Influence of Phosphate and Glucose Addition on some Important Spoilage Bacteria in Vacuum Packed Bologna-Type Sausage

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ABSTRACT

Studies were done on the influence of phosphate and glucose addition on some selected spoilage bacteria in vacuum packed sliced bologna-type sausage during refrigerated storage. Batches with low pH phosphate mixture or sodium tripolyphosphate were used along with batches without phosphate addition. Addition of low pH phosphate had a pronounced influence on Brochothrix thermosphacta and Serratia liquefaciens, while the influence of glucose addition on these bacteria was small. No marked effect of phosphate type could be observed with the lactic acid bacteria, but the most profound growth happened in sausages without phosphate, and at 2°C was stimulated by glucose addition. Lactic acid accumulated more rapidly in batches without phosphate addition.

Several attempts have been made during the years to find an effect of phosphate addition to foods on the microflora. In broth cultures studies by Kelch and Bühmann (18) with Staphylococcus aureus, Streptococcus faecalis and sporeformers as Bacillus subtilis and Clostridium bifermens, inhibition was observed by adding 0.5 and 1% of phosphate mixtures (Curafoss and Fibrisol). The studies were done at 37°C. Heating the cultures increased the inhibition. Firstenberg-Eden et al. (9) studied the influence of added phosphate on colony formation of Moraxella-Acinetobacter on PCA. The effectiveness of inhibiting colony formation decreased in the following order: sodium tripolyphosphate, pyrophosphate and orthophosphate. A filter-sterilized solution was more effective than if the phosphate solution was heated. The inhibitory action caused by polyphosphate addition to bacteriological media is not related to ion strength (8). Inhibition in media could be at least partially reduced by adding divalent metal ions (Mg²⁺), and inhibition might therefore be caused by chelating of essential metal ions (8). A patent (30) has claimed an effect of phosphate added to nutrient broth at 37°C. There was an increased inhibition of S. aureus with increasing chain length, but even tripolyphosphate showed an effect at a concentration of 1%. Medium chain length (C₁₆₋₃₄) polyphosphates were inhibitory towards both gram-negative (Pseudomonas fluorescens, Salmonella thyphimurium) and gram-positive (S. faecalis and Clostridium sporogenes) bacteria. Polyphosphate addition to egg white, ground fish and beef broth is also said to have an effect (30). Another patent (34) claims an effect of long-chained polyphosphates on yeast and fungi. Several studies have been concerned with the effect of chilling chickens in solutions of phosphate mixtures (8,28,32,34). Dipping in phosphate solutions (3% 8%) did not significantly affect total bacterial counts, while counts were lower with a combined phosphate dipping and heat treatment (35). The shelf-life of phosphate injected poultry did not improve (21).

Experiments with phosphate addition to a bologna-type sausage (0.3% of a mixture of pyro- and orthophosphates) showed no inhibition of the aerobic mesophile count, lactic acid bacteria or enterococci but stimulated growth of Enterobacteriaceae was observed at 4 and 10°C (3). Experiments with matured bacon injected with phosphate brine showed lower microbial counts than brine without phosphate, while the opposite was true for vacuum packed bacon stored at 5 and 10°C (19). In studies (27) with Clostridium butylicum, reduced toxin production was observed in a high pH meat emulsion product but not at low pH.

Blending polyphosphate and meat in an emulsion may result in hydrolysis of the added phosphate. Added sodium tripolyphosphate is rapidly hydrolysed by meat enzymes even at refrigeration temperatures (14,22,33). An inhibitory effect of tripolyphosphate addition might therefore be questionable.

Tripolyphosphates are often added to minced meat products as bologna-type sausage to increase the water binding capacity. This study was undertaken to determine if the phosphate addition has an influence on some important spoilage organisms in the vacuum packed product. Brochothrix thermosphacta and Serratia liquefaciens are bacteria often found in chilled foods associated with spoilage (4,11,15,17,22,24,26). The study includes an acid phosphate mixture which sometimes is used in salami production. Moreover, the influence of glucose addition to the sausage was examined.
SAUSAGE PRODUCTION

Dried onions and sodium ascorbate were added at the 55 batches 3 and 4 was added a commercial phosphate product, a mixture of 0.3% sodium tripolyphosphate. The phosphates were added at a level of 0.3% calculated as P₂O₅. Even numbered batches also received 1% glucose. After stuffing the sausage emulsion into casings, the sausages were cooked in a steam cabinet until a temperature of 75°C was reached in the center. After cooling (water spraying) the sausages were stored overnight at 2-4°C. The sausages were sliced using aseptic conditions and 5 slices (50 g) were inserted in premade polyethylene/polyamide bags (Riloplaste, Otto Nielsen A/S, Denmark) with an oxygen permeability of c.52 mm³/m²/24 h/1 atm. at 25°C and 75% R.H. The packs were inoculated with a mixture of 3 strains of *B. thermosphacta* and an atypical streptobacterium. All strains were isolated from vacuum packed bologna-type sausage. Five-tenths ml of a suspension of the bacteria grown in all purpose medium with tween (APT, Difco) at 8°C was used as inoculum. Other packs were inoculated with a mixture of 3 strains of *S. liquefaciens* and 3 strains of *B. thermosphacta* in the same manner as described above. Packs were vacuum packed using a Multivac machine at a vacuum level of 98%.

An experiment was done to determine if an effect of low pH phosphate on *B. thermosphacta* resulted from a specific phosphate or if it merely was pH dependent. Bologna-type sausage without added phosphate and glucose was sliced and packed in the bags as usual and 0.7 ml of sterile 1 N HCl was added. The bags were placed at 0°C for some hours for equilibration, pH was checked with pH paper. The packs were inoculated along with packs from the low pH phosphate batch (pH 5.9) with *B. thermosphacta*, vacuum packed as above and placed at 8°C until sampling. Duplicates of packs were examined at each sampling time.

**Sample preparation**

The entire content of the packs was aseptically put in sterile bags and homogenized in a Stomacher with 150 ml of sterile peptone water (0.1% peptone and 4% sodium chloride). The sodium chloride was added to protect the bacteria from an osmotic shock during homogenizing and dilution. The slurry was subsequently plated and serially diluted in peptone water with 4% sodium chloride (0.1%). *B. thermosphacta* was determined on streptomycin-thallous-acetate-actidione agar (STAA) (10), the lactic acid bacteria on nitrite-actidione-polymyxin agar (NAP) (4) and the gram-negative bacteria on all-purpose-medium with tween (AP). APT medium was always included in the experiments to compare growth on the selective media and to check for contamination. Contamination by unwanted microorganisms was never observed. Plates were incubated at 20°C.

**Chemical analyses**

The bologna-type sausages were analyzed for sodium chloride by the Volhard method (2) and for moisture by drying at 104°C to constant weight (1). The pH was determined on food homogenates using a combination electrode (Radiometer, Denmark).

**Sample dilution**

D-lactic acid was determined by substituting L-lactate dehydrogenase with D-lactate dehydrogenase. The remainder of the samples from the bacteriological analyses, which were stored until use at -24°C, were thawed in plastic bags at 60°C on a water bath and minced in a meat mincer. About 6 g was put into a centrifuge tube and the sample was mixed with an Ultra-Turrax (Janke & Kunkel KG, Germany) together with water for 1 1/2 min. After mixing, the tube was placed in a water bath at 60°C for 20 min, cooled in an ice bath, and the sample was then centrifuged for 30 min at 3500 r.p.m. The supernatant liquid was filtered and diluted as necessary. Measurements were made in disposable polymethacrylate cuvettes using a Beckmann DB-G spectrophotometer operating at 340 nm.

**Sensory analyses**

Sensory analyses were done on packs in the experiment with *B. thermosphacta* and *S. liquefaciens*. Samples were evaluated for odor by a trained panel (5 members) using an 11-point scale (+5, ideal 0, neither good nor bad, -5 extremely undesirable). Data were analysed by analyses of variance, when significant mean separation was accomplished using Duncan's Multiple Range test (5).

**RESULTS AND DISCUSSION**

Chemical analyses revealed that the sausages contained: sodium chloride 2.26-2.30%, moisture 56.52-57.50% i.e., a salt/water ratio of 3.96-4.05. The pH of batches 1, 2, 5 and 6 was 6.39-6.41 and of batches 3 and 4 pH was 5.93. The initial microbial load of uninoculated packs was less than 3/g consisting of sporogenic bacteria, which did not grow during storage.

Microbial growth is shown as growth curves for *B. thermosphacta*, lactic acid bacteria and *S. liquefaciens*. Differences in growth of *B. thermosphacta* in packs of low-pH phosphate and packs in which pH was lowered with hydrochloric acid was observed during 1 week of storage at 8°C (Fig. 1), i.e. the influence of phosphate is not merely due to the lowering of pH. Only after 5 d of storage, however, was the difference significant (99.9%).

There was a great influence of low pH phosphate addition to bologna-type sausage on growth of *B. thermosphacta* compared to sausages with added tripolyphosphate or without phosphate addition. At 2°C, the counts of *B. thermosphacta* were several log₁₀ units lower in the low-pH phosphate batches (3 and 4) compared with the other batches (Fig. 2) during a 5-week storage period. While counts in batches 3 and 4 had just exceeded 10⁶/g after 5 weeks of storage, counts of more than 10⁷/g were reached within 2 weeks in the other batches, and about 10⁸/g, counts which very rapidly results in organoleptic deterioration (6), were reached during 3 weeks of storage. No influence of addition of tripolyphosphate was observed and counts were not affected by glucose addition. At the higher temperature, 8°C, an effect of low pH phosphate could still be observed, with counts up to several log₁₀ units lower in batches 3 and 4 than in the other series (Fig. 3).

Growth of the lactic acid bacteria, an atypical streptobacterium, was slow at 2°C (Fig. 4), counts of 10⁷-10⁹/g not being reached before the end of the storage period. No marked influence of phosphate or glucose addition was observed, except that counts in batch 2, without added phosphate but with added glucose, were considerably higher during storage than in the other batches. At 8°C lactic acid bacteria counts were higher in batches without added phosphate during logarithmic growth (Fig. 4) regardless of glucose addition.

At 2°C, no change in pH was observed before the 19th day, where pH in batches 1 and 2 had decreased to 6.1 and in batches 5 and 6 to 6.2. After 33 d the pH had decreased to 5.9 in batches 1 and 2 and to 6.1 in batches 5 and 6, while the pH in the series 3 and 4 was still 5.9. After 38 d of storage the pH in all batches was 5.9.
At 8°C, the pH naturally decreased faster during storage. After 1 week, the pH had decreased to 6.28 in batches 1 and 2 and to 6.33 in batches 5 and 6. After 2 weeks of storage, the pH had decreased 5.78, 5.58, 5.79, 5.81, 6.01, and 5.96 in batches 1, 2, 3, 4, 5, and 6, respectively. A further decrease in pH was observed during storage and after 4 weeks the difference between batches with and without added glucose was 0.4 pH unit for batches 1, 2, 5, and 6 and 0.15 unit for batches 3 and 4. It is obvious that phosphate addition had a stabilizing influence upon pH in the sausage. After only 2 weeks of storage, the pH in the sausage without added phosphate was the lowest.

The examination of lactate, shows (Table 2) that there was a steady increase in L- as well as D-lactate during storage at 8°C. The lactate production was more rapid in glucose-added batches and higher terminal concentrations were observed in these batches. While some L-lactate originating from the meat, is present in the sausage at the beginning of the storage period, the D-lactate is entirely produced by the bacteria. Although no marked difference in growth between lactic acid bacteria in batches 3, 4, 5, and 6 was observed, the increase in lactate was slower in batches in which low pH phosphate was added. A study by Luke (20) stated that a concentration of more than 0.5 mg of D-lactate/g of meat was related to a decreased organoleptic quality. Table 2 shows that this would be true at an earlier time in the batches 1 and 2 than in batches with added phosphate. At 2°C (Table 1) the D-lactate concentration was higher during storage in batches with added glucose, and the D-lactate concentration was much lower in batches 3 and 4 than in the other batches. This could mean that although the counts of lactic acid bacteria were not markedly different, except for batch 2, the low pH phosphate nevertheless had an influence on the metabolic activity. One might therefore expect a better organoleptic quality. Furthermore, although the end product of glucose for *B. thermosphacta* is primarily L-lactate under anaerobic conditions (12,16), the lactate produced in vacuum packed cured meat products is more related to lactic acid bacteria than to *B. thermosphacta* (according to present work in the laboratory). Under aerobic conditions the end product of glucose is acetoin (L. E. Brownlie, M. Sc. Thesis, University of Sydney, Sydney 1969), and acetoin is also produced in vacuum packed cured meat (29). Since this was not measured, it is not possible to say anything about the influence of phosphate addition on the metabolic activity of *B. thermosphacta* but the numbers were, as the growth curves show, greatly reduced by low pH phosphate addition.

The gram-negative bacterium, *S. liquefaciens* was greatly influenced by low pH phosphate addition at 2°C (Fig. 5). Counts in batches 3 and 4 increased from 10^2/g at the beginning of the storage period to about 10^5/g after 3 weeks regardless of glucose addition. In all other batches, counts of more than 10^7/g were reached within 2 weeks.

![Figure 1. Growth of *B. thermosphacta* at 8°C. Open symbols, pH controlled with low pH phosphate; closed symbols, pH controlled with hydrochloric acid.](image1)

**Figure 1.** Growth of *B. thermosphacta* at 8°C. Open symbols, pH controlled with low pH phosphate; closed symbols, pH controlled with hydrochloric acid.

![Figure 2. Growth of *B. thermosphacta* at 2°C. Open symbols without added glucose, closed symbols with 1% glucose added.](image2)

**Figure 2.** Growth of *B. thermosphacta* at 2°C. Open symbols without added glucose, closed symbols with 1% glucose added.
The gram-negative bacteria used in this inoculation experiment are common in vacuum packed cured meat products and they are important spoilage organisms (24). One might therefore be interested in reducing their growth in these products, and this could be accomplished by adding low pH phosphate. It is, however, necessary to use a low storage temperature, at 8°C (Fig. 5) growth of S. liquefaciens was very much the same in all batches. The study involved both S. liquefaciens and B. thermosphacta. Growth curves of B. thermosphacta are not shown, they were identical with the growth curves obtained in combination with the lactic acid bacteria. The pH fell at 2°C to 6.0 in batches 1 and 2 and to 6.27 and 6.22 in batches 5 and 6, respectively, while the pH remained constant in the low pH phosphate batches. At 8°C, the pH in the packs decreased to 6.10 in batches 1 and 2, and to 6.30 and 6.25 in batches 5 and 6, respectively, while the pH remained constant during storage in batches 3 and 4. Comparing the drop in pH in this experiment with those in the study with B. thermosphacta and the lactic acid bacteria it is obvious that considerably less lactic acid was produced by B. thermosphacta than by the lactic acid bacteria. While glucose addition did not affect growth of B. thermosphacta or S. liquefaciens in the present study, naturally contaminated products showed lower counts for these bacteria in batches with triphosphate and without glucose than in batches with glucose addition (25).

The organoleptic analyses of odor showed that throughout the 3-week storage period at 2°C a trend towards a better organoleptic quality was obtained by adding low pH phosphate. Both the batch with added glucose and the batch without sugar addition had higher mean odor values than the other batches (Table 3). In naturally contaminated products significantly different odor scores between low pH phosphate batches and batches without phosphate or with triphosphate were observed (25).

<table>
<thead>
<tr>
<th>Batch</th>
<th>L</th>
<th>D</th>
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<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>2.51 0.02</td>
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<tr>
<td>2</td>
<td>14</td>
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<tr>
<td>3</td>
<td>19</td>
<td>2.34 0.00</td>
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<tr>
<td>4</td>
<td>25</td>
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<td>33</td>
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<tr>
<td>6</td>
<td>38</td>
<td>3.16 0.73</td>
</tr>
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</table>

**Figure 3.** Growth of B. thermosphacta at 8°C. ○, batches without phosphate; Δ, batches with low pH phosphate; □, batches with triphosphate. Open symbols without added glucose, closed symbols with 1% glucose.

**Figure 4.** Growth of lactic acid bacteria at 2°C (-----) and 8°C (----). O, batches without phosphate; Δ, batches with low pH phosphate; □, batches with triphosphate. Open symbols without added glucose, closed symbols with 1% glucose.

**Table 1.** Concentration of L- (L) and D- (D) lactate in packs during storage at 2°C. Batches 1 and 2 without phosphate, 3 and 4 with low pH phosphate and 5 and 6 with triphosphate. Even numbered batches were added glucose.
TABLE 2. Concentration of L- (L) and D- (D) lactate in packs during storage at 8°C. Batches 1 and 2 without phosphate, 3 and 4 with low pH phosphate and 5 and 6 with tripolyphosphate. Even numbered batches were added glucose.

<table>
<thead>
<tr>
<th>Days</th>
<th>Batch 1 (L)</th>
<th>Batch 1 (D)</th>
<th>Batch 2 (L)</th>
<th>Batch 2 (D)</th>
<th>Batch 3 (L)</th>
<th>Batch 3 (D)</th>
<th>Batch 4 (L)</th>
<th>Batch 4 (D)</th>
<th>Batch 5 (L)</th>
<th>Batch 5 (D)</th>
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<tr>
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<td>0.01</td>
<td>1.36</td>
<td>0.02</td>
<td>1.86</td>
<td>0.02</td>
<td>1.76</td>
<td>0.03</td>
<td>1.82</td>
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<td>6</td>
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<td>0.11</td>
<td>1.86</td>
<td>0.08</td>
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<td>0.02</td>
<td>1.56</td>
<td>0.03</td>
<td>1.86</td>
<td>0.03</td>
<td>1.85</td>
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<td>13</td>
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<td>0.92</td>
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<td>3.79</td>
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<td>4.77</td>
<td>1.27</td>
<td>5.47</td>
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CONCLUSIONS

The study showed that sodium tripolyphosphate addition had negligible influence on growth of B. thermosphacta and S. liquefaciens. The bacteria were strongly inhibited by addition of a low pH phosphate mixture. Odor assessment showed that this resulted in a better quality of the product especially when glucose addition was omitted. The lactic acid bacteria showed only minor inhibition by phosphate, with stimulated growth by glucose at 2°C in batches without phosphate addition. Analyses of lactate in packages during storage showed that metabolic activity was inhibited by phosphate addition and stimulated lactate production was observed in glucose added batches.

ACKNOWLEDGMENTS

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REFERENCES


TABLE 3. Means of odor assessments of packs inoculated with a mixture of B. thermosphacta and S. liquefaciens during storage at 8°C.

<table>
<thead>
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<th>Days</th>
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<td>0</td>
</tr>
<tr>
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<td>-1.40</td>
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<tr>
<td>7</td>
<td>-1.80</td>
</tr>
<tr>
<td>19</td>
<td>-1.40</td>
</tr>
</tbody>
</table>

*Mean in the same row followed by a common or no letter are not different (P>0.05).


