Inhibitory Effect of Provian® (A Co-spray Dried Mixture of Sodium Lactate and Sodium Acetate) on the Growth of *Listeria monocytogenes* in Frankfurters Stored at 4, 7, or 10°C

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Abstract—**In meat processing, powdered ingredients are preferred over liquids due to ease in handling, mixing, and storing. This study was designed to assess the impact of Provian® powder (PP, a co-spray dried mixture of sodium lactate and sodium acetate) and Provian®D powder (PD, a mixture of Provian® powder and sodium diacetate) from Kemira ChemSolutions b.v., Netherlands, as well as two liquid inhibitors [60% (w/v) sodium lactate (SL), and 60% (w/v) SL and sodium diacetate (SL-SD)] on *Listeria* inhibition, organoleptic properties, and physicochemical characteristics of frankfurters. In each of three replications, six batches of frankfurters – control, 0.5% PP, 1% PP, 0.65% PD, 2.5% of 60% SL, and 2.5% of 60% SL-SD were dip-inoculated with a 6-strain cocktail of *L. monocytogenes*. Populations of *Listeria* and mesophilic aerobic bacteria (MAB) were then assessed during storage at 4, 7, and 10°C for up to 90 days. *Listeria* populations increased < 2 log CFU/g on frankfurters containing PD, 1% PP, or SL-SD during 90 days of storage at 4 and 7°C, whereas minimal inhibition was seen at 10°C. Using these same inhibitors, MAB populations reached 4 – 5 logs CFU/g compared to 7 log CFU/g in the control at 4 and 7°C after 45 days with minimal inhibition again seen at 10°C. No significant differences (*P* > 0.05) were found in appearance, texture, flavor, or overall acceptability, regardless of frankfurter formulations. Given the observed antilisterial activity with no adverse organoleptic issues, both of these powders (PP and PD) appear to be viable alternatives to liquid inhibitors.**

Keywords— **Powder, *Listeria* Inhibitor, Frankfurter.**

I. INTRODUCTION

Listeria monocytogenes has been recognized as a hazardous organism likely to occur in cooked (ready-to-eat) meat and poultry products. Regarding listeriosis, studies indicated that deli meats and frankfurters both pose a major per serving risk of illness/death from this organism with a high fatality up to 25% [1] Three major concerns among meat processors include the ability of *L. monocytogenes* to survive in processing environments, re-contaminate products during post-thermal processing, and subsequently grow in the finished product at refrigeration temperatures.

Research has indicated that the salts of lactic acid and acetic acid have significant antimicrobial activity against *Listeria*. Incorporation of lactate and/or diacetate into product formulation has been widely used to prevent growth of *Listeria* with the advantages of low cost and assurance of residual activity after opening the package [2]. However, lactate which is highly hydroscopic needs to be prepared in a liquid form with diacetate, negatively impacting flavor. Recently, Kemira ChemSolutions developed these salts in powder form, named Provian® (lactate and acetate salts) and Provian®D (lactate, acetate and diacetate salts). The purpose of this research was to evaluate the effect of Provian® powders and liquid inhibitors (sodium lactate and sodium lactate/sodium diacetate) on *Listeria* and mesophilic aerobic bacteria inhibition, organoleptic quality, and physicochemical properties of frankfurters.

II. MATERIALS AND METHODS

A. Frankfurter Preparation: Raw meat (pork butt, pork back fat and 85% lean beef) and non-meat ingredients were purchased locally except for Provian and other antimicrobial ingredients that were shipped from Kemira ChemSolutions b.v. (Tiel, The Netherlands). Two Provian® products were "50%

sodium acetate and 50% sodium lactate co-spray dried mixture" (Provian®) and "38% sodium acetate, 38% sodium lactate and 23% sodium acetate mixture" (Provian®D). The two liquid inhibitors were a 60% solution of sodium lactate (SL) and a 60% solution of 56% sodium lactate and 4% sodium diacetate (SL-SD). In each of three replications, six different formulations (22.7kg/batch) of frankfurters were prepared in a bowl chopper as follows: 1) no inhibitor (control), 2) 0.5% Provian® powder (PP), 3) 1% PP, 4) 0.65% ProvianD® powder (PD), 5) 2.5% of a 60% sodium lactate (SL) solution, and 6) 2.5% of a 60% sodium lactate and sodium diacetate (SL-SD) solution.

B. Listeria monocytogenes strains and frankfurter inoculation: A cocktail (~1 x 10^6 CFU/ml) of six *L. monocytogenes* strains, representing different serotypes and pulsed field gel electrophoresis (PFGE) patterns, was used in this study. Fifty frankfurters from each treatment were aseptically transferred to a mesh bag and submerged in the *L. monocytogenes* cocktail with gentle stirring. After 1 min, the mesh bag was removed and drained for a minute prior to transferring the frankfurters on the tray to a bio-safety hood. After the inoculum absorbed into the product for 25 minutes, two frankfurters were aseptically transferred into a 4×6 O.D. Shanvac vacuum bag and vacuum sealed. Eight bags from each formulation were stored at 4, 7, and 10°C for up to 90 days. Uninoculated frankfurters from each treatment were also prepared in the same manner for quantification of background microflora and sensory analysis.

C. Microbial Analysis: Initially and after 15, 30, 45, 60, 75 and 90 days of storage at 4, 7, and 10°C, *L. monocytogenes* and mesophilic aerobic bacteria (MAB) were, respectively, quantified in the inoculated and uninoculated samples. All 25-g samples were diluted in sterile phosphate buffer solution (PBS) and homogenized by stomaching for 1 min. Appropriate serial dilutions in PBS were then plated on Modified Oxford Agar (Difco, Becton Dickinson, Sparks, MD, USA) and trypticase soy agar supplemented with 0.6% (w/v) yeast extract to enumerate *L. monocytogenes* and MAB, respectively, after 48 h of incubation at 35°C.

D. Sensory Analysis: In each of three replications, about 50 consumer panelists were recruited to assess 5 samples for appearance, texture, flavor, and overall acceptability based on a 9-point hedonic scale. Samples containing 1% Provian were not included since current USDA regulations do not permit the use of sodium diacetate (or acetate) at levels above 0.25%. Frankfurters were heated (72°C) in boiled water and kept in a warmer (63°C) after inserting into a sealable bag. Upon serving, samples from five different treatments were cut (4 cm cross-sections) and presented to each panelist. Data were combined for a total of n = ~ 150.

D. Physicochemical Analysis: Seven physiochemical parameters were evaluated for frankfurters after chopping. pH, protein, fat, moisture, and water activity were measured using a pH meter, nitrogen analyzer, fat extractor, dry oven, and AquaLab water activity meter, respectively. Sodium content was analyzed using a pH/ion analyzer after calibration, and cooking yield was calculated by the weight difference before and after cooking.

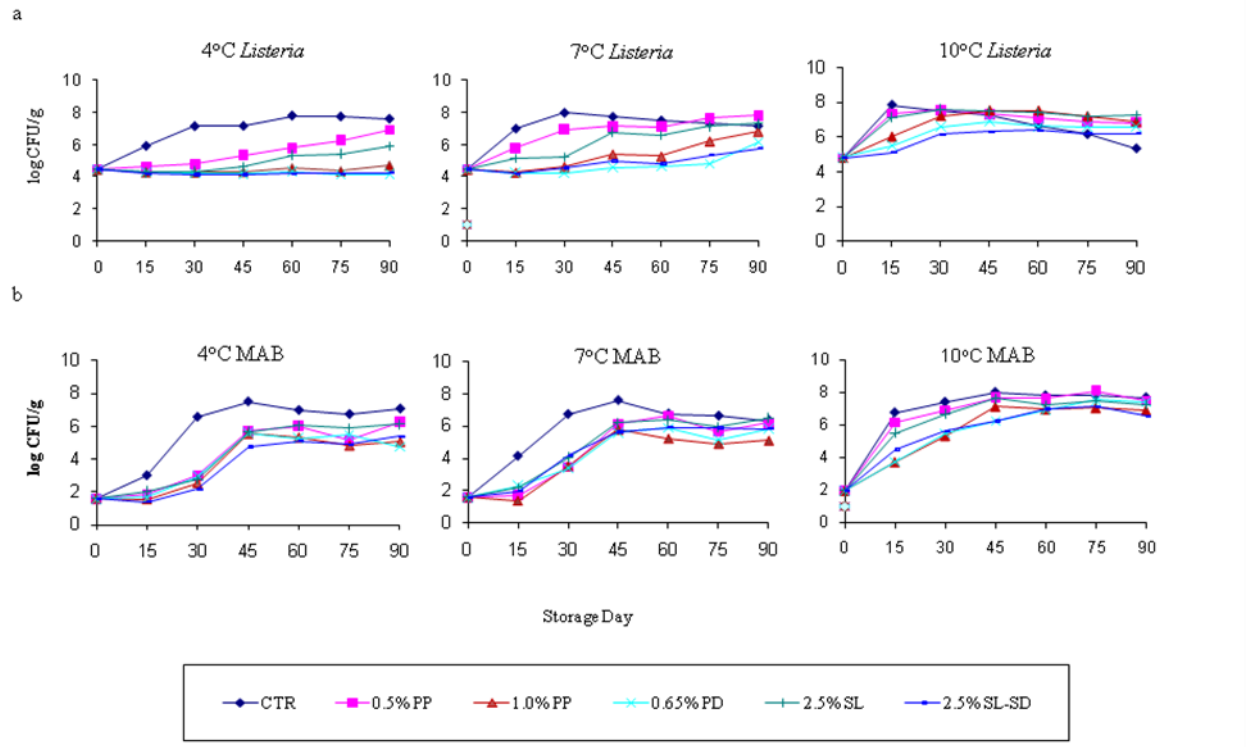
E. Statistical analysis: Data were subjected to a mixed model ANOVA procedure of SAS (SAS Institute, 2002). Overall means were separated with Tukey's Test at the $P < 0.05$ level. When replication and treatment were significant ($P < 0.05$), the differences between treatments were separately reported for each replication using a Bonferroni adjustment based on the number of such comparisons within each replication.

III. RESULTS

A. Microbial Growth: During 90 days of storage at 4°C (Fig. 1a), 1.0% PP, 0.65% PD, and 2.5% SL-SD suppressed *Listeria* growth while 2.5% SL, 0.5% PP, and CTR allowed the organism to reach populations of 5, 6, and 7 log CFU/g, respectively. At 7°C, the same three formulations (1% PP, 0.65% PD, and 2.5% SL-SD) similarly suppressed *L. monocytogenes* (4 - 5 log CFU/g) up to 60 days with the remaining formulations allowing a rapid growth after 15 or 30 days of storage. When frankfurters were stored at 10°C, *Listeria* rapidly grew in all formulations to 7 log CFU/g after 15 to 30 days, except for formulations containing 0.65% PD and 2.5% SL-SD.

Initial mesophilic aerobic bacteria (MAB) populations in uninoculated frankfurters were < 2 log CFU/g (Fig. 1b). MAB populations in the control

reached 7.5 log CFU/g after 45 days at 4°C and remained at this level throughout storage. Five treated samples also exhibited peak growth at 45 days but with significantly lower levels (4.7 to 5.7 log CFU/g) than the control. After the peak, 1% PP and 0.65% PD decreased MAB populations by 0.5 – 0.9 log CFU/g, whereas 0.5% PP, 2.5% SL, and 2.5% SL-SD led to population increases of 0.4 – 0.7 log CFU/g at the end of storage. At 7°C, the control yielded a maximum



[1]CTR – 0% control
0.5% PP – 0.5% Provian® powder
1% PP – 1% Provian® powder
0.65% PD – 0.65% Provian®D powder
2.5% SL – 2.5% of a 60% sodium lactate solution
2.5% SL-SD – 2.5% of a 60% sodium lactate and sodium diacetate 60% solution

Figure 1. *Listeria monocytogenes* (a) and mesophilic aerobic bacteria (b) populations (log CFU/g) in frankfurters

population of 7.6 log CFU/g at 45 days and then declined to 6.4 log CFU/g. In all five formulations containing inhibitors, MAB populations increased to 5.5 – 6.2 log CFU/g after 45 days of storage and then remained at these levels until the end of storage. At 10°C, all treatments permitted rapid growth to > 7 log CFU/g after 30 – 75 days of storage.

B. Sensory analysis: Five formulations were evaluated for sensory characteristics (Table 1). No significant differences ($P < 0.05$) were found in appearance, texture, flavor, and overall acceptability regardless of the formulation. However, both Provian® powders (PP and PD) received numerically higher scores than liquids (SL and SL-SD), especially for flavor and overall acceptability.

C. Physicochemical analysis: Seven physicochemical parameters (sodium, pH, a_w, cooking yield, moisture, protein, and fat) were evaluated for frankfurters (Table 2). The two liquid inhibitors (2.5% SL and 2.5% SL-SD) yielded the highest values for sodium (1345 and 1328 mg/100g), followed by the three Provian® powders (1172 – 1279 mg/100g), and the control (1031 mg/100g). Regarding pH, SD-containing inhibitors (0.65% PD and 2.5% SL-SD) had higher scores (pH 6.21 – 6.26) than those without SD (1% PP, 0.5% PP and 2.5% SL) (pH 6.39 – 6.41). Proximate analysis (protein, fat, moisture) and cooking yield showed differences of less than 1%, with no significant difference seen for water activity.

Table 1. Appearance, texture, flavor, and overall acceptability of frankfurters containing *Listeria* growth inhibitors[1].

ATTRIBUTE	CTR	0.5% PP	0.65% PD	2.5% SL	2.5% SL-SD
APPEARANCE	6.35 ± 0.19^{a}	6.5 ± 0.19^{a}	6.44 ± 0.19^{a}	6.44 ± 0.19^{a}	6.35 ± 0.19^{a}
TEXTURE	6.52 ± 0.21^{a}	6.49 ± 0.21^{a}	6.76 ± 0.21^{a}	6.49 ± 0.21^{a}	6.37 ± 0.21^{a}
FLAVOR	6.51 ± 0.21^{a}	6.52 ± 0.21^{a}	6.80 ± 0.21^{a}	6.38 ± 0.21^{a}	6.26 ± 0.21^{a}
OVERALL	6.51 ± 0.20^{a}	6.43 ± 0.20^{a}	6.74 ± 0.20^{a}	6.30 ± 0.20^{a}	6.15 ± 0.20^{a}

[1]Inhibitor treatments as in Fig 1.

Table 2. Physicochemical properties of frankfurters containing *Listeria* growth inhibitors[1].

PROPERTY	CTR	0.5% PP	1.0% PP	0.65% PD	2.5% SL	2.5% SL-SD
Sodium	1031^{d}	1178^{c}	1279^{b}	1172^{c}	1345^{a}	1328^{ab}
pH	6.35^{c}	6.39^{ab}	6.41^{a}	6.21^{e}	6.39^{ab}	6.26^{d}
a_w	0.956^{a}	0.955^{a}	0.950^{a}	0.956^{a}	0.951^{a}	0.949^{a}
Cooking yield	89.50^{b}	88.51^{c}	90.03^{ab}	90.69^{a}	88.03^{c}	88.01^{c}
Moisture	60.79^{ab}	60.02^{ed}	60.50^{abc}	60.61^{ab}	60.07^{cde}	59.71^{e}
Protein	14.86^{ab}	15.15^{a}	14.44^{bc}	14.58^{bc}	14.18^{c}	14.65^{abc}
Fat	18.67^{ab}	18.59^{ab}	18.84^{a}	18.47^{ab}	17.03^{cd}	17.42^{cd}

[1]Inhibitor treatments as in Fig 1.

IV. CONCLUSIONS

In this study, various powdered forms of these acid salts containing lower levels of organic acid salts showed either bacteriostatic or bacteriocidal activity against *Listeria* in frankfurters. Compared to the liquids, these powders similarly inhibited *Listeria* in frankfurters during 90 days of storage at 4, 7, or 10 °C with similar sensory scores also seen in consumer panels. Based on these findings, both Provian® and Provian®D powders could be alternatives to current liquid products with the powder likely to be preferred due to ease in handling, mixing, storing, and shipping.

ACKNOWLEDGMENT

The authors would like to thank Kemira ChemSolutions b.v. for funding of this research.

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