Reprinted from *J. Food Sci.*
55(3): 655–657
Purchased by USDA for official use only

Comparison of Homogenization by Blending or Stomaching on the Recovery of *Listeria monocytogenes* from Beef Tissues

JAMES S. DICKSON

ABSTRACT

Homogenization by blending or stomaching was compared for the recovery of *Listeria monocytogenes* from inoculated intact beef tissue. There were no differences in numbers of recovered bacteria ($P>0.10$) attributable to either homogenization time or method. Fewer viable bacteria ($P<0.05$) were recovered in phosphate buffer than either buffered peptone water or 2% trisodium citrate buffer. Tween 80 increased the numbers of bacteria recovered from fat tissue ($P<0.05$). Stomaching is an acceptable method for homogenizing samples for Listeria analysis.

INTRODUCTION

MICROBIOLOGICAL ANALYSES of solid or semi-solid food items are dependent on proper homogenization of the sample in diluent. Several methods of homogenization have been used for sample preparation, and of these the two that have gained wide acceptance are blending and stomaching. Homogenization in a blender has long been accepted as the standard method of preparation. The Stomacher was introduced for food analyses in 1972 (Sharpe and Jackson, 1972), and has gained acceptance based on both performance and ease of use. The standard procedure for homogenizing solid samples is blending a 1:10 dilution of the sample at 8000 rpm (low speed) for 2 min (Andrews, 1984; Gabis et al., 1984). Stomaching is performed in a similar manner, except that the suggested homogenization time is 30 to 60 seconds (Gabis et al., 1984).

Andrews et al. (1978) reported that when stomaching and blending were compared with split samples, there was considerable variation of the stomacher results by food type. When aerobic plate counts of 37 samples of ground beef and pork sausage were compared, stomaching only recovered from 51% to 94% of the bacteria recovered by blending. However, Tuttlebee (1975) reported that homogenization by stomaching generally recovered more bacteria than homogenization by blending for aerobic plate counts of meat samples. Emswiler et al. (1977) also reported generally good correlation between blending and stomaching for a variety of meat products for aerobic plate count, *Staphylococcus aureus*, and coliforms.

In recent years, there has been an increasing an awareness of *Listeria monocytogenes* as a food-borne pathogen (Doyle, 1985). Although primarily associated with dairy products, *L. monocytogenes* has been associated with animal products intended for human consumption (Brackett, 1988). Stomaching commonly has been used for homogenization of meat samples in several studies involving listeriae (Truscott and McNab, 1988; Buchanan et al., 1987). However, Yousef et al. (1988) reported that significantly more *L. monocytogenes* were recovered from cheese when samples were homogenized by blending than by stomaching. The method of sample preparation apparently has an effect on the recovery of this bacterium from cheese products.

The objective of this study was to evaluate the effect of sample homogenization technique on the recovery of *L. monocytogenes* from intact beef tissue.

Author Dickson is with the USDA-ARS, Roman L. Hruska Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933.

MATERIALS & METHODS

Bacterium

Listeria monocytogenes strain Scott A (FDA, Bacteriology Physiology Branch, Cincinnati, OH) was used as the test organism for all experiments. The bacterium was maintained on tryptic soy agar (TSA, Difco). The culture was transferred to tryptic soy broth (TSB, Difco) and grown for 18 hr at 23°C. Cells were harvested by centrifugation (3000 × *g*, 10 min; 5°C) and the pellet resuspended in Butterfield's phosphate buffer (Pertel and Kazanas, 1984). The buffer suspension typically contained approximately 10^8 colony forming units per mL (CFU/mL).

Tissue preparation and inoculation

Lean beef and fat tissue was separated by tissue type and sliced into 0.5 cm thick pieces. The tissue was frozen until required, and then sliced into 1.0 × 1.0 cm pieces (final sample size 1.0 × 1.0 × 0.5 cm). An inoculum was prepared in sterile beakers containing 20 mL phosphate buffer. The tissue samples were immersed in the inoculum for 5 min, drained briefly, and then transferred for enumeration. Alternately, some samples were attached to sterile "alligator" clips and suspended in empty sterile beakers, such that the tissue did not contact the side of the beaker. These beakers were loosely covered and incubated at 5°C for up to 7 days.

Recovery methods and enumeration

Mechanical homogenization was accomplished in either a Waring Blendor (New Hartford, CT) or a Stomacher 400 (Tekmar Inc, Cincinnati, OH), using 99 mL phosphate buffer, 0.1% buffered peptone water (BPW, Difco), or 2% sodium citrate buffer (Marth, 1978) as the diluent. Homgenization times were varied from 30 sec to 5 min. Where indicated, Tween 80 (1% vol/vol; Sigma) or sterilized sea sand (5% wt/vol; Fisher Scientific) were added to the phosphate buffer. Alternately, the buffer was adjusted to pH 8, 9, or 10 with 1N NaOH, or tempered to 37°C. Samples were enumerated using the pour plate technique (Busta et al., 1984) and TSA. Plates were incubated at 32°C for 24 to 48 hr.

Statistical analysis

The counts were expressed as the number of bacteria per cm^2 of tissue surface, and the data were analyzed using the analysis of variance methods in the General Linear Model of SAS (1982). The models were appropriate to the completely randomized design of the experiments. The means reported are the $\log_{10}$ transformations of three independent replications.

RESULTS & DISCUSSION

Effects of homogenization time and method

There was no effect ($P>0.10$) of homogenization time on recovery with either stomaching or blending (Table 1). The higher counts associated with fat tissue for blending are a function of higher inoculum levels (as determined by plate counts of the inoculum), and not inherent differences in bacterial attachment to the tissues. When blending and stomaching were compared directly with a homogenization time of 2 min, there were no differences in recovery ($P>0.10$) over time between the two methods (Table 2). These results differ from those of Yousef et al. (1988), who reported that blending of Colby

Table 1—Effect of homogenization time by blending method on recovery of L. monocytogenes

Method	Tissue type	Time (min) 0.5	1.0	2.0	3.0	4.0	5.0
Blendor	Lean	ND[c]	6.56[a]	6.72[a]	6.71[a]	6.56[a]	6.67[a]
	Fat	ND	7.04[b]	7.04[b]	7.28[b]	7.15[b]	7.08[b]
Stomacher	Lean	6.53[a]	6.59[a]	6.27[a]	6.12[a]	ND	6.11[a]
	Fat	6.15[a]	6.36[a]	6.35[a]	6.45[a]	6.20[a]	6.19[a]

[a,b] Log CFU/cm². Means with different superscripts within homogenization method are significantly different (P<0.05).
[c] Not done

Table 2—Comparison of homogenization by blending and stomaching on recovery of L. monocytogenes[a]

Method	Tissue type	Mean $\log_{10}$ CFU/cm² Time (days)[b] 0	1	7
Blendor	Lean	7.18[e]	6.26[f]	5.41[f]
Stomacher		7.27[e]	6.54[f]	5.23[f]
Blendor	Fat	6.65[c]	5.97[c]	5.23[d]
Stomacher		6.59[c]	6.16[c]	5.68[d]

[a] Homogenization time = 2 min.
[b] Time of incubation at 5°C; Day 0 is immediately after inoculation.
[c-f] Means with different superscripts within rows and columns are significantly different (P<0.05).

Table 3—Effect of diluent and temperature on recovery of L. monocytogenes by stomaching[a]

Tissue	Diluent[b]	Mean $\log_{10}$ CFU/cm² diluent temperature 23°C	37°C
Lean	Phosphate	7.03[c]	6.70[c]
	Peptone	7.28[d]	7.16[c,d]
	Citrate	7.22[d]	7.23[d]
Fat	Phosphate	6.83[c]	6.76[c]
	Peptone	7.08[a,d]	6.94[c,d]
	Citrate	7.06[a,d]	7.18[d]

[a] Homogenization time = 2 min.
[b] Diluents: Phosphate = Butterfield's phosphate buffer; Peptone = 0.1% buffered peptone water; Citrate = 2% trisodium citrate.
[c-d] Means with different superscripts within tissue type are significantly (P<0.05) different.

Table 4—Effects of Tween 80 and sterile sand on the recovery of L. monocytogenes from meat tissue samples homogenized by blending or stomaching.

Tissue	Buffer[c]	Mean $\log_{10}$ CFU/cm² Homogenization Blendor	Stomacher
Lean	Control	6.83[a]	6.73[a]
	Tween 80	6.72[a]	6.72[a]
	Sand	6.72[a]	6.92[a]
Fat	Control	6.95[a]	6.90[a]
	Tween 80	7.13[a,b]	7.27[b]
	Sand	6.85[a]	6.78[a]

[a-b] Means with different superscripts within tissue type are significantly different (P<0.05).
[c] Buffer type; Control = Butterfield's phosphate buffer; Tween 80 = phosphate buffer + 1% Tween 80; Sand = phosphate buffer + 5% sterile sand.

cheese samples produced slightly higher, although statistically significant, counts than stomaching.

The reported differences may be attributable to the physical nature of the samples and distribution of bacteria within each sample. The cheese was inoculated with *L. monocytogenes* at the beginning of the manufacturing process, i.e., prior to the addition of the starter culture. Given the fluid nature of the milk and the subsequent mixing during addition of the starter culture, the bacteria would be expected to be uniformly distributed through the cheese. Samples obtained from this cheese would contain *L. monocytogenes* cells uniformly distributed throughout the entire sample. Intact animal tissue, however, is generally contaminated only on the outer edges (crevices) and exposed tissue surfaces. Because of this, it may not be necessary to completely homogenize the entire sample, as long as the surfaces are sufficiently disrupted to release the bacteria. While blending visually produced a more homogenized sample, stomaching is apparently sufficient to disrupt the tissue surfaces, where the majority of bacteria are attached. In addition, because of the potential for generation of aerosols during blending, the stomacher may be a more desirable method for processing samples for biosafety concerns.

Effects of diluent type, temperature, and pH

The type of buffer used to homogenize the samples had an effect (P<0.05) on total numbers of recovered *L. monocytogenes* (Table 3). The lowest populations were found when phosphate buffer was used as a diluent. There were no differences in the populations recovered with either peptone or citrate buffers. The pH of the buffer (phosphate) did not affect the counts (P>0.10) over a range of pH 7 to pH 10 (data not shown). The lower counts recovered with phosphate buffer at 23°C, although statistically significant, were only marginally less than those of the other diluents. The differences became more pronounced at 37°C. Yousef et al. (1988) reported a mean log CFU/g of 3.78 for cheese samples stomached with 2% trisodium citrate at 20°C, versus 3.59 for those stomached with tryptose broth at the same temperature. While these values were statistically different (P<0.05), the actual biological significance of these findings is open to question.

The differences in recovery between phosphate buffer and the other diluents would be expected if the bacteria had been subjected to stress prior to the enumeration process. However, the only stresses that the bacteria had been subjected to were chilling (5°C) during harvesting and the homogenization procedure itself. These may have been sufficient to affect viability of the cells, although other treatments would have been expected to detect differences attributable to stress as well (i.e., use of sand).

Effects of Tween 80 and sand

Tween 80 increased (P<0.05) the numbers of *L. monocytogenes* recovered from fat tissue samples (Table 4). Emswiler et al. (1977) reported that the addition of Tween 80 increased the numbers of bacteria recovered from a variety of meat products, but that this increase was generally not significant. The addition of sterile sand visually resulted in a more complete homogenization of the samples by stomaching, although it did not affect (P>0.10) the numbers of recovered bacteria. As discussed earlier, this is most likely a result of the distribution of bacteria on the sample, and does not necessarily preclude the use of sand as an agent for processing other samples, since it did not decrease the numbers of recovered bacteria.

CONCLUSIONS

THERE WAS NO DIFFERENCE between blending and stomaching on the recovery of *L. monocytogenes* from intact beef tissue, nor was there an apparent difference with homogenization time over a range of 0.5 to 5 min. Phosphate buffer was slightly inferior to buffered peptone water or 2% trisodium citrate buffer as a diluent.

REFERENCES

Andrews, W.H. 1984. Food sample handling in the laboratory and preparation of the sample homogenate. Ch. 13. In "Bacteriological Analytical Manual." 6th ed. Association of Official Analytical Chemists, Arlington, VA.

Andrews, W.H., Wilson, C.R., Polema, P.L., Romero, A., Rude, R.A., Duran, A.P., McClure, D., and Gentile, D.E. 1978. Usefulness of the stomacher

in a microbiological regulatory laboratory. Appl. Environ. Microbiol. 35: 89.
Brackett, R.E. 1988. Presence and persistence of Listeria monocytogenes in food and water. Food Technol. 42(4): 162.
Buchanan, R.L., Stahl, H.G., and Archer, D.L. 1987. Improved plating media for simplified, quantitative detection of Listeria monocytogenes in foods. Food Microbiol. 4: 269.
Busta, F.F., Peterson, E.H., Adams, D.M., and Johnson, M.G. 1984. Colony count methods. Ch. 4. In "Compendium of Methods for the Microbiological Examination of Foods," 2nd ed. M.L. Speck, (Ed.). American Public Health Association, Washington, DC.
Doyle, M.P. 1985. Food-borne pathogens of recent concern. Ann. Rev. Nutr. 5: 25.
Emswiler, B.S., Pierson, C.J., and Kotula, A.W. 1977. Stomaching vs. blending. Food Technol. 31(10): 40.
Gabis, D.A., Messer, J.W., Midura, T.R., and Peeler, J.T. 1984. Sampling plans, sample collection, shipment, and preparation for analysis. Ch. 2. In "Compendium of Methods for the Microbiological Examination of Foods," 2nd ed. M.L. Speck (Ed.). American Public Health Association, Washington, DC.
Marth, E.H. (Ed.) 1978. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, DC.
Pertel, R. and Kazanas, N. 1984. Stains, reagents, and diluents. In "Bacteriological Analytical Manual," 6th ed. Association of Official Analytical Chemists, Arlington, VA.
SAS. 1982. User's Guide: Statistics. SAS Institute, Cary, NC.
Sharpe, A.N. and Jackson, A.K. 1972. Stomaching: a new concept in bacteriological sample preparation. Appl. Microbiol. 24: 175.
Truscott, R.B. and McNab, W.B. 1988. Comparison of media and procedures for the isolation of Listeria monocytogenes from ground beef. J. Food Protect. 51: 626.
Tuttlebee, J.W. 1975. The stomacher - its use for homogenization in food microbiology. J. Food Technol. 10: 113.
Yousef, A.E., Ryser, E.T., and Marth, E.H. 1988. Methods for improved recovery of *Listeria monocytogenes* from cheese. Appl. Environ. Microbiol. 54: 2643.
Ms received 6/7/89; revised 9/20/89; accepted 10/6/89.

The author thanks Ms. Terri Alberts and Ms. Tammy Stuehm for their technical assistance in the laboratory.

Mention of a trade name, proprietary product or specific equipment does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.