INCIDENCE OF POTENTIALLY PATHOGENIC MICROORGANISMS IN FURTHER-PROCESSED TURKEY PRODUCTS[1]

M. M. Hagberg[2], F. F. Busta, E. A. Zottola, and E. A. Arnold[3]
Department of Food Science and Nutrition
University of Minnesota, St. Paul, Minnesota 55101

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Abstract

Numbers of certain pathogenic microorganisms associated with turkey and turkey products were determined at three Minnesota turkey eviscerating and processing plants. The influence of processing and freezing was investigated. Using a predetermined sampling plan, skin and meat samples were obtained from 96 turkeys. Numbers of *Clostridium perfringens*, *Staphylococcus aureus*, salmonellae, and coliform organisms in each sample were determined. All types of organisms studied were found in 10 to 1000-fold higher levels in the skin than in meat samples. *Clostridium perfringens* and S. *aureus* organisms were recovered from samples obtained during each stage in the processing of the birds. Salmonellae were not found as frequently. Of 85 fresh skin and meat samples, 53 (62%) yielded *C. perfringens* and 46 (54%) yielded S. *aureus*. Salmonellae were recovered from 11 of 74 (15%) fresh samples and coliform organisms from 74 of 85 (85%) fresh samples. Frozen storage (31 days at −20 F) reduced recovery of the organisms to 56, 53, 9, and 67% respectively.

The increased production and consumption of turkey and further-processed turkey products, plus the continued reporting of food-borne illness attributed to turkey products, have created a need for more information on their microbiological quality. While there is information available on the occurrence of selected potentially pathogenic microorganisms associated with turkey products, there is relatively little information on the numbers of these organisms associated with turkeys processed under current commercial manufacturing conditions.

The first systematic study undertaken to determine the total numbers of selected microorganisms on turkey carcasses was that of Walker and Ayres *(19)*. They reported the presence and prevalence of coliform bacteria, staphylococci, fecal streptococci, and salmonellae on processed birds from five turkey processing plants. Other studies have dealt primarily with turkey products or selected organisms. Bryan et al. *(4, 5, 6)* investigated salmonellae associated with turkey products; Nivas et al. *(12)* identified salmonellae associated with various turkey processing plants in Minnesota; Salzer et al. *(15, 16, 17)* reported on salmonellae and other bacteria associated with turkey giblets. In these studies, the main emphasis was on detection of salmonellae, but some data were presented on numbers of mesophilic bacteria and the presence of other potentially pathogenic organisms such as *Staphylococcus aureus* or *Clostridium* spp. Recently, Mercuri et al. *(11)* published on the bacterial quality of precooked turkey rolls, and Zottola and Busta *(21)* reported on the microbiological quality of further-processed turkey products. Other related reports include the isolation of salmonellae and other air-borne microorganisms in turkey processing plants *(22)*, the microbiology of commercial turkey deboning *(3)*, the microbiological evaluation of mechanically deboned poultry meat *(14)*, distribution of clostridia in poultry processing plants *(10)*, and quantifying bacteria on poultry carcass skin and on subcutaneous bacteria in turkey carcasses *(1, 2)*. However, none of these reports contained quantitative or comprehensive information on the microbiological quality of turkeys being processed and further-processed in commercial operations.

The objectives of this study were to collect incidence data on numbers of *Clostridium perfringens*, S. *aureus*, salmonellae, and coliforms associated with turkey and turkey products produced under current manufacturing practices, and to evaluate the influence of processing and frozen storage on the numbers and incidence of these bacteria.

Materials and Methods

Sampling

Eight visits were made to three Minnesota turkey processing plants. Plant visitations were scheduled so that all samples from each visit could be obtained from a single flock during midday operations. Visits were at least one week but no more than two weeks apart. The plants visited were considered typical of commercial turkey evisceration and further-processing plants located in Minnesota.

Ninety-six turkeys were sampled, 12 during each plant visit. Sample birds were identified through use of predetermined sampling plans. At plants A and B, three birds about 100 birds apart on the eviscerating line were identified (1-3) before spin-chilling. In the same manner, three birds were identified (4-6) after spin-chilling and six birds were identified (7-12) at the end of the eviscerating line for sampling after overnight iced storage. Birds 10, 11, and 12 were sampled

[1]Scientific Journal Series Paper No. 8385, Minnesota Agricultural Experiment Station, St. Paul.
[2]Present address: Headquarters, U. S. Army Health Services Command, Fort Sam Houston, Texas 78234.
[3]Present address: Dalbo Cheese Company, Dalbo, Minnesota 55017.

by plant personnel. At plant C, three sets of four birds each were identified about 100 birds apart on the eviscerating line. The first bird in each set was sampled before spin-chilling, the second bird after spin-chilling, and the third and fourth birds after overnight iced storage. The fourth bird in each set was sampled for breast and thigh meat by plant personnel; skin samples were taken experimentally.

Scald-tank and spin-chill tank water was sampled at the same time the sample birds were exposed to these steps in the processing. On four occasions, breast feathers were obtained from sample birds before they were scalded.

Skin samples

Skin samples were obtained by dissecting a flap of skin from the posterior ventral median sternum laterally approximately 7.5 cm on the right and left sides of the breast and anteriorly to include the neck skin. Skin samples collected from birds before spin-chilling, after spin-chilling, and after overnight iced storage were removed by using a sterile knife and sterile gloves for each bird to prevent carry-over of organisms from one bird to the next. The fourth skin sample was removed by personnel at plants A and B who used the same equipment (knives and gloves) as in their commercial deboning operations. The fourth set of skin samples from plant C was obtained experimentally using a sterile knife and gloves for each bird. All samples were placed in either commercially sterile polyethylene type bags or in sterile blender jars. The commercially removed skin samples from plants A and B and the fourth set of experimentally removed skin samples from plant C were frozen and held for 31 days before sampling. Frozen samples from plants A and B were held at −20 F in laboratory freezer space. Plant C frozen samples were held in plant freezer space at −30 F.

Breast samples

Breast samples were collected from birds after overnight iced storage. The breast of the third bird in each sample set from plant C and from birds 7, 8, and 9 at plants A and B was collected using the same knife and gloves as used in removing the skin sample. The breast of the fourth bird in each sample set from plant C and birds 10, 11, and 12 at plants A and B was commercially removed immediately after removal of the skin samples. Breasts from each bird were then placed into two sterile two-quart jars with one sample being retained for 31 days frozen storage.

Thigh samples

Thigh samples were collected in a manner similar to breast samples, except that single thighs were collected from two birds only on each plant visit. Samples were obtained from the first bird with the same knife and gloves used to collect the skin and breast samples from that bird. The second bird was sampled using commercial techniques by plant personnel. Samples were halved and placed in sterile jars for immediate culturing and for 31 days of frozen storage.

Water samples

Scald-tank and spin-chill water samples (200 ml) were collected in sterile jars at the times indicated above. Sodium thiosulfate was not added to the jars to neutralize residual chlorine. Identical water temperatures were being maintained at all three processing plants: scald-tank water, 144 ± 1 F; and chill-tank water, 59 ± 1 F.

Transport of samples

Samples were kept on ice and transported to the laboratory within 2 h of collection, except for frozen samples from plant C which were immediately placed in the plant freezer at −30 F and held for 31 days.

Deboning procedures

Experimental deboning was accomplished by using a sterile knife and gloves for each individual sample turkey. Samples were not permitted to touch other plant equipment such as conveyor belts and cutting boards. Likewise, commercial samples were taken from the fourth bird in each sample set of four birds, in the same manner except that the plant employees used their own routinely used knives and gloves. Therefore, carry-over of microbial contamination from one bird to the next was permitted with the commercially deboned birds by knives and gloves, but again, these samples were not permitted to touch other plant equipment.

These procedures should have permitted measurement of numbers of surface contaminants (*C. perfringens*, S. *aureus*, salmonellae, and coliforms) and of contaminants carried onto the meat samples by knives and gloves. They should have prevented, in the case of sanitarily deboned samples, carry-over from one bird to the next and in both instances minimized contamination by other environmental factors. Future studies will consider the contribution of other deboning equipment. The probability that the sample birds had systemic disease produced by *C. perfringens*, S. *aureus*, salmonellae, and coliform organisms was considered small; therefore, all of the organisms recovered from the meat samples were assumed to be due to surface (skin) sources.

Numbers of organisms

The numbers of organisms are reported as arithmetic ranges. Means and standard deviations were omitted, since variations in counts from one sample to another were relatively small with a small sampling. Whenever a > (greater than) or < (less than) value appears with one significant figure, e.g., <3, this indicates that all replicates were below the minimum for measurement.

The lower limits for the numbers of organisms (minimum quantity measurements) determined during this study were as follows: *C. perfringens* (MPN/g) <0.03, *C. perfringens* (CFU/g) <1.0, S. *aureus* (MPN/g) <3.0, salmonellae (MPN/g) <0.03, and coliforms (CFU/g) <1.0.

Laboratory procedures

Where applicable, laboratory procedures were identical to those previously reported by Zottola and Busta *(21)*. Numbers of *C. perfringens*, S. *aureus*, *Salmonella* spp., and coliforms were assessed. Portions of the sampled product (skin and meat) were weighed into sterile, tared, blender jars. A 1:10 dilution in sterile 0.1% peptone water was made and blended for 2 min in the case of skin and 1 min in the case of meat in an electric blender (Oster blender, operated at >3000 rpm.)

Clostridium perfringens

Enumeration and confirmation of *C. perfringens* was carried out using the procedure suggested by Hall *(9)*. Sulfite-Polymyxin-Sulfadiazine agar (SPS) incubated at 45 C was used. In addition, a triplicate tube dilution Most Pobable Number (MPN) determination and an enrichment technique both employing fluid thioglycollate were used. All enrichments were incubated at 45 C for 24 h. At the end of that time, a generous loopful was streaked onto SPS agar medium and the plates incubated anaerobically at 35 C for 24 h. The presence of *C. perfringens* was confirmed in the same manner as above. SPS agar medium was made from individual constituents immediately before use.

Staphylococcus aureus

The presence of S. *aureus* was determined as suggested by Olson *(13)* where Vogel Johnson medium (VJ) was used as

TABLE 1. *Clostridium perfringens* IN ENVIRONMENT AND TURKEY OBTAINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (CFU/g - range)[b, c] Visit no.				Plant B (CFU/g - range)[b, c] Visit no.				Plant C (CFU/g - range)[b, c] Visit no.				Total
	1	2	3	Fraction[a]	1	2	Fraction[a]	1	2	3	fraction[a]
Feathers	6500	4500	*	2/2	18	20	*	*	*	*	4/ 4(100%)
Scald-tank water	(5.5-7.2)	(.8-2.3)	(43-55)	4/4	3000	(65-140)	9/9	(2-3)	(34-48)	(39-47)	19/19(100%)
Spin-chill water	<1	<1	<1	4/4	(3-6)	(1-3)	5/9	<1	<1	(.1-.3)	10/19(53%)
Skin:											
Before spin-chill	(1-<1)	(1-<1)	(1-<1)	6/6	(3500-10000)	<3000	7/9	<1	(<1-18)	(1-5)	21/24(87%)
After spin-chill	(2-<1)	(1-<1)	(.3-4.3)	6/6	(9-26)	<3000	7/9	<1	(<1-3)	(1-2)	20/24(83%)
After overnight storage in ice	<1	<1	(1.1-<1)	6/6	(5-99)	<1	6/9	<1	<1	<1	19/24(79%)
After 31 days frozen storage	<1	<1	<1	6/6	<1	<1	6/9	<1	<1	<1	18/24(75%)
Meat - after overnight storage in slush ice:											
Experimentally deboned breast	<1	<1	<1	6/6	<1	<1	4/9	<1	<1	<1	11/24(46%)
Commercially deboned breast	<1	<1	<1	6/6	<1	<1	7/9	<1	<1	<1	15/23(65%)
Experimentally deboned thigh	<1	<1	<1	2/2	<1	<1	1/2	*	<1	<1	4/ 7(57%)
Commercially deboned thigh	*	<1	<1	2/2	<1	<1	2/3	<1	<1	<1	4/ 7(57%)
Meat - after 31 days frozen storage:											
Experimentally deboned breast	<1	<1	<1	4/6	<1	<1	2/9	<1	<1	<1	9/24(38%)
Commercially deboned breast	<1	<1	<1	6/6	<1	<1	6/9	<1	<1	<1	14/23(61%)
Experimentally deboned thigh	<1	<1	<1	2/2	<1	<1	0/2	*	<1	<1	3/ 7(43%)
Commercially deboned thigh	*	<1	<1	2/2	<1	<1	1/3	<1	<1	<1	4/ 7(57%)

*Not determined

[a]Number of samples positive/number of samples tested

[b]Colony Forming Units per gram (CFU/g)

[c]Minimum quantity measurement <1 organisms per gram of sample

the plating medium and as suggested by Elliott *(7)* where Trypticase Soy Broth (TSB) with 6.5% NaCl was used in the triplicate tube per dilution Most Probable Number (MPN) determination. Data were reported when black colonies on VJ were observed and selected isolates proved to be coagulase-positive.

Salmonellae

Salmonella analysis was done as outlined by Galton et al. *(8)*. Triplicate tube, 10-fold dilutions (10, 1, and 0.1 g) were pre-enriched in lactose broth, enriched in tetrathionate broth, and streaked on Xylose Lysine Deoxycholate agar (XLD). Suspect colonies were tested biochemically in triple sugar iron, lysine iron agar, dulcitol broth, urea broth, and presumptive salmonellae were confirmed serologically with O-antigen and Spicer Edwards H-antigen antisera schemes. Serotypes were determined by the National Animal Disease Laboratory, Ames, Iowa.

Coliforms

Numbers of coliform organisms were determined by direct plating and Violet Red Bile agar (VRB) as suggested in Sharf *(18)*. Media were purchased from BBL or Difco.

RESULTS AND DISCUSSION

Clostridium perfringens

Table 1 presents a summary of data on the range of numbers (Colony Forming Units per gram) of *C. perfringens* found during the study and the fraction of total samples that yielded organisms for each category of sample. *C. perfringens* organisms were recovered from turkey skin and meat samples obtained at each stage tested. Numbers on feather samples ranged from 18 to 6500/g; numbers from scald water ranged from < 1 to 3000/ml; numbers from spin-chill water were from < 1 to 6/ml; a range of < 1 to 10,000/g was observed on fresh skin samples; and in all instances < 1 CFU/g of sample was recovered from frozen skin and from either fresh or frozen experimentally or commercially deboned turkey meat. Similar results were obtained using enrichment techniques and triplicate tube dilution Most Probable Number determinations. These data indicate that current processing techniques are able to rapidly reduce total numbers of *C. perfringens*, but also indicate that all turkey products may contain viable organisms. Even by experimentally deboning breast and thigh meat, thereby preventing carry-over of organisms from one bird to the next and contamination of the meat by processing equipment, 20 of 48 (40%) breast samples and 7 of 14 (50%) thigh samples yielded *C. perfringens* organisms. A similar occurrence of *C. perfringens* was reported by Mead and Impey *(10)* who studied poultry and turkey plants in England and found that clostridia could be readily isolated from carcasses at each stage tested in the processing of the birds.

It was evident in this Minnesota study that the numbers of *C. perfringens* present during initial pro-

TABLE 2. *Staphylococcus aureus* IN ENVIRONMENT AND TURKEY SAMPLES DETERMINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (MPN/g - range)[b, c] Visit no.				Plant B (MPN/g - range)[b, c] Visit no.			Plant C (MPN/g - range)[b, c] Visit no.				Total
	Fraction[a]	1	2	3	Fraction[a]	1	2	Fraction[a]	1	2	3	fraction[a]
Feathers	2/2	3.6	460	*	2/2	7.2	27	*	*	*	*	4/ 4 (100%)
Scald-tank water	2/5	3.6	<3	(<3-3.6)	3/4	(3.6-9.1)	(<3-23)	1/6	*	<3	(<3-3.6)	(%)
Spin-chill water	1/6	(<3-3.6)	<3	<3	2/4	(<3-3.6)	(<3-14)	1/9	(<3-9.1)	<3	<3	4/19 (22%)
Skin - breast:												
Before spin-chill	5/9	(3.6-43)	(<3-460)	(<3-3)	5/6	(<3-460)	(3.6-240)	7/9	(<3-23)	(15-21)	9.1	17/24 (71%)
After spin-chill	2/9	(<3-460)	(<3-3.6)	<3	6/6	(240-1100)	(7.3-23)	8/9	(9.1-23)	(24-93)	(<3-9.1)	16/24 (67%)
After overnight storage in ice	3/9	(<3-3.6)	(<3-3.6)	<3	6/6	(21-1100)	(23-1100)	8/9	(3.6-75)	(3.6-64)	(<3-43)	17/24 (71%)
After 31 days frozen storage	8/9	(7.3-39)	(23-43)	(9.1-43)	6/6	(43-150)	(23-150)	8/9	(<3-43)	(7.3-23)	(15-210)	22/24 (91%)
Meat - after overnight storage in slush ice:												
Experimentally deboned breast	0/9	<3	<3	3	4/6	(3.6-23)	(<3-36)	2/9	(<3-3.6)	3	(<3-3.6)	6/24 (25%)
Commercially deboned breast	1/8	<3	<3	(<3-91)	6/6	(9.1-93)	(14-43)	8/9	(<3-3.6)	(3.6-15)	3.6	15/23 (65%)
Experimentally deboned thigh	0/3	<3	<3	3	1/2	<3	3.6	1/2	*	9.1	<3	2/ 7 (28%)
Commercially deboned thigh	1/2	*	<3	3.6	2/2	1100	9.2	3/3	3.6	3.6	3.6	6/ 7 (87%)
Meat - after 31 days frozen storage:												
Experimentally deboned breast	3/9	<3	<3	(15-93)	4/6	(<3-9.1)	(<3-36)	3/9	<3	(<3-3.6)	(<3-9)	9/24 (38%)
Commercially deboned breast	2/8	<3	(23-93)	<3	4/6	(<3-9.1)	(<3-23)	4/9	3	(<3-9.1)	(<3-3.6)	10/23 (43%)
Experimentally deboned thigh	1/3	<3	<3	3.6	0/2	<3	3	0/2	*	<3	<3	1/ 7 (14%)
Commercially deboned thigh	1/2	*	<3	3.6	2/2	3.6	>1100	0/3	<3	<3	<3	3/ 7 (43%)

*Not determined
[a]Number of samples positive/number of samples tested
[b]Range per gram Most Probable Number (MPN/g)
[c]Minimum quantity measurement <3 organisms per gram of sample

cessing of turkeys varied from plant to plant. Data in Table 1 show that the number of organisms recovered from plant B sample birds, before and after spin-chilling, was from 100 to 1000 times greater than the number recovered from plants A and C sample birds. Week to week variations in numbers of organisms recovered were not obvious, indicating processing techniques may be directly responsible for the higher numbers of *C. perfringens* recovered from plant B birds.

Staphylococcus aureus

Estimates of the numbers of S. *aureus* organisms recovered from scald-tank water, spin-chill water, and turkey skin and meat samples are in Table 2. The fractions of total samples that yielded organisms for each category of sample are also presented. S. *aureus* organisms were recovered from skin and meat samples obtained at each stage tested. Numbers of 3.6 to 460/g were found on feather samples; numbers from scald water or chill water did not exceed 23/ml; numbers of < 3 to 1100/g were obtained from fresh skin samples; frozen skin samples yielded < 3 to 210/g; meat samples yielded < 3 to 1100/g.

These data imply that processing techniques, as used by these plants, did not materially reduce the numbers of S. *aureus* organisms present on turkey; however, a buildup of organisms did not occur during processing. Turkey meat samples, fresh or frozen, experimentally or commercially deboned, yielded numbers of organisms reflecting the numbers on their respective skin samples. There is evidence that carryover of organisms between birds and between skin and meat by deboning knives and gloves did take place. Recovery of S. *aureus* was made from 25 of 46 (54%) commercially deboned breast samples, while 15 of 48 (31%) experimentally deboned breast samples yielded the organism. Likewise, 9 of 14 (64%) commercially deboned thigh samples yielded organisms, while only 3 of 14 (21%) of the experimentally deboned thighs were found positive for S. *aureus* organisms.

This observed occurrence of S. *aureus* in the three Minnesota turkey processing plants is in sharp contrast to lack of this organism reportedly being recovered by Brant and Guion *(3)* in their survey of four commercial California turkey processing plants. They *(3)* reported that two of the California plants surveyed produced no samples that were positive for S. *aureus* organisms. However, they did indicate that their sample numbers were low and that a less inhibitory enrichment medium perhaps would have yielded more positive cultures. Other reasons for our more frequent recovery of S. *aureus* compared to the California study may include sampling and quantitating techniques. For example, in this study a whole skin blending technique was used, while Brant and Guion *(3)* used a swab sampling technique. As indicated by Avens and Miller *(1)*, swab techniques for sampling turkey products, especially skin, measure only the microorganisms on or under the loose outer surface skin cells. S. *aureus* isolated from feather follicles may pose just as serious a potential hazard as surface organisms.

Walker and Ayres *(19)* estimated the numbers of staphylococci associated with commercially produced turkeys during processing at < 10 to 3100/cm^2 on skin surfaces, and chill or scald water samples at < 500/ml. They estimated that 38% of their colonies were coagulase-positive. Two recent studies of further-processed turkey products have reported the presence of S. *aureus* organisms. Mercuri et al. *(11)* reported 8 of 28 (28%) foil-wrapped, baked, Eastern-type turkey rolls contained coagulase-positive staphylococci. Zottola and Busta *(21)* recovered coagulase-positive staphylococci from 24 of 35 (65%) raw further-processed turkey products. This emphasizes the necessity for adoption of standardized methods to isolate and enumerate S. *aureus* organisms in turkey products.

Salmonellae

Estimates of the numbers and incidence of salmonellae recovered during this study are in Table 3. Three (5%) isolations of salmonellae were made from 62 samples obtained during visits to plant A. One salmonellae-positive sample was a commercially removed skin sample frozen for 31 days at −20 F. Two were isolated from experimentally removed skin samples obtained from sample birds stored overnight in slush ice. No salmonellae were isolated from plant B samples. In contrast to this, 30 of 100 samples collected during three visits to plant C yielded salmonellae. Three (9.4%) of 32 samples were from the first visit to plant C. Two of these isolations were made from fresh commercially deboned breast meat. In the second visit to plant C, salmonellae were recovered from 14 (41%) of 34 samples and involved all 12 of the sample birds (100%). Isolations were made from: scald-tank water, 1 of 3 (33.3%); spin-chill water, 1 of 3 (33.3%); skin samples, 10 of 12 (83.8%); and from breast meat, 2 of 12 (16.6%). A similar wide distribution of salmonellae was found during the third visit to plant C where 13 (38%) of 34 samples and 9 (75%) of 12 sample birds yielded organisms. Isolations were accomplished from spin-chill water, 2 of 3 (66.6%); skin samples, 8 of 12 (66.6%); breast meat, 2 of 12 (16.6%); and from 1 of 4 (25%) thigh samples.

TABLE 3. *Salmonella* IN ENVIRONMENT AND TURKEY OBTAINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (MPN/g - range)[b, c] Visit no.			Plant B (MPN/g - range)[b, c] Visit no.			Plant C (MPN/g - range)[b, c] Visit no.				Total
	Fraction[a]	1	2	Fraction[a]	1	2	Fraction[a]	1	2	3	fraction[a]
Feathers	0/2	<.03	<.03	0/2	<.03	<.03	*	*	*	*	0/ 4 (0%)
Scald-tank water	0/4	<.03	<.03	0/4	<.03	<.03	1/9	<.03	(<.03-.11)	<.03	1/17 (6%)
Spin-chill water	0/4	<.03	<.03	0/4	<.03	<.03	3/9	<.03	<.03	(<.03-.36)	3/17 (18%)
Skin - breast:											
Before spin-chill	0/6	<.03	<.03	0/6	<.03	<.03	6/9	<.03	(.036-.93)	(.091-.21)	6/21 (28%)
After spin-chill	0/6	<.03	<.03	0/6	<.03	<.03	5/9	<.03	(.036-.15)	(<.03-.03)	5/21 (24%)
After overnight storage in ice	2/6	<.03	(<.03-.036)	0/6	<.03	<.03	5/9	<.03	(.091-.39)	(<.03-4.6)	7/21 (33%)
After 31 days frozen storage	1/6	<.03	<.03	0/6	<.03	<.03	2/9	<.03	(<.03-.15)	(<.03-.15)	3/21 (14%)
Meat - after overnight storage in slush ice:											
Experimentally deboned breast	0/6	<.03	<.03	0/6	<.03	<.03	1/9	<.03	<.03	<.03	1/21 (4.8%)
Commercially deboned breast	0/5	<.03	<.03	0/6	<.03	<.03	2/9	(<.03-.1)	<.03	<.03	2/20 (10%)
Experimentally deboned thigh	0/2	<.03	<.03	0/2	<.03	<.03	0/2	*	<.03	<.03	0/ 6 (0%)
Commercially deboned thigh	0/1	*	<.03	0/2	<.03	<.03	1/3	<.03	<.03	.07	1/ 6 (17%)
Meat - after 31 days frozen storage:											
Experimentally deboned breast	0/6	<.03	<.03	0/6	<.03	<.03	0/9	<.03	<.03	<.03	0/21 (0%)
Commercially deboned breast	0/5	<.03	<.03	0/6	<.03	<.03	4/9	(<.03-.036)	<.03	<.03	4/20 (20%)
Experimentally deboned thigh	0/2	<.03	<.03	0/2	<.03	<.03	0/2	*	<.03	<.03	0/ 6 (0%)
Commercially deboned thigh	0/1	*	<.03	0/2	<.03	<.03	0/3	<.03	<.03	<.03	0/ 6 (0%)

*Not determined
[a]Number of samples positive/number of samples tested
[b]Range per gram Most Probable Number (MPN/g)
[c]Minimum quantity measurement <.03 organisms per gram of sample

Fifteen salmonellae isolates from plant C were serotyped by the National Animal Disease Laboratory, Ames, Iowa. The serotypes identified from the first visit were *Salmonella newport* and *Salmonella saint-paul;* from the second visit, *Salmonella san-diego, Salmonella chester,* and six isolates of *Salmonella heidelberg;* and from the third visit, S. *chester* and four isolates of S. *heidelberg.*

These data indicate a possible contribution to the salmonellae "load" of turkey products by the deboning equipment (knives and gloves). Only 1 (2.4%) of 42 experimentally deboned breasts had recoverable salmonellae while organisms were recovered from (15%) of 40 commercially deboned breast samples. None of 12 experimentally deboned thigh samples yielded salmonellae while 1 (8.3%) of 12 commercially deboned thighs were found positive. Accompanying skin samples yielded salmonellae 10 (24%) of 42 times. Theoretically, contamination from the skin to the breast meat samples via knives or gloves would be the same in either experimentally deboned or commercially deboned samples, because the same knife and gloves were used to obtain experimentally deboned breast and thigh samples from an individual sample bird as was used to obtain the skin sample. Therefore, the increased recovery of organisms from the commercially deboned meat, compared to that of experimentally deboned meat, might be attributable to the buildup and/or transfer of salmonellae on the employees' knives and gloves. Ironically, this procedure of using the same knife and gloves to remove skin and meat samples from individual birds was not used in Plant C where all six of the salmonellae recoveries from commercially deboned breast meat were made. At Plant C, the breast skin was removed by the experimentalist from all sample birds using a sterile knife for each bird without excising the breast meat. Consequently, the salmonellae recoveries from these breast samples must be attributed to carry-over on the knives and/or gloves of the plant deboning personnel from previously deboned birds and not from the skin of the test bird. These findings are in agreement with the observations of Bryan et al. *(5)* who reported an increase in isolations of salmonellae from finished products compared to carcasses before further processing.

Coliforms

Enumeration of coliform organisms was included in this study, based on their role as sanitation indicator organisms in the food industry, although their use in the poultry processing industries has not been extensive. According to Zottola and Busta *(21),* use of coliforms as a sanitation index in further-processed turkey products has limitations because these organisms are part of the natural flora indigenous to turkeys. Wilkerson et al. *(20)* indicated that coliforms were of equal value to enterococci in indicating initial contamination. However, coliforms failed to survive freezing and, therefore, lacked value as indicators on frozen turkey products. It is possible that coliforms could be used as indicator organisms for cooked further-processed turkey products where isolation of these bacteria should be an indication of contamination after cooking. Brant and Guion *(3)* reported that the results of their study of California turkey processing plants confirm that coliforms are not a good index of sanitation. They found that sanitation measures which appear to reduce total counts also appear to encourage coliforms. Walker and Ayres *(19)* in 1959 noted that if coliforms occur in unusually great numbers, undue contamination from fecal material and filth and the lack of careful handling during processing would be indicated.

Coliform organisms were recovered from all of the turkey carcasses sampled and from 204 (80%), of 257 various samples, 4 (100%) of 4 feather samples, 3 (19%) of 16 scald-tank water samples, 18 (95%) of 19 spin-chill water samples, and from 23 (82%) of 28 thigh meat samples (see Table 4).

Enumeration procedures indicated that coliforms were present at each stage tested in the processing of the birds. The greatest numbers of coliforms were recovered from skin samples before and after spin-chilling. Subsequent skin samples, after the birds had been stored overnight in slush ice, generally showed a reduction in numbers of organisms. These samples yielded from 4 to 550 organisms per gram, excluding samples from visit one to plant B where undue contamination was evident in numbers ranging from 570 to 4400/g. These coliform counts appear higher than those reported by Walker and Ayres *(19)* who found coliform numbers on the skin surface of turkeys to range from 10 to 70/cm^2 with the usual range from 10 to 30. The significance of these observations may have been reduced because Walker and Ayres utilized cotton swab sampling techniques over 2 cm^2 areas delineated by metal guides, while our results were obtained by determining the number of organisms in 200 to 500 g of blended skin tissue. According to Avens and Miller *(1),* the cotton swab method for quantifying bacterial populations on turkey carcass skin enumerated only 22% of the bacteria measured by the skin tissue blending method. Considering this difference in recovery, the coliform counts obtained in our study were even nearer to the values of Walker and Ayres *(19)* and to those of Brant and Guion *(3)* where they found the mean coliform counts from various plants ranged from 0 to 1010/cm^2.

Results of this study substantiate the limitations on

TABLE 4. COLIFORMS IN ENVIRONMENT AND TURKEY OBTAINED IN MINNESOTA TURKEY PROCESSING PLANTS

Sample	Plant A (CFU/g - range)[b, c] Visit no.			Plant B (CFU/g - range)[b, c] Visit no.			Plant C (CFU/g - range)[b, c] Visit no.			Total
	Fraction[a]	1	2	3	Fraction[a]	1	2	Fraction[a]	1	2	3	fraction[a]
Feathers	2/2	680	440	*	2/2	10000	2300	*	*	*	*	4/ 4 (100%)
Scald-tank water	0/6	<1	<1	<1	1/4?	<1	(<1-70)	2/6	*	(<.1-1.6)	(<.1-.2)	3/16 (19%)
Spin-chill water	5/6	(<1-6)	(13-64)	(11-39)	4/4	(1000-1300)	(1700-3100)	9/9	(11-20)	(31-43)	(71-93)	18/19 (95%)
Skin - breast:												
Before spin-chill	9/9	(9-24)	(26-170)	(4-220)	6/6	(5600-28000)	(90-280)	9/9	(310-470)	(290-2800)	(490-640)	24/24 (100%)
After spin-chill	9/9	(9-45)	(100-120)	(55-690)	6/6	(1500-16000)	(100-250)	9/9	(100-370)	(270-480)	(350-560)	24/24 (100%)
After overnight storage in ice	9/9	(4-19)	(14-290)	(12-76)	6/6	(570-4400)	(45-100)	9/9	(100-550)	(360-530)	(110-210)	24/24 (100%)
After 31 days frozen storage	6/9	(<1-27)	(2-7)	(14-310)	6/6	(140-680)	(24-52)	7/9	(<1-1)	(3-6)	(15-43)	19/24 (80%)
Meat - after overnight storage in slush ice:												
Experimentally deboned breast	3/9	(<1-1)	(<1-1)	(<1-3)	5/6	(9-30)	(<1-8)	9/9	(2-26)	(5-17)	(3-5)	17/24 (71%)
Commercially deboned breast	6/8	(<1-7)	(6-25)	(<1-14)	6/6	(26-930)	(5-28)	9/9	(4-70)	(14-72)	(6-15)	21/23 (95%)
Experimentally deboned thigh	1/3	1	<1	<1	2/2	18	13	2/2	*	1	8	5/ 7 (70%)
Commercially deboned thigh	2/2	*	11	2	2/2	40	47	3/3	4	47	17	7/ 7 (100%)
Meat - after 31 days frozen storage:												
Experimentally deboned breast	0/9	<1	<1	<1	5/6	(2-8)	(<1-2)	5/9	(<1-1)	(<1-1)	(2-3)	10/24 (41%)
Commercially deboned breast	5/8	(<1-1)	(1-5)	(<1-28)	6/6	(55-70)	(3-27)	6/9	(1-5)	<1	(1-3)	17/23 (73%)
Experimentally deboned thigh	1/3	<1	<1	1	2/2	13	4	2/2	*	1	4	5/ 7 (70%)
Commercially deboned thigh	2/2	*	3	6	2/2	47	25	2/3	<1	6	4	6/ 7 (86%)

*Not determined

[a]Number of samples positive/number of samples tested

[b]Colony Forming Units per gram (CFU/g)

[c]Minimum quantity measurement <1 organism per gram of sample

TABLE 5. FREQUENCY OF ISOLATION OF SELECTED MICROORGANISMS FROM FRESH AND FROZEN TURKEY SKIN, BREAST, AND THIGH MEAT

Sample	*C. perfringens**	*S. aureus**	Salmonellae*	Coliforms*
Skin:				
Before spin-chilling	21/24 (87%)	17/24 (71%)	6/21 (28%)	24/24 (100%)
After spin-chilling	20/24 (83%)	16/24 (67%)	5/21 (24%)	24/24 (100%)
After overnight storage of whole birds in slush ice	19/24 (79%)	17/24 (71%)	7/21 (33%)	24/24 (100%)
After 31 days frozen storage	18/24 (75%)	22/24 (91%)	3/21 (14%)	19/24 (80%)
Breast meat:				
After overnight storage of whole birds in slush ice	26/47 (55%)	21/47 (45%)	3/41 (7%)	38/47 (80%)
After 31 days frozen storage	23/47 (50%)	19/47 (40%)	4/41 (10%)	27/47 (57%)
Thigh meat:				
After overnight storage of whole birds in slush ice	8/14 (55%)	8/14 (55%)	1/12 (8%)	12/14 (86%)
After 31 days frozen storage	7/14 (50%)	4/14 (29%)	0/12 (0%)	11/14 (79%)

*Number of positive samples/number of samples tested.

the use of coliforms as indicators of potentially pathogenic organisms. Recoveries of salmonellae were successfully accomplished from four of nine Plant C commercially deboned, frozen breast meat samples, yet coliform numbers were found to be only <1 to 5/g. Likewise, no salmonellae were recovered from Plant B commercially deboned, frozen breast meat samples, yet the number of coliforms were estimated from 3 to 70/g. Thus, the lack of apparent coliforms cannot guarantee a freedom from potentially pathogenic organisms, nor can the presence of coliforms necessarily indicate the presence of potentially pathogenic organisms. Obviously, the numbers of coliforms may be used to indicate relative sanitation and processing conditions in the plant.

The levels of all types of microorganisms studied were greater in skin samples than in equivalent meat samples. This differential ranged from 10-fold to 1000-fold greater populations from skin compared to the meat. Frozen skin samples yielded potential pathogens from 14 to 91% of the time (Table 5): *C. perfringens*, 18 (75%) of 24; *S. aureus*, 22 (91%) of 24; salmonellae, 3 (14%) of 21; and coliforms, 19 (80%) of 24. The implications of these findings may be obvious, yet the solution to the problem is not readily evident. One cannot discard skin from further-processed turkey products without adversely affecting flavor and profits any more than one can market skinless turkeys. All types of microorganisms studied were recoverable from fresh and frozen meat samples at a sufficiently high level that even if skin was not included in further-processed turkey products, essentially all raw further-processed turkey products would contain some potentially pathogenic microorganisms.

The numbers of organisms recovered from different sources varied from plant to plant. Plant B samples yielded from 10- to 100-fold more microorganisms than did samples from Plants A or C; however, no salmonellae were recovered from Plant B samples. Plant A samples yielded the fewest numbers of organisms. Plant C appeared to have been processing salmonellae infected birds. During the second visit, 12 (100%) of 12 and during the third visit, 9 (75%) of 12 carcasses sampled yielded salmonellae. During the first visit to plant C, salmonellae were recovered from three breast meat samples, yet no skin sample recoveries were accomplished, indicating that either infected birds were being processed or a build-up of organisms from previously processed infected birds had occurred. Recovery levels from breast and thigh meat obtained by the experimentalist, when compared to levels with similar commercially deboned meat, indicate that there probably was a contribution by processing personnel to the microbial "load" of the samples. Coliform bacteria isolated from the various plants tended to be associated with higher numbers of *C. perfringens* and *S. aureus;* however, no obvious correlation between numbers of salmonellae and coliform bacteria can be made from this study. The effect of freezing on the frequency of recovery and on the apparent numbers of these unwanted microorganisms does not appear significant. Although, after 31 days of frozen storage there is a trend towards reduced incidence of recoverable organisms, especially with *C. perfringens* and coliforms, the incidence rates were still considerable. Consequently, product freezing and storage at currently available commercial freezer temperatures will provide no assurance of complete destruction of all potentially pathogenic organisms.

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