Efficacy of Lactates on *Listeria monocytogenes* and *Salmonella* Typhimurium in Buffalo Meat Cooked Salami during Refrigerated Storage

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ABSTRACT

The effect of 0 and 1% addition of lactates viz., sodium lactate, potassium lactate and calcium lactate on the pH, titratable acidity and growth of *Listeria monocytogenes*, 4b (MTCC - 1143) and *Salmonella* Typhimurium experimentally inoculated (~ 4 log cfu/g) separately in buffalo meat cooked salami samples stored at 4 ± 1°C was assessed at weekly interval. The pH of lactate treated salami samples either with *L. monocytogenes* or *S. Typhimurium* were significantly (P<0.05) lower than their respective control. Significant (P<0.05) decrease in pH values were also recorded throughout the storage period. The pH and titratable acidity of control as well as lactate treated samples either of the organism showed exactly reverse trend due to treatment as well as storage period. Moreover, marked (P>0.05) inhibition of *L. monocytogenes* and *S. Typhimurium* in lactate treated samples was recorded over control. Calcium lactate (1%) in cooked salami showed optimum inhibitory effect on *L. monocytogenes* and *S. Typhimurium* as compared to other lactates. Nevertheless, 1% level of any of these lactates was not sufficient to exhibit bacteriostatic effect on either of these food borne pathogenic bacteria under refrigerated storage.

Keywords: Buffalo meat cooked salami, lactates, *Listeria monocytogenes*, *Salmonella* Typhimurium, refrigerated storage

Introduction

*Listeria monocytogenes* and *Salmonella* Typhimurium are important foodborne pathogens of zoonotic importance. Under the optimum conditions, these organisms grow in various foods when contaminated during production, processing and/or handling operations. Therefore, certain ingredients / substances like lactates may be added to provide microbial safety as well as other beneficial effects to them. Several workers (Bacus and Bontenbal, 1991; Shelef and Yang, 1991; Chen and Shelef, 1992; Weaver and Shelef, 1993; Miller and Acuff, 1994; Shelef and Potluri, 1995 and Stekelenburg and Kant-Mueranans, 2001) have documented that higher concentrations (2 - 4%) of various lactates viz., sodium lactate (SL), potassium lactate (KL) and calcium lactate (CL) had inhibited these food borne pathogens in various meat products under different conditions of processing and storage. However, these levels (>1%) have adversely affected the sensory quality of the products (Brewer et al., 1991 and 1993). Our preliminary trials of preparation of salami with 2% and 3% levels of lactates also showed similar adverse effects on sensory attributes. In addition, higher levels did not attribute any added advantage to the physicochemical attributes of the products. On the other hand, the information on this aspect pertaining to lower level (< 2%) incorporation of these lactates in meat product is scanty. Hence, present study was undertaken to assess the efficacy of 1% level of various lactates on the *L. monocytogenes* and *S. Typhimurium* added...
exogenously in buffalo meat cooked salami under refrigerated storage (4±1°C).

**Materials and Methods**

**Preparation of cooked salami**

Cooked salami mix (control) was prepared by using lean ground buffalo meat (85.44%) and non meat ingredients viz., salt 1.54%, sodium nitrate 0.02%, sodium nitrite 0.01%, sodium ascorbate 0.04%, crushed black pepper 0.09%, cane sugar 0.21%, sodium tripolyphosphate 0.26%, spice mix 0.85%, condiments 3.85%, ice-water 7.69% in a Hobart mixture. In addition, the treated salami mixes had 1% of SL or KL or CL. About 500 g of the mix from control as well as treated samples was stuffed manually into weasands separately. The encased mass was tied with cotton thread in such a way so as to have a product of an uniform length. Raw salami sticks were hanged in the smoke oven (Enviro-Pak, Model CVU-350T, USA) for drying of casings at 55°C for 30 min. This was followed by smoking of the product at 60°C for 2½ h for development of a desired colour. The product was cooked at a core temperature of 85°C for 30 min, promptly cooled in the oven and then shifted to refrigerator at 4±1°C for overnight chilling.

**Test organisms**

The cultures of *L. monocytogenes* (4b MTCC-1143) and *S. Typhimurium*, after testing their purity, morphological and biochemical characteristics were inoculated separately in brain heart infusion (BHI) broth. The cells were pelleted by centrifugation and washed twice with sterile NSS. The final cell pellet was suspended in normal saline solution (NSS) and opacity of the bacterial suspension was adjusted to a count of approx. 1x10^8 cells/ml with the help of nephelometer tubes (Paik and Suggs, 1974). Further 10-fold dilutions were made in NSS so as to have cell concentration of 1x10^4 cells/ml.

**Inoculation of cooked salami slices**

Cooked product was sliced by following the aseptic conditions. Twelve cooked salami slices (10 g) each of control and different lactate were inoculated with approximately 1x10^4 cells/g *L. monocytogenes*. Each slice was packaged separately in pre-sterilized low density polyethylene (LDPE) bags and stored in refrigerator at 4±1°C. Similar procedure was followed separately for inoculation of cooked salami slices with *S. Typhimurium*.

The effect of different treatments was determined on the basis of pH (Trout et al., 1992), titratable acidity (Koniecko, 1979) and *Listeria* count and *Salmonella* count at weekly interval. The experiment was replicated thrice.

**Listeria count**

One LDPE bag from each treatment and control containing inoculated slice was randomly drawn for enumeration of organism. The bag was cut opened and the slice was cut into pieces and blended in stomacher (Seward Laboratory, London, Model BA6021) with 90 ml NSS and 0.1 ml of inoculum from these dilutions was spread over Domincuez Rodriguez agar (DRA) plates in duplicate and incubated at 37°C for 48 h. The number of colonies were expressed as log_{10} cfu/g of sample.

**Salmonella count**

The same procedure as described for *Listeria* count was followed for making the serial dilutions and inoculation on bismuth sulphite agar (BSA) plates. The plates were incubated at 37°C for 48 h. The number of colonies were expressed as log_{10} cfu/g of the sample.

**Statistical analysis**

The data obtained were analysed by using statistical software package developed at Computer Centre of the Institute for analysis of variance (Snecedor and Cochran, 1989). Critical difference and Duncan’s multiple range tests were used for comparing the means to find out the effects of treatments and storage period for various parameters in different experiments.

**Results and Discussion**

The results pertaining to the effect of 1%
lactates viz., SL, KL and CL in buffalo meat salami on the pH, TA and Listeria counts are presented in Table 1. It was observed that pH of control and samples with 1% SL or KL did not reveal any significant (P>0.05) variations. However, pH of CL treated samples was significantly (P<0.05) lower (r 0.3 units) among the lactates. There was significant (P<0.05) reduction in pH throughout the study (14 days). Chen and Shelef (1992) have recorded similar trend in pH of cooked beef treated with 4% of either SL or KL, whereas Shelef and Potluri (1995) observed slightly elevated pH in 3 and 4% SL treated cooked liver sausages and low pH in CL treated samples. Thus the deviations of pH might be either due to type of meat or levels of lactates.

Titratable acidity of the control and the samples treated with SL did not reveal any variations (P>0.05). However, significant (P<0.05) increase in TA was observed in samples treated with 1% CL. Similarly, there was significant (P<0.05) increase in TA of the samples during the storage period. Thus results on pH and titratable acidity have confirmed inverse relationship between them.

Significantly (P<0.05) lower listerial counts were recorded in lactate treated samples than that of the control. However, significant (P<0.05) increase in the counts was observed throughout the refrigerated storage. It is interesting to note a slight inhibition in listerial growth by all the lactates and this inhibition was consistently more in CL.

Table 1: Effect of lactates on pH, titratable acidity and Listeria counts of buffalo meat cooked salami during refrigerated storage (4±1°C) (n=6)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>Treatment mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>6.06±0.02</td>
<td>6.00±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1% SL</td>
<td>6.07±0.02</td>
<td>6.01±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1% KL</td>
<td>6.07±0.02</td>
<td>6.02±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1% CL</td>
<td>5.77±0.03</td>
<td>5.71±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall mean (days)</td>
<td>5.99±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.93±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

|            | 0.057±0.002           | 0.068±0.002<sup>a</sup> | 0.077±0.002<sup>a</sup> | 0.067±0.002<sup>a</sup> |
| Titratable acidity (%) | 0.058±0.002           | 0.067±0.002<sup>a</sup> | 0.075±0.002<sup>a</sup> | 0.067±0.002<sup>a</sup> |
|            | 0.058±0.002           | 0.065±0.002<sup>a</sup> | 0.073±0.002<sup>a</sup> | 0.066±0.002<sup>a</sup> |
|            | 0.063±0.002           | 0.068±0.002<sup>a</sup> | 0.078±0.002<sup>a</sup> | 0.070±0.002<sup>a</sup> |
| Overall mean (days) | 0.059±0.001<sup>c</sup> | 0.067±0.001<sup>d</sup> | 0.076±0.001<sup>e</sup> | |

| Control    | 4.61±0.03             | 7.40±0.01<sup>a</sup> | 8.20±0.02<sup>a</sup> | 6.73±0.37<sup>a</sup> |
| 1% SL      | 4.59±0.02             | 6.67±0.02<sup>a</sup> | 7.75±0.05<sup>a</sup> | 6.34±0.32<sup>a</sup> |
| 1% KL      | 4.64±0.04             | 7.07±0.06<sup>a</sup> | 7.73±0.03<sup>a</sup> | 6.48±0.32<sup>a</sup> |
| 1% CL      | 4.58±0.02             | 6.62±0.03<sup>a</sup> | 7.28±0.03<sup>a</sup> | 6.15±0.28<sup>a</sup> |
| Overall mean (days) | 4.61±0.01<sup>e</sup> | 6.94±0.07<sup>d</sup> | 7.74±0.07<sup>d</sup> | |

Means with common superscripts in a row and in a column do not differ significantly (P>0.05)
Treatment means are the means of 14 days only
samples than the other two lactates. However, effect of these lactates studied up to 14 days cannot be called listeriostatic. These results are in agreement with the previous reports, wherein lower levels of lactates (<2%) did not show listeriostatic effect (Miller and Acuff, 1994) whereas only higher levels (>2.5%) had an inhibitory effect on the growth of \textit{L. monocytogenes} (Shelef and Yang, 1991; Chen and Shelef, 1992; Shelef and Pottiuri, 1995; Stekelenburg and Kant-Muermans, 2001). The study was discontinued after 14 days (2 weeks) as there was no listeriostatic effect of various lactates and \textit{Listeria} count almost doubled reaching approximately 8 log cfu/g during 14 days storage.

The results on the effect of 1% lactates (viz., SL, KL and CL) in buffalo meat cooked salami on the pH, TA and \textit{Salmonella} counts are presented in Table 2. There was apparent decrease in pH values of control as well as lactate treated samples. However, the decline was more significant (P<0.05) in control (6.08-5.86) as compared to lactate treatments. There was no difference in pH of samples treated with either SL or KL. As compared to control, the decrease in pH (5.79 to 5.64) of CL treated samples was very less. However significant (P<0.05) decrease in pH values were recorded throughout the storage period. Weaver and Shelef (1993) observed the rapid decline in pH of cooked liver sausages and stable pH in sausages treated with 3 and 4% of either SL or CL. The variations in this study in respect of pH of lactate treated samples might be due to lower level of these lactates.

Table 2: Effect of lactates on pH, titratable acidity and \textit{Salmonella} counts of buffalo meat cooked salami during refrigerated storage (4±1°C) (n = 6)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>Treatment mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 7 14 21</td>
<td></td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Control    | 6.08±0.01 5.99±0.003 5.89±0.004 5.86±0.003 | 5.96±0.02
| 1% SL      | 6.07±0.01 6.01±0.004 5.96±0.010 5.93±0.010 | 5.99±0.012
| 1% KL      | 6.07±0.01 6.01±0.010 5.95±0.010 5.92±0.004 | 5.99±0.012
| 1% CL      | 5.79±0.02 5.72±0.012 5.69±0.011 5.64±0.014 | 5.71±0.014
| Overall mean (days) | 6.00±0.03 5.93±0.03 5.87±0.022 5.84±0.025 |
| **Titratable acidity (%)** |               |                |
| Control    | 0.065±0.002 0.077±0.002 0.085±0.002 0.092±0.002 | 0.080±0.002
| 1% SL      | 0.062±0.002 0.068±0.002 0.077±0.002 0.082±0.002 | 0.072±0.002
| 1% KL      | 0.062±0.002 0.067±0.002 0.078±0.002 0.080±0.000 | 0.072±0.002
| 1% CL      | 0.067±0.002 0.075±0.002 0.088±0.002 0.092±0.002 | 0.080±0.002
| Overall mean (days) | 0.064±0.000 0.072±0.011 0.082±0.021 0.086±0.001 |
| **Salmonella counts (log\textsubscript{10} cfu/g)** |               |                |
| Control    | 4.21±0.05 5.15±0.05 7.14±0.07 8.12±0.07 | 6.16±0.33
| 1% SL      | 4.15±0.04 4.76±0.07 6.21±0.04 7.79±0.07 | 5.73±0.29
| 1% KL      | 4.13±0.04 4.78±0.06 6.26±0.03 7.90±0.06 | 5.77±0.30
| 1% CL      | 4.04±0.06 4.66±0.05 6.06±0.08 7.27±0.05 | 5.51±0.26
| Overall mean (days) | 4.13±0.03 4.84±0.05 6.42±0.09 7.77±0.07 |

Means with common superscripts in a row and in a column do not differ significantly (P>0.05)
Treatment means are the means of 21 days only.
Significant (P<0.05) increase in TA was observed in control as compared to either SL or KL samples. Although TA was same for control and samples treated with CL, it may be due to pH lowering effect of latter. TA significantly (P<0.05) increased throughout the storage period.

Trends on effect of lactates on growth of S. Typhimurium as depicted in table clearly show that all the lactates had a significant (P<0.05) inhibitory effect on Salmonella growth. Among lactates, CL had the lowest count indicating its superiority with respect to inhibition of S. Typhimurium as compared to other two lactates. The growth inhibition of S. Typhimurium was more pronounced up to storage period of 7 days with only marginal increase in the counts. However, there was significant (P<0.05) increase in Salmonella count on 14 and 21 days storage period. The study has revealed that 1% concentration of all the lactates used were not bacteriostatic with respect to S. Typhimurium despite lower counts being observed in lactate treated samples as compared to control. These views were supported by the findings of Miller and Acuff (1994) who reported continued growth of S. Typhimurium in the cooked beef roasts with 0 and 2% SL even after 28 days of storage at 10°C. However, the growth was significantly (P<0.05) arrested by 3 and 4% SL. Al-Sheddy (1995) also observed that addition of 1% either SL or KL had a limited effect on growth of S. Typhimurium at 37°C. The study was discontinued after 21 days (3 weeks) as there was no inhibitory effect of various lactates and Salmonella count almost doubled reaching approximately 8 log cfu/g during 21 days storage.

From the study it was concluded that 1% level of any of these lactates was not sufficient to exhibit bacteriostatic effect on either of these food borne pathogenic bacteria in buffalo meat cooked salami under refrigerated storage.

References