Asymmetric Distribution and Growth of Bacteria in Sliced Vacuum-Packaged Ham and Bologna

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ABSTRACT

Commercially sliced vacuum-packaged cooked ham and bologna were found to contain significantly greater numbers of total and lactic acid bacteria (LAB) on package surface slices than on internal slices. This asymmetric distribution persisted in most samples to beyond the manufacturer’s “best before” date. Enterobacteriaceae and bacterial spores were detected infrequently. Bacterial spores were found most often on the surface slices of commercially packaged sliced bologna. Where they occurred, aerobic and anaerobic spores were detected in equal numbers. Center slices of bologna were less hospitable sites for spores and LAB than were surface slices in vacuum packages. When freshly cooked ham and bologna were sliced together with uncooked fermented sausage, LAB from the sausage (pediococci and thermotolerant homofermentative lactobacilli) contaminated the cooked meats immediately causing an equal distribution of bacteria throughout the slices. Pediococci did not survive in the vacuum-packaged cooked meats more than 2 weeks at 7°C. The pH of co-sliced bologna prematurely dropped. The shelf life of refrigerated co-sliced ham was reduced by 44%, probably because of adventitious lactobacilli from the sausage. Brochothrix thermosphacta was not present in co-sliced or sliced control meats. Homofermentative lactobacilli predominated in co-sliced and control samples packaged in the laboratory, but in commercially packaged sliced cooked ham and bologna heterofermentative LAB species were dominant. Prolonging cooked ham and bologna shelf life is possible if handling of uncooked fermented sausage is kept separate from the slicing and packaging of cooked cured products.

Key words: Lactic acid bacteria, cooked cured meats, fermented sausage, shelf life, packaging

Bacterial growth in unsliced vacuum-packaged cooked cured meat products held at refrigerator or abuse temperatures occurs asymmetrically at the meat-surface-packaging-film interface (2, 9, 19). The predominant growth of bacteria at the meat surface was believed to be due in part to greater water activity (a_w) at the surface (19), but in our work this relationship could not be established (9). Bell and Gill (2) and Korkeala and Lindroth (19) suggested that the difference might also result from greater oxygen concentrations in the microenvironment at the meat surface. This may happen following the slow diffusion of oxygen through the packaging material during storage and may be facilitated even with barrier films as a result of heat-induced mechanical distortion and stretching (thinning) during thermoforming of the package (29).

Product handling after cooking plus slicing prior to vacuum repackaging are believed to be major factors which influence the level of bacterial contamination in freshly packaged cured meat products. In commercial practice the slicing procedure itself has been shown to add between 0.5 to 2.0 log CFU of bacteria per g to the meat (17, 24). Holley et al. (9) found that when delicatessen meats were sliced and repackaged at the retail level (rather than at the manufacturer’s) the increase due to slicing could be kept as low as 0.5 log CFU of bacteria per g through the use of accepted sanitation practices. When wholesale packaged unsliced cooked cured meats nearing the manufacturer’s coded “best before” date were sliced, repackaged under vacuum and the top slices then sampled, products initially appeared to have reduced bacterial loads. This contradictory result was due to the differences in sampling site—sampling from the top slice was equivalent to sampling from an internal core sample of the unsliced meat. These latter are known to yield lower numbers of bacteria than surface samples (2, 18, 19). When packaged cooked meats were sliced and repackaged, their shelf life was more closely related to the number of days the slices were in the final consumer package rather than to the length of time wholesale packages were held prior to slicing (13). This suggested, provided Enterobacteriaceae and Brochothrix thermosphacta were absent from the unsliced meat, that slicing and repackaging under vacuum could have the effect of extending the available combined (unsliced plus sliced) shelf life under vacuum.

Lactic acid bacteria (LAB) dominate the microorganisms present in vacuum-packaged chill-stored cured meats and initially serve a protective function by excluding less desirable and pathogenic species (6, 7). As their numbers reach levels of >8 log CFU/g (3, 20) the end of product shelf life is signalled by the development of off or sour odors, sour

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flavors, the presence of cloudy purge, and visually detectable slime. Heterofermentative lactobacilli and *Leuconostoc* spp. also produce CO₂, which accumulates in packages and causes loss of vacuum and package swelling. Whether products are spoiled by *Leuconostoc* (30) or *Lactobacillus* spp. (15, 20, 27) is significantly influenced by the microflora resident at the place of manufacture (26) and even by differences in levels of processing-line sanitation within the same plant (24).

The organisms which frequently dominate during refrigerated storage of cooked delicatessen meats (12) are often similar to the major species (*Lactobacillus curvatus, L. sake*) present in fully ripened fermented sausages (15, 18, 27). Suspicion that these organisms might be responsible for the early spoilage of vacuum-packaged cooked meats prompted a precautionary statement that raw fermented dry sausages and cooked cured meat products be handled in two different areas of the processing plant (24). Makela (22) demonstrated that ropy-slime-forming homofermentative lactobacilli, including a strain of *L. sake* isolated from spoiled vacuum-packaged cooked cured meat, could successfully compete with starter cultures during dry sausage manufacture and reach high levels by the end of sausage fermentation. Clear evidence for their transfer to cooked meats has not been developed.

The current work was undertaken to determine whether in commercial vacuum packages of machine-sliced cooked cured meats, surface growth would predominate as it does in unsliced meats during refrigeration storage. Asymmetric distribution of bacteria in packages of sliced meats may be an important reason that conventional sampling procedures yield results which are variable and not correlated with product shelf life. This asymmetry was also of interest from the perspective of developing strategies for the use of antimicrobial film materials for sliced vacuum-packaged meats. In addition, it was noted that the practice of co-slicing two or more different cured meats on a slicing machine platform at the same time is popular at both retail and processing plants and often involves semiautomated slicing equipment. In one large processing plant, ≤20% of vacuum packages of cured meats contain two types of meat prepared by simultaneous slicing. This procedure was viewed as having the potential to substantially reduce shelf life of packaged cooked products, particularly if one of the meats handled were a fermented sausage containing live starter and adventitious LAB. It was of interest to validate this concern and evaluate the effect of co-slicing cooked cured meats with fermented sausage by testing the subsequent microbiological condition and potential shelf life of the resulting vacuum-packaged products.

**MATERIALS AND METHODS**

All meat samples used in these experiments were manufactured at federally registered establishments in Canada according to standard recipes.

*Retail samples of bologna at “best before” (expiry) date*

As preliminary tests, 4 samples of one brand (manufacturer A) of sliced vacuum-packaged all-beef bologna were purchased from a retail meat display case, held in the laboratory at 7°C until the coded “best before” date was reached, and analyzed. Three samples weighed 500 g and one weighed 175 g. Slices measured 2 to 4 mm thick in the same package and 10.5 to 11 cm in diameter and were arranged in a stack. The transparent flexible packaging film material was fabricated with a nonformed top (lidding material) bearing the identity of the manufacturer joined by a heat seal to a formed seamless flexible bottom panel closely adherent to the meat surfaces.

At sampling, the packages were aseptically opened and a 10-cm² disk was cut through the slice with an aseptically prepared 12-cm length of stainless-steel pipe having a sharpened edge and an internal cross-sectional area of 10 cm². One sample was taken from the center of both top and bottom slices; the stack of slices was then divided in half and a similar sample was taken from the center slice. The samples, which weighed between 2.81 and 3.85 g, were separately placed in a Stomacher bag with 90 ml of 0.1% (wt/vol) peptone water and massaged in a Stomacher 400 (A. J. Seward, Canlab,Toronto, ON) for 60 s. The samples were serially diluted in 0.1% peptone and surface spread on prepoured plate count agar (PCA) (BBL, Cockeysville, MD); duplicate plates of each dilution were incubated at 25 and 35°C for 48 h. Aerobic and anaerobic spores were enumerated on PCA at 35°C by heating 10 ml of a 10⁻¹ dilution of meat suspension at 75°C for 20 min and cooling for 10 min before plating (21). *Enterobacteriaceae* were enumerated by using overlaid pour plates of violet red bile agar (BBL) containing 1% (wt/vol) glucose (VRBGA) and incubating for 24 h at 35°C (24). Lactic acid bacteria were counted on spread plates of de Man Rogosa Sharpe agar medium (BBL) containing 0.1% thallium acetate (MRST) (24) and on M5 medium (31) following incubation at 25°C for 48 h. Anaerobic spores and LAB were grown anaerobically in a BBL GasPak® anaerobic system with a CO₂ plus H₂ generator. Numbers of viable bacteria were reported as CFU per square centimeter, taking into account that each disk sampled had two 10-cm² surfaces. Results throughout were divided by 2 to obtain numbers of bacteria per square centimeter of surface. The pH of all samples was measured in a 10⁻¹ dilution of meat homogenate (20) using a combination glass electrode and an Accumet model 910 pH meter (Fisher Scientific Ltd., Winnipeg, MB).

*Testing asymmetry of bacterial growth in sliced bologna*

On three occasions six 500-g packages of sliced, vacuum-packaged, branded (manufacturers A to C) all-beef bologna from the same lot were purchased at retail. Each lot was held for several days in the laboratory at 7°C and shelf-life studies were started when 35 days remained until coded product “best before” date. Sliced geometry, arrangement, package format and appearance were as described previously. Sampling and microbiological and pH analyses were conducted as previously described at 0, 14, 21, 28, 35, and 60 days of storage at 7°C. A freshly opened package was used each sampling date.

*Testing asymmetry of bacterial growth in sliced cooked ham*

Two sets of 4 samples of vacuum-packaged sliced reformulated cooked ham (375-g packages) were obtained at retail. Each set, consisting of packages from the same lot (manufacturers C and D), were packaged with flexible opaque lidding (top) material displaying brand identification. The top was joined by a heat seal to a rigid transparent heat-formed base. Slices measured 2 to 4 mm thick and were 121 cm² in cross section (11 by 11 cm). Samples were held at 7°C until 2 weeks prior to the “best before” date, when single packages from each set were opened and sampled. New packages were sampled at weekly intervals until 1 week
Co-slicing experiments

Freshly cooked wholesale packages of ham and bologna weighing 5.1 and 4.3 kg, respectively, were obtained directly from the same meat plant (manufacturer B). The ham measured 12.5 by 12.5 cm in cross section and had 101 days left to reach the coded “best before” date. The bologna measured 11 cm in diameter and 59 days remained in product freshness code. Two intact fermented sausages (produced by manufacturer E) stuffed in collagen casings and weighing 1.2 kg each were purchased at retail. These Hungarian salami measured 8 cm in diameter; they were from the same manufacturer’s lot and had 122 days left before reaching the “best before” date. The lactic acid bacteria starter culture used in salami manufacture (Trumark LTH) was obtained from both the meat processor and the culture supplier (Canada Compound Western Ltd., Winnipeg, MB) and analyzed for active bacterial species: for total bacteria on APT at 32 and 25°C both aerobically and anaerobically; for micrococci on Baird Parker agar and mannitol salt agar, (Difco Laboratories, Detroit, MI) at 35°C for 48 h; for streptococci on KF streptococcus agar (BBL) at 35°C for 48 h; for Brochothrix thermosphacta on streptomycin–thallous acetate–acetidione agar (STAA) (15) at 21°C for 3 days; for Enterobacteriaceae on VRBGA, and for lactic acid bacteria on MRSD at 25°C for 72 h, and 45°C for 3 to 5 days anaerobically, using the GasPak® System.

Prior to beginning the co-slicing experiments, half of the intact meat samples were separately sliced using a Hobart 410 slicer (Hobart Manufacturing Co. Ltd., Mississauga, ON) to yield slices 2 to 3 mm thick. These control samples were “stack packed” in 400-g amounts (ham and bologna) and 200-g amounts (salami) and aseptically packaged under vacuum using a model GM 2002 modified-atmosphere packaging machine (Bizerba Canada, Mississauga, ON) and SchurBar® oxygen barrier bags (Schurpack Inc., St. Joseph, MO), consisting of ethylene vinyl alcohol, EVOH, coextruded with polyethylene and nylon, (1.91 capacity) and stored at 7°C. The slicer was cleaned and sanitized between the use of different meats.

For the co-slicing experiments, the remaining ham or bologna was placed next to the salami on the cutting platform of the slicing machine in a manner such that the blade passed through the salami and then through the ham or bologna. The co-sliced salami, ham, and bologna were packaged separately under vacuum as previously described. The slicing machine was again dismantled, cleaned, and sanitized between each use of different meats. Samples were stored at 7°C and analyzed on day 0 (slicing) and after 7, 14, 21 and 28 days of storage. Co-sliced and control packages of bologna, ham, and salami were sampled at day 0 using the slice-sampling system previously described. On subsequent days the surface area sampled was increased from 10 cm² to 40 cm² by taking four disks per slice, increasing the pooled sample weight to 9.70 ± 1.56 g. Control samples of salami were taken at days 0 and 28 only; control ham and bologna were sampled and analyzed in parallel with co-sliced samples. At each sampling time a new package was used. All meat samples were analyzed for total bacteria on APT at 25°C for 48 h anaerobically, for lactic acid bacteria on MRSD at 25°C for 48 h anaerobically, for Enterobacteriaceae on VRBGA at 35°C for 24 h, and for Brochothrix thermosphacta on STAA at 21°C for 3 days. In addition, duplicate MRSD plates were incubated at 45°C for 3 to 5 days to detect thermotolerant LAB such as Lactobacillus plantarum, L. sake, and L. curvatus (18, 27).

Statistical analyses

One- and two-way analyses of variance were conducted using the general linear model procedure of SAS with comparison of means by Duncan’s multiple range test (1). Differences were considered statistically significant at the 5% level.

Oxygen permeability of film packaging

The O₂ permeability of packaging film materials was measured by using an Ox-Tran 10/50 (Mocron, Minneapolis, MN) as previously described using film samples cut from the lid and thermoformed bottom of the same package (9).

RESULTS

Bologna at coded “best before” date (preliminary tests)

In samples of vacuum-packed sliced bologna prepared by one manufacturer, purchased at retail and held at 7°C until the “best before” date was reached, packages contained large numbers of bacteria which were almost exclusively LAB. Analysis revealed there was no significant difference among the numbers of bacteria recovered on PCA (at 32 or 25°C), M5, or MRST (at 25°C) agars. Therefore the results of these tests were combined. The numbers of bacteria on top and bottom slices did not differ (P > 0.05), even though the oxygen permeability of the films at the top and bottom of the packages was very different (Table 1). Numbers of bacteria on outside slices were significantly higher than the number present on the middle slice. These latter represented <0.01% of the number present on outside slices. Bacterial colonies on M5 medium were exclusively white (heterofermentative LAB) whether the sample was from an inside or outside slice. No colonies were found on VRBG agar from any of the samples tested. Three samples

TABLE 1. Bacterial distribution in vacuum-packaged sliced bologna at termination of shelf life

<table>
<thead>
<tr>
<th>Slice location in package</th>
<th>Total + LAB (log CFU/cm², mean ± SD)</th>
<th>pH</th>
<th>Dry packaging film O₂ permeability (cm³/m²/day/ atm, 23°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>7.20 ± 0.28a</td>
<td>6.50 ± 0.50a</td>
<td>6.1</td>
</tr>
<tr>
<td>Middle</td>
<td>3.13 ± 1.56c</td>
<td>6.33 ± 0.28c</td>
<td>NAa</td>
</tr>
<tr>
<td>Bottom</td>
<td>7.30 ± 0.18b</td>
<td>5.85 ± 0.60b</td>
<td>85.7</td>
</tr>
</tbody>
</table>

* Total bacteria (PCA, 35°C, 25°C) and lactic acid bacteria (M5, MRST, 25°C).

a Values in a column followed by the same letter are not statistically different (P > 0.05).

a NA, not applicable.
did not contain bacterial spores but when they occurred in the fourth they were <3.0 log10 CFU/cm² and were present only on the outside slices.

The pH measured at each location in the packages was not significantly different but tended to be lower in package extremities. At the anticipated end of product shelf life, packages of bologna were not discolored and did not have any off odors, but they contained milky purée and a noticeable amount of smeared fat from slicing which detracted from product appearance. None of the packages contained material which would be described as ropy or slimy. While meats were not discolored, color had faded and this tended to accentuate the presence of fat deposits at the edge of slices in the stack.

Asymmetric bacterial growth in sliced bologna

Again, numbers of total bacteria recovered on PCA at both 35 and 25°C as well as numbers of LAB present on M5 and MRST agars at 25°C were almost identical. Results from PCA analyses at the two temperatures and LAB results from the two media were each combined separately for different package locations. The bologna products from the three manufacturers spoiled in slightly different ways, which precluded grouping the data together in one figure. Differences in growth kinetics were unrelated to differences in ingredients used by the manufacturers. In each of the trials reported in Figures 1 to 3, manufacturer’s coded “best before” or estimated end of shelf life occurred at day 35 of the tests.

Numbers of both total bacteria and LAB were consistently higher on outside slices throughout storage at 7°C to the end of the bologna shelf life at 35 days. This difference was also noted at day 60, but in one trial the number of organisms present internally increased to almost the same level as found on outside slices (Fig. 1).

Results from analyses for bacterial spores and Enterobacteriaceae are reported in the text only because their numbers were low and not all successive samples yielded positive results following their initial discovery. Bacterial spores (aerobic and anaerobic) were conspicuously absent from samples taken from the middle package slices except for product A sampled at day 60 where numbers of spores from both inside and outside were equal (3.1 log CFU/cm²). Other observations of spores on internal slices of product from manufacturers B and C were also only made at day 60 but numbers were very low (≤0.7 log CFU/cm²).

Enterobacteriaceae were not found in samples from manufacturers A and B but were found at low levels (≤2 log CFU/cm²) at day 14 in both inside and outside slices of product C and again in both inside (1.4 log CFU/cm²) and outside (3.70 log CFU/cm²) slices at day 60.

The nature of changes in bologna pH during storage also varied among the three manufacturers’ products and are illustrated in Figures 1–3.

FIGURE 1. Asymmetric bacterial growth and pH changes in commercially sliced and vacuum-packaged bologna (product A) stored at 7°C. Results from PCA at 35°C and 25°C are combined. Results from M5 and MRST (25°C) are combined to yield numbers of lactic acid bacteria. Data points are means of 4 (outside) and 2 (inside) tests done in duplicate. Outside pH results are means from observations at top and bottom of packages; SD were ≤0.04 pH units.

FIGURE 2. Asymmetric bacterial growth and pH changes in commercially sliced and vacuum-packaged bologna (product B) stored at 7°C. Data for PCA and lactic acid bacteria are each combinations as in Fig. 1. For outside slice pH, SD were ≤0.42 pH units.

FIGURE 3. Asymmetric bacterial growth and pH changes in commercially sliced and vacuum-packaged bologna (product C) stored at 7°C. Data for PCA and lactic acid bacteria are each combinations as in Fig. 1. For outside slice pH, SD were ≤0.08 pH units.
Asymmetric bacterial growth in sliced ham

Results from analyses for total bacteria (on PCA and APT at 25°C) and LAB (on M5, MRST, and MRSD at 25°C) did not differ and therefore these were pooled as were results from both trials. Colonies on HHD medium were small and difficult to count on IsoGrid® filters and therefore these results were not included; HHD use was discontinued in further testing. Results from other media are reported in Figure 4.

Common commercial practice for these meat products is to code the “best before” date to be 50 days after slicing by the manufacturer (personal communication, manufacturer B). The abscissa on Figure 4 has been adjusted to take this into account; thus day 0 of the trials was actually 36 days following slicing by the manufacturer. This is the only experiment reported here where this change was made and it was necessitated by the closeness of the expiry date when the samples were purchased.

Numbers of bacteria had reached peak levels by the time the “best before” date was reached and increased only slightly during the next week of storage (Fig. 4). Bacterial numbers on the outside slices at each time samples were taken during storage were significantly (∝ 0.05) higher than numbers present on inside slices. These latter never exceeded 5% of those present on the outside slices. No growth occurred on the three LAB media (M5, MRST, and MRSD) when they were incubated at 45°C for as long as 5 days. Samples for these analyses were plated at a dilution as low as 10⁻¹.

It is notable that with ham samples plated on M5 medium, initially most colonies were blue colored, indicating homofermentative LAB. Within 2 weeks of storage and thereafter, colonies recovered on this medium were white, reflecting a change to heterofermentative LAB in the packages (31) (Table 2).

It was significant that no Enterobacteriaceae were detected in any samples (<3 CFU/cm²). Aerobic spore-forming bacteria were detected in one outside slice (1.55 log CFU/cm²) on day 43 but otherwise were absent from samples. Analyses for anaerobic spores and B. thermosphacta were not conducted on these ham samples. Preliminary screening indicated low numbers would be detected.

Ham pH progressively declined during the study from its initial levels of > 6.0 at day 36 and was substantially lower within 2 weeks (Fig. 4). Although the pH of internal ham slices was consistently higher than that of the outside slices, these differences were not significant.

Products from manufacturer D showed visible liquid in the packages at day 36. Contents wereropy at day 43, showed evidence of package bloating at day 50, and also had a sour smell at that time. Samples from manufacturer C showed the same characteristics but these occurred 1 week later in each case. Although not discolored, the ham products displayed faded color at day 43.

Co-slicing

The fermented sausage used in these experiments initially contained 6.1 log CFU/cm² of LAB in equal numbers at the surface and internally. Numbers of bacteria on both APT and MRSD were the same at both sites. After 28 days of storage following slicing and vacuum packaging this number had decreased to 5.7 log CFU/cm² at both the surface and internally. Thermotolerant LAB were present at levels of ≥ 4.95 log CFU/cm² at day 0 and these numbers remained stable during storage to 28 days (4.75 log CFU/cm²) (Fig. 5).

Analysis of the starter culture used in fermented sausage manufacture indicated that it consisted mainly of a nonpathogenic Staphylococcus sp. (probably S. carnosus) at a concentration of 8.52 log CFU/g, a Pediococcus sp. (8.23 log CFU/g), and a smaller number of thermostolerant lactobacilli (6.48 log CFU/g). No Enterobacteriaceae or Brochothrix thermosphacta were present in any of the samples analyzed. On KF agar both presumptive streptococci and pediococci were recovered as previously reported for this product (11).

Since streptococci do not generally grow on MRSD (14) and since the numbers of bacteria on MRSD and APT were identical, it was concluded that streptococci were not a major component of the starter-culture microflora. Microbial

![FIGURE 4. Asymmetric bacterial growth and pH changes in commercially sliced and vacuum-packaged ham. Data presented are means ± SD (vertical bars) of observations made with products from manufacturers C and D. Total numbers of bacteria are means following growth on PCA, APT, MRST, and MRSD media incubated at 25°C. Data points are means of ≥ 17 (outside) and ≥ 9 (inside) duplicate measurements.](http://meridian.allenpress.com/jfp/article-pdf/60/5/510/1665290/0362-028x-60_5_510.pdf)

<table>
<thead>
<tr>
<th>Location of slicing</th>
<th>Meat</th>
<th>Initial</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>60</th>
</tr>
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<tbody>
<tr>
<td>Commercial</td>
<td>ham</td>
<td>95–100</td>
<td>0–95</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Commercial</td>
<td>bologna</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Laboratory</td>
<td>ham</td>
<td>75</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Total colonies present on M5 and MRSD agars and confirmed as described in the text. Remaining organisms were heterofermentative lactobacilli.
* ND, not determined.
The effect of co-slicing salami with bologna or ham upon the microflora of the salami was also studied. Since there were no differences in microbiological content of salami following slicing with bologna or ham, these results were combined and presented as “co-sliced” in Figure 5. Numbers of bacteria recovered from salami on APT and MRSD media at the 10−1 dilution samples were plated on MRSD and incubated at 45°C were unaffected by contact with the slicing blade which had passed through these other meats. Pediococci (blue colonies on MRSD at 25°C) were dominant in samples up to day 7 but thereafter were not found in co-sliced salami (Table 3), and as with the control salami at day 28, colonies present on MRSD at 25°C were green.

In contrast with control salami samples, at day 0 no bacteria were recovered from 10−1 sample dilutions of salami sliced together with ham or bologna plated on MRSD and incubated at 45°C. By day 7 numbers of these thermotolerant LAB were almost restored to initial control levels and recovery was complete by day 14 (Fig. 5). Also at day 7, 25% of colonies from salami sliced with other meats plated on MRSD incubated at 45°C were pediococci. These disappeared by day 14 in all samples and were replaced by green colonies (thermotolerant lactobacilli) which predominated for the remainder of the study. Numbers of these organisms tended to be lower on internal slices of repacked salami but a statistical comparison was not made (Fig. 5). No Enterobacteriaceae or Brochothrix thermosphacta were found in any of the co-sliced meat samples throughout the study.

No bacteria were recovered at day 0 from control bologna (Fig. 6) and control ham (Fig. 7) samples plated on APT and MRSD media at the 10−1 dilution. Bacteria grew faster on outside slices of control cooked meats than on inside slices. Bacteria were not recovered from control ham or bologna throughout the study when 10−1 dilution samples were plated on MRSD and incubated at 45°C. Co-slicing ham and bologna with salami led to the immediate transfer of cultures from the supplier and meat processor were identical.

The effect of co-slicing salami with bologna or ham upon the microflora of the salami was also studied. Since there were no differences in microbiological content of salami following slicing with bologna or ham, these results were combined and presented as “co-sliced” in Figure 5. Numbers of bacteria recovered from salami on APT and MRSD media at the 10−1 dilution samples were plated on MRSD and incubated at 45°C. Bacteria were not recovered from control ham and bologna at day 0 and control bologna at day 7 where no bacteria were recovered.

**TABLE 3. Changes in the population of pediococci present in sliced vacuum-packaged cured meats during storage**

<table>
<thead>
<tr>
<th>Meat</th>
<th>Treatment</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salami</td>
<td>control</td>
<td>60</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Salami</td>
<td>co-sliced</td>
<td>75</td>
<td>70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bologna</td>
<td>control</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bologna</td>
<td>co-sliced</td>
<td>50–80</td>
<td>10–70</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ham</td>
<td>control</td>
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</tr>
<tr>
<td>Ham</td>
<td>co-sliced</td>
<td>50–80</td>
<td>10–70</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*The remaining organisms were lactobacilli except for control ham and bologna at day 0 and control bologna at day 7 where no bacteria were recovered.

*ND, not determined.

**FIGURE 5. Changes in bacteria present in fermented sausage (salami) following co-slicing with ham and bologna, repackaging meats separately under vacuum, and storage at 7°C. Total bacteria are combined results from PCA and MRSD at 25°C. Thermotolerant lactic acid bacteria were recovered on MRSD at 45°C.**

**FIGURE 6. Growth of bacteria in bologna after co-slicing with fermented sausage and repackaging meats separately under vacuum and storage at 7°C. Total bacteria are combined results from PCA and MRSD at 25°C. Thermotolerant lactic acid bacteria were recovered on MRSD at 45°C.**

**FIGURE 7. Growth of bacteria in ham after co-slicing with fermented sausage and repackaging separately under vacuum and storage at 7°C. Total bacteria are combined results from PCA and MRSD at 25°C. Thermotolerant lactic acid bacteria were enumerated on MRSD at 45°C.**

Control samples were not co-sliced.
FIGURE 8. Changes in sliced meat pH with or without co-slicing, followed by repackaging and storage under vacuum at 7°C. With control salami, pH measurements were made only at the beginning and at day 28 of the study (dashed line). Results from salami co-sliced with ham and bologna were combined. For bologna, ham, and salami controls (not co-sliced), inside and surface pH values were combined. Co-sliced ham surface and inside pH values were also combined. SD were ≤0.07 pH units.

of bacteria from the salami to these meats. Results from APT and MRSD at 25°C were the same in these samples and have been combined in Figures 6 and 7.

Co-slicing ham and bologna with salami not only increased the numbers of bacteria present but also induced reductions in cooked meat pH. These changes for meats used in these trials are presented in Fig. 8.

When sliced and repackaged under vacuum in the laboratory, control ham, bologna, salami, and co-sliced bologna and salami retained their appearance throughout the 28-day study period at 7°C. None of the packages of co-sliced meats lost vacuum during storage. However, the appearance of co-sliced ham deteriorated, and while it was not discolored at day 14, significant milky purge was present. This developed into a ropy liquid at day 28.

DISCUSSION

Greater numbers of bacteria on surface slices of bologna (Table 1) occurred independently of package size (Table 1). Eight other packages of bologna ranging in weight from 120 to 500 g and sliced by three different manufacturers yielded similar differences between inside and outside slices at or beyond the coded “best before” date (results not reported). Almost exclusively, bacteria present in both surface and internal bologna slices were LAB. This situation differed from that in unsliced cooked cured meats where it has been shown that 95% of bacteria in the surface layer of packages were LAB but only 55% present internally were LAB. A large proportion of the remainder were Bacillus spp. (18). In only one of the sliced preliminary test samples examined were bacterial spores present (<2.8 log CFU/cm², aerobically or anaerobically cultured) and these were present at the same level in both outside slices but were absent from the inside slice. Bacterial spores were found in a core sample of unsliced bologna and represented 0.8% of the population of LAB present (results not reported). These results may reflect differences in cooking regimens used in manufacture of meat products.

In sliced bologna analyzed at product expiry (Table 1), all of the LAB colonies on M5 medium were heterofermentative (31) and results from further testing revealed they were lactobacilli. No ropiness or discoloration was noted but milky purge was visible and vacuum was lost from these packages. The decreased pH of outside slices was coincident with greater bacterial growth at those locations.

In the subsequent experiment where multiple packages from the same lot were tested up to shelf-life expiration it was found that although there were differences in the initial numbers of bacteria present on slices of bologna from the different manufacturers (Fig. 1 to 3), consistently higher numbers of bacteria on surface slices was common to the trials.

The more frequent occurrence of bacterial spores, both aerobic and anaerobic, on the surface slices and their infrequent occurrence on internal slices was unexpected but consistent with the results obtained in the preliminary testing with single packs at product expiry. Spores were found in outside slices of bologna in all three trials at day 35, but their inconsistent recovery in subsequent samples and almost complete absence from internal slices except at day 60 in product C suggests the slicing action either contaminated the outside surface or perhaps stimulated germination of spores on internal slices and facilitated the lethal action of nitrite.

Thickness of commercially prepared and laboratory sliced samples varied between 2 and 4 mm, and therefore the weights of 10-cm² samples varied. Some variation in bacterial numbers might have been a direct result of this difference, but the influence of sample location was far greater upon bacterial recovery than sample weight within the range of weights analyzed (3 to 4 g). In experiments with co-sliced meats, sample area was increased from 10 cm² to 40 cm² to avoid this potential difficulty and improve recovery of low numbers of bacteria from control samples of cooked meats. The occasional observation of similar numbers of bacteria on internal and external slices (Fig. 1, day 60; Fig. 2, day 21; Fig. 3, day 60) suggests that the difference between numbers of bacteria on inside and outside slices may be a transient occurrence and reflect the dynamic changes of growth, death, and generic succession taking place in sliced-meat packages during refrigerated storage. Nonetheless, the difference persisted in most samples to well beyond the estimated shelf life and was clearly evident in a commercially sliced package of ham held at 7°C for 3.5 months after shelf-life expectation was reached (results not reported). Differences among trials were unrelated to packaging film O₂ permeability and are more likely related to product handling and plant practices of the different manufacturers (26).

Stiebing (29) recommended that for optimized shelf life of these types of meat products, packaging films should have an O₂ permeability of <15 cm³/m²/day/ atm. Grini et al. (8) found films with >40 cm³/m²/day/ atm O₂ transmission caused unacceptably large decreases in slice redness during
storage at 4°C for 28 days. Color fading in commercial bologna samples used in our study was a significant factor affecting product appearance and could be avoided if better O2 barrier film were used. Microbiological results were reported by Grini et al. (8) on bologna slices stored in film with an O2 transmission of 1.5 cm3/m2/24 h/atm. Bacteria present were almost exclusively LAB, with L. sake dominating. There were no B. thermosphacta and coliforms were rarely detected in samples. Unfortunately, the method of sampling bologna for microbiological analyses was not specified in this study.

The initial levels of bacteria found in commercial ham samples 36 days following slicing and packaging (Fig. 4) were low (9, 13, 28) and significant differences in numbers of bacteria between inside and outside slices were maintained throughout the study. The peak populations reached were similar to those found in the previous studies. In contrast with commercially sliced bologna, where heterofermentative LAB dominated from sample entry into the study, in commercially sliced ham a succession from primarily homofermentative to heterofermentative types took place and was complete within 2 weeks of storage (Table 2). These organisms were not Leuconostoc spp. (they produced CO2 from glucose but were arginine positive). The transition may have been a true change in the type of organisms present or may have occurred following induction of heterofermentative metabolism as a result of glucose limitation in the anaerobic environment of the ham (4). Commercial films used for packaging sliced ham did have low O2 transmission properties (<8 cm3/m2/day/atm). Glucose limitation in the low-fat ham used in the study is a real possibility based on carbohydrate analyses of similar products in an earlier study (9).

The appearance of ropiness at day 43 and 50 in the two trials where manufacturer-packed products were examined (Fig. 4) and at day 28 in the co-sliced ham (Fig. 7) illustrate the importance of preventing access by LAB capable of producing this defect (22). Boerema et al. (3) reported that lactobacilli dominated in vacuum packages of sliced ham and eventually caused spoilage. Our results with manufacturer-sliced products are consistent with this conclusion.

Techniques to identify specific LAB groups are tedious when large numbers of samples are involved. To follow the transfer of organisms from the salami to cooked meats during co-slicing, the approach taken was to incubate plated samples on MRSD at 42 to 45°C to detect thermotolerant LAB such as L. plantarum, L. sake, and L. curvatus, which occur in fermented sausages (14, 18, 27) but which were not found in the control cooked-meat samples tested here. MRSD was also used at 25°C to separate pediococci from other LAB. MRSD does not distinguish between lactobacilli and Leuconostoc spp. but when used in conjunction with morphology, CO2 production, and arginine hydrolysis it was possible. The Staphylococcus component of the starter culture used for salami manufacture was not followed in co-slice experiments because these organisms do not tolerate well the acidic conditions in mature sausage (10).

Almost negligible differences were seen in the number of bacteria recovered from inside and outside salami slices (Fig. 5). This result had been anticipated since these fermented sausages were formulated from ground meats inoculated before stuffing with a Pediococcus- and Staphylococcus-containing bacterial starter culture. However, Papa et al. (25) examined fermented Italian sausage and found numbers of LAB were higher at the external surface by the end of ripening (70 days); the number of internal bacteria represented 87% of those present at the surface.

The Pediococcus component of the starter culture in the present study was followed by monitoring a combination of carbohydrate fermentation and arginine hydrolysis on MRSD medium and by microscopic observation (22). At the start of the experiment half or more than half of the LAB present were pediococci, but by day 28 none were found in control slices. The transition to a homofermentative lactobacilli population in fully mature sausages has been reported previously for samples where nonpediococci starters were used (10, 27). The negative effect of slicing upon pediococci survival was unexpected.

Co-slicing of salami with cooked cured bologna and ham caused a temporary decrease in pH, but otherwise the stability of the sliced salami was unaffected (Fig. 5). Decline of the Pediococcus starter also occurred in these samples and none were present at day 28. The fate of pediococci in fully mature, starter-fermented sausages has not been previously reported. They are not normally isolated from adequately cooked cured vacuum-packaged meats (5).

The salami also contained a viable population of thermotolerant homofermentative lactobacilli which were tracked along with the pediococci in the transfer of organisms to the ham and bologna during co-slicing. Neither the control ham or bologna contained pediococci or thermotolerant lactobacilli but both were evident in the co-sliced products. Growth of pediococci was limited by the low-temperature (<10°C) storage of samples (10). In contrast, thermotolerant lactobacilli began to grow 7 days after the...
ham (Fig. 7) and 14 days after the bologna (Fig. 6) were co-sliced. While these organisms made up a significant proportion of those present in the control salami they were not the dominant organism present in the co-sliced cooked meats. It is suspected that other homofermentative lactobacilli that were transferred from the salami dominated in these products. They could not have been streptococci, since MRSD and APT bacterial recoveries were the same for all the samples. Suspect organisms were probably strains of L. sake or L. curvatus, which are commonly present in fermented sausage at maturation (15, 16, 18, 27) but not able to grow at 45°C. Although Schillinger and Lücke (27) found that 77 and 87% of L. curvatus and L. sake strains, respectively, could grow at 45°C, Hugas et al. (16) found that <35% of these species grew at this temperature.

The starter culture used in manufacture of the salami used in the current study contained 6.74 log CFU of lactobacilli per g capable of growth at 45°C. Both arginine-positive and negative strains of L. sake and L. curvatus (27) would have been included in the total Lactobacillus count as green colonies on MRSD at 25°C (14). Therefore, strains of these organisms incapable of growth at 45°C could represent the major fraction of lactobacilli present in the co-sliced cooked meats. Although not conclusive, it is an important result because L. sake has been implicated as the cause of spoilage in vacuum-packed cooked cured meats (8, 22, 27).

In view of the substantial transfer of LAB which took place from the salami to the bologna and ham during co-slicing, considerable reduction of cooked product shelf life is probable. Even though the storage part of the co-slicing experiment ran 28 days and used fresh meat products obtained directly from the manufacturer, co-sliced ham showed signs of ropiness at 28 days. This represented a 44% loss in normal shelf-life expectation. The ropiness defect had not been found in similar products packaged by the same manufacturer (manufacturer B) even when held 3 weeks after the 50-day “best before” code used by the manufacturer for sliced products. Although a substantial drop in pH (≤0.5 pH units) occurred by day 28 in co-sliced bologna, shelf life was not compromised within the storage period of this study, perhaps because of the greater stability of NaNO₂ in bologna than ham (9). Occurrence of premature ropiness has been a problem with other types of cooked delicatessen meats in vacuum packages (22), and in view of the results obtained here, the practice of co-slicing these meats is ill advised. It is almost impossible to visually detect any difference between delicatessen meats with 3.0 log CFU/g from those with 7.0 log CFU/g or cm². If a fermented sausage or a “stale-dated” product were to be inadvertently included on the slicing machine platform with a freshly cooked cured product, the latter would be contaminated by organisms already adapted to environmental conditions generated by vacuum packaging, shortening the time to spoilage. The possible survival of E. coli O157:H7 during fermented sausage manufacture and its transfer to cooked meats during co-slicing is an additional concern.

The results presented indicate that asymmetric distribution of organisms in vacuum-packed sliced meats is the normal expectation where good practices are followed. Bacterial distribution in sliced-meat packages may be a useful indication of handling practices prior to final packaging of slices. In addition, standardized surface-sampling techniques should be used to evaluate the microbiological condition of these products and reduce variability of results.

The significantly faster rate of bacterial growth on outside slices of vacuum-packaged meats may indicate that antimicrobial film materials can serve to delay time to spoilage of sliced delicatessen meats handled and packaged according to good manufacturing practices.

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