Attachment of *Salmonella typhimurium* to Skins of Chicken Scalded at Various Temperatures

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ABSTRACT

Microtopography of chicken skin was studied by varying scalding temperature to determine the least favorable skin surface for salmonellae attachment. Birds were scalded at 52, 56, and 60°C, and the changes of skin morphology were examined by light and transmission electron microscopy throughout the whole processing. Breast skins obtained immediately after picking were inoculated with *Salmonella typhimurium*, and the attachment was quantified by using scanning electron microscopy and microbiological plating techniques. Skins scalded at 52 and 56°C retained most of the epidermis, although the latter temperature caused the loss of twice as much stratum corneum layers and produced a smoother surface than the former. Skins at 60°C began to lose most of epidermal layers during scalding and exposed dermal surface after picking, which was sometimes covered with thin fragmental epidermis or basal tissue. The number of salmonellae attached to 60°C-processed skins was 1.1-1.3 logs higher than those attached to the skins processed at 52 and 56°C, as measured by scanning electron microscopy. Microbiological plating, however, showed no significant difference in attachment among three skins processed at different temperatures. This was probably due to the insensitivity of the plating method to differentiate attachment strengths of salmonellae to the skin. The above results suggest that removal of whole epidermis should be avoided in processing to reduce salmonellae attachment to the skin.

Since some foodborne illness including salmonellosis have been traced to poultry products (2, 9), reduction of bacterial contamination during poultry processing is one of the major goals of poultry processors and food safety researchers. Jones et al. (4) reported that salmonellae contamination in the U.S. broiler production and processing has not been changed over 20 years and indicated the necessity of a comprehensive effort to control salmonellae in poultry. Gradual increase in salmonellae contamination was also reported in Canada (20). Therefore, new methods to reduce or eliminate salmonellae from chicken carcasses need to be developed (16, 17).

Skin microtopography was suggested to be an important factor affecting bacterial attachment to poultry skin (18). Recently, Kim and Doores (6, 7) also showed the importance of skin microtopography on the attachment of *Salmonella typhimurium* to turkey skin. Different defeathering systems produced disparate skin microtopographies, and the adhesion of *S. typhimurium* was significantly influenced by resulting skin morphology. These studies suggested the possibility of controlling bacterial attachment/contamination by changing poultry skin microtopography. Because skin microtopography is mainly determined by the scald/picking step, scalding temperature was selected as a parameter to manipulate skin microtopography in combination with varying the picking time.

Therefore, the objective of this study was to find the least favorable skin microtopography for salmonellae attachment by changing scalding temperature during chicken processing.

MATERIALS AND METHODS

Processing condition and chicken skin sampling

Seven-week-old chickens (75 male Cobb Vantress) were divided into three groups of 25 by weight, and each group was processed at three levels of scalding temperature (52, 56, and 60°C) in the pilot plant of the University of Arkansas. The scalding times were 2.0, 1.5, and 1.0 min, and picking times were 1.3, 1.0, and 1.0 min for 52, 56, and 60°C scalding temperature, respectively. The whole breast skins were aseptically removed from the carcasses immediately after picking, put into sterile plastic bags, and stored in a freezer (-20°C) for future inoculation studies. In order to monitor the changes of skin morphology for the entire processing procedure, breast skins were also obtained after killing, scalding, and chilling from each group and immediately fixed for observation using electron microscopy. At least six different samples per stage were used in each group.

Scanning electron microscopy (SEM)

The procedure described by Kim and Doores (7) was followed with some modification. Briefly, skin samples were fixed in Karnovsky's fixative (pH 7.2) overnight at 4°C (5), dehydrated with graded ethanols (30, 50, 70, 80, 95%, and three changes of 100% vol/vol) for 15 min per bath. Then, skin pieces (-1/6 cm²) were washed with hexamethydisilazane for 5 min three times and air-dried. Skins were mounted, gold-coated, and viewed by ISI-60 scanning electron microscope (Pleasanton, CA) at 15 kV.

Light and transmission electron microscopy (TEM)

Replicate skin pieces that had been fixed in Karnovsky's fixative were postfixed with 1% osmium tetroxide in 0.05M cacodylate buffer (pH 7.2) for 2 h and prestained overnight with 0.5% uranyl acetate in distilled water at 4°C. They were dehydrated with graded ethanols as described above and treated with propylene oxide (15 min x 2 times) to ensure complete dehydration. Dried samples were infiltrated and embedded with Spurr mix. Thick sections were cut with glass knives and stained with 1% uranyl acetate and 0.05% lead nitrate in ethanol (1 h) prior to observation in TEM (Marion, OH) at 80 kV.
sections (1 μm) were obtained by using LKB-2088 ultramicrotome (LKB Instrument Inc., Rockville, MD), stained with 1% toluidine blue in water, and the cross sections of the skins were observed using a light microscope (Olympus Vanox, Tokyo, Japan). Thin sections (0.05 μm) were put on a 300-mesh grid and stained with 2% uranyl acetate and lead citrate in distilled water. The cross sections of each skin were examined by JEM-100 CX TEM (Jeol electron microscope, Boston, MA).

Salmonella typhimurium inoculum

Lyophilized S. typhimurium (ATCC 14028) was suspended in brain heart infusion broth and incubated at 37°C for 18 to 20 h. The culture was centrifuged at 3,000 x g for 10 min at 4°C and resuspended in physiological saline (0.85%). Cell culture was adjusted to ~10^8 CFU/ml and used for inoculation studies.

Inoculation of chicken skin

Procedure described by Kim and Doores (7) was followed. Briefly, two pieces of breast skins (1 and 10 cm²) taken from each chicken immediately after the picking step were stretched and pinned on the sterile corksheets. Then skin surfaces were inoculated with S. typhimurium. Inoculum (20 μl of 10^8 CFU/ml/cm² of skin) was gently dropped and spread without touching skin surface by using a micropipette. For 10-cm² pieces, a 100 μl of inoculum was delivered twice. Inoculated skins were incubated in a moisture chamber for 30 min at room temperature and gently rinsed with 0.85% physiological saline (1 ml/cm² of skin) to remove unattached cells. The 1 cm² pieces were fixed for electron microscopic observation, and the 10-m² pieces were used for enumerating attached salmonellae by conventional plating technique.

Enumeration of attached salmonellae by SEM and plating

Attached cells were also enumerated by directly counting the cells from the SEM screen (7). Thirty randomly selected areas were counted at 3,000X in each sample. The number was then converted to the CFU/cm² of chicken skin. Because the number of natural microflora before inoculation was about 10^4 CFU/cm² of each skin, their effect was negligible compared to the predominating inoculum level.

For enumeration by plating, inoculated skin pieces (10 cm²) were put into sterile plastic bags containing 10 ml of physiological saline and macerated in a stomacher (Stomacher 400, Tekmar Co., Cincinnati, OH) for 1 min. Serial dilutions were made and surface plated on xylose lysine desoxycholate agar. Plates were incubated at 37°C for 24 h before counting. At least six different skin samples/group were used for both SEM and plating method.

Statistical analysis

Data were statistically analyzed using the general linear models procedure of the Statistical Analysis System (15) for completely randomized design.

RESULTS

Morphological changes of chicken skin during processing

The changes of skin morphology after major processing stages, i.e., scald, picking, and chilling, were monitored in three processes using 52, 56, and 60°C scalding temperatures by light microscopy and TEM. These changes were compared with intact morphology of the skin, which had been obtained immediately after kill (Fig. 1). After scalding at both 52 and 56°C, the keratinized epidermal layers (stratum corneum) began to lose their laminated structure; however, the general structure of intact skin still remained. The 52°C-scalded skin lost a certain amount of stratum corneum layers after picking, and the skin surface appeared to be very rough with delaminated layers (Fig 2a). Skins from 56°C scalders lost twice as much stratum corneum layers after picking, and the surface was much smoother than the 52°C-processed skins (Fig. 2b). Although the extent of delamination in the 52°C-processed skin after picking subsequently decreased due to shrinking caused by low temperature of chill water, the chilling step did not change the general morphology of skin produced after picking.

At 60°C process, skins lost large portion of epidermis (all stratum corneum and part of stratum germinativum) during scalding, and the dermal surface was often exposed. After picking, although a few fragments of stratum germinativum were observed, the residual epidermal layers were completely removed and the dermis was exposed (Fig. 2d). Exposure of dermis can be identified by the characteristic upper dermal layers showing orthogonal arrangement of collagen fibers (12). Another characteristic of 60°C-processed skin was that the skins began to swell during scalding and became significantly thicker than others after chilling. The higher heat treatment also obscured the observation of intact skin structure and sometimes shriveled the surface layers (Fig. 2c).
As expected from the cross-sectional observation, the laminated stratum corneum layers of unprocessed skin (Fig. 1) began to delaminate during scalding at either 52 or 56°C. After picking, while 52°C-processed skins still showed a rough surface (Fig. 3a), 56°C-processed skins revealed a smooth surface (Fig. 3b) which looked similar to the surface morphology of unprocessed skin except having less contaminants. Skin morphologies produced after picking were maintained throughout the whole processing for both skins. Relamination of delaminated stratum corneum layers after chilling in 52°C-processed skins was not evident when observed by SEM.

After 60°C scalding, skins lost all stratum corneum layers and exposed basal membrane or the fragments of lower epidermis (stratum germinativum). Unlike the 52°C- and 56°C-processed skins, unidentified cocci and rod-shaped cells were observed, which reflected the adhesive characteristics of bacteria to these layers (Fig. 3c). After picking, a thin basal membrane covering the dermis was exposed, which appeared clean due to loss of most of the epidermal fragments (Fig. 3d). The morphology of chilled skins was similar to that after...
Figure 3c.

Figure 4b.

Figure 3d.

Figure 4. Attachment of salmonellae to skin surfaces after picking. (a) 52- or 56°C-processed skins. Both skins showed a few cells attached to stratum corneum surfaces, (b) 60°C-processed skin. A significantly high number of cells were attached to dermal surface.

Figure 3b.

picking except that dermal collagen fibers of chilled skins often formed a tangled network and the surfaces looked more wrinkled probably due to the shrinkage of collagen fiber bundles located below the basal membrane.

Salmonellae attachment enumerated by SEM and plating

When the attached cells were enumerated by SEM, a significant difference was noticed among the skins from different processings (p < 0.05). A 60°C-processed skin had about 10 times higher numbers of attached cells than others when observed directly from the SEM screen at 3,000X (Fig. 4). The converted data showed 1.1 and 1.3 log higher number of attachment than to the skins processed at 52 and 56°C, respectively (Fig. 5). In addition, salmonellae produced attachment fibrils connecting the cells to the skin or to each other (Fig. 6). However, stratum corneum surfaces of 52- and 56°C-processed skins had fewer attached cells with no fibrils observed.

Conventional plating, however, did not show any significant differences in the number of salmonellae attached to the skins processed at the three temperatures (Fig. 5).

DISCUSSION

The microtopographies of chicken skin produced after scald/picking were generally maintained throughout the processing at each temperature. However, it appeared to be very important which skin layers were exposed during scalding. The fact that the dermal layer was exposed during 60°C scald is a major problem, since our results indicated that salmonellae attached more readily to the dermis than to epidermis (especially stratum corneum layers). In addition, the scalding tank is the critical contamination point due to its high bacterial load. It has been known that once bacteria attach to the surface, they are hard to remove (10,11,13,14,18). Therefore, the high temperature-scald conditions perhaps should be
reconsidered to reduce bacterial attachment, which will be carried over throughout the processing.

The 52- and 56°C-processed skins showed similar changes except the thickness of lost keratinized epidermis. Although salmonellae revealed poor attachment to hydrophobic stratum corneum surface, the surfaces may entrap the cells within their rough layers when the birds are processed at a lower temperature (8). Also, because bacteria are attached or entrapped within upper layers of stratum corneum prior to processing, it is desirable to remove enough upper stratum corneum layers (19). For the above reasons, a 56°C processing, which removed enough stratum corneum layers as well as produced less delaminated surface, is more efficient than 52°C processing for reducing contamination to the skin.

A significantly high number of S. typhimurium were attached to the dermis when observed by SEM. A similar phenomenon had been observed in a study comparing the skin microtopography produced by three different defeathering systems for turkey (7). A steam-spray defeathering system removed the whole epidermis, and the exposed dermis allowed salmonellae to attach in three times higher numbers than the skins from the cold-water scal (kosher system). Because it is difficult to remove feathers from cold-water scalded birds, the kosher system needs a three times longer picking line, which subsequently causes damages or cracks to the skin. This damage was believed to allow bacterial penetration into the dermis, although kosher processed skin kept stratum corneum layers on the surface (8). However, high-temperature scal/picking, which allows a partial retaining of stratum corneum layers (such as 56°C process in this study), would not produce such crack and would prevent the high attachment of bacteria during processing. Also, the formation of attachment fibrils only on the surface of dermis was the same observation as in a previous study (6), which reflects a high selectivity of salmonellae for attachment to dermis.

The different results between two methods (SEM versus conventional plating) for recovery of attached cells (Fig. 5) can be explained by the fact that perhaps the conventional plating technique is not sensitive enough to differentiate the attachment strengths. In other words, when the inoculated skin is macerated with a stomacher, most of the cells are recovered, regardless of whether they are strongly or loosely attached, entrapped or just involved with water film on the skin surface. Because there is no standard measure for the strength of bacterial attachment, it is difficult to differentiate the cells in disparate stages of attachment. However, SEM might show only the cells that were firmly attached via formation of some kind of bond toward the skin surface. If the cells did not form any attachment ligand, they would be removed by serial washing steps involved in SEM preparation procedure. A previous study (7) showed a similar result and explained the difference with an indirect measurement of attachment strength, $S_{in}$ value, which is based on releasing attached cells with two different forces; first, stomaching skins, second, blending stomached skins. For the above reasons, the results obtained by SEM is considered to be significant. The differences (1.1-1.3 logs) present in 52- and 56°C-processed skins in Fig. 5 would suggest that most cells were loosely attached to hydrophobic stratum corneum surfaces, and the similar counts in 60°C-processed skins in both methods (SEM and plating) indicate that most cells were strongly attached to the dermal surface.

The above results were based on the inoculation study conducted with small scale-skin samples; however, it will be meaningful to process birds in the scale of a real processing plant and examine the contamination level and the shelf life of whole carcasses. Previous reports showing longer shelf life of soft-scalded birds than of hard-scalded ones (3,21) may be explained by our results, since fewer bacteria would have attached to soft-scalded chicken skins than to hard-scalded ones. There is also a report attempted to lower scaling bath temperature for decrease of bacterial contamination (7).

This study clearly demonstrated that the reduction of bacterial attachment can be achieved by changing scald temperature only. It suggests that the removal of whole epidermis should be avoided to reduce salmonellae attachment during processing as in a previous study (6). When a skin microphotography similar to the one of 56°C processing is produced, further reduction of bacterial contamination might be achieved by additional water spraying or surfactant treatment during processing, because loosely attached bacteria will be more easily removed.

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levels in the foods ranged from 2-8 CFU/g. As in the naturally contaminated foods, Salmonella was recovered within 24 h after incubation by both the stabilized and lab-prepared BSA. Based upon the performance parameters used, overall results were better on the stabilized BSA (Table 4). Stabilized BSA plates inoculated from TT performed better than lab-prepared BSA plates similarly inoculated in 12 of 44 comparisons; equivalent performance was obtained in the remaining 32 comparisons. In addition, stabilized BSA produced enhanced blackening of Salmonella colonies in 10 of 22 instances using TT or SC enrichment cultures; in no case did the lab-prepared BSA perform better for this parameter.

Results comparing the two types of BSA for the detection of Salmonella in ground turkey were equivalent. In NFDM and chicken samples, stabilized BSA performance was better than that of lab-prepared BSA for 18 of 84 Salmonella and nonsalmonellae comparisons under both enrichment conditions; only three of 84 comparisons were better with lab-prepared BSA (Table 4). Ten of the 18 superior ratings on stabilized BSA were for enhanced blackening of Salmonella colonies and the surrounding medium.

This study shows that the stabilized BSA (DuraPak) can be utilized to detect Salmonella in foods, instead of lab-prepared plates. The utilization of DuraPak BSA will assure consistent quality of plates which requires less quality control than laboratory-prepared plates. In addition to BSA, 30 other plated media have been successfully stabilized in the DuraPak format to a shelf-life of at least 8 months room temperature storage. One medium, tryptic soy agar with 5% sheep blood, has been stabilized for 8 months at 2-8°C (8).

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