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Optimum Skin Blending Method for Quantifying Poultry Carcass Bacteria[1]

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Optimum blending fluids and blending times for use in quantifying bacteria on poultry carcass skin by the skin "blending" method were determined. Butterfield's buffered-phosphate diluent, physiological saline solution (0.85% NaCl), peptone water (0.1% peptone), and deionized water, each at four different skin blending times of 1, 2, 3, and 4 min, were compared. The comparison was based on relative numbers of bacteria per cm² of skin, enumerated by each combination on turkey carcasses. Peptone water and physiological saline solution each yielded significantly ($P < 0.01$) higher bacteria counts from turkey carcass skin samples than did Butterfield's buffered-phosphate diluent or deionized water. There were no significant differences among the four skin blending times and no significant interaction effect between the two factors tested.

The skin "blending" and dilution method for quantifying bacteria on poultry carcass skin consists of two factors, blending fluid and blending time. Either factor could affect the number of bacteria recovered and enumerated by this method.

Straka and Stokes (2) demonstrated rapid and extensive destruction of bacteria in distilled water, tap water, phosphate water, and physiological saline solution. Their data indicated that water or even physiological saline was most destructive. Therefore, their use as a diluent can lead to serious errors in quantitative determination of bacteria in food. The degree of error depends on the number of sensitive bacteria present, exposure time to the fluid, and quantity of protective organic food material in the fluid. They warned that these diluent fluids can cause large errors in quantitative bacterial counts by plating. They found that 0.1% peptone in distilled water as a diluent fluid did not cause any appreciable destruction of bacteria for 1 hr.

Previously, sterile water had been commonly used as a food diluent for bacteriological plate counts; also, physiological saline and phosphate-buffered distilled water have been used to a lesser extent (2). The Official Methods of Analysis of the Association of Official Analytical Chemists (1) specifies using Butterfield's buffered-phosphate diluent as a blending fluid for all frozen, chilled, precooked, or prepared foods. A survey of recent literature indicated that 0.1% peptone water is most frequently used as a diluent fluid for determining bacteria counts on poultry meat.

Subjecting a sample of poultry carcass skin in diluent fluid to the agitation of a laboratory blendor partially disintegrates the skin, physically removes bacteria from the skin sample, and uniformly distributes the bacteria in the fluid so representative samples can be plated. Consequently, duration of this agitation could possibly affect the number of bacteria determined by plate count.

The objective of this study was to compare four skin blending fluids and four skin blending times to determine the optimum combinations for use in quantifying bacteria on poultry carcass skin samples. The experiment was designed to detect whether blending fluids or blending times significantly affect bacteria counts. The null hypotheses were that no significant difference in bacteria counts occurs among the four blending fluids or among the four blending times and that no interaction occurs. These hypotheses were tested in a factorial experiment by analysis of variance.

MATERIALS AND METHODS

Four blending fluids, Butterfield's buffered-phosphate diluent, physiological saline solution (0.85% NaCl), peptone water (0.1% peptone in distilled water), and deionized water (A, B, C, and D, respectively, in Table 1), were compared, each at four

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TABLE 1. *Pattern of random selection of blending fluids for turkey carcass skin samples*

Breast								Legs								Replicate
Left side (part I)				Right side (part II)				Left side (part III)				Right side (part IV)				
1[a]	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
A[b]	C	B	D	A	B	D	C	D	C	B	A	D	A	B	C	1
B	C	D	A	A	B	C	D	C	A	B	D	C	B	D	A	2
B	D	C	A	B	D	C	A	B	D	C	A	C	B	D	A	3
B	C	D	A	C	D	A	B	D	C	A	B	A	D	B	C	4
A	B	D	C	A	B	D	C	B	A	D	C	B	A	C	D	5

[a] Sample number.

[b] Blending fluids: (A) Butterfield's buffered phosphate, (B) physiological saline (0.85% NaCl), (C) peptone water (0.1% peptone), (D) deionized water.

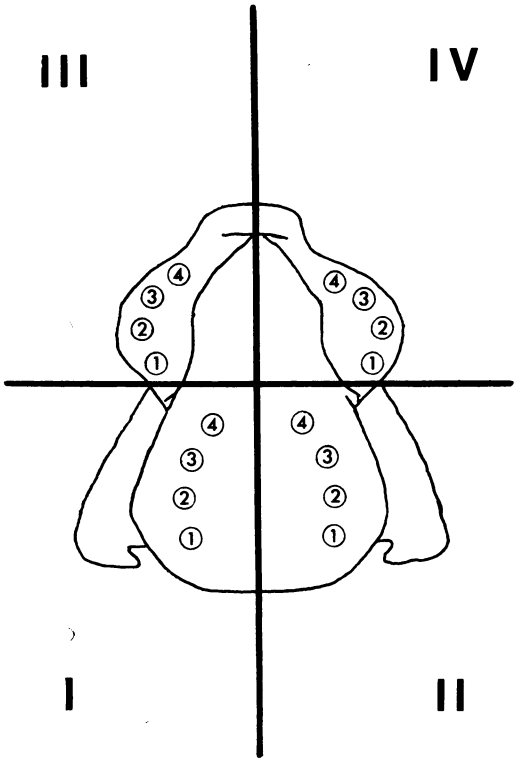

FIG. 1. *Skin sample locations on the turkey carcass. Carcass parts are: (I) left side breast, (II) right side breast, (III) left leg, and (IV) right leg. Skin samples (7.145 cm² discs) are numbered 1, 2, 3, and 4.*

different skin blending times of 1, 2, 3, and 4 min. The basis of comparison was the relative number of bacteria per cm² of skin on turkey carcasses, determined by plate count for each blending fluid-time combination. Male turkey carcasses ranging in weight from 15 to 22 lb (6.8 to 10 kg) were used.

For each replicate, one frozen (−29 C) turkey carcass was thawed in its plastic bag for 16 to 24 hr at room temperature (20 to 27 C). Skin samples (7.145 cm² discs) from four parts of the carcass (breast, left and right side; and legs, left and right) were sampled for each of the four blending fluids. Thus, four skin samples were removed from each part of a single carcass (Fig. 1). Random selection of blending fluids for carcass skin samples is shown in Table 1. Sample 1 was removed consecutively from each of parts I through IV; then sample 2 was removed in the same consecutive order, as were samples 3 and 4. Each of the four blending fluids was used once for each carcass part and was randomly selected for the four skin samples in each part by use of a random number table. Skin sample 1 was removed from the same location on each leg and the same location on each side of the breast for all replicate carcasses; but the blending fluid used on skin sample 1 for each area was randomly selected, as it was for skin samples 2, 3, and 4.

Each skin sample was agitated in a sterile laboratory blendor jar with 100 ml of blending fluid. The blending fluid was plated in duplicate at the 10^{-2} and 10^{-3} levels in Trypticase Soy Agar plus 2% Yeast Extract after blending each skin sample for 1, 2, 3, and 4 min. After each blending time, 2.2 ml was removed from the blendor jar for plating. This volume change was adjusted by the appropriate dilution factors. Plates were incubated at 35 to 37 C for 48 hr, 25 to 28 C for 60 hr, and stored at 4 C for at least 48 hr prior to colony counting.

The aerobic plate count (APC) representing each carcass with any one blending fluid-time combination was the geometric mean of APC values from the four samples, each representing one of four carcass parts.

The experiment was replicated with five carcasses. The geometric means were subjected to analysis of variance and Duncan's multiple range test to determine optimum blending fluids and optimum blending times for use in quantifying bacteria on poultry carcass skin by the skin "blending" method.

TABLE 2. *Turkey carcass aerobic plate counts (APC/cm² of skin) for each blending fluid-time combination*

Blending		(APC/cm² of skin) on carcass (geometric mean)[a]					
Fluid[b]	Time	Replicate carcasses					Avg
		1	2	3	4	5	
	min						
A	1	570	1,600	970	600	210	790
	2	740	1,800	750	620	260	830
	3	760	1,700	900	880	370	920
	4	690	1,600	690	670	280	790
B	1	2,000	1,400	480	2,400	360	1,300
	2	1,900	1,700	520	2,000	420	1,300
	3	1,900	1,600	420	2,400	510	1,400
	4	1,900	1,600	380	2,000	430	1,300
C	1	2,100	1,200	420	1,400	1,600	1,300
	2	2,200	1,300	370	1,100	2,000	1,400
	3	2,500	1,300	370	1,300	1,800	1,500
	4	2,200	1,200	360	1,200	1,700	1,300
D	1	830	1,100	880	960	730	900
	2	820	1,100	750	880	610	830
	3	770	920	520	860	640	740
	4	670	850	590	800	450	670

[a] Each value represents a geometric mean of four skin sample aerobic plate counts per carcass.

[b] Blending fluids: (A) Butterfield's buffered phosphate, (B) physiological saline (0.85% NaCl), (C) peptone water (0.1% peptone), (D) deionized water.

RESULTS

APC/cm² of skin for each carcass with each blending fluid-time combination is shown in Table 2. Average APC values per cm² of skin for each blending fluid-time combination are shown in Table 3. Peptone water and physiological saline solution yielded significantly ($P < 0.01$) higher APC values from turkey carcass skin than did Butterfield's buffered-phosphate diluent or deionized water. There was no significant difference in APC values between peptone water and physiological saline solution or between Butterfield's buffered-phosphate diluent and deionized water. There were no significant differences in APC values among the four skin blending times. No significant interaction occurred between the two factors tested.

Results of this study are illustrated graphically in Fig. 2. With the agar-plate count technique, turkey carcass skin samples "blended" in peptone water or physiological saline solution yielded significantly higher carcass bacteria counts than skin samples "blended" in Butterfield's buffered-phosphate diluent or deionized water, regardless of blending time. It made no significant difference

TABLE 3. *Average turkey carcass aerobic plate counts (APC/cm² of skin) for each blending fluid-time combination*

Blending time (min)	Avg APC/cm² of skin per carcass[a]				
	Blending fluid[b]				Grand avg[c]
	D	A	B	C	
1	900	790	1,300	1,300	1,100
2	830	830	1,300	1,400	1,100
3	740	920	1,400	1,500	1,100
4	670	790	1,300	1,300	1,000
Grand avg[d]	790	830	1,300	1,400	

[a] Each value represents an arithmetic mean (average) of five carcass aerobic plate counts, each of which represents a geometric mean of four skin sample counts per carcass.

[b] Blending fluids: (A) Butterfield's buffered phosphate, (B) physiological saline (0.85% NaCl), (C) peptone water (0.1% peptone), (D) deionized water.

[c] No significant difference ($P < 0.05$).

[d] D and B, D and C, A and B, and A and C are significantly different ($P < 0.01$); D and A, and B and C, are not significantly different ($P < 0.05$).

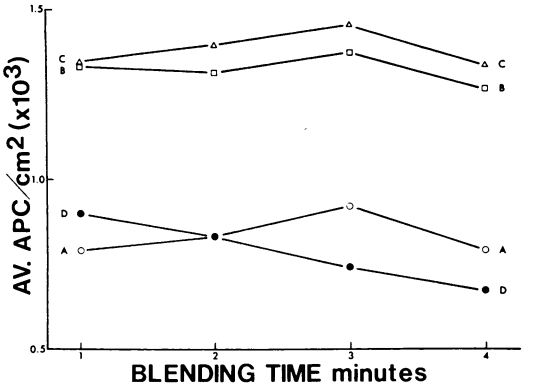

FIG. 2. *Average turkey carcass aerobic plate counts (APC/cm² of skin) for each blending fluid-time combination. Each point represents an arithmetic mean (average) of five carcass aerobic plate counts, each of which represents a geometric mean of four skin sample counts per carcass. Blending fluids used are: (A) Butterfield's buffered phosphate, ○; (B) physiological saline (0.85% NaCl), □; (C) peptone water (0.1% peptone), △; and (D) deionized water, ●.*

whether skin samples were "blended" for 1, 2, 3, or 4 min. In fact, in many cases (Table 2) lower counts were obtained after "blending" for 4 min, but this was not significant.

DISCUSSION

Prolonged "blending" could possibly rupture some bacterial cells or result in a toxic effect from overexposure to the fluid. A blending time of 1 or 2 min was sufficient for optimum results. Apparently, chains or clumps of bacteria were broken into individual cells before 1 min of blending time, or, if not, they were not further broken to any appreciable extent by blending for 2, 3, or 4 min.

Based on these results and on the work of Straka and Stokes (2), it can be concluded that peptone water (0.1% peptone) is the optimum blending and diluent fluid of those tested and that the customary 2 min is an optimum blending time for use in quantifying bacteria on poultry carcass skin by the skin "blending" method.

Butterfield's buffered-phosphate diluent is recommended (1) and is commonly used as a blending and diluent fluid for many types of food in microbiological analyses. With some foods, this may be a preferable blending and diluent fluid, perhaps because of its buffering capacity. However, with many materials it may not yield the maximum number of viable bacteria on plate count, as shown by this study with poultry carcass skin. If it is important to determine the maximum number of viable bacteria present in a food material by plate count, the particular food being tested should be considered as an individual case which perhaps will require a different blending and diluent fluid than some other food material.

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LITERATURE CITED

1. Association of Official Analytical Chemists. 1966. Changes in official methods of analysis made at the seventy-ninth annual meeting, October 11–14, 1965. Microbiological methods. J. Ass. Offic. Anal. Chem. 49:246–250.
2. Straka, R. P., and J. L. Stokes. 1957. Rapid destruction of bacteria in commonly used diluents and its elimination. Appl. Microbiol. 5:21–25.