Fates of Foodborne Pathogens in Raw Hams Manufactured Rapidly Using a New Patented Method

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ABSTRACT

To manufacture raw ham in an efficient manner, we recently developed a new system in which presliced pork loin was used, and the processing time was reduced to 5% of the conventional method. This study aimed to examine whether this raw ham could be as safe as ham produced by the conventional method. Pork loin spiked with enterohemorrhagic Escherichia coli serotype O157:H7, Listeria monocytogenes serotype 1/2c, Salmonella enterica serovar Enteritidis, and Staphylococcus aureus were processed using either the new or conventional method. The fate of the foodborne pathogens and behavior of hygiene indicator bacteria were examined. Whereas nitrite had disappeared during the conventional packaging process, the reduced processing time in the new system allowed for the ham to be vacuum packed with retention of the nitrite (6.9 ± 1.2 ppm, P < 0.01). This accounts for the prominent decrease in L. monocytogenes (2.3 log reduction in 35 days) and S. aureus (3.3 log reduction in 13 days) counts during storage. E. coli O157 and Salmonella Enteritidis were likely resistant to the nitrite in the ham. However, they were unable to multiply in the ham and decreased gradually as in the conventionally produced ham. The bacteriostatic nature of the raw ham was also indicated by the gradual decrease in coliforms (1.3 log reduction in 13 days) in nonspiked ham. In conclusion, the raw ham produced using presliced pork loin is practically as safe as conventionally produced raw ham.

Raw ham, such as German Lachsschinken, is manufactured by brining raw meat (i.e., pork loin) with subsequent exposure to a natural air/smoke-curing process that dries, flavors, and preserves the meat. However, it takes more than 1 month to produce the packed sliced ham from raw meat loaf. Quality control issues such as salt distribution and contamination with foodborne bacteria are potential problems involved in the production of raw ham.

Kato et al. (19) developed a new processing method for raw ham. In this method, pork loin is sliced prior to brining and curing. The new method dramatically shortened the manufacturing time from 15 days or more for the conventional processing method to less than 1 day (18.5 h) because the brining solution (pickle) infiltrates the sliced loin from the surface quickly and evenly. Moreover, this method allows for the easy control of concentrations of salt, nitrites, and seasonings, as well as the resultant water activity (aw).

It is expected that the shortened manufacturing time of the new method will contribute to decreased opportunity for bacterial contamination and proliferation. However, if manufacturers handle the sliced loins under poor hygienic conditions, the risk of contamination could be higher in the new method, because the slices have greater surface area per weight compared with pork loin loaf.

Listeria monocytogenes has been recognized as a foodborne pathogen since the early 1980s, with reports of listeriosis cases caused by the consumption of foods such as vegetables, cheese, and meat (2, 13, 15, 18, 21, 27). Epidemiological investigations have revealed that L. monocytogenes is ubiquitous throughout the environment and in foods. The characteristics of this pathogen that are of particular relevance to its role as a foodborne agent are its ability to grow at refrigeration temperatures, over a wide pH range (4.3 to 9.5), and at salt concentrations above 10% (7, 11, 12). As a consequence, a diverse range of foods has been reported to be associated with both sporadic cases and outbreaks of listeriosis, and even preserved foods, such as smoked products, can be carriers (23, 24). In 2008, a large outbreak due to L. monocytogenes–contaminated meat products occurred in Canada and resulted in the deaths of 22 people (5). The incident impelled Japanese manufacturers to voluntarily recall raw ham products in which L. monocytogenes was detected, regardless of the number of contaminating organisms.

To assess the risk of raw hams produced by our new method, we sought to determine whether representative foodborne pathogens could be controlled in the raw hams as effectively as in the hams produced by the conventional...
method. *L. monocytogenes*, enterohemorrhagic *Escherichia coli* serovar O157:H7, *Salmonella enterica* serovar Enteritidis, and *Staphylococcus aureus* were used to contaminate pork loin. The fates of these representative foodborne pathogens were longitudinally traced during the processing. The numbers of standard plate counts, coliforms, clostridia, and lactic acid bacteria were also examined as hygiene indicator bacteria.

**MATERIALS AND METHODS**

**Bacterial strains.** Strains used in this study were as follows. *E. coli* serotype O157:H7 strain 96-98-83, *Salmonella enterica* serovar Enteritidis strain PT-1, and *S. aureus* strain 96-55-11A were all originally isolated from fecal specimens of diarrheal patients at Osaka City Institute of Public Health and Environmental Sciences. *L. monocytogenes* ATCC7644 serotype 1/2c was purchased from the American Type Culture Collection.

**Bacterial inocula.** All bacteria, except *L. monocytogenes*, were transferred from stock cultures to tryptic soy broth (TSB; Oxoid Ltd., Hampshire, UK) and cultured at 37°C for 24 h. *L. monocytogenes* was cultured in TSB supplemented with 0.6% yeast extract (TSBYE; Oxoid Ltd.) at 37°C for 24 h. A loopful of each fresh culture broth was transferred to either new TSB or TSBYE, as required, and incubated at 37°C for 18 h. Cultures were mixed with 0.5% dimethyl sulfoxide (Sigma Aldrich Co., Ltd., Ayshire, UK) and were dispensed to be stored frozen at −45°C until use. After thawing at 37°C, a loopful of suspension was transferred to TSB or TSBYE and incubated at 37°C until the bacterial concentration reached 8.0 CFU/ml. Cultures were transferred into centrifuge tubes and washed twice with PBS. The bacterial pellets were resuspended in PBS at a final concentration of approximately 5.0 log CFU/ml.

**Preparation of spiked pork.** Pork loins imported from Denmark were used throughout this study. The frozen pork used for the conventional processing method was thawed so that the temperature of the meat did not exceed 10°C. After confirming that the pH of the pork was less than 6.0, the pork loin was cut into 400-g sections (10.0 ± 0.7 by 6.1 ± 0.5 by 6.8 ± 0.8 cm, length by width by thickness) and kept at 5°C or below.

The meats used for the new method were partially thawed at 10°C or less in a refrigerator and then sliced to a thickness of about 1 mm per piece (super deluxe slicer WSD-2P&3P, Watanabe Foodmach Co., Ltd., Aichi, Japan). Three pieces were layered, with a 5-mm offset. The three pieces were wrapped in kitchen wrap and kept at less than −3°C.

**Bacterial inoculation.** In both the conventional processing method and the new processing method, inoculation was performed by immersing the meat in a double volume of bacterial suspension for 1 min. Excess liquid was allowed to run off, and the meat was air dried for 1 min in a safety cabinet.

**Ham production method.** The conventionally processed ham was manufactured according to the Food Sanitation Law of Japan (1). Meat sections were immersed in pickle (15% NaCl, 10% starch syrup, 20% glucose, 2% sodium glutamate, 0.25% sodium nitrate acid preparation containing 10% sodium nitrate acid, 0.5% sodium ascorbic acid, 52.25% water) so that the amount of pickle constituted 60% of the meat section weight, and the meat sections remained in the solution until the *a*<sub>p</sub> became less than 0.97 at 5°C or less (pickle cure). Subsequently, meat sections were dried at 30°C and kept at less than 3°C.

The processed pickled pork was cut into round slices (about 25 g) perpendicular to the minor axis with a sterilized knife to prepare specimens of the conventional processing method. Each sample of the new processing method was composed of three sliced pieces (about 20 to 30 g). All samples were aseptically transferred to a sterile filter stomach bag and then diluted 10-fold with PBS. Each sample was pummeled for 90 s in a stomacher (Masticator Classic 400 ml, IUL, S. A., Barcelona, Spain).

**Chemical analyses.** Determinations of salt concentration, *a*<sub>p</sub>, pH, and nitrite acid concentration were performed on noninocu-
TABLE 1. Chemical characteristics of noninoculated pork at each processing step

<table>
<thead>
<tr>
<th>Processing step</th>
<th>pH</th>
<th>$a_w$</th>
<th>NaCl (%)</th>
<th>Nitrous acid (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork loin</td>
<td>5.36 ± 0.14</td>
<td>0.990 ± 0.002</td>
<td>0.06 ± 0.01</td>
<td>ND</td>
</tr>
<tr>
<td>Pickle cure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pickle</td>
<td>7.01 ± 0.08</td>
<td>0.794 ± 0.002</td>
<td>14.97 ± 0.17</td>
<td>146.4 ± 4.7</td>
</tr>
<tr>
<td>N</td>
<td>5.40 ± 0.02**</td>
<td>0.957 ± 0.001</td>
<td>3.43 ± 0.15**</td>
<td>23.7 ± 1.4**</td>
</tr>
<tr>
<td>C</td>
<td>5.48 ± 0.07</td>
<td>0.956 ± 0.004</td>
<td>3.96 ± 0.33</td>
<td>1.8 ± 1.3</td>
</tr>
<tr>
<td>Dry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5.50 ± 0.04*</td>
<td>0.925 ± 0.004</td>
<td>5.62 ± 0.41*</td>
<td>6.9 ± 1.2**</td>
</tr>
<tr>
<td>C</td>
<td>5.55 ± 0.04</td>
<td>0.930 ± 0.005</td>
<td>5.21 ± 0.34</td>
<td>ND</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5.56 ± 0.08</td>
<td>0.922 ± 0.009</td>
<td>5.79 ± 0.46</td>
<td>1.8 ± 0.8**</td>
</tr>
<tr>
<td>C</td>
<td>5.51 ± 0.08</td>
<td>0.924 ± 0.008</td>
<td>5.47 ± 0.42</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Data are represented as mean ± standard deviation. The number of specimens is nine, except for the pickle ($n = 6$). *, $P < 0.05$; **, $P < 0.01$; ND, not detected.

a, N, pork processed by the new system; C, pork processed by the conventional system.

RESULTS

Chemical analyses. In the conventional processing method, it took 8 days or more until the $a_w$ of the pork loin in pickle decreased to less than 0.97. The pork was then stored for about 7 days in the maturater to reach an $a_w$ of less than 0.95 (Fig. 1). In the new processing method, the $a_w$ of the sliced pork dropped to less than 0.97 within 35 min in the pickle and reached less than 0.95 by drying for 18 h in the maturater (Fig. 1). Between the conventional method and the new method, there were few differences in salt concentration, pH, and $a_w$ of the products (Table 1). However, the concentration of nitrite was significantly
Populations of P,

Salmonella O157 and Enteritidis and S. aureus Enteritidis (Salmonella, S. aureus E. coli & E. coli, S. O157 decreased further, O157, and Enteritidis at the dry processing step and on days 7 to OMORI ET AL.

Fates of the foodborne pathogens in the ham. Each point represents the mean ± standard error (n = 15, three experiments in pentaplicate). * and ** indicate significant differences at P < 0.05 and P < 0.01, respectively, compared with the mean at the inoculation process. The numbers of S. aureus were significantly fewer than E. coli O157 and Salmonella Enteritidis at the dry processing step and on days 7 to 12 of storage. Each point represents the mean ± standard error (n = 15, three experiments in pentaplicate).

Throughout the 15 days of conventional processing, the number of Salmonella Enteritidis, E. coli O157, and S. aureus in the pork decreased by 3.0, 2.9, and 0.7 log CFU/g, respectively (Fig. 3). During the subsequent 7-day storage, Salmonella Enteritidis and E. coli O157 decreased further, by 0.6 and 0.3 log CFU/g each. A reduction in S. aureus numbers was observed only at the pickle cure step.

After 18.5 h in the new process, Salmonella Enteritidis, E. coli O157, and S. aureus numbers in the pork decreased by only 0.4, 0.5, and 0.6 log CFU/g, respectively (Fig. 4). At 12 days of storage the number of Salmonella Enteritidis, E. coli O157, and S. aureus in the pork decreased by 2.1, 1.2, and 3.3 log CFU/g, respectively.

The fate of L. monocytogenes during storage of the ham was examined. The number of organisms in the conventionally produced ham continued to gradually decrease, and L. monocytogenes was undetectable after 70 days of storage (Fig. 5). In contrast, L. monocytogenes numbers were clearly higher in the stored ham produced using the new method than in the conventionally produced product, because the processing after inoculation took only 18.5 h to reach the storage stage. However, the listeria became undetectable in the ham processed by the new method by 35 days, although as an exception, the organism was detected in one sample on day 49.

DISCUSSION

In our opinion, the present study proved that raw ham produced by our new method was as safe as, or safer than, ham produced by the conventional method. The new method required less than 1 day (18.5 h), a significant time reduction compared with the conventional processing.
method (15 days or more). The fact that the hygiene indicator bacteria were controlled and coliform numbers decreased gradually also supports the shelf-stable properties of the ham (Fig. 2). Chemical analysis showed that there was no problematic difference between the hams produced by the new method and those produced by the conventional method. The concentration of nitrite in the ham was higher in the new method than in the conventional method. Furthermore, since the sliced pork quickly passed through the pickle cure and drying processing steps and was then vacuum packaged, the nitrite could remain at the inhibitory level against bacteria until storing the ham. However, consumers are not exposed to excess amounts of nitrite, since it breaks down by the time of consumption, as suggested in Table 1.

*S. aureus* numbers quickly dropped in the new method compared with the conventional method. This unexpected inhibitory effect was presumably brought about by the higher nitrite level, as discussed above; several studies have shown that nitrite is very effective against *S. aureus* under anaerobic conditions (3, 6, 9, 10). On the other hand, in the conventional method the nitrite concentration had decreased by the time of vacuum packaging.

The number of *L. monocytogenes* was also decreased efficiently in the new method. Although this organism is well known to be resistant to physical and chemical stresses, it requires the pH and *a*<sub>v</sub> of the environment to be more than 5.6 and 0.94, respectively, in order to multiply (16, 28); the levels of both in our ham were below these values, irrespective of the method. Furthermore, the higher nitrite during the curing process in the new method presumably brought about earlier diminution of the listeria. Nitrite can reportedly suppress *L. monocytogenes* in combination with proper pH, *a*<sub>v</sub>, NaCl concentration, temperature, packaging method, and storage time (4, 22, 25). In this study, a 400-g piece of pork loin was used to produce the raw ham by the conventional method. However, a 3-kg pork loin is usually used in our manufacturing plant; this suggests that the pH, *a*<sub>v</sub>, and salinity would vary unexpectedly. Since the new method can control the variability of these factors more easily than the conventional method, nitrite will work as expected in the new method.

In the case of *Salmonella* Enteritidis and *E. coli* O157, the organisms decreased gradually compared with *S. aureus* in the new method (Fig. 4). The fate of these pathogens was similar to the behavior of coliforms in the ham (Fig. 2). As nitrite is less effective against these gram-negative bacteria (6, 14, 26), the higher concentration of nitrite in the new method could not result in a prominent antimicrobial effect. Gibson and Roberts (14) reported that *Salmonella* was unable to grow in an environment where pH, temperature, and salinity were <6.8, 10<sup>°</sup>C, and >4%, respectively. Reportedly, *Salmonella* does not grow when the *a*<sub>v</sub> is less than 0.95 (17), and *E. coli* O157 cannot multiply at less than 10<sup>°</sup>C (8). Since the properties of our ham fit these conditions, *Salmonella* Enteritidis and *E. coli* O157 did not appear to grow. However, the organisms were not killed by nitrite and thus decreased gradually. The reason why the numbers of both pathogens on the 7th day of storage were higher than those of the conventional method could be as follows. To reach the 7th day of storage, the conventional method required 528 h from the onset of processing of the pork loin, whereas the new method required only 186.6 h (Figs. 3 and 4).

In conclusion, our new method was able to quickly produce safe and shelf-stable raw ham. Prompt processing allowed the nitrite to remain until the product was vacuum packed. Consequently, *S. aureus* and *L. monocytogenes* were efficiently decreased by the nitrite under a vacuum-packed condition. Although it took the same amount of time for *Salmonella* and *E. coli* organisms to decrease as in the conventional method, they did not grow in the ham. This method is worth validating in a small-scale production setting for future manufacturing use.

**REFERENCES**


