inoculated slices were immersed in a solution of LCaB (8533, 33 UA/g) or in acetic or lactic acid (1, 3, 5 g/100 ml), sodium acetate or diacetate (3, 5 g/100 ml), and potassium benzoate or sorbate (3 g/100 ml) for five minutes and stored at 4°C in vacuum packages. Additional slices containing *Listeria* (10² cfu/g) were immersed for five minutes in LCaB solutions enriched with organic acids or salts before storage at 4°C in vacuum packages. The LCaB solution without acid or salt added reduced *L. monocytogenes* by 1.0 log cfu/g after two weeks. Thereafter, the pathogen multiplied in samples, with growth being faster beyond the third week. LCaB in combination with 3 or 5 g/100 ml acetic acid or sodium diacetate or 3 g/100 ml benzoate. Applied individually, it did not permit growth before five weeks. In untreated (control) smoked salmon, *L. monocytogenes* increased to achieve 4 log cfu/g at the end of experience.

Keywords. *Listeria monocytogenes*; bacteriocin; organic acids; smoked salmon; antimicrobials

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1. Introduction

The occurrence and distribution of listeriae in a fish due to post-processing contamination by *Listeria monocytogenes* represents one of the highest fish safety risks (Centers for Disease Control & Prevention (CDC), 2002). It appears that HACCP programs applied in the fish industry are often insufficient to prevent the presence or inhibit the growth of *L. monocytogenes* in processed fish; therefore, post-packaging hurdle technologies are needed for its control (Bernard and Scott 1999). The inclusion of chemical antimicrobials in cured fish formulations remains the most effective hurdle against *L. monocytogenes* (Sofos 1989; Shelef 1994). Several studies have shown increased antilisterial efficacy when

organic acid salts are used in combinations (Wederquist et al., 1994; Qvist et al., 1994; Blom et al., 1997; Samelis et al., 2002). The antimicrobial activity of post-processing dipping solutions of organic acids or salts may be increased even if used at lower concentrations by combining them with additional inhibitors, such as bacteriocins or other natural antimicrobials. Recent research in our laboratory (Kouakou et al., 2008, 2009) has indicated that, at concentrations of 1g/100ml of bacteriocin produced by strains of *Lb. curvatus* CWBI-B28 may provide short inhibition against *L. monocytogenes* of two weeks in refrigerated slices of pork meat. The combination of

chemical antimicrobials, such as lactates, benzoates, sorbates, potassium, acetates, and diacetates with LCaB in cured meat or fish matrix formulations might be the most effective hurdle against *L. monocytogenes*. The antilisterial effects of such combined solutions have not been examined in these cured products contaminated post-processing with *L. monocytogenes*. Therefore, the objective of this study was to evaluate the LCaB of *Lactobacillus curvatus* CWBI-B28, applied singly or in combination with organic acids or salts as aqueous dipping solutions, for control of *L. monocytogenes* introduced artificially on smoked salmon after slicing and before vacuum packaging and storage at 4°C.

2. Materials and Methods

2.1 Antimicrobial Solutions Preparation

Antimicrobial solutions, (100ml each) except lyophilized cell-adsorbed bacteriocin (LCaB), were obtained commercially. LCaB has been prepared in our laboratory (Kouakou et al., 2008). During preparation, bacteriocin activity was assessed at each step according to an agar well diffusion assay described by Parente and Hill (1992). Molten agar was seeded with *L. monocytogenes* and dispensed into Petri dishes (20 mL per plate). Wells approximately 4.6 mm in diameter were bored in the agar. Two-fold serial dilutions of the bacteriocin homogenate were prepared, and 60-μL aliquots of the various dilutions were loaded into separate wells. The plates were incubated overnight at 37°C, and bacteriocin activity was determined in arbitrary units (AU) defined as the reciprocal of the highest dilution showing a definite zone of inhibition. Bacteriocin activity in arbitrary units per millilitre (AU mL⁻¹) was calculated by multiplying the reciprocal of the critical dilution by 1000 / 60. This result was then converted into AU g⁻¹.

The bacteriocin activity was 2133.33 AU/ml after 24 hours. The fermentate was heat-treated for cell and protease inactivation. Treatment at 70°C had no effect on the bacteriocin activity. Concentration of the fermentate from 81 to 150 ml (performed by means of two centrifugations-resuspensions) resulted in increasing the bacte-

riocin activity to 4266.66 AU/ml. During overnight crystallization, the activity of the concentrate remained stable. Freeze-drying of the concentrate resulted in production of an active powder (8533.33 AU/g dry matter).

The cell-adsorbed bacteriocin activity expressed in AU/g dry matter remained constant at 8533.33 AU/g in the freeze-dried material.

This LCaB was used alone or combined with each of lactic acid (1, 3, and 5 g/100 ml, DL-lactic acid, 85 g/100 g syrup, Sigma), acetic acid (1, 3 and 5 g/100 ml glacial 100% acetic acid, Mallinckrodt), potassium benzoate (3 g/100 ml, Sigma), potassium sorbate (3 g/100 ml, Sigma), sodium acetate (3 and 5 g/100 ml, Sigma), or sodium diacetate (3 and 5 g/100 ml, Niacet, Niagara Falls, NY, USA).

2.2 Salmon Inoculation and Treatment with Antimicrobials

Slices of smoked salmon were placed on aluminum foil under a biohazard hood and inoculated with 100μL of *L. monocytogenes* (10² cfu/g) before all antimicrobials. One ml of each antimicrobial solution was then deposited on one side of each slice and spread with a sterile bent glass rod on the surface. Inoculated slices were left to stand separately at 4°C for 10 minutes for inoculum attachment. The same procedure was repeated for the other side of each slice. Then, inoculated slices were vacuum packaged and stored at 4°C for six weeks.

2.3 Salmon Sampling

Slices of smoked salmon were sampled at regular intervals (1, 2, 3, 4, 5, and 6 weeks) of incubation. At each sampling, 20-g samples were taken aseptically from the smoked salmon blocks, diluted with 10 mL sterile saline solution (0.85% sodium chloride), and pressed manually in a Stomacher bag to extract as much liquid as possible. This liquid (called the “smoked salmon juice” hereafter) was then used for microbiological analyses.

2.4 Microbiological Analysis

Growth of the inoculated strains was determined on the basis of cfu counts after homogenisation of 1 mL meat juice in 9 mL peptone water, as described by Katla and collaborators. (2001). A decimal dilution series was prepared, and 1-mL

aliquots were plated. *L. monocytogenes* was plated on Palcam agar and enumerated after incubation for 48–72 hours at 37°C.

2.5 Statistical Analysis

Each trial was repeated twice and each determination was done in triplicate. Statistical analysis (analysis of variance $\alpha = 0.05\%$ and Student's t-test) was done with Excel software.

3. Results

3.1 Effect of LCaB Combined with Acetic or Lactic Acid on *L. monocytogenes* in Smoked Salmon

Figure 1 shows the evolution of the *L. monocytogenes* cfu count in smoked salmon co-inoculated with LCaB and either organic acids or salts during storage at 4°C. The count initially decreased, whatever the quantity and type of the organic acids or salts added, albeit much less markedly and more briefly in the presence of the lactic acid at

1, 3, or 5 g/100 ml or acetic acid at 1 g/100 ml than in the presence of 3 or 5 g/ml acetic acid. In the latter case, bacteria was undetectable after one week for acetic acid (5 g/ml) and undetectable after two weeks for acetic acid (3 g/ml), but re-growth occurred thereafter only for acetic acid (3 g/ml) after the fifth week.

As shown in Figure 1, the lactic acid at 1, 3, or 5 g/100 ml or acetic acid at 1 g/100 ml control displayed a bacteria decrease at the start of the experiment. The bacteria cfu count decreased slower, down to one log at two weeks followed growth rebound to reach rapidly 8.3×10^3 cfu/g by the end of the experiment (at six weeks). However, when 3 or 5 g/ml acetic acid was added, the presence of the LcaB combined with the high organic acids or salt content resulted in a 99,98% kill within one and two weeks. This was also followed by a growth rebound much later after the fifth week for the acetic acid (3 g/ml), reaching only 10 cfu/g by the end of the experiment.

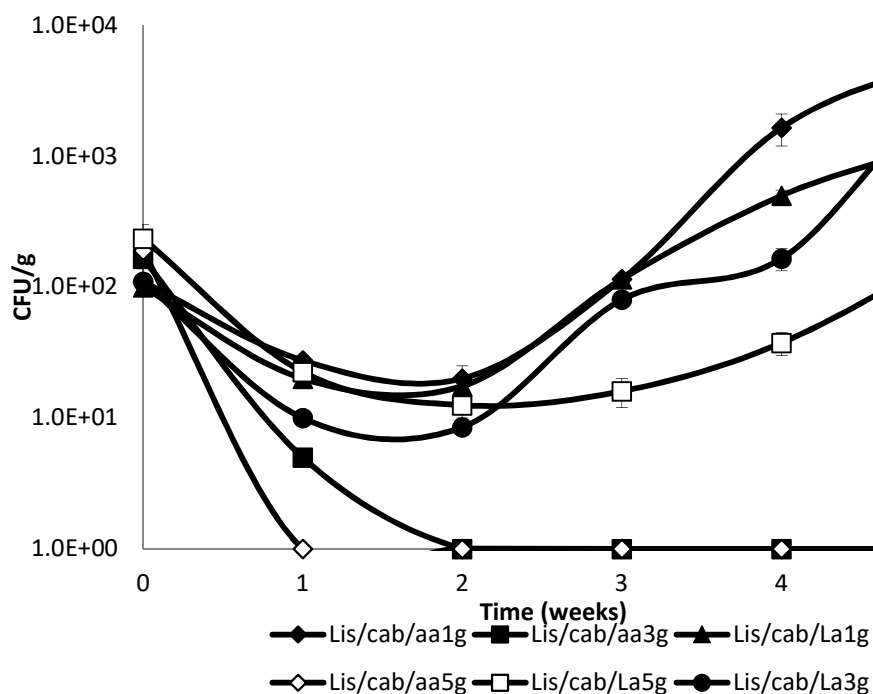


Figure 1: Survival/growth of inoculated *Listeria monocytogenes* (PALCAM agar) on smoked salmon slices containing Lyophilized cell-adsorbed bacteriocin (cab) with acetic acid (◆) (aa1g) or acetic acid (■) (aa3g) or acetic acid (◇) (aa5g) treatment added, or with lactic acid (▲) (La1g) or lactic acid (●) (La3g) or lactic acid (□) (La5g) treatment added vacuum packaging and storage at 4°C.

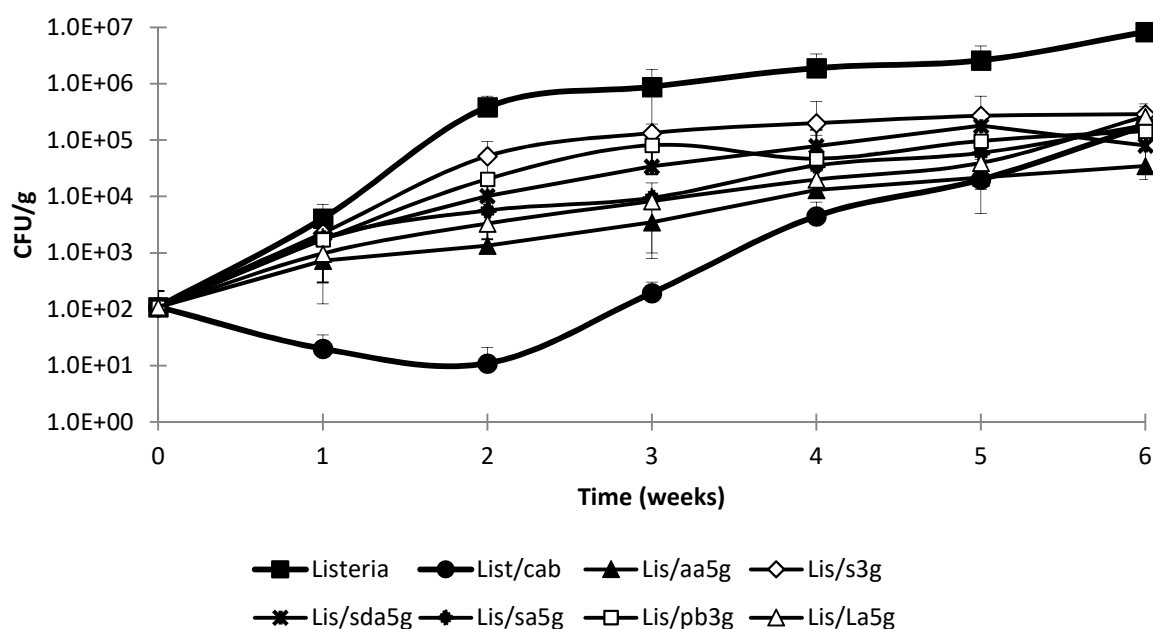


Figure 2: Survival/growth of inoculated *Listeria monocytogenes* (PALCAM agar) on smoked salmon slices with no treatment (■), or with cell-absorbed bacteriocin (cab) (●) added (4266.66 UA/ml), or with acetic acid (aa) (▲) added (5g), or with lactic acid (La) (△) added (5g), or sodium diacetate (sda) (*) added (5g), or sodium acetate (sa) (◆) added (5g), or potassium benzoate (pb) (□) added (3g), or sorbate (s) (◇) added (3g) vacuum packaging and storage at 4°C.

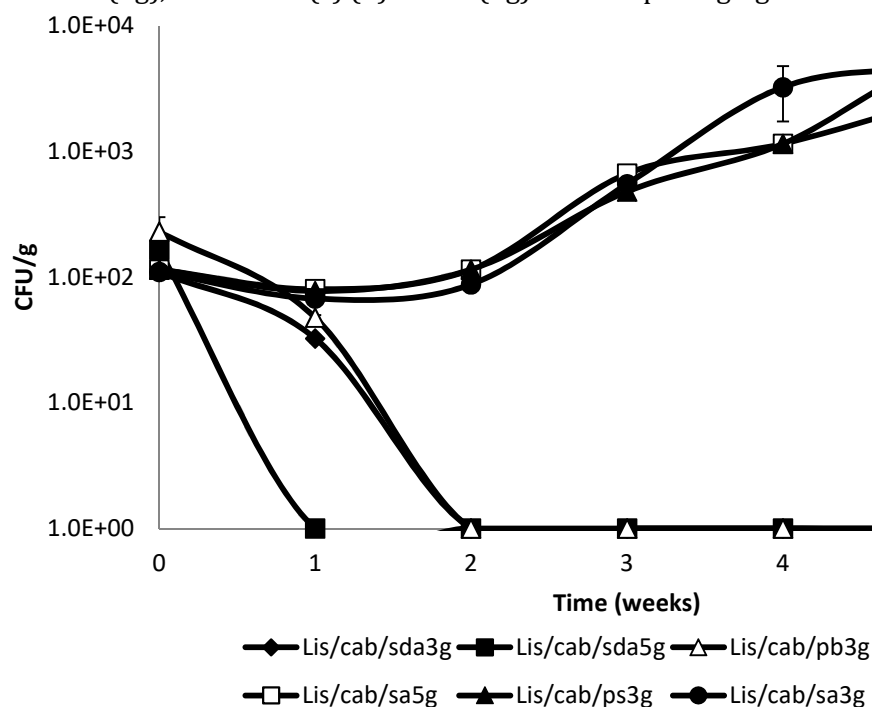


Figure 3: Survival/growth of inoculated *Listeria monocytogenes* (PALCAM agar) on smoked salmon slices containing Lyophilized cell-adsorbed bacteriocin (cab) with sodium diacetic (◆) (sda3g) or sodium diacetic (■) (sda5g) or potassium benzoate (△) (pb3g) or sodium acetate (□) (sa5g) or potassium sorbate (▲) (ps3g) or sodium acetate (●) (sa3g) treatment added, vacuum packaging and storage at 4°C.

3.2 Behavior of *L. monocytogenes* in Untreated or LCaB-Treated or Acids/Salts-Treated Smoked Salmon

Figure 2 shows the evolution during storage of the *L. monocytogenes* cfu count in smoked salmon with or without either LCaB or organic acids or salts treatment. In smoked salmon stored at 4°C, the presence of LCaB resulted in a 90% kill within two weeks; this was also followed by a growth rebound. As shown in Figure 1, the LCaB control displayed a bacteria growth rebound after two weeks with the cfu count rising rapidly to 4.3×10^5 cfu/g by the end of the experiment (at six weeks). In parallel untreated controls, the bacteria cfu count increased five-fold during the course of the experiment. In organic acids or salts treated controls, the bacteria cfu count increased three-fold over the first three weeks (this increase was significant, $p < 0.05$). Figure 2 shows that whatever the type of organic acids or salt content of the smoked salmon, and whether organic acids or salts were present or not, the *L. monocytogenes* cfu count increased from 10^2 to approximately 1.2×10^5 cfu g⁻¹ over the first three weeks of the experiment. Thus, *L. monocytogenes* can grow in organic acids or salts-treated smoked salmon. However, from week one to week six, a negative effect of those salts was observed. After six weeks, the *L. monocytogenes* count was about 1–2 log units lower in organic acids or salts-treated samples than in untreated samples (Figure 2). This is weak, but a significant negative effect of organic acids or salts on *L. monocytogenes* over the six-week storage period means that these organic acids and salts must be considered obstacles, having the ability to counteract the in situ development of *L. monocytogenes* in fish products.

3.3 Effect of LCaB Combined with Sodium Acetate or Diacetate or Potassium Sorbate or Benzoate on *L. monocytogenes* in Smoked Salmon

Figure 3 shows the evolution of the *L. monocytogenes* cfu count in smoked salmon co-inoculated with LCaB with either sodium acetate or diacetate or potassium sorbate or benzoate during storage at 4°C. The count initially decreased, whatever the quantity and type of the organic acids or salts added, albeit much less markedly and

more briefly in the presence of the sodium acetate at 3 or 5 g/100 ml or potassium sorbate at 3 g/100 ml than in the presence of 3 or 5 g/ml sodium diacetate or 3 g/ml potassium benzoate. In the latter case, *Listeria* was undetectable after one week for sodium diacetate (5 g/ml) and undetectable after two weeks for both sodium diacetate (3 g/ml) and potassium benzoate (3 g/ml). Re-growth occurred thereafter only for sodium diacetate (3 g/ml) (after the fifth week).

As shown in Figure 3, the sodium acetate at 3 or 5 g/100 ml or potassium sorbate at 3 g/100 ml control displayed a bacteria decrease at the start of the experiment. The bacteria cfu count decreased slower, down 1.1×10^2 cfu/ml to 85 cfu/ml at the first week followed by a growth rebound to rapidly reach 1.3×10^4 cfu/ml by the end of the experiment (at six weeks). But when 3 or 5 g/ml sodium diacetate or 3 g/ml potassium benzoate was added, the presence of the LCaB combined with these specific organic acids or salts resulted in a 99, 98% kill within one and two weeks. This was also followed by a growth rebound much later after the fifth week only for the sodium diacetate (3 g/ml), reaching approximately 5 cfu/g by the end of the experiment.

4. Discussion

Transient inhibition of *L. monocytogenes* by *Lb. curvatus* CWBI-B28 strain has been observed previously (Kouakou et al., 2008) and is observed again here. This inhibition is mostly due to the bacteriocin produced by *Lb. curvatus* CWBI-B28, since a bacteriocin-negative mutant derivative of this strain is unable to inhibit *Listeria* growth (Kouakou et al., 2008, 2009). In the present work, we show that the inhibition of *Listeria* caused by the LCaB in our fish system is also transient. This leads us to believe that the use of single bacteriocinogenic starter cultures or single bacteriocins in meat or fish systems has been somewhat disappointing.

The present study was an attempt to inhibit bacteria growth and prevent its rebound in artificially contaminated smoked salmon during storage at 4°C by adding different types of organic acid and salts in hope that the combination of a low pH with the presence of bacteriocin would

be sufficient to prevent bacteria rebound.

Overall, results indicated that post-processing LCaB treatments combined with organic acids or salts as dipping solutions (Figures 2 and 3) may provide greater antilisterial protection compared to treatments with solutions (e.g., 8533,33 UA/ml) of LCaB alone (Figure 1). Although LCaB had an immediate killing effect (≥ 1 log) on *L. monocytogenes* populations for approximately two weeks during subsequent storage at 4°C, growth was thereafter restored progressively (Figure 1). Similar results were reported for *L. monocytogenes* exposed to a nisin-containing (400 IU/ml) broth of pH 5.5 at 4°C (Buncic et al., 1995). Several factors may have contributed to the ineffectiveness of our LCaB solutions to inhibit or provide sufficient prevention of *L. monocytogenes* growth on smoked salmon for longer periods of time. First, the high average smoked salmon pH of 6.5 may have reduced the activity of LCaB on treated slices. Huot and collaborators (1996) have shown that high pH range, (from 6.2 to 6.4) may cause significant reductions in nisin activity. Also, unspecific binding of nisin on lipid components (Henning et al., 1986) of the sliced bologna may have caused partial inactivation of the bacteriocin. Another potential reason may have been an increased resistance of some of the inoculated strains to our LCaB, which may have been intrinsic or induced during storage. For example, several studies about nisin have shown that nisin sensitivity varies greatly among different *L. monocytogenes* strains and is enhanced at low pH, while resistant mutants may arise when nisin is used as an antimicrobial in foods (Harris et al., 1991; Ming and Daeschel 1993; De Martinis et al., 1997). Thus, Gonzales and Robert-baudouy (1996) think that bacterial enzymes, fish proteases, or both might be responsible for bacteriocin degradation.

Organic acids or salts at concentrations of 3–5 g/100 ml in dipping solutions, applied in combination with 8533,33 UA/ml LCaB, controlled growth of *L. monocytogenes*, with each compound being more effective at the higher concentration except lactic acid (Figures 2 and 3). At equal concentrations in dipping solutions, acetic acid (Figure 2), sodium diacetate, or potassium benzoate (Figure 3) were more effective against

the pathogen than were lactic acid (Figure 2), sodium acetate, or potassium sorbate, respectively (Figure 3). Thus, the long-term antilisterial effect observed was primarily dependent on the type and concentration of the acid or salt, rather than the inclusion of bacteriocin in the fish. Indeed, when the concentrations of lactic (1 to 5g/100ml) or acetic acid (Figure 2) and potassium benzoate or sorbate (data not shown) were lowered to 1 g/100 ml, their residual antilisterial effects were minimized, irrespective of LCaB presence.

Acid or salt marinades are becoming more popular as antimicrobial ingredients, particularly for their ability to reduce *L. monocytogenes* in ready-to-eat fish products. According to US regulations, sodium acetate and diacetate are approved as flavoring compounds at a maximum level of 0.25% of the formulation weight (Keeton 2001). In general, sodium lactate and diacetate are thought to inhibit bacterial growth by extending the lag phase (Doyle 1999). Although acid marinades may act as antimicrobials, they also affect fish quality and functionality. For instance, potassium lactate functions in a similar manner as sodium lactate but is less preferred because of its slightly bitter taste (Weaver et al., 1992).

Various investigators have concluded that the rate of *Listeria* inactivation is dependent not only on the pH of the environment but also on the identity and concentration of the acidulant used to modify the pH (Buchanan et al., 1993). Many investigators have studied and reported the inhibitory effects on low pH and organic acids on *L. monocytogenes*. Two inhibitory mechanisms have been proposed: 1) an intracellular acidification (loss of homeostasis) and 2) a specific effect on the acid (nondissociated form) on metabolic activities. Ita and Hutkins (1991) observed that low cellular pH was not the major factor in the inhibition of *L. monocytogenes* at acid pH. Cells treated with organic acids at pH values as low as 3.5 were able to maintain their cytoplasmic pH at a value near 5. Therefore, the efficiency of the treatments using organic acids would be due to the nondissociated fraction rather than to proton toxicity. The inhibitory effect of these acids can be correlated with their dissociation constant (pKa) and with the greater permeability of the

cell membrane to weak acids in their undissociated form. The highest inhibitory effect of acetic acid or diacetate can be explained by their ability to diffuse through the cell membrane, which is permeable to nondissociated, nonprotonated, and lipophilic weak acids. This leads to an accumulation of the acid within the cell cytoplasm, acidification of the cytoplasm, disruption of the proton-motive force, and inhibition of substrate transport (Vasseur et al., 1999). However, lactic acid may be less inhibitory, because it cannot passively penetrate the cell membrane (Vasseur et al., 1999). This is certainly what explains the low inhibition of *Listeria* in our present study when lactic acid was applied (Figure 2).

In view of our results, it seems that acetic acid, sodium diacetate, and potassium benzoate were present in their undissociated form so that their combination with LCaB may be more effective against *L. monocytogenes*. Specifically, LCaB with 5 g/100 ml sodium diacetate or 5 g/100 ml acetic acid or 3 g/100 ml potassium benzoate may be the most promising combinations in dipping solutions. Preventive spraying with, or dipping in, cell-adsorbed bacteriocin solutions may assist in controlling *L. monocytogenes* during slicing, packaging, and storage of processed fish, especially if combined as appropriate, with organic acids or salts.

References

- Bernard, D.T. and Scott, V.N. (1999), "*Listeria monocytogenes* in meats: New strategies are needed", *Food Technology*, 53, p. 124
- Blom, H., Nerbrink, E., Dainty, R., Hagtvedt, T., Borch, E., Nissen, H. and Nesbakken, T. (1997), "Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum-packed, sensory-acceptable serelat sausage and cooked ham at 4°C", *International Journal of Food Microbiology*, pp. 38, 71–76
- Buchanan, R. L., Golden, M. H. and Whiting, R. C. (1993), "Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation", *Journal of Food Protection*, pp. 56, 474–478
- Buncic, S., Fitzgerald, C.M., Bell, R.G. and Hudson, J.A. (1995), "Individual and combined listericidal effects of sodium lactate, potassium sorbate, nisin and curing salts at refrigeration temperatures", *Journal of Food Safety*, pp. 15, 247–264
- Centers for Disease Control & Prevention (CDC) (2002), "Public Health dispatch: Outbreak of listeriosis—Northeastern United States, (2002)", *Morbidity and Mortality Weekly Report*, pp. 51, 950–951
- De Martinis, E.C.P., Crandall, A.D., Mazzotta, A.S. and Montville, T.J. (1997), "Influence of pH, salt and temperature on nisin resistance of *Listeria monocytogenes*", *Journal of Food Protection*, pp. 60, 420–423
- Doyle, M. E. (1999), FRI briefings: Control of *Listeria* in meat. www.wisc.edu/fri/briefs.htm (Accessed: November 2006)
- El Soda, M., Said, H., Desmazeaud, M. J., Le Bars, D. and Zevaco, C. (1986), "Cell-wall associated proteinases in *L. casei* and *L. plantarum*", *Journal of Food Protection*, pp. 49(5), 361–365
- Gonzales, T. and Robert-baudouy, J. (1996), "Bacterial aminopeptidases: Properties and functions", *FEMS Microbiology Reviews*, pp. 18(4), 319–344
- Harris, L.J., Fleming, H.P. and Klaenhammer, T.R. (1991), "Sensitivity and resistance of *Listeria monocytogenes* ATCC 19115, Scott A, and UAL500 to nisin", *Journal of Food Protection*, pp. 54, 836–840
- Henning, S., Metz, R. and Hammes, W.P. (1986), "Studies on the mode of action of nisin", *International Journal of Food Microbiology*, pp. 3, 121–134
- Huot, E., Barrena-Gonzalez, C. and Petitdemange, H. (1996), "Comparative effectiveness of nisin and bacteriocin J46 at different pH values", *Letters Applied Microbiology*, pp. 22, 76–79
- Ita, P. S., and Hutkins, R. W. (1991), "Intracellular pH and survival of *Listeria monocytogenes*

- Scott A in tryptic soy broth containing acetic, lactic, citric, and hydrochloric acids", *Journal of Food Protection*, pp. 54, 15–19
- Katla, T., Moretro, T., Aasen, I.M., Holck, A., Axelson, L. and Naterstad, K. (2001), "Inhibition of *Listeria monocytogenes* in cold smoked salmon by addition of sakacin P and/or live *Lactobacillus sakei* cultures", *Food Microbiology*, pp. 18, 431–439
- Keeton, J. T. (2001), Formed and emulsion products. Pages 195–226 in *Poultry Meat Processing*. A. R. Sams, ed. CRC Press, Boca Raton, FL.
- Kouakou, P., Ghalfi, H., Destin, J., Dubois-Dauphin, R., Evrard, P. and Thonart, P. (2008), "Enhancing the antilisterial effect of *Lactobacillus curvatus* CWBI-B28 in pork meat and co-cultures by limiting bacteriocin degradation", *Meat Science*, pp. 80, 640–648
- Kouakou, P., Ghalfi, H., Destin, J., Dubois-Dauphin, R., Evrard, P. and Thonart, P. (2009), "Effects of curing sodium nitrite additive and natural meat fat on growth control of *Listeria monocytogenes* by the bacteriocin-producing *Lactobacillus curvatus* strain CWBI-B28", *Food Microbiology*, pp. 26, 623–628
- Kunji, R. S., Mierau, I., Hagting, A., Poolman, B. and Koning, W. N. (1996), "The proteolytic systems of lactic acid bacteria", *Antonie van Leeuwenhoek*, pp. 70, 187–221
- Ming, X. and Daeschel, M.A. (1993), "Nisin resistance of foodborne bacteria and the specific resistance response of *Listeria monocytogenes* Scott A", *Journal of Food Protection*, pp. 56, 944–948
- Qvist, S., Sehested, K. and Zeuthen, P. (1994), "Growth suppression of *Listeria monocytogenes* in a meat product", *International Journal of Food Microbiology*, pp. 24, 283–293
- Samelis, J., Bedie, G.K., Sofos, J.N., Belk, K.E., Scanga, J.A. and Smith, G.C. (2002), "Control of *Listeria monocytogenes* with combined antimicrobials after post-process contamination and extended storage of frankfurters at 4°C in vacuum packages", *Journal of Food Protection*, pp. 65, 299–307
- Shelef, L.A. (1994), "Antimicrobial effects of lactates: A review", *Journal of Food Protection*, pp. 57, 445–450. View Record in Scopus | Cited By in Scopus (92)
- Sofos, J.N. (1989), *Sorbate food preservatives* (237pp), CRC Press, Inc, Boca Raton, Florida
- Vasseur, C., Beverel, L., Hebraud, M., and Labadie, J. (1999), "Effect of osmotic, alkaline, acid, or thermal stresses on the growth and inhibition of *Listeria monocytogenes*", *Journal Applied Microbiology*, pp. 86, 469–476
- Weaver, R. A., and Shelef, L. A. (1992), "Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage", *Journal of Food Safety*, pp. 13, 133–146
- Wederquist, H. J., Sofos, J. N. and Schmidt, G. R. (1994), "*Listeria monocytogenes* inhibition in refrigerated vacuum packaged turkey bologna by chemical additives", *Journal of Food Science*, pp. 59, 498–516