Bacteriology and Storage Life of Moisture-Enhanced Pork

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ABSTRACT

This study was undertaken to determine the impact of the moisture enhancement process on the bacterial contamination and storage life of vacuum-packaged pork loins. Bone-in and boneless pork loins injected with brine (sodium chloride, sodium phosphate, lemon juice) were obtained from a commercial processing facility and stored for 5 weeks in vacuum packaging at 2 and 5°C. At weekly intervals, samples were excised to determine numbers of spoilage bacteria and pathogens. The loins were subjectively evaluated by a sensory panel to quantify appearance and odor acceptability. Moisture-enhanced loins were initially contaminated with a population of psychrotrophic bacteria that was approximately 2 log units higher than that for noninjected boneless loins. This difference was largely due to contamination by larger numbers of pseudomonads in the brine-injected loins. There were no significant differences in the initial numbers of lactic acid bacteria, Enterobacteriaceae, or Brochothrix thermosphacta. Similar trends in spoilage bacterial populations were observed for moisture-enhanced loins with bones, but Enterobacteriaceae counts were also found to be approximately 1 log unit higher for the injected product. Brine-injected loins generally had larger bacterial numbers at each storage time, but there were no consistent injection treatment effects on bacterial growth. Brine injection did not affect color or odor deterioration, and the storage life for vacuum-packaged loins was the same as that for noninjected controls. The incidence of Listeria monocytogenes was 21% for control loins and 27% for moisture-enhanced loins. Although the brine injection process resulted in an increase in bacterial contamination, there was no evidence that this contamination would affect the storage life of vacuum-packaged loins, and further research is necessary to determine the significance of the increased incidence of L. monocytogenes.

The meat industry has recently begun injecting liquid in the form of brine into fresh meat to provide consumers with a consistent, quality product that can tolerate some cooking abuse (14). Moisture-enhanced meat is produced through multineedle injection of a brine solution that may contain ingredients such as salt, phosphates, sodium lactate, and lemon juice solids. The moisture enhancement process produces a retail product with increased juiciness and tenderness and with substantially improved sensory quality (4).

To meet customer demands, pork processors must consistently produce a high-quality product with a guaranteed storage life. Pork processors are currently using the moisture enhancement process to produce both boneless and bone-in pork products for the retail market, and these products have been well received by consumers. However, there is concern that the procedure involved in producing such products may introduce bacteria that normally are present only on meat surfaces (7) into the interior of the muscle. There is evidence that recirculating brines from multiple-injection-needle machines harbor large bacterial populations (6). The introduction of bacteria into the interior of meat has the potential to reduce storage life and to increase the risk of foodborne illness for the consumer (10, 11), but data in this area are limited and generally restricted to mechanical tenderization (15).

In a recent review of meat-processing technologies, Kastner et al. (12) provided preliminary data that demonstrated the translocation of inoculated Salmonella spp. into the interior of needle-injected pork loins, and the U.S. Department of Agriculture is now considering the safety of such “non-intact” products. Since data are sparse, there is a need for research involving commercial trials with more realistic bacterial populations to assist regulatory authorities in making informed decisions. The objectives of the current study were to determine the impact of a commercial injection process on spoilage microflora and to determine the storage life of moisture-enhanced pork. The incidences of specific pathogens were also determined.

MATERIALS AND METHODS

Pork loins. Vacuum-packaged, brine-injected boneless and bone-in pork loins were processed on separate processing lines at a major pork-processing facility. The brine mix used was a commercial product (UFL Food Corp., Edmonton, Alberta, Canada) containing sodium chloride, sodium phosphate, and lemon juice solids (47% salt, 0.2% protein). A solution was prepared by adding 145 kg of the powdered mix to 1,100 liters of water. This solution was used to replenish a smaller, chilled (block ice) reservoir connected to the needle injection apparatus on the processing floor. The injection apparatus used a pump to recirculate brine through the needles as the loins were conveyed through the system. Loins were packaged with a Cryovac shrink-wrap packaging
system with a barrier film with an oxygen transmission rate of 30 to 50 cm²/m²/24 h/atm at 25°C. On the same processing day, vacuum-packaged boneless and bone-in pork loins that had not been injected with brine were processed to serve as control samples.

**Storage and samples.** The quality assurance staff at the commercial facility selected loins on 6 different days (3 days for boneless loins and 3 days for bone-in loins). On each sampling day, 44 loins (22 control and 22 moisture-enhanced loins) of one product type (boneless or bone-in) were retained and delivered to the laboratory. Loins were selected at random throughout each day’s production run and randomized within storage treatments to eliminate any possible effects of production time. Two moisture-enhanced loins and two control loins were sampled immediately (day 0), and the rest were divided and stored at 2 and 5°C. Two samples of both the control and the moisture-enhanced loins for each storage temperature were analyzed at each sampling interval. Samples were analyzed weekly for 35 days. Loins of each product type (boneless or bone-in) were tested for three separate processing dates.

**Bacteriology.** Ten-gram portions comprising both surface and subsurface muscle were aseptically excised from four different areas of each packaged pork loin with a sterile cork borer (surface area, 10 cm²; thickness, ca. 5 mm). The four 10-g samples were combined and homogenized for 2 min in 360 ml of 0.1% peptone water with a Colworth Stomacher 400 (A. J. Seward, London, UK). The samples were plated on agar media for the identification and enumeration of bacteria. Additional 25-g samples were collected in the same manner as the 10-g samples and were homogenized in preenrichment media for the isolation of pathogenic bacteria.

After serial 10-fold dilutions in 0.1% peptone water, levels of total psychrotrophic bacteria, lactic acid bacteria, *Brochothrix thermosphacta*, and pseudomonads were determined by the spread plate technique, and *Enterobacteriaceae* levels were determined by the pour plate technique.

Total psychrotrophic bacteria were enumerated on plate count agar (Difco Laboratories, Becton Dickinson and Co., Sparks, Md.) after incubation for 10 days at 5°C. Lactic acid bacteria were enumerated on deMan Rogosa Sharpe agar (Difco) after incubation in 5 to 10% CO₂ (BBL anaerobic system; Becton Dickinson and Co., Sparks, Md.) for 72 h at 25°C. *B. thermosphacta* cells were enumerated on streptomycin thallous acetate actidione (STAA) agar after incubation for 72 h at 25°C. Pseudomonads were isolated on cephaloridine fucidin cetrimide agar (Oxoid, Nepean, Ontario, Canada) after incubation for 48 h at 25°C. Enterobacteriaceae were enumerated on violet red bile glucose agar (Difco) after incubation for 18 to 24 h at 35°C. The performances and applications of these media have been described in detail (2). The lower limit of sensitivity for the enumeration of *Enterobacteriaceae* was 1 log CFU/g, and that for all other organisms was 2 log CFU/g. To identify pathogenic bacteria, reagents, media, and protocols were used as outlined in Health Protection Branch Methods of Microbiological Analysis of Foods (9) with slight modifications.

Coagulase-positive *Staphylococcus aureus* was isolated by spread plating 0.1 ml of diluted sample on Baird Parker agar (Difco) and were enumerated after 2 days of incubation at 35°C. Presumptive colonies were tested for coagulase production. *Clostridium perfringens* was isolated by spread plating 1 ml of diluted sample on tryptose sulfite cycloserine agar, overlaying it with tempered molten tryptose sulfite cycloserine agar, and incubating it anaerobically for 24 h at 35°C. *Salmonella* spp. were isolated through preenrichment of the samples in universal enrichment broth (Difco) followed by selective enrichment in tetrathionate and selenite cystine broth (1). Selective enrichments were streaked for isolation on brilliant green sufa agar and xylose lysine tergitio-1 agar. Isolates were biochemically identified with API strips (bioMerieux Canada Inc., St. Laurent, Quebec, Canada) and serologically identified with poly-O antigen. *Listeria monocytogenes* was isolated by preenrichment in universal enrichment broth followed by selective enrichment in Fraser broth. Selective enrichments were streaked on Oxford (Oxoid) and PALCAM (Difco) agar for isolation. Isolates were identified by hemolysis on horse blood agar and by nucleic acid hybridization probe tests (Accuprobe, Gen-Probe Inc., San Diego, Calif.). *Escherichia coli* O157:H7 was isolated by enrichment in modified EC broth and was streaked for isolation on modified sorbitol MacConkey agar with tellurite, cefsulodin, and cefixime and on sorbitol MacConkey agar with cefixime and tellurite (Dynal Inc., Lake Success, N.Y.). Isolates were serologically identified with latex agglutination (Prolex, Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada).

**Sensory evaluation.** Odor and appearance were evaluated on the basis of hedonic scales by an untrained panel consisting of five laboratory staff members. Appearance and odor assessments were made after the packages had been opened for a minimum of 15 min. The acceptability of a loin’s appearance was determined on the basis of a 7-point scale (1 = extremely undesirable; 7 = extremely desirable). The acceptability of a loin’s odors was determined on the basis of a 5-point scale (1 = acceptable; 5 = unacceptable). Storage life was arbitrarily defined as the time required for a loin to reach an average value of 3.5 on the scales (8).

**pH.** The pH values of the loins were measured at each sampling interval after samples were excised for bacteriological analyses. The pH was determined by inserting the electrode of a calibrated meter (Accumet AR50, Fisher Scientific, Edmonton, Alberta, Canada) into the muscle tissue.

**Statistical analyses.** Data were analyzed by two-way analysis of variance according to the general linear model procedures of the SAS Institute (17). The significance of injection treatment differences in bacterial growth curves was determined on the basis of a model that included time and log CPU/g as classification variables. The significance of injection treatment differences in the storage lives of loins were determined on the basis of a model that included time and the main effects of treatment for both odor and appearance. Data were expressed as least-squares means and standard errors of least-squares means.

**RESULTS**

**Bacterial growth: boneless pork loins.** Numbers of total psychrotrophic bacteria (Fig. 1A), pseudomonads (Fig. 1B), lactic acid bacteria (Fig. 1C), and *Enterobacteriaceae* (Fig. 1D) were significantly larger (*P < 0.05*) for the moisture-enhanced pork loins than for the control pork loins during storage. There were no significant differences (*P > 0.05*) in the numbers of *B. thermosphacta* (Fig. 1E) that could be attributed to the injection treatment.

Total psychrotrophic bacteria were initially more numerous (*P < 0.05*) in the moisture-enhanced product than in the control product, and this difference was maintained until day 21 of the storage period, at which time bacterial population sizes were not significantly different (*P > 0.05*)
and had reached numbers approaching 8 log CFU/g (Fig. 1A). The kinetics of bacterial growth were similar for both control and moisture-enhanced loins.

The initial number of pseudomonads for the moisture-enhanced product was substantially larger ($P < 0.05$) than that for the control samples; however, the number of pseudomonads did not increase for the enhanced product during the storage period (Fig. 1B). There was an increase of slightly more than two orders of magnitude in the number of pseudomonads in the control loins during storage. The numbers for both products reached 4 log CFU/g by the end of the storage period.

There was no significant difference ($P > 0.05$) between the initial numbers of lactic acid bacteria in moisture-enhanced loins and those in control loins, and the numbers for both products reached $>7$ log CFU/g by the end of the storage period (Fig. 1C).

The initial numbers of Enterobacteriaceae were the same ($P > 0.05$) for both moisture-enhanced and control boneless loins (Fig. 1D). Growth of Enterobacteriaceae in the enhanced product was observed within 7 days, whereas measurable growth was delayed in the control samples until the 14th day of storage. Counts for both the control and the moisture-enhanced loins reached $>4$ log CFU/g by the end of the storage period.

The initial numbers of $B.\ thermosphacta$ for the moisture-enhanced boneless loins and the control samples were not significantly different ($P > 0.05$), and substantial $B.\ thermosphacta$ growth was not observed in either product during the storage period (Fig. 1E).

Control and moisture-enhanced products were also stored in a vacuum at $5^\circ$C (data not shown). In general, for all bacterial groups analyzed, storage at $5^\circ$C resulted in larger numbers than did storage at $2^\circ$C during the earlier...
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FIGURE 2. Effects of moisture enhancement processing on the acceptability of boneless pork loins during storage at 2°C. ○, control loins; ▲, moisture-enhanced loins. (A) Acceptability based on appearance; standard error (SE) = 0.09. (B) Acceptability based on odor; SE = 0.09. Each point on the graph represents the mean value for six loins. The horizontal line at 3.5 on the subjective scale represents the point of rejection.

part of the storage period, likely as a consequence of a faster rate of bacterial growth at the higher temperature. However, the final bacterial numbers after 35 days of storage were very similar for samples at both storage temperatures.

Storage life: boneless pork loins. There was a slight decline in the acceptability of the appearance of samples during the storage period, with the moisture-enhanced boneless pork loins having a lower rating ($P < 0.05$) than the control samples after 28 and 35 days (Fig. 2A). None of the samples reached an appearance rating of 3.5 to signify the end of storage life. No significant discoloration for either control or moisture-enhanced boneless pork loins was observed during storage. The control loins had a higher unacceptable-odor rating ($P < 0.05$) after 35 days of storage; however, neither control nor moisture-enhanced products reached an odor rating of 3.5 to signify the end of storage life (Fig. 2B).

Bacterial growth: bone-in pork loins. Numbers of total psychrotrophic bacteria (Fig. 3A), pseudomonads (Fig. 3B), lactic acid bacteria (Fig. 3C), Enterobacteriaceae (Fig. 3D), and $B.\ thermosphacta$ (Fig. 3E) were significantly larger ($P < 0.05$) for moisture-enhanced bone-in pork loins than for control bone-in pork loins during storage at 2°C.

Numbers of psychrotrophic bacteria were initially larger ($P < 0.05$) for the moisture-enhanced bone-in loins than for the control loins, and this difference was maintained until storage days 28 and 35, when bacterial populations reached about log 8 CFU/g (Fig. 3A).

The initial numbers of pseudomonads for moisture-enhanced and control samples were not significantly different ($P > 0.05$) on day 0; however, pseudomonads were significantly more numerous in moisture-enhanced samples than in control samples ($P < 0.05$) on day 7, and this difference was maintained for the remainder of the storage period (Fig. 3B). The kinetics of growth of pseudomonads were similar for both control and moisture-enhanced bone-in loins.

The initial numbers of lactic acid bacteria for moisture-enhanced and control bone-in loins were not significantly different ($P > 0.05$), and the growth kinetics for the moisture-enhanced and control loins were similar throughout the storage period (Fig. 3C).

The numbers of Enterobacteriaceae were significantly larger for moisture-enhanced products than for control products ($P < 0.05$) from storage day 7 through storage day 28, and the growth kinetics of Enterobacteriaceae were similar for control and moisture-enhanced products except that measurable growth was observed at 7 days of storage for moisture-enhanced loins but did not occur until day 14 for control loins (Fig. 3D).

The initial numbers of $B.\ thermosphacta$ for the injected bone-in loins and the control loins were not significantly different ($P > 0.05$). However, unlike the boneless loins, the bone-in loins exhibited $B.\ thermosphacta$ growth at 2°C in moisture-enhanced and control bone-in products (Fig. 3E).

As was the case for the boneless loins, for all bacterial groups analyzed, storage of the bone-in loins at 5°C resulted in larger numbers than did storage at 2°C because of a faster rate of bacterial growth at the higher temperature, with the kinetics of growth for control and moisture-enhanced products being similar (data not shown).

Storage life: bone-in pork loins. The appearance acceptability ratings were similar ($P > 0.05$) for the control and moisture-enhanced bone-in pork loins for the entire 2°C storage period (Fig. 4A). None of the samples reached an appearance rating of 3.5 to signify the end of storage life. No significant discoloration for control or moisture-enhanced bone-in pork loins was observed during the 35-day storage period. The odor acceptability ratings for the control and the moisture-enhanced products were very similar ($P > 0.05$) (Fig. 4B). Both control and moisture-enhanced products had a storage life of about 20 days.
FIGURE 3. Effects of moisture enhancement processing on the growth of spoilage bacteria on bone-in pork loins during vacuum storage at 2°C. ●, control loins; ▲, moisture-enhanced loins. (A) Total psychrotrophic bacteria; standard error (SE) = 0.12. (B) Pseudomonads; SE = 0.15. (C) Lactic acid bacteria; SE = 0.11. (D) Enterobacteriaceae; SE = 0.17. (E) B. thermosphacta; SE = 0.18. Each point on the graph represents the mean log count for six loins.

Pathogenic bacteria: vacuum-packaged pork loins.
No Salmonella spp., C. perfringens, or E. coli O157:H7 were isolated from 264 control and moisture-enhanced samples of boneless and bone-in pork loins. Sixty-four of 264 samples (24.2%) tested positive for L. monocytogenes. Twenty-eight (21%) of the control loins tested positive for L. monocytogenes, and 36 (27%) of the moisture-enhanced loins tested positive for L. monocytogenes. Two of 264 samples (0.76%) contained coagulase-positive S. aureus. One moisture-enhanced bone-in pork loin contained 2 log CFU of coagulase-positive S. aureus per g, and one control bone-in pork loin contained almost 4 log CFU of coagulase-positive S. aureus per g.

pHs of vacuum-packaged pork loins. The average pH of the boneless pork loins was 5.67 and the average pH of the bone-in loins was 5.80. The moisture-enhanced boneless loins had an average pH of 5.68, and the boneless control loins had an average pH of 5.66. The moisture-enhanced bone-in loins had an average pH of 5.88, while the bone-in control loins had an average pH of 5.72.

DISCUSSION

When innovative processing technologies are introduced to "add value" to meat products, it is essential that the microbial safety and storage life are not sacrificed to improve sensory properties. It is conceivable that processes involving blades or needles (mechanical tenderization, brine injection) may introduce bacterial contaminants into a product and/or alter the meat environment to influence the rates of growth of the contaminating bacteria. The limited research in this area has focused on mechanical tenderization, and although some have suggested that mechan-
Figure 4: Effects of moisture enhancement processing on the acceptability of bone-in pork loins during storage at 2°C. (A) Acceptability based on appearance; standard error (SE) = 0.12. (B) Acceptability based on odor; SE = 0.08. Each point on the graph represents the mean value for six loins. The horizontal line at 3.5 on the subjective scale represents the point of rejection.

Critical tenderization does not affect bacterial levels in beef steaks (16), others have concluded that the procedure can introduce bacteria into meat and has the potential to reduce storage life and increase the risk of foodborne illness (10, 11).

A recent survey (5) of retail pork in the United States showed little difference in total plate counts when moisture-enhanced and conventional pork cuts were compared, and mean bacterial counts were approximately 4 log CFU/g. Similar levels of bacterial contamination were found in the current study, but more detail on the composition of the spoilage microflora was provided. Thus, moisture-enhanced pork loins obtained from a commercial facility were contaminated with considerably more psychrotrophic bacteria than conventionally processed loins were, primarily because of increased levels of pseudomonads. In moisture-enhanced boneless pork loins, this increase was substantial (ca. 2 log cycles).

A similar trend toward the recovery of larger initial numbers of bacteria from moisture-enhanced boneless and bone-in pork loins was observed, but the increase in bacteria in bone-in loins was less extensive. This difference could be due to differences in processing lines, injection apparatuses, or the times at which the loins were obtained.

With one exception, the growth of spoilage bacteria on stored pork loins was unaffected by brine injection. When initial bacterial numbers were larger in moisture-enhanced pork, these numbers generally remained larger throughout 35 days of storage in a vacuum. For reasons that are not apparent, pseudomonads did not grow in moisture-enhanced boneless pork, while a 2-log increase in bacterial numbers was observed in control loins during storage at 2°C for 35 days.

It is tempting to speculate that the brine injected was bacteriostatic and that this may have accounted for differences in bacterial growth. However, the commercial brine used by the processor contained only sodium chloride, phosphates, and lemon juice solids at concentrations that would not be expected to have any significant impact on bacterial growth in injected pork. Moreover, the lack of pseudomonad growth in injected loins was an exception to the general trends observed. That is, brine injection had no effect on the growth of pseudomonads in bone-in loins during storage at 2°C or in boneless and bone-in loins stored at 5°C (data not shown). The functionality of the ingredients used for the production of enhanced pork has previously been examined in laboratory studies and has been extensively reviewed (13). It is evident that lactates and diacetates would be more potent antimicrobial agents, but these agents are currently not approved for use in moisture-enhanced pork production in Canada.

It was also interesting that B. thermosphacta did not grow in boneless pork but increased by about 2 log cycles in bone-in product. Bacterial growth rates for all spoilage bacteria seemed faster for bone-in products (data not shown), and this may explain why objectionable odors were detected earlier in bone-in pork than in boneless pork. Other researchers have also found variation in the growth of spoilage bacteria on different cuts of pork stored in vacuum packs, and this finding has been attributed to intrinsic differences in pH, fat, and lactate (3). Thus, B. thermosphacta grew more rapidly in muscle with a higher pH and more adipose tissue. In the present study, the pHs of the bone-in loins (5.72 for control loins and 5.88 for injected loins) were higher than those of the boneless loins (5.66 for control loins and 5.68 for injected loins), and this may have influenced the rates of bacterial growth.

Despite increased bacterial numbers in moisture-enhanced pork, the storage life of moisture-enhanced pork loins at 2 or 5°C did not differ from that of pork loins processed in a conventional manner. The appearance of the loins remained acceptable to the sensory panel for 35 days, irrespective of injection treatment. Odor remained accept-
able for 35 days in a vacuum for boneless loins but was limited to 20 days for bone-in product. Apart from these product differences, the onset and intensity of spoilage odors were only marginally influenced by the moisture enhancement treatment.

It is noteworthy that the populations of lactic acid bacteria were unaffected by the injection procedure, growing to maximum numbers of about 8 log CFU/g within 28 days of vacuum storage at 2°C, irrespective of treatment. These bacteria are known to prevail in vacuum-packaged meat and can effectively compete with other spoilage flora, and they may have displaced any spoilage changes that would have been anticipated with larger populations of other spoilage bacteria in injected pork (3).

There are serious concerns about the safety of needle-injected meat, and preliminary results of laboratory trials involving artificially inoculated pork and beef have been published. Phebus et al. (15) observed the translocation of E. coli O157:H7 from the surface of blade-tenderized beef steaks, and Kastner et al. (12) reported that Salmonella spp. could be recovered from the interior of needle-injected pork loins. Kastner et al. (12) noted that a certain percentage of the Salmonella population survived during the cooking of chops to temperatures of 60 to 82.2°C.

In a survey of retail pork products, Duffy et al. (5) found that L. monocytogenes was the most common pathogen isolated, with overall incidences of 26.7% in plant samples and 19.8% in retail samples. The highest incidence of L. monocytogenes was in ground pork products. Both whole-muscle enhanced pork and whole-muscle conventional pork were found to have L. monocytogenes incidences of ca. 15%.

The present study was not designed to examine the translocation of pathogens to the interior of pork loins during the moisture enhancement process. However, the commercial injection process did not alter the incidence of Salmonella spp., C. perfringens, E. coli O157:H7, or coagulase-positive S. aureus. The incidence of L. monocytogenes in moisture-enhanced loins was 27%, compared with 21% in conventionally processed loins. These values are higher than the 15% incidence reported by Duffy et al. (5) but do not necessarily suggest that moisture-enhanced pork would pose a greater human health risk than pork processed in the traditional manner. Further studies are needed to determine whether naturally occurring L. monocytogenes are translocated to the interior of the muscle and if they can survive conventional cooking procedures.

This is the first known report of the bacteriological consequences of a commercial process for the production of moisture-enhanced pork, but the present study was limited to a single processing establishment that used an apparatus that recirculated brine. Although the brine injection process dramatically increased the population of spoilage bacteria in pork, this increase had no effect on the storage life of vacuum-packaged loins. Further research is necessary to determine the sources of the increased bacterial numbers in the injection process, to introduce control measures, and to quantify the impact on the case life of aerobically stored retail products. With adequate processing hygiene, the moisture enhancement process would result in a superior, consistent-quality product with a guaranteed storage life and safety.

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REFERENCES