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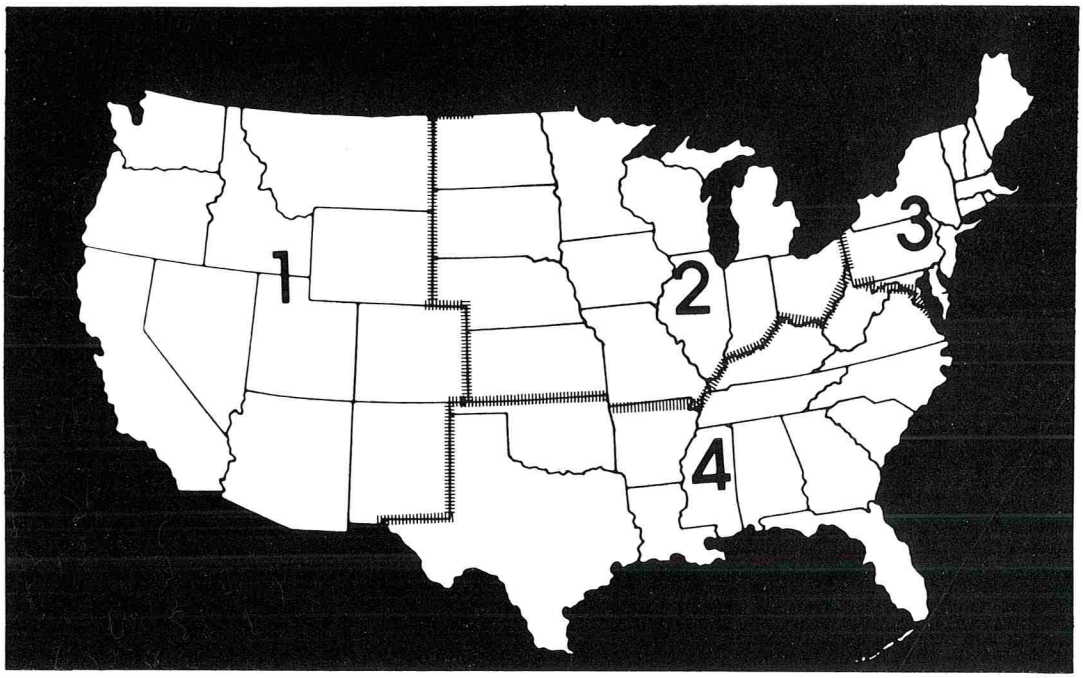

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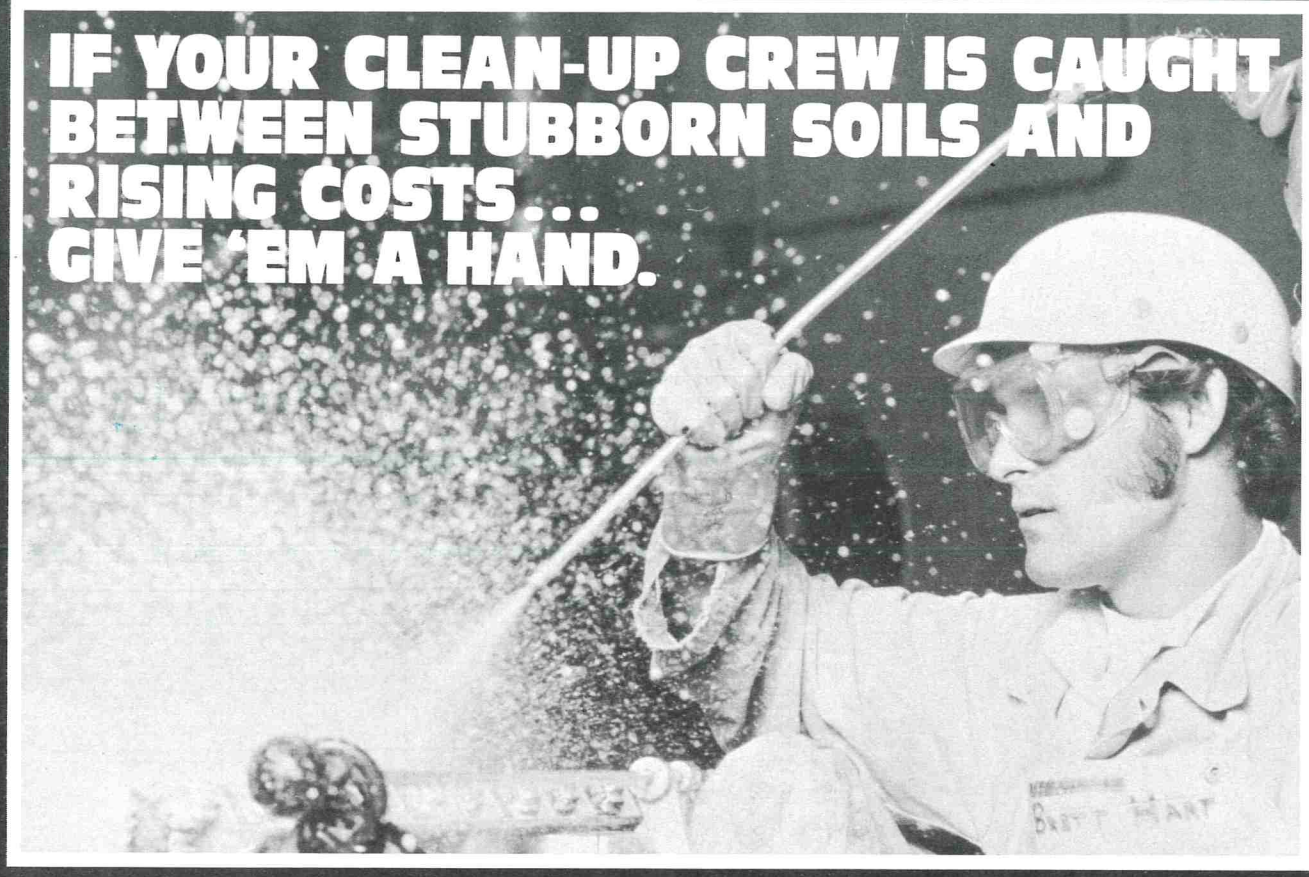

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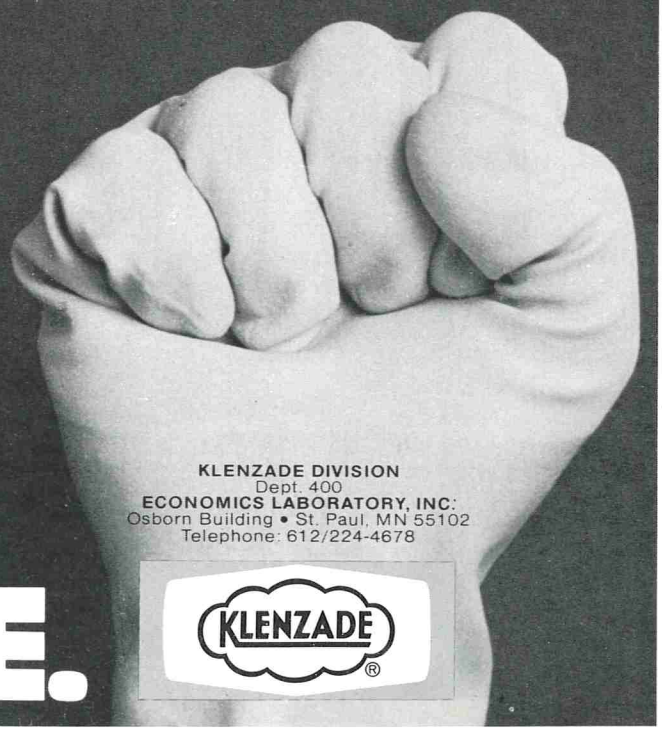

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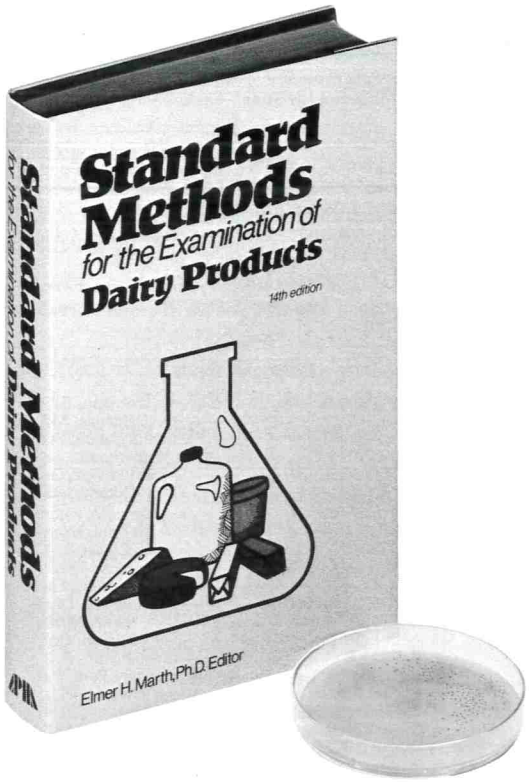

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Fate of *Salmonella* Inoculated into Beef for Cooking

S. J. GOODFELLOW* and W. L. BROWN

American Bacteriological and Chemical Research Corporation
P.O. Box 1557, Gainesville, Florida 32602

(Received for publication April 17, 1978)

ABSTRACT

D-values for *Salmonella* in a ground beef system were established at 125, 135 and 145 F (51.6, 57.2 and 62.7 C). The D-values determined at these temperatures were 61-62, 3.8-4.2 and 0.6-0.7 min, respectively. These values were used to develop a processing table that insured elimination of 10 million inoculated *Salmonella* in the center of beef roasts at temperatures sufficiently low to maintain the characteristic red color of "rare" roast beef. The effectiveness of the processing values in eliminating 10^7 *Salmonella* was confirmed by processing inoculated beef roasts under commercial conditions. Additional processing procedures necessary to insure elimination of *Salmonella* from the surface of dry roasted beef were also developed, since it was found that *Salmonella* could survive on the dry roasted beef surfaces under some circumstances.

Recent outbreaks of *Salmonella* food-poisoning caused by consumption of "rare" roast beef (*9,10,15,18*) prompted the USDA to issue a regulation requiring that all cooked beef and beef roasts be processed to a minimum internal temperature of 145 F (62.7 C) (*1*). Processing of beef to this internal temperature precluded the manufacture of "rare" roast beef. Representatives of industry requested that the USDA state its requirements for use of processing procedures other than a minimum internal temperature of 145 F (62.7 C). The USDA responded that it would consider amendments to this regulation if industry provided data on D-values for *Salmonella* serotypes in a meat system, as well as safe time-temperature processes for elimination of *Salmonella* from both water or steam cooked and dry roasted beef.

Several studies had established the D-values for *Salmonella* in broth systems (*4,11,13,16,19*). Considerable experimentation had also been done on the fate of *Salmonella* in various cooked meat systems (*2,3,5-8, 12,14,17,21-23*). However, D-values for *Salmonella* serotypes in beef systems had not been determined and time-temperature processes for manufacture of *Salmonella*-free, "rare" roast beef had not been established.

This study, contracted for by several professional associations of the meat packing industry, with a USDA-approved protocol, was conducted to determine D-values for *Salmonella* in a beef system and to establish time-temperature processes for manufacture of "rare" roast beef that was free from *Salmonella*.

MATERIALS AND METHODS

Products and processing

All meat used in this study was purchased from a local supplier (Est. No. U.S. 9157). Unless otherwise stated, all beef rounds were 16-18 lb. All processing was done in the ABC Research USDA-inspected pilot plant (Est. No. U.S. 7336).

Salmonella cultures

Salmonella typhimurium strain TMI used in this study was a reference strain which had been used in previously reported D-value determinations (*19*). This strain was obtained from the USDA Western Regional Research Laboratory. A *Salmonella* strain isolated from ground beef was obtained from the University of Florida (*20*). *Salmonella newport, Salmonella agona, Salmonella bovis-morbificans* and *Salmonella muenchen* strains (all isolated from food poisoning outbreaks associated with meat) were obtained from the USDA in Beltsville, Maryland. All stock cultures were maintained on slants of Plate Count Agar (PCA, Difco) stored at 4 C. Working cultures were maintained by daily transfer in Brain Heart Infusion Broth (BHI, Difco).

Enumeration of Salmonella

Two methods were used to enumerate *Salmonella*. The first was direct plating of the culture or product with PCA followed by adding an overlay of XL Agar base (Difco) supplemented with sodium thiosulfate and ferric ammonium citrate. Incubation was at 37 C for 48 h. Plates were counted at both 24 and 48 h to insure that the hydrogen sulfide indicator system in the XL Agar did not obscure individual colonies. The second method used was a most probable number enumeration technique using *Salmonella* methodology set forth in the Bacteriological Analytical Manual (B.A.M.) of the FDA.

Inoculation of ground meat

The ground meat employed in the D-value determinations and in the water-cooked beef experiment was inoculated with the six serotypes of *Salmonella* listed previously. The ground meat was prepared by extensive trimming of a freshly boned beef round followed by aseptic grinding. The total aerobic plate count of the uninoculated ground meat was less than 10 viable organisms per g. The ground meat was inoculated with a mixture of 48 h cultures of the six *Salmonella* serotypes. Before inoculation, each culture was centrifuged, washed with phosphate-buffered 0.85% saline solution, resuspended in the buffered saline solution and plated in triplicate. After incubation, the average of the triplicate plates showed that all cultures had a viable count of 1 to 3×10^9 colony forming units (CFU) per ml. A mixture of

the six cultures was prepared by combining a 1.0-ml portion of each washed culture in a sterile tube.

A 200-g portion of the ground meat was inoculated with 2.0 ml of the *Salmonella* culture mixture. The meat was placed in a presterilized, Waring mixer bowl, and the inoculum was added dropwise while mixing. The inoculated meat was reground to insure random distribution of the *Salmonella* organisms. The inoculated ground meat was analyzed in triplicate for total aerobic plate count and found to contain 1.7×10^7 CFU/ml. The inoculated meat was kept refrigerated below 32 F (0 C) during the day the D-value experiments were done. The meat was reanalyzed for total aerobic plate count before the last D-value experiment. The average of the triplicate counts was 1.3×10^7 CFU/ml.

D-value determinations

The D-values for the *Salmonella* cultures in ground meat were determined at 125, 135 and 145 F (51.6, 57.2 and 62.7 C). One-gram portions of the inoculated ground meat described previously were placed in sterile 10 × 60 mm screw-cap test tubes and the tubes placed immediately in an ice bath. At each test temperature, tubes were completely submerged in a water bath preadjusted to that temperature. Duplicate tubes were monitored for temperature increase. The timing for each experiment was not started until the 1-g portions of inoculated meat had reached the same temperature as the water bath. This was designated as time "zero." Duplicate tubes were removed from the water bath and placed immediately in an ice bath at time "zero" and at subsequent selected time intervals. After cooling, sterile 0.85% saline solution was added to the tubes and the meat mixed thoroughly before diluting and plating as described previously. All D-value experiments were performed in triplicate.

Internal inoculation and sampling—water-cooked beef

Beef rounds used in the water-cooking experiment were inoculated with *Salmonella* employing the dialysis tubing technique developed by Willardsen et al. (*24*). Twenty-gram portions of the inoculated ground meat described previously were placed in approximately 10-inch presoaked pieces of dialysis tubing [4.8-mm pore size 0.87 inch (ca. 2.2 cm) diameter, regenerated cellulose, A. H. Thomas]. The tubing was tied at both ends and was pushed to the geographic center of rounds cored previously with a sterile knife. Rounds were cored parallel to the long axis of the roast. After the tubing was inserted, coring tunnels were repacked with the meat removed during the coring operation. In eight randomly selected rounds, thermocouple leads were inserted into the end of the round opposite the coring tunnel to a location approximately 0.25 inch (6 mm) from the dialysis tubing. All rounds were then inserted into commercial cooking bags and vacuum-packaged with a Model Z-400 Tipper Clipper. Rounds containing thermocouples were packaged with the thermocouple wire exiting the bag at the point where the clip was applied.

Before cooking all rounds were pretempered to approximately 40 F (4.4 C). After pretempering, 38 rounds were placed on racks in a stainless steel cook tank filled with water. All rounds were completely submerged during the experiment. The cook tank was steam-sparged, and the temperature was controlled with a new Trerice controller precalibrated to 165 F (73.8 C). The water was continuously agitated for the duration of the experiment by means of a commercial water pump. All temperatures, including cook water temperature, were monitored with thermocouples. Rounds containing thermocouples were positioned in random locations throughout the cook tank.

Initial temperatures of all thermocouple leads were recorded, two rounds were removed from the cook tank for time "zero" analyses, and the steam supply was activated. Times and temperatures were recorded during the duration of the cooking and cooling time. Duplicate rounds were removed from the cool tank and placed in slush-ice at the following intervals: (*a*) Initial Time 0, (*b*) 120 F (48.8 C) — internal temperature, (*c*) 125 F (51.6 C) — internal temperature, (*d*) 125 F (51.6 C) — 1-h holding time, (*e*) 125 F (51.6 C) — 3-h holding time, (*f*) 125 F (51.6 C) — 5-h holding time, (*g*) 125 F (51.6 C) — 7-h holding time, (*h*) 130 F (54.4 C) — internal temperature, (*i*) 130 F (54.4 C) — 10-min holding time, (*j*) 130 F (54.4 C) — 30-min holding time, (*k*) 130 F (54.4 C) — 60-min holding time, (*l*) 130 F (54.4 C) — 120-min holding time, (*m*) 135 F (57.2 C) — internal temperature, (*n*) 135 F (57.2 C) — 3-min holding time, (*o*) 135 F (57.2 C) — 6-min holding time (*p*) 135 F (57.2 C) — 10-min holding time (*q*) 135 F (57.2 C) — 20-min holding time, (*r*) 140 F (59.9 C) — internal temperature, and (*s*) 145 F (62.7 C) — internal temperature. Rounds which were scheduled for holding at a given temperature were transferred from the cook tank to constant temperature baths at the given holding temperature. Slush-ice was added to the cooling bath on a continuous basis. Since all rounds were not exactly the same size and since it was impossible to determine exact thermocouple location in a given round, pull times were determined by averaging the eight readings from the thermocouples in the rounds.

After rounds had cooled to an internal temperature of approximately 50 F (10 C), the dialysis tubing was removed and the contents analyzed for *Salmonella*. The meat in the dialysis tubing was analyzed employing both methods described previously. At least five dilutions were employed for each sample analyzed by the MPN method. One single 10-g sample per piece of tubing was analyzed for *Salmonella*.

Surface inoculation and sampling — dry roasted beef

Beef rounds used in the dry roasting experiments were surface-inoculated with the suspension of the mixed *Salmonella* cultures described previously. Each round was wrapped with cord as in commercial operations and inoculated by injecting 0.1 ml of the mixed bacterial suspension 1/8 inch below the surface with an intradermal inoculation syringe. Ten different sites, including both fat and lean areas, were inoculated per round.

Sampling of the round surfaces consisted of using a surgical scalpel to cut off the top 1/8 inch of the meat over an area approximately 1 inch square around an inoculated site. Each sample consisted of a portion of round cut from a lean-inoculated area and a fat-inoculated area. All samples in the dry roasting experiments were analyzed for *Salmonella* employing both methods described previously.

Dry roasting experiments were conducted in a commercial kitchen oven (Frigidaire) at 200, 225, 250, and 275 F (93.2, 107.1, 121.0 and 134.9 C). Surface samples were collected immediately before placing the rounds in the oven and after internal temperatures had reached 125, 130 and 135 F (51.6, 54.4 and 57.2 C). All temperatures were monitored with thermocouples.

Mixed steam injected-dry roasting experiments were conducted in a Vortron smokehouse at 175 F (79.4 C). One experiment was conducted with steam injected during the first 30 min of processing. A second experiment was conducted with steam injected during approximately the last 30 min of processing. Steam was injected through the steam vents at the lower back of the smokehouse. In both experiments, surface samples were collected immediately before placing the rounds in the smokehouse and after reaching internal temperatures of 120, 125, 130 and 135 (48.8, 51.6 54.4 and 57.2 C).

A final experiment was designed to determine the effect of size of the beef roast on the time-temperature process necessary to eliminate *Salmonella* from the surface of meat. This experiment employed 10-lb. and 5-lb. portions of beef round and oven temperatures of 250 (121.0 C) and 275 F (134.9 C). Roasts were inoculated, cooked, sampled and analyzed as described previously.

RESULTS AND DISCUSSION

D-values for Salmonella in a ground meat system

Results of D-value experiments done at 125 F (51.6 C) are in Fig. 1. This plot of log survivors versus heating time was obtained from linear regression analysis of the data. The $D_{125\ F\ (51.6\ C)}$ value for *Salmonella* in a ground meat system was found to be 61-62 min.

In similar manner, triplicate experiments were conducted at water bath temperatures of 135 F (57.2 C) and 145 F (62.7 C). Results of these experiments are in Fig. 2 and 3, respectively. The plots obtained from linear regression analysis show a *Salmonella* $D_{135\ F\ (57.2\ C)}$

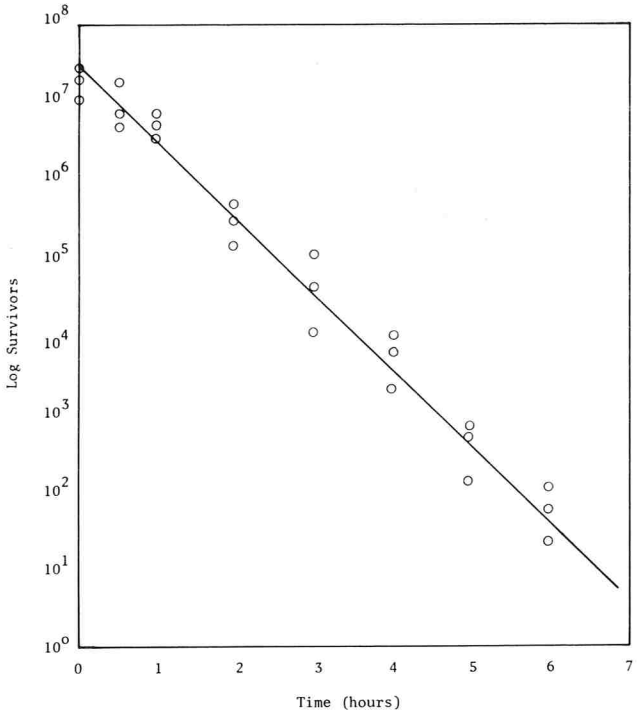

Figure 1. *Thermal death curve of* Salmonella *in ground meat at 125 F (51.6 C).*

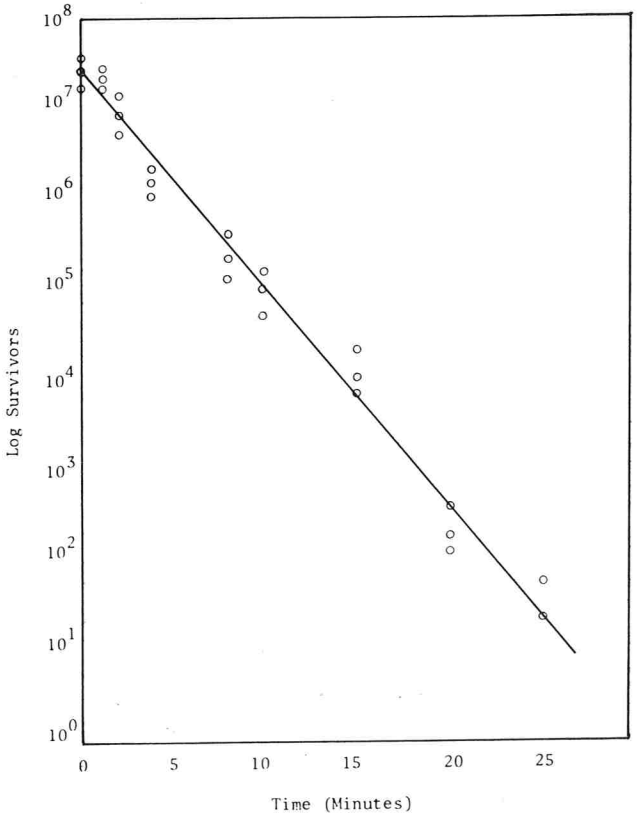

Figure 2. *Thermal death curve of* Salmonella *in ground meat at 135 F (57.2 C).*

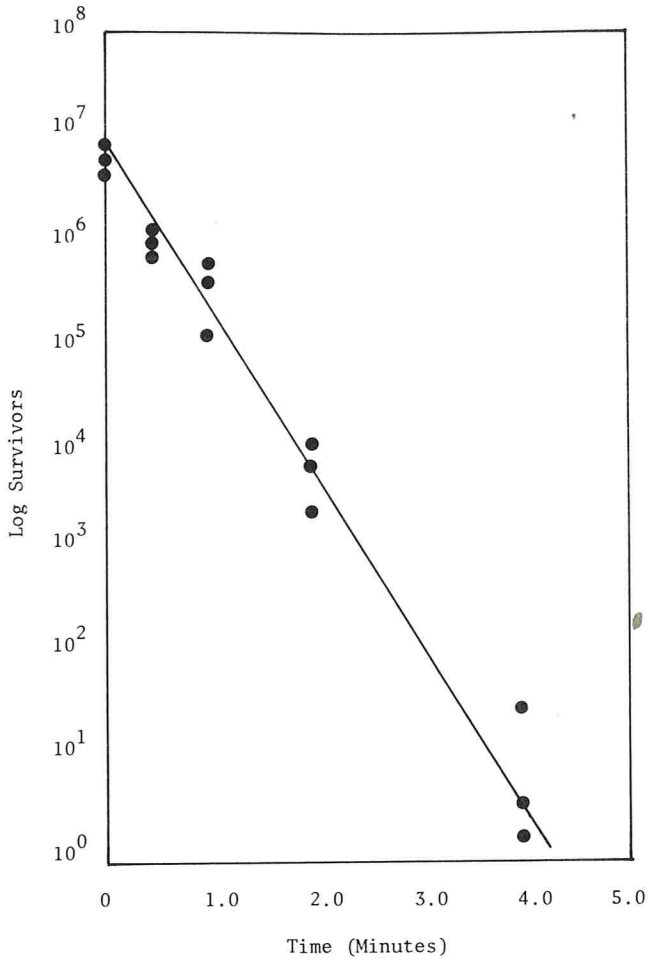

Figure 3. *Thermal death curve of* Salmonella *in ground meat at 145 F (62.7 C).*

value of 3.8-4.2 min and a $D_{145\ F}$ (62.7 C) value of 0.6-0.7 min.

Two composite "thermal death curves" for 1 log and 7 logs of *Salmonella* destruction are in Fig. 4. The z-value of the *Salmonella* serotypes used in this experiment was found to be 10. It was found that the D-values for the *Salmonella* serotypes used in this experiment were significantly higher in ground meat than D-values obtained from experiments with *Salmonella* in broth systems (*7,8,19*).

Fate of Salmonella inoculated into beef for water cooking

After D-values for *Salmonella* in a ground meat system were established employing beef emulsion in small tubes, an experiment was conducted with beef rounds inoculated internally with *Salmonella* and cooked using a commercial procedure. The time-temperature curves for the beef rounds used in this experiment are in Fig. 5. These results demonstrated that beef rounds processed to a given internal temperature under commercial conditions actually attained a final internal temperature of 1 to 3 degrees higher than the temperature at which the product was removed from the cook tanks. This was true even for rounds placed directly into slush ice. It was

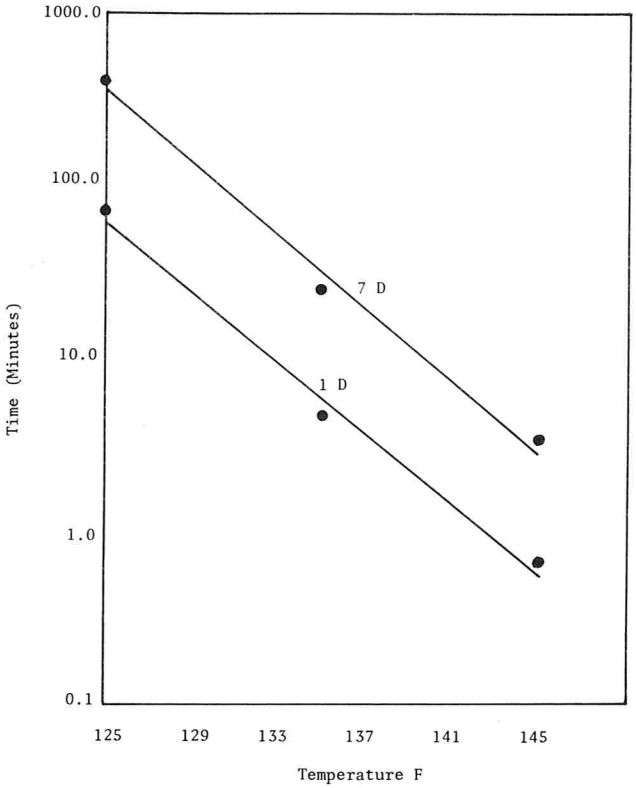

Figure 4. *Thermal death curves for* Salmonella *in ground meat.*

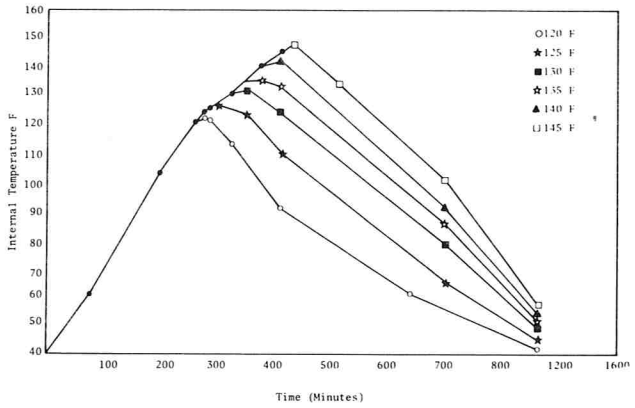

Figure 5. *Heat-cooling curves for water cooked beef.*

also noted that the internal temperature of a 16-18-lb. beef round removed from the cook tank at a given temperature was equal to or above that temperature for a minimum of 30 min.

Analytical results for the presence of *Salmonella* are in Table 1. As would be expected, the MPN method employing a larger sample size and preenrichment techniques was more sensitive than the plate count method for detecting small numbers of *Salmonella*.

Results of this experiment indicated that 125 F (51.6 C) was a marginal processing temperature for beef rounds since very low levels of survivors were detected after 7 h of holding at this temperature. However, slightly longer processing times would undoubtedly have eliminated the very low level of survivors detected. A final processing temperature of 130 F (54.4 C) resulted in no detectable *Salmonella* survivors if the rounds were held at this internal temperature for a time interval between 30 and 60 min. Rounds processed to an internal temperature of 135 F (57.2 C) contained no detectable *Salmonella* if they were held for a minimum of 3 min at this temperature. Rounds processed to final internal temperatures above 135 F (57.2 C) were all negative for *Salmonella*.

Results of this experiment demonstrated that the commercial practice of water-cooking beef was capable of reducing 10 million inoculated *Salmonella* to undetectable levels at a variety of temperatures lower than 145 F. In actual practice, commercial water or steam cooking of beef rounds to a given internal temperature in the 125 F (51.6 C) to 145 F (62.8 C) range resulted in more rapid death of *Salmonella* at that internal

TABLE 1. *Effect of time-temperature on* Salmonella *destruction in water-cooked beef.*

Sampling intervals	Aerobic plate count/g	MPN *Salmonella*/g	Sampling intervals	Aerobic plate count/g	MPN *Salmonella*/g
Initial	10,000,000	11,000,000	130F - 60 min	< 10	< 0.3* *
Initial	14,000,000	> 24,000,000	130F - 60 min	< 10	< 0.3* *
120 F Internal	10.000,000	11,000,000	130F - 120 min	< 10	< 0.3* *
120 F Internal	7,100,000	11,000,000	130F - 120 min	< 10	< 0.3* *
125 F Internal	11,000	24,000	135F Internal	< 10	0.4
125 F Internal	31,000	24,000	135F Internal	< 10	< 0.3* *
125 F - 1 h	600	11.0	135F - 3 min	< 10	< 0.3* *
125 F - 1 h	100	23.0	135F - 3 min	< 10	< 0.3* *
125 F - 3 h	100	4.0	135F - 6 min	< 10	< 0.3* *
125 F - 3 h	80	7.0	135F - 6 min	< 10	< 0.3* *
125 F - 5 h	< 10	1.0	135F - 10 min	< 10	< 0.3* *
125 F - 5 h	< 10	< 0.3* *	135F - 10 min	< 10	< 0.3* *
125 F - 7 h	< 10	< 0.3* *	135F - 20 min	< 10	< 0.3* *
125 F - 7 h	< 10	0.3*	135F - 20 min	< 10	< 0.3* *
130 F Internal	1,600	2,100	140F Internal	< 10	< 0.3* *
130 F Internal	6,000	4,600	140F Internal	< 10	< 0.3* *
130 F - 10 min	< 10	15.0	145F Internal	< 10	< 0.3* *
130 F - 10 min	< 10	30.0	145F Internal		
130 F - 30 min	< 10	0.3*			
130 F - 30 min	< 10	< 0.3* *			

* Positive 10 g.
* * Negative 10 g.

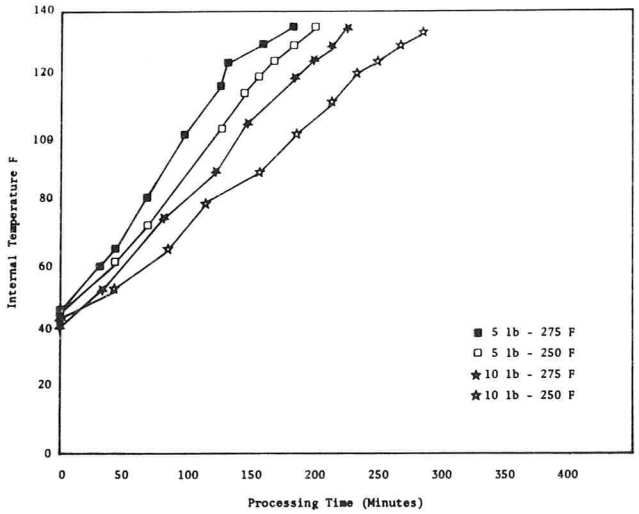

Figure 8. *Time-temperature curves for five and ten pound beef rounds dry roasted at 250 and 275 F (121 and 134.9 C).*

TABLE 5. *Processing times required for a 7-D kill of* Salmonella *in cooked beef at various temperatures.*

Internal Temperature, F	Processing time - Min[a]
128	195
129	153
130	121
131	97
132	77
133	62
134	47
135	37
136	32
137	24
138	19
139	15
140	12
141	10
142	8
143	6
144	5

[a]D-values employed in calculating processing times were taken from regression line curves provided by the USDA Statistical Division based on submitted data.

was established. The various values established from D-value experiments are in Table 5. Experimental results found in the water-cooked beef portion of this study demonstrated that the values given in Table 5 would definitely eliminate 10^7 *Salmonella* from the center of a beef round. The processing values in the table would also apply to the internal portion of dry roasted beef. However, it was found that elimination of *Salmonella* from the surface of dry roasted product could

TABLE 4. *Effect of time-temperature and product size on* Salmonella *destruction at the surface of dry oven roasted beef*

Sampling interval	Product size (lb.)	Oven temperature, F	Total cooking time	Aerobic plate count/g	MPN *Salmonella*/g
Initial	5	—	—	8,600,000	> 11,000,000
Initial	5	—	—	5,000,000	> 11,000,000
Initial	5	—	—	1,300,000	> 11,000,000
Initial	5	—	—	9,300,000	> 11,000,000
Initial	10	—	—	3,500,000	4,600,000
Initial	10	—	—	2,600,000	> 11,000,000
Initial	10	—	—	5,900,000	4,600,000
Initial	10	—	—	7,200,000	> 11,000,000
120 F Internal	5	250	2 h 35 min	17,000	> 24,000
120 F Internal	5	250	2 h 35 min	13,000	> 24,000
120 F Internal	5	275	2 h 10 min	4,600	11,000
120 F Internal	5	275	2 h 10 min	2,000	4,600
120 F Internal	10	250	3 h 53 min	30	110
120 F Internal	10	250	3 h 53 min	< 10	70
120 F Internal	10	275	3 h 6 min	< 10	75
120 F Internal	10	275	3 h 6 min	< 10	9.0
125 F Internal	5	250	2 h 50 min	2,500	> 240.0
125 F Internal	5	250	2 h 50 min	700	> 240.0
125 F Internal	5	275	2 h 15 min	60	240.0
125 F Internal	5	275	2 h 15 min	110	1,100.0
125 F Internal	10	250	4 h 10 min	< 10	2.4
125 F Internal	10	250	4 h 10 min	< 10	< 0.03
125 F Internal	10	275	3 h 23 min	< 10	0.11
125 F Internal	10	275	3 h 23 min	< 10	< 0.03
130 F Internal	5	250	3 h 5 min	140	> 240.0
130 F Internal	5	250	3 h 5 min	< 10	7.5
130 F Internal	5	275	2 h 40 min	< 10	24.0
130 F Internal	5	275	2 h 40 min	90	46.0
130 F Internal	10	250	4 h 29 min	< 10	< 0.03
130 F Internal	10	250	4 h 29 min	< 10	< 0.03
130 F Internal	10	275	3 h 33 min	< 10	< 0.03
130 F Internal	10	275	3 h 33 min	< 10	< 0.03
135 F Internal	5	250	3 h 23 min	190	> 240.0
135 F Internal	5	250	3 h 23 min	20	24.0
135 F Internal	5	275	3 h 5 min	20	4.6
135 F Internal	5	275	3 h 5 min	30	> 24.0
135 F Internal	10	250	4 h 48 min	< 10	< 0.03
135 F Internal	10	250	4 h 48 min	< 10	< 0.03
135 F Internal	10	275	3 h 47 min	< 10	< 0.03
135 F Internal	10	275	3 h 47 min	< 10	< 0.03

be accomplished only if the product was at least 10 lb. in size and was cooked to a minimum internal temperature of 130 F (54.4 C) in an oven set at 250 F (121.0 C) or above. An alternative procedure developed for dry roasting beef products consisted of cooking product in a high humidity chamber or injecting steam for a minimum of 30 min during the cooking process.

In summary, the color, or degree of doneness, of roast beef is directly related to the maximum internal temperature attained in the product. Destruction of microorganisms, including the *Salmonella,* by heat is a time and temperature phenomenon. This study has established manufacturing processes that have internal temperatures sufficiently low to maintain the characteristic red color of "rare" roast beef but with sufficient processing time to insure the destruction of as many as ten million *Salmonella*/g. Any of the processes developed for temperatures less than 136 F would produce true "rare" roast beef. Processes have been developed for both water (steam) cooked beef and dry (oven) roasted beef.

The data included in this study have been accepted by the USDA and will be the basis of a future expansion of the regulation governing the cooking of roast beef.

ACKNOWLEDGMENTS

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REFERENCES

1. Angelotti, R. 1977. Cooking requirements for cooked roast beef. Fed. Reg. 42(171):44217.
2. Angelotti, R., M. J. Foter, and K. H. Lewis. 1961. Time-temperature effect on salmonellae and staphylococci in foods. Appl. Microbiol. 9:308-315.
3. Angelotti, R., M. J. Foter, and K. H. Lewis. 1961. Time-temperature effects on salmonellae in foods. II. Behavior at warm holding temperatures. Am. J. Public Health 51:76-88.
4. Baird-Parker, A. C., M. Boothroyd, and E. Jones. 1970. The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of salmonellae. J. Appl. Bacteriol. 33:515-522.
5. Bayne, H. G., J. A. Garibaldi, and H. Lineweaver. 1965. Heat resistance of *Salmonella typhimurium* and *Salmonella senftenberg* 775W in chicken meat. Poultry Sci. 44:1281-1284.
6. Brown, D. F., and R. M. Twedt. 1972. Assessment of the sanitary effectiveness of holding temperatures on beef cooked at low temperature. Appl. Microbiol. 24:599-603.
7. Bryan, F. L., J. C. Ayres, and A. A. Kraft. 1968. Destruction of salmonellae and indicator organisms during thermal processing of turkey rolls. Poultry Sci. 47:1966-1978.
8. Castellani, A. G., R. R. Clarke, M. I. Gibson, and D. F. Meisner. 1952. Roasting time and temperature required to kill food poisoning microorganisms introduced experimentally into stuffing in turkeys. Food Res. 18:131-138.
9. Checko, P. J., J. N. Lewis, R. Altman, K. Black, H. Rosenfeld, and W. Parkin. 1976. *Salmonella bovis-morbificans* in precooked roasts of beef. Morbidity Mortality Weekly Rep. 25(42):333.
10. Checko, P. J., J. N. Lewis, R. Altman, G. Halpin, R. Inglis, M. Pierce, K. Pilot, J. Prince, W. Rednor, M. Fleissner, D. O. Lyman, and W. E. Parkin. 1977. Multi-state outbreak of *Salmonella newport* transmitted by precooked roasts of beef. Morbidity Mortality Weekly Rep. 26(34):277-278.
11. Daoud, A. Z. 1977. Cooking requirements for roast beef.(Eckrich Food Service Division — Private Report).
12. Davidson, C. M., and J. K. Witty. 1977. Fate of salmonellae in vacuum-packaged ground meat during storage and subsequent cooking. J. Inst. Can. Sci. Technol. Aliment. 10:223-225.
13. Dega, C. A., J. M. Goepfert, and C. H. Amundson. 1972. Heat resistance of salmonellae in concentrated milk. Appl. Microbiol. 23:415-426.
14. Goepfert, J. M., and K. C. Chung. 1970. Behavior of salmonellae during the manufacture of a fermented sausage product. J. Milk Food Technol. 33:185-191.
15. Jordan, D., R. Martini, D. McClosky, J. Surowiec, P. Capparelli, D. J. Grun, R. Altman, C. Coleman, R. Dennis, M. Goldfield, K. Pilot, W. Rednor, H. Rosenfeld, A. Stemhagen, O. Sussman, and F. Timko. 1976. *Salmonella saint-paul* in pre-cooked roasts of beef. Morbidity Mortality Weekly Rep. 25:34-39.
16. Lee, A. C., and J. M. Goepfert. 1975. Influence of selected solutes on thermally introduced death and injury of *Salmonella typhimurium.* J. Milk Food Technol. 38:195-200.
17. Masters, B. A., S. J. Goodfellow, J. Bacus, W. L. Brown, and J. Oblinger. 1977. Fate of salmonellae in commercially processed Wisconsin summer sausage (in preparation).
18. Mayer, L., B. Freeman, J. C. Watson, and C. Carraway. 1975. *Salmonella singapore* — New Orleans. Morbidity Mortality Weekly Rep. 24(47):397-398.
19. Ng, H., H. G. Bayne, and J. A. Garibaldi. 1969. Heat resistance of salmonella: The uniqueness of *Salmonella seftenberg* 775W. Appl. Microbiol. 17:78-82.
20. Shoup, J. G., and J. L. Oblinger. 1976. Microbiological evaluation of retail ground beef: Centralized and traditional preparation. J. Milk Food Technol. 39:179-183.
21. Smith, J. L., S. A. Palumbo, J. C. Kissinger, and C. N. Huhtanen. 1975. Survival of *Salmonella dublin* and *Salmonella typhimurium* in Lebanon bologna. J. Milk Food Technol. 38:150-154.
22. Vermilyea, B. L., C. Dzuik, and H. Krull. 1977. Salmonella lethality study in rare cooked beef roasts. (Reuben Packing Co., — private report).
23. Wilkinson, R. J., W. L. Mallmann, L. E. Dawson, T. F. Irmiter, and J. A. Davidson. 1964. Effective heat processing for the destruction of pathogenic bacteria in turkey rolls. Poultry Sci. 44:131-136.
24. Willardson, R. R., F. F. Busta, and C. E. Allen. 1977. Dialysis technique for containment of microbial populations inoculated into food systems. Appl. Environ. Microbiol. 34:240-241.

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Microbial Persistence on Inoculated Beef Plates Sprayed with Hypochlorite Solutions

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ABSTRACT

Exterior surfaces of beef plates were inoculated with aerobic mesophilic, psychrotrophic, coliform, fecal coliform and *Staphylococcus aureus* bacteria before spraying with city water (7.03 kg/cm²) or with 100, 150 or 200 ppm hypochlorite solutions applied at 3.75, 5.25 or 7.03 kg/cm² for 12 sec. Surface strips excised from the inoculated carcass regions were analyzed for bacterial persistence at 2 h and 2,4,8,16 and 20 days after spray treatment. A one log, or greater, reduction in coliforms, fecal coliforms and *S. aureus* counts occurred within 4 days for each spray treatment. Initial aerobic mesophilic and psychrotrophic counts were reduced by < 1 log within 2 h but increased to original inoculum levels within 8 days for each treatment. Mesophilic and psychrotrophic counts increased to > 3.5 logs higher than inoculated levels after 16 days at 4-6 C. Spray pressure ($P < .01$) was more effective in reducing mesophilic, psychrotrophic and *S. aureus* counts than was hypochlorite concentration ($P < .05$) while both variables were about equally effective against coliforms ($P < .01$) and fecal coliforms ($P < .05$). Surprisingly, reductions of psychrotrophic, coliform and *S. aureus* counts on the beef carcass surface sprayed with hypochlorite solutions were not significantly ($P < .05$) different from those observed on carcasses sprayed with city water. However, a significantly ($P < .05$) greater reduction in aerobic mesophile and fecal coliform counts at 2 days after treatment was obtained when using the intermediate spray pressure of 5.25 kg/cm² containing 200 ppm hypochlorite, rather than city water. No undesirable change in beef grade, muscle shear or color property was observed for carcasses treated with the hypochlorite solutions.

Microbial counts exceeding one million/cm² on beef carcasses received at a central breaking plant have been reported by Emswiler et al. (*7*). Subsequent processing of beef carcasses with high microbial counts may shorten the shelf life of processed meats and retailed cuts due to development of slime and off-odors (*3,4,15*).

Kotula (*12*) reviewed the use of several techniques to reduce the microbial loading on animal carcasses. One method, aqueous hypochlorite spray, has been reported to reduce the bacterial contamination on surfaces of beef carcasses by greater than 3 log counts/cm² (*14*) while other researchers (*16*) reported reductions of only 0.44 to 1.18 log counts/cm². Such disagreements are understandable considering the number of variables reported to influence the effectiveness of hypochlorite sprays: hypoclorite strength (*5, 7, 13, 17*); chemical source of hypochlorite (*7, 9, 16*); temperature (*11, 13, 14, 18*); time of exposure (*1, 5, 8, 9, 16*); spray pattern (*5*); pressure (*1, 14, 16*); and solution pH (*9, 14, 19*). Of these variables, most sources agree that hypochlorite sprays are the most effective in reducing microbial counts when applied at pH 6-7, but more often they disagree on the optimum combinations of other variables.

Variability in results could also be attributed to differences in experimental procedures. For example, a given conclusion about the effectiveness of a particular hypochlorite spray combination may only be valid for the type of microorgansim(s) being monitored, and it is not unreasonable to expect a particular set of spray conditions to have a greater effect upon one group of bacteria, yeast or mold than another. Secondly, considerable variation in the reported effectiveness of hypochlorite sprays may be attributed to the various carcass sampling methods used to estimate microbial persistence. A discussion of the relative merits of sampling by excision, scraping, swabbing, and adhesion and washing techniques has been presented by Ingram and Roberts (*10*). Of these sampling methods, Marshall et al. (*16*) recommended use of excision rather than surface swabbing to best quantitate bacterial survivors on carcasses at 48 h after hypochlorite spraying. Finally, Kotula et al (14) indicated that it becomes difficult to judge the full effectiveness of chlorine spray solutions on carcasses having initially low surface bacterial numbers because treatment "overkill" effects reduced counts to levels too low to enumerate accurately.

This study was conducted to (*a*) compare the effectiveness of city water and eight pressure × aqueous hypochlorite concentration combinations to reduce the bacterial numbers on beef carcass surfaces inoculated with aerobic mesophiles, psychrotrophs, coliforms, fecal coliforms and *Staphylococcus aureus* by periodically excising strips from the carcasses during a 20-day period and (*b*) check carcass grade, muscle shear and color

properties for undesirable changes that could affect the marketability of beef carcasses that had been treated with aqueous hypochlorite.

EXPERIMENTAL

Carcasses

Ten U.S. choice grade forequarters were randomly selected from a 64-head lot of quartered beef cattle received at a central breaking plant 5 days post-slaughter. Each coded forequarter was separated into corresponding rib eye and plate sections. Approximately 4-cm thick lean muscle slices from the shoulder-rib interface were removed for color and shear analysis before spray treatments. The plate surfaces to be inoculated with bacteria were aseptically circumscribed by a 25.4 × 35.56 cm rectangle having an area of 903 cm². All the forequarter sections were received and maintained at 4-6 C during handling, preparation and storage after hypochlorite spray treatment.

Inoculation

Cultures of bacteria previously isolated from beef carcasses as received at the plant were separately grown in trypticase soy broth at 30 C/48 h (aerobic mesophiles), 7 C/72 h (psychrotrophs) and 37 C/48 h (coliforms, fecal coliforms and *S. aureus*). These cultures were combined to give 150 ml of mixed culture inoculum to provide 1.2×10^8 to 2.1×10^8 counts/ml of each organism. Ten ml of this mixed inoculum were applied to 903 cm² of the exterior surface of each beef plate using sterile brushes. The plates were laid horizontally during inoculation and the surface then gently dried by flushing with air from a hand held hair dryer. All the inoculated plates were maintained at 4-6 C until they were spray treated within 3 h.

Treatments

Each inoculated beef plate was randomly assigned to the spray treatment combinations shown in Table 1. Hypochlorite spray treatments were applied to inoculated plates hung in a vertical position on hooks as they were mechanically conveyed through the spray cabinet having a bank of 14 spray nozzles arranged on each side as shown in Fig. 1. Each nozzle delivered 29.1 ml/sec at 3.75 kg/cm², 36.9 ml/sec at 5.25 kg/cm² and 41.7 ml/sec at 7.03 kg/cm². The nozzles were located an average of 40 cm from the beef plate surface and delivered spray patterns approximately 30 cm high by 15 cm wide to the carcass surface as the carcass traveled at 24.8 cm/sec through the tunnel. Total beef plate residence time in the spray cabinet was 12 sec.

The treated beef plates and prime rib sections were permitted to drip dry 10 min before moving to a 5-C and 88 ± 2% humidity holding room.

Sampling

At each sampling time, two strips 2.54-cm wide × 12.7-cm long × approximately 0.5 cm-thick were aseptically excised at random from the inoculated region of each beef plate surface according to the procedure of Thomas (*20*). Duplicate samples were excised from the inoculated carcasses immediately before, 2 h after and at 2, 4, 8, 16 and 20 days after the hypochlorite or non-sprayed control treatments. Each strip was placed into separate sterile plastic bags and immediately transported on ice to the laboratory for bacterial analysis within 3 h.

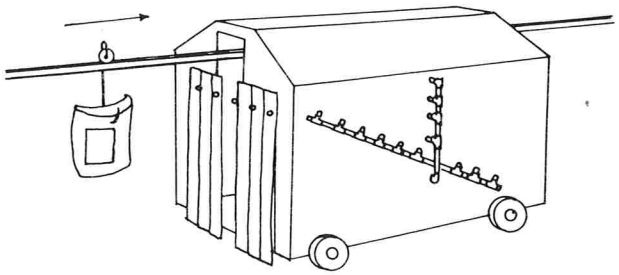

Figure 1. *Cabinet tunnel used to spray beef carcasses with aqueous hypochlorous acid (length 2.97 m; width 1.04 m; side wall height 2.03 m).*

Bacteriological

At the laboratory 100 ml of sterile 0.85% NaCl were added to each sample strip bag, the bag was kneaded 5 min and decimal serial dilutions were made in saline. One-ml aliquots of the serial diutions were pour-plated in duplicate; or three 0.5-ml amounts of each dilution were pipetted into each of three tubes of broth (4.5 ml) to determine the most probable number (MPN) of the bacterial groups as summarized in Table 2.

TABLE 2. *Enumeration procedures used to determine bacterial counts on surfaces of beef plates.*

Bacterial group	Medium and method reference[a]	Temperature and incubation time	Enumeration procedure
Aerobic mesophiles	Standard Plate Count agar	30 C 72 h	CFU[b]
Psychrotrophs	Standard Plate Count agar	7 C 7 day	CFU
Colforms	Lauryl Sulfate Tryptose broth and Brilliant Green Lactose Bile (2%) broth	35 C 48 h	MPN[c]
Fecal coliforms	EC broth and Levine Eosin Methylene Blue agar	35 C 48 h	MPN
S. aureus	Trypticase Soy broth +10% NaCl and Baird-Parker agar	35 C 48 h	MPN

[a]See reference (*2*), Anonymous, 1972.
[b]Colony forming units.
[c]Most Probable Number, 3 tube.

Bacteria counts obtained from the strips taken after spray treatment minus the inoculated counts from strips taken before spray treatment provided the log mean count differences (log 10 per 6.45 cm²) for Analysis of Variance and Duncan's Multiple Range Test (*6*) mean comparisons.

Carcass evaluation

Portions of the prime rib eye sections (5-6th rib) corresponding to each plate were examined for color values before and at 20 days after spray treatment. Color determinations were conducted with a Garner Color Difference Meter, Model C4 (Gardner Laboratory, Inc., Bethesda MD) which was standardized with a pink standard plate (No. CG-6632;

TABLE 1. *Combined hypochlorite concentrations and pressure spray treatments randomly assigned to inoculated beef plates and corresponding prime rib section.*

Treatment combination	Treatment code									
	A[a]	B[b]	C	D	E	F	G	H	I	J
Hypochlorite (ppm)[c]	0	<5	100	100	150	150	150	200	200	200
Pressure (kg/cm²)[d]	0	7.03	5.23	7.03	3.75	5.25	7.03	3.75	5.25	7.03

[a]Control, no spray treatment.
[b]City water at 16 C.
[c]At 16 C and adjusted to pH 6.0-6.5 with acetic acid; electrically generated from NaCl brine (Quad-Chlor Model T-200, Quad Corp., Highland Park, Ill).
[d]At nozzle, No. 4 1/2, SS 1104 (Spraying Systems, Co., Wheaton, Ill.).

$L = 52.9$, $a_L = 31.9$, $b_L = 11.4$). Freshly cut surfaces of the fat and lean muscle surfaces and a minced muscle sample were prepared for measurement. Results were expressed in terms of L, a_L and b_L values.

Shear resistance of rib eye lean muscle cubes having a surface area of 16.43 cm² was measured with a Food Texture Press (FTC) equipped with a 3000 lb texture gauge. The press was operated at a downstroke of 30 sec and range 1000. Cubes were approximately 2.2 cm in thickness. The shearing force was calculated as kg/g/cm² of surface area exposed to the shear blades.

The exposed loin eye muscle (12th rib) of each section was visually scored for marbling, grade, fat color, lean and fat firmness immediately before and 20 days after spray treatment.

RESULTS AND DISCUSSION

Aerobic Mesophiles

An Analysis of Variance applied to the mean squares of $\log_{10}$ differences between inoculated counts and aerobic mesophilic counts remaining on beef plates after spray treatments (Table 1) showed survival was significantly ($P < 0.01$) affected by the time after treatment and hypochlorite concentration × pressure interaction (Table 3). Within the hypochlorite concentration and spray pressure interaction variables, spray pressure was found to be the more significant ($P < .05$) variable while hypochlorite concentration was indicated to impart some, but a less significant ($P < .10$), effect upon the number of surviving aerobic mesophiles. However, statistical comparison of the counts of survivors at each of the six sampling intervals showed that spray pressure was the only treatment producing a significant ($P < .01$) reduction 2 days after treatment (Table 9).

Changes in the mean counts ($\log_{10}$) of aerobic mesophiles that occurred at each examination time for each spray treatment are presented in Table 4. Duncan's Multiple Range Mean test showed none of the eight hypochlorite and spray pressure treatments were more effective than any other, including the non-spray control, at 2 h after treatment, even though all the inoculated beef plates showed some reduction (< 1 log) in aerobic mesophilic numbers. But, at 2 days after treatment a significant ($P < .05$) reduction of 1.69 logs in the number of aerobic mesophiles was observed for treatment I (200 ppm hypochlorite at 5.25 kg/cm²), which was substantially better than the reductions of counts on non-sprayed or city water sprayed beef plates. Within 4 days after treatment the non-sprayed and city water treated beef plate counts had increased to greater than their original inoculated numbers as did plates receiving hypochlorite treatments D, F, G, H and J. By 8 days, post treatment growth of aerobic mesophiles on all 10 beef plates produced surface counts that had exceeded original inoculated levels and at 16 days there were no significant ($P < .05$) differences among treatments. In general, the lowest mean counts of aerobic mesophiles were usually associated with beef plates that had been treated with 200 ppm hypochlorite applied at 5.25 or

TABLE 3. *Analysis of variance of hypochlorite spray treatment combinations applied to inoculated beef plates.*

Source	df	Mean squares[a]				
		Aerobic mesophiles	Psychrotrophs	Coliforms	Fecal coliforms	*S. aureus*
		($\log_{10}$)				
Total	119					
HOCL conc. (A)	4	1.68[b]	0.95	3.78[d]	1.58[c]	0.84[c]
Pressure (B)	2	2.63[c]	2.09[c]	7.48[d]	2.16[c]	3.85[d]
Time (C)	5	120.65[d]	129.20[d]	6.83[d]	5.58[d]	5.84[d]
A × B	3	4.89[d]	4.27[d]	0.98	3.28[d]	1.05[b]
A × C	20	0.30	0.42	0.44	0.69	0.24
B × C	10	0.85	0.85	0.70	1.06[c]	0.34
A × B × C	15	0.81	0.75	0.54	0.63	0.41
Error	60	0.77	0.66	0.39	0.47	0.32

[a]Per 6.45 cm² calculated as the difference between bacterial counts measured after treatment less inoculated counts measured before treatments.
[b]($P < .10$)
[c]($P < .05$)
[d]($P < .01$)

TABLE 4. *Log mean changes[a] in aerobic mesophilic counts[b] on sample strip surfaces of inoculated beef plates 2 h to 20 days after hypochlorite spray treatments.*

Time	Treatments[c]										Order[d] of means
	A	B	C	D	E	F	G	H	I	J	
2 h	−0.25[e]	−0.02[e]	−0.66[e]	−0.35[e]	−0.34[e]	−0.89[e]	−0.79[e]	−0.39[e]	−0.62[e]	−0.81[e]	BAEDHICGJF
2 day	−0.12[e]	−0.04[e]	−0.62[e]	0.16[e]	−0.21[e]	−0.71[e]	0.20[ef]	0.47[e]	−1.69[e]	−0.37[e]	HGDBAEJCFI
4 day	0.86[e]	0.75[e]	1.36[e]	1.85[f]	−0.24[e]	1.45[f]	0.56[eg]	0.61[e]	−0.49[ef]	0.27[e]	DFABHGJEIC
8 day	3.31[f]	2.31[f]	2.02[f]	3.89[g]	1.61[e]	3.01[g]	3.03[ef]	3.05[f]	1.24[f]	1.51[ef]	DAHGFBCEJI
16 day	4.34[f]	5.30[g]	4.73[g]	5.57[h]	5.10[g]	5.53[h]	3.80[f]	5.10[g]	4.26[g]	4.38[f]	DFBEHCJAIG
20 day	4.73[f]	5.14[g]	5.08[g]	5.76[h]	5.51[f]	5.40[h]	4.01[f]	4.84[g]	4.12[g]	3.64[f]	DEFBCHAIGJ

[a-b]Per 6.45 cm² calculated as the measured $\log_{10}$ count at each sampling time less the measured inoculated $\log_{10}$ count before spray treatment.
[c]see Table 1.
[d]Any 2 treatments in the same row underscored by a common line did not differ significantly ($P < .05$).
[e-h]Any 2 means in the same column bearing the same superscript did not differ significantly ($P < .05$).

7.03 kg/cm², treatments I and J, respectively.

Psychrotrophs

As observed for aerobic mesophiles, the time lapse after spraying, spray pressure and hypochlorite concentration × spray pressure interaction were the more important ($P < .05$) variables contributing to psychrotrophic count reduction, Table 3. But unlike that for the mesophiles, hypochlorite strength up to 200 ppm was not significant ($P < .10$).

Though not significantly ($P < .05$) more effective in controlling psychrotrophic numbers than non-spraying or city water spraying, treatment C, 100 ppm hypochlorite applied at 5.25 kg/cm², generally produced the greatest reduction of psychrotrophic mean counts through 4 days while the plate surface of treatment J (200 ppm hypochlorite @ 7.03 kg/cm²) had the lowest psychrotrophic count at 20 days of storage (Table 5).

Coliforms

The number of coliform bacteria surviving on the surface of spray-treated beef plates was found to be significantly ($P < .01$) affected by the hypochlorite concentration, spray pressure and lapse time after spraying, but not ($P < .10$) by any of the interactions between these variables (Table 3).

Though none of the coliform counts were significantly different ($P < .05$), it should be pointed out in Table 6 that at 2 h all nine of the inoculated and spray-treated beef plates exhibited some reduction in coliform counts while the non-sprayed control, treatment A, demonstrated a slight increase in coliform counts. By 4 days the coliform counts on all the sprayed beef plates had decreased 1-2 logs more than the non-sprayed plate. Continued reduction in the non-sprayed plate coliform numbers through 20 days may be attributed to the inability of this bacterial group to compete with the other microflora and maintain its numbers at the 4-6 C storage temperature.

At 2 and 4 days after spraying, the greatest reduction in coliform counts was provided by treatment I (200 ppm hypochlorite at 5.25 kg/cm²) and then by treament C (100 ppm hypochlorite at 5.25 kg/cm²) at 8, 16 and 20 days. However, at no time were the reductions in mean coliform counts for treatments I and C significantly ($P < .05$) different than those observed for the beef plate in spray treatment B, city water at 7.03 kg/cm² (Table 6).

Fecal Coliforms

Table 3 indicates that time after spray treatment and the interaction of hypochlorite concentration × spray pressure significantly ($P < .01$) affected the detectable counts of fecal coliforms surviving on the surface of treated beef plates. To a lesser degree ($P < .05$), hypochlorite concentration and spray pressure (by themselves) and the interaction of spray pressure × time after spraying also affected the survival of fecal coliforms. In any event, no more than about a 1.2-log reduction in fecal coliform numbers was observed after

TABLE 5. *Log mean changes[a] in psychrotrophic counts[b] on sample strips from the surfaces of inoculated beef plates 2 h to 20 days after hypochlorite spray treatments.*

Time	Treatments[c] A	B	C	D	E	F	G	H	I	J	Order[d] of means
2 h	0.08^{e}	−0.13^{e}	−0.62^{e}	0.04^{e}	−0.21^{e}	−0.50^{e}	−0.31^{e}	−0.65^{e}	0.29^{e}	−0.14^{e}	AIDBJEGFCH
2 day	−0.21^{e}	0.02^{e}	−0.97^{e}	0.50^{e}	0.09^{ef}	−0.53^{e}	0.48^{ef}	0.47^{ef}	−0.87^{e}	0.15^{e}	DGHJEBAFIC
4 day	1.32^{e}	1.27^{f}	−0.67^{e}	2.69^{f}	0.07^{ef}	1.78^{f}	1.23^{ef}	1.18^{f}	0.21^{e}	0.81^{e}	DFABGHJIEC
8 day	4.29^{f}	3.15^{g}	2.42^{f}	4.64^{g}	1.96^{f}	2.95^{f}	3.43^{efg}	3.24^{g}	2.13^{f}	2.25^{ef}	DAGHBFCJIE
16 day	4.82^{f}	5.79^{h}	5.11^{g}	6.20^{h}	5.49^{g}	5.91^{g}	4.31^{fg}	5.13^{h}	5.27^{g}	5.03^{f}	DFBEIHCJAG
20 day	5.10^{f}	5.40^{h}	5.48^{g}	6.28^{h}	5.88^{g}	5.71^{g}	4.90^{g}	4.95^{h}	5.08^{g}	4.33^{f}	DEFCBAIHGJ

[a-b]Per 6.45 cm² calculated as the measured $\log_{10}$ count at each sampling time less the measured inoculated $\log_{10}$ count before spray treatment.
[c]See Table 1.
[d]Any 2 treatments in the same row underscored by a common line did not differ significantly ($P < .05$).
[e-h]Any 2 means in the same column bearing the same superscript did not differ significantly ($P < .05$).

TABLE 6. *Log mean changes[a] in coliform counts[b] on the surface of strip samples from inoculated beef plates over 2 h to 20 days after hypochlorite spray treatments.*

Time	Treatments[c] A	B	C	D	E	F	G	H	I	J	Order[d] of means
2 h	0.11^{e}	−0.78^{e}	−1.58^{e}	−0.43^{e}	−0.71^{e}	−0.76^{e}	−0.38^{e}	−0.67^{e}	−0.56^{e}	−0.24^{e}	AJGDIHEFBC
2 day	−0.26^{eg}	−1.58^{ef}	−1.70^{ef}	−0.16^{e}	−1.11^{e}	−1.76^{ef}	−1.38^{e}	−0.45^{e}	−2.10^{f}	−0.57^{e}	DAHJEGBCFI
4 day	−0.16^{eg}	−1.90^{f}	−2.35^{efg}	−1.14^{c}	−2.34^{e}	−2.56^{f}	−1.32^{e}	−2.44^{f}	−2.58^{f}	−1.30^{ef}	ADJGBECHFI
8 day	−0.56^{fg}	−2.60^{f}	−3.08^{fg}	−2.50^{e}	−2.52^{e}	−2.26^{f}	−0.88^{e}	−2.37^{f}	−2.30^{f}	−2.41^{f}	AGFIHJDEBC
16 day	−0.99^{fh}	−2.16^{f}	−2.67^{g}	−1.49^{e}	−2.44^{e}	−2.08^{f}	−1.61^{e}	−1.93^{f}	−1.09^{e}	−1.31^{ef}	AIJDGHFBEC
20 day	−1.31^{h}	−2.49^{f}	−3.04^{g}	−0.08^{e}	−0.76^{e}	−2.97^{f}	−1.96^{e}	−2.37^{f}	−2.10^{f}	−2.02^{f}	DEAGJIHBFC

[a-b]Per 6.45 cm² calculated as the measured $\log_{10}$ count at each sampling time less the measured inoculated $\log_{10}$ count before spray treatment.
[c]See Table 1.
[d]Any 2 treatments in the same row underscored by a common line did not differ significantly ($P < .05$).
[e-h]Any 2 means in the same column bearing the same superscript did not differ significantly ($P < .05$).

2 h for any of the spray treatments (Table 7). At 2 days after spraying the two highest pressures, 5.25 and 7.03 kg/cm², at 200 ppm of hypochlorite, treatments I and J, provided ($P < .05$) the largest reductions in fecal coliform numbers, –2.35 and –2.49 logs, respectively. But by 4 days and at 8 days treatment I and J were not significantly ($P < .05$) more effective than city water spray, treatment B. Generally, hypochlorite spray treatments provided a greater reduction, 0.75 to 2 logs, in fecal coliform numbers when compared to the non-sprayed plate at 4 and 8 days. By 16 days the reduction in numbers on the untreated control beef plate A approximated the counts for the treated plates so that at 20 days there was no significant ($P < .05$) difference between the reduction of fecal coliform counts on the surface of sprayed or non-sprayed beef plates. The fairly rapid reduction in fecal coliform numbers on the non-sprayed control plate surface after 8 days was attributed to the 4-6 C storage conditions which appeared to retard fecal coliform survival.

S. aureus

Analysis of Variance (Table 3) showed *S. aureus* survival to be more influenced ($P < .01$) by spray pressure and time after spraying than by hypochlorite concentration or hypochlorite concentration × spray pressure interaction ($P < .05$). However, a comparison of the *S. aureus* residual $\log_{10}$ mean difference counts in Table 8 showed that the greatest reduction obtained with any of the eight hypochlorite spray treatments was not significantly ($P < .05$) lower than the reductions observed for the non-sprayed beef plate, treatment A, or for plates sprayed with city water, treatment B. Generally, the greatest reduction in *S. aureus* counts on the surface of beef plates, whether treated or not, occurred within 4 days of storage. Apparently, the inhibitory effects of storage at 4-6 C and of competitive bacteria were as effective in reducing the numbers of *S. aureus* on the surface of beef plates as was spraying with city water or solutions containing up to 200 ppm hypochlorite at pressures up to 7.03 kg/cm².

Spray pressure and concentration

Separate Analyses of Variance for $\log_{10}$ mean count difference observed for each of the five bacterial groups within each sampling time were used to statistically test for the separate effects of spray pressure, hypochlorite concentration or pressure × concentration interactions. The significant spray pressure and hypochlorite effects obtained from these 30 Analyses of Variance have been summarized in Table 9.

Hypochlorite concentrations studied between < 5 and 200 ppm did not substantially ($P < .01$) reduce the numbers of aerobic mesophiles, psychrotrophs or *S. aureus* (except at 20 days) on beef plate surfaces up to 20 days after spray treatment. But hypochlorite did help reduce the number of fecal coliforms at 2 h and at 8 days. Eight days after treatment hypochlorite concentration was more effective in reducing the coliforms than the other four bacterial groups tested.

TABLE 7. *Log mean changes[a] in fecal coliform counts[b] on sample strips from the surfaces of inoculated beef plates 2 h to 20 days after hypochlorite spray treatments.*

Time	Treatments[c] A	B	C	D	E	F	G	H	I	J	Order[d] of means
2 h	0.17[e]	-0.52[e]	-1.17[e]	-0.70[e]	-0.26[e]	0.23[e]	-0.70[e]	-0.62[e]	-0.70[e]	-0.66[e]	FAEBHJDIGC
2 day	-0.70[f]	-0.27[e]	-0.94[e]	-0.67[e]	-0.43[e]	-0.41[f]	-1.40[e]	0.10[e]	-2.35[f]	-2.49[e]	HBFEDACGIJ
4 day	-0.53[f]	-1.62[ef]	-2.08[f]	-1.26[e]	-1.29[efg]	-0.94[g]	-1.51[e]	-1.92[e]	-2.58[f]	-2.15[e]	AFDEGBHCJI
8 day	-0.76[f]	-2.03[f]	-2.11[f]	-2.30[e]	-1.97[fg]	-0.61[fg]	-1.76[e]	-1.92[e]	-2.19[f]	-2.62[e]	FAGHEBCIDJ
16 day	-1.58[g]	-1.14[ef]	-1.40[ef]	-1.87[e]	-1.26[ef]	-0.65[fg]	-1.88[e]	-1.78[e]	-1.09[e]	-1.54[e]	FIBECJAHDG
20 day	-1.83[g]	-2.42[f]	-2.07[f]	-1.83[e]	-2.50[g]	-1.82[h]	-1.99[e]	-1.51[e]	-2.58[f]	-2.20[e]	HFADGCJBEI

[a-b]Per 6.45 cm² calculated as the measured $\log_{10}$ count at each sampling time less the measured inoculated $\log_{10}$ count before spray treatment.
[c]See Table 1.
[d]Any 2 treatments in the same row underscored by a common line did not differ significantly ($P < .05$).
[e-h]Any 2 means in the same column bearing the same superscript did not differ significantly ($P < .05$).

TABLE 8. *Log mean changes[a] in* S. aureus *counts[b] on sample strips from the surfaces of inoculated beef plates 2 h to 20 days after hypochlorite spray treatments.*

Time	Treatments[c] A	B	C	D	E	F	G	H	I	J	Order[d] of means
2 h	0.14[e]	0.10[e]	-1.00[e]	0.13[e]	-0.53[e]	-0.31[e]	0.18[e]	-0.02[e]	-0.35[e]	-0.00[e]	GADBJHFIEC
2 day	-0.76[ef]	-0.69[ef]	-1.40[e]	-0.31[e]	-0.67[e]	-1.31[ef]	-0.58[ef]	-0.00[e]	-1.37[ef]	-0.40[e]	HDJGEBAFIC
4 day	-1.83[f]	-1.97[f]	-2.01[e]	-1.03[f]	-1.83[e]	-2.75[efg]	-0.98[ef]	-2.15[f]	-1.57[f]	-0.87[ef]	JGDIEABCHF
8 day	-1.06[ef]	-1.20[f]	-1.67[e]	-0.87[f]	-1.91[e]	-1.50[fg]	-0.46[ef]	-0.81[e]	-1.06[ef]	-1.75[f]	GHDAIBFCJE
16 day	-1.18[ef]	-0.75[ef]	-1.45[e]	-0.87[f]	-1.33[e]	-1.82[fg]	-0.70[ef]	-0.80[e]	-0.33[e]	-0.49[ef]	IJGBHDCAEF
20 day	-1.46[f]	-1.95[f]	-1.89[e]	-0.15[e]	-1.87[e]	-2.03[g]	-2.02[f]	-0.70[e]	-0.70[ef]	-1.30[ef]	DHIJAECBGF

[a-b]Per 6.45 cm² calculated as the measured $\log_{10}$ count at each sampling time less the measured inoculated $\log_{10}$ count before spray treatment.
[c]See Table 1.
[d]Any 2 treatments in the same row underscored by a common line did not differ significantly ($P < .05$).
[e-g]Any 2 means in the same column bearing the same superscript did not differ significantly ($P < .05$).

At 2 days after treatment, spray pressures of 3.75 to 7.03 kg/cm² were highly effective ($P < .01$) in reducing the number of aerobic mesophiles, coliforms, fecal coliforms and *S. aureus*, and were fairly effective ($P < .05$) in reducing psychrotrophic counts. The effectiveness of spray pressure in reducing counts of aerobic mesophiles, psychrotrophs and fecal coliforms (with exception of the 16-day data) was no longer apparent after 4 days, for *S. aureus* at 8 days or for coliforms at sometime between 8 and 16.

In only two of the 30 Analyses of Variance was the hypochlorite concentration × spray pressure interaction ($P < .05$) found to significantly affect residual bacterial numbers (coliforms at 2 h and *S. aureus* at 20 days).

These data imply that the ability of bacteria to persist on the surfaces of beef carcasses was generally less affected by hypochlorite concentration than by pressure of the spray when exposure time, spray pattern and temperature were held constant. More bacteria were apparently physically "scrubbed-off" of the beef plate surfaces by the spray action itself than were inactivated by hypochlorite concentration. Secondly, the organic nature of beef carcass surfaces was thought to substantially reduce any hypochlorite residual from the spray solutions that would theoretically be available on non-proteinous surfaces to exert any bactericidal effects.

Carcasses

Subjective ratings of prime rib eye sections before and 20 days after hypochlorous spray treatment are presented in Table 10. No major changes in visible grade or marbling were observed. Slight increases at 20 days in fat and lean firmness were judged not to be objectionable while slight lightening in the fat color for two of the hypochlorite spray-treated sections was no greater than observed for the non-sprayed control.

Gardner color values (Table 11) of surface lean and minced (interior) lean tissues showed no major changes from the initial stage to the final 20-day samplings. Surface fat color values showed a lightening (increased L) and shift from the redness (decreased a_L) hue in comparison of the 0 days and 20 day samples. The visual effect was noted as a change from light yellowness to more whiteness which is in agreement with the ratings reported in Table 10. Variations for color values found within treatment groups were as large as those among groups.

Shear values were not affected by treatment with hypochlorite sprays. The average shear value at 0 day was 0.32 ± 0.04 kg/cm² and the average value at 20 days was 0.39 ± 0.03 kg/g/cm².

TABLE 11. *Gardner color values of muscle tissue and surface fat from beef plates.*

Sample	Gardner color values L	^aL	^bL
0 day			
Surface lean	30.0 ± 2.9	15.4 ± 5.5	6.7 ± 2.1
Minced lean	41.3 ± 2.7	22.9 ± 1.7	11.0 ± 0.6
Surface fat	60.8 ± 3.0	15.4 ± 6.6	14.0 ± 2.0
20 days			
Surface lean	31.9 ± 3.2	18.2 ± 4.6	8.6 ± 3.4
Minced lean	44.7 ± 1.3	20.5 ± 1.2	11.2 ± 0.3
Surface fat	68.7 ± 5.9	7.3 ± 4.3	13.7 ± 2.2

TABLE 9. *Significant[a] time effects observed for the hypochlorite concentration and pressure combinations of spray treatments applied to beef plates.*

Time	Aerobic mesophiles HOCL conc	Aerobic mesophiles Pres	Psychrotrophs HOCL conc	Psychrotrophs Pres	Coliforms HOCL conc	Coliforms Pres	Fecal coliforms HOCL conc	Fecal coliforms Pres	S. aureus HOCL conc	S. aureus Pres
2 h					* *	* *	* *			
2 day		* *		*	* *	* *		* *		* *
4 day						*				*
8 day					* *	*	*			
16 day								* *		
20 day									* *	

[a]Determined by Analysis of Variance of the log mean counts, corrected for pretreatment inoculated counts, for each indicator bacterial group at each of the 6 sampling times. Total, df =19; HOCl conc (A), df=4; ressure (b), df=2; a × B, df=3; Error, df=10.
* $P < .05$
* * $P < .01$

TABLE 10. *Ratings assigned to beef prime rib eye muscle immediately before and 20 days after aqueous hypochorite spray treatment.*

Prime rib eye	Ratings: Before treatment A	B	C	D	E	F	G	H	I	J	20 day after treatment A	B	C	D	E	F	G	H	I	J
Grade[1]	6	6	6	5	6	6	7	6	6	6	6	6	6	5	6	6	7	6	6	6
Lean firmness[2]	2	4	2	2	3	2	3	2	2	2	3	4	3	4	4	2	4	3	3	3
Marbling[3]	13	13	11	11	13	12	14	11	12	13	13	13	11	11	13	12	14	11	12	13
Fat color[4]	2	3	4	3	4	4	4	2	2	3	3	4	4	3	4	4	4	3	3	3
Fat firmness[5]	4	5	5	3	5	4	5	3	3	5	4	5	5	4	5	4	5	4	4	5

[1]Grade: 9 = Good −; 10 = Good; 11 = Good +; 12 = Choice, −; 13 = Choice; 14 = Choice +; 15 = Prime
[2]Lean firmness: 1 =Soft: 2 =Slightly soft; 3 =Moderately firm; 4 =Firm; 5 =Very firm.
[3]Marbling: 10=Modest +; 11 =Moderate −; 12 =Moderate; 13 =Moderate +; 14 =Slight abundant.
[4] Fat color: 1 =Yellow; 2 =Slightly yellow; 3 =Moderately white; 4 =White; 5 =Very white.
[5] Fat firmness: 1 =Soft; 2 =Slightly soft; 3 =Moderately firm; 5 =Very firm.

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REFERENCES

1. Anderson, M. E., R. T. Marshall, H. D. Naumann, and W. C. Stringer. 1975. Design criteria for spray cleaning of beef — a model system. Paper No. 75-6010 presented at the Ann. Meeting of Am. Soc. Agric. Engineers, Davis, CA.
2. Anonymous. 1972. Bacteriological analytical manual for foods. 3rd ed. Division of Microbiology, Food and Drug Administration.
3. Ayres, J. C. 1951. Some bacteriological aspects of spoilage of self-service meats. Iowa St. College J. 26:31-48.
4. Ayres, J. C. 1959. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Res. 25:1-18.
5. Bailey, C. 1971. Spray washing of lamb carcasses. Bull. No. 75, Meat Res. Inst., Landford, Bristol, England.
6. Duncan, D. B. 1955. New multiple range for multiple F test. Biometrics 11:1-42.
7. Emswiler, B. S., A. W. Kotula, and D. K. Rough. 1976. Bactericidal effectiveness of three chlorine sources used in beef carcass washing. J. Animal Sci. 42:1445-1450.
8. GRAS Affirmation Petition No. 3 G0029. 1973. Ltr. from Swift and Co. to S. Gardner, Food and Drug Adm.
9. GRAS Affirmation Petition No. 5 G0055. 1975. Ltr. from Quad Corp. to D. Larry, Food and Drug Adm.
10. Ingram, M., and T. A. Roberts. 1976. Recent advances in hygiene of meat production. Paper presented at Congress of Royal Society of Health, Veterinary Public Health Session, p. 95-101.
11. Kelly, C. A., and J. F. Dempster. 1974. The effects of washing lamb carcasses. Proc. European Meat Res. Workers Conf. 20:244-251.
12. Kotula, A. W. 1975. Researchers work on microbial growth control. National Provisioner 173(20):124-130.
13. Kotula, A. W. 1975. Washing carcasses with chlorinated water. Proc. Recip. Meat Conf. 28:274-287.
14. Kotula, A. W., W. R. Lusby, J. D. Crouse, and B. deVries. 1974. Beef carcass washing to reduce bacterial contamination. J. Animal Sci. 39:674-679.
15. Kraft, A. A., and J. C. Ayres. 1952. Post-mortem changes in stored meats. IV. Effect of packaging materials on keeping quality of self-service meats. Food Technol. 6:8-12.
16. Marshall, R. T., M. E. Anderson, H. D. Naumann, and W. C. Stringer. 1977. Experiments in sanitizing beef with sodium hypochlorite. J. Food Prot. 40:246-249.
17. Patterson, J. T. 1968. Chlorination of water used for poultry processing. Brit. Poul. Sci. 9:129-133.
18. Patterson, J. T. 1969. Hygiene in meat processing plants. 4. Hot-water washing of carcasses. Record of Agr. Res., Ministry of Agric., North Ireland 18:85-89.
19. Sheltmire, W. H. 1962. Chlorinated bleaches and sanitizing agents. In J. S. Sconce (ed.) Chlorine: its manufacture, properties and uses. Reinhold Publ. Corp., New York.
20. Thomas, J. D., D. M. Allen, M. C. Hunt, and C. L. Kastner. 1977. Nutitional regime, post-slaughter conditioning temperature, and vacuum packaging effects on bacteriology of beef carcasses and retail meat cuts. J. Food Prot. 40:678-682.

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Nematodes in Fresh Market Fish of the Washington, D.C. Area

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ABSTRACT

For this study 1,010 fresh whole fish belonging to 14 families, 20 genera, and 23 species were bought in retail markets of the Washington, D.C. area. Most of the fish had been caught in Chesapeake Bay and adjacent waters. Examination for parasitic roundworms, i.e., nematodes, by dissection, candling or digestion of the flesh, and elution of nonedible viscera produced 6,547 nematodes, mostly alive. Among fish species of which 25 or more were examined, spotted hake (*Urophycis regius*) was the most wormy and white perch (*Morone americana*) the least. Only two of the nematodes were recovered from fish flesh; both were *Anisakis* sp. larvae. Recovered from fish viscera were nine *Anisakis* sp. larvae, 41 *Porrocaecum* sp. larvae, 3,221 *Thynnascaris* spp. larvae and adults, 21 *Goezia* sp. larvae, and 1,220 *Raphidascaris acus* larvae. All the aforementioned nematodes are considered to be anisakines; in addition, 225 anisakines were too damaged to be identified more precisely. The other nematodes recovered in the survey were 71 *Spinitectus* spp. adults and larvae, 114 *Bulbodactinis* sp. adults and larvae, 108 *Metabronema* sp. adults and larvae, 111 *Spirurinae* larvae, 662 *Philometra* sp. adults and larvae, one *Capillaria* sp. larva, 447 similar small larvae so undeveloped that they could not be identified, and 294 other nematodes too damaged even for general classification. Only the *Anisakis* sp. larvae are considered pathogenic to human consumers of raw or semiraw fish. The low incidence of pathogenic anisakines in these fish intermediate hosts is attributed to the absence of definitive hosts (marine mammals) from Chesapeake Bay and adjacent waters. Thirty genera of anisakine nematodes are distinguished morphologically.

Roundworms occurring in the edible fish of U.S. waters are currently of concern. In the 1950s it became apparent that health problems could be associated with some of these parasitic nematodes (*13,62*). Certain anisakine nematode larvae were discovered in patients, several of whom had acute abdominal pain, and in the remaining portions of fish they had eaten raw (*56*). During the following decade increasingly frequent reports of this "new" human disease were published. Cases were recognized in Europe, especially in the Netherlands (*56*), as well as in Japan (*50*). In the United States, raw and semiraw fish recipes appeared to be gaining popularity with consumers (*29*), but at the time, comprehensive nematode surveys of seafoods had not been done with either market samples or fresh catch. This situation prompted new work on the anisakines and anisakiasis, of which the Washington, D.C. area samplings presented here are a part. Reports of what were thought to be the first verified human infections in North America (*33,37,38*) gave additional impetus to the research efforts. It was discovered later that there had been earlier cases (*16,51*). At the present time actual cases of human infection with some anisakine species are no longer considered to be unusual (*31*).

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MATERIALS AND METHODS

Fish samples

For this study 1,010 whole and apparently fresh fish were bought in retail markets of the Washington, D.C. area. Most purchases were made during a 3-year period, 1972 to 1974, but two small additional samples were bought for a minisurvey in 1975. As far as could be determined, the fish had been refrigerated but not frozen, and most had been caught in Chesapeake Bay and adjacent waters. After purchase they were taken to the laboratory as quickly as possible and either examined for nematodes immediately or stored at ca. 5 C in a food refrigerator until examined.

Fish identification

Fish were selected by common name and gross features at the time of purchase. Before dissection, they were identified by the revised specific morphological criteria of Hildebrand and Schroeder (*26*) and classified according to current nomenclature (*3*).

Examining fish for nematodes

Fish being examined were eviscerated, and the viscera and the eviscerated portions of each fish were examined separately. The eviscerated portion, consisting mostly of edible flesh, was dissected grossly. Specimens were then either dissected minutely, digested for 4 h at 35 to 37 C in a 1% pepsin solution adjusted to pH 3 with 3 N HCl after addition of 200 g of fish per liter, or candled (*52*). The efficiency of the three methods for nematode recovery (minute dissection, digestion, candling) could not be compared because of the apparently few parasites in the eviscerated portions (see Results). When nematodes were found in these examinations, they were washed in Ringer's solution and placed in clean Ringer's to be tested for viability and potential infectivity.

For isolating the nematodes from fish viscera, each set of viscera was placed on an 8 × 8 cm wire grid of mesh size 6 × 6 mm. The grid was suspended in a funnel with a top diameter of 15 cm. Funnels were closed at the bottom with tubing and a clamp and were filled with ca. 600 ml of Ringer's solution to cover the viscera on the grid. Nematodes from the viscera settled to the funnel's stem during several hours at controlled room temperature, ca. 24 C. Early results of the survey

TABLE 1. *Survey of nematodes in market fish of Washington, D.C. area*[a].

Fish host	No. infected fish	No. examined fish	% infected fish	Parasitic nematodes (No. nematodes/No. fish)														Nematodes/ fish
				A	P	G	T	R	AN	SPN	B	M	SPR	PH	C	L	N	
BOTHIDAE																		
Paralichthys dentatus	85	134	63	0/0	0/0	0/0	556/69	383/35	54/16	5/4	4/2	54/15	9/3	0/0	0/0	47/1	79/18	8.9
CLUPEIDAE																		
Alosa aestivalis	55	76	72	0/0	0/0	0/0	319/51	19/4	13/5	0/0	0/0	0/0	0/0	0/0	0/0	1/1	13/9	4.8
Alosa sapidissima	9	13	69	0/0	0/0	0/0	96/9	16/1	0/0	0/0	2/1	0/0	0/0	0/0	0/0	0/0	1/1	8.9
Clupea harengus harengus	2	2	100	1/1	0/0	0/0	8/1	7/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	8.5
GADIDAE																		
Gadus morhua	1	1	100	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1.0
Melanogrammus aeglefinus	0	1	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
Urophycis regius	141	149	95	9/5	9/1	0/0	1220/128	240/25	54/19	52/25	106/39	7/3	66/21	0/0	0/0	8/2	71/16	12.4
ICTALURIDAE																		
Ictalurus catus	0	3	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
Ictalurus punctatus	1	8	12	0/0	0/0	0/0	0/0	0/0	3/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.4
LUTJANIDAE																		
Lutjanus campechanus	0	3	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
MUGILIDAE																		
Mugil cephalus	0	1	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
PERCICHTHYIDAE																		
Morone americana	5	28	18	0/0	0/0	0/0	7/3	0/0	0/0	0/0	0/0	1/1	2/1	0/0	0/0	0/0	2/2	0.4
Morone saxatilis	189	225	74	0/0	1/1	20/1	95/32	25/7	4/2	14/3	1/1	4/2	3/3	662/176	0/0	0/0	32/6	3.4
PERCIDAE																		
Perca flavescens	13	28	46	0/0	0/0	0/0	88/9	99/6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	5/2	6.9
POMATIDAE																		
Pomatomus saltatrix	0	5	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
SCIAENIDAE																		
Cynoscion regalis	43	61	70	0/0	5/1	1/1	123/35	42/7	3/3	0/0	0/0	0/0	3/1	0/0	1/1	1/1	23/12	3.3
Leistomus xanthurus	4	4	100	0/0	0/0	0/0	7/2	20/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	180/1	0/0	51.8
Micropogn undulatus	122	184	66	0/0	26/9	0/0	541/102	367/37	87/30	0/0	0/0	29/8	28/5	0/0	0/0	210/12	61/23	7.3
Sciaeops ocellata	0	1	0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0.0
SCOMBRIDAE																		
Scomber scombrus	6	12	50	1/1	0/0	0/0	14/6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1.3
SERRANIDAE																		
Centropristis striata	24	36	67	0/0	0/0	0/0	143/22	2/2	6/4	0/0	1/1	7/2	0/0	0/0	0/0	0/0	5/3	4.6
SPARIDAE																		
Stenotomus chrysops	2	2	100	0/0	0/0	0/0	3/2	0/0	0/0	0/0	0/0	6/2	0/0	0/0	0/0	0/0	0/0	4.5
STROMATEIDAE																		
Peprilus triacanthus	1	3	33	0/0	0/0	0/0	0/0	0/0	0/	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/1	0.7
Total number of *nematodes: 6547*				11	41	21	3221	1220	225	71	114	108	111	662	1	447	294	6.5
Total number of examined fish: 1010																		(Average)
Total number of infected fish: 703				7	12	2	468	128	81	32	44	33	34	176	1	18	93	
Infected no. of fish species: 17				3	4	2	15	11	9	3	5	7	6	1	1	6	11	

[a]A = *Anisakis;* P = *Porrocaecum;* G = *Goezia;* T = *Thynnascaris;* R = *Raphidascaris;* AN = Anisakines (U); SPN = *Spinitectus;*
B = *Bulbodactinis;* M = *Metabronema;* SPR = Spirurinae; PH = *Philometra;* C = *Capillaria;* L = Larve (u); N = Nematodes (U); U = Unidentified.

showed that with a settling time of 4 h, almost all nematodes could be recovered live in the bottom 50 ml of liquid in a funnel. Longer settling times, 8 and 12 h, slightly increased the number of nematodes but drastically decreased their viability. Consequently, 4 h became the standard settling time. The 50 ml of liquid was drained into a container by opening the clamp at the bottom of the funnel. Aliquots of the 50 ml were then removed from the container, placed in petri dishes, and examined grossly as well as microscopically. The nematodes in each dish were counted, washed in clean Ringer's solution, and tested for viability by their movement in clean Ringer's at room temperature, and for potential infectivity in a 35 to 37 C incubator (*25*).

Parasites other than nematodes

Besides nematodes, internal fish parasites recovered and observed frequently during these examinations included cestodes, acanthocephalans and digenetic trematodes. External parasites included monogenetic trematodes and copepods. No attempt was made to detect protozoa.

Identification of nematodes

After being recovered, washed, and tested for viability plus infectivity, the nematodes were fixed either in hot (55 C) 70% ethanol or in room temperature (ca. 24 C) formalin buffered with phosphate to pH 7. Specimens fixed in ethanol were preserved in 70% ethanol - 10% glycerol; those fixed in formalin were preserved in formalin. All specimens were stored in tightly screwcapped glass vials. Relevant information was written in India ink on a small slip of bond paper and placed with the nematode in the vial.

For microscopic examination, nematodes were poured from the vial into a petri dish containing a clearing solution of 70% ethanol - 10% glycerol. (Specimens fixed and stored in formalin were transferred into the petri dish through 30 and 50% ethanol). Petri dishes were placed in a vacuum desiccator at ca. −1 atmosphere to permit evaporation of the alcohol and water. In glycerine, with a refractive index of 1.473 at 20 C, the following morphological features of nematodes are visible: digestive tract, reproductive organs, and nerve ring on the inside; cuticular striations, cuticular appendages, sensory papillae, and lips on the outside. The internal excretory duct and its surface opening are not easily or usually visible in glycerine. Since the position of the pore is important for classification, nematodes were recleared in phenol (Mallinckrodt loose crystals liquefied by addition of 10 ml of 95% ethanol per 100 g of the crystals). In this phenol solution with its refractive index of 1.501 at 26 C, the excretory duct and pore are visible, as are other structures visible in glycerine, except for the nerve ring. In glycerine, nematodes are pliable and easily manipulated; in phenol or other heterocyclic hydrocarbons such as xylene (refractive index = 1.505 at 20 C) they become brittle.

Cleared nematodes in a small amount of clearing solution were placed on microscope slides and covered with a glass slip. Morphological examinations were conducted at various magnifications using bright field, phase contrast, and interference contrast microscopy. The general morphological criteria used for identifying the specimens were those of Stiles and Hassal (*61*), Baylis (*8*), York and Maplestone (*66*), Chitwood and Chitwood (*15*), Hoffman (*27*), Hartwich (*23,24*), and Myers (*47*). During the course of this study, certain criteria were amended (see Results).

After examination, the nematodes cleared in phenol were washed overnight in 70% ethanol; all nematodes were returned to their storage vials in glycerine or in 70% ethanol with glycerine. Information gathered during the microscopic examination and the previous information were written on new paper slips and placed in the vials. Samples of the collection have been deposited with the U.S. National Museum, Animal Parasite Institute, U.S. Department of Agriculture, Agriculture Research Center, Beltsville, Maryland 20705.

RESULTS

In the examination of 1,010 fish belonging to 14 families, 20 genera, and 23 species, 6,547 parasitic nematodes were found. The types and numbers of fish are given in Table 1 and Table 1a.

TABLE 1a. *Common name of fish listed in Table 1.*

Scientific name	Common name
Paralichthys dentatus	summer flounder
Alosa aestivalis	blueback herring
Alosa sapidissima	American shad
Clupea harengus harengus	Atlantic herring
Gadus morhua	Atlantic cod
Melanogrammus aeglefinus	haddock
Urophycis regius	spotted hake
Ictalurus catus	white catfish
Ictalurus punctatus	channel catfish
Lutjanus campechanus	red snapper
Mugil cephalus	striped mullet
Morone americana	white perch
Morone saxatilis	striped bass
Perca flavescens	yellow perch
Pomatomus saltatrix	bluefish
Cynoscion regalis	weakfish
Leiostomus xanthurus	spot
Micropogon undulatus	Atlantic croaker
Sciaenops ocellata	red drum
Scomber scombrus	Atlantic mackerel
Centropristis striata	black sea bass
Stenotomus chrysops	scup
Peprilus triacanthus	butterfish

Categories and numbers of nematodes recovered from fish. All nematodes recovered in the fish survey (Table 1) were assigned to one of the 14 categories. The following six categories meet the criteria for anisakines (Table 2).

TABLE 2. *General characteristics of anisakine nematodes*[a]

1) Three lips
   a) No lip ornaments known
   b) May have interlabia
2) Esophagus in 2 or more distinct parts
   a) Part 1: anterior muscular tube
   b) Part 2: posterior glandular sac, i.e., ventriculus
   c) Ventricular appendices: 0 — several
3) Excretory system
   a) The gland is a band, tube or both but not a normal Renette cell
   b) Excretory pore opens at base of subventral lips or on ventral surface just posterior to nerve ring
4) Female genital opening in anterior half of body
5) Host requirements
   a) Larval anisakines probably require an invertebrate as intermediate host (unlike their "terrestrial" ascarid relatives)
   b) A second intermediate host, invertebrate or vetebrate, may be required
   c) Additional paratenic or transport hosts are often involved in the life cycle

[a]Emendation of Baylis (*8*)

1. *Anisakis* sp. Eleven larvae, each with a boring tooth, met the criteria for this category. They could be identified more precisely than Myers' *sensu lato* type (*47*), which includes nine genera. By morphological criteria (Tables 2 and 3), the specimens were differentiated from larvae of the genera *Viverranisakis, Paranisakis, Ichthyanisakis, Belanisakis, Acanthocheilus, Pseudanisakis*, and *Paranisakiopsis.* A physiological criteroin (*25*), survival for 24 h or more at 35 to 37 C, differentiated these larvae from the morphologically similar but incompletely described genus *Heligmus,* which matures in ectotherms and presumably cannot survive for long at and above 35 C. No marked diffences were noted among the 11 larvae, and it is thought that they may belong to a single *Anisakis* sp.

these larvae are indistinguishable from Berland's *Anisakis* type-1 larva (*11*).

2. *Porrocaecum* sp. Forty-one boring tooth larvae could have been members of the genera *Paradujardinia, Dujardinascaris, Pseudoterranova, Porrocaecum,* and *Aliascaris* according to the criteria of Table 3. However, at least two additional characteristics eliminate the genera *Paradujardinia* and *Dujardinascaris* as appropriate categories: the spherical shape of the ventriculus and the extreme narrowness of the caecum (*47*). Because the caecum of our specimens was much longer than the ventriculus, we concluded that these larvae were not members of the genus *Pseudoterranova,* which have a caecum only slightly longer than the ventriculus. The genus *Aliascaris* is characterized by cuticular cordons and "trilobed wart-like" structures all along the body (*32*). Thus, the characteristics of these 41 boring tooth larvae are those described for the genus *Porrocaecum*. Our *Porrocaecum* sp. larvae lacked alae and had a boring tooth more prominent and cuticularized than that of *Phocanema* sp. larvae from Canadian cod. Cuticularization gives the tooth a darker brown color than that of *Phocanema*, and the color is noticeable even at low magnifications. Since no significant differences were noticed among the 41 larvae, they are thought to belong to a single *Porrocaecum* sp. Tested specimens of the *Porrocaecum* sp. did not survive incubation at 35 to 37 C for more than 4 h.

3. *Thynnascaris* spp. and *sensu lato* type. From 15 species of fish, 3,221 nematodes, adults as well as larvae, were distinguished as belonging to the genus *Thynnascaris* or to Myers' (*47*) larval type. Recovery of more than one type of toothed infective larva suggested the possibility of different species. The reported *Contracaecum* sp. found in white perch (*9*) may have been a misidentified *Thynnascaris* sp.

The genus *Thynnascaris* cannot be distinguished easily from the genera *Lappetascaris, Goezia, Pulchrascaris,* or *Heterotyphlum* by the criteria of Table 3. There are, however, additional criteria. The genus *Heterotyphlum* has a very small ventriculus and older stages have no interlabia; the genus *Lappetascaris* has a long ventricular appendix that extends almost to midbody; there are spines on the cuticle of the genus *Goezia*; separation of ventriculus and ventricular appendix is definite in *Thynnascaris* but not in *Pulchrascaris* (*64*).

4. *Goezia* sp. As mentioned, the presence of cuticular spines distinguishes the genus *Goezia* from the genus *Thynnascaris* and from other related anisakine nematodes. Twenty-one larval specimens of what is probably a single *Goezia* sp. were recovered in this survey with striped bass the principal host.

Because of its spines and flattened lips, the genus *Goezia* is not grouped with the anisakines by some authorities (*23,24,47*) who argue that no other anisakines have these features. However, cuticular rings are occasionally present on some larvae and on some adults which are clearly in the anisakine genus *Thynnascaris*. We believe these features justify the reinstatement of this anisakine-like nematode genus, *Goezia,* with its ornamented cuticle, among the true anisakines.

5. *Raphidascaris acus.* Only the older larvae and adults of the genera *Raphidascaris* and *Raphidascaroides* can be distinguished by the criteria of Table 3. Because the 1,220 specimens recovered in this survey all had a boring tooth, they were judged to be infective larvae, i.e., younger stages. Initially we classified them tentatively as belonging to the *Raphidascaris sensu lato* type, which also included *Raphidascaroides* spp. However, *Raphidascaris acus* is the only species of either genus which has a long ventricular appendix that extends past the nematode's midbody. Since all our specimens had such an appendix, they appeared to be *Raphidascaris acus.* They were recovered from most species of fish that were parasitized by *Thynnascaris* spp.

6. *Unidentified (U) anisakines.* Because of damaged or missing parts or degenerative changes before fixation, 225 nematodes could be identified only as anisakines but could not be identified in any greater detail.

The following eight categories do not meet the criteria for anisakines.

7. *Spinitectus* spp. According to the criteria of York and Maplestone (*66*), 71 adult and larval nematodes were classified as belonging to the genus *Spinitectus.* These nematodes, which mature in fish, were recovered from spotted hake, striped bass, and summer flounder.

8. *Bulbodactinis* sp. The criteria of York and Maplestone (*66*) were used to classify 114 adult and larval nematodes in their monospecific genus, *Bulbodactinis.* These nematodes, which mature in fish, were found mostly in spotted hake.

9. *Metabronema* sp. According to the criteria of York and Maplestone (*66*), 108 nematodes were classified as a species in the genus *Metabronema.* Members of this genus mature in fish. Our specimens, mostly adults, were recovered primarily from summer flounder and Atlantic croaker.

10. *Spirurinae.* By the general criteria of York and Maplestone (*66*), 111 larval nematodes were identified as belonging to the subfamily Spirurinae. The principal fish sources were the summer flounder and the Atlantic croaker.

11. *Unidentified (U) larvae.* Atlantic croaker, spot, and summer flounder were the principal source fishes for 447 similar specimens of small, fairly featureless, and therefore apparently still young nematode larvae categorized as Larvae (U). They could not be anisakines because they lacked a boring tooth.

12. *Philometra* sp. The criteria of Hoffman (*27*) were used to classify 662 adult and immature nematodes from striped bass as members of the genus *Philometra.* As in our previous study (*12*) there appeared to be only one species of *Philometra* in the striped bass.

13. *Capillaria* sp. A single specimen recovered from the weakfish was classified according to Hoffman's criteria (*27*) as a *Capillaria* sp. larva. Nematodes of this

TABLE 3. *Morphological differentiation of the anisakine nematodes*[a].

Genera[b]	Mouth		Neck	Body	Excretory system				Digestive system		Reproductive system			
					Pore		Gland		Appendages		Male		Female	
	Interlabia	Dentigerous ridges	Modifications behind lips	Alae	Lip base	Nerve ring	Band	Tube	Ventriculus	Intestine	Spicules	Cubernaculum	Vulva: anterior to midbody	Definitive host
Anisakis (17,19)	—	+	—	—	+	—	+	—	—	—	2	—	+	marine mammals
Viverranisakis (58)	+	+	?	?	?	?	?	?	—	—	2	—	+	land mammals
Paranisakis (7,47)	+	—	—	—	—	+	?	?	—	—	2	+	+	elasmobranchs
Ichthyanisakis (20,47)	+	—	+	+	?	?	?	?	—	—	2	—	+	fish
Belanisakis (40,47)	+	—	—	+	?	?	?	?	—	—	2	+	+	birds
Heligmus (19,47)	?	?	?	?	?	?	?	?	—	—	1	—	+	fish
Acanthocheilus (21,43)	—	+	—	—	—	+	?	?	—	—	2	—	+	sharks
Pseudanisakis (21,34)	—	+	—	—	—	+	L	R	—	—	2	—	+	skates & rays
Paranisakiopsis (36,65)	+	+	—	±	+	—	+	—	—	—	2	—	+	macrourid fish
Paradujardinia (47,63)	+	—	—	—	—	+	?	?	—	+	2	—	+	sirens
Duhardinascaris (4, 47)	+	+	—	±	—	+	?	?	—	+	2	+	+	reptiles
Phocanema (47,48)	—	+	—	—	+	—	+	—	—	+	2	—	+	marine mammals
Terranova (24,35)	—	+	—	+	+	—	+	—	—	+	2	±	+	elasmobranchs & crocodiles
Sulcascaris (22,24)	+	+	—	—	+	—	L	R	—	+	2	—	+	marine turtles
Pseudoterranova (44,47)	—	+	—	?	—	+	?	?	—	+	2	+	+	marine mammals
Porrocaecum (47,54)	+	+	—	±	—	+	—	+	—	+	2	±	+	fish, birds & rodents
Aliascaris (32)	—	?	—	+	—	+	?	?	—	+	2	—	+	marine fish
Cloeoascaris (5,24)	—	+	+	?	?	?	+	—	+	+	2	—	+	otter
Pelicanascaris (1)	+	+	+	—	+	—	?	?	+	+	2	—	+	birds
Phocascaris (24,28)	—	+	—	?	+	—	+	—	+	+	2	—	+	marine mammals
Contracaecum (24,54)	+	—	—	±	+	—	+	—	+	+	2	—	+	birds & marine mammals
Lappetascaris (55)	—	—	+	—	—	+	?	?	+	+	2	—	+	fish
Goezia (24,67)	—	—	—	—	—	+	—	+	+	+	2	—	±	fish, (?) reptiles
Pulchrascaris (64)	—	+	—	—	?	?	?	?	?	+	2	—	+	fish
Thynnascaris (18,24)	+	+	—	±	—	+	—	+	+	+	2	—	+	fish, (?) prawn (42)
Heterotyphlum (47,59)	—	—	—	?	—	+	?	?	+	+	2	—	+	fish, (?) sea snakes
Raphidascaris (24,53)	—	—	—	+	—	+	—	+	+	—	2	—	+	fish
Raphidascaroides (47, 65)	+	±	—	?	—	+	?	?	+	—	2	—	+	fish
Multicaecum (6,47)	+	?	—	?	—	+	?	?	4	+	2	+	+	reptiles
Polycaecum (39,47)	—	?	+	?	—	+	?	?	5	+	?	?	+	reptiles

[a]Definitions: Interlabia (raised structures between lips); dentigerous ridges (raised structures on lips' inner surface); modifications behind lips (vertical or horizontal cuticular structures, but not cuticular folds as described for *Phocanema* sp.); alae (longitudinal cuticular "wings"); excretory pore (surface opening of excretory system); excretory gland (tube type of gland is associated with lateral cords and only the nuclear region extends into the pseudocoelom; band type of gland fills the ventral pseudocoelom and contains a tubule plus a large nucleus); ventricular appendage (solid projection of posterior esophagus); intestinal appendage or caecum (anterior projection of the Intestine with lumen, ending blindly at a ligament to the body wall); spicule (moveable accessory male structure, normally paired); gubernaculum (immobile accessory male structure); vulva (surface opening of female genitalia).

[b]*Viverranisakis* is the only anisakine genus reported exclusively from land mammals (civet cat, mongoose). *Acanthocheilus* can be distinguished by its round ventriculus and "small number of distinct teeth" from *Pseudanisakis* which has an oblong or oval ventriculus and "one to two complete rings of denticles on a vellum surrounding the mouth" (*21*). *Metanisakis* (*45*) is a synonym of *Pseudanisakis* (*19*). Bier, Lichtenfels and Campbell are preparing a manuscript which describes the excretory system of *Paranisakiopsis* as shown in this table. *Aliascaris* is distinguished from other anisakine genera by cordons and "trilobed papilla-like" structures in longitudinal rows (*32*). *Pelicanascaris* lacks ventriculus according to text of Ali and Farooqui (*1*) but not according to their illustration. *Phocascaris* not as redefined by Berland (*10*). The genus *Cerascaris* Cobb 1929 (*2*) is rejected for *Contracaecum* because the major distinguishing characteristic is a larval feature, the boring tooth. The subgenus *Acollaris* is also rejected because the genus *Thynnascaris* has priority for these distinguishing characteristics. *Heterotyphlum* is distinguished from *Paraheterotyphlum* (*30*) only by spicule proportions and host (*28*); Hartwich (*24*) synonymizes these two genera but Schmidt and Kuntz (*57*) resurrected *Paraheterotyphlum*. Numbers following the name of a genus refer to the original description of the genus and, if existent, to a recent redescription in the reference cited. Symbols are as follows: + (existence of a feature); — (absence of a feature); ± (conflicting reports of existence of a feature); ? (not mentioned); L (exists on left side); R (exists on right side); 1 (only one of a pair reported); 2 (pair reported); 4 (2 anterior and 2 posterior); 5 (2 anterior, 3 posterior).

genus mature in fish.

14. *Unidentified (U) nematodes.* Because of missing or damaged parts or degenerative changes before fixation, 294 nematodes could not be classified.

Viability of recovered nematodes

The vast majority of nematodes recovered from these whole, unfrozen fish were viable when first observed. Only a few (less than 1%) nematodes from fish that had begun to decay appeared moribund although active nematodes were also recovered from these fish. Many of the nematode specimens reported as broken or damaged appeared to be alive; their injuries probably occurred during the isolation process.

Distribution of nematodes in edible and usually discarded portions of fish.

The vast majority of nematodes recovered from the whole, unfrozen fish in this survey were located in the viscera. Only two were isolated from the eviscerated portion consisting primarily of edible flesh. Both were *Anisakis* sp. larvae, occurring in Atlantic herring and spotted hake.

DISCUSSION

In this study, 6,547 nematodes were recovered from 703 of 1,010 fresh market fish bought in the Washington, D. C. area. The mean nematode burden was calculated as 6.48 roundworms per fish, and the overall nematode infection rate of the fish as 69.60%. Averages, however, tend to erase differences. Both the intensity and the distribution of the nematode burden differed among different types of fish, and a variety of nematodes caused the infections. Because of these differences, it is necessary to discuss the incidence of particular nematodes in the various fish groups.

Nematodes of the anisakine genera *Thynnascaris* and *Raphidascaris* were the parasites most frequently recovered in the survey. No fish species of which a minimum of 25 specimens was examined proved to be free of a *Thynnascaris* sp., and only one, white perch, was free of *Raphidascaris acus*. These nematodes have not been identified as pathogens for human consumers of raw or semiraw fish for two reasons. First, specimens of these genera are not usually found in what are considered to be the edible portions of fish. Second, if swallowed live by mammals, the infective larvae recovered in this survey die without attaching to the digestive mucosa and are evacuated with the feces. Only some *Thynnascaris* sp. larvae from warm waters are known to survive long enough to attach to host tissue at mammalian temperatures (*49*). Maturation of *Thynnascaris* spp. and *Raphidascaris* spp. occures only in fish hosts.

Overall, 3,221 *Thynnascaris* spp. specimens were recovered from 486 fish belonging to 15 species (Table 1). The majority were larval forms; adults were found with greatest frequency in Atlantic mackerel and weakfish. Of the *Raphidascaris* sp., 1,220 specimens were recovered from 128 fish belonging to 11 species; no adult nematodes of the genus were found.

Anisakis and *Porrocaecum* were the two genera of anisakines identified in the survey which include *potential* pathogens. As expected, the *Anisakis* sp. larvae recovered from our samples did survive the digestion test (24 h at 35 to 37 C); the *Porrocaecum* sp. larvae did not, and presumably are a species of the genus that matures in fish rather than in birds or mammals. Consequently, the *Anisakis* sp. larvae but not the *Porrocaecum* sp. larvae are considered to be pathogens.

Specimens of these genera were found: 11 *Anisakis* sp. larvae from seven fish belonging to three species (spotted hake, Atlantic herring, Atlantic mackerel). Only two specimens occurred in the edible portions of fish. Forty-one *Porrocaecum* sp. larvae were found in the viscera of 12 fish belonging to four species (spotted hake, striped bass, weakfish, Atlantic croaker).

Other than the specimens of one anisakine genus, *Anisakis,* no other nematode identified in the survey is considered to be pathogenic to human consumers. The seasonal periodicity of the *Philometra* sp. life cycle in striped bass (Chesapeake Bay rockfish) has been documented and discussed previously (*12*). Because many fish species represented in this study are not uniformly available year round or, if available, are caught at different locations depending on the time of year, one cannot make a valid analysis of seasonal fluctuation in nematode incidence other than for rockfish.

The unidentified larvae are probably a heterogeneous collection of very young, undifferentiated nematodes; even if some belonged to potentially pathogenic species, they would not be directly infectious for mammals so early in the life cycle.

Among fish species of which 25 or more were examined, spotted hake was the most wormy and white perch the least wormy in both terms of the percentage of infected fish and number of worms per fish. Spotted hake was also the largest source of pathogenic anisakines.

The true or Atlantic herring, *Clupea harengus,* deserves special mention although few specimens were examined. What is sold as herring in Washington, D.C., markets is usually a mixture of species. The majority are *Alosa* spp. but occasional *Clupea harengus* occur (less than 2% of lots). The only pathogen (one specimen of the *Anisakis* sp. larva) recovered from a herring in this survey was from such a true herring.

The incidence of pathogenic nematodes in fresh market fish of the Washington, D.C. area — 11 *Anisakis* sp. larvae of 6,547 nematodes — was low compared to the number of nonpathogenic nematodes in the same fish. It was also low compared to the number of potentially pathogenic nematodes in fish along the U.S. Pacific Coast (*46,60*) and in fish from more northern Atlantic coastal waters (*14,41*). This infrequency of potential pathogens is attributed to the paucity in Chesapeake Bay and adjacent waters of marine mammals that are the definitive hosts for most of the

anisakines which can infect humans. Along the U.S. Pacific Coast where there is a substantial population of marine mammals close to shore, many fish are infected with pathogenic *Anisakis* sp. and *Phocanema* sp. larvae. Seal hosts which occur along the Atlantic Coast north of Cape Cod account for the high incidence of *Phocanema decipens,* the pathogenic codworm anisakine, in the catch of those waters.

In the absence of marine mammal hosts in Chesapeake Bay, the question arises as to why the fish in the Washington, D.C. area markets contain any pathogenic *Anisakis* sp. larvae. Three possibilities are likely: First, some fish may not have been caught locally, contrary to the assurances of the fishmongers. (Red snapper is definitely not from the area and Alantic cod, probably not.) Second, fish from elsewhere may have migrated or been swept into the area accidentally. This probably happens to the occasional Atlantic true herring that join schools of *Alosa* spp. Third, migrating whales that usually travel off North America's eastern continental shelf come close to shore on occasion and could be transient sources of infection.

Although the prevalence of pathogenic anisakines in fish is determined by the distribution of the parasites' definitive hosts and the feeding and migrating habits of the fish intermediate hosts, none of these are invariable patterns. Chances for human infection with an anisakine nematode from such dishes as sashimi or ceviche may be greater on the West Coast, but there is some risk even in Washington, D.C. For example, whereas only 0.05% of spotted hake in our major collection during 1972 to 1974 were infected with the *Anisakis* sp. larvae, 0.2% were infected in the 1975 minisurvey mentioned earlier. Changes of climate, of offshore fishing limits, and of marketing practices could change the future number of pathogenic nematodes found in fresh market fish of the Washington, D.C. area.

REFERENCES

1. Ali, S. M., and N. Farooqui. 1970. A new ascarid from the grey pelican, *Pelecanus philippensis* from India, p. 139-141. *In* K.S. Singh and B. K. Tandan (ed.), H. D. Srivastava commemoration volume. Indian Veterinary Research Institute, Izatnager.
2. Araujo, P. 1970. Consideracoes sobre o genero *Contracaecum* Railliet and Henry, 1912 (Nematoda: Ascaroidea). Rev. Farm. Bioquim. 8:103-113.
3. Bailey, R. M., J. E. Fitch, E. S. Herald, E. A. Lachner, C. C. Lindsay, C. R. Robins, and W. B. Scott. 1970. A list of common and scientific names of fishes from the United States and Canada. Special publication 6. American Fisheries Society, Washington, D.C.
4. Baylis, H. A. 1947. The nematode genus *Dujardinascaris* (nom. nov. pro *Dujardinia*) in Crocodilia, with a description of a new species. Ann. Mag. Nat. Hist. 14:123-134.
5. Baylis, H. A. 1923. A new ascarid from an otter. Ann. Mag. Nat. Hist. 11:459-463.
6. Baylis, H. A. 1923. On the classification of the Ascaridae. III. A revision of the genus *Dujardinia* Gedoelst, with a description of a new genus of Anisakinae from a crocodile. Parasitology 15: 223-232.
7. Baylis, H. A. 1923. Report on a collection of parasitic nematodes mainly from Egypt. Parasitology 15:1-38.
8. Baylis, H. A. 1920. On the classification of the Ascaridae. I. The systemic value of certain characters of the alimentary canal. Parasitology 12:253-264.
9. Beacham, B. E., and A. J. Haley. 1976. Some parasites of the white perch, *Morone americana* (Gmelin) in Chesapeake Bay. Proc. Helminthol. Soc. Wash. 43:232-233.
10. Berland, B. 1963. *Phocascaris cystophorae* sp. nov. (Nematoda) from the hooded seal, with an emendation of the genus. Mat.-Naturv. Serie No. 17. Arbok for Universitetet I. Bergen, Bergen.
11. Berland, B. 1960. Nematodes from some Norwegian marine fishes. Sarsia 2:1-50.
12. Bier, J. W., W. L. Payne, and G. J. Jackson. 1974. Seasonal periodicity of a *Philometra* sp. (Nematoda) infection in the rockfish *Morone saxatilis*. p. 1631-1632. *In* Proceedings of the Third International Congress of Parasitology, vol. 3. Facta Publication, H. Egermann Publishers, Vienna.
13. Buckley, J. J. C. 1951. Immature *Porrocaecum* recovered from the human mouth. Trans. R. S. Trop. Med. Hyg. 44:362.
14. Cheng, T. C. 1976. The natural history of anisakiasis in animals. J. Milk Food Technol. 39:32-46.
15. Chitwood, B. G., and M. B. Chitwood. 1974. Introduction to nematology. University Park Press, Baltimore
16. Davey, J. T. 1971. A case of *Anisakis* sp. infection in a brown bear from Alaska (and a larva in vomitus from an Eskimo). Trans. R. S. Trop. Med. Hyg. 65:433-434.
17. Davey, J. T. 1971. A revision of the genus *Anisakis* Dujardin 1845 (Nematoda: Ascaridata). J. Helminthol. 45:51-72.
18. Dollfus, R. P. 1933. *Thynnascaris legendrei,* n. gen., n. sp., de l'estomac du germon, *Germo alalonga* (Gmelin). Bull. Soc. Zool. Fr. 58:7-12.
19. Dujardin, F. 1845. Histoire naturelle des helminths ou vers intestinaux, p. 180-221. *In* Libr. Encyclopedique de Roret, Paris.
20. Gendre, E. 1928. Anisakinae Railliet et Henry, 1912, p. 56-60. *In* C. Joyeaux, E. Gendre and J. G. Baer (ed.), Recherches sur les helminthes de l'Afrique occidentale Francaise. Monographie II Soc. Path. Exotique, Paris.
21. Gibson, D. I. 1973. The genus *Pseudanisakis* Layman and Borovkova, 1926 (Nematoda: Ascaridida). J. Nat. Hist. 7:319-340.
22. Gibson, D. I., and A. L. Taylor. 1976. A comment upon the taxonomic significance and function of the excretory system of ascaridoid nematodes. Parasitology 73(2):v.
23. Hartwich, G. 1974. Keys to the genera of Ascaridoidea. 2:1-15. *In* R. C. Anderson, A. G. Chabaud and S. Willmott (ed.), CIH Keys to the nematode parasites of vertebrates. Commonwealth Agricultural Bureau, Farnham Royal, England.
24. Hartwich, G. 1957. Zur Systematik der Nematoden-Superfamilie Ascaroidea. Zool. Jahrbuch. 85:211-252.
25. Healy, G. R., G. J. Jackson, J. R. Lichtenfels, G. L. Hoffman and T. C. Cheng. 1975. Compendium of methods for the microbiological examination of foods. p. 471-483. *In* M. L. Speck (ed.), American Public Health Association, Washington, D. C.
26. Hildebrand, S. F., and W. C. Schroeder. 1928. Fishes of Chesapeake Bay. Bulletin XLIII, Part 1, Document 1024. Bureau of Fisheries. U. S. Government Printing Office, Washington, D.C. (1972. Revised reprint by Smithsonian Institution.)
27. Hoffman, G. L. 1970. Parasites of North American freshwater fishes. p. 241-269. University of California Press, Berkeley.
28. Host, P. 1932. *Phocascaris phocae,* n.g., n. sp. eine neue Askaridenart aus *Phoca groenlandica* Fabr. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1. Orig. 125:335-340.
29. Jackson, G. J. 1975. The "new disease" status of human anisakiasis and North American cases: a review. J. Milk Food Technol. 38:769-773.
30. Johnston, T. H., and P. M. Mawson. 1948. Some new records of nematodes from Australian snakes. Rec. South Aust. Mus. (Adelaide) 9:101-106.
31. Juels, C. W., W. Butler, J. W. Bier, and G. J. Jackson. 1975. Temporary human infection with a *Phocanema* sp. larva. Am. J. Trop. Med. Hyg. 24:942-944.

32. Kalyankar, S. D. 1971. On a remarkable new nematode, *Aliascaris indica* gen. et sp. nov. (Ascarididea: Heterocheilidae), from a marine fish, *Trygon sephen.* Riv. Parassitol. 32:85-88.
33. Kates, S., K. A. Wright, and R. Wright. 1973. A case of human infection with the cod nematode, *Phocanema* sp. Am. J. Trop. Med. Hyg. 22:606-608.
34. Layman, E. M., and M. M. Borovkova. 1926. Parasitic worms of the skate (*Raja radiata* Don.) from material of the 15th federal helminthological expedition. (In Russian). Rab. Parazit. Lab. Mosk. Gos. Univ. 9-26. (Cited in reference 45).
35. Leiper, R. T., and E. L. Atkinson. 1914. Helminths of the British Antarctic expedition, 1910-1913, p. 222-226. Proc. Zool. Soc. London.
36. Lichtenfels, J. R., F. G. Kern, III, D. E. Zwerner, J. W. Bier, and P. A. Madden. 1976. Anisakid nematode in shellfish of Atlantic continental shelf of North America. Trans. Am. Microsc. Soc. 95:265-266.
37. Little, M. D., and J. C. Macphail. 1972. Large nematode larva from the abdominal cavity of a man in Massachusetts. Am. J. Trop. Med. Hyg. 6:948-950.
38. Little, M. D., and H. Most. 1973. Anisakid larva from the throat of a woman in New York. Am. J. Trop. Med. Hyg. 22:609-612.
39. Maplestone, P. A. 1932. Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens. Records Indian Mus. 34:229-261.
40. Maplestone, P. A. 1930. Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens. Records Indian Mus. 32:385-412.
41. Margolis, L. 1977. Public health aspects of "codworm" infection: a review. J. Fish. Res. Board Can. 34:887-898.
42. Margolis, L., and T. H. Butler. 1954. An unusual and heavy infection of a prawn, *Pandalus borealis* Kryer by a nematode, *Contracaecum* sp. J. Parasitol. 40:649-655.
43. Molin, R. 1858. Prospectus helminthum, quacin prodromo faunae helminthologicae venetiae continentur. Sitzungsber. Akad. Wiss. Wien. 30:127-158.
44. Mozgovoi, A. A. 1950. Ascaridata of animals (morphology, biology, systematics and an attempt to construct the phylogenetics of their zoogeography). 1. Anisakoidea. (In Russian). Tr. Gel'mintol. Lab. Akad. Nauk. SSSR. 4:263-269.
45. Mozgovoi, A. A. 1950. Diagnosis of the genus *Pseudanisakis* (Layman and Borovkova, 1926) nov. gen. (In Russian). Tr. Gel'mintol. Lab. Akad. Nauk SSSR. 4:17-24.
46. Myers, B. J. 1976. Research then and now on the Anisakidae nematodes. Trans. Am. Microsc. Soc. 95:137-142.
47. Myers, B. J. 1975. The nematodes that cause anisakiasis. J. Milk Food Technol. 38:774-782.
48. Myers, B. J. 1959. *Phocanema,* a new genus for the anisakid nematode of seals. Can J. Zool. 37:459-465.
49. Norris, D. E., and R. M. Overstreet. 1976. The public health implications of larval *Thynnascaris* nematodes from shellfish J. Milk Food Technol. 39:47-54.
50. Oshima, T. 1972. Anisakis and anisakiasis in Japan and adjacent area. p. 303-393. *In* K. Morishita, Y. Komiya and H. Matsubayashi (ed.). Progress of medical parasitology in Japan, vol. 4. Meguro Parasitol. Mus., Tokyo.
51. Pfaff (Dr.). 1967. Unpublished findings discussed by R. Leuckart. 1876 (Leipzig). Die menschlichen Parasiten und die von ihnen herruhrenden Krankheiten, vol. 2, p. 98, 116-124, 877. C. F. Wintersche Verlagshandlung, Leipzig. Also discussed by O. Martin. 1921. Über Ascaridenlarven aus dem Fleische von Seefischen. Z. Infektionskr. Haustiere. 22:13-36.
52. Power, E. H. 1958. The effect of various lighting conditions on the efficiency of "candling" cod fillets for the detection of parasites. J. Fish. Res. Board Can. 15:537-542.
53. Railliet, A., and A. Henry. 1915. Sur les nematodes du genre *Goezia* Zeder. Bull. Soc. Pathol. Exot. 8:270-275.
54. Railliet, A., and A. Henry. 1912. Quelques nematodes parasites des reptiles. Bull. Soc. Pathol. Exot. 5:251-259.
55. Rasheed, S. 1965. On a remarkable new nematode, *Lappetascaris lutjani* gen. et sp. nov. (Anisakidae: Ascaridoidea) from marine fishes of Karachi and an account of *Thynnascaris inguies* (Linton, 1901) n. comb. and *Goezia intermedia* n. sp. J. Helminthol. 39: 313-342.
56. Ruitenberg, E. J. 1970. Anisakiasis: pathogenicity, serodiagnosis and prevention. Published doctoral dissertation. Rijksuniversiteit te Utrecht, The Netherlands.
57. Schmidt, G. D., and R. E. Kuntz. 1973. Nematode parasites of Oceania.. XX. *Paraheterotyphlum ophiophagos* n. sp. (Heterocheilidae), from the banded yellow-lip sea snake, *Laticauda colubrina.* Am. Midl. Nat. 89:481-484.
58. Soota, T. D., and Y. Chaturvedi. 1971. The nematode fauna of Goa. Indian J. Helminthol. 23:11-20.
59. Spaul, E. A. 1927. A new nematode of the subfamily Anisakinae. Ann. Mag. Nat. Hist. 19:627-635.
60. Stern, L., B. J. Myers, R. A. Amish, W. G. Knollenberg, and K. Chew. 1976. Anisakine nematodes in commercial marine fish from Washington State. Trans. Am. Microsc. Soc. 95:264.
61. Stiles, C. W., and A. Hassal. 1899. Internal parasites of the seal. *In* D. S. Jordan (ed.), The fur seals and fur seal islands of the North Pacific Ocean. U.S. Government Printing Office, Washington, D.C. 3:99-177.
62. Straub, M. 1955. Unpublished findings discussed by P. Van Thiel, F. C. Kuipers, and R. T. Roskam. 1960. A nematode parasitic to herring, causing acute abdominal syndromes in man. Trop. Geogr. Med. 12:97-115.
63. Travassos, L. 1933. Sobre os Ascaroidea parasitos dos crocodilos Sul-Americanos. Ann. Acad. Bras. Cienc. 5:153-163.
64. Vicente, J. J., and E. dos Santos. 1972. Sobre um novo genero da subfamilia filocapsulariinae Yamaguti, 1961 (Nematoda, Ascaridoidea). Atas Soc. Biol. Rio de Janeiro. 16:17-19.
65. Yamaguti, S. 1941. Studies on the helminth fauna of Japan (Part 33). Nematodes of fishes, II. Jpn. J. Zool. 9:343-395.
66. York, W., and P. A. Maplestone. 1926. The nematode parasites of vertebrates. J. A. Churchill, London. (1969. Reprint by Hafner, New York).
67. Zeder, J. G. 1800. Erster Nachtrag zur Naturgeschichte der Eingeweidewürmer, mit Zufassen und Anmerkungen herausgegeben. Privately printed, Leipzig.

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Effect of Potassium Sorbate on Toxinogenesis by *Clostridium botulinum* in Bacon

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ABSTRACT

In an effort to reduce the initial levels of nitrite used to cure bacon and still supply the botulinal inhibition expected in cured meats, bacon was produced at nitrite levels of 0 and 40 ppm $NaNO_2$ with and without 0.13 and 0.26% potassium sorbate. This bacon was inoculated with 1100 spores per g of a mixture of five Type A and five Type B strains of *Clostridium botulinum*. The time for occurrence of the first swollen package and number of toxic swells were recorded over 110 days of incubation at 27 C. The above variables were compared to bacon containing 80 and 120 ppm $NaNO_2$ as well as a commercial sample. Presence of potassium sorbate in the cure significantly reduced the number of toxic swollen packages occurring during incubation and lengthened the time before a toxic swollen package was observed. The presence or absence of 40 ppm $NaNO_2$ appeared to have no significant effect on the sorbate inhibition of *C. botulinum* in bacon in this study. Microbial growth of uninoculated samples was also retarded by addition of potassium sorbate to the brine. Flavor panel evaluations indicated that potassium sorbate decreased preference slightly using experienced judges. Also, reduced occurrence of nitrosopyrrolidine with reduced nitrite was observed.

Due to the potential reaction of nitrite and amines producing carcinogenic nitrosamines, addition of nitrite to foods has been questioned. Fried bacon is the one product in which nitrosamines have been routinely found at parts per billion (ppb) levels (*9*). Therefore, while the need to reduce the addition of nitrite and its precursor, nitrate, may exist in all cured meats, to eliminate nitrosamines, the urgency is greater with bacon.

Nitrite has three essential functions in cured meat products which make its removal difficult. Recent studies have shown that as little as 25 to 50 ppm sodium nitrite ($NaNO_2$) is required to give typical color and flavor (*8*). However, other studies consistently indicate that at least 100 to 150 ppm $NaNO_2$ is required to inhibit outgrowth and toxin production by *Clostridium botulinum* (*3,4*).

Potassium sorbate is a GRAS-listed food ingredient which finds application in many foods from dairy products to baked goods. Sorbate is an antimicrobial additive which has excellent inhibitory effects on molds, yeasts and some bacteria. Sorbate has been reported to have either no effect or a stimulatory growth effect on *C. botulinum* (*6,13*). However, in a recent report, Tompkin et al. (*12*) evaluated sorbate in uncured, cooked sausage and found that sorbate apparently delayed or retarded *C. botulinum* outgrowth. The purpose of this study was to determine the possibility of reducing nitrite in cured meats to the level needed for color and flavor development while supplying the botulinal inhibition with potassium sorbate.

MATERIALS AND METHODS

Bacon preparation

Bacon was manufactured under conditions simulating commercial practices. Different pickle solutions were used to produce a total of 18 different experimental lots of bacon. The basic pickle contained 13.3% sodium chloride and 1.1% sucrose in water. Potassium sorbate, $NaNO_2$, and a polyphosphate blend (2:1 ratio of sodium tripolyphosphate and sodium acid pyrophosphate to minimize pH effects which may occur due to addition of potassium sorbate to some test lots and not to others) were incorporated into the pickle solutions to give the desired levels in each finished lot of bacon (Table 1). All lots containing $NaNO_2$ also contained 550 ppm sodium erythrobate. The pickle solutions were pumped into the bellies to give an 11% gain and drained to an approximate 10% gain. Lot 18 was commercially pumped and the bellies frozen after pumping for further processing with the other lots. The bacon was smoked to an internal temperature of 53 C, cooled and sliced. Slices from each lot were taken at random from the finished bacon in that lot and vacuum packed.

Chemical analysis

Bacon from experimental lots 1, 4, 6, 8, 11,13,16,17 and 18 were analyzed for nitrite, sorbate, phosphate and pH. Salt, moisture, protein and fat analyses were conducted on lots 1, 6, 11, 16 and 18. Nitrite, phosphate, salt, moisture, protein and fat were analyzed by the methods of A.O.A.C (*1*). Sorbate was determined by the method of Allerton and Lewis (*2*) and pH was determined by blending 10 g of product in *50* ml of distilled water and measured with a pH meter.

Flavor evaluation

Vacuum-packed bacon from each lot was immediately frozen after packaging. The bacon was maintained in the frozen condition until just before preparation for flavor evaluation. Bacon slices were cut in half

[1]*Monsanto Company*
[2]*Swift and Company*

TABLE 1. *Lot identity and treatment levels of experimental bacon*

Experimental lot number	$NaNO_2$ (ppm)	Potassium sorbate (% by wt.)	Phosphate (% by wt.)
1	0	0	0
2	0	0	0.4
3	0	0.13	0
4	0	0.13	0.4
5	0	0.26	0
6	0	0.26	0.4
7	40	0	0
8	40	0	0.2
9	40	0	0.4
10	40	0.13	0
11	40	0.13	0.2
12	40	0.13	0.4
13	40	0.26	0
14	40	0.26	0.2
15	40	0.26	0.4
16	80	0	0
17	120	0	0
18	Commercial Product		

and fried on Teflon® coated 10-inch Club Aluminum skillets, the gas burners turned to medium heat, and the bacon cooked 2 min on each side. The cooked samples were held in the top of double boilers for serving.

All samples were served in paper portion cups coded with three digit random numbers. In all tests, experimental lot #18, the commercially pumped product, was served as a known reference in a cup labeled "Ref" and was also included among the five coded samples for scoring. The reference sample was placed in the upper left corner and the five coded samples randomly placed on the serving tray. Trays were served to judges seated in individual testing booths lighted with red lights. Twenty-five experienced judges were used and there were two replicates per test. Judges scored the five coded samples in direct relation to the reference sample using a scale from 7, same flavor, to 1, extremely different flavor; and then scored the desirability of the samples from 7, very desirable, to 1, very undesirable.

Botulinal testing

Ten pounds of bacon from each experimental lot were inoculated with five type A and five type B strains of *C. botulinum* spores using sterile sand as a carrier as described by Christiansen et al. (*4*). An inoculum of 1000 spores per gram was applied to randomly selected slices from each test lot. Six slices of inoculated bacon (125-150 g) were stacked and placed into plastic pouches (Curpolene 200, Curwood, Inc., New London, Wisc.) and vacuum-sealed.

Twenty-five packages were prepared per experimental lot. The packages were held at 27 C and observed daily for swells and removed from incubation when visible swollen. The first 10 or more swollen packages from each lot were tested for the presence of botulinal toxin by mouse assay. The days to first swell, days to first toxic swell, and number of tested swells which were toxic were recorded, as well as total swells over the 110-day incubation period. At the end of the 110-day incubation, 10 non-swollen packages from lots having no toxic swells were also tested for toxin.

Toxin testing was done by mixing (not blending) the bacon with an equal weight of water. The mixture was held refrigerated overnight and a portion of the liquid injected into mice. Toxin assays were conducted as described by Christiansen et al. (*4*). Three mice were injected with unheated liquid (0.5 ml/mouse) and two additional mice were injected with liquid which had been boiled for 15 min. Death of mice receiving the unheated material with the typical signs of respiratory distress coupled with survival of mice receiving the boiled extract was considered presumptive evidence for the presence of botulinal toxin. Samples which were non-toxic following this procedures were blended, again refrigerated overnight, centrifuged, and the supernatant fluid tested as just described.

Shelf-life evaluation

Selected experimental lots of bacon were incubated at 7 C under aerobic and anaerobic conditions. For aerobic storage, three packages of each lot tested were aseptically slit at the edge and the resultant flap was opened to promote aerobiosis. The anaerobic samples were vacuum-packed. A different package from each lot was used for counting each week. Lots no. 1, 2, 4, 5, 6, 7, 10, 12, 13, 15, 16, 17 and 18 were incubated anaerobically up to 4 weeks and lots 1, 4, 5, 6, 7, 12, 15 and 17 were incubated aerobically up to 3 weeks.

Microbial counts were done on six selective media. Assays were made by blending 22-g samples in phosphate buffer plus 1% Tergitol 15-5-7 nonionic surfactant, diluting in the same menstruum, and inoculating or plating onto the appropriate media. These media were: Tryptone Glucose Yeast Extract Agar for total aerobes; Azide Blood Agar base without blood for catalase-negative organisms; Lactobacillus Selective Agar for lactobacilli; Mac Conkey's Agar for gram negative organism; acidified Potato Dextrose Agar for yeasts and molds; deaerated peptone colloid for putrefactive anaerobes.

Nitrosamine analysis

Five pounds of bacon from each experimental lot were fried at 171 C for 3 min on a side, drained, wrapped in aluminum foil and frozen. Lots 7, 9, 10, 12, 13, 19 and 17 were selected for analysis, thawed and analyzed for presumptive nitrosopyrrolidine by GLC using the method of Fazio et al. (*7*)

RESULTS AND DISCUSSION

Table 2 contains the results of the chemical analyses made on the bacon samples. The data show that the levels of salt, sorbate and phosphate are approximately as expected, indicating that preparation of the bacon was normal. Residual nitrite levels, measured just after processing, were within expected commercial ranges. The residual $NaNO_2$, was lower than the amount added and increased as initial nitrite levels increased. The analysis of lot 18 indicated that it was different from others, having a higher brine (salt in the moisture) concentration. This lot was pumped by a commercial process, frozen and held to be smoked and sliced with the other lots. Because this lot was prepared using a commercial brine, this product was used as the standard during flavor evaluation.

Three sets of fried bacon samples were organoleptically evaluated for difference and preference (Table 3). When tested with bacon containing no nitrite (set I), 40 ppm $NaNO_2$ containing bacon was not judged to be significantly different from the commercially processed control. When tested without the no-nitrite bacon, the 40 ppm $NaNO_2$ treatment was judged significantly different from the control (set II). In set III, even the 120 ppm $NaNO_2$ bacon was found significantly different from the control. The preference testing was also varied in results. Data from Table 3 indicate that in set II, one sorbate-containing sample (#10) was judged significantly less desirable than the commercial control while another sorbate sample (#13) was not. The desirable sample had 0.26% while the less desirable sample had 0.13%. In set III, both sorbate-treated samples were judged less desirable but the 120 ppm $NaNO_2$ sample (#17) was also less desirable than the commercial product. This indicated that differences between the samples could be detected by an experienced panel, but those differences were very slight. Consumer acceptability of bacon would probably not be affected by addition of sorbate since the panel did not consistently judge the sorbate treatments

less desirable.

Levels of *C. botulinum* were determined after packaging in four test lots by a three-tube Most Probable Number method. Recoverable spore levels were 1100, 460, 2400 and 1100 with a log average of 1100/g of meat.

The days of incubation until the first observed swollen package in each test lot were recorded (Table 4). However, differentiation between "swelled" and "non-swelled" packages was not a clearcut matter. Many packages lost vacuum within a few days of incubation, so that loss of vacuum could not be used as a criterion for removing packages for toxin testing. Also, few packages

TABLE 2. *Chemical analysis of experimental bacon*

Experimental lot	$NaNO_2$ (ppm)	Potassium sorbate (ppm)	Phosphorus (%)	Salt (%)	Moisture (%)	Protein (%)	Fat (%)	Brine (%)	pH
1	1	80	0.09	1.3	39.6	10.6	48.0	3.18	6.2
4	1	998	0.17	—	—	—	—	—	6.25
6	1	2653	0.18	1.4	30.9	8.3	57.5	4.33	6.25
8	12	380	0.18	—	—	—	—	—	6.15
11	18	1148	0.14	1.3	34.5	8.8	52.0	3.63	6.35
13	19	2240	0.07	—	—	—	—	—	6.25
16	26	200	0.09	1.2	37.3	10.4	50.5	3.12	6.35
17	31	147	0.10	—	—	—	—	—	6.10
18	21	166	0.12	1.4	25.8	9.6	63.5	5.15	6.35

TABLE 3. *Organoleptic evaluation of experimental bacon*

Experimental lot	Set I		Set II		Set III	
	Diff[1]	Pref[2]	Diff[1]	Pref[2]	Diff[1]	Pref[2]
1 ON,OS, OP[3]	4.52*	3.92*				
7 4ON,OS, OP	5.96	5.68	5.78*	5.78		
9 4ON,OS,.4P					6.10	5.82
10 40N,.13S,OP			5.54*	5.42*		
12 40N,.13S,.4P					5.72*	5.60*
13 40N,.26S,OP			5.72*	5.64		
15 40N,.26S,.4P					5.32*	5.30*
16 8ON,OS, OP	5.86	5.64	6.02	5.84		
17 12ON,OS, OP	5.88	5.44			5.58*	5.46*
18 Commercial	6.06	5.70	6.22	5.96	6.38	6.08
LSD (.05)	0.38	0.47	0.41	0.47	0.38	0.44

[1]Score range 7, same flavor as reference (#18) to 1, very extremely different flavor.
[2]Score range 7, very desirable to 1, very undesirable.
[3]N-ppm. $NaNO_2$, S - %potassium sorbate, P - %sodium polyphosphate.
* Significantly different from reference (#18) at the five percent level.

TABLE 4. *Effect of potassium sorbate on toxin production at 27 C in vacuum-packed bacon[1] inoculated with Clostridium botulinum*

Lot no.				Days to first swell	Days to first toxic swell	Total no. of swells[2]	No. swells toxic/ no. swells tested	No. nonswells toxic/no. tested[3]
6	ON	0.26S	0.4P[4]	32	>110	1	0/1	0/10
13	40N	0.26S	OP	33	>110	1	0/1	0/10
5	ON	0.26S	OP	28	> 110	2	0/2	0/10
3	ON	0.13S	OP	28	>110	4	0/4	0/10
12	4ON	0.13S	0.4P	26	>110	4	0/4	0/10
14	4ON	0.26S	0.2P	29	>110	6	0/6	1/10
18	Comm.	plant	product.	32	70	7	1/7	0/10
10	4ON	0.13S	OP	33	39	8	4/8	3/10
4	ON	0.13S	0.4P	20	33	13	4/10	—
17	12ON	OS	OP	27	33	25	8/20	—
15	40N	0.26S	0.4P	27	33	18	8/10	—
9	4ON	OS	0.4P	20	33	20	14/20	—
7	4ON	OS	OP	27	27	19	8/10	—
11	4ON	0.13S	0.2P	19	26	22	8/10	—
8	4ON	OS	0.2P	20	22	20	9/10	—
16	8ON	OS	OP	20	20	25	8/10	—
1	ON	OS	OP	19	19	25	9/10	—
2	ON	OS	0.4P	19	19	25	10/10	—
U statistic (sorbate inhib. ≥ control)[5]				13.0*	3.0* * *	5.5* *	1.5* * *	

[1]Inoculated with 1100 spores/g. of a mixture of 5 strains each of *C. botulinum* type A and B.
[2]No. of swollen packages out of 25 per test lot after 110 days of incubation at 27 C.
[3]Packages which did not appear swollen after 110 days of incubation.
[4]Level of test variables: N-ppm $NaNO_2$, S-%potassium sorbate, P-%sodium polyphosphate
[5]Computed Mann-Whiting U statistic comparing lots with sorbate-free test lots with 80 ppm $NaNO_2$ or less. Critical values of U ($N_1 = 10, N_2 = 10, N_2 = 6$); 14 at $a = 0.05$* , 8 at $a = 0.01$* * , 3 at $a = 0.001$* * * (*10*).

swelled extensively and most, even toxic packages, swelled only slightly beyond the loss of vacuum. Date to first swollen package found to be toxic was also recorded and felt to be a better measure of botulinal inhibition.

Three other indicators of botulinal inhibition observed were: (*a*) number of swollen packages after incubation, (*b*) number of toxic swollen packages out of the 110-day the first 10 or more tested, and (*c*) if few swells were observed during incubation, the number of toxic non-swollen packages were reported of 10 randomly selected after 110 days of incubation. Only samples containing potassium sorbate had no toxic swollen packages over the 110-day incubation. Six of the 10 sorbate-treated lots had no toxic swollen packages. Three of these contained no $NaNO_2$ and three contained 40 ppm $NaNO_2$. One of the remaining four sorbate-treated lots (#10) exhibited delayed toxin production longer than the 120 ppm nitrite test lot (#17), while two sorbate-treated lots were roughly equivalent to the 120 ppm nitrite sample (#4 and #15). One test lot (#11) containing 0.13% potassium sorbate and 40 ppm $NaNO_2$ had toxic swells before the 120 ppm $NaNO_2$ sample. The commercial plant sample (#18) contained only one toxic swell after 110 days. The reason for the unexpectedly poor botulinal growth in the commercial product is not known. One possibility is the higher brine level in this product.

Variability in outgrowth is expected. The three test lots containing 40 ppm $NaNO_2$ without sorbate had toxic swells after 27, 33 and 22 days with 0, 0.2% and 0.4% polyphosphate, respectively. A period of 19 days passed before the two test lots containing no added nitrite or sorbate were observed to have toxic samples, which was very slow compared to previous reports (*4,5*).

The inhibiting effect of sorbate on *C. botulinum* was statistically significant, even with the variability of these results. All sorbate-treated lots were compared with all test lots that did not contain sorbate, but did contain sorbate, 80 ppm $NaNO_2$ or less. Sorbate had a significant effect on *C. botulinum* toxinogenesis by the Mann Whiting U Test (*10*) at a 0.001 confidence level (Table 4) whether evaluation was based on time to first toxic swell or total number of toxic swells observed, and at the 0.1 level when evaluated by total number of swells. The data were significant by this test even by days to first swell which was extremely subjective. Further, there were no significant differences in inhibition between sorbate samples which had no $NaNO_2$ and which had 40 ppm $NaNO_2$. This indicates that in bacon, inhibition was apparently due to sorbate alone and not to a synergistic combination of nitrite and sorbate. Linear regression analysis (*11*) indicated that the days to first toxic swell were significantly related to sorbate treatment level at a 0.05 confidence level. There could be little doubt that under the conditions of this test, sorbate had a significant inhibiting effect on *C. botulinum* outgrowth and toxin production. Neither phosphate level nor nitrite level at 80 ppm or below had a significant effect on botulinal toxin production.

Inhibition of natural microbial flora was also achieved by sorbate addition to bacon (Table 5). There was no significant growth of putrefactive anaerobes or gram negative organisms in either condition and no yeast and mold growth in the vacuum packed products, so these data were omitted. However, significant differences after

TABLE 5. *Effect of potassium sorbate* (PS) *on log average counts of selected organisms in experimental bacon stored at 7 C*

Test	Log Average Plate Count During Anaerobic Storage			Log Average Plate Count During Aerobic Storage		
	0% PS	0.13% PS	0.26% PS	0% PS	0.13% PS	0.26% PS
Total aerobic plate count						
Initial	2.83	3.24	2.85	2.80	3.32	2.89
1 Week	4.36	4.43	4.04	6.39	4.51	4.70
2 Weeks	6.15	2.92* *	4.18*	6.08	3.31	2.79*
3 Weeks	5.69	4.49	4.16*	8.87	5.43* *	3.97* *
4 Weeks	7.11	5.41* *	3.84* *	—	—	—
Catalase negative organisms						
Initial	2.23	2.42	2.27	2.30	2.63	2.26
1 Week	3.26	3.46	3.97	5.27	3.20	3.97
2 Weeks	5.97	2.42* *	3.26* *	5.86	2.89	2.57*
3 Weeks	5.48	4.50	2.90* *	8.94	4.26* *	3.29* *
4 Weeks	6.88	4.25*	3.48* *	—	—	—
Lactobacillus						
Initial	2.0	2.0	2.0	2.0	2.0	2.0
1 Week	2.64	2.51	2.0	3.15	2.0	2.35
2 Weeks	4.52	2.0	2.0*	5.20	2.56	2.0*
3 Weeks	4.91	2.74*	3.01* *	7.50	2.15* *	2.0* *
4 Weeks	6.80	2.42*	2.71* *	—	—	—
Yeasts and molds						
Initial	—	—	—	2.00	2.53	2.0
1 Week	—	—	—	2.73	3.17	2.57
2 Weeks	—	—	—	4.16	2.24	2.00*
3 Weeks	—	—	—	5.32	3.31	2.43* *

* Significantly different from the 0% potassium sorbate samples at $a = 0.10$.
* * Significantly different from the 0% potassium sorbate samples at $a = 0.05$.

2 and 3 weeks were seen by comparing the sorbate-free samples with all samples containing 0.13 and 0.26% potassium sorbate, respectively, by the Student's t test (*11*).

Results of nitrosamine analyses are given in Table 6. None of the samples contained enough nitrosopyrrolidine for mass spectral confirmation so these data are only presumptive and indicate that sorbate addition does not increase the occurrence of nitrosopyrrolidine in fried bacon. Previous work by Sen et al. (*9*) has shown a relationship between nitrite level and nitrosopyrrolidine content after frying. While these data followed this trend, data were insufficient to confirm this.

Production of bacon with reduced nitrite level and potassium sorbate appears, from this study, to be a potential method for producing acceptable bacon of low nitrite content with good antibotulinal protection. However, work to replicate these observations and further research should be conducted to confirm the botulinal inhibition using several processing techniques.

TABLE 6. *Presumptive nitrosopyrrolidine in experimental bacon after frying*[1]

Lot No.				NOPYR[2]
7	4ON	OS	OP[3]	1 ppb
9	4ON	OS	.4P	3 ppb
10	4ON	13S	OP	0 ppb
12	4ON	13S	.4P	0 ppb
13	4ON	.26S	OP	1 ppb
15	4ON	.26S	.4P	1 ppb
16	8ON	OS	OP	3 ppb
17	12ON	OS	OP	4 ppb

[1]Fried at 171 C, 3 min side.
[2]No attempt made to confirm on GC-MS.
[3]N-ppm. $NaNO_2$, S-% Potassium sorbate P-% sodium polyphosphate in the bacon.

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REFERENCES

1. AOAC. 1970. Official methods of analysis, 11th ed. Association of Official Analytical Chemists, Washington, D.C.
2. Alderton, G., and J. C. Lewis. 1958. Determination of sorbic acid and its disappearance from pickle brines. Food Res. 23:338-342.
3. Christiansen, L. N., R. W. Johnston, D. A. Kautter, J. W. Howard, and W. J. Aunan. 1973. Effect of nitrite and nitrate on toxin production by *Clostridium botulinum* and on nitrosamine formation in perishable canned comminuted cured meat. Appl. Microbiol. 25:357-362.
4. Christiansen, L. N., R. B. Tompkin, A. B. Shaparis, T. V. Kueper, R. W. Johnston, D. A. Kautter, and O. E. Kolari. 1974. Effect of sodium nitrite on toxin production by *Clostridium boltulinum* in bacon. Appl. Microbiol. 27:733-737.
5. Collins-Thompson. D. L., P. C. Chang, C. M. Davidson, E. Larmond, and H. Pivnick. 1974. Effect of nitrite and storage temperature on the organoleptic quality and toxinogenesis by *Clostridium botulinum* in vacuum-packed side bacon. J. Food Sci. 39:607-609.
6. Emard, L. O., and R. H. Vaughn. 1952. Selectivity of sorbic acid media for the catalase-negative lactic acid bacteria and Clostridia. J. Bacteriol. 63:487-494.
7. Fazio, T., J. W. Howard, and R. H. White. 1971. Proceedings of the Heidelberg meeting on nitrosamines (IARC). Oct. 13-15, 1971. Co-sponsored by IARC-WHO and German Cancer Center.
8. Hustad, G. O., J. G. Cerveny, H. Trenk, R. H. Deibel, D. A. Kautter, T. Fazio, R. W. Johnston, and O. E. Kolari. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. Appl. Microbiol. 26:22-26.
9. Sen, N. P., J. R. Iyengar, B. A. Donaldson, and T. Panalaks. 1974. Effect of sodium nitrite concentration on the formation of nitrospyrrolidine and dimethylamine in fried bacon. J. Agr. Food Chem. 22:540-541.
10. Siegal, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co., Inc., New York.
11. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York.
12. Tompkin, R. B., L. N. Christiansen, A. B. Shaparis, and H. Bolin. 1974. Effects of potassium sorbate on salmonellae, *Staphylococcus aureus, Clostridium perfringens*, and *Clostridium botulinum* in cooked, uncured sausage. Appl. Microbial. 28:262-264.
13. York, G. K., and R. H. Vaughn, 1954. Use of sorbic acid enrichment media for species of *Clostridium*. J. Bacteriol. 68:739-744.

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Media for Confirming *Clostridium perfringens* from Food and Feces

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ABSTRACT

Several media recommended for confirming isolates of *Clostridium perfringens* from selective plating media were evaluated. Media for testing motility, reduction of nitrate to nitrite, fermentation of lactose, and liquefaction of gelatin were found to be the most useful. A modified motility-nitrate medium, developed during the study, and lactose-gelatin medium were the most satisfactory for doing these tests. Fermentation of salicin and raffinose in peptone-yeast extract medium was also useful for differentiating atypically reacting strains of *C. perfringens* from a variety of culturally similar clostridia.

Because several *Clostridium* species are able to grow in the selective plating media used for enumerating *Clostridium perfringens* in foods and feces, a minimum number of confirming tests should be done to assure that the colonies counted actually are *C. perfringens* (*1*). The inadequacy of procedures commonly used during the past decade to distinguish this organism from a variety of culturally similar clostridia (*5*) was in part due to the poor performance of the media used and to a misunderstanding of the adequacy of the tests to differentiate *C. perfringens* from other *Clostridium* species. The tests recommended by Angelotti et al. have been used extensively in the United States and elsewhere (*1*). The antitoxin half-plate method, rarely used in the United States, has also been used for this purpose, primarily in Great Britain (*12*). Results obtained by Nakamura et al. suggest that this method would yield false-positive results with *Clostridium paraperfringens* and possibly with *Clostridium absonum* because lecithinases of these species react with *C. perfringens* antiserum (*8*). Angelotti et al. believed that gram-positive, obligately anaerobic spore-formers which reduced sulfite, were non-motile, and reduced nitrate to nitrite were confirmed as *C. perfringens*. However, the results of recent studies show that these criteria are inadequate to differentiate *C. perfringens* from culturally similar clostridia (*5,7,8*). The culture media recommended were also difficult to use routinely but are more practical since they have been modified (*6,11*).

Recognition of the culturally similar species, *C. paraperfringens*, *C. absonum*, and *Clostridium celatum*, which like *C. perfringens* are non-motile and reduce nitrate to nitrite, has resulted in efforts to modify confirmatory tests for *C. perfringens* to prevent false results. Suggested additional or alternative criteria include fermentation tests for lactose, salicin, and raffinose, and a test for liquefaction of gelatin (*5,6*).

The purpose of this study was to determine which of these tests are the most useful and to investigate the factors that affect test results obtained with both the original and the modified confirmatory media.

MATERIALS AND METHODS

Cultures

All *C. perfringens* cultures and many of the culturally similar clostridia used were from the Food and Drug Administration (FDA) stock culture collection. The type strains of *C. absonum* ATCC 27555 and *C. paraperfringens* ATCC 27639 were obtained from the American Type Culture Collection, Rockville, MD. Additional strains of these species and five atypical *C. perfringens* strains isolated from spices and other foods in our laboratory were classified by Lillian V. Holdeman at the Anaerobe Laboratory of VPI and SU, Blacksburg, VA. Ms. Holdeman also supplied authenticated strains of *C. absonum* VPI 6903, *C. paraperfringens* 4446, and *Clostridium sardiniensis* strains VPI 2975 and VPI 2984 for use in the study. The *C. celatum* GD-1 culture was supplied by A. H. W. Hauschild, Health and Welfare, Canada. Cultures were maintained at room temperature in a medium prepared from fresh cooked beef. Test cultures were propagated in the same medium or in fluid thioglycollate medium (*4*).

Motility-nitrate media.

Indole-nitrite (IN) medium was obtained from BBL as the complete dehydrated medium and was prepared according to directions. All of the other media were prepared from ingredients as specified by the original authors. Modifications of the various media were made as indicated in the formulas given below: Motility-nitrate (MN) medium consisted of 0.3% beef extract, 0.5% peptone (Difco), 0.3% agar, and 0.1% potassium nitrate (*1*). Supplemented motility-nitrate (SMN) medium was prepared from MN medium by adding 0.5% each of D-galactose and glycerol (*6*). Buffered supplemented motility-nitrate (BSMN) medium was prepared in the same manner as SMN medium except that 0.25% disodium phosphate was added and the pH was adjusted to 7.3 ± 0.1. To prepare buffered motility-nitrate (BMN) medium, the potassium nitrate level in BSMN was increased from 0.1% to 0.5% (*5*). Tryptose-phosphate-nitrate (TPN) medium consisted of 1% tryptose (Difco), 0.25% Na_2HPO_4, 0.5% potassium nitrate, and 0.3% agar.

Ingredients were dissolved in distilled water and the pH was adjusted to 7.3. All motility-nitrate media were sterilized by autoclaving for 15 min at 121 C.

Nitrite test reagents.

Solution A for the nitrite test was prepared by dissolving 1 g of sulfanilic acid in 125 ml of 5 N acetic acid. Solution B consisted of either 1 g of *a*-naphthol or N-(1-naphthyl) ethylene diamine dihydrochloride in 200 ml of 5 N acetic acid. To test for nitrite, 0.5 ml of solution A and 0.2 ml of solution B were added to the culture.

Nitrite destruction

Destruction of nitrite by *C. perfringens* was determined by adding filter-sterilized $NaNO_2$ to IN base without nitrate to obtain 200 and 400 μg of nitrite/ml of medium. A 0.5-ml inoculum of fresh fluid thioglycollate culture was used per 11 ml of IN base containing nitrite and the culture was incubated at 35 C. At 30-min intervals, 0.5 ml of culture was diluted to 3 ml with distilled water, and 0.25 ml each of sulfanilic acid and *a*-naphthylamine solution was added to detect nitrite (*1*). When color intensity was greatest, usually within 5 min, percent absorbance was measured at 540 nm with a Cary model 118 spectrophotometer. The approximate nitrite level was determined by comparing the result with a standard curve derived with basal medium containing various concentrations of nitrite.

Gelatin media

Lactose-gelatin was prepared as specified by Hauschild and Hilsheimer (*6*). The salicin-gelatin and raffinose-gelatin media were prepared by adding 1% salicin or raffinose, 12% gelatin, 0.5% Na_2HPO_4, and 0.005% phenol red to polypeptone yeast extract (PY) medium (*8*). The pH was adjusted to 7.4 and the medium was autoclaved for 15 min at 121 C. The basal PY medium consisted of 2% polypeptone (BBL), 0.5% yeast extract, and 0.5% NaCl. Thioglycollate-gelatin (thio-gel) medium was obtained from Difco. This medium and the basal medium of lactose-gelatin used for preliminary experiments were prepared with 1% salicin or raffinose substituted for lactose.

Fermentation tests

Salicin and raffinose fermentations were determined in PY medium as specified in the AOAC method (*5*). Production of acid from salicin was checked after 24 h of incubation and again after 3 days. PY-raffinose was checked for acid after 3 days. The spot plate method with phenol red indicator was used during the early part of the study, but for subsequent testing, 15.0-cm Whatman No. 31 filter paper disks were saturated with a 0.2% bromthymol blue solution made slightly alkaline with ammonium hydroxide. The disks were air dried and stored for later use. A 2-mm platinum loop was used to transfer 0.04 ml of culture to the indicator disk. The precision of the color change was checked with a variety of cultures ranging in pH from 5.8 to 7.2. These determinations were confirmed with a Fisher Accumet 420 electronic digital pH meter equipped with a microprobe combination electrode. Salicin and raffinose fermentations in PY-gelatin media were observed daily for acid and gas production.

Antitoxin half-plate

The lactose egg yolk medium was prepared as indicated by Willis and Hobbs (*12*) and plated in 15 × 100-mm culture dishes. Test cultures were grown overnight in fluid thioglycollate medium. Test plates were prepared by the even application of 0.05 ml of *Clostridium welchii* (*perfringens*) type A diagnostic serum (obtained from Burroughs-Wellcome and Co., Greenville, NC) to one-half of the medium. The medium was inoculated by streaking once across the plate with a 4-mm loopful of culture from the untreated side. After anaerobic incubation for 24 h, plates were checked for lecithinase activity and neutralization of this enzyme by the antiserum. Lactose fermentation was also determined by observing a color change of the neutral red from red to yellow and then to fuchsia after holding the plates at room temperature for about 4 h.

RESULTS AND DISCUSSION

Motility-nitrate media

Figure 1 shows that the rate of nitrite accumulation after 18 h of incubation was greater in supplemented motility nitrate (SMN) medium and in buffered motility-nitrate (BMN) medium than in the indole-nitrite (IN) and tryptose-phosphate-nitrate (TPN) media. The latter two media have been used extensively in FDA laboratories for making motility and nitrate reduction determinations with *C. perfringens*. The higher nitrite levels in SMN and BMN media appeared to be due to better growth and probably to other factors which resulted from the addition of galactose and glycerol. Buffering the SMN medium with Na_2HPO_4 also resulted in increased nitrite levels as shown in Table 1. The reason for the lower levels of nitrite in the IN and TPN media (Fig. 1) was not determined, but further

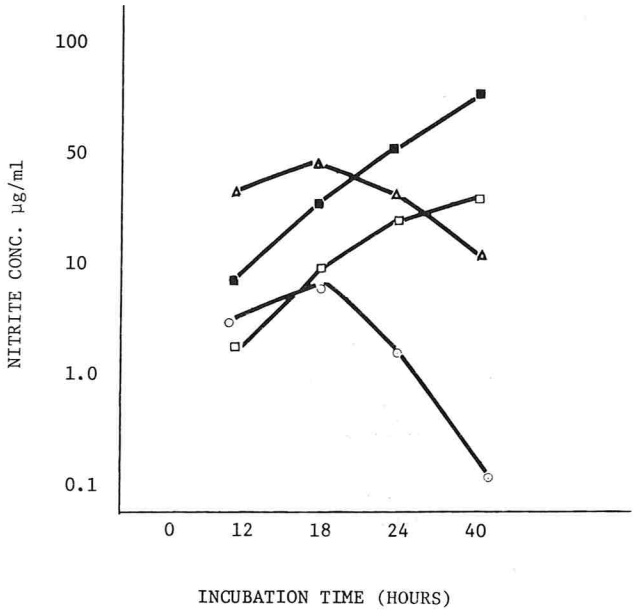

Figure 1. *Nitrite levels produced by* Clostridium perfringens *in different media, as determined by the method used for measuring nitrite destruction. Indole-nitrite medium, BBL, (open circles); supplemented motility-nitrate medium (open squares); buffered motility nitrate medium (solid squares); tryptose phosphate nitrate medium (open triangles).*

testing of the IN medium suggested that it was due to rapid destruction of nitrite. When nitrite was added to IN basal medium without nitrate at 200 and 400 μg/ml, it decreased steadily and was undetectable in many *C. perfringens* cultures after 4 h of incubation. The rapidity of nitrite destruction in this medium appeared to be related to addition of glucose to encourage better anaerobe growth.

Because of these effects it was concluded that modification of SMN to the medium referred to as BMN was desirable to obtain consistently positive results with *C. perfringens* when the safe *a*-naphthol test reagent was used as recommended by the AOAC (*5*). Although this reagent is preferable from a safety standpoint, it requires a higher level of nitrite in the medium to obtain a positive

TABLE 1. *Effects of potassium nitrate concentration and buffering with disodium hydrogen phosphate on nitrite levels produced by* Clostridium perfringens *and 7 other clostridia in supplemented motility-nitrate medium.*

Organism	strain	Nitrite level obtained in[a] SMN (KNO_3 0.1%)	SMN (KNO_3 0.5%)	BMN[b] (KNO_3 0.5%)
C. perfringens	FD-1	±[c]	+	++++
	FD-2	+	++	++++
	S-40	++	+++	++++
	S-45	+	++	++++
	S-80	+	+	+++
	IU-686	+	+	++++
	IU-3344	±	±	++++
	CDC-3131	+	+	++++
	79393	±	+	++
	NCTC 8238	+	±	++
	NCTC 8798	++	+++	++++
	NCTC 10240	+	++	++++
C. absonum	VPI 6903	+	±	+
C. bifermentans	FDA-1	–	-	–
C. butyricum	E-33	–	-	–
C. celatum	GD-1		+	++
C. paraperfringens	ATCC 27639	+	++	++
C. sardiniensis	VPI 2984	–	-	±
C. sporogenes	E-17	–	-	–

[a]As measured with the *a*-naphthol test reagent after 24 h incubation at 35 C.
[b]Same as SMN except 0.25% Na_2HPO_4 was added.
[c]Symbols: Maximum nitrite concentration > 50 μg/ml = ++++; strong nitrite test equivalent to 25 μg/ml = +++; positive test equivalent to 6 μg/ml = ++; weak positive test equivalent to 3 μg/ml = +; trace, limit of the test = ±; no detectable nitrite, i.e. <1 μg/ml = -.

test as shown in Table 1. Comparative testing also showed that the performance of SMN medium was improved by the addition of 0.25% Na_2HPO_4 (BSMN) and that satisfactory results were obtained in the modified medium referred to as BSMN when N-(1-naphthyl) ethylene diamine dihydrochloride was used as the test reagent instead of *a*-naphthol. Both of these reagents have been recommended as suitable substitutes for *a*-naphthylamine, a carcinogen which could represent a hazard to laboratory personnel (*3,5*).

The results of the comparative tests were substantiated by further testing SMN and the two buffered modifications with a total of 108 *C. perfringens* isolates and 21 strains of culturally similar clostridia. Results of these tests indicated that the BMN and BSMN media were more suitable for conducting the motility and nitrate reduction tests with *C. perfringens* because of the better growth response and higher nitrite levels produced by many of the strains.

Results of these tests also showed that like *C. perfringens*, all of the *C. absonum*, *C. celatum*, and *C. paraperfringens* cultures were non-motile and reduced nitrate to nitrite; thus, they could not be distinguished from *C. perfringens* without doing additional tests. However, these two tests were important for distinguishing the *C. sardiniensis* strains which were weakly motile and usually produced only a small amount of nitrite.

Carbohydrate fermentation and gelatin liquefaction

The data presented in Table 2 show the response of 43 strains of *C. perfringens* and 21 strains of other clostridia in lactose-gelatin (*6*) and in a salicin-gelatin medium

TABLE 2. *Reactions of 43* C. perfringens *strains and 8 other clostridia in lactose-gelatin and salicin-gelatin media.*

Organism	No. of strains	Reactions in[a] Lactose-gleatin: Lactose	Lactose-gleatin: Gelatin	Salicin-gelatin: Salicin	Salicin-gelatin: Gelatin[b]
C. perfringens	38	+[c]	+	–	+
	3	+	+	+	+
	2	+	–[d]	–	–
C. absonum	1	+	–	+	–
C. acetobutylicum	1	+	–	+	–
C. bifermentans	2	–	+	–	+
C. butyricum	2	+	–	+	–
C. celatum	1	+	–	+	–
C. paraperfringens	6	+	–	+	–
C. sardiniensis	3	+	+	+	+
	3	+	–	+	–
C. sporogenes	2	–	+	–	+

[a]After 48 h of incubation at 35 C.
[b]Sterile lactose was added after 24 h of incubation to encourage gelatin liquefaction.
[c]+Means carbohydrate was fermented or gelatin was liquefied.
[d]–Means carbohydrate was not fermented or gelatin was not liquefied.

which was developed during this study. Lactose was fermented by all the test organisms except *Clostridium bifermentans* and *Clostridium sporogenes*, which were included as negative controls. Gelatin was liquefied within 48 h by all except two strains of *C. perfringens* but was usually not liquefied by the culturally similar clostridia. A few strains labeled *C. absonum* or *C. sardiniensis*, as well as six recent isolates characteristic of these species, liquefied gelatin slowly in lactose-gelatin medium. It was also noted that lactose-gelatin cultures of *C. perfringens* were densely turbid while those of culturally similar species were clear, with cells sedimented to the bottom. The phenol red indicator in lactose-gelatin cultures was often completely reduced, giving a false impression that acid was produced from lactose. Because we encountered difficulty in obtaining gelatin which retained a suitable viscosity after autoclaving the lactose-gelatin medium, we recommend expanded testing of isolates of special interest which react atypically in this medium.

An attempt was made to combine tests for fermentation of either salicin or raffinose with that for gelatin liquefaction. Raffinose was utilized too slowly to allow rapid liquefaction of gelatin. Peptone yeast extract (PY) medium supplemented with salicin and gelatin could be used for performing these tests at the same time, but sterile lactose had to be added to the culture after 24 h of incubation to encourage gelatin liquefaction. Because of this requirement, there appears to be little advantage in use of a salicin-gelatin medium. Similar results were also obtained when thio-gel or lactose-motility (LM) broth (*11*) was used as the basal medium.

However, rapid fermentation of salicin in PY medium was found to be useful for differentiating *C. perfringens* from culturally similar species (*5*). All of the culturally similar organisms tested were invariably positive, while only 13% of 108 *C. perfringens* isolates produced acid from salicin and most of those were weakly positive. To test for acid, we preferred spot testing a 2-mm loopful of

culture on filter paper saturated with a 0.2% solution of bromthymol blue. Production of acid from raffinose in PY medium was even more consistent with the *C. perfringens* strains. Only one *C. perfringens* strain failed to produce acid from raffinose. Of the culturally similar clostridia, one strain, *C. paraperfringens* 4446, produced acid from raffinose, but it also rapidly fermented salicin. Thus, it was concluded that fermentation tests in PY medium were useful for differentiating *C. perfringens* from culturally similar clostridia when the tests were used in combination with BMN medium and lactose-gelatin.

Antitoxin half-plate

Results in Table 3 show the response of representative strains of *C. perfringens* and the culturally similar *Clostridium* species on the antitoxin half-plate of Willis and Hobbs (*12*). The response of *C. paraperfringens* was almost identical to that of some *C. perfringens* strains. *C. perfringens* antiserum completely inhibited the lecithinase activity of these strains, thus yielding a false-positive test for *C. perfringens*. The lecithinase activity of

TABLE 3. *Response of* C. perfringens *and five culturally similar clostridia on the antitoxin half-plate*[a]

Organism	No. of strains	Lactose fermentation	Lecithinase production	Lecithinase inhibition by *C. perfringens* antiserum (type A)
C. perfringens	40	+	+	complete
C. absonum	2	+	+	partial
C. bifermentans	2	–	+	partial
C. celatum	1	+	–	NA[b]
C. paraperfringens	6	+	+	complete
C. sardiniensis	6	+	+	partial

[a]As determined by the method of Willis and Hobbs.
[b]Not applicable.

strains labeled *C. absonum* and *C. sardiniensis* was only partially inhibited by *C. perfringens* antiserum but in other respects the strains were much the same as *C. perfringens*. The same was true for *C. bifermentans* although this species was lactose-negative. As noted by Nakamura and his colleagues, the partial inhibition of *C. absonum* was an indication that this species was not *C. perfringens,* and we found this to be true of strains designated as *C. sardiniensis* (*10*). The antitoxin half-plate was capable of differentiating *C. celatum* from *C. perfringens* because *C. celatum* invariably failed to produce lecithinase. However, this test would give false negative results with the lecithinase-negative *C. perfringens* strains. Some workers have referred to lecithinase-negative strains as *C. plagarum* (*9*).

When adequate controls were utilized, the antitoxin half-plate differentiated several culturally similar clostridia from *C. perfringens*; however, both false positive and false negative tests occurred with some culturally similar clostridia. In fact, the response of some of our cultures was so simialr to *C. perfringens* that we suggest that supplementary tests be performed with isolates whenever it is at all likely that culturally similar species are present.

Application of the confirmatory tests

Results obtained in this study show that BMN medium and BSMN medium in combination with lactose-gelatin were the most satisfactory media for confirming isolates of *C. perfringens* from selective sulfite agars. Additional tests may be occasionally necessary to assure that isolates actually are *C. perfringens* because of the similarity of reactions obtained with other species such as *C. absonum, C. celatum,* and *C. paraperfringens*. These species were distinguished from *C. perfringens* only by their inability to liquefy gelatin within the 44-h incubation period specified for this test. In addition, the *C. sardiniensis* strains fermented lactose, and three of the six strains liquefied gelatin within 48 h. These strains reduced nitrate to nitrite and could only be distinguished from *C. perfringens* by a weakly positive motility test. For these reasons, it is suggested that supplementary tests for fermentation of salicin and raffinose be employed when doubtful results are obtained with either the motility-nitrate or lactose-gelatin media. Isolates which rapidly ferment salicin and fail to produce acid from raffinose in PY medium within 3 days may be assumed to be one of the culturally similar species.

However, when test results are clear-cut, gram-positive, obligately anaerobic non-motile bacteria which reduce nitrate to nitrite, ferment lactose, and liquefy gelatin within 48 h should be provisionally identified as *C. perfringens*. Only two of 108 *C. perfringens* isolates tested failed to give this result on repeated testing. These two strains failed to liquefy gelatin in the lactose-gelatin medium in fewer than 3 days and then did so feebly. Our results with these "atypical" *C. perfringens* strains were confirmed by Lillian V. Holdeman at the Anaerobe Laboratory, VPI and SU, Blacksburg, VA (personal communication). As more experience is gained with the newly defined species, other tests may prove to be more appropriate for confirming isolates as *C. perfringens;* however, at the present time the tests recommended as part of the AOAC and APHA methods appear to be adequate (*2,5*).

REFERENCES

1. Angelotti, R., H. E. Hall, M. J. Foter, and K. H. Lewis. 1962. Quantitation of *Clostridium perfringens* in foods. Appl. Microbiol. 10:193-199.
2. APHA Intersociety Agency Committee on Microbiological Methods for Foods. 1976. Compendium of methods for the microbiological examination of foods. M. L. Speck (ed.). American Public Health Association, Washington, D.C.
3. Committee on Laboratory Standards and Practices. 1975. American Public Health Association. Health Lab. Sci. 12:131.
4. Food and Drug Administration, U.S. Department of Health, Education, and Welfare. 1972. Bacteriological analytical manual. 3rd ed. U. S. Government Printing Office, Washington, D.C.
5. Harmon, S. M. 1976. Collaborative study of an improved method for the enumeration and confirmation of *Clostridium perfringens* in foods. J. Assoc. Off. Anal. Chem. 59:606-612.
6. Hauschild, A. H. W., and R. Hilsheimer. 1974. Evaluation and modifications of media for enumeration of *Clostridium perfringens.* Appl. Microbiol. 27:78-82.
7. Hauschild, A. H. W., and L. V. Holdeman. 1974. *Clostridium*

celatum sp. nov., isolated from normal feces. Int. J. Syst. Bacteriol. 24:478-481.

8. Nakamura, S., T. Shimamura, M. Hayase, and S. Nishida. 1973. Numerical taxonomy of saccharolytic clostridia, particularly *Clostridium perfringens*-like strains: description of *Clostridium absonum* sp. nov. and *Clostridium paraperfringens*. Int. J. Syst. Bacteriol. 23:419-429.
9. Nakamura, S., M. Sakurai, S. Nishida, T. Tatsuki, Y. Yanagase, Y. Higashi, and T. Amano. 1976. Lecithinase-negative variants of *Clostridium perfringens:* the identity of *C. plagarum* with *C. perfringens*. Can. J. Microbiol. 22:1497-1501.
10. Prevot, A. 1966. Manual for the classification and determination of the anaerobic bacteria. 1st Am. ed., Lea and Febiger, Philadelphia.
11. Shahidi, S. A., and A. R. Ferguson. 1971. New quantitative, qualitative, and confirmatory media for rapid analysis of food for *Clostridium perfringens*. Appl. Microbiol. 21:500-506.
12. Willis, A. T., and G. Hobbs. 1958. A medium for the identification of clostridia producting opalescence in egg yolk emulsions. J. Pathol. Bacteriol. 75:299-305.

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Potential Transmission of Foot-and-Mouth Disease in Whey Constituents[1]

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ABSTRACT

Whey and whey constituents were prepared from the milk of cows infected with foot-and-mouth disease (FMD) virus. The virus was detected in the sweet whey by-product of Cheddar and Camembert cheese but was not detected in the acid whey by-product of casein manufacture. Whey constituents, α-lactalbumin, β-lactoglobulin, and lactose, produced from sweet whey were noninfectious when inoculated into cattle. These products do not appear as likely candidates for the transmission of FMD.

The discharge of whey, the by-product of cheese manufacture, into sewage systems is a major concern in the control of environmental pollution. This concern has accelerated numerous studies on the utilization of whey and whey constituents. Included in such studies is the utilization of whey as a source of human and animal food. Progress in this area, especially use in solid animal feeds, appears promising (*6*). However, animal health concerns have arisen concerning the transmission of disease agents through these products. Earlier studies have shown that foot-and-mouth disease (FMD) virus survived in many dairy products produced from the milk of infected cows (*2,3,5*). The manufacture of these products included both high processing temperatures and low acidities. Because the FMD virus survived Cheddar and Camembert cheese manufacture (*3*), the survival of FMD virus in whey and whey constituents and their possible role in the transmission of FMD were examined.

MATERIALS AND METHODS

Processing of milk

Milk was collected from grade lactating dairy cows that had been inoculated with 7 $\log_{10}$ plaque-forming units (PFU)/ml of a tissue suspension of the Mecklenburg strain of FMD virus, type A, subtype 3 (A_3); techniques used were described previously (*2,3*). .

Milk was collected at days 1, 2, and 3 postinoculation and heated at 67 C/1.0 min or 72 C/0.25 min for Cheddar or Camembert cheese manufacture, respectively. Animal housing and maintenance procedures, as well as methods of milk and cheese manufacture, have been described previously (*3*).

Preparation of whey constituents

Sweet whey samples were obtained as a by-product of Cheddar and Camembert cheese manufacture (*3*). Acid whey was obtained as a by-product of isoelectrically precipitated (pH 4.7) hydrochloric acid casein as described by Cunliffe and Blackwell (*5*).

Samples of α-lactalbumin and β-lactoglobulin were prepared on a laboratory scale. The principles of the method used were described by Aschaffenburg and Drewry (*1*); the method incorporated the separation of the two proteins on the basis of solubility in hydrochloric acid (pH 2.0). The β-lactoglobulin fraction remains in solution whereas the α-lactalbumin fraction is precipitated rapidly. The precipitated fraction is solubilized in 0.1 N ammonium hydroxide solution and the two proteins are processed independently and crystallized as described in Fig. 1.

Crystallization was enhanced by seeding with purified crystals of commercial α-lactalbumin and β-lactoglobulin. Upon crystallization, α-lactalbumin was dissolved in 0.7 saturated ammonium sulfate solution; however, β-lactoglobulin crystals were dissolved in sterile distilled deionized water.

The precipitated fractions were separated at the various preparative stages, more efficiently by centrifugation at 800 × g for 20 min than by filtration. When centrifugation temperatures were maintained at 26 C or above, sodium sulfate crystals remained in solution (Fig. 1).

Lactose was prepared from deproteinized (ammonium sulfate precipitated) whey samples by the evaporation of the solutions to 50% of their original volume by use of a rotary evaporator or on a hot plate (Fig. 1). The concentrated whey solution was crystallized by holding at 4 C for 18 h. Lactose crystals were pelleted by centrifugation at 800 × g for 20 min at 4 C. This procedure is a modification of procedures described by Nickerson (*7*). Lactose crystals were then resuspended in sterile deionized water for virus assay.

Serial ten-fold dilutions of test samples prepared in chilled Eagle's minimal essential medium (f-15, GIBCO, Inc., USA) and 0.1 ml of these preparations were inoculated onto each of three primary bovine kidney cell culture monolayers. The cell cultures were incubated at 37 C for 1 h followed by an overlay medium of methylcellulose. The monolayer cell cultures were incubated at 37 C for 24 h, then stained with a 20% crystal violet solution in formaldehyde. Plaques were enumerated macroscopically. Samples negative for FMD virus by plaque assay in cell culture were inoculated into two steers; each steer received 2 ml intradermally into the tongue and 8 ml intramuscularly. Cattle were observed daily for clinical signs of disease.

When vesicular liesons developed, tongue tissue samples were collected for identification by complement fixation (*4*). Cattle remaining free of clinical signs of disease 14 days postinoculation were

[1] *Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.*

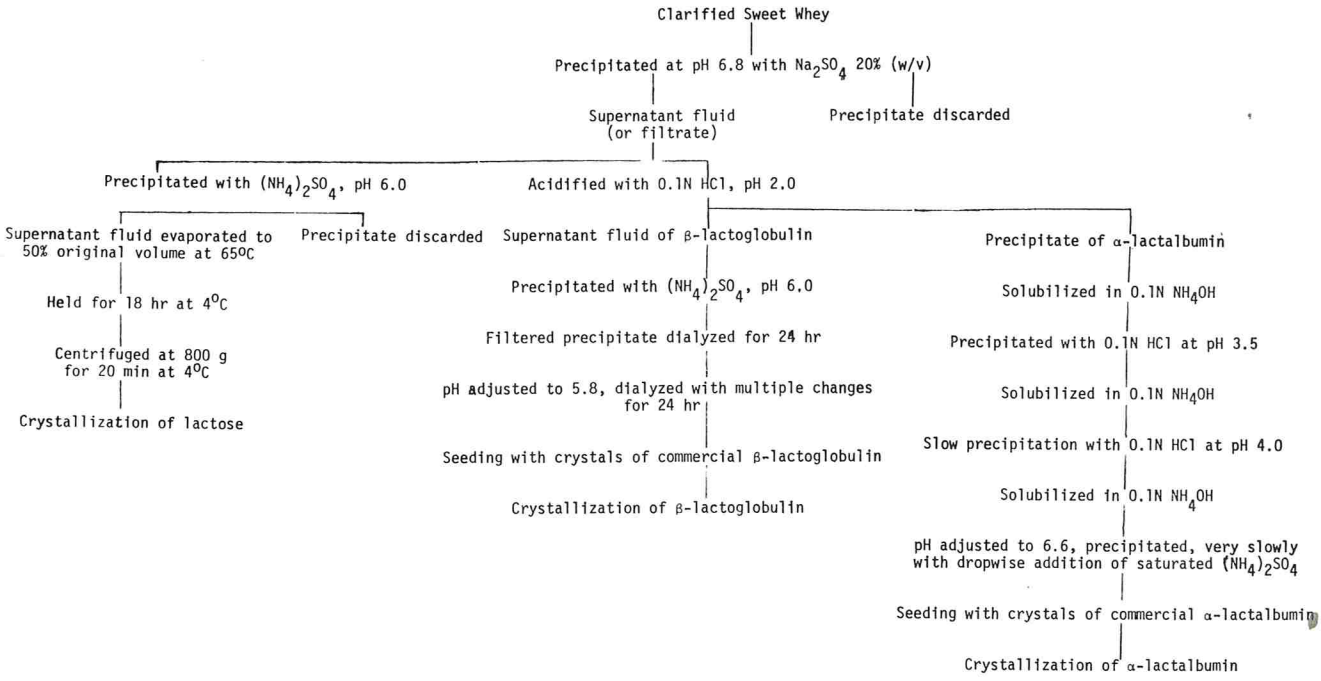

Figure 1. *Procedures used in the preparation of whey constituents.*

later inoculated intradermally into the tongue with 2 ml of suspension containing 6 $\log_{10}$ PFU/ml of FMD virus A_3 (Mecklenburg) to determine susceptibility to the disease.

RESULTS

Survival of FMD virus in whey

Sweet whey obtained as a by-product of either Cheddar or Camembert cheese manufacture contained infective FMD virus in all lots tested. (Table 1). Acid whey (pH 4.7) obtained as a by-product of casein manufacture did not contain detectable FMD virus by cell culture assay or cattle inoculation. Virus, however, was detected in the above-mentioned casein samples.

TABLE 1. *Survival of foot-and-mouth disease (FMD) virus in sweet whey.*[a]

Processing temperature of milk (C)	Mean virus concentration[b]		Response in cattle to whey inoculation
	Unheated milk	Whey	
67 C/1.0 min	5.8	0.05	+[d]
72 C/0.25 min	4.4	< 0.4[c]	+

[a]By-product of Cheddar and Camembert cheese production.
[b]Log_{10}PFU/ml primary bovine kidney cell culture monolayer; mean of 6 test lots.
[c]Plaques not observed in 0.3 ml of inoculum in the 6 test trials.
[d]FMD produced in cattle, 2 steers/test, 6 test lots, total of 12 steers.

Because infective FMD virus was not demonstrable in acid whey, only sweet whey was used as starting material (mother liquor) in the extraction of whey proteins and lactose.

Survival of FMD virus in whey constituents

The virus was not detected by cell culture assay or cattle inoculation in either the α-lactalbumin or β-lactoglobulin preparations (Table 2). In addition, the virus was not detectable in resuspended lactose crystal preparations.

TABLE 2. *Survival of foot-and mouth disease (FMD) virus in sweet whey constituents.*

Days post-inoculation milk collected	Virus concentration in cell culture		Response in cattle to whey product inoculation[a]		
	Milk[b]	Whey[c]	a-lactal-bumin	B-lacto-globulin	lactose
1	7.3	0.2	—	—	—
2	4.5	< 0.4[d]	—	—	—
3	3.3	< 0.4	—	—	—

[a]FMD not produced in cattle; 2 steers/sample.
[b]Log_{10}PFU/ml of unpasteurized milk.
[c]Log_{10}PFU/ml of sweet whey.
[d]Plaques not observed in 0.3 ml of inoculum; 3 bottles/dilution set.

DISCUSSION

Even though the manufacture of dairy products involves various combinations of heat and low pH, FMD virus has been detected in many of these products prepared from the milk of FMD virus infected cows (*3,5*). In this report, however, FMD virus was not detected in whey constituents produced from FMD virus positive milk. Curd formation and fat mobilization are not preparative steps in production of whey constituents, such as α-lactalbumin, β-lactoglobulin, and lactose. However, these factors are characteristic of those dairy products in which FMD virus has survived (*2,3,5*) and, as observed here, by the survival of FMD virus in casein but not in the acid whey by-product. Whey constituents contain negligible fat and casein concentrations and in their manufacture undergo continuous heating, precipitation, and solubilization at pH extremes. Thus, FMD virus in such products is apparently more sensitive to inactivation than FMD virus in products containing high concentrations of casein, and/or fat. This report therefore suggests minimal potential for the transmission of FMD through whey constituents α-lactalbumin,

β-lactoglobulin, and lactose manufactured as described from sweet or acid whey.

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REFERENCES

1. Aschaffenburg, R., and J. Drewry. 1957. Improved method for the preparation of crystalline β-lactoglobulin and α-lactalbumin from cow's milk. Biochem. J. 65:273-277.
2. Blackwell, J. H., and J. L. Hyde. 1976. Effect of heat on foot-and-mouth disease virus in the components of milk from FMDV-infected cows. J. Hyg. Camb. 77:77-83.
3. Blackwell, J. H. 1976. Survival of foot-and-mouth disease virus in cheese. J. Dairy Sci. 59:1574-1579.
4. Cowan, K. M., and R. Trautman. 1976. Immunochemical studies of foot-and-mouth disease. I. Complement fixation reactions with isolated antigenic components. J. Immunol. 99:729-736.
5. Cunliffe, H. R., and J. H. Blackwell. 1977. Survival of foot-and-mouth disease virus in casein and sodium caseinate. J. Food Prot. 40:389-372.
6. Hargrove, R. E., and G. P. Lynch. 1974. Solid animal feeds from whey and whey products. Proc. Whey Products Conf. 1:38-47.
7. Nickerson, T. A. 1970. Lactose. pp. 356-380. In B. H. Webb and E. O. Whittier (eds.) By-products from milk. AVI Publishing Co., Westport, CT.

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A Survey of Histamine Levels in Sausages[1,2]

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ABSTRACT

A survey of 390 sausage samples of nine different types obtained at the retail level revealed the following mean histamine levels on a mg/100 g basis: bologna – 0.55, cooked salami – 0.83, kosher salami -0.50, beef summer sausage - 1.07, thuringer-cervelat -2.35, thuringer – 1.19, Italian dry salami Brand A – 24.5, Brand B– 2.14, Brand C — 19.1, pepperoni Brand D — 1.03, Brand B —1.42, Brand C — 38.1, and chorizo — 2.29. The dry, fermented sausages (Italian dry salami and pepperoni) had higher and more variable histamine levels than either the cooked or the semi-dry sausages. The brand specific differences seen in Italian dry salami and pepperoni, which are significant at the 0.005 level, would suggest that proper control of the natural fermentations could largely prevent histamine accumulation. These differences also indicate that the microflora of each processing plant influences the microflora and the resultant histamine levels of the naturally fermented sausage. Since the semi-dry sausages (beef summer sausage, thuringer-cervelat, and thuringer) are fermented by addition of commercial lactic acid starter cultures, these bacteria must produce only small amounts of histamine. The presence of rather high histamine levels in some samples of Italian dry salami and pepperoni suggests that the potential exists for formation of toxic levels of histamine in dry, fermented sausage if any gross mishandling of the product occurs.

Histamine has been associated with several outbreaks of foodborne illness in the U.S. (*1,2,11*) and numerous episodes in other countries (*3,10,15*). Histamine accumulates in foods via microbial decarboxylation of histidine. Therefore, histamine is a normal constituent of fermented foods such as cheese (*18*), wine (*12*), and sauerkraut (*10*). The presence of high concentrations of free histidine in certain food products, such as tuna fish, which are exposed to microbial degradation, also enhances the possible formation of large amounts of histamine. The small amounts of histamine that occur in such foods under normal circumstances do not pose a public health hazard. However, if certain microbial species, such as *Proteus morganii*, which possess considerable capacities for histamine formation (*8*), are allowed to proliferate during handling or processing, the histamine content may rise to a deleterious level. The minimal concentration of histamine in food which would elicit a toxic response has been estimated to be 100 mg/100 g (*3,15*).

Little definitive information exists on the levels of histamine in meat products. Comminuted beef (*14,16*), beef-soy (*16*), pork (*16*), turkey (*16*), and cured hams (*9, 14*) have low histamine levels. However, a preliminary survey of sausages indicated the occurrence of occasionally high histamine concentrations (*16*). Fermentation may be important in the formation of histamine in certain types of sausages. Semi-dry sausages are fermented for short periods often with lactic acid cultures added, while dry sausages are allowed to ferment from the action of natural microflora for longer periods (*6*). During this sausage ripening process, the histamine concentration increases at least 10-fold during the first 3 days of ripening, while the concentration of histidine correspondingly decreases (*5*). The histamine concentration in a fermented sausage can be variable and dependent on the length of the ripening process (*4*). This survey was undertaken to determine the amount of histamine in various types of sausages. The histamine levels between various classes of sausage, including cooked, semi-dry, and dry are compared.

MATERIALS AND METHODS

Thirty samples each of bologna, cooked salami, kosher salami, beef summer sausage, thuringer, thuringer-cervelat, and chorizo and 90 samples each of Italian dry salami and pepperoni were obtained from retail markets in the San Francisco Bay area. Samples were kept refrigerated until analysis.

Sample preparation and histamine analysis were done as described previously (*16*). Assays were done on duplicate samples with the results being averaged. The error calculated from the duplicate samples was within 5%. The histamine recovery calculated from an internal standard (*16*) was usually within the range of 90 to 110%.

[1] Reprint requests to: Commander, LAIR, ATTN: Medical Research Library, Presidio of San Francisco, CA 94129.

[2] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

RESULTS AND DISCUSSION

The histamine content of the tested sausages was dependent on the processing treatment that the particular types of sausage received (Table 1). Cooked sausages had the lowest mean histamine levels with bologna -0.55 mb/g, cooked salami -0.83 mg/100 g, and kosher salami -0.50 mb/100 g. The range of histamine concentrations for cooked sausages was relatively narrow with most samples having less than 1.00 m1100 g of sausage. One sample of cooked salami had a histamine content of 5.86 mg/100 g which may indicate faulty processing or storage to allow microbial growth at some stage. These histamine concentrations are similar to those found in fresh comminuted meats (*16*), which is not surprising since these products are not allowed to ferment. Rice et al. (*14*) found somewhat lower histamine concentrations in bologna. However, these investigators also found lower histamine levels in comminuted beef than those previously reported (*16*), which would indicate that their extraction procedure may have been somewhat less efficient.

Semi-dry sausages had slightly higher mean histamine concentrations than cooked sausages with beef summer sausage — 1.07 mg/100 g, thuringer-cervelat — 2.35 mg/100 g, and thuringer — 1.19 mg/100 g. The range of histamine concentrations for semi-dry sausages was also relatively narrow with samples containing between 0.31 mg/100 g and 3.63 mg/100 g. Rice et al. (*14*) reported histamine contents of semi-dry sausages to range from 0.18 to 0.55 mg/100 g. These lower histamine levels may again reflect a less efficient extraction procedure or analysis of a different type of semi-dry sausage. Most of the semi-dry sausages had lactic acid fermentation cultures added. The presence of low histamine levels suggests that the commercial starter cultures produce very little histamine. Rice and Koehler (*13*) reported a lock of histidine decarboxylase activity and histamine production by *Lactobacillus* starter cultures commonly used in fermented sausages. The rapid growth rate of the starter cultures would lessen the likelihood of substantial growth by any microorganisms capable of greater histamine production.

By comparison to cooked and semi-dry sausages, dry sausages had larger and more variable amounts of histamine. A brand specific variation in histamine content was noted for both types of dry sausage, Italian dry salami and pepperoni. Three brands of each type of dry sausage were tested. A paired t-test comparison of Brand B Italian dry salami with either Brand A or C showed significantly lower histamine content at the 0.005 level of significance. The range of histamine concentrations was rather wide for all three brands, especially Brand B. One sample of Brand B contained 20.2 mg histamine per 100 g which compares closely with the levels found in Brands A and C. A paired t-test comparison of Brand C pepperoni with either Brand D or B showed significantly higher histamine content at the 0.005 level of significance. The range of histamine concentrations found with Brands D and B was narrow while the range of concentrations found with Brand C was much wider. These results would indicate that the natural fermentation process used with dry sausages can result in accumulation of high histamine levels. The brand specific variation suggests that different sausage processors subject the products to different environmental conditions, which can have a dramatic effect on histamine content. Pepperoni Brands D and B and Italian dry salami Brand B had histamine levels only slightly higher than fresh comminuted beef (*16*). The natural fermentation process used in preparation of these sausages probably did not involve growth by any major histamine-producing bacteria. The other brands

TABLE 1. *Histamine content of sausages.*[a]

Range of histamine content[b]	Cooked sausages			Semi-dry sausages			Dry Sausages						
				Beef			Italian dry salami			Pepperoni			
	Bologna	Cooked salami	Kosher salami	summer sausage	Thuringer cervelat	Thuringer	A[c]	B[c]	C[c]	D[c]	B[c]	C[c]	Chorizo
⩽ 1.00	30[d]	26	30	14		12		15		15	1		6
1.01 - 2.00		3		15	5	15		7		15	24		17
2.01 - 3.00				1	19	3		4			5		2
3.01 - 5.00					6			1					1
5.01 - 7.50		1						2					1
7.51 - 10.0													3
10.1 - 15.0									7				
15.1 - 20.0							4		10				
20.1 - 30.0							22	1	11			1	
30.1 - 40.0							4		2			17	
40.1 - 50.0												10	
50.1 - 60.0												2	
Mean	0.55	0.83	0.50	1.07	2.35	1.19	24.5	2.14	19.1	1.03	1.42	38.1	2.29
Standard deviation	0.19	0.96	0.17	0.39	0.73	0.64	6.2	3.7	6.8	0.28	0.58	9.7	2.3
Range	0.19 to 0.84	0.47 to 5.86	0.33 to 0.97	0.69 to 2.40	1.03 to 3.63	0.31 to 2.56	17.5 to 36.4	0.42 to 20.2	10.6 to 35.0	0.72 to 1.60	1.00 to 2.72	28.2 to 55.0	0.60 to 8.08
Number of different brands	6	5	3	2	1	6	1	1	1	1	1	1	4

[a]Thirty samples of each type of sausage were tested.
[b]Histamine content in mg/100 g.
[c]Capital letters refer to different brands.
[d]Number of items within range.

of Italian dry salami and pepperoni had such high levels of histamine in comparison to the other fermented sausages that involvement of *P. morganii* or other histamine-producing bacteria in fermentation must have occurred. It is doubtful that growth by *P. morganii* or other known histamine-producing bacteria is desirable during the sausage ripening process, since proteolysis would lead to off-flavors. However, the lack of quality control in production of these sausages (*6*) and the use of natural fermentation makes selection of desirable organisms difficult. The consistent association of high histamine levels with certain brands would suggest that the microbial flora of dry, fermented sausage is a reflection of the microbial flora of the processing plant. Consequently, while a marked difference was noted between brands, Brand B of Italian dry salami was the only product that showed distinct differences in histamine content among different samples of the same brand. Since only three brands of each type of dry, fermented sausage were analyzed, the overall incidence of brands with consistently high histamine levels is unknown.

The levels of histamine in dry, fermented sausage previously reported were also quite variable (*4,5,7,14, 16,17*). Rice et al. (*14*) found the histamine content of dry sausage to range from 0.07 to 0.78 mg/100 g. Henry (*7*) reported levels of histamine ranging from 0.2 to 10 mg/100 g, while Cantoni et al. (*4*) found histamine concentrations ranging from 0.20 to 2.49 mg/100 g. Vandekerckhove (*17*) reported histamine levels ranging from non-detectable to 19.7 mg/100 g for Belgian dry, fermented sausages and 2.6 to 28.6 mg/100 g in French air-dried sausages, while sausages prepared under controlled conditions in a pilot plant had histamine concentrations below 4 mg/100 g. Again, these findings suggest that the conditions of fermentation may affect the amount of histamine produced. It also suggests that the histamine levels can be controlled by proper fermentation conditions.

Chorizos exhibited a wide range of histamine concentrations. While the mean histamine level was 2.29 mg/100 g, concentrations in the range of 7.51 to 10.0 mg/100 g were found in 10% of the samples (Table 1). Chorizos are not fermented, but the lack of cooking may lead to potential microbial spoilage if improperly stored. Microbial growth is regulated to some extent by the presence of spices. However, the presence of relatively high levels of histamine in 10% of the samples would suggest that chorizos are susceptible to microbial action.

None of the 390 tested samples had histamine concentrations above the level of 100 mg/100 g, which has been suggested as the minimal level necessary to precipitate clinical symptoms of toxicity in humans (*3,15*). The cooked and semi-dry sausages as well as most of the dry, fermented sausages were well below this level of histamine. The highest concentration of histamine found in any of the sausage was 55.0 mg/100 g in a pepperoni. Henry (*7*) has stated that 70 to 1000 mg of histamine ingested in a single meal is necessary for the onset of toxicity, depending on individual sensitivity. Therefore, 130 g of the pepperoni sample that contained 55.0 mg histamine per 100 g would be necessary to cause problems in the most sensitive individuals. Of course, the amounts necessary for development of clinical symptoms of toxicity are merely guesses based on previous outbreaks. However, the rather high levels of histamine in certain brands of pepperoni and Italian dry salami would suggest that the potential exists for formation of toxic levels of histamine in dry, fermented sausage, especially if any gross mishandling occurred. It is apparent from the brand-specific variations that acceptable Italian dry salami and pepperoni can be prepared without undue accumulation of histamine. Since the histamine accumulation is probably due to the presence of undesirable bacteria in the early stages of fermentation, imposition of more rigorous quality control procedures should circumvent any possible public health problems. These quality control procedures should be imposed at both the production and retail levels, since storage conditions at the retail level may allow microbial growth with resultant histamine formation.

REFERENCES

1. Anonymous. 1973. "Scombroid" poisoning from mahi-mahi. Calif. Morbidity. No. 23.
2. Anonymous. 1974. Scombroid poisoning from Spanish mackerel. Calif. Morbidity. No. 13.
3. Arnold, S. H., and W. D. Brown. 1977. Histamine (?) toxicity from fish products. Adv. Food Res. (In Press.)
4. Cantoni, C., M. A. Bianchi, and G. Beretta. 1974. Amino acids, histamine and tyramine variation during ripening of dry sausage (salami). Ind. Aliment. 13:75-78.
5. Dierick, N., P. Vandekerckhove, and D. Demeyer. 1974. Changes in nonprotein nitrogen compounds during dry sausage ripening. J. Food Sci. 39:301-304.
6. Genigeorgis, C. A. 1976. Quality control for fermented meats. J. Am. Vet. Med. Assoc. 169:1220-1228.
7. Henry, M. 1960. Dosage biologique de l'histamine dans les aliments. Ann. Fals. Exp. Chim. 53:24-33.
8. Kimata, M. 1961. The histamine problem. pp. 329-352. In G. Borgstrom (ed.) Fish as food. Vol. I. Academic Press, New York.
9. Lakritz, L., A. M. Spinelli, and A. E. Wasserman. 1975. Determination of amines in fresh and processed pork. J. Agr. Food Chem. 23:344-346.
10. Mayer, K., and G. Pause. 1972. Biogenic amines in sauerkraut. Lebensm.-Wiss. Technol. 5:108-109.
11. Merson, M. H., W. B. Baine, E. J. Gangarosa, and R. C. Swanson. 1974. Scombroid fish poisoning. Outbreak traced to commercially canned tuna fish. J. Am. Med. Assoc. 228:1268-1269.
12. Ough, C. S. 1971. Measurement of histamine in California wines. J. Agr. Food Cem. 19:241-244.
13. Rice, S. L., and P. E. Koehler. 1976. Tyrosine and histidine decarboxylase activities of *Pediococcus cerevisiae* and *Lactobacillus* species and the production of tyramine in fermented sausages. J. Milk Food Technol. 39:166-169.
14. Rice, S., R. R. Eitenmiller, and P. E. Koehler. 1975. Histamine and tyramine content of meat products. J. Milk Food Technol. 38:256-258.
15. Simidu, W., and S. Hibiki. 1955. Studies on putrefaction of aquatic products. XXIII. On the critical concentration of poisoning for

histamine. Bull. Japan. Soc. Sci. Fish. 21:365-367.
16. Taylor, S. L., E. R. Lieber, and M. Leatherwood. 1978. A simplified method for histamine analysis of foods. J. Food Sci. 43:249-250.
17. Vandekerckhove, P. 1977. Amines in dry fermented sausage. J. Food Sci. 42:283-285.
18. Voigt, M. N., R. R. Eitenmiller, P. E. Koehler, and M. K. Hamdy. 1974. tyramine, histamine, and tryptamine content of cheese. J. Milk Food Technol. 37:377-381.

Blood Protein Offers Huge Source of Nutritious Human Food

Protein is essential to human nutrition and is in growing demand from an increasing world population. Yet even though millions of people suffer from malnutrition, there is a source of many million pounds of nutritious protein that is largely unused for human food.

This source is blood from slaughtered animals. Blood proteins can now be recovered in the form of white powder by a method used by scientists with the Texas Agricultural Experiment Station.

"This powder is more than 95 percent pure protein and is odorless and tasteless in the bargain. But added to some tasty food, it can radically raise the protein content," says Dr. W. A. Landmann, who led the laboratory research on ways to recover blood protein. He heads the Department of Biochemistry and Biophysics at Texas A&M University.

Nutritionists call elements in protein the "building blocks" of the body. Protein is necessary for the normal development of the young, and without enough to keep the body repaired, the adult ages too rapidly.

Yet the supply of protein now available in the world for human nutrition is in short supply, and decreasing. But increased use of slaughter animal blood could help ease this shortage.

Experiment Station scientists at Texas A&M started this research because of these figures: From January to May of 1971, 8.829 billion pounds of cattle were slaughtered by the U.S. *Over this 5-month period, a good portion of the 12.5 million pounds of available blood protein was wasted insofar as its potential of being converted to food for humans.*

The term "wasted" is not too strong because the world currently is more than 20 percent short of the protein it needs to feed its existing population. And we're told this population will double in the next 20 to 25 years.

"Think what it will mean to the State of Texas and the world if this poorly used resource can be converted into high quality food. The necessary technology has been worked out; a small pilot plant (continuous) operation has been built. It can be readily enlarged to a commercial-sized operation at a later date," says Dr. C. W. Dill who headed its development team. Dill, a food chemist, is with the Department of Animal Science at Texas A&M University.

"Blood protein powder blends readily with many foods," says Dill. "It has desirable characteristics of solubility, whipping ability and emulsification.

"For example, take emulsification: this is of utmost importance to the use of such protein in salad dressings. We're also optimistic that these powders can be used in baking high-protein bread and there are many other possibilities.

"One of these is the use of blood protein in edible meat products. As many of you know this use is not new; blood has been collected for many years in Europe, generally for the production of speciality goods such as blood sausage. And some blood is collected in this country for the same purposes.

"But the odorless, tasteless white powder we extract from blood, with 95 percent protein, permits use in a wide range of food products," added Dill.

"We have determined that up to 10 percent of the plasma isolated can be substituted for the flour in bread dough," says Dr. Lloyd Rooney, cereal chemist with the Experiment Station.

"Not only is loaf volume increased by 16 to 18 percent but there is also an improvement in crumb texture and most of the other characteristics of a quality loaf of bread.

Adding only 1.5 ounces of the plasma protein to the ingredients of a 1-pound loaf boosts protein content by 50 percent, compared to regular white pan bread," according to Rooney and Dr. M. N. Khan of the Cereal Chemistry Laboratory at Texas A&M University.

These results have very significant implications in terms of world-wide nutrition because some form of bread is eaten almost universally.

Still another use for the blood plasma protein is as a food binder in ground meat products. Even 1 percent of the plasma protein makes a hamburger patty hold together better.

"From preliminary studies on frankfurters and extended meat products, we can see a unique role for plasma proteins. In addition to the characteristics already mentioned, plasma forms a gel that is heat stable," Dill says.

"It will restore the 'bite' or 'snap' of all-meat products while assisting in emulsifying, water-binding, and improving the nutritional qualities.

"Such protein-to-protein interaction can be of significance in stabilization of fluid systems, cheese-making, puddings, gels, and frankfurter manufacture.

"We should emphasize that the food industry, and particularly the meat industry, are still far away from full utilization of the available volumes of blood.

"To be used as a 'food' grade protein, any product such as these isolates must be approved by the Animal and Plant Health Inspection Service for a specific use. When this is done, the technology is now available to make much better use of this immense store of quality protein," Dill concluded.

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Selective Action of Sodium Cholate-$MgCl_2$ Broth and its Possible Use in Isolation of Salmonellae and Other Enteric Pathogens

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ABSTRACT

Numerous chemical and biological compounds were screened for possible use as selective inhibitors in *Salmonella* enrichment broths. Efforts were directed primarily at certain strains of *Enterobacter* which often interfere with isolation of *Salmonella* from some food products. Tests of various bile compounds showed that sodium cholate had such a potential and that its selective qualities were enhanced when used in conjunction with $MgCl_2$. Studies were conducted with various proportions of the two compounds in tryptic-soy broth, using pure cultures and mixed cultures in broth alone and in the presence of food material. In general, *Salmonella, Arizona*, and *Shigella* were less inhibited that other *Enterobacteriaceae*. Levels of cholate-$MgCl_2$ which strongly inhibited other organisms also prevented growth of some strains of *Salmonella* when only a few cells were inoculated into the medium. When the concentrations of sodium cholate and magnesium chloride were adjusted to permit growth of the very low inocula of all 39 test strains of *Salmonella*, the selectivity was reduced; however, growth and inhibitory results still compared favorably with those of other presently recommended selective broths. In tests using chicken and ground beef as typical food products materials, the need for more than one selective medium was reaffirmed.

During the course of experiments involving recovery of *Salmonella* on selective agar formulations, we examined various food products seeded with *Salmonella*. When foods such as dried chicken soup, non-fat dry milk, egg powders and fresh poultry were seeded and carried through recommended enrichment procedures (*7*), detection of *Salmonella* on selective agars was often difficult. Plates were frequently crowded with extraneous organisms that either masked or overgrew the smaller number of salmonellae. The predominant organisms present in these instances caused an overall acid reaction on selective agars such as brilliant green (BBL), brilliant green sulfa (Difco), Hektoen enteric (Difco) and one or more of the new formulations of the authors (*6*). Microscopic examination and biochemical tests indicated that these organisms were predominatly strains of *Enterobacter aerogenes*. The presence of *Citrobacter* and *Proteus* also was noted.

In view of the relatively poor performance of conventional enrichment broths, we surveyed a variety of chemical compounds to determine if better inhibition of interfering bacteria could be attained. We found that a combination of sodium cholate and magnesium chloride was inhibitory to many of the types of bacteria that interfere with detection of *Salmonella* and was relatively well-tolerated by intestinal pathogens such as *Salmonella, Shigella* and *Arizona*. Neither sodium cholate nor magnesium chloride was by itself particularly inhibitory, however. To our knowledge, sodium cholate by itself has not been previously used as a component of bacteriological media nor has the synergistic effect of the sodium cholate-magnesium chloride combination previously been reported. Leifson (*3*) reported a synergistic effect with sodium desoxycholate and sodium chloride and Tutumi et al. (*17*) recently reported a synergistic action between sodium cholate and sodium chloride in inhibiting *Staphylococcus epidermidis*. Magnesium chloride has been used by Rappaport et al. (*11,12*), and Padron and Dockstader (*9*) in media for isolation of salmonellae.

The present paper describes investigations on the inhibitory properties of the sodium cholate-magnesium chloride medium and its application as an enrichment broth for isolation of *Salmonella*.

MATERIALS AND METHODS

Reagents and media

The following were used: sodium cholate (Nutritional Biochemicals Co.), magnesium chloride ($MgCl_2 \cdot 6H_20$) reagent grade from several sources, tryptic-soy broth (TSB) (Difco), lactose broth (Difco), selenite brilliant green sulfa broth (Baltimore Biological Laboratories) (BBL), tetrathionate broth base (Difco), selenite-cystine (SC) broth (Difco), and Hektoen enteric agar (HE) (Difco). Tryptic-soy brilliant green (TSBG) and tryptic-soy xylose lysine (TSXL) agars were prepared as previously described (*6*), TSBG with lactose (8 g/l) and brilliant green (BG) (5 mg/l), TSXL without lactose and with BG (8 mg/l). Neutral red-lysine-iron-cystine (NRLIC) broth was prepared as described by Hargrove et al. (*2*).

Preparation of sodium cholate-magnesium chloride broths

Separate stock solutions of magnesium chloride and sodium cholate were used to permit adjustment of formulas. Sodium cholate (20 g) was dissolved in 60 ml of hot distilled water, giving a clear dark yellow

solution which was diluted to 100 ml in a volumetric flask. $MgCl_2 \cdot 6H_2O$ (80 g) was dissolved in about 40 ml of distilled water and diluted to 100 ml in a volumetric flask. The solutions were stable for several weeks if refrigerated. The final pH values of the sodium cholate and $MgCl_2$ solutions were 7.92 and 5.10, respectively, at 23 C.

To formulate sodium cholate-$MgCl_2$ broths, tryptic-soy broth was prepared, sterilized at 121 C for 15 min and cooled. Sodium cholate and $MgCl_2$ were aseptically added to the tryptic-soy broth from the stock solution in amounts calculated to give the desired final concentrations. Final pH values ranged from 6.5 (0.3%-4.4%) to 7.5 (4.0%-1.8%).

Bacterial cultures

Stock cultures used included 39 strains of *Salmonella* and representative strains of *Arizona, Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Serratia, Shigella,* and *Pseudomonas aeruginosa.*

Screening of test media

The test broths (10 ml in tubes) were inoculated with a loopful of 24-h TSB cultures of representative strains of the test bacteria. Tubes of TSB were similarly inoculated as controls. The tubes were observed after 18-24 h of incubation at 37 C for growth as indicated by appearance of turbidity.

Recovery of salmonella grown in competition with other organisms in various enrichment broths.

Broths used were lactose, selenite-cystine (SC), selenite-brilliant green-sulfa (SBGS), tetrathionate (TT), and sodium cholate $MgCl_2$ (0.2%-0.2%) in tubes containing ca. 10 ml. Test *Salmonella* were *S. typhimurium, S. senftenberg,* and lactose (+) *S. anatum*. Competir organisms were *Escherichia coli, Enterobacter aerogens* (food isolate, *Enterobacter cloacae, Proteus mirabilis,* and *Citrobacter* sp. These were grown 24 h at 37 C in TSB and diluted in 0.1% peptone. For each experiment tubes were inoculated with 0.2 ml of a 10^{-8} dilution of *Salmonella* and 0.2 ml of either 10^{-6} or a 10^{-2} dilution of the other organisms. This gave ratios of *Salmonella:* competing organisms of approximately 1:100 and $1:10^6$, respectively. Each broth was also inoculated with each organism alone at the same dilutions. These broths were incubated 24 h at 37 C and streaked onto TSBG, TSXL, and Hektoen and enteric agars. The plates were observed after 24 h at 37 C and the appearance of characteristic *Salmonella* colonies on one or more of the agars was considered a positive test. Since pure cultures were used, further identification of characteristic *Salmonella* colonies was considered unnecessary.

Application to food products

Concurrently, 25-g samples of raw chicken giblets and raw ground beef were inoculated with 0.2 ml of a 10^{-8} dilution of *Salmonella typhimurium* (approximately 10 cells). The samples were covered and refrigerated for 30 min, allowing the bacterial suspension to permeate the food material. Uninoculated controls were treated similarly. The meat samples were blended for 2 min in sterile blender jars with the following broths: lactose, SBSG, TT, SC, NRLIC (*2*), sodium cholate (0.4%)-$MgCl_2$(0.4%) and sodium cholate (2.0%)-$MgCl_2$ (0.6%). The mixtures were then incubated at 35-37 C for 18-24 h. Following incubation the broths were streaked onto TSBG agars. Also, 0.5 ml of the lactose broth was transferred to each of 10-ml tubes of SBGS, TT, SC, NRLIC and the two sodium cholate-$MgCl_2$ formulas as selective enrichments. These were incubated at 35-37 C for 24 h, after which time they were streaked onto TSBG and TSXL selective agars. Typical and atypical colonies were picked from the agar plates and transferred to triple sugar-iron and lysine-iron agar slants, and further confirmed by biochemical tests.

RESULTS AND DISCUSSION

During the general screening procedure for an inhibitory medium, older, accepted broths as well as more recent formulations were given consideration. In addition, a variety of metal salts, dyes, antibiotics and various bile salt preparations were tested. Of these, certain bile salts showed the most promise.

According to MacConkey (*5*) bile salts have been used in bacteriological media since about 1900. Since then numerous media have been described which contain such ingredients as "bile", "bile salts", "bile salts mixture" and "oxgall." Some workers have selected specific refined versions such as sodium desoxycholate, sodium glycotaurocholate or sodium taurocholate. According to Lominiski and Lendrum (*4*) the surface active properties of bile salts are responsible for suppression of swarming of *Proteus* by these compounds.

We tested the inhibitory properties of all these bile compounds and also sodium cholate at concentrations ranging from 1.0-20.0% in basal broth. Most all of the compounds tested either demonstrated little or no inhibition of *Enterobacter* and other non-salmonella organisms, or were insoluble at the higher concentrations used. Sodium desoxycholate demonstrated some apparent inhibition but was not as effective as sodium cholate. Also, the functional properties of the desoxycholate were adversely affected with the addition of $MgCl_2$. Usually precipitation and solidification occurred, rendering the compound useless as a broth. This did not occur at the same concentration levels with sodium cholate.

We found no published reports of the previous use of sodium cholate as a standard microbiological ingredient. Levels of 5.0, 10.0 and 20.0% tended to inhibit the *Enterobacter* strain. Of all compounds examined, sodium cholate and bile salts #3 (Difco) appeared to show the most promise. The need for concentrations of up to 20% prompted the search for some other material which would complement the inhibitory effect of these bile salts, thereby reducing the quantity needed. Pilot tests in which $MgCl_2$ was combined with sodium cholate and bile salts #3 showed that at certain relative concentrations of each, desirable selectivity could be retained using substantially less bile salts. Problems of solubility and precipitation arose with the use of bile salts #3-$MgCl_2$ combination. Therefore sodium cholate was chosen as the more acceptable compound.

Many tests were conducted to determine the most effective ratio of sodium cholate to $MgCl_2$, and the best method of combining the two materials. The chemicals could not be heated together in solution since a heavy precipitate formed. The most feasible method was to sterilize the tryptic-soy broth (Difco) at 121 C for 15 min and then add sodium cholate and $MgCl_2$ to the desired concentration from stock solutions of 20% w/v respectively. At the onset of the work the stock solutions were stream-steamed, but this procedure was later eliminated when proven unnecessary.

Individually, sodium cholate inhibited only one of the non-salmonellae and $MgCl_2$ had little if any effect (Table 1). Combinations of the two, sodium cholate (4.0%)-$MgCl_2$ (1.8%) and sodium cholate 0.3%)-$MgCl_2$ (4.4%) were far more inhibitory than either compound alone

at that same respective concentration, and showed desirable selectivity for *Salmonella*. Higher combined levels of sodium cholate-$MgCl_2$ (not shown) were excessively toxic for *Salmonella* and tended to form precipitates. When $MgCl_2$ levels exceeded 1.0%, any increase in the sodium cholate concentration above 4.0% did not increase the selectivity of the broth.

An ideal enrichment broth should support vigorous growth of low levels of all strains of *Salmonella* as well as inhibit growth of competing organisms. Table 2 summarizes the results obtained when small numbers (1-6) of 39 strains of *Salmonella* were inoculated into various enrichment broths. All grew well in lactose and sodium cholate-$MgCl_2$ (0.2%-0.2%). In other broths, the number of strains allowing no growth were only one in tetrathionate, seven in SBGS, 14 in SC, and eight in the more selective sodium cholate-$MgCl_2$ (2.0-0.6%). At the low levels of inoculum used, it appears possible that some tubes might have failed to receive any of the few viable organisms present. However, no skips occurred with lactose or the weaker sodium cholate-$MgCl_2$ formulations, which would suggest that this was not a factor in the results.

The effect of various enrichment broths on growth of *Salmonella* in competition with other organisms also were evaluated (Table 3). Recovery of characteristic *Salmonella* colonies on one or more of the agars was considered a positive test. The mildly selective sodium cholate-$MgCl_2$ (0.2%-0.2%) was used along with SC, TT, SBGS and lactose broths. At the 1:100 ratio, salmonellae were recovered from the sodium cholate broth in all instances and in all but two instances from the lactose broth. More recoveries were made from this sodium cholate broth at the $1:10^6$ ratio than with any of the other enrichments broths. Recovery of *Salmonella* from enrichment broths seemed to depend both on the *Salmonella* strain present and the nature of the competing organisms.

In two instances, salmonellae were recovered at the 10^6 level, but not at the 10^2. This may reflect the occasional failure of small numbers of salmonellae to grow in some enrichment broths. At the level of inoculum used, the *Salmonella* cultures invariably grew in lactose broth in the absence of competing organisms.

The sodium cholate formulations were also evaluated in comparison with other enrichment broths for recovery of *Salmonella* from artifically and naturally contaminated food samples. Sodium cholate-$MgCl_2$ (0.2%/0.2%) did not prove sufficiently inhibitory to other organisms in food samples to be useful, so two more selective formulations, 0.4%-0.4% and 2.0%-0.6%, were used.

Table 4 presents data obtained from attempts to recover *Salmonella* from food material to which known numbers of the organisms were seeded. The chicken was ground giblet material in which spreading, lactose positive organisms were abundant. For this material both

TABLE 1. *Inhibition characteristics of sodium cholate and magnesium chloride at two levels, separate and combined[a] in Tryptic Soy Broth (TSB), (Difco).*

Organism[b]	TSB alone	Sodium cholate (0.3%)	Sodium cholate (4.0%)	$MgCl_2$ (1.8%)	$MgCl_2$ (4.4%)	Sodium cholate (0.3%), $MgCl_2$ (4.4%)	Sodium cholate (4.0%), $MgCl_2$ (1.8%)
Arizona spp	+++[c]	+++	++	+++	+++	+++	++
Citrobacter spp	+++	+++	++	+++	+++	—	—
Enterobacter aerogenes (ATCC 15038)	+++	+	—	++	++	—	—
Enterobacter aerogenes (food isolate)	+++	+++	+++	+++	+++	—	—
E. cloacae	+++	+	—	++	++	—	—
Escherichia coli	+++	++	++	+++	+++	—	—
Klebsiella pneumoniae	+++	+++	+++	+++	+++	—	—
Proteus vulgaris	+++	+++	+	+++	+++	—	—
Pseudomonas aeruginosa	+++	+++	++	+++	+++	+	+
Salmonella anatum	+++	+++	+++	+++	+++	+++	+++
S. typhimurium	+++	+++	+++	+++	+++	+++	+++
Serratia marcescens	+++	+++	+++	+++	+++	—	—
Shigella sonnei	+++	+++	+++	++	++	+	+

[a]Incubated for 18-24 h at 35-37 C.
[b]Inoculation rate: One loopful undiluted 24-h TSB culture per 10 ml of broth.
[c]Abbreviations: based on turbidity of broth, +++, heavy growth; ++, moderate growth; +, slight growth; —, no growth.

TABLE 2. *Survival and/or growth of laboratory strains of* Salmonella *in several broths incubated 18-24 h at 35-37 C.*

	Lactose	SBGS[a]	SC	TT	Sodium cholate (0.2%)/ $MgCl_2$ (0.2%)	Sodium cholate (2.0%)/ $MgCl_2$ (0.6%)
No. strains tested[b]	39	39	39	39	39	38[c]
No. showing good growth	39	31	23	36	39	22
No. showing fair growth	0	1	2	2	0	8
No. showing no growth	0	7	14	1	0	8

[a]SBGS - selenite brilliant sulfa broth (BBL); SC - Selenite-cystine broth (Difco); TT - Tetrathionate broth base (Difco).
[b]Inoculation rate: 0.2 ml of 10^{-8} dilution per 100 ml of broth (1-6 cells).
[c]One species lost and unrecoverable during course of experiment.

formulas of the sodium cholate-$MgCl_2$ were effective as direct enrichments and allowed recovery of *Salmonella*. SBGS also yielded a few salmonellae on one type of agar. The results were quite different following lactose pre-enrichment. There TT broth gave good recovery on both agars as did sodium cholate-$MgCl_2$ (2.0%-0.6%) on one agar. Scant growth of *Salmonella* was noted from the two selenite media and one from the remaining two broths. All streaked plates were crowded with extraneous organisms. Pre-enrichment in lactose broth is designed to permit recovery of *Salmonella* stressed by heat or drying (*8*) and may not be advantageous in all situations (*14*). The ground beef samples, which were treated in the same manner as the chicken, contained substantially less interfering flora. The degree of *Salmonella* recovery reflects this, both with the pre-enrichment and enrichment from lactose. Salmonellae were isolated at both stages with the sodium cholate-$MgCl_2$. These data are indicative of the problems associated with *Salmonella* isolation from food products; the type of food, initial

TABLE 3. *Comparative recovery of* Salmonella *in mixed cultures from several broths.*

Organsm	Broths									
	Lactose[a]		SBGS[b]		SC		TTw/OBG		Sodium cholate $MgCl_2$-0.2%/0.2%	
	1:100	$1:10^6$[c]	1:100	$1:10^6$	1:100	$1:10^6$	1:100	$1:10^6$	1:100	$1:10^6$
S. typhimurium with:										
Escherichia coli	+[d]	—	+	+	+	—	+	+	+	+
Enterobacter cloacae	+	+	+	+	+	+	+	+	+	+
Enterobacter aerogenes	+	—	+	+	+	+	+	+	+	+
Proteus mirabilis	+	+	+	+	+	—	+	—	+	+
Citrobacter spp.	+	+	—	+	+	+	+	+	+	+
S. senftenberg with:										
E. coli	+	—	+	+	+	—	+	+	+	+
E. cloacae	+	+	+	+	+	—	+	+	+	+
E. aerogenes	+	—	+	+	+	—	+	+	+	+
P. mirabilis	+	+	+	—	+	+	—	—	+	+
Citrobacter spp.	+	+	+	—	+	—	—	—	+	+
S. anatum (lactose *positive*) *with:*										
E. coli	+	—	—	—	+	—	+	+	+	—
E. cloacae	—	—	+	—	—	+	+	+	+	+
E. aerogenes	—	—	—	—	—	—	+	+	+	+
P. mirabilis	+	+	+	—	+	+	+	+	+	+
Citrobacter spp.	+	+	—	—	+	—	+	—	+	—

[a]Incubation: 18-24 h at 35-37 C.
[b]See Footnote, Table 2.
[c]Test organisms grown 24 h at 35-37 C in tryptic soy broth. 1:100 = 0.2 ml. 10^{-8} dilution *Salmonella*, and 0.2 ml 10^{-6} dilution other organisms in 10 ml indicated broth; $1:10^6$ = 0.2 ml 10^{-8} dilution *Salmonella*, 0.2 ml 10^{-2} other organism in 10 ml indicated broth.
[d]Recovery of *Salmonella* determined by streaking on TSXL, TSBG and Hektoen agars after 18-24 h. Appearance of characteristic *Salmonella* colonies on one or more agars +; no characteristic colonies —.

TABLE 4. *Recovery of* Salmonella *from two inoculated food products using several broths.*[a/]

	Chicken[b]		ground beef[b]	
	Salmonella recovered on:		*Salmonella* recovered on:	
	TSBG[c/]	TSXL	TSBG	TSXL
Pre-enrichment[d]				
Lactose	—[e]	—	—	—
SBGS	+	—	++	++
SC	—	—	++	++
TT w/o BG	—	—	++	++
NRLIC	—	—	—	—
Sodium cholate/ $MgCl_2$ (0.4/4.0%)	++	++	—	++
Sodium cholate/ $MgCl_2$ (0.2/0.6%)	++	++	++	++
Enrichment from lactose[d/]				
SBGS	—	+	—	—
SC	—	+	++	++
TT w/o BG	++	+	++	++
NRLIC	—	—	—	—
Sodium cholate/ $MgCl_2$ (0.4/0.4%)	—	—	++	++
Sodium cholate/ $MgCl_2$ (2.0/0.6%)	—	++	++	++

[a]Inoculation rate: 0.1 ml 10^{-8} dilution of *S. typhimurium* per 25-g sample.
[b]Food samples were 25 g in 225 ml broth.
[c]TSBG-Tryptic-soy brilliant green agar; TSXL-Tryptic soy xylose lysine agar.

bacterial load, type of competing organism and individual characterisitics of the strain of *Salmonella* encountered (*1,13,14*). The different preferences and sensitivities of the many microorganisms encountered in such work illustrate the need for more than a single procedures or medium; hence the recommendations of workers in the field for use of complementary enrichment media (*7,10,15*).

We feel that enough work has been done thus far to show that sodium cholate-$MgCl_2$ broth may be a useful tool for isolation of *Salmonella* and to warrant further investigation in this direction. Shigellae, which are strongly inhibited in most selective broths (*16*), appear to grow fairly well in sodium cholate-$MgCl_2$, indicating that this medium may be useful for isolation of this group of bacteria.

REFERENCES

1. Banwart, G. J., and J. C. Ayres. 1953. Effect of various enrichment broths and selective agars upon the growth of several species of *Salmonella*. Appl. Microbiol. 1:296-301.
2. Hargrove, R. E., F. E. McDonough, and R. H. Reamer. 1971. A selective medium and presumptive procedure for detection of *Salmonella* in dairy products. J. Milk Food Technol. 34:6-11.
3. Leifson, E. 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. 4:581-599.
4. Lominiski, I., and A. C. Lendrum. 1942. The effect of surface active agents on *B. proteus*. J. Pathol. Bacteriol. 54:421.
5. MacConkey, A. T. 1908. Bile salt media and their advantages in some bacteriological examinations. J. Hyg. (Camb.) 8:322-334.
6. Moats, W. A., and J. A. Kinner. 1976. Observations on brilliant green agar with an H_2S indicator. Appl. Environ. Microbiol. 31:380-384.
7. National Academy of Science - National Research Council. 1971. Reference methods for the microbiological examination of foods. NAS publ.
8. North, W. R., Jr. 1961. Lactose pre-enrichment method for isolation of *Salmonella* from dried egg albumen. Its use in a survey of commercially produced albumen. Appl. Microbiol. 9:188-195.
9. Padron, A. P., and W. B. Dockstader. 1972. Selective medium for hydrogen sulfide production by salmonellae. Appl. Microbiol. 32:1107-1112.
10. Randon, M., V. Fuchs, D. Dejerano, and S. F. Finsterbusch. 1970. The comparative efficacy of selenite and tetrathionate media in th isolation of *Salmonella* from some foods and feeds of animal origin. Refuah. Vt. 27:14-18.
11. Rapport, F., N. Konforti, and B. Navon. 1956. A new enrichment for certain salmonellae. J. Clin. Pathol. 9:261-266.
12. Rapport, F., and N. Konforti. 1959. Selective enrichment for paratyphoid bacteria. Appl. Microbiol. 7:62-66.
13. Silliker, J. H., and W. I. Taylor. 1958. Isolation of salmonellae from food samples. II. Effect of added food samples upon the performance of enrichment broths. Appl. Microbiol. 6:228-232.
14. Silliker, J. H., R. H. Deibel, and P. T. Fagan. 1964. Isolation of salmonellae from food samples. VI. Comparison of methods for isolation of *Salmonella* from egg products. Appl. Microbiol. 12:224-228.
15. Taylor, W. I., and J. H. Silliker. 1961. Isolation of salmonellae from food samples. IV. Comparison of methods of enrichment. Appl. Microbiol. 9:484-486.
16. Taylor, W. I., and B. Harris. 1965. Isolation of shigellae. II. Comparison of plating media and enrichment plates. Amer. J. Clin. Pathol. 44:476-479.
17. Tutumi, M., S. Takoda, E. Shozaki, and W. Watanabe. 1976. The effect of cholate on *Staphylococcus epidermidis*. I. Synergistic action of cholate and sodium chloride. Eiyo to Shokunyo 29:17-21.

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Staphylococcal Enterotoxin A and C Production with Various Sugars as Energy Source [1]

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ABSTRACT

One percent added sugar (sucrose, maltose, lactose, glucose and glucose plus fructose) to casein hydrolysate (NAK) medium with or without starch or low methoxyl pectin did not significantly affect enterotoxin C production but did significantly decrease the pH at 12 and 24 h. The increased viscosity of colloidally thickened media resulted in decreased aeration in shaken cultures and decreased quantities of enterotoxin C. Sugars tested did not differ practically in their effects on the parameters of multiplying *Staphylococcus aureus* 361 and 265-1 which were measured. Addition of 1% of glucose, sucrose, or maltose prevented the decreased growth rate observed when strain 265-1 was grown in 20% O_2 + 80% N_2 instead of air.

Staphylococcus aureus enterotoxins appear to be the leading cause of foodborne illness in the United States, although documentation is difficult since the actual number of cases is impossible to estimate (*2*). Foods which are involved include not only cooked meats and protein-containing salad mixtures but also starch-thickened products such as sauces and gravies and pie and other cream fillings. Improper holding temperatures are the most common contributing factor (*2*).

Starch-thickened dessert products generally contain sugar as one ingredient. The effect of sugars on production of enterotoxin has been most extensively studied for type B. The repressive effect of glucose on staphylococcal enterotoxin B production has been well documented for growing cells (*7,8,12,13*). Jarvis et al. (*8*) found strains producing A, B, and C formed less enterotoxin in a medium with 0.1 M glucose with either controlled or uncontrolled pH.

Since an increasing variety of sugars is being used in dessert-type products, we decided to study the effects of sucrose, maltose, glucose, glucose plus fructose, and lactose on production of enterotoxins which have been frequently implicated in foodborne illness, types A and C. All of these sugars are metabolized by *S. aureus*. A degree of colloidal complexity typical of a food product was provided by adding cornstarch or a low methoxyl pectin to the conventional liquid medium for growing a strain producing type C enterotoxin.

In addition, it was of interest to determine whether or not the effect of sugars in a liquid medium varied with the level of oxygenation as determined by growth and enterotoxin production of a strain producing type A enterotoxin. The gaseous environment was varied by bubbling of gas mixtures containing 5 and 20% oxygen in nitrogen through the cultures during incubation.

MATERIALS AND METHODS

Strains

The *S. aureus* 361 culture, a strain that produces enterotoxin C_2, obtained from M. S. Bergdoll (Food Research Institute, University of Wisconsin, Madison), was preserved on porcelain beads (*6*) for use in this study. The 265-1 culture, a strain producing enterotoxin A, obtained from R. W. Bennett (Food and Drug Administration, Washington, D.C.), was used for the limited oxygen study and also held on beads.

Colloidal ingredients

Cornstarch and pectin pastes were made with 4% NZ Amine-NAK (Humko Sheffield, Lyndhurst, N.J.) supplemented with 0.5 μg of thiamin per ml and 10 μg of niacin per ml. The pH of the medium was 6.8. The cornstarch was obtained from Corn Products Refining Co. (Argo, Ill.) and low methoxyl pectin (LMP) (unstandardized product with no compounds added) was obtained from Sunkist Growers (Corona, Calif.).

Starch paste was made by adding 14.6 g of cornstarch to 300 ml of NAK medium which had been preheated to 80 C and raising the temperature to 90 C while stirring constantly. Samples of 50 ml were autoclaved for 15 min at 121 C. Reducing sugars, expressed as glucose, were present at 0.75% level after this treatment. For pectin paste, a slurry of 8 g of LMP in 95% ethanol was made before addition to 388 ml of medium. Samples were divided so as to be 50 g after adjustment of pH and calcium ion concentration. After 1 min of autoclaving at 121 C, the pH was adjusted to 6.8 with a predetermined amount (7.3 ml) of standardized sterile 0.1 N NaOH. Sterile 0.05 M $CaCl_2$ (4.0 ml) was added to each flask.

Both the cornstarch and pectin dispersions were pastes which did not gel. Viscosities as determined with a Brookfield viscometer were 2100 cp before shaking and 400 cp with shaking for the cornstarch system and 510 cp and 89 cp, respectively, for pectin.

Sugars

The sugars chosen for this study were glucose, sucrose, maltose, lactose and an equal molar mixture of glucose and fructose. (All were from Difco, Detroit, Mich.) Only the first three sugars were used in the

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oxygen study. Sterile sugar solutions were added aseptically to sample flasks after autoclaving so that the final concentration of added sugar would be 1%.

Inoculation

Media which were 24 h old and had been held at 37 C a minimum of 15 h, either shaken or still, were inoculated with 0.5 ml of a dilution of a 24-h NAK broth culture to give an inoculum level of 700 organisms per ml in each flask. In the oxygen study, all flasks were equilibrated at 37 C with the appropriate gases for 1 h before being inoculated with strain 265-1 to the level of 1,000 organisms per ml in each flask.

Incubation

After inoculation, flasks were incubated at 37 C for both still and shaken (gyratory water bath shaker, New Brunswick Scientific Co., New Brunswick, N. J., 200 rpm) samples. For shaken samples, 300-ml triple-baffled shake flasks (Bellco Glass, Inc., Vineland, N.J.) were used and for still samples, 250-ml Erlenmeyer flasks. In the aeration study in which all samples were shaken, 250-ml Erlenmeyer flasks fitted with two-hole rubber stoppers for inlet and outlet of gases were used. Gas mixtures of 5% and 20% oxygen in nitrogen, saturated with water vapor, were filtered and bubbled through the flasks.

Sampling

Six-ml samples of the strain 361 culture were removed at 6, 12, and 24 h. For the oxygen study, data are reported for sampling hours 3, 5, 7, 11, and 16.

Colony-forming units (CFU) were determined by direct plating of appropriate dilutions on Plate Count Agar (Difco). After the pH was determined, samples were heat-treated at 50 C for 10 min and centrifuged for 10 min at 1,200 × *g* to obtain supernatant fractions for enterotoxin determinations. Crowle's micro-slide gel double-diffusion technique, as modified by Casman et al. (*1*), was used to estimate enterotoxin levels before Oudin assay and for samples containing too little enterotoxin to quantitate by the single gel diffusion method. Samples giving negative results were concentrated with Aquacide (Calbiochem, Los Angeles, Calif.) and retested. The detection limit for the micro double-diffusion technique was 0.2 μg/ml in the concentrated supernatant.

The Oudin single gel diffusion method, as described by Hall et al. (*5*), was used for quantitative assay. The minimal concentration for which this method could be used reliably was 2 μg/ml.

The purified enterotoxins A and C used for these assays were provided by E. J. Schantz and M. S. Bergdoll (Food Research Institute, University of Wisconsin, Madison), respectively. Antiserum for A was produced in our laboratory; that for C was provided by M. S. Bergdoll.

Statistical analyses

The experiment with strain 361 and five carbohydrate variables was designed as a split plot with a balanced incomplete block design as whole plot units. Specifically, the whole plots were Plan 11.4 of Cochran and Cox (*4*). The incomplete block design was necessary because shaker space was sufficient to allow only one-half of the desired experiment to be done on a given day. The six treatments were four sugars, a combination of two sugars, and no sugar. There were five replications. The analysis recovered the interblock information on the effects of sugars. The split plot treatments were combinations of three media (NAK alone, NAK + LMP, and NAK + cornstarch) and shaken or still incubation. Observations included pH and CFU at 6, 12, and 24 h and enterotoxin level at 12 and 24 h. Each of the eight measurements was analyzed separately. A logarithmic transformation was applied to CFU. Comparisons between treatments were made using the Hartley sequential variant of Newman-Keuls method of multiple comparisons (*15*).

The aeration study with strain 265-1 was designed as a complete block with three replications. An analysis of variance was done.

RESULTS

Effect of sugars

The most marked effect of addition of sugars was observed on the pH. The special nature of the sample with no sugar could be expected to, and did, produce significant interactions of sugars and combinations of media and incubation state. The interaction of sugar with incubation and that of sugar with incubation and media were essentially eliminated in analyses excluding the no-sugar samples.

The presence of 1% of any of the sugars added, all of which are commonly used as food ingredients, resulted in significantly (1% level) decreased pH of the staphylococcal cultures after both 12 and 24 h of incubation (Table 1). Several statistically significant differences among sugars were also observed.

CFU were significantly greater (1% level) for all cultures in sugar-containing media at 12 h (Table 1). However, the difference was of little practical importance since in all instances excellent growth occurred. After 24 h, the differences were no longer significant.

However, production of enterotoxin at 12 or 24 h was not significantly different in the presence of sugars, as is evident from the mean values for enterotoxin (Table 1). Differences were also not significant at the 5% level. Toxin was detectable after 12 h in all media. After 6 h of incubation, enterotoxin was detected at very low levels occassionally in the five replications. The eight samples in this group all contained an added sugar. No one sugar appeared to support earlier production of enterotoxin.

Effect of colloidal ingredient

In the presence of LMP, the pH was significantly (1% level) higher at 12 h although not at 24 and the CFU significantly (1% level) lower at 12 and 24 h (Table 2). Correspondingly, the enterotoxin production was also least for LMP containing media. However, levels

TABLE 1. *pH, CFU, and enterotoxin in cultures of* S. aureus *361 with sugars (1%) added to culture media (means of 5 replications for each of 3 media).*[a]

Factor	Incubation time (h)	None	Lactose	Maltose	Sucrose	Glucose	Glucose + fructose
pH	6	6.84	6.79	6.80	6.81	6.77	6.74
	12	6.99^{D}	6.38^{C}	6.08^{B}	5.87^{AB}	5.75^{AB}	5.55^{A}
	24	7.80^{C}	5.52^{B}	5.29^{AB}	5.06^{A}	5.17^{A}	5.01^{A}
CFU log	6	5.45^{A}	5.84^{AB}	6.01^{BC}	6.07^{BC}	6.51^{C}	6.59^{C}
	12	8.62^{A}	9.11^{B}	9.17^{B}	9.37^{B}	9.24^{B}	9.41^{B}
	24	9.28	9.45	9.39	9.47	9.29	9.28
Enterotoxin	12	1.00	0.79	0.98	1.09	0.74	1.16
(μg/ml)	24	6.15	5.69	5.95	6.04	4.67	5.28

[a]Means, within a row, with different superscript letters are significantly different at the 1% level.

of enterotoxin at 24 h were significantly lower for each of the added colloidal ingredients only on the shaken samples. In contrast, early production may have been favored by the presence of cornstarch since seven of the eight samples which contained detectable enterotoxin C at 6 h (two shaken and four unshaken) had cornstarch as an ingredient.

TABLE 2. *pH, CFU, and enterotoxin in cultures of* S. auresu *361 with cornstarch or low methoxyl pectin added to media containing or not containing added sugar (means of 5 replications for each of 6 media).*[a]

Factor	Incubation time (h)	NAK only	NAK + Cornstarch	NAK + pectin
pH	6	6.74^{A}	6.70^{A}	6.93^{B}
	12[b]	5.82^{A}	5.72^{A}	6.24^{B}
	24[b]	5.20	5.28	5.17
CFU log	6	6.04^{A}	6.58^{B}	5.62^{A}
	12	9.24^{B}	9.55^{C}	8.67^{A}
	24	9.54^{B}	9.61^{B}	8.92^{A}
Enterotoxin (μg/ml)	12	1.19^{B}	1.17^{B}	0.52^{A}
Shaken	24	12.85^{C}	8.17^{B}	3.22^{A}
Still	24	4.04	4.08	1.43

[a]Means, within a row, with different superscript letters are significantly different at 1% level.
[b]Data only from culture media containing sugar.

Aeration

Significantly lower pH at 12 h and a more rapid rise by 24 h were found in shaken samples (Table 3). At 24 h there was a significant interaction between the colloidal ingredient and aeration. Therefore, the significance of the presence of cornstarch or LMP was separated as shown in Table 2. Shaking did not favor early production. One unshaken sample for each of the five sugars contained a trace amount of enterotoxin at 6 h; shaken samples for only two of the five sugars were also positive.

Further study of aeration was done with a strain producing staphylococcal enterotoxin A. Addition of any one of the three sugars had little effect on growth in air. However, 1% sugar greatly increased the growth in the $O_2 + N_2$ mixtures (Table 4). The sugars did not differ significantly (0.05 level) in their effect on CFU or pH at 7, 11, or 16 h nor was there a significant interaction of sugar with oxygenation or time. The atmosphere affected the

TABLE 3. *Comparison of shaken and still incubation for cultures of* S. aureus *361 (means of 5 replications for 5 added sugars in 3 media).*[a]

Factor	Incubation time (h)	Still	Shaken
pH	6	6.79	6.79
	12[b]	6.13^{B}	5.73^{A}
	24[b]	5.12^{A}	5.31^{B}
CFU log	6	6.02	6.14
	12	8.93^{A}	9.37^{B}
	24	9.14^{A}	9.58^{B}
Enterotoxin (μg/ml)	12	0.47^{A}	1.45^{B}

[a]Means, within a row, with different superscript letters to the right are significantly different at 1% level.
[b]Data only from culture media containing sugar.

CFU significantly (.01 level) and the change in pH with time (.01 level) when sampling times of 7, 11, and 16 h were compared. Because enterotoxin was at a very low level in the early sampling periods, which are of special interest in foodborne illness application, the number of positive samples are reported in Table 4.

DISCUSSION

A lower pH was produced by the cultures growing with any one of the sugars added as an energy source and cell numbers increased more rapidly, but the effect on enterotoxin C production was not statistically significant. Earlier production of enterotoxin C but not of A as indicated by levels detectable at 5-6 h by the immunological assay used was found. Although differences between sugars were statistically significant in some comparisons of pH and CFU, these were not of practical importance.

Morse et al. (*13*) reported that at glucose concentrations of 0.35% or greater enterotoxin B synthesis was repressed even if pH was controlled. Jarvis et al. (*8*) reached the same conclusion that the effect on production of enterotoxin A, B, and C was only partially due to pH changes.

Total amounts of enterotoxin C produced by 12 and 24 h increased two-to-three-fold with aeration. The lowered quantities of enterotoxin C in the systems containing cornstarch or LMP also appeared to be mediated through a depressing effect of the increased viscosity on the incorporation of air since the colloidal

TABLE 4. *CFU, pH and enterotoxin production of* S. auresu *strain 265-1 during growth in 3 atmospheres in medium with added sugars (1%).*

		Sugars											
		None			Glucose			Sucrose			Maltose		
Atmosphere	Sampling time (h)	CFU (log)	pH	Enterotoxin[a]	CFU (log)	pH	Enterotoxin[a]	CFU (log)	pH	Enterotoxin[a]	CFU (log)	pH	Enterotoxin[a]
Air	3	5.98	6.7	3/3	6.26	6.7	0/3	5.84	6.7	0/3	6.27	6.6	0/3
	5	7.62	6.8	3/3	7.91	6.6	0/3	7.82	6.7	0/3	7.89	6.5	0/3
	7	8.83	6.7	3/3	9.28	5.9	1/3	9.37	6.1	1/3	9.41	6.2	2/3
20% O_2 in N_2	3	N.D.[b]	N.D.[b]	N.D.[b]	5.25	6.6	1/3	5.56	6.6	1/3	5.41	6.6	1/3
	5	N.D.[b]	N.D.[b]	N.D.[b]	6.71	6.6	1/3	7.21	6.6	1/3	6.07	6.6	1/3
	7	4.43	6.8	3/3	8.49	6.3	3/3	8.90	6.3	3/3	8.76	6.4	3/3
5% O_2 in N_2	3	N.D.[b]	N.D.[b]	N.D.[b]	5.43	6.6	0/3	5.60	6.7	0/3	5.51	6.7	0/3
	5	N.D.[b]	N.D.[b]	N.D.[b]	7.05	6.6	0/3	7.18	6.6	0/3	6.02	6.6	2/3
	7	4.71	6.8	3/3	8.00	6.1	2/3	8.90	6.2	2/3	8.94	6.2	2/3

[a]Replications with enterotoxin production/total replications.
[b]Not determined.

effect was not evident in unshaken samples. Greater yields of enterotoxin B with the usual conditions of aeration have been generally found for growing cultures (*9,12,16*). Carpenter and Silverman (*3*), however, found an optimal dissolved oxygen level of 10% for enterotoxin and nuclease productivity.

There was a marked effect of sugars in counteracting the slower growth rate that may have been due to limitation of CO_2 in cultures with $O_2 + N_2$ atmospheres. In unpublished experiments in this laboratory, addition of 0.5 or 1.0% CO_2 to the $0_2 + N_2$ gas mixture increased growth rates to levels obtained in air for strains 265-1 and S-6 when compared at 5 and 7 h. By 24 h growth was also good in the absence of CO_2.

The influence of the composition of a food is more complex because growth of competing microflora may be affected as well as that of the staphylococci. Peterson et al. (*14*) found that 0.25 to 18% dextrose, lactose, or sucrose generally had greater inhibitory effects on the competing psychrophilic bacteria than on numbers of *S. aureus*, especially above 20 C. The differing effect of type of sugar also appeared to be related to growth of the other bacteria.

However, in cooked products such as starch-thickened puddings, the staphylococci introduced by human food handlers may greatly outnumber other non-spore forming microorganisms and competition becomes less of a factor. Instances of such a route to food poisoning have been reviewed by Minor and Marth (*11*).

A trend to earlier apparent production of enterotoxin C if cornstarch had been added to the basic protein hydrolysate medium indicates a need for further study of such food components. Very small amounts of glucose such as 0.2% (*10*) have been found to favor enterotoxin B production in fermenter cultures. The cornstarch medium contained 0.75% reducing substances expressed as glucose due to slight hydrolysis.

Perishable food which is mishandled is often eaten within less than 12-24 h at a temperature permitting staphylococcal multiplication. Therefore, the earlier detection of enterotoxin C under nonshaken conditions in the more complex media indicates that this portion of the growth period needs to be studied more extensively by food microbiologists.

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REFERENCES

1. Casman, E. P., R. W. Bennett, A. E. Dorsey, and J. E. Stone. 1969. The micro-slide gel double diffusion test for the detection and assay of staphylococcal enterotoxin. Health Lab. Sci. 6:185-198.
2. Center for Disease Control. 1976. Foodborne and waterborne disease outbreaks annual summary 1975. HEW Publ. CDC 76-8185. Atlanta, Ga.
3. Carpenter, D. F., and G. J. Silverman. 1974. Staphylococcal enterotoxin B and nuclease production under controlled dissolved oxygen conditions. Appl. Microbiol. 28:628-637.
4. Cochran, W. G., and G. M. Cox. 1957. Experimental designs, 2nd ed. John Wiley & Sons, Inc., New York.
5. Hall, H. E., R. Angelotti, and K. H. Lewis. 1963. Quantitative detection of staphylococcal enterotoxin B in food by gel-diffusion method. Public Health Rep. (U.S.) 78:1089-1098.
6. Hunt, G. A., A. Gourevitch, and J. Lein. 1958. Preservation of cultures by drying on porcelain beads. J. Bacteriol. 76:453-454.
7. Iandolo, J. J., and W. M. Shafer. 1977. Regulation of staphylococcal enterotoxin B. Infect. Immun. 16:610-616.
8. Jarvis, A. W., R. C. Lawrence, and G. G. Pritchard. 1975. Glucose repression of enterotoxins A, B and C and other extracellular proteins in staphylococci in batch and continuous culture. J. Gen. Microbiol. 86:75-87.
9. McLean, R. A., H. D. Lilly, and J. A. Alford. 1968. Effects of meat-curing salts and temperature on production of staphylococcal enterotoxin B. J. Bacteriol. 95:1207-1211.
10. Metzger, J. F., A. D. Johnson, W. S. Collins, II, and V. McGann. 1973. *Staphylococcus aureus* enterotoxin B release (excretion) under controlled conditions of fermentation. Appl. Microbiol. 25:770-773.
11. Minor, T. E., and E. H. Marth. 1976. Staphylococci and their significance in foods. Elsevier Scientific Publishing Co., New York.
12. Morse, S. A., and J. N. Baldwin. 1973. Factors affecting the regulation of staphylococcal enterotoxin B. Infect. Immun. 7:839-846.
13. Morse, S. A., R. A. Mah, and W. J. Dobrogosz. 1969. Regulation of staphylococcal enterotoxin B. J. Bacteriol. 98:4-9.
14. Peterson, A. C., J. J. Black, and M. F. Gunderson. 1964. Staphylococci in competition. IV. Effect of starch and kind and concentration of sugar on staphylococal growth in mixed populations. Appl. Microbiol. 12:77-82.
15. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods, 6th ed. The Iowa University Press, Ames, Iowa. pp. 273-274.
16. Woodburn, M., T. N. Morita, and S. Z. Venn. 1973. Production of staphylococcal enterotoxins A, B, and C in colloidal dispersions. Appl. Microbiol 25:825-833.

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Bacterial Populations of Ground Beef, Textured Soy Protein and Ground Beef Extended With Soy Protein After 3 and 10 Days of Refrigerated Storage [1,2]

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ABSTRACT

A survey of the microbial populations of 31 samples of ground beef (GB), textured soy protein (TSP), and ground beef extended with TSP (SGB) after 3 and 10 days of storage at 4 C was done. Analyses included aerobic plate count (APC), psychrotrophic plate count (PPC), coliform Most Probable Number (CMPN) and plate determinations (CPC), *Escherichia coli* MPN (EMPN) and plate determinations (EPC), *Staphylococcus aureus* MPN, and fecal streptococcus plate count. Statistical analyses of data from the enumeration procedures showed significant increases in the total microbial flora after 10 days of storage. PPCs were significantly higher than APCs. CMPNs were significantly higher than CPCs for GB and SGB. The EMPNs were significantly higher than EPCs in SGB only. These products contained a variety of microorganisms many in large numbers; however if properly handled and cooked before consumption, these products should present no public health problems.

Use of soy protein as an extender in ground meat products has increased considerably in recent years, especially in institutional feeding systems. There have been many factors, mostly economic, which have had an impact on increased use of soy protein in meat products. Red meat costs have increased to the processor and consumer; the cost of soybeans has decreased. Dollar savings are as much as 21% on a raw basis and 30% on a cooked basis when ground beef is properly extended with soy protein (*20*). Research has shown that ground beef patties containing 20% soy protein concentrate are about equal in flavor, appearance, aroma, juiciness, and overall acceptability when compared with all-beef patties (*5*). Ground beef extended with soy protein has less total shrinkage when it is cooked than regular ground beef (*3, 14, 19*). Nutritionally, ground beef extended with soy protein is equal to regular ground beef (*20*).

Although the physical and nutritional characteristics of beef/soy have been well investigated, the question of wholesomeness from a bacterial standpoint has not been answered. A review of the literature found only three studies (*4,14,16*) where bacterial analyses of ground beef extended with soy protein had been done.

In this study regular ground beef, textured soy protein, and ground beef extended with textured soy protein were analyzed to determine the microbial populations present. Additional analyses were done to determine the changes in total bacterial number after storage at at 4 C for 3 and 10 days.

MATERIALS AND METHODS

Samples

Duplicate units from 31 production lots of ground beef (GB), textured soy protein (TSP), and TSP-extended ground beef (SGB) were obtained from a production facility in the San Francisco Bay Area. The SGB was composed of 75% trim (30% fat) and 25% rehydrated textured soy protein (8.33% TSP and 16.67% water). All TSP used in this study was of one commercial type. Product temperatures during processing were: ground beef after the first grind 7 C and rehydrated TSP 16 C. These components were then mixed, chilled by injection with CO_2, and reground. The final temperature of the extended product was maintained at 2 to 4 C. After rehydration, the TSP was held from 4 to 6 h before addition to the GB. The processing plant temperature was maintained at 1 to 4 C. All units were transported to the laboratory in insulated containers. Temperature upon arrival ranged from 2 to 5 C. These units were maintained at 4 ± 1 C and analyzed after 3 and 10 days of storage from the date of production.

Sample preparation

A 25-g portion of each unit was weighed into a sterile 1-liter blender. Following addition of 225 ml of sterile phosphate buffered water, the sample was blended at high speed for 3 min. Serial dilutions from 10^{-2} through 10^{-7} were prepared.

Aerobic plate count (APC)

Duplicate plates for dilutions 10^{-1} through 10^{-7} were prepared and poured in accordance with *Bacteriological Analytical Manual for Foods* (BAM) (*2*). Plates were incubated at 32 C for 72 ± 2 h.

Psychrotrophic plate count (PPC)

Duplicate plates for dilutions 10^{-1} through 10^{-7} were prepared as in the APC procedure. Plates were incubated at 7 C for 10 days.

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Total coliform (CPC) and Escherichia coli plate count (EPC)

CPC and EPC counts were made in accordance with the procedures described in *Reference Methods for the Microbiological Examination of Foods* (*15*).

Total coliform (CMPN) and Escherichia coli MPN (EMPN] *count*

CMPN and EMPN determinations were made using the techniques described in *BAM* (*2*).

Staphylococcus aureus analyses

S. aureus MPN (SMPN) determinations were done in accordance with the AOAC method (*13*) except that tellurite polymyxin egg yolk (TPEY) agar was substituted for Baird-Parker agar. The tube coagulase test (*13*) was done as needed.

Fecal streptococcus analyses

The fecal streptococcus analyses were done in accordance with the procedures outlined in *BAM* (*2*). In addition, representative colonies from the KF streptococcal agar plates were inoculated into ethyl violet azide broth. After incubation at 35 C for 48 ± 2 h, tubes exhibiting a yellow color and sediment were reported as confirmed fecal streptococci.

Statistical analyses

Friedman's two-way analysis of variance with multiple comparisons based upon rank sums was applied to data obtained by the enumeration procedures (*12*). The Wilcoxon matched-pairs signed-ranks test was applied to determine if significant differences existed between APC and PPC, and CPC and CMPN, and EPC and EMPN (*12*). All statistical analyses were done with $\alpha = .05$ level of significance.

RESULTS

The aerobic plate count (APC) distributions for ground beef (GB), textured soy protein (TSP), and TSP-extended ground beef (SGB) for the 3- and 10-day storage times are in Table 1. Analyses of the data show that 96.8% of the GB samples at 3 days had APCs of less than 5×10^6 organisms per gram while 90.3% of the TSP and SGB samples evaluated had APCs of less than this value. After 10 days of storage at 4 C this pattern was significantly altered with 26.7, 24.1, and 10.0% of the GB, TSP, and SGB samples having APCs of less than 5×10^6 organisms per gram, respectively.

Statistical analyses of these data showed a significant increase in APCs after 10 days of storage for all products (Table 2). It is noteworthy that significant differences

TABLE 1. *Aerobic plate counts for ground beef, textured soy protein (TSP) and TSP-extended ground beef.*

Log_{10} plate count range	Ground beef				Textured soy protein				TSP Extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤ 2.7					19	61.3	1	3.5				
2.8 to 3.0					5	77.4	1	6.9				
3.1 to 3.7					1	80.7	1	10.3				
3.8 to 4.0	1	3.2			1	83.9	1	13.8				
4.1 to 4.7	7	25.8			1	87.1	1	17.2				
4.8 to 5.0	1	29.0			1	90.3						
5.1 to 5.7	13	70.9							12	38.7		
5.8 to 6.0	4	83.9					2	24.1	8	64.5		
6.1 to 6.7	4	96.8	8	26.7					8	90.3	3	10.0
6.8 to 7.0			2	33.3	1	93.5	3	34.5	1	93.6	3	20.0
7.1 to 7.7			7	56.7	1	96.8	7	58.6			9	50.0
7.8 to 8.0			4	70.0			2	65.5			6	70.0
8.1 to 8.7			5	86.7			7	89.7			7	93.3
8.8 to 9.0			2	93.3			1	93.1				
> 9.0	1	100.0	2	100.0	1	100.0	2	100.0	2	100.0	2	100.0
Total units	31		30		31		29		31		30	
Range[c]	3.9 to 9.5		6.2 to 9.5		1.5 to 9.5		1.8 to 9.5		5.3 to 9.5		6.5 to 9.5	
Mean[c]	5.4		7.5		3.0		7.0		6.1		7.7	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts.

TABLE 2. *Friedman two-way analysis of variance with multiple comparisons for ground beef (GB), textured soy protein (TSP), and TSP-extended ground beef (SGB) after 3 and 10 days of storage.*[a]

Aerobic Plate Count - TSP-3*	GB-3	SGB-3	TSP-10	GB-10	SGB-10
Psychrotrophic Plate Count - TSP-3	GB-3	SGB-3	TSP-10	GB-10	SGB-10
Coliform Plate Count - TSP-3	TSP-10	GB-3	SGB-3	SGB-10	GB-10
Coliform MPN Count - TSP-3	TSP-10	GB-3	SGB-3	SGB-10	GB-10
E. coli Plate Count - TSP-10	TSP-3	GB-10	GB-3	SGB-10	SGB-3
E. coli MPN Count - TSP-10	TSP-3	GB-10	GB-3	SGB-10	SGB-3
S. aureus MPN Count - TSP-3	TSP-10	GB-3	GB-10	SGB-10	SGB-3
Fecal streptococci Count - TSP-10	TSP-3	GB-10	GB-3	SGB-10	SGB-3

[a]Mean counts are ranked from lowest to highest (left to right).
* Products underscored by the same line are not significantly different at the .05 level.

existed between products at 3 days of storage; however, after 10 days of storage no significant differences in APCs existed for all products analyzed.

Psychrotrophic plate count (PPC) (Table 3) distributions were similar to the APC determinations. After the 3-day storage, 93.6% of the GB, 90.3% of the TSP and 87.1% of the SGB units resulted in PPCs of less than 5×10^6 organisms per gram. The 10-day determinations resulted in 10.0, 24.1, and 0.0% of the GB, TSP, and SGB with counts of less than 5×10^6/g. As found in the APC determinations, significantly higher PPCs were demonstrated after storage for 10 days at 4 C. The same pattern of differences among 3-day and 10-day determinations for APC was also found in the PPCs (Table 2).

With the exception of the 3-day GB and the 10-day TSP, statistical comparison of the APC and PPC data showed that the PPC determinations were significantly higher than the corresponding APC determinations for the products at both 3- and 10-day storage times.

Coliform plate count (CPC) distributions are in Table 4. Following the 3-day storage period 90.3, 100.0 and 80.7% of the GB, TSP and SGB samples contained fewer than 1×10^3 coliforms per gram. These percentages decreased somewhat with an additional 7 days of refrigerated storage resulting in 53.3, 96.7 and 63.3% of the GB, TSP and SGB samples, respectively, having less than 1×10^3 coliforms per gram. With the exception of the 10-day GB all of the products had total coliform counts of less than 1×10^4/g. Statistical analyses of these data showed that no significant difference existed between the counts obtained from the 3- or 10-day storage periods. Additionally, no significant difference was indicated in the coliform counts from GB and SGB (Table 2).

Coliform most probable number (CMPN) determinations (Table 5) were similar to the CPC results, however, some interesting differences were noted. Statistical analyses of these data indicated that no significant difference existed between 3- and 10-day determinations

TABLE 3. *Psychrotrophic plate counts for ground beef, textured soy protein (TSP) and TSP-extended ground beef.*

Log_{10} plate count range	Ground beef				Textured soy protein				TSP extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤ 2.7					23	74.2	1	3.5				
2.8 to 3.0					1	77.4						
3.1 to 3.7	1	3.2			2	83.9	2	10.3				
3.8 to 4.0					1	87.1	1	13.8				
4.1 to 4.7	7	25.8					1	17.2				
4.8 to 5.0	2	32.3			1	90.3						
5.1 to 5.7	12	71.0							10	32.3		
5.8 to 6.0	1	74.2							9	61.3		
6.1 to 6.7	6	93.6	3	10.0			2	24.1	8	87.1		
6.8 to 7.0	1	96.8	1	13.3	1	93.5	2	31.0			2	6.7
7.1 to 7.7			8	40.0	1	96.8	6	51.7	3	96.8	2	13.3
7.8 to 8.0			7	63.3			3	62.0			7	36.7
8.1 to 8.7	1	100.0	8	90.0	1	100.0	6	82.8			13	80.0
8.8 to 9.0			2	96.7			3	93.1			5	96.7
> 9.0			1	100.0			2	100.0	1	100.0	1	100.0
Total units	31		30		31		29		31		30	
Range[c]	3.3 to 8.4		6.3 to 9.1		<0 to 8.7		2.9 to 9.1		5.1 to 9.3		6.8 to 9.2	
Mean[c]	5.4		7.7		2.7		7.1		6.1		8.2	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts

TABLE 4. *Coliform plate counts for ground beef, textured soy protein (TSP) and TSP-extended ground beef.*

Log_{10} plate count range	Ground beef				Textured soy protein				TSP extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
< 0	5	16.1	3	10.0	28	90.3	25	83.3	1	3.2	2	6.7
0 to 0.7												
0.8 to 1.0												
1.1 to 1.7	6	35.5	1	13.3			1	86.7	2	9.7	1	10.0
1.8 to 2.0	5	51.6	5	30.0	1	93.6			6	29.0	3	20.0
2.1 to 2.7	11	87.1	6	50.0	2	100.0	3	96.7	13	70.9	10	53.3
2.8 to 3.0	1	90.3	1	53.3					3	80.7	3	63.3
3.1 to 3.7	3	100.0	7	76.7			1	100.0	5	96.8	7	86.7
3.8 to 4.0									1	100.0	4	100.0
4.1 to 4.7			5	93.3								
> 4.7			2	100.0								
Total units	31		30		31		30		31		30	
Range[c]	<0 to 3.5		<0 to 5.5		<0 to 2.3		<0 to 3.2		<0 to 3.9		<0 to 4.0	
Mean[c]	1.8		2.8		0.1		0.4		2.4		2.6	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts.

from TSP and SGB, however, the 10-day GB coliform determinations were significantly higher than the 3-day results (Table 2).

Statistical comparison of the CPC and CMPN data showed, except for the 3-day TSP determinations, the CMPN procedure resulted in significantly higher counts than the CPC procedure. Although the CMPN counts for the 3-day TSP were higher than the CPC results there was no significant difference in the counts at the 95% confidence level.

E. coli determinations were made using the plate and MPN procedures. Results of the *E. coli* plate count (EPC) analyses are in Table 6. Among the 3-day analyses only 64.5, 93.6 and 48.4% of the GB, TSP and SGB samples resulted in counts of less than 50/g. The 10-day analyses were similar with 63.3, 96.7 and 46.7% of the GB, TSP and SGB samples containing less than 50 *E. coli* per gram.

Statistical analyses (Table 2) revealed that no significant differences were present when comparing the EPCs after 3 and 10 days of storage. No significant difference was indicated between counts from GB and SGB, and GB and TSP. However, a significant difference in counts from TSP and SGB was noted.

The *E. coli* MPN (EMPN) determinations produced count distributions similar to those found in the EPC procedure (Table 7). The 3-day data show that 74.2, 100.0 and 35.5% of the counts for GB, TSP and SGB had less than 50 *E. coli* per gram. The 10-day determinations resulted in 66.7, 100.0 and 46.7% of the GB, TSP and SGB with *E. coli* counts of less than 50/g. Only 8.2% of all TSP samples tested contained *E. coli*, while 78.7 and 96.7% of the GB and SGB samples were *E. coli*-positive.

Statistical analyses of the EMPN data showed that there was no significant difference in the counts obtained after 3 and 10 days of storage; however, the 10-day determinations were lower than the 3-day determinations in all instances (Table 2). *E. coli* counts from TSP were significantly lower than counts from GB and SGB.

Comparison of the EPC and EMPN procedures by the Wilcoxon test (*12*) indicated: (a) no significant differences were shown for GB and TSP at the 3- and 10-storage intervals; (*b*) the EMPN counts for the 3- and 10-day SGBs were significantly higher than the EPC counts for the same intervals; (*c*) with the exception of the 3-day TSP, all of the EMPN determinations were higher than the corresponding EPC determinations.

S. aureus MPN (SMPN) distributions are presented in Table 8. Less than 7% of the samples for all product types and both storage times had SMPN counts in excess of 150/g. Statistical analyses (Table 2) indicated that no significant differences were found between GB and SGB

TABLE 5. *Coliform Most Probable Number (MPN) counts for ground beef, textured soy protein (TSP) and TSP-extended ground beef.*

MPN Count Range	Ground beef				Textured soy protein				TSP Extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
≤ 3	1	3.2			27	87.1	20	66.7				
3.6 to 10					1	90.3	2	73.3				
20 to 42	2	9.7					2	80.0				
43 to 64	4	22.6	1	3.3					1	3.2	1	3.3
72 to 150	3	32.3	1	6.7	1	93.5			3	12.9		
160 to 460	11	67.7	4	20.0			2	86.7	14	58.1	3	13.3
530 to 1100	2	74.2	3	30.0	1	96.7	1	90.0	7	80.7	4	26.7
1200 to 9500	5	90.3	9	60.0	1	100.0			5	96.8	7	50.0
> 11000	3	100.0	12	100.0			3	100.0	1	100.0	15	100.0
Total units	31		30		31		30		31		30	
Range[c]	<0 to 4.7		<0 to 6.0		<0 to 3.4		<0 to 4.4		1.6 to 4.2		1.6 to 6.0	
Mean[c]	2.5		3.7		0.3		0.9		2.8		3.7	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts.

TABLE 6. Escherichia coli *plate counts for ground beef, textured soy protein [TSP] and TSP-extended ground beef.*

Log_{10} plate count range	Ground beef				Textured soy protein				TSP extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
<0	13	41.9	18	60.0	29	93.6	28	93.3	9	29.0	14	46.7
0 to 0.7												
0.8 to 1.0												
1.1 to 1.7	7	64.5	1	63.3			1	96.7	6	48.4		
1.8 to 2.0	4	77.4	2	70.0			1	100.0	2	54.8	3	56.7
2.1 to 2.7	5	93.6	4	83.3	2	100.0			7	77.4	8	83.3
2.8 to 3.0			1	86.7					2	83.9	1	86.7
3.1 to 3.7	2	100.0	4	100.0					4	96.8	3	96.7
3.8 to 4.0									1	100.0	1	100.0
Total units	31		30		31		30		31		30	
Range[c]	<0 to 3.2		<0 to 3.7		<0 to 2.3		<0 to 1.7		<0 to 3.8		<0 to 3.7	
Mean[c]	1.1		0.9		0.1		0.1		1.7		1.2	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts.

for the 3- or 10-day storage time and the 3- and 10-day TSP determinations were significantly different from all other determinations.

Fecal streptococci plate count (FSC) distributions are outlined in Table 9. Only 6.4, 0.0 and 9.7% of the GB, TSP and SGB 3-day analyses produced FSCs in excess of 1000/g. For the 10-day analyses, 0.0, 3.3 and 10% of the GB, TSP and SGB samples exceeded 1000 fecal streptococci per gram. There were no significant differences indicated between counts obtained after 3 and 10 days of storage for all product types (Table 2). However, 3- and 10-day TSP determinations differed significantly from all others.

DISCUSSION

The microbial quality of raw ground beef has been well documented. In a recent report by Foster et al (*8*), studies of the microbial quality of raw ground beef for the past 63 years were tabulated. Although numerous reports pertaining to the microbial quality of raw ground beef are available, few studies have investigated the microbial quality of ground beef extended with soy protein (SGB) (*4,14,16*).

Statistical analyses of the data in Table 1 show that there was no significant difference in the APCs from GB and SGB at either 3 or 10 days of storage. However, a significant difference existed between 3-day APCs and

TABLE 7. Escherichia coli *Most Probable Number (MPN) counts for ground beef, textured soy protein (TSP) and TSP-extended ground beef.*

MPN count range	Ground beef				Textured soy protein				TSP extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
⩽ 3	4	12.9	9	30.0	28	90.3	28	93.3	1	3.2	2	6.7
3.6 to 19	12	51.6	6	50.0	2	96.8			4	16.1	1	10.0
20 to 42	6	70.9	3	60.0	1	100.0	1	96.7	2	22.6	5	26.7
43 to 64	1	74.2	2	66.7			1	100.0	4	35.5	6	46.7
72 to 150	3	83.9	5	83.3					7	58.1	7	70.0
160 to 460	5	100.0	1	86.7					6	77.4	3	80.0
530 to 1100			2	93.3					4	90.3	4	93.3
1200 to 9500			2	100.0					3	100.0	2	100.0
Total units	31		30		31		30		31		30	
Range[c]	<0 to 2.7		<0 to 3.2		<0 to 1.4		<0 to 1.6		<0 to 3.7		<0 to 3.4	
Mean[c]	1.3		1.3		0.1		0.1		2.1		1.9	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts.

TABLE 8. Staphylococcus aureus *Most Probable Number (MPN) counts for ground beef, textured soy protein (TSP) and TSP-extended ground beef.*

MPN count range	Ground beef				Textured soy protein				TSP extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
⩽ 3	11	35.5	6	20.0	30	96.8	28	93.3	2	6.5	3	10.0
3.6 to 19	8	61.3	13	63.3	1	100.0	1	96.7	7	29.0	7	33.3
20 to 42	3	70.9	5	80.0			1	100.0	7	51.6	10	66.7
43 to 64	3	80.7	3	90.0					8	77.4	4	80.0
72 to 150	4	93.6	1	93.3					6	96.8	5	96.7
160 to 460	2	100.0	1	96.7					1	100.0	1	100.0
1200 to 9500			1	100.0								
Total units	31		30		31		30		31		30	
Range[c]	<0 to 2.7		<0 to 3.4		<0 to 0.6		<0 to 1.4		<0 to 2.7		<0 to 2.4	
Mean[c]	0.9		1.0		.02		0.1		1.4		1.3	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts.

TABLE 9. *Fecal streptococci counts for ground beef, textured soy protein (TSP) and TSP-extended ground beef.*

Log_{10} plate count range	Ground beef				Textured soy protein				TSP extended ground beef			
	3 Day		10 Day		3 Day		10 Day		3 Day		10 Day	
	U[a]	CP[b]	U	CP	U	CP	U	CP	U	CP	U	CP
< 0	1	3.2	3	10.0	28	90.3	27	90.0	1	3.2	3	10.0
0 to 1.7	12	41.9	3	20.0	2	96.8	2	96.7			5	26.7
1.8 to 2.0	3	51.6	14	66.7			4	16.1	3	36.7		
2.1 to 2.7	10	83.9	8	93.3	1	100.0			15	64.5	13	80.0
2.8 to 3.0	3	93.6	2	100.0					8	90.3	3	90.0
3.1 to 3.7	2	100.0					1	100.0	3	100.0	3	100.0
Total units	31		30		31		30		31		30	
Range[c]	<0 to 3.3		<0 to 3.0		<0 to 2.2		<0 to 3.0		<0 to 3.3		<0 to 3.4	
Mean[c]	2.0		1.8		0.2		0.2		2.4		2.1	

[a]U - Number of samples within each count range.
[b]CP - Cumulative percentage of samples.
[c]Log_{10} of counts.

10-day APCs for both products. This indicates that the addition of TSP had no effect on the APC even after a 10-day storage period. This same result was found in the analyses of the PPC (Tables 2 and 3). Comparison of the APC and PPC data by Wilcoxon test (*12*) indicated that the PPCs were significantly higher than the APCs in all but two instances. This indicates that the predominant microbial flora in raw beef products is psychrotrophic in nature and that the current incubation temperatures (i.e., 30, 32 and 35 C) for testing raw meat products are questionable. This point is supported by Goepfert (*11*), Westhoff and Feldstein (*18*) and Foster et al. (*7*).

The APCs for products stored at 4 C in this study were in agreement with the findings of Judge et al. (*15*) and Craven and Mercuri (*4*). Analysis of the 3- and the 10-day APC data in this study agrees with the findings of Judge et al. (*14*) who reported significant increases in plate counts with ground beef extended with soy protein after 7 days of storage at 4 C. Additionally, they reported significant differences in plate counts initially and no significant difference in plate counts after 7 days of storage when comparing soy-extended and regular ground beef. Craven and Mercuri (*4*) showed that the APC increased faster in beef patties extended with textured soy protein than in regular ground beef patties. Also they found that the counts increased over storage time for all samples tested. Craven and Mercuri (*4*) found 2.5 to 3 $\log_{10}$ increases in the APC for hydrated textured soy protein over an 11-day storage period at 4 C.

The State of Oregon currently uses the APC guideline of 5×10^6 organisms per gram in the sanitary inspection of ground beef in retail meat stores. This guideline includes all raw meat products including ground beef extended with soy protein. Comparing the APC data to the Oregon guideline, we found that 96.8% of the GB and 90.3% of the SGB samples were in compliance after the 3-day storage period (Table 1). Comparison of the PPC data showed that 93.6% of the GB and 87.1% of the SGB samples would comply after the 3-day storage period (Table 3). After 10 days of storage the percent of samples which comply with the Oregon guideline was dramatically reduced (Tables 1 and 3). This shows that ground meat products can be produced in compliance with what some consider an extremely rigid guideline; however, as expected, these percentages are reduced with increased storage time. At this time use of microbiological standards, with all their legal and enforcement complications, to ensure the quality of various food items seem questionable. Alternatively, use of microbiological guidelines coupled with increased sanitary inspection and laboratory testing, and cooperation with the industry will result in a product of improved microbial quality and longer shelf-life.

The coliform analyses presented in Tables 4 and 5 show distributions similar to those previously reported for ground beef (*8, 11, 18*). Statistical analyses showed that no significant differences in counts existed when comparing ground beef with or without soy protein. These results are in conflict with the findings of Craven and Mercuri (*4*) who reported that coliform counts increased with the addition of soy protein in beef patties. Only with raw ground beef without soy protein did the coliform count increase significantly during refrigerated storage.

Statistical comparison of the coliform plate and MPN procedures showed that the most probable number procedure yielded significantly higher counts for all products except the 3-day TSP. Since the manufacturing steps necessary to produce TSP include high pressure heat extrusion, the result should be a product with a low bacterial load. Therefore, the fact that no significant difference existed between the MPN and plate determinations for the 3-day TSP was not unexpected.

Currently, eight states have microbial guidelines based upon coliform counts (*17*). These guidelines range from 1×10^2 to 1×10^4 organisms per gram. Fowler et al. (*9*) recently reported that a coliform plate count limit of 1×10^4/g for ground beef appears to be feasible. This recommendation was based on the analyses of 1856 samples of ground beef. Comparing the data from this study to the limit of 1×10^4/g, 100% of the GB and SGB samples after the 3-day storage period were in compliance, using the coliform plate method (Table 4). However, when utilizing the most probable number procedure, it was found that only 90.3% of the GB and 96.8% of the SGB samples would comply with the limit of 1×10^4 coliforms per gram. The percent of samples in compliance was reduced after the 10-day storage time. This finding re-emphasizes the point that ground beef is a product of limited shelf-life and that improved sanitary conditions during processing which favor lower microbial populations would be beneficial to both the consumer and producer.

The *E. coli* counts were determined by both the plate and MPN procedures. The results of the *E. coli* analyses, as determined by both procedures, indicated that addition of TSP had no effect on the *E. coli* count. Comparisons of the different procedures indicated that the MPN method gave higher counts but these counts were not significantly higher.

There are 11 states that have microbiological guidelines for *E. coli* in ground beef (*17*). These guidelines range from 0 to 1×10^3 organisms per gram. The majority of the states use the value of 50 *E. coli* per gram as their guideline. If the *E. coli* plate count data were compared to this guideline, 64.5% of the GB and 48.4% of the SGB samples would be acceptable after the 3-day storage time. These percentages changed very little after an additional 7 days of storage. Comparison of the *E. coli* MPN data was quite different, with 74.2% of the GB and 35.5% of the SGB samples in compliance after the 3-day storage time. The 10-day storage data showed 66.7% of the GB and 46.7% of the SGB samples having *E. coli* counts of 50 or less. The wide difference in the 3-day values of GB and SGB was probably due to the dilution of the GB with TSP. Only 8.2% of all TSP

samples tested contained *E. coli*, and 100% of the samples tested by the MPN method had values less than 50/g at both 3 and 10 days' storage (Table 7).

S. aureus as determined by the MPN method was found in limited numbers. Less than 7% of all the samples tested had SMPN counts in excess of 150/g. The recovery of *S. aureus* in low numbers from ground beef is in concurrence with the findings of other investigators (*1,6,8,17*). Currently, eight states have guidelines pertaining to the numbers of *S. aureus* allowable in ground beef (*17*). These limits range from 0 to 2.5×10^2 *S. aureus* organisms per gram. The samples tested in this study compared favorably to these guidelines. Although *S. aureus* is recognized as a potential food poisoning organism, to date there have been no reported cases of *S. aureus* food poisoning from ground beef. This could be attributed to the fact that this organism's ability to compete with the microbial flora of ground beef is questionable (*1,10*). However, *S. aureus* is a potential hazard and product should be handled in a manner which will minimize the possibility of the growth of this organism.

The fecal streptococcus determinations indicated that addition of TSP to GB and/or refrigerated storage did not effect an increase in this group of organisms.

CONCLUSIONS

1. Addition of textured soy protein appears to have no effect on the total microbial load of regular ground beef.
2. The coliform most probable number method produced significantly higher counts than the plate method.
3. The microflora of the products tested appears to be psychrotrophic in nature since the PPCs were significantly higher than the APCs.
4. Additional studies to evaluate the effect of TSP on specific foodborne pathogenic organisms are warranted.
5. Evaluation of currently accepted incubation times and temperatures for meat analyses is needed.

REFERENCES

1. Al-Delaimy, K. S., and M. E. Stiles. 1975. Microbial quality and shelf-life of raw ground beef. Can. J. Pub. Health 66:317-320.
2. Anonymous. 1976. Bacteriological analytical manual for foods (Fourth edition). Association of Official Analytical Chemists, Washington, DC.
3. Bowers, J. A., and P. O. Engler. 1975. Freshly cooked and cooked frozen reheated beef and beef-soy loaves. J. Food Sci. 49:624-625.
4. Craven, S. E., and A. J. Mercuri. 1977. Total aerobic and coliform counts in beef-soy and chicken-soy during refrigerated storage. J. Food Prot. 40:112-115.
5. Drake, S. R., L. C. Hinnergart, R. A. Kluter, and P. A. Prell. 1975. Beef patties: The effect of textured soy protein and fat levels on quality and acceptability. J. Food Sci. 40:1065-1067.
6. Emswiler, B. S., C. J. Pierson, and A. W. Kotula. 1976. Bacteriological quality and shelf life of ground beef. Appl. Environ. Microbiol. 31:826-830.
7. Foster, J. F., J. L. Fowler, and J. Dacey. 1977. A microbial survey of various fresh and frozen seafood products. J. Food Prot. 40: 300-303.
8. Foster, J. F., J. L. Fowler, and W. C. Ladiges. 1977. A bacteriological survey of raw ground beef. J. Food Prot. 40:790-794.
9. Fowler, J. L., D. L. Stutzman, J. F. Foster, and W. H. Langley, Jr. 1977. Selected food microbiological data collected through a computerized program. J. Food Prot. 40:166-169.
10. Goepfert, J. M., and H. U. Kim. 1975. Behavior of selected foodborne pathogens in raw ground beef. J. Milk Food Technol. 38: 449-452.
11. Goepfert, J. M. 1976. The aerobic plate count, coliform and *Escherichia coli* content of raw ground beef at the retail level. J. Milk Food Technol. 39:175-178.
12. Hollander, M., and D. A. Wolfe. 1973. Non-parametric statistical methods (First edition). John Wiley and Sons, New York. pp 27-33, 139-146, 151-158.
13. Horowitz, W. (editor). 1975. Official methods of analysis of the Association of Official Analytical Chemists (Twelfth edition). Association of Official Analytical Chemists, Washington, DC.
14. Judge, M. D., C. G. Haugh, G. L. Zachariah, C. E. Parmelee, and R. L. Pyle. 1974. Soya additives in beef patties. J. Food Sci. 39: 137-139.
15. National Research Council. 1971. Reference methods for the microbiological examination of foods. National Academy of Sciences, Washington, DC.
16. Pivnick, H., I. E. Erdman, D. Collins-Thompson, G. Roberts, M. A. Johnston, D. R. Conley, G. Lachapelle, U. T. Purvis, R. Foster, and M. Milling. 1976. Proposed microbiological standards for ground beef based on a Canadian survey. J. Milk Food Technol. 39:408-412.
17. Wehr, H. M. 1977. Microbiological standards for foods — Attitudes and policies of state government. 37th Annual Meeting, Institute of Food Technologists.
18. Westhoff, D., and F. Feldstein. 1976. Bacteriological analysis of ground beef. J. Milk Food Technol. 39:401-404.
19. Williams, C. A., and M. A. Zabik. 1975. Quality characteristics of soy-substituted ground beef, pork and turkey meat loaves. J. Food Sci. 40:502-505.
20. Wolford, K. M. 1974. Beef/soy: Consumer acceptance. J. Am. Oil Chem. Soc. 51:131A-133A.

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Natural (Unhydrolyzed) Milk Versus Lactose-Hydrolyzed Milk for Cultured Dairy Products: Physiological and Practical Implications for the Starter Industry

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ABSTRACT

As a microbiological medium, lactose-hydrolyzed milk is quite different from natural milk in that the restrictive role of milk lactose (the majof energy source for microorganisms) is eliminated. To emphasize the restrictive role of lactose, the enzymatic and genetic mechanisms involved in the utilization of this beta-galactoside are discussed. Elimination of this selectivity leads to certain manufacturing and storage difficulties with cultured dairy products. This important change in the raw material (milk) should be recognized in selection of starter strains for use in conversion of lactose-hydrolyzed milk to cultured dairy products.

In considering the implications of lactose-hydrolyzed milk for the starter industry, emphasis should be directed at the difference(s) between natural (untreated) and enzyme-treated milk as microbiological media. Milk is indeed an excellent medium for microbial growth and metabolic activity. The moisture content and the pH of milk are ideal for most microorganisms to thrive, and the solid components of milk, i.e. milk-fat, casein, whey proteins, lactose, ash and minor components, can more than adequately provide the carbon, nitrogen, mineral and vitamin (accessory growth-factor) requirements of microorganisms. The only difference between natural milk and lactose-hydrolyzed milk lies in the relative concentrations of the disaccharide, lactose, and its monosaccharide components. It is important to examine how this change influences the course and rate of microbial growth and activity in the enzyme-treated milk.

MILK SUGAR-LACTOSE

Lactose is the major carbohydrate in milk and it accounts for about 4.9% of the weight of this biological fluid. From the viewpoint of chemical structure, lactose is designated as 4-0-β-D-galactopyranosyl-D-glucose pyranose. It is a disaccharide made up of D-glucose and D-galactose, linked together by a 1, 4, β-linkage. the first moiety in the linkage is galactose and the second, glucose. The other free sugars in milk—glucose, galactose and certain phosphate esters of hexoses—occur in relatively trace amounts (*26, 47*) that lactose is for all practical purposes the major energy and carbon source for microorganisms (*32*).

LACTOSE UTILIZATION AMONG MICROORGANISMS

In examining the role of lactose as the sole carbon source for microorganisms in milk, one has to consider the mechanism(s) of utilization of lactose by microorganisms. The major metabolic pathways involved in energy — trapping and in producing short chain carbon intermediates (for synthesis), accomodate simple sugars or their derivatives, primarily the aldohexose, glucose (*30*). So in the utilization of lactose, microorganisms have to first convert the disaccharide into its hexose components before they are fed into the relevant energy-yielding pathways. From an operational standpoint, use of any substrate involves two phases — viz: the uptake or transport of the substrate into the cell; and, the actual breakdown of substrate. The uptake of the substrate may involve specific permeases or reactions involving expenditure of bond energy—energy dependent active transport systems (*57*). he breakdown of disaccharide substrates usually involves enzyme systems capable of cleaving the bond between the monoaccharide components. In the case of utilization, the mechanisms needed are the specific permease or other transport systems and the specific enzyme system to cleave the 1,4 β-linkage between the galactose and glucose moieties. Conversely, only those microorganisms that possess the specific transport system(*s*) and the enzyme capable of cleaving the β-linkage can derive their carbon requirements from lactose. This in effect is the selective or restrictive role of lactose as far as microbial growth in milk is concerned (*32*).

Four enzymatic mechanisms are recognized for use of lactose by microorgnisms:

1. β-Galactosidase (β-gal), formerly known as lactase, catalyzes the hydrolysis of lactose to glucose and

galactose. This system is linked either to an inducible galactoside permease or an energy-requiring active transport system. This enzyme is widely distributed among microorganisms and has been described in *Aeromonas formicans, Shigella sonii, Escherichia coli, Bacillus megaterium, Bacillus subtilis, Kluyveromyces fragilis, Neurospora crassa, Aspergillus niger* (*38*), lactobacilli (*50*), *Streptococcus thermophilus* (*35*), and *Propionibacterium shermanii* (*18*).

2. Lactose dehydrogenase system (*38*) found among pseudomonads which converts lactose to lactobionic acid through two steps with the intermediate formation of lactobionic-ζ-lactone. The intermediate is then cleaved to gluconate and galactose.

3. In the linked PEP-phosphotransferase-β-D-phosphogalactoside galactohydrolase system (PEP-β-D-gal) lactose is first phosphorylated at carbon 6 of the galactose moiety during its passage through the transferase system, and the phosphorylated disaccharide is cleaved to galactose-6-PO_4, and glucose by β-D-phosphogalactoside galactohydrolase (β-P-gal). This integrated system is found among lactic streptococci (*36,37*), lactobacilli (*50*), and *Staphylococcus aureus*(*27*).

4. The fourth is a plausable system, but for which no experimental evidence is now available. It is a scheme similar to that involved in maltose utilization, where there is direct phosphorylation of the disaccharide (*11,46*). In the case of lactose, this would involve the transfer of a phosphate group to the first carbon in the glucose moiety.

The known lactose utilization mechanisms among dairy starter bacteria are summarized in Table 1. For detailed discussion of these systems, the reader should consult excellent reviews by McKay et al. (*38*) and Lawrence et al. (*31*). An excellent treatment of the transport systems involving carbohydrates is given in a recent article by Kornberg (*28*).

TABLE 1. *Lactose utilization mechanisms among dairy starter bacteria.*

Bacterial species	Dairy fermentation	Lactose utilization mechanism	Reference
S. lactis	Ripened cream butter	PEP-β-gal[a]	*36, 37*
S. cremoris	Buttermilk, sour cream		
S. lactis subsp. *diacetylactis*	Cottage cheese American cheese varieties, etc.		
S. thermophilus	Yogurt	β-gal[b]	*35, 52*
	Swiss cheese	PEP- β-P-gal (?)	
	Italian cheeses		
Lactobacillus spp.	Yogurt	β-gal and/or	50
	Swiss cheese	PEP-β-P-gal	
	Italian cheese		
	Acidophilus milk		
Propioni bacterium shermanii	Swiss cheese	β-gal	*18*
	varieties	β-P-gal (?)	

[a]PEP-Phosphotransferase β-D-Phosphogalactoside galactohydrolase.
[b]β-Galactosidase.

GENETIC ASPECTS OF LACTOSE UTILIZATION AMONG STARTER BACTERIA

Phenotypic characteristics such as lactose utilization reflect the genetic make-up of the microorganisms, and the genetic control mechanisms that govern their expression. For a more complete understanding of lactose utilization among starter bacteria, the genetic aspects of this function needs to be surveyed here. Currently, only something of the genetics of this function among lactic streptococci is known. This system will be examined here to understand the complexities involved in lactose utilization by starter bacteria.

INSTABILITY OF LACTOSE UTILIZATION

One of the very first observations made about the lactose fermentative characteristics (as manifested by lactic acid accumulation) of lactic streptococci was that it is relatively unstable. As early as 1931, Harriman and Hammer (*17*) reported on the emergence of slow acid-producing variants within pure cultures of lactic streptococci. Later Hunter (*21*) reported on occurrence of slow variants within pure cultures of *Streptococcus cremoris*. Recently McKay et al. (*39*) and McDonald (*34*) observed the spontaneous and dominant emergence of slow lactose-fermenting or lactose-negative variants when fast acid producing single strains of lactic streptococci were propagated in a continuous fermentation system. The numbers of such variants were very high as the fermentation progressed beyond 120 h.

These observations plus the data showing the dramatic increase of lactose-negative variants within a pure lactic *Streptococcus* culture treated with acriflavin (a mutagen that selectively induces the loss of extrachromosal elements within cells), prompted McKay et al. (*39*) to propose that the genetic information for lactose utilization in these bacteria resides in extrachromosomal elements.

LACTOSE UTILIZATION - CODED ON TRANSIENT GENETIC ELEMENTS

The first evidence for a transient genetic element-linked lactose fermentation function among lactic streptococci was reported by McKay et al. (*40*). They found that certain UV-induced prophages from *Streptococcus lactis* C_2 can *transduce* the ability for active lactose fermentation to spontaneous and acriflavin-induced lactose-negative mutants of the same strain. Transduction is the process by which genetic transfer among bacteria is mediated by bacteriophages. This was confirmed by induction of high frequency transducing particles from lactose-positive transductants. Transductants are bacteria that have been genetically altered by acquiring genetic markers transferred via bacteriophages. Later, Cords et al. (*6*) presented the first evidence for a plasmid-linked lactose utilization function in *S. cremoris* B_1. Plasmids are characteristically small, covalently closed, circular duplex DNA molecules which may confer upon the host known genetic functions (*6*).

RELATIONSHIP BETWEEN PROTEOLYTIC FUNCTION AND LACTOSE UTILIZATION IN MILK

Another interesting sidelight to the loss of β-galactoside utilization by lactic streptococci is the observation that this phenotypic expression is accompanied by the relative loss of efficient proteolytic function. In milk, slow acid production from lactose is invariably accompanied by considerable weakening or complete loss of caseolytic ability (*4,44*). The relatedness of these functions was clearly demonstrated in the analysis of transductants derived from lactose and proteinase-negative *S. lactis* C_2 mutants by treating them with prophages induced from lactose and proteinase-positive wild-type *S. lactis* C_2 culture (*41*). In publications that followed (*9,43*), further experimental proof was presented to show that both lactose utilization and proteinase functions are genetically coded on plasmids carried by lactic streptococci.

COMPLEXITY OF GENETICS OF LACTOSE UTILIZATION AMONG LACTIC STREPTOCOCCI

In a recent paper Anderson and McKay (*1*) demonstrated the complexities of the genetics of lactose utilization in *S. cremoris* B_1. They used wild-type *S. cremoris* B_1, its mutants and revertants in complementation studies with site-specific mutants of *S. aureus* and showed the following:

1. The location of genetic determinants for lactose-specific transport enzymes of PEP-phosphotransferase system appears to be on a plasmid with a molecular weight of 36×10^6 daltons.
2. The site for the induction of β-D-phosphogalactoside galactohydrolase by galactose-6-PO_4 also appears to reside in the same plasmid.
3. There appears to be an alternative site for the genetic information for the elaboration of β-P-gal enzyme on the bacterial chromosome which is expressed under special conditions. Figure 1 depicts the complex genetic system governing use of lactose by lactic streptococci.

So far, the mechanism(s) and genetics of lactose utilization among starter bacteria have been surveyed at length, because in natural milk, lactose presents the major hurdle to rapid and uniform acid producing function of starter cultures. In lactose-hydrolyzed milk, this impediment is eliminated. Depending upon the conditions of hydrolysis, concentration of enzyme, and the purity of enzyme used, 90% hydrolysis of the disaccharide in fluid milk can be achieved (*16*). Hence in such enzyme-treated milk, free glucose and galactose are readily available for starter bacteria.

GLUCOSE AND GALACTOSE UTILIZATION BY LACTIC STREPTOCOCCI

One important observation that needs to be stressed here is that in all the studies cited earlier involving spontaneous or induced loss of lactose utilization function by lactic streptococci, none of the lactose-negative mutants lacked the ability to utilize glucose. So glucose utilization appears to be a stable phenotypic characteristic. This observation assumes great significance in dealing with lactose-hydrolyzed milk.

Lactic streptococci preferentially utilize glucose (*4,44*) and when this hexose is available, rapid lactic acid accumulation is observed. Galactose, on the other hand, is slowly metabolized. Gilliland et al. (*13*), using glucose, galactose and lactose individually in a broth system, showed that the fastest growth of lactic streptococci is obtained with glucose; the growth rate was the lowest with galactose. The same workers also found that in milk supplemented with *E. coli* β-galactosidase, and inoculated with a lactic starter, there was rapid use of glucose, but galactose accumulated gradually. Other authors (*15,19,31*) have also reported on the lingering presence of galactose in milk and cheese during and after active fermentation by lactic bacteria. Recently O'Leary and Woychick (*48*) made similar observations with yogurt bacteria in natural and lactose-hydrolyzed milk.

IMPLICATIONS OF LACTOSE-HYDROLYZED MILK TO STARTER INDUSTRY

What implications do these observations have in selection of starters for lactose-hydrolyzed milk? It may be safely assumed that removal of the restrictive role of lactose in the hydrolyzed milk makes it easier to select and formulate starters. In all the published work relating to the manufacture of cultured products from lactose-hydrolyzed milk (*16,48,58,61*) with one exception (*3*), the major observation was that the rate of acid production in this substrate was much faster than in untreated milk. This is definitely because of the ready availability of glucose in free form in the enzyme-treated milk. Slow use of galactose is not of much significance because in most fermentations in dairy manufacturing only a very small portion, about 16-18% of the available sugar, is utilized (*53,54*). In a recent article, Kosikowski (*29*) has proposed the possible application of a suitable galactose isomerase to stimulate the rate of dissipation of this persistant hexose.

Based on the foregoing discussion, several important implications can be projected for the starter industry:

1. Lactose-hydrolyzed milk affords a wider selection of conventional starter strains because many of the so called "slow strains" can be included in mixed-strain starters.

2. Variation in rate of acid production either due to inherent "slowness" or genetic changes may not be as critical as in untreated milk.

3. Wider selection of strains would offer a greater number of strain combinations to provide multiplicity of and flexibility in culture rotation to protect against phage failures.

4. Faster rate of acid production in itself (as found in hydrolyzed milk in comparison with untreated milk) may provide greater phage protection, because the faster

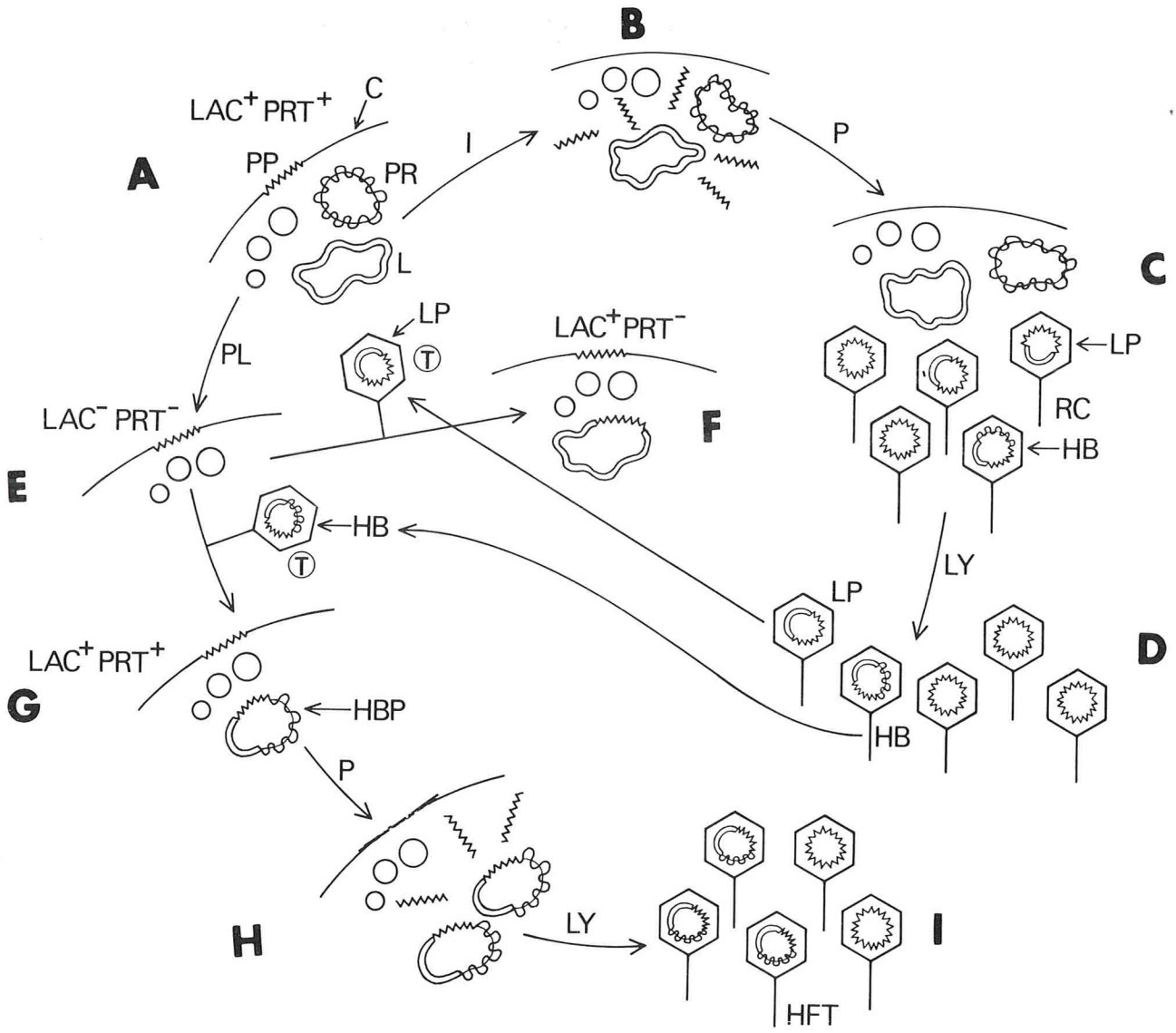

FIGURE 1. *Diagrammatic representation of genetic recombination of Lac and Prt markers in* S. lactis C_2[a]. [a]*Lac-Lactose; Prt - Proteinase; C-Bacterial chromosome:*

HB¶Hybridphage containing phage DNA, small pieces of Lac and Prt plasmids caused by copy error during proliferation of prophage (Spontaneous or induced).

HBP-Hybrid plasmid containing portions of Lac and Prt plasmids plus a small piece of phage DNA. Ths plasmid is produced in large numbers because it contains phage DNA. As phage DNA is replicated, enzymes are furnished which allow increased replication of the hybrid plasmid.

HFT-High frequency transductants produced as a result of replication on HBP. These HFT phages transfer Lac and Prt markers at high frequency.

I-Induction-spontaneous or UV; L-Lactose plasmid; LP-Phage containing small piece of lac plasmid plus the phage DNA; LY-Lysis of cell liberating mature phages; P-Proliferation of phage; PL-Loss of plasmid(s)-spontaneous or induced by agents like acriflavin; PP-Phophage DNA; PR-Proteinase plasmid; RC-Phage-Lac/Prt plasmid recombination; T-Transduction.

A. Lactose fermenting and proteinase producing S. lactis C_2 *cell containing phage DNA (PP) which inserted into the bacterial chromosome and the five identified plasmids. Three of the plasmids of unknown function have been designated as open circles, the proteinase plasmid as (PR), and the lactose plasmid as (L).*

B. Exposing the cell to UV irradiation or other inducing agents will release the phage DNA from the chromosome. The phage DNA then begins self-replication to form mature phage particles.

C. S. lactis C_2 *cell containing plasmid DNA and mature phage particles. Most of the phage particles will contain phage DNA but a few, due to errors in recombination events, will contain phage DNA plus lactose plasmid DNA (LP) or phage DNA plus lactose plasmid DNA and proteinase plasmid DNA (HB). These particles serve as transducing phage.*

D. The bacterial cell has lysed releasing the phage particles.

E. An S. lactis C_2 *cell is unable to ferment lactose or produce the proteinase enzyme system because it has lost the lactose and proteinase plasmids. This cell is infected with the phage lysate from D.*

F. If the Lac^-Prt^- *cell becomes infected with LP phage only lactose fermenting ability is added back and the cell is now able to ferment lactose but does not produce the proteinase enzyme system.*

G. If the Lac^-Prt^- *cell becomes infected with HB phage both lactose fermenting ability and proteinase activity are added back and the cell now regains ability to ferment lactose and produce the proteinase enzyme system.*

H. Steps B and C are repeated upon release of the phage from the chromosome.

I. A phage lysate is formed which has a higher rate of transducing ability than observed in D because of replication of HB. This plasmid is produced in large numbers because it contains some phage DNA. As phage DNA is replicated, enzymes are furnished which allow increased replication of the hybrid plasmid. Since HB would be present in higher numbers than L, a higher precentage would be incorporated into the phage head, thus resulting in a high frequency transducing lysate.

fermentation attains pH values lower than 5.0, the smaller is the chance for massive phage infection (*49*).

5. Use of lactose-hydrolyzed milk for cultured products would probably offer wider application of "protective cultures." The term "protective cultures" refers to specific lactic *Streptococcus* and *Leuconostoc cremoris* cultures that are used to extend the shelf-life of products like cottage cheese (*33,45,59*). Some of these cultures also have inhibitory properties against certain foodborne pathogens (*2,7,51,56*). With removal of the restrictive lactose barrier, and the easy availability of glucose, a sugar that is readily fermentable by a wide variety of flora, spoilage of lactose-hydrolyzed fluid milk and certain cultured products made from it will be a problem. Currently, a highly concentrated culture of *S. lactis* subspecies *diacetylactis* is available for direct application to cottage cheese curd to lengthen shelf-life (*55*). There is further need to develop procedures and technology for application of such protective cultures to lengthen the shelf-life of fluid lactose-hydrolyzed milk and products made from it (*14*).

6. Production of concentrated culture for application in lactose-hydrolyzed milk will be simpler and cheaper. For use in natural milk, it is recommended that milk or milk derivatives be used in fermentation media to retain the caseolytic function in starter bacteria, which would ensure production of good acid-producing cells (*60*). The need to retain specific proteolytic function in milk necessary for lactose metabolism is not critical when glucose is available. So it would be possible to substitute milk or milk derivatives with more soluble, non-opaque and more heat-stable components. Also a wider variety of ingredients and stimulants can be used in the fermentation media to obtain high cell count without the loss of acid-producing activity (*60*). Removal of milk or milk derivatives from the fermentation media would make separation of bacterial cells (centrifugation or filtration) easier and more efficient. However, care should be taken to include strains with good caseolytic function in combining mixtures for the production of ripened natural cheeses.

7. The availability of free glucose in sufficient amounts in lactose-hydrolyzed milk would allow the use of safe, clean lactic acid producing bacteria used in non-dairy fermentations. This will provide additional safeguards against phage failures. For example, selected strains of *Pediococcus cerevisiae*, which are widely used in sausage fermentations (*10*), can be employed. The procedure for applying such cultures in lactose-hydrolyzed milk has been patented (*20*). Certain selected low temperature lactobacilli like *Lactobacillus plantarum*, which is used in sausage, pickle, sauerkraut and silage fermentations (*12*), could also be considered.

Another possibility is the application of carefully screened strains of enterococci, which are capable of fast acid production. Recently a series of papers was published on production of safe, excellent quality cheese using selected strains of enterococci as supplemental starters (*23-25*). Although the relationship between enterococci and food poisoning outbreaks is considered tenuous (*8*), extensive testing of strains for the absence of any trace of enteropathogenicity should be done before large scale application.

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REFERENCES

1. Anderson, D. G., and L. L. McKay. 1977. Plasmids, loss of lactose metabolism, and appearance of partial and full lactose-fermenting revertants in *Streptococcus cremoris* B_1. J. Bacteriol. 129: 367-377.
2. Babel, F. J. 1977. Antibiosis by lactic culture bacteria. J. Dairy Sci. 60:815-821.
3. Cardwell, J. T., and N. Prombutara. 1976. Effect of partial hydrolysis of lactose on quality of certain dairy products. J. Dairy Sci. 59:26.
4. Citti, J. E., W. E. Sandine, and P. R. Elliker. 1965. Comparison of slow and fast acid-producing *Streptococcus lactis*. J. Dairy Sci. 48:14-18.
5. Citti, J. E., W. E. Sandine, and P. R. Elliker. 1965. β-Galactosidase of *Streptococcus lactis*. J. Bacteriol. 89:937-942.
6. Cords, B. R., L. L. McKay, and P. Guerry. 1974. Extrachromosomal elements in group N streptococci. J. Bacteriol 117: 1149-1152.
7. Daly, C., W. E. Sandine, and P. R. Elliker. 1972. Interactions of food starter cultures and food-borne pathogens: *Streptococcus diacetilactis* versus food pathogens. J. Milk Food Technol. 35: 349-357.
8. Deibel, R. H., and P. A. Hartman. 1976. The enterococci. pp. 370-372. In : M. L. Speck (ed.) Compendium of methods for the microbiological examination of foods. American Public Health Association; Washington, D.C.
9. Efstathiou, J. D., and L. L. McKay. 1976. Plasmids in *Streptococcus lactis*: Evidence that lactose metabolism and proteinase activity are plasmid linked. Appl. Environ. Microbiol. 32:38-44.
10. Emerson, C. W., W. E. Danner, and P. A. Hammes. 1970. Bacterial starter cultures in sausage products. Agr. Food Chem. 18: 570.
11. Fitting, C., and M. Doudoroff. 1952. Phosphorolysis of maltose by enzyme preparations from *Neisseria meningitis*. J. Biol. Chem. 19:153
12. Frazier, W. C. 1958. Food microbiology, 1st ed. McGraw-Hill Book Co., Inc., New York. pp. 167-174, 337.
13. Gilliland S. E., M. L. Speck, and J. R. Woodard, Jr. 1972. Stimulation of lactic streptococci in milk by β-galactosidase. Appl. Microbiol. 23:21-25.
14. Gilliland, S. E., and M. L. Speck. 1975. Inhibition of psychrotrophic bacteria by lactobacilli and pediococci in non-fermented refrigerated foods. J. Food Sci. 40:903-905.

15. Goodenough, E. R., and D. H. Kleyn. 1976. Qualitative and quantitative changes in carbohydrates during the manufacture of yogurt. J. Dairy Sci. 59:45-47.
16. Gyricsek, D. M., and M. P. Thompson. 1976. Hydrolyzed lactose cultured dairy products. II. Manufacture of yogurt, buttermilk and cottage cheese. Cultured Dairy Prod. J. 11(3):12-13.
17. Harriman, L. A., and B. W. Hammer. 1931. Variation in the coagulation and proteolysis of milk by *Streptococcus lactis.* J. Dairy Sci. 14:40-49.
18. Hartley, J. C., and E. R. Vedamuthu. 1975. α-Galactosidase of *Propionibacterium shermanii.* Appl. Microbiol. 29:74-80.
19. Hettinga, D. H., A. H. Miah, E. G. Hammond, and G. W. Reinbold. 1970. Sensitive enzymatic method for determination of glucose, galactose and lactose in Cheddar cheese. J. Dairy Sci. 53:1377.
20. Humphreys, T. W., and D. S. Titus. 1963. Coagulation of milk U.S. Patent 3,086,866.
21. Hunter, G. J. E. 1939. Examples of variation within pure cultures of *Streptococcus cremoris.* J. Dairy Sci. 102:464-470.
22. Hurst, A. 1972. Interactions of food starter cultures and food-borne pathogens: The antogonism between *Streptococcus lactis* and spore forming microbes. J. Milk Food Technol. 35:418-422.
23. Jensen, J. P., G. W. Reinbold, C. J. Washam, and E. R. Vedamuthu. 1973. Role of enterococci in Cheddar cheese: Growth of enterococci during manufacturing and curing. J. Milk Food Technol. 36:613-618.
24. Jensen, J. P., G. W. Reinbold, C. J. Washam, and E. R. Vedamuthu. 1975. Role of enterococci in Cheddar cheese: Proteolytic activity and lactic acid development. J. Milk Food Technol. 38:3-7.
25. Jensen, J. P., G. W. Reinbold, C. J. Washam, and E. R. Vedamuthu. 1975. Role of enterococci in Cheddar cheese: Organoleptic considerations. J. Milk Food Technol. 38:142-145.
26. Johnson, A. H. 1974. The composition of milk. pp. 43-44. In: B. H. Webb, A. H. Johnson, and J. A. Alford (eds.) Fundamentals of dairy chemistry. The AVI Publishing Co., Inc. Westport, CN.
27. Kennedy, E. P., and G. A. Scarborough. 1967. Mechanism of hydrolysis of O-nitrophenyl-β-galactoside in *Staphylococcus aureus* and its significance for theories of sugar transport. Nat. Acad. Sci., Proc. 58:225-228.
28. Kornberg, H. L. 1976. Genetics in the study of carbohydrate transport by bacteria. J. Gen. Microbiol. 96:1-16.
29. Kosikowski, F. V. 1977. The character, movement and future of the United States dairy industry. J. Dairy Sci. 60:635-642.
30. Krebs, H. A., and H. L. Kornberg. 1957. Energy transformations in living matter — A survey. Springer Verlag, Berlin. 298 p.
31. Lawrence, R. C., T. D. Thomas, and B. E. Terzaghi. 1976. Reviews of the progress of dairy science: Cheese starters. J. Dairy Res. 43:141-193.
32. Marth, E. H. 1974. Fermentations. pp 813-815. In: B. H. Webb, A. H. Johnson, and J. A. Alford (eds.) Fundamentals of dairy chemistry. The AVI publishing Co., Inc. Westport, CN
33. Mather, D. W., and F. J. Babel. 1959. Inhibition of certain types of bacterial spoilage in creamed cottage cheese by use of a creaming mixture prepared with *Streptococcus citrovorus.* J. Dairy Sci. 42:1917.
34. McDonald, I. J. 1975. Occurrence of lactose-negative mutants in chemostat cultures of lactic streptococci. Can. J. Microbiol. 21:245-251.
35. McKay, L. L. 1970. Mechanisms of lactose utilization by lactic acid bacteria: enzymatic and genetic studies. Ph. D. Thesis. Oregon State University, Corvallis, Oregon.
36. McKay, L. L., L. A. Walter, W. E. Sandine, and P. R. Elliker, 1969. Involvement of phosphoenolypyruvate in lactose utilization by group N streptococci. J. Bacteriol. 99:603-610.
37. McKay, L. L., A. Miller, III, W. E. Sandine, and P. R. Elliker, 1970. Mechanisms of lactose utilization by lactic acid streptococci: enzymatic and genetic analyses. J. Bacteriol. 102:804-809.
38. McKay, L. L., W. E. Sandine, and P. R. Elliker. 1971. Lactose utilization by lactic acid bacteria: A review. Dairy Sci. Abstr. 33:493-499.
39. McKay, L. L., K. A. Baldwin, and E. A. Zottola. 1972. Loss of lactose metabolism in lactic streptococci. Appl. Microbiol. 23: 1090-1096.
40. McKay, L. L., B. R. Cords, and K. A. Baldwin. 1973. Transduction of lactose metabolism in *Streptococcus lactis* C_2. J. Bacteriol. 115: 810-815.
41. McKay, L. L., and K. A. Baldwin. 1974. Simultaneous loss of proteinase — and lactose — utilizing enzyme activities in *Streptococcus lactis* and reversal of loss by transduction. Appl. Microbiol. 28:342-346.
42. McKay, L. L., and K. A. Baldwin. 1975. Plasmid distribution and evidence for a proteinase plasmid in *Streptococcus lactis* C_2 Appl. Microbiol. 29:546-548.
43. McKay, L. L., K. A. Baldwin, and J. D. Efstathiou. 1976. Transductional evidence for plasmid linkage of lactose metabolism in *Streptococcus lactis* C_2. Appl. Environ. Microbiol 32:45-52.
44. Molskness, T. A., D. R. Lee, W. E. Sandine, and P. R. Elliker. 1973. β-D-Phosphogalactoside galactohydrolase of lactic streptococci. Appl. Microbiol. 25:373-380.
45. Moseley, W. K., P. R. Elliker, and W. E. Sandine. 1967. Method for preparing a creaming composition for dairy products and the resulting product. U.S. Patent 3,323,921.
46. Moustafa, H. H., and E. B. Collins. 1968. Role of galactose or glucose-1-phosphate in preventing the lysis of *Streptococcus diacetilactis.* J. Bacteriol. 95:592-602.
47. Nickerson, T. A. 1974. Lactose. pp 273-278. In: B. H. Webb, A. H. Johnson, and J. A. Alford (eds) Fundamentals of dairy chemistry. The AVI Publishing Co., Inc. Westport, CN.
48. O'leary, V. S., and J. H. Woychik. 1976. A comparison of some chemical properties of yogurt from control and lactase-treated milks. J. Food Sci. 41:791-793.
49. Overcast, W. W., F. E. Nelson, and C. E. Parmelee. 1951. Influence of pH on proliferation of lactic *Streptococcus* bacteriophage. J. Bacteriol. 61:87.
50. Premi, L., W. E. Sandine, and P. R. Elliker. 1972. Lactose-hydrolyzing enzymes of *Lactobacillus* species. Appl. Microbiol. 24:51-57.
51. Pinheiro, A. J. R., B. J. Liska, and C. E. Parmelee. 1968. Properties of substances inhibitory to *Pseudomonas fragi* produced by *Streptococcus citrovorus* and *Streptococcus diacetilactis.* J. Dairy Sci. 51:183-187.
52. Reddy, M. S., F. D. Williams, and G. W. Reinbold. 1973. Lactose transport in *Streptococcus thermophilus.* J. Dairy Sci. 56:634.
53. Reineccius, G. A., T. E. Kavanagh, and P. G. Keeney. 1970. Identification and qualification of free neutral carbohydrates in milk products by gas-liquid chromatography. J. Dairy Sci. 53: 1018-1022.
54. Richter, R. L., G. A. Reineccius, and L. L. McKay. 1973. Acid production by *Streptococcus lactis* in low-lactose skim milk. J. Food Sci. 38:796-798.
55. Sing, E. L. 1976. Preparation of cottage cheese. U.S. Patent 3,968,256.
56. Speck, M. L. 1972. Control of food-borne pathogens by starter cultures. J. Dairy Sci. 55:1019-1022.
57. Stanier, R. Y., M. Doudoroff, and E. A. Adelberg. 1970. The microbial world, 3rd ed. Prentice-Hall Inc. Englewood Cliffs, NJ. pp 354.
58. Thompson, M. P., and D. P. Brower. 1976. Hydrolyzed lactose cultured dairy products. I. Manufacture of Cheddar Cheese. Cultured Dairy Prod. J. 11(1):22-23.
59. Vedamuthu, E. R., B. A. Hauser, D. R. Henning, W. E. Sandine, P. R. Elliker, and E. A. Day. 1966. Competitive growth of *Streptococcus diacetilactis* in mixed strain lactic cultures and cheese. Inter. Dairy Congress. Proc. Munich, Sec D(2):611-618.
60. Vedamuthu, E. R. 1976. Getting the most out of your starter. Cultured Dairy Products. J. 11(1):16-20.
61. Woodward, G. J., and F. V. Kosikowski. 1975. Manufacture of Cheddar cheese from milk with added glucose and from hydroylzed lactose milk. J. Dairy Sci. 58:792.

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Environmental Health Surveillance at the 1976 Festival of American Folklife[1]

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ABSTRACT

Overall the 1976 Festival of American Folklife was a highly successful affair both from a cultural and environmental health standpoint. Although a number of health-related problems arose on a day-to-day basis, close surveillance and prompt action on the part of the onsite sanitarian made it possible to secure early resolution of them with no ill effects among either visitors or the Festival staff. Logistically, planning and conducting of the comprehensive Smithsonian Institute (SI) food operations involving 50 different ethnic groups was an accomplishment worthy of note. Even more important, however, was the fact that during the entire time the Government Services, Inc. and SI food service operations were in progress, there was not a single case of foodborne illness associated with foods and beverages served to an estimated 500,000 persons onsite. This was in large measure due to the excellent cooperative assistance furnished by health agency personnel in the District of Columbia and the surrounding counties in Maryland and Virginia. These personnel inspected the many food establishments within their jurisdictions which served as sources for foods served at the Festival. All individuals and groups assisting directly or indirectly with this aspect of the Festival may justly be proud of the achievement.

One of the major attractions for visitors celebrating the Bicentennial in the Nation's capital was the Festival of American Folklife which was held June 16 to September 6, 1976, on the Mall beside the Lincoln Memorial Reflecting Pool.

Providing adequate environmental health protection for visitors and staff, particularly as it related to foods served onsite, was a matter of major concern to the National Park Service (NPS) in planning for this event. Toward this end, staff of the NPS Environmental Sanitation Program (NPS/ESP) who are on detail to the National Park Service from the Public Health Service, Center for Disease Control, were extensively involved in planning needed sanitary facilities and provided day-to-day environmental health surveillance onsite throughout the operating period.

This report sets forth in detail the steps taken to assure the safety and wholesomeness of foods served onsite. It can serve as a guideline for regulatory agency personnel responsible for planning and implementing environmental health programs at similar affairs of the same magnitude.

While foodservice at most temporary affairs such as fairs and carnivals is normally on a scale far smaller than the 1976 Festival, the basic principles of food protection remain the same. However, the sophistication of the facilities used at the Festival may not be needed at smaller and shorter affairs. This report attempts to provide guidance in arriving at the minimal facilities needed to provide adequate consumer protection.

HISTORY OF THE FESTIVAL

The Festival of American Folklife is a presentation of the Smithsonian Institution (SI) and the National Park Service (NPS), and is sponsored by several commercial organizations. Its purpose is to increase public appreciation of the various cultures of this country and to demonstrate to the people of this Nation the vital and continuing folk traditions, arts, and skills of Americans. This is achieved through use of eight different Regional themes or activities. They are: Native Americans, Old Ways in the New World, African Diaspora, Regional Americans, Working Americans, Transportation, Family Folklore, and Children's Folklore. Five of the eight Regional activities, in addition to presenting their many arts, skills, and crafts, demonstrated a wide variety of ethnic foods, including the specific methods and procedures to be followed in their preparation and serving.

This year was the 10th consecutive year for the Festival which in recent years has been held on the Mall beside the Lincoln Memorial Reflecting Pool. It has grown from a 3-day affair to a 2-week affair, with expansion to 12 weeks for the 1976 Bicentennial celebration.

The 1976 Festival was one of the largest and most prolonged outdoor events of this type ever to be held in this country. Over 5,000 performers from the United

[1] *The festival was held in Washington, D.C. from June 16 to September 6, 1976.*

States and 38 foreign countries, in addition to members from 55 trade unions and organizations, 116 Native American Tribal groups, and over 600 musicians and crafts people from every Region of the United States participated. The estimated visitor attendance at this event was 4 million. The cost of producing it was $7.4 million.

Although the Festival required implementation of an environmental control program covering water supply, solid and liquid waste disposal, insect and rodent control, and food protection, the latter was the area of primary concern from a public health standpoint.

In this regard, there were two types of foodservice available onsite. The Government Services Inc. (GSI) provided basic foodservice through one small and two large operations. Such service has been considered necessary at these festivals since offsite food establishments are too far removed for convenient use by visitors spending extended periods taking in the Festival events. In addition, the SI, as a part of the Festival, sponsored specialized foodservice operations intended to give the visitor an opportunity to partake of ethnic foods representing the culture in many of the American ethnic communities, e.g., Native American, Old Ways in the New World, and African Diaspora. In all, there were approximately 50 different ethnic groups and 900 individuals involved in transporting, preparing, displaying, and serving foods from 21 ethnic and food demonstration stands and the GSI food stands.

ENVIRONMENTAL SANITATION PLANNING FOR THE 1976 FESTIVAL

In looking ahead to the 1976 Festival, experiences of NPS/ESP staff with the provision of environmental health control at prior festivals were evaluated to determine changes which would be needed to assure adequate protection for what was expected to be a greatly increased visitation during the Bicentennial festival. While the problems of solid and liquid waste disposal were expected to increase substantially, food protection continued to be the major concern, with vector control and its direct impingement on food protection being a secondary yet very important environmental health consideration.

In this regard, the somewhat limited foodservice facilities available in the ethnic food stands at previous festivals made it difficult to provide a satisfactory level of food protection. Generally speaking, the equipment and facilities available for storage, preparation, display, and serving of foods and for washing of utensils and worker's hands were minimal. This made it necessary at times to limit menus, provide improvised sanitation facilities, and require special handling procedures to assure that foods to be served were prepared and handled in a satisfactory manner. This situation also required close day-to-day environmental health surveillance to compensate for the quality of the physical facilities available and to cope with food handling problems associated with the use of untrained volunteer food handlers.

While it was a difficult task to provide adequate environmental health protection at previous festivals, the extended length of this Festival, together with the anticipated increase in visitation, was expected to seriously intensify these problems, particularly as they relate to facility construction and maintenance, operating practices, and vector control. Thus, it was agreed that the food service facilities to be installed for the 1976 Festival would have to be much more sophisticated than in prior years if adequate environmental health protection was to be provided.

Accordingly, in early November 1975 it was recommended to the National Capitol Parks (NCP), the organizational unit within the National Park Service responsible for the Festival, that meetings be held with representatives of the GSI and the SI to discuss all environmental health implications of the Festival, develop plans for installation of adequate sanitary facilities, and establish minimum operational criteria. Subsequently seven major conferences and many individual discussions were held with NPS, SI, and GSI personnel. At these meetings, environmental health problems encountered at previous festivals and the effect of lengthening it from 10 days to 12 weeks were discussed in depth and the actions needed to be taken to correct both actual and potential environmental health problems were identifed.

These meetings proved to be invaluable when viewed retrospectively. For one thing, they provided an opportunity to discuss in detail the "Tentative Guidelines for Establishing and Operating Temporary Food Service Operations on National Park Service Controlled Areas," developed by the NPS/ESP, which contained the criteria to be applied to all Festival food operations. This was particularly important in regard to the design and construction of stands since it resulted in development of detailed plans by the concessioners which were submitted to, and approved by, NPS in advance of construction. With but a few exceptions, the stands were installed according to the plans thereby eliminating a major problem frequently encountered with temporary foodservice operations, i.e., inadequate facilities.

As a result of the above actions, the foodservice facilities installed included the major equipment items needed to conduct a satisfactory operation such as refrigeration; cooking and hot food holding equipment; separate hand and utensil washing facilities; hot water tanks; concrete floors with sewer connections; screening; formica or painted counters with storage shelves; and storage space for staples. In addition, each ethnic food stand had an ice maker, and three backup walk-in refrigerator/freezer units were made available to such stands by the SI for storage of excess food purchased for peak weekend demands. A series of photographs (Fig. 1-9) show both general and detailed pictures of those stands under construction and after completion and also show other sanitary facilities. Without a doubt, few if any

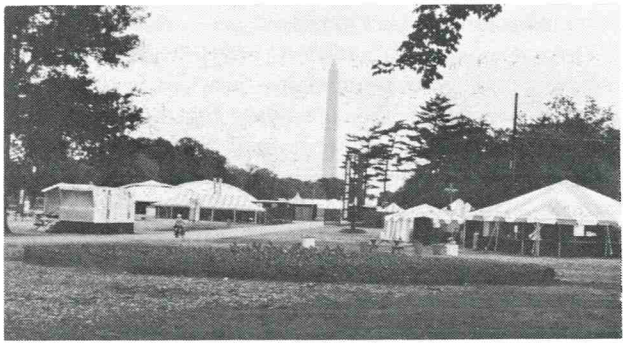

Figure 1. *General view of Festival site.*

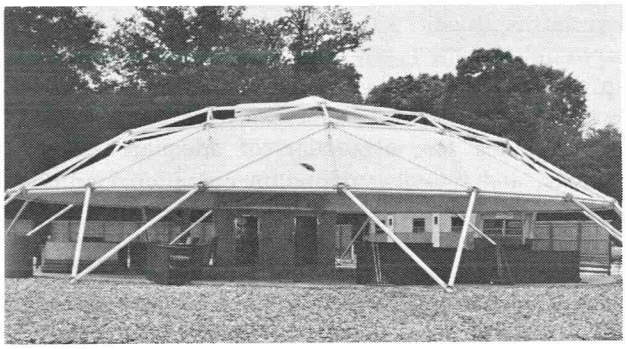

Figure 2. *GSI Food Stand under construction.*

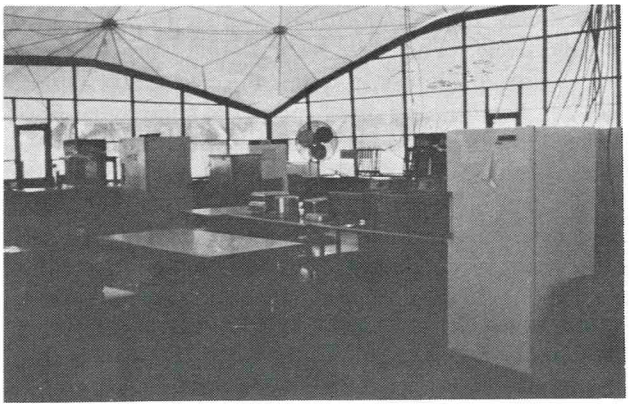

Figure 3. *Inside view of completed GSI stand.*

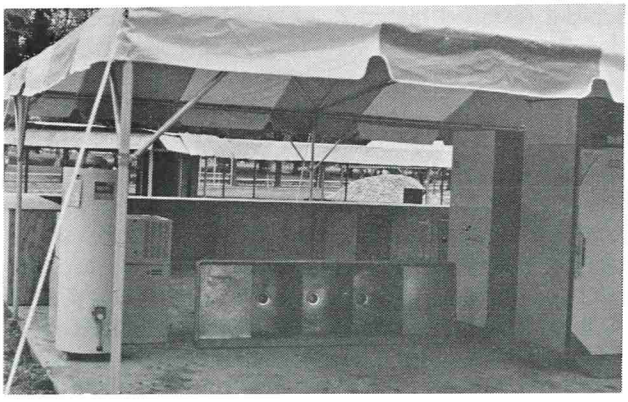

Figure 4. *Ethnic food stand under construction.*

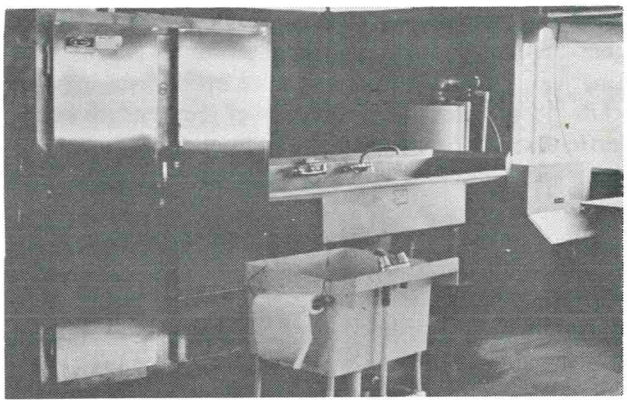

Figure 5. *Inside view of completed ethnic food stand.*

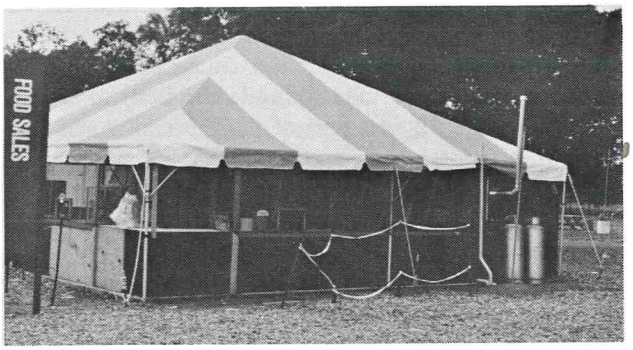

Figure 6. *Exterior view of ethnic food stand.*

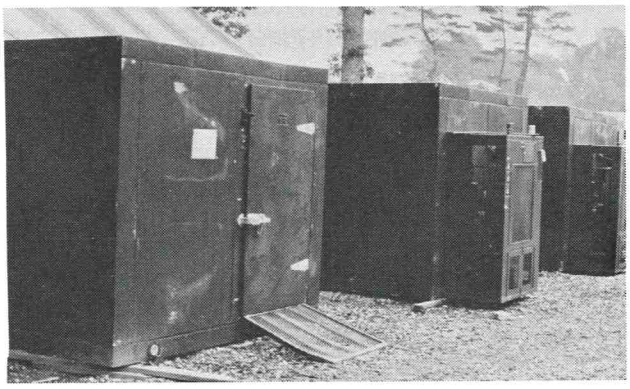

Figure 7. *SI backup refrigerator/freezer units.*

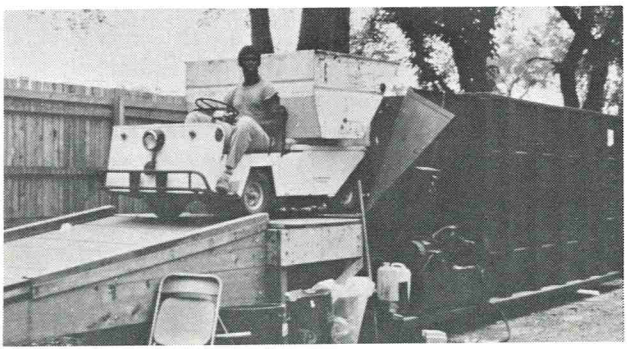

Figure 8. *On-site solid waste compactor unit.*

Figure 9. *Temporary comfort station.*

of the temporary foodservice establishments across the country have ever been better equipped. Further, the provision of these facilities was extremely important in providing a high degree of food protection.

A number of other decisions which were made and the actions planned were also very important. One was to provide a full-time highly qualified PHS food sanitarian for continuous surveillance during operating periods. As will be shown later under Festival operations, his presence onsite was a major factor, along with good facilities, in helping assure an effective foodborne illness prevention program.

Another important decision was to require that the SI staff furnish menus for all ethnic food operations (including foods to be handled in demonstration programs) showing a list of the specific ingredients to be used in the food to be served. There were approximately 75 separate menus involved. Due to the ethnic characteristics of many of these foods, it was necessary to obtain a complete description of the ingredients to be used in each menu item, as well as preparation and storage methods to be employed by the concessioners. This information was supplied in advance of the dates the foods were to be served to permit adjustment of the menu where the facilities onsite for preparing and serving a specific potentially hazardous food were not considered adequate.

Further, the SI was required to identify all offsite food sources to the NPS/ESP personnel so such sources could be officially inspected by the appropriate health agencies. If the source did not meet accepted public health standards, the concessioner was told to find an approved source. It was further required that specific information be submitted as to the methods and facilities to be used in transporting the food from offsite sources to the Festival area and the approximate time the food was scheduled to arrive onsite. It was also agreed that the NPS, with assistance of the PHS sanitarian, would supervise installation of all food facilites and, in addition, would provide needed utilites, collect and dispose of all trash and garbage generated onsite, and provide insect and rodent control as needed.

The SI's planned ethnic food program created yet another potential problem. As previously stated, approximately 50 different volunteer groups agreed to prepare and serve, or demonstrate preparation of, special foods reflecting their ethnic background or industry processing in the 16 stands set aside for this purpose. This required, in some instances, more than one group operating in a given stand simultaneously, and in other situations, two or more groups used the same facility within a particular week's operating period (Wednesday through Sunday). To assure that the stand was left clean and ready for the next group's use, the SI staff assumed responsibility for seeing that each group maintained the equipment and facilities in a sanitary manner and left the food site clean and in an operative condition. If they failed to do so, it was the SI's responsibility to correct deficencies before a new group took over.

Yet another major step in planning was to provide for the prompt investigation of any illnesses, particularly foodborne, alleged to have occurred as a result of Festival operations. In this regard, the District of Columbia Department of Human Resources (DHR) set up four first-aid stations at strategic locations on the Mall, and the Red Cross manned one such facility on the Festival grounds. Through a coordinated effort of the NPS, the Center for Disease Control, DHR, Red Cross, and the NPS/ESP, a carefully worked out contingency plan was set up to handle any reported illnesses. Fortunately, it was not necessary to test the effectiveness of this plan.

1976 FESTIVAL OPERATIONS

On the opening day of the Festival the onsite PHS representatives found all of the GSI and SI foodservice units were in substantial compliance with the applicable foodservice establishment guidelines and that they would be acceptable for preparing and serving food and drink. During the first few days of the Festival, it was necessary to require some changes in the foodservice facilities and operations primarily due to malfunction of equipment, overloading of food storage equipment, and lack of sufficient utensils and equipment to prepare, store, or serve foods or drinks properly.

Conditions found subsequently that warranted improvement were generally corrected immediately or within a very short time of their occurrence. For example, if the refrigeration facilities (freezer, refrigerator or ice maker) were not operating satisfactorily, a correction notice was issued by the onsite PHS sanitarian and the appropriate service group would repair the units or replace the faulty equipment. The same basic procedure was also used for correction of defects found with other facilities, such as plumbing, cooking, and water heating equipment; food tent repair; and the replacement or repair of cabinets, tables, doors, and screening. With regard to screening, doors and serving windows on the ethnic food stands were not of proper or durable construction and required constant attention throughout the Festival.

In several instances as many as three or four different SI volunteer groups of from 10 to 20 persons each had agreed to prepare and serve food for from a few hours to

1 or 2 days during a given 5-day period of operation. This large turnover of foodservice personnel resulted in the need to provide very close scrutiny of the food handling methods employed by these inidivduals.

It was further observed that due to the closing of commercial meat, poultry, and produce establishments over weekends and holidays there was a tendency for the ethnic food service stand operators to purchase large amounts of foods to cover their needs during these periods. This resulted at times in overstocking of refrigerators and freezers in the ethnic food stands as well as straining the capacity of their bulk food storage facilities. On two occasions, it was necessary to destroy over 150 lb. of decomposed meat due to overstocking of the refrigeration facilities in the stands to the point that the meat could not be cooled effectively. These and similar problems were corrected to a great degree by requiring that all backup stocks of foods be placed in the supplementary cold storage units provided by the SI group and that more accurate projections of food needs be made before additional food was shipped to the site. Further, to preclude overstocking of the backup cooling facilities, it was recommended that to the extent possible daily food shipments to the site be made by the commercial food suppliers.

INSPECTIONS OF THE FOOD ESTABLISHMENTS

A comprehensive surveillance program covering inspection of the Festival food stands and monitoring operating practices was established as set forth below. During the operating period of Wednesday through Sunday of each week, an early morning inspection was made of each site at least 2 or 3 h before actual preparation of the food. It was possible during this inspection to determine if the establishment had been properly cleaned, if the refrigeration and ice-making equipment were in satisfactory operating condition, and if hot and cold water were available. It was also noted at this time if any repairs were needed for the tent or for the food preparation equipment or facilities. If repairs were required, they were identified to the SI or GSI officials for correction of the condition. Since most of the food deliveries arrived at an early hour, this inspection also provided an opportunity to determine the temperature of incoming food and the protection given to the food in transit.

A daily record was made and posted at each food site stating the refrigerator and freezer temperatures, the condition of the hot water heating facilities, and whether the ice-making units were operating. When necessary, a written notice was posted in the food site advising the operator to correct the unsatisfactory condition before any food was prepared or served.

A second visit was made at the opening of the serving period, which varied from 11 a.m. to 12.m, at which time it could be determined if the food was being properly prepared, stored, and served. It also afforded an opportunity to detect any additional corrective measures that appeared necessary. A third inspection was conducted during the mid-afternoon food operations. Improper food service practices generally occurred about this time and the problems encountered could be quickly corrected before the food was to be served during the afternoon rush period.

In most instances food operations were required to be closed before 7 p.m. each day, and generally, depending on customer demands, the concessioners completed their operations by 5:30 p.m. A fourth inspection was conducted at about the end of the serving period to determine if proper cleanup procedures were being followed and to see if the stored foods were being correctly placed under effective refrigeration.

When necessary, a daily report or notice was submitted directly to the concessioners to advise them on any insanitary condition that was observed. Also an official inspection report was prepared for each food establishment during each work week, and a recap of the violations that had occurred was reported to the SI officials and their concessioners and to the GSI, respectively. Copies of inspection reports relating to sanitation problems were also furnished to National Capital Parks officials.

In those instances where it was necessary to suspend a foodservice operation until a problem was corrected, a notification of the suspension was submitted to both the SI and National Capital Parks which delegated this authority to the onsite sanitarian. In this regard, it was necessary on five occasions to restrict or suspend operations of a foodservice establishment until such time as the violated conditions were corrected.

In addition to the daily inspection of all ethnic and regular food facilities, a scheduled visit was made at least once each day to the Children's Folklore area to observe the methods employed in preparation and serving of beverages and to assure that methods used at the Arabbers fruit and melon cutting operation for storage of the equipment and fruit, sanitizing of the knives and cutting boards, and wrapping and icing of the cut fruit were satisfactory.

Also, during this visit the pony cart locations were inspected to assure that the area was being cleaned satisfactorily. The horse and pony stables were inspected to assure that cleaning of the stalls, removal of manure, and correction of any rodent or insect infestation at the stables were properly done. Inspections of insect, rodent, manure, and odor problems that occurred from Festival events using animals such as sheep, cattle, work horses, dairy cows, and calves were included.

Daily inspections were made at all trash collection sites and very few defects were observed during the entire Festival period. In fact, the collection and disposal of garbage and trash from the Festival area was perhaps one of the most efficient and well organized portions of the Festival environmental operations.

A number of special food demonstrations were featured by the Festival, and daily inspections were

required during their presentation. These events included baking and smoking salmon, preparing chuck wagon type foods (beans, barbecue beef, and biscuits), demonstrating meat cutting and baking, barbecuing beef and poultry, cooking catfish, milking cows, making sorghum, and other specialty food handling activities such as restaurant and bar operation techniques.

In all such special events, the food could not be offered to the public unless it was properly protected during preparation, storage, and serving. In those instances where the food was not properly protected and therefore not considered acceptable for human consumption, following the demonstration the food had to be destroyed under supervision of the PHS Sanitarian. As an example of the preventive measures taken where the food was considered unacceptable, after the cow milking demonstration the milk had to be discarded to waste because the cows were milked under an open tent and the milk was not properly protected when stored. Another example relates to manufacture of a specialty cheese using unpasteurized milk. The cheese was permitted to be exhibited but was not acceptable for human consumption. The specialty type foods prepared by the restaurant association group were discarded as garbage following each demonstration. Appropriate signs were displayed advising that the food was to be used only for demonstration purposes and not to be consumed by the public.

A series of water and ice samples were collected from selected distribution points throughout the Festival area and submitted for bacteriological analysis — no unsafe sample results were obtained. It was necessary, however, to reject shipments of ice from two sources due to presence of foreign matter.

TRAINING

Food sanitation training courses were conducted for most of the food service supervisors and a number of their personnel. The training was generally conducted at the food stands and held just before the start of each new operating period. The course included a brief discussion of the needs for safe-guarding the food during preparation and serving and was followed by a film discussing the sanitation aspects of food handling. A total of 158 persons, mostly supervisors and cooks, attended these training sessions.

PLANNING FOR FUTURE FESTIVALS

The design and construction features of the food-service stands for the 1976 Festival were generally satisfactory and provided the operators with a facility for preparing and serving foods and drinks which was much superior to those available in past events. Despite this, it is believed the following facility improvements should be considered in designing facilities for future festivals to provide greater food protection and/or improved cleaning:

1. All serving counters and cabinets should be sealed to the floor or installed on legs or supports to provide at least 6 inches of clearance between the floor and the bottom of the counters to provide for complete improved cleaning of the floors at the food sites and to avoid harborage areas for vermin. The space will require closure against insect and rodent entry between the bottom of the counters and the floor.

2. The tent roof overhang should be extended to at least 8 inches beyond the outside edge of the counters to avoid rain water from dripping on the counters and shelves.

3. The plastic mesh screening installed in the 1976 ethnic food stands was not entirely satisfactory and should be replaced with galvanized or aluminum type screening. Further, all doors and serving windows should be constructed of durable materials that will withstand extreme usage. Also, food demonstration stands should be equipped with outward opening self-closing doors of either screened or solid type construction.

4. Hot water facilities should be installed in all food demonstration sites for use in cleaning operations and for washing of the hands of the foodservice workers. Hot water should also be made available at all hand-washing facilities at the other food sites.

5. All indirect sewer connections from hand-washing and utensil-washing sinks should be equipped with a funnel-type splash guard to avoid splash.

6. There were a number of pots and pans used at all food operations which were too large to permit proper washing and sanitizing in the sinks provided. Sinks installed at future festivals should be of such size as to accommodate the utensils to be washed.

7. A variety of beverage dispensers were brought onsite by concessioners, many of which were not of acceptable design or construction. Approved type beverage dispensers should be provided in each food stand as a part of the installed facilities.

8. A 4-ft to 6-ft hard-surface walkway of concrete or asphalt material should be installed at the entrance to the food tents to prevent pooling of water which causes a soggy condition at the entrance to food tents.

9. A raised platform or rack should be installed outside each food site for storage of trash containers.

10. Each stand should be equipped with conventional hot-holding equipment where hot foods are displayed for serving. Use of "canned heat" and chafing dishes for heating foods cannot be accepted since sufficient heat cannot be applied to the food with such equipment in this type situation to maintain the food temperature at 140 F or above.

11. All food stands should have a separate water hose connection installed for use in floor cleaning operations. Also, vacuum breakers should be installed on all hose connections at the food stands, animal watering troughs, and at the cleaning facility for garbage trucks and equipment.

12. All sewer connections need to be properly trapped

to avoid odors.

13. All sources of ice should be investigated for compliance with public health standards before being considered acceptable for use at the Festival.

14. Changes in the location of a number of facilities should be considered at the next Festival. The foodservice sites, first aid stations, and employee feeding facilities should not be located near areas such as horse stables and garbage, trash-dumping, or collection areas. These should be located in areas of sufficient distance from other facilities so that the problems of flies, odors and rodents will be minimized.

Some improvements in operating practices which should be considered in planning future festivals include the following:

1. All bakery products or other food staples not packed in rodent-proof containers should be removed from the stand each night or rodent proof containers should be provided for their storage during nonoperating periods. It was necessary on several occasions during the last 3 weeks of the Festival to destroy large quantities of bakery products which were contaminated by rodents.

2. Concessioners should be required to provide an adequate supply of dippers of assorted size, large spoons, long-handled scoops, plastic gloves, and similar utensils needed to minimize contact with foods.

3. Concessioners should be required to provide approved single-service cup dispensers for all sizes of single-service cups used.

4. The use of horses by the National Capital Park Police in the main visitor areas of the Festival needs to be reevaluated. In view of the amount of horse manure deposited at these areas and near the food sites and the manure not being disposed of properly, it is suggested that the mounted police be stationed only at the fringe areas and that motor bikes or electric golf carts be used to patrol the main areas of the Festival and that, even then, more frequent manure collection be established.

5. When not in use, grates for outdoor grills should be stored inside the stand following cleaning to protect the food contact surfaces from dust, bird droppings, and rodents.

As previously stated, this report was intended in part to provide guidance to regulatory agencies responsible for planning and implementing a comprehensive environmental health program at long-term temporary gatherings such as fairs and community festivals. It should be noted, however, that the unusual length of the Festival of American Folklife dictated the installation of more sophisticated facilities than would be economically feasible or perhaps even necessary for many short term (3-10 day) affairs of this type. For example, installation of concrete floors and sewers may not be justified in such cases unless the same site is used several times in a year or annually over a long period. Well constructed wooden platforms would be adequate flooring for such stands where the affair does not exceed 10 days. Likewise, improvised handwashing facilities can be used in lieu of conventional sewer-connected lavatories if there is adequate monitoring during the operating periods to assure proper use.

Accordingly, it is suggested that where the facilities to be used do not meet fully the requirements set forth in current PHS-FDA *Food Service Sanitation Manual,* the regulatory agency involved meet with the concession operators and establish jointly the facilities needed to assure adequate food protection, waiving or modifying the FDA criteria when it is determined that such action will not result in a health hazard. In this process, it is highly recommended that all menus be approved by the regulatory agency during the planning stage to serve as a basis in part for establishing what facilities will be needed. At times it may be necessary to impose even more stringent requirements to assure that the proposed operations are acceptable from a food protection standpoint.

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Progress in Palmito (Heart-of-Palm) Processing Research

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ABSTRACT

Palmito is a gourmet product obtained from the young and tender leaves of certain palm trees. Brazil is the main producer and exporter of canned, acidified palmito. In this paper the main aspects of raw material procurement, processing and packaging are reviewed. Most of the original work was done at the Instituto de Tecnologia de Alimentos (ITAL) in Campinas, Brazil. The palmito is mainly produced from two species of naturally growing palms. There are two main technical problems in palmito processing: discoloration of the naturally white product and safe acidification to inhibit *Clostridium botulinum* growth. Both problems have been solved adequately in laboratory and pilot plant work. Acceptance and implementation of these technological developments by the small palmito canneries is essential to obtain products acceptable to the international market.

Palmito is a gourmet product obtained from the young and tender leaves of certain palm trees. In some areas of Brazil the fresh palmito can be bought in open markets and even in supermarkets. However, due to the perishable nature of the product, most of the product is presently canned and heat processed. Canning of palmito is done in very primitive plants located in the palm-growing areas. The Instituto de Tecnologia de Alimentos (ITAL) was the first institution to study the raw materials and processing techniques (*3,5,15*). However, it was only recently that an intensive program was initiated on various aspects of palmito processing: types of raw materials, acidification, browning, heat processing, packaging. In this paper the most significant findings will be discussed.

PRODUCERS AND CONSUMERS

The world production and consumption of canned palmito was recently surveyed by Renesto (*13*). Presently Brazil and Paraguay are the main producing countries. In 1975 Paraguay exported 2464 tons while Brazil exported 7012 tons.

Most of the product of Paraguay is exported to Argentina while France consumes approximately 70% of the Brazilian export. The volumes exported to the United States were quite variable during the last years. In 1974 it amounted to 841 tons and in 1975 to 571 tons.

In addition to the export, palmito is a valued product for Brazilian dishes. No reliable statistics on the domestic consumption of palmito are available. However, the available data indicate that approximately two-thirds of the canned palmito is consumed in Brazil.

Due to the great demand for canned palmito and the scarcity of the raw material, the export price has increased steadily from US$ 508.00 per ton in 1965 to US$ 1,294.00 per ton in 1975.

RAW MATERIALS

Palmito from the following species of palms has been processed successfully:

Common name	*Scientific name*	*Reference*
Juçara	*Euterpe edulis* Mart.	(*4,9*)
Açaí	*Euterpe oleracea*	(*9*)
Indaiá	*Attalea dubia*	(*9*)
Babacú	*Orbignya oleifera* Burret	(*3*)
Guariroba (bitter palmito).	*Syagrus oleracea*	(*9*)
Bacuri	*Scheelea phalerata*	(*9*)

In addition there exist several species of palms in Brazil and in other countries, some of which may not even have been studied adequately botanically. Some of these can possibly be economical sources of palmito.

There is considerable interest in the processing of the palmito of the babaçú palm which is also a major source of palm oil in Brazil. The babaçú can be found as the predominant feature of the vegetation of 10 million hectares in the central and northern part of the country (*3*).

Presently only the palmitos of the Jucara and Acai palms are processed in large amounts. There are small areas along the coast of Brazil where the giant palmito (Indaiá-palm) is processed.

The geographic distribution of the two main palmito producing palms in Brazil is shown on Fig. 1. Until

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recently the Jucara palm (*Euterpe edulis*), found in the south, was the main source of palmito. Since 1973 more palmito is being exported through the northern ports (Belém) than through the southern ports (mainly Santos) of Brazil. This is due to the abundant supply of palmito from the Açaí palm (*Euterpe oleracea*) in the north while the Juçara in the south is getting depleted.

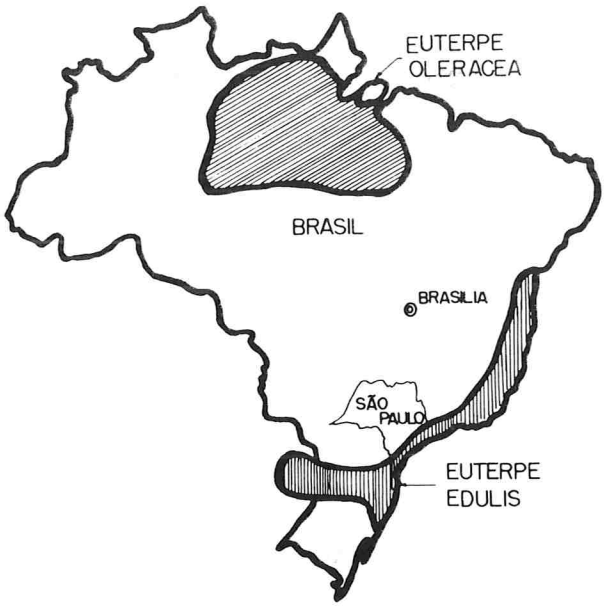

Figure 1. *Approximate geographic distribution of two palmito producing palms.*

Presently all the palmito harvested is from natural seeding. It is only recently that some companies have replanted some of the palms, mainly of the Jucara species, along the southern coast of Brazil. The oldest plantations are now approximately 7 years old and should be ready for harvesting. Unfortunately the commercial plantations of palmito-producing palms have not been very successful due to lack of bacis knowledge involving the following: preparation of the seeds and seedlings, type of shading required, spacing between plants, and time between successive harvests.

Figure 2 shows a typical Juçara palm in its natural habitat. This plant may be 6 to 12 years old. Figure 3 shows the upper part of the palm, from where the palmito is obtained. The edible part starts just above the stem and ends where the tissue becomes hard and fibrous due to its transformation into adult leaves. Roughly one-third of the length shown in Fig. 3 is edible. While the outside diameter of the section is typically 10 to 20 cm, the inner edible part has typically a diameter of 3 to 6 cm.

Figure 4 shows a typical young plant of the Acaí palm. Unlike the Juçara palm, the acaí plant grows in a cluster of shoots of different ages. If only the older shoots are harvested, the plant continues its growth and the adult palmitos can be harvested at regular intervals. Due to lack of information and control, frequently the whole plant is harvested at once. In this case the Açaí plant may die.

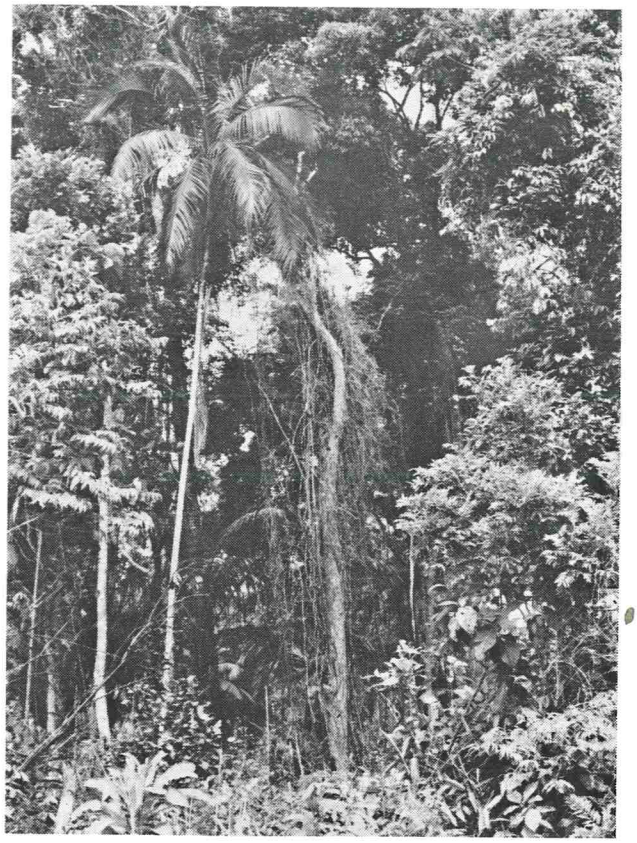

Figure 2. *Juçara palm* (Euterpe edulis) *in its natural habitat.*

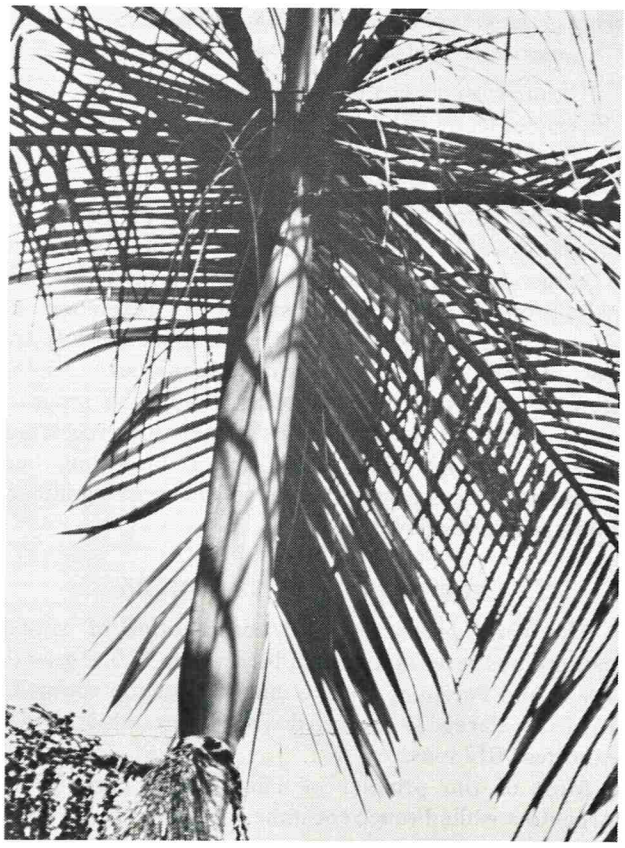

Figure 3. *Palmito — containing region of the Juçara palm* (Euterpe edulis).

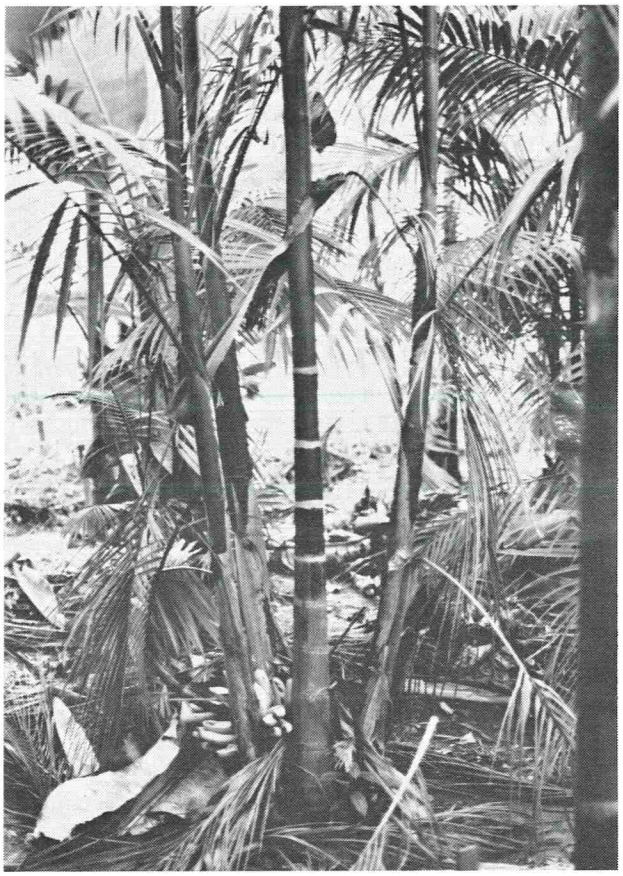

Figure 4. *Young Acai palm* (Euterte oleracea)

The Juçara plant, having only one stalk, is always killed when the palmito is harvested. New plants have to grow from seeds. Due to the intensive harvesting in the southern part of Brazil, the factories processing Juçara palmito are being transferred to the Amazon region where they now process Açaî palmito.

The palmitos of the Juçara and Açai palms are quite similar. No special mention of the species is made on the labels of the canned product. Usually the diameter of the palmito from the Juçara palm is larger than that of the Açaî palm.

Present Brazilian Government regulations require that no palmitos with less than 2.5 cm diameter be canned. These regulations were made to prevent the harvest of palms before the adult stage. However, harvesting is done by poorly qualified personnel and therefore many small palmitos are brought to the factories.

After cutting the palm, the harvester cuts the edible part which usually has a length of 40 to 60 cm. Next he removes the outer shells (sheaths) but leaves several nonedible layers for protection of the palmito during transportation. A typical bundle of product at this stage is shown in Fig. 5. In the southern part of Brazil the raw material is frequently transported by mules while in the Amazon, transportation is mainly by boat. The time from harvest to delivery at the factory sometimes takes 1 week, causing extensive product loss and quality

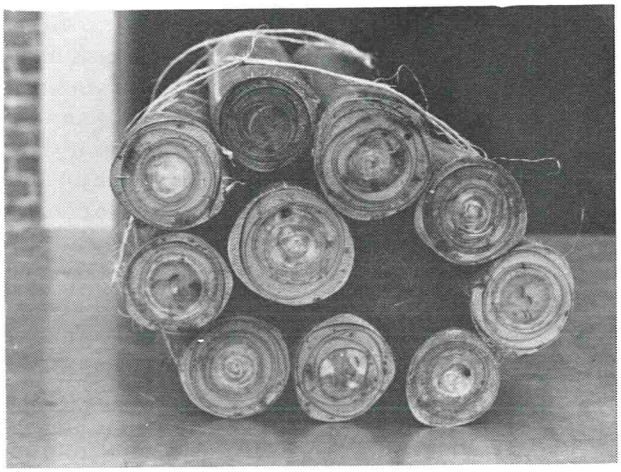

Figure 5. *Bundle of palmitos as delivered at the factory*

deterioration. Depending on the moisture conditions, dehydration or microbial spoilage occurs, starting from the ends.

Immersion of the ends into a disinfectant solution after cutting could help maintain the quality of the product but is difficult to implement under present conditions.

CHEMICAL COMPOSITION

The chemical composition of the Jucara and Acai is given in table 1 (*14*). The differences between the two palms may in part be due to the differences in their ages, since the Acai palmito is frequently harvested at an earlier age. It can be seen that no important nutritional value can be claimed for palmito. Just like asparagus, the palmito is consumed for its pleasant flavor and texture.

TABLE 1. *Chemical composition of palmito* (*14*).

Component	Juçara	Acai
Protein (%)	2.42	1.72
Ash (%)	1.43	0.83
Crude fiber (%)	0.89	0.27
Fat (%)	0.33	0.08
Total sugars (%)	0.86	0.70
Reducing sugars (%)	0.49	0.30
Tannins (%)	0.06	0.06
Vitamin C (mg/100 g)	1.8	1.4

PROCESSING

The traditional processing of palmito is summarized in Fig. 6. The preliminary peeling is usually done outside the factory. Up to 50% of the weight is eliminated at this stage. The process is manual and is illustrated in Fig. 7. The final peeling has to be done more carefully to avoid damage to the soft inner tissues. The fully peeled palmito is shown on the right side of Fig. 8. Then the peeled palmito stem is cut with a knife into pieces slighty shorter than the can height, starting from the lower end.

The palmito cutter evaluates the hardness of the tissue by the cutting force required. Usually, as the upper end (towards the leaves in the palm) is approached, the outer

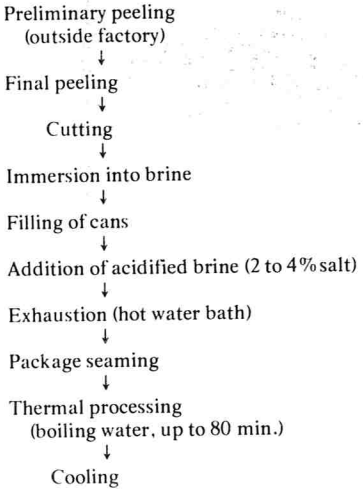

Figure 6. *Traditional process of palmito canning.*

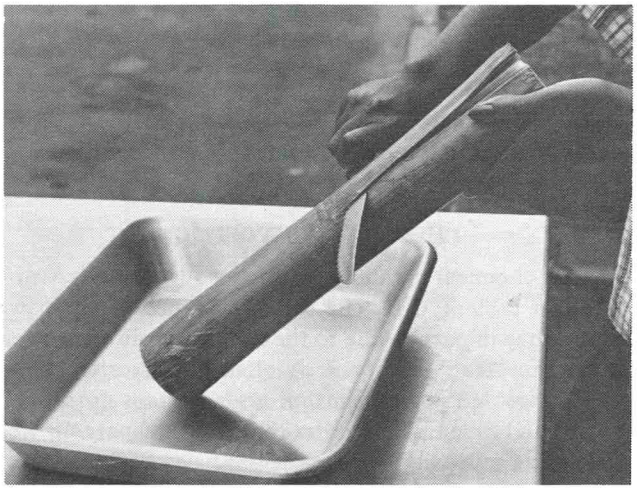

Figure 7. *Preliminary peeling of palmito*

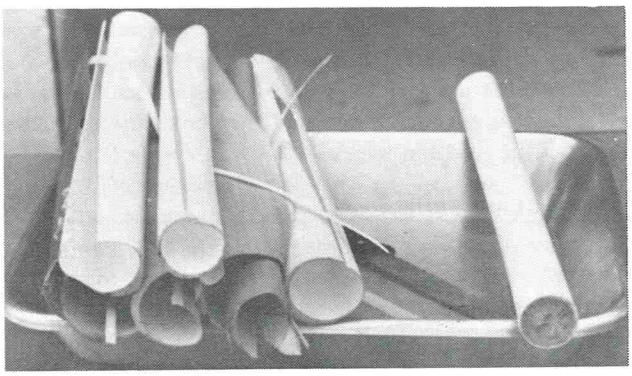

Figure 8. *Palmito (on right side) and wastes from preliminary (dark) and final (white) peeling.*

shell (sheathing of the leaf) becomes gradually harder and needs to be removed to yield a soft inner part of smaller diameter. The cutting process in continued up to a point where the whole section becomes unacceptably hard.

After cutting, the damaged tissues show rapid enzymatic browning in the presence of oxygen. Therefore the pieces are usually immersed in a sodium chloride brine with citric acid (0.5 to 0.8%) and which may also contain sulfur dioxide. Usually some browning takes place at this stage but most of the browning occurs during the exhaustion. This process is slow and at this time the tissue oxygen is expelled through the damaged tissues at the cut surfaces. Some browning also occurs between the sheaths of the palmito. The discoloration is typically bluish-gray rather than brown.

Since the palmito is an acidified product (*2*), thermal processing is usually done in a boiling water bath. If the product is not well exhausted, additional browning may occur during heat processing.

Some experiments have been done on the heat processing of palmito at its natural pH of approximately 6.0. A pink discoloration of the product was observed when a retort temperature of 121 C was employed, even for well-exhausted cans. However, there is no special interest in producing a low acid canned palmito since it is usually consumed in salads.

IMPROVED PROCESSING

The main technological problem encountered in processing of palmito is browning. Preliminary work at ITAL indicated that enzymatic browning was particularly fast in the temperature range of 40 to 60 C (*16*). Contact between the substrate, the oxygen and the enzyme in this temperature range had to be either avoided or reduced to a minimum. Faster heating by rotating the can during heating was tested but was found to be too slow to avoid browning.

Next the possibility of eliminating oxygen from the plant tissue was studied (*8*). It was found that the tissue contianed 11 to 17 cm^3 of gas per 100 g. Composition of the gas was similar to that of air. This gas could be removed readily by applying vacuum to the palmito pieces immersed in the acid brine for 10 to 30 sec.

Based on these laboratory experiments on deaeration of the tissue, pilot plant experiments were done using commercially available equipment (*7*). The palmito was cut, immersed in 3% NaCl +0,85% citric acid brine and transferred to the cans. It was covered by cold canning brine of adequate acidity (citric acid). Then the cans were sealed in a vacuum closer (120 mm Hg residual pressure for 15 sec). The product in 300 × 406 cans was heat-processed for 40 min in boiling water.

The canned palmito was compared with a similar product in which gas (oxygen) removal was done by the traditional method (thermal deaeration or exhaustion). Both processes gave a final can vacuum of approximately 15 inches of mercury. Figure 9 shows the two products obtained. It can be seen that the thermally deaerated product is distinctly brown at the surfaces while the vacuum-seamed product is white. It must be mentioned

that thermal deaeration employed in this experiment was relatively fast. Under commercial processing conditions by the traditional method, browning is frequently much stronger.

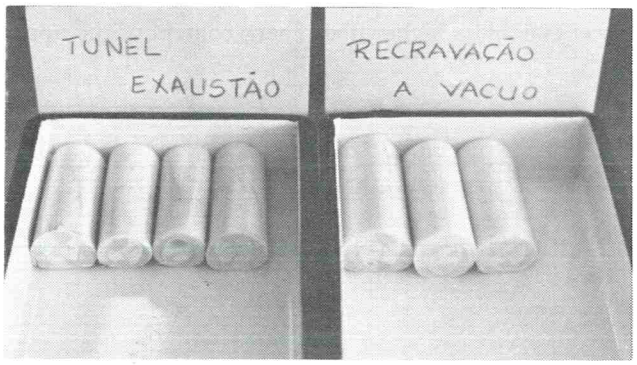

Figure 9. *Canned palmito obtained by traditional (left) and improved (right) processes.*

DEFECTS OF PROCESSED PALMITO

From experience of the authors and surveys by the Ministry of Foreign Relations (*6*) and by Renesto and Vieira (*13*), the following appear to be the most common defects of the product presently produced in Brazil: (*a*) inadequate acidification, (*b*) hard pieces and too soft pieces, (*c*) browning, (*d*) poor vacuum, (*e*) poor control of drained weight, and (*f*) packaging problems such as rusty cans, loose labels, and weak cardboard boxes.

From Table 2 it can be seen that extremely wide variations of key control factors can be observed between manufacturers. Slightly smaller variations can be observed within products of the same manufacturer.

TABLE 2. *Typical values for 21 No. 2 1/2 cans of palmito from southern Brazil (13).*

Determination	Minimum	Maximum	Average
pH	4.3	4.8	4.5
Vacuum (in Hg)	0.0	9.0	3.2
Headspace (mm)	8	20	11.6
Total weight (g)	885	968	945
Drained weight (g)	395	587	486

Proper acidification of the palmito is a very important factor for safety of the product. This subject will be treated separately.

Inclusion of hard pieces results from poorly trained personnel and poor quality control. Some producers think that longer cooking will render these pieces softer. Although this is true, the additional cooking does not destroy the fibers. In addition, the regular raw material becomes too soft when overcooked, losing its distinctive character. The delicate flavor of the palmito is also affected by excessive cooking. For this reason the processing time in the improved method was reduced to 40 min from 80 min in the traditional process.

Browning and poor vacuum are usually related. Some manufacturers make almost no exhaustion since they fear browning during this process. However, in this case browning occurs during heat processing. The improved process always gives adequate vacuum and a product without any discoloration.

Control of the drained weight for palmito is quite difficult, especially in small cans, since the individual pieces are relatively large. The problem becomes even more difficult if uniformity of length and diameter is sought. In addition, small variations of the drained weight may occur during storage. Paschoalino and Berhardt (*7*) found a 4% decrease in the weight of the palmito canned in a 2.5% NaCl +0.85% citric acid brine after 30 days of storage (very close to equilibrium). This decrease may be larger for more concentrated brines.

The packaging problems presently encountered are the consequence of the primitive conditions under which palmito is processed. Rusty cans result from cooling in water contaminated with canning brine. Inside corrosion of the cans is rarely a problem unless SO_2 is used in the canning brine. Plain tin cans are to be preferred over lacquered cans. The palmito in lacquered cans is usually slightly darker than the palmito in plain tin cans.

ACIDIFICATION OF PALMITO

Until recently no clear limit of the pH for dividing foods into "low acid foods" and "acid foods" was established. It was accepted by various authors to lie in the range of 4.4 to 4.7. Recently the Food and Drug Administration (*2*) established the limit at pH = 4.6. The natural pH of palmito is approximately 5.8.

Presently the acidification of palmito is done by using a brine containing a fixed percentage of the acid (almost always citric). Different concentrations are employed by the various manufacturers, usually without any scientific reason. Sometimes the manufacturers consider this as proprietory information. As a result of the misinformation many lots of the product reach the consumer with an equilibrium pH well above 4.6. Several lots of canned palmito have been retained by the Food and Drug Administration in the U.S., due to inadequate acidification. Presently the European countries do not appear to have any legal restrictions with regard to acidification and thermal processing of imported canned foods. The authors do not know of any documented case of botulism in canned palmito that was not properly acidified. They have heard about suspected botulism in a product exported from Paraguay to Argentina.

However, since the danger of botulism exists, proper acidification is a fundamental requirement for safety of the product. To allow acidification to a specified pH, it is necessary to know the titration curve of the product. Figure 10 shows the titration or acidification curves of the Juçara palmito with different acids. Not surprisingly, hydrochloric acid lowers the pH to 4.3 with the smallest amount of acid. Unfortunately this acid is not allowed for

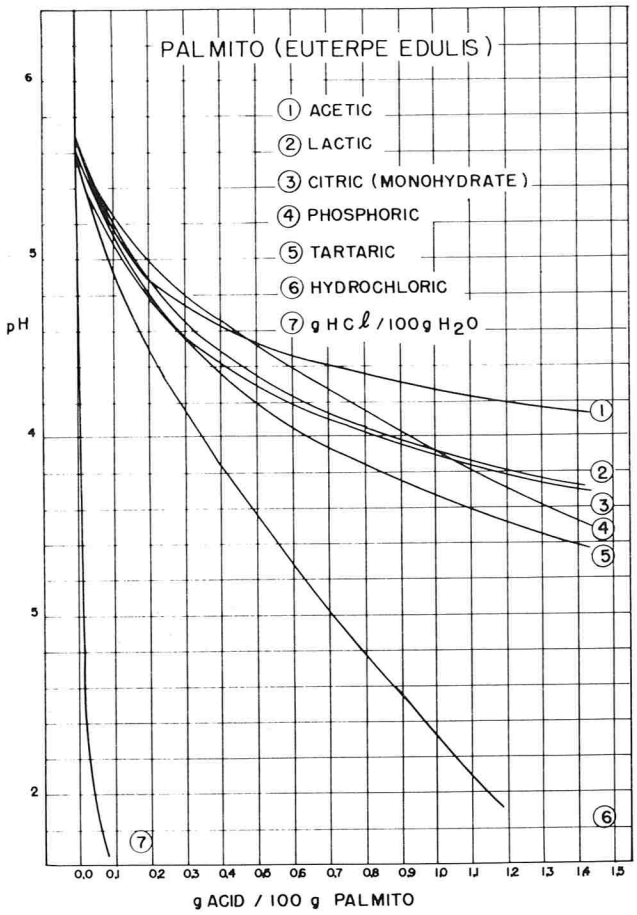

Figure 10. *Titration curves of palmito with different acids (4).*

the Juçara palmito with different acids. Not surprisingly, hydrochloric acid lowers the pH to 4.3 with the smallest amount of acid. Unfortunately this acid is not allowed for food products in most countries. The authors see no reason for this restriction since the amount of chloride from the salt is approximately 10 times larger than that from the acid.

Citric and lactic acid showed very similar acidification curves. Approximately 0.3 g of acid per 100 g of palmito are required to lower the pH to 4.6. Therefore, this amount has to be present in a can to obtain a pH of 4.6. The acid concentration of the brine depends on the product-to-brine ratio. If this ratio is not controlled closely, considerable variations in the pH can occur.

The great variability of the equilibrium pH of canned palmito prepared with the same brine and with the same product-to-brine ratio always intrigued the authors. A search for the source of this variability showed that the different parts of the palmito had remarkably different buffer capacities. From Fig. 11 it can be seen that 0.35% citric acid was required to reach pH 4.3 for the upper part (towards the leaves) of the palmito. For pieces from the lower end, 0.67% acid was required. Pieces between the extremities gave intermediate results. The same canning brine which gives a very safe pH of 4.3 for the upper part, would yield an unsafe product of pH 4.65 if used in a can containing only pieces from the lower end of the palmito. These differences were found to be quite consistent and were observed for all the five species studied (*9*). The reasons for this behavior are not fully established. It could be due to higher concentrations of free amino-acids and salts in the younger tissue of the lower end and a higher fiber (inert) content of the upper end.

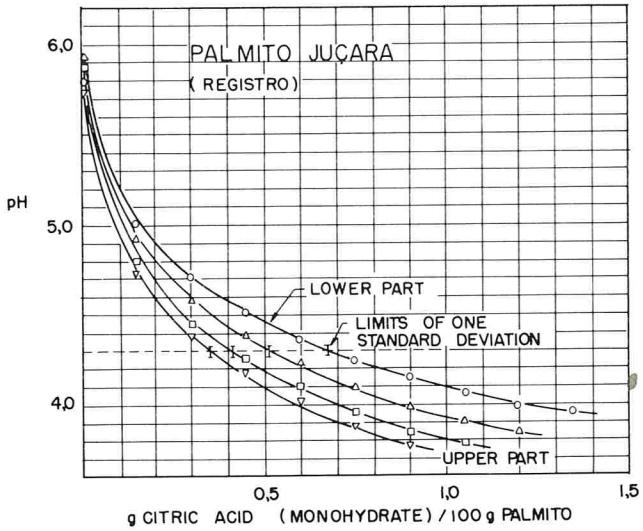

Figure 11. *Titration curves of different parts of the palmito (9).*

The variation of the buffer capacity along the palmito brings an unexpected complexity to the commercial acidification process. Clearly, three alternatives are available. (*a*) Use enough acid brine for the lower end of palmito; cans containing only upper end will be quite sour; range of variation of pH will be about 0.4 pH-unit. (*b*) Carefully mix pieces from upper and lower end into the same can; use brine of intermediate acid concentration; this procedure is the most recommended one. (*c*) Separate product into "lower end" and "upper end"; use different brines.

While the first alternative is the only safe one for primitive processing conditions, the last method would be recommended for well controlled operations. In present commercial practice the product is frequently divided into two groups, since the lower end is the softer, higher quality part. Unfortunally the same brine is used for the two product groups.

In addition to the differences between the upper and the lower end of the palmito, large differences in the acidification curves were also found between species (*9*). Figure 12 shows that the Juçara palmito required an average of 0.53% citric acid to reach pH 4.3, while the Açaí palmito required only 0.34%. Fortunately these two types of palmitos are usually not processed at the same factory, due to their geographic distribution.

Currently the Brazilian legislation does not permit use of more than 0.2% citric acid in vegetables, and palmito is included in this category. Fortunately this requirement is virtually not enforced and some palmito canners are now starting to implement satisfactory acidification

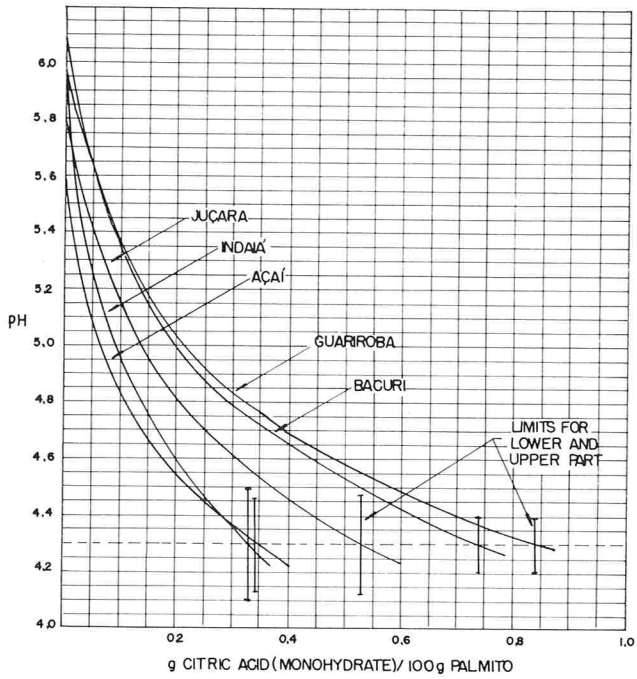

Figure 12. *Titration curves of palmito from five species of palms (9).*

procedures.

An additional problem for safe acidification involves the rate of diffusion of the acid of the brine into the low-acid food. Quast et al. (*10*) found that 33 days after canning there was still a small difference between the average pH of the product and the pH in the center of the palmito pieces. Similar results were obtained by Ferreira (*1*) for palmitos of different species of palms. Figure 13 shows in cross-section the pH distribution in a piece of palmito of 5-cm diameter and 10-cm length, 8 days after canning. It can be seen that in this case after 8 days the pH in the center was still sufficiently high for growth of *Clostridium botulinum*. Of course, it is very unlikely that spores of *C. botulinum.* would be present at the center of a piece of palmito. Since the question of safety under conditions of acidification by diffusion has not been solved for other foods, the best that can be done is to acidify to a specified equilibrium (average) pH. It must be mentioned that the pH of the brine containing 0.5% citric acid is approximately 2.5. Two days after processing the pH of the brine was 3.8 to 4.0, while the average pH of the palmito was 4.3 (*1*). In this case the average pH of the can contents was 4.1.

With the purpose of accelerating the acidification, palmito pieces were immersed into 5% citric acid solutions and vacuum was applied to deaerate the product. It was hoped that breaking of the vacuum would cause acid penetration. However, this procedure did not give significant acidification in the interior of the product.

Palmito stems with freshly cut lower ends were placed into solutions containing acid. The regular biological transport of the fluid did not take place, probably because there was no evaporation on the other end as it occurs when there are leaves on the upper end.

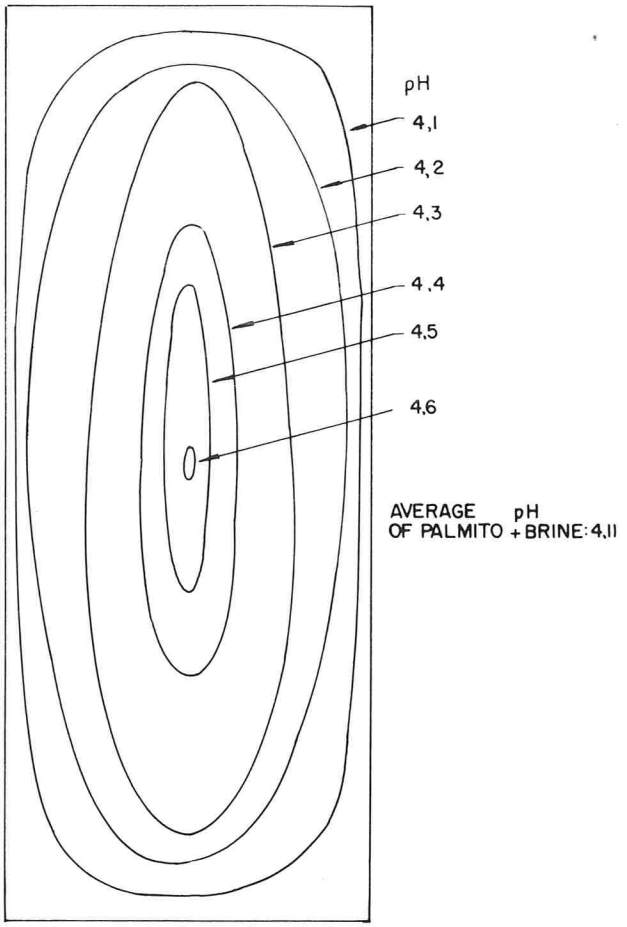

Figure 13. *pH at different locations of a 5-cm diameter piece of palmito, 8 days after processing (11).*

ACKNOWLEDGMENT

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REFERENCES

1. Ferreia, V. L. P. 1975. Comparação fisico-quimico-sensorial do palmito de três especies de palmeria. (Comparison of the physical, chemical and sensory properties of the palmito of three species of palms). Final report, project 46.5/75., Instituto de Tecnologia de Alimentos, Campinas, Brazil.
2. Food and Drug Administration. 1976. Proposed good manufacturing practices: Pickled, Fermented, Acidified and Low Acid Foods, Federal Register, July 23, 1976 p 30441-30462.
3. Martin, Z. de, C. G. Teixeira, E. Bleinroth, V. C. Scarbieri, T. B. de Menezes, J. P. Nery. 1969. Estudos preliminares sobre o processamento do palmito da palmeira de babaçú (*Orbignya oleifera* Burret) (Preliminary study on the processing of the palmito from the babaçú palm). Coletânea de Tecnologia de alimentos e Alimentos 3:435-452.
4. Mejia Zapata, M. and D. G. Quast. 1975. Curvas de titulação do palmito-doce (*Euterpe edulis* Mart) (Titration curves of sweet palmito), Coletânea do Instituto de Tecnologia de Alimentos 6:167-187.
5. Menezes, T. B. and M. F. F. Leitão. 1967. Estudo comparativo do

palmito enlatado (Comparative study of canned palmito), Coletânea do Instituto de Tecnologia de Alimentos, 2:11-22.
6. Ministry of Foreign Relations (Brazil). 1971. O mercado norte-maericano do palmito (The American market for palmito), Coleção do Comércio Exterior-Mercados para o Brasil, série I, No. 27.1971.
7. Paschoalino, J. E., and L. W. Bernhardt. 1977. Influência dos méto-dos de exaustão sôbre a qualidade do palmito enlatado (Influence of exhausting methods on the quality of canned palmito), accepted for publication in Coletânea do Instituto de Tecnologia de Alimentos, Campinas, Brazil.
8. Paschoalino, J. E., L. W. Bernhardt, D. G. Quast. 1977. Volume e composição da mistura de gases oclusos nos tecidos do palmito (Volume and composition of the mixture of gases occluded in the palmito tissue), accepted for publication in the Coletânea do Instituto de Tecnologia de Alimentos, Campinas, Brazil.
9. Quast, D. G., and L. W. Bernhardt. 1976. Curvas de titulaçao do palmito de cinco espécies de palmeiras (Titration curves of the palmito of five species of palms), Coletânea do Instituto de Technologia de Alimentos 7:241-263.
10. Quast, D. G., M. Mejia Zapata, and L. W. Bernhardt. 1975. Estudos preliminares sobre a penetraçao da acidez no palmito enlatado (Preliminary study on the acid penetration into canned palmito), Coletânea do Instituto de Tecnologia de Alimentos 6:341-349.
11. Quast, D. G. 1976. Unpublished results, Instituto de Tecnologia de Alimentos, Campinas, Brazil.
12. Renesto, O. V. 1976. Data presented at the First Seminar on Palmito Processing, April 26 to 27. Instituto de Tecnologia de Alimentos, Campinas, Brazil.
13. Renesto, O. V., and L. F. Vieria. 1977. Análise econômica da produção e processamento de palmito na região sudeste-sul do Brasil, (Economic analysis of palmito production in southeastern Brazil), submitted for publication at Instituto de Technologia de Alimentos, Campinas, Brazil.
14. Shimokamaki, M., C. Abdalla, J. F. Franca, I. S. Draeta, I. B. Figueiredo, E. Angelucci. 1975. Estudos com parativos entre os palmitos de sabor doce e sabor amargo I: Composicao quimica: péptides e aminoácidos livres (Comparative study on sweet and bitter palmitos: Peptide and free amino-acid content), Coletanea do Instituto de Tecnologia de Alimentos 6:69-79.
15. Teixeira, C. G. 1966. Fino alimento vegetal brasileiro é industrializado em condições precárias (A fine Brasilian vegetable is processed under precarious conditions) Alimentos e Bebidas 2(3):12-13.
16. Yang, J. F. 1976. Estudo sobre escurecimento do palmito (Study of the browing of palmito), Progress report of project 46.3, Instituto de Tecnologia de Alimentos, Campinas, Brazil.

Whey Land Application Safe, Benefits Crops

Whey, the liquid by-product of the cheese-making process, can be an excellent source of nutrients for crop plants, according to University of Wisconsin-Madison soil scientists Arthur E. Peterson and William G. Walker. In addition, it can be applied to the soil in relatively large amounts without posing a threat to the environment.

Peterson told the American Chemical Society meeting in Chicago Aug. 31 that studies on whey application to fields indicate the material provides all the nitrogen, phosphorus and potassium needed by growing crops, and that phosphorus in whey, considered the most serious potential pollutant substance, remains in the root zone even after many years' application without inhibiting plant growth.

Dried whey has found several uses as human food—in cake and bread mixes, puddings, etc.—and as a high protein addition to animal feeds. But the drying equipment is expensive, impractical for small cheese plants, which must find other suitable ways to dispose of liquid whey.

State environmental protection regulations prohibit disposal of whey in waterways. Disposal into municipal sewage treatments facilities is costly and wastes nutrients.

Many plants are turning to nearby land disposal of whey, Peterson said, saving energy needed to transport the material.

Peterson said corn yields in test plots increased dramatically with applications of 4 and 8 acre-inches (an acre-inch is 28,000 gallons) of whey. Per acre yields were 100 bushels higher with 4 acre-inches and 110 bushels higher with 8 acre-inches in the first year of tests after fall and spring applications. Average increases for four years of tests (including two poor corn years) were 56 bushels with 4 inches and 70 bushels with 8 inches. Yields begin to fall off somewhat with heavier applications, Peterson noted.

Corn plant leaves in whey-treated plots were "a deep, dark green," Peterson reported, "indicating an abundance of nitrogen throughout the growing season."

An acre-inch of whey contains about 320 pounds of nitrogen, 100 pounds of phosphorus and 400 pounds of potassium.

Phosphorus and potassium build up in the soil, sometimes moving below the principal root zone. But tests of groundwater quality indicate no threat from phosphorus. Peterson says the reason is that the soil serves "as a sink, preventing downward movement of appreciable amounts of phosphorus."

Potassium moves deeper into the soil than phosphorus and could eventually enter the groundwater, Peterson said, but not in amounts to make it a serious pollutant.

For the many small cheese factories around the nation, the most satisfactory application method is truck spreading on crops needing nitrogen, such as grasses and corn, but not on alfalfa, Peterson said.

Cranberry Juice used for food coloring

Cranberry juice concentrate probably will be used as a replacement for red dyes in food colorings. It gives a satisfactory coloring for pie fillings, says Mrs. Sally Coble, foods and nutrition specialist with the Texas Agricultural Extension Service, The Texas A&M University System.

Thomas A. Nickerson, 1921-1978

A memorial scholarship fund was recently established by the Department of Food Science and Technology at U.C. Davis to honor Thomas A. Nickerson, who died unexpectedly following a heart attack on May 29. He was 56.

Nickerson, who had won international recognition for his research on lactose, began his career at UCD in 1950 with the Department of Dairy Industry. He was Associate Dean of the College of Agricultural and Environmental Sciences at Davis from 1964 to 1969, and acting Associate Dean in 1976. Also active in the University's academic senate, he was Chairperson of the Faculty of the College at the time of his death.

In 1969-70 he was a visiting scholar at the New Zealand Dairy Research Institute, and in 1976-77 he worked with the Faculty of Agriculture, Novi Sad, Yugoslavia through the Fulbright-Hays Program. Also in that same year he did work at the Division of Food Research, CSIRO, Highett, Australia.

Nickerson was a member of the American Dairy Science Association and President of its Western Division in 1961-62. He was also a member of the Institute of Food Technologists and Sigma Xi.

His research activities over the years, in addition to investigations of the chemical and physical properties of lactose, included butter packaging, preservation of milk, chemical composition of milk and milk constituents, and ice cream problems—particularly "sandiness." He wrote some 80 publications concerning lactose, butter, frozen milk, and concentrated milk.

More recently his work had concentrated on lactose utilization. His projects included studies of acid and enzymatic hydrolysis of lactose, adsorption of flavor volatiles by lactose, control of lactose crystallization, production of different forms of lactose by organic solvents or heat, and functional properties of lactose and its hydrolysis products in foods. His work lead to contributed chapters on lactose in both the first and second editions of *Fundamentals of Dairy Chemistry* and the second edition of *Byproducts From Milk.*

His most recent report of lactose utilization was "Why Lactose and Its Derivatives in Food?" published in the January issue of *Food Technology.* He recently presented invitational papers at technical meetings of the American Dairy Science Society and Western Food Industry Conference. He was to have presented an invited paper, "Biochemistry and Biophysics of Lactose," at the International Dairy Congress meeting in Paris at the end of June.

Nickerson was a Davis native. He attended the local schools and received his bachelor's degree in Dairy Industry in U.C. in 1943. He earned his M.S. and Ph.D. degrees in Dairy Manufacturing at the University of Minnesota in 1948 and 1950.

He was active in Cub Scouts, Boy Scouts, and Little League, as well as 4-H from which he received an honorary membership in 1971 in recognition of his 15 years' advisory committee service. He was a member of Kappa Sigma, and had served with the Navy in World War II.

He is survived by his wife, Katinka, two sons, Mark and Eric, and a daughter, Natalie.

Contributions for the Thomas A. Nickerson Memorial Scholarship Fund may be sent to the Department of Food Science and Technology, University of California, Davis, 95616. Checks should be made payable to The Regents of the University of California.

1978 ACDPI Conference to Feature International Symposium and Forum for Dairy Editors

Plans for the 1978 American Cultured Dairy Products Institute Annual Meeting and Conference have been finalized, reports Institute Secretary Dr. C. B. Lane. Approximately 300 cultured product processors, allied tradesmen, regulatory agency personnel, and university staff members from 40 states are expected for the Sept. 12-13 conclave at the Marriott Hotel, Atlanta, GA.

Robert Elliott, editor of *American Dairy Review,* will launch the conference with a presentation, "Innovative Marketing and Merchandising Programs for Cultured Dairy Foods." A forum, "The Editors Speak Out," will feature Herb Saal, editor of *Dairy Record,* and Jay Sandler, publisher of *Dairy and Ice Cream Field.*

An International Symposium the afternoon of Sept. 12 will feature cultured product marketing and technology experts from Europe and Canada. General presentations will be given both days and will include a report on an ACDPI-sponsored national survey on yogurt manufacture and preferences.

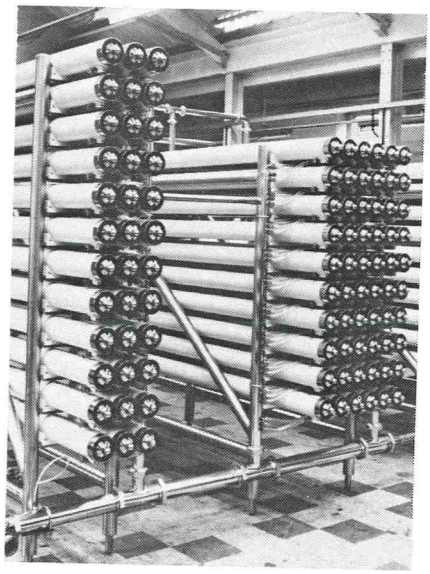
A "mini-clinic" on "Quality Concerns" will conclude the conference.

Additional information and advance registration materials may be obtained from Dr. C. B. Lane, ACDPI, P.O. Box 7813, Orlando, FL 32854, or Margie Franck, ACDPI, 910 17th St., N.W., Washington, D.C. 20006.

In France, it appears that milk will get top billing in a new French Health Ministry campaign aimed at educating the public about the importance of a well-balanced diet. In the $156,000 campaign, the French Health Ministry will promote breads, cereals and milk in an effort to teach French citizens what to eat.

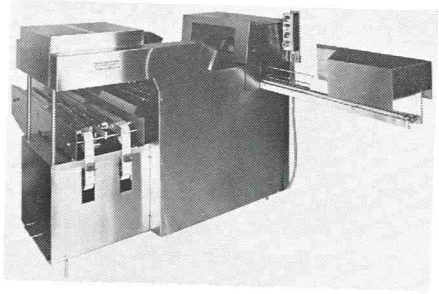
•*The marketing, installation* and servicing of PCI Reverse Osmosis and Ultrafiltration systems for food and dairy industries in North America is being handled by Damrow Company, Fond du Lac, Wisconsin. Reverse osmosis is particularly useful in cheese whey and skim milk concentration and in sugar and antibiotic concentration. Ultrafiltration finds application in protein recovery from whey protein enrichment of skim milk, and enzyme concentration and purification. Damrow Company is offering in-plant and portable pilot plant operating demonstrations and product pretesting. For more information on the operation and application of PCI reverse osmosis and ultrafiltration systems, contact the Damrow Company, Fond du Lac, Wisconsin, a Division of DEC International.

•*A new bulletin on high-volume* reverse osmosis systems describes two central water purification systems designed to produce 125 to 250 liters per hour (800 and 1600 gallons per day). These high purity water purification systems are especially designed and engineered for central building distribution, boiler feed or pyrogen-free pharmaceutical production. The capital and operating costs are much lower than, and the water quality is comparable to, distillation. The Millipore Milli-RO systems are available for immediate delivery, and service is available regionally. Millipore bulletin PB843 on "Milli-RO High Volume Reverse Osmosis Systems" is available free, on request, from Millipore Corporation, Water Systems Division, Bedford, Massachusetts, 01730. Or, by calling toll-free 800-225-1380. In Massachusetts, call 617-275-9200.



•*A new concept* in fine particulate removal, the Bird vortex clarifier, is described in a brochure now available from Bird Machine Company, Inc. The equipment, covered in the four-page bulletin (BVK101), uses a new concept in centrifugation to remove up to 97% of the suspended solids in municipal and industrial waste waters. The recovery, or cleaning out, of the accumulated particulate and solid matter is accomplished by a periodic shut down and back-flushing operation. The entire process is fully automatic. For more information contact Bird Machine Company, Inc., South Walpole, Mass. 02071.

Product Potpourri



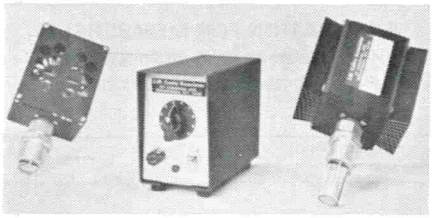
•*"Significant reductions in labor costs"* have been reported by Favorite Foods, Fullerton Califronia, through the installation of General Dairy's Multi-Packer 24. Spokesmen for the 43-year-old Minneapolis-based General Dairy Company stress that their Multi-Packer system has met with enthusiastic reception because it meets design and sanitary requirements of the dairy and food industry. The system consists of a cup inspector; the Multi-Packer 24; a case or tray former; a case sealer and a stacker-accumulator. Among the system's major advantages is that it requires only one operator.

•*A line of improved* swivel joints is being marketed by L. C. Thomsen and Sons. The joints assure smooth, flawless, leakproof filling operations when transferring product from one container to another in food, beverage and chemical plants. Made of sanitary, polished stainless steel construction, Thomsen swivel joints have teflon bearing surfaces for smooth action. They are available with Acme thread, clamp or butt weld ends for welding into existing assemblies. Stock sizes are 1½" and 2". Special assemblies can be made in numerous combinations. The joints are compatible with all type of similar size tubing. For additional information contact L. C. Thomsen and Sons, Inc., 1303 Forty Third Street, Kenosha, Wisconsin 53140, Phone (414) 652-8755.

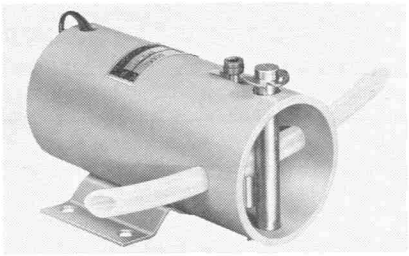
•*Citation Mfg. Co., Inc.* of Siloam Springs, Arkansas, has introduced the smallest gasoline engine driven, cold pressure washer they've ever manufactured. The unit, known as the MODEL 210, produced 132 gallons per hour at 1000 PSI. The main components of the washer include a Hypro positive displacement pump and a 5 hp Briggs & Stratton engine. The unit ships complete with portable gear, discharge hose, wand and detergent injection kit. For more information, contact Citation Mfg. Co., Inc., Box 550, Siloam Springs, AR 72761.

•*Catalog TF-78* provides full specifications and dimension data on two series of 3A approved TRI-FLO® Sanitary Centrifugal stainless steel pumps manufactured by Ladish Co., Tri-Clover Division of Kenosha, Wisconsin. Four models of C Series Close-Coupled and five models of SP Series Base-Mounted pumps are described. According to the manufacturer, this catalog supersedes Catalog TF-73, published in 1973. New features included in the TF-78 catalog include an update and revision of the Head Capacity Curve Charts, new data on the Net Positive Suction Head Required, along with viscosity and specific gravity tables of various materials. Pump and motor selection tables along with capacity curves that rate each pump size in: Head in Feet, PSI and Capacity in FPM and pounds per hour are included in this extensive new 44-page catalog. Five types of pump seals, cited to handle 99% of most processing applications are also described along with a seal selection chart. Send for Catalog TF, Ladish Co., Tri-Clover Division, 9201 Wilmot Road, Kenosha, Wisconsin 53141.

•*Surge Solid State Pulsation Control.* coupled with either single acting or alternating direct acting pulsators, is designed for maximum efficiency with a minimum of labor. Safe, accurate pulsation control is provided over a wide-range of temperatures and operating conditions. A 24 volt impulse is produced by the solid state pulsator controller which will handle 24 milker units using single pulsators or 12 units on alternating pulsators. Direct-acting pulsators are designed without a diaphragm to offer dependability with a minimum of moving parts. The solid state circuitry is housed and protected by a roomy, gasketed, corrosion-proof fiber-glass cabinet. For more information contact a Surge dealer or write Babson Bros. Co., 2100 South York Road, Oak Brook, Illinois 60521.



•*The HYL-80 pressure chamber light* is a specially designed unit to transmit light across a wall/barrier through a small opening (1" pipe thread) in pressure vessels, chambers, autoclaves etc., illuminating difficult, confined, hazardous gas or fire areas without encroachment on inside space, according to John M. Canty, president of J. M. Canty Associates, Tonawanda, N.Y. It is suitable for high pressure 1200 PSI at 110°F. or high temperature model for 1000 PSI at 400°F. All models are complete with a light source, light pipe and prewired power supply, optional weatherproof light source. Units are prewired ...80 watts of power is required-120 volt-50/60 HZ. Send for a free catalog. No. HYL-80, to J. M. Canty Associates, Dept. 29, 117 Cornwall Avenue, Tonawanda, New York 14150.



•*Three solenoid pinch valve* models for use with customers flexible tubing up to 1-1/8" O.D. have been introduced by the Trombetta Corporation of Milwaukee, Wisconsin. The new valve is designed to pinch off the flow in vacuum, low line pressure and liquid transfer lines. Its self-aligning pinching mechanism permits flow in either direction. The solenoid coil can be wound for AC or DC power. When the coil is energized, the valve is open—when de-energized, spring action pinches the tubing shut to control the flow. (Power-on valve open—power-off valve closed). Since a continuous length of tubing can be used, there are no clamps, fittings, or valve mechanisms to contaminate the materials being transferred. The new Trombetta pinch valve is suitable for numerous applications such as research lab control and sampling functions, waste treatment, and food processing plants. The valve operates quietly and efficiently with low power consumption. It can be customized for different types and thicknesses of tubing and a variety of flowing materials. For further information, write for Bulletin 124, Trombetta Corporation, 1633 E. North Avenue, Milwaukee, Wisconsin 53202 for more details.

•*The Spiral System* for performing microbial assays will, in many applications, increase laboratory productivity while reducing operating costs. Originally developed by the Bureau of Foods of the U.S. Food and Drug Administration, the Spiral System is now available commercially. The heart of the Spiral System is a precision built dispenser which distributes the liquid sample on the surface of a rotating agar plate. The dispensing arm moves from the near center of the plate toward the outside while a cam-activated syringe dispenses a continuously decreasing volume of sample, resulting in a concentration range of up to 10,000:1 along the spiral tract of a single plate. Counting can be done manually using a specially designed viewing grid, or automatically. The automatic laser colony counter is especially designed to rapidly count spiral plates and measure the area counted. An electronically guided laser beam scans the plate in a spiral from the edge toward the center to determine the annular area containing a preselected number of colonies. Total plate count can also be obtained. Spiral plating and counting instruments are available from Spiral Systems Marketing, 1200 Quince Orchard Boulevard, Gaithersburg, MD. 20760.

•*A new thread sealant* which assures leak-proof joints in stainless steel piping systems has been introduced by Loctite Corporation, Newington, CT. Called "Stainless Steel PST," the new product is designed for use in the chemical processing, marine, phosphate processing, petroleum refining, pulp and paper, waste treatment, food, drug and other industries. It will resist the working pressure of any pipe, including high-pressure hydraulic systems. Further information from Loctite Corporation, North Mountain Road, Newington, Connecticut 06111.

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Fennema Wins Teaching Award

For the second time since 1977, University of Wisconsin - Madison food scientist Owen Fennema is being honored for his teaching.

The Institute of Food Technologists (IFT) has selected Fennema to receive the 1978 William V. Cruess Award for Excellence in Teaching. Each year the award is presented to an outstanding food science educator. Fennema received the award and a cash honorarium in June at IFT's annual meeting in Dallas.

He was one of two professors to receive the UW-Madison College of Agricultural and Life Sciences Excellence in Teaching Award in 1977.

One of Fennema's goals in teaching is to help students keep pace with the changing food industry. To do this he has designed two new food science courses.

One course focuses on major food processing procedures including canning, drying, freezing and packaging. The second course was designed to familiarize students with the chemical and physical changes that occur in food during processing and packaging.

Fennema is the editor and co-author of a two part textbook, "Principles of Food Science."

News and Events

Book Review

"Developments in Food Analysis Techniques-1"
ed. R. D. King. Published by Applied Science Publishers Ltd. London. 323 pp. $55.

This is an interesting, but expensive, addition to the literature on food analysis. The number 1 in the title suggests that this is the first of a series, yet there is no indication in the preface, on the dust cover, or covering letter that the publisher plans a series of books on this topic. This volume covers only physical and chemical aspects of food analysis and contains nothing on microbiological analysis of foods.

There are ten well-written chapters by authors from government and university laboratories, all in England. The organization of the book suffers from some of the chapters being written about analytical techniques and other chapters are about analysis of specific food components. Thus, there is a chapter on "Developments in Vitamin Analysis" that covers the use of gas chromatography and high pressure liquid chromatography (among other techniques) for vitamin analysis, and there are chapters on "The Use of Gas Chromatography in Food Analysis" and on "Applications of High Pressure Liquid Chromatography in Food Analysis."

Chapter 3 on "The Significant Role Played by Water Present in Food Stuffs" is not on moisture analysis but discusses the interesting question of the degree of binding of water in food. This chapter also covers the methods, such as NMR and calorimetry, for analyzing the state of water in food rather than the quantity.

A listing of the chapter titles of the other six chapters will give the reader an appreciation of the range of topics:

Determination of Nitrogen and Estimation of Protein in Foods

Enzymic Methods in Food Analysis

The Application of Ion Selective Electrodes to Food Analysis

Automatic Methods of Food Analysis

The Determination of Carbohydrates in Foods

Atomic Absorption Spectroscopy in Food Analysis

The audience for this book would seem to be the analytical chemist working on food analysis in a government, industrial or university laboratory. It is not suitable as a textbook or as a methods book such as the "Official Methods" of the Association of Official Analytical Chemists. In spite of the fact that all authors are from English laboratories, the material covered is of world wide interest. Emphasis is on techniques and current literature rather than on specific analytical work called for by a specific set of food regulations.

I was favorably impressed by this book despite the mild objections on price and organization. For the practitioner, this is a worthwhile overview of the current literature, techniques, and problems of food analysis.

I hope it is the beginning of a series.

HARRY E. SNYDER
Department of Food Technology
Iowa State University
Ames, Iowa 50011

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Coming Events

Sept. 12-13 AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE ANNUAL MEETING AND CONFERENCE. Marriott Hotel, Atlanta, GA. Contact: Dr. C. B. Lane, ACDPI, P.O. Box 7813, Orlando, FL 32854, or Margie Franck, ACDPI, 910 17th St. N.W., Washington, D.C. 20006.

Sept. 24-28 NATIONAL ENVIRONMENTAL SANITATION AND MAINTENANCE EDUCATIONAL CONFERENCES AND EXPOSITION. Baltimore Hilton Hotel, Baltimore, MD. Contact: Environmental Management Association, 1701 Drew Street, Clearwater, FL 33515.

Sept. 26-28 INTERNATIONAL SEPARATION, FILTRATION AND DUST CONTROL EXPOSITION '78. O'Hare Trade and Exposition Center, Rosemont, IL. Contact: Aaron Kozlov, ISCM, Inc., 222 West Adams St., Chicago, IL 60606.

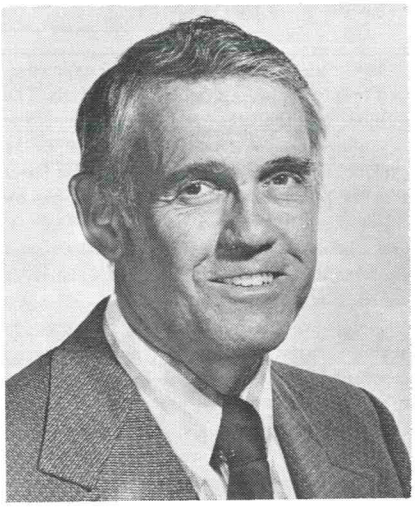
Oct. 31-Nov. 2 FIFTH NATIONAL CONFERENCE ON INDIVIDUAL ONSITE WASTEWATER SYSTEMS. Marriott Inn, Ann Arbor, MI. Contact: National Sanitation Foundation, NSF Building. Ann Arbor, MI 48105.

Nov. 5-9 FOOD AND DAIRY EXPO '78. Anaheim Convention Center, Anaheim, CA. Contact: Dairy and Food Industries Supply Association, 5530 Wisconsin Ave., Suite 1050, Washington, D.C. 20015.

Dec. 4-6 THIRD ANNUAL TOXIC SUBSTANCES CONTROL CONFERENCE. Contact: Government Institutes, Inc., 4733 Bethesda Ave. N.W., Washington, D.C. 20014.

Dec. 11-13 MICROBIOLOGY AND ENGINEERING OF STERILIZATION PROCESSES, three-day intensive course. Marquette Inn, Minneapolis, MN. Course director: Irving J. Pflug, University of Minnesota. Sponsored by the Parenteral Drug Association and Environmental Sterilization Services. $330 fee. Contact: T. E. Odlaug, Environmental Sterilization Services, 306 Foshay Tower, Minneapolis, MN 55402.

Sept. 23-29, 1979 XV INTERNATIONAL CONGRESS OF REFRIGERATION. Cini Foundation, Venice, Italy. Contact: The Organizing and Scientific Committee, Laboratorio per la Technica del Freddo, P.O. Box 1075, 35100-PADOVA (Italy). Tel. 049-760933. Telex 43302.



Larry Gordon Receives Walter F. Snyder Award

Larry J. Gordon, deputy secretary for the New Mexico Health and Environment Department, was the seventh recipient of the Walter F. Snyder Award for achievement in attaining environmental quality. He received the award June 27 in Snowmass, Colorado at the 1978 annual dinner of the National Environmental Health Association (NEHA).

The Walter F. Snyder Award is a joint presentation of the National Sanitation Foundation (NSF) and NEHA. Walter Snyder was a co-founder of NSF and achieved national recognition as its executive director until his death in 1965.

Gordon began his work in the field of public health in 1950 as county sanitarian in Silver City, New Mexico. He received his master of science degree from the University of New Mexico and an MPH from the University of Michigan.

In the 28 years since Silver City he has distinguished himself as an innovative environmentalist. He created and administered the nation's first local comprehensive environmental health department in Albuquerque. It was later broadened to a city/county environmental health agency. In addition to developing the traditional health programs, Gordon was instrumental in developing a model cities program, an urban renewal program, a housing conservation program and a low-rent/leased housing program.

At the state level, he was the prime mover in creating the New Mexico Environmental Improvement Agency, which is recognized as one of the nation's most comprehensive state environmental health agencies.

In 1973, Gordon was instrumental in recreating the state health agency, again with emphasis on comprehensive programmatic coverage and service. He was also responsible for planning, organizing and administering the nation's only comprehensive scientific laboratory system, which offers service to all tax-supported agencies in New Mexico.

Gordon has served as assistant editor of the *Journal of Environmental Health* and is a consulting editor of the *Environment News Digest.* For six years he was a member of the Council of Public Health Consultants of the National Sanitation Foundation and also served as chairman of the NSF joint committee on food service equipment standards. From 1957 to the present he has had various periods of duty as a commissioned officer or consultant to the U.S. Public Health Service.

He is on the executive board of the American Public Health Association. He is a past president of the New Mexico Public Health Association and past president of the New Mexico Environmental Health Association. In addition, Gordon has received honors for distinguished service in public health from many professional organizations including the APHA, NEHA, IAMFES and the New Mexico Health Association.

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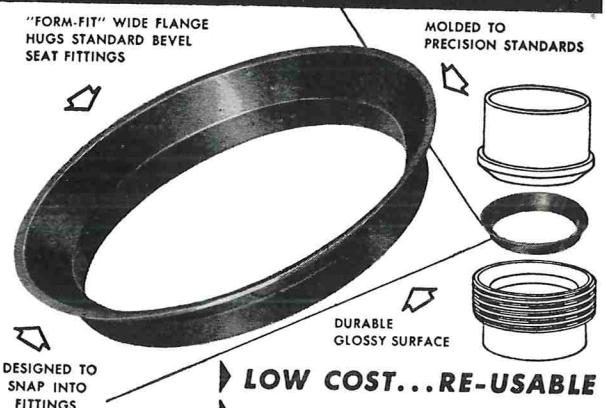

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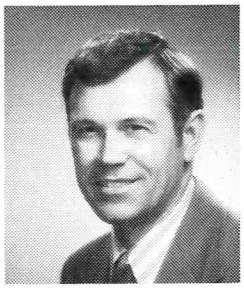
A Better Milk Harvest Through Good Milking Practices

By Dr. John R. Campbell
Professor of Dairy Husbandry
University of Missouri—Columbia



For the corn producer, the most important harvest he makes occurs only once a year—when he goes into the fields with his corn picker. But, for the dairyman, the most important harvest takes place two, and in some cases, three times a day, every day of the year. And, the use of good milking practices helps dairymen to have a good harvest every time they milk their cows. Additionally, a complete milk harvest today will help the cow produce more milk tomorrow.

Milk-making Cells Work Harder With Use

The milk-making (epithelial) cells work the hardest immediately following milking because that is when intramammary pressure is the lowest. At each milking a hormone called prolactin or lactogen is released and its effect is to cause the milk-making cells to go back to work. But, if through poor milking practices, some of the milk is left in the udder, intramammary pressure mounts faster and this, in turn, slows down milk secretion. Research indicates that milk secretion each hour following milking is approximately 90 to 95 percent of that of the preceding hour. But as the udder fills, this percentage decreases. Naturally, milk left in the udder following milking will shorten the period of time that the milk-making cells work at maximal capacity.

Repeated failure to remove milk from mammary glands causes the milk-making cells to become inactive. Thus, for maximal milk production, the milk secreting cells must be challenged... and that means removing all of the milk possible at each and every milking. Although incomplete milking will not have a big detrimental effect in one or two milkings, it sure will over a period of several days. Not only will the milk left in the udder not be harvested and, therefore, not be sold, it will, in addition, accelerate the cow's decline in level of production and, thereby contribute to unprofitable dairying.

Persistency: A Slower Decline Means More Profit

A cow reaches her peak production about two months into the lactation. After this, a natural, gradual decline in milk production occurs. The relationship between milk given one month compared to that produced the next is known as persistency. Persistency can be improved if good milking practices are used to assure a full harvest of the milk crop at each milking.

Eight Steps Toward Getting a Full Milk Harvest

Good cow milking practices include eight steps which, when done properly, will achieve the fullest possible harvest of your valuable milk crop.

1. Environment: Provide a comfortable, stress-free environment.
2. Proper Stimulation: A vigorous massage of the mammary glands will help insure complete letdown of milk.
3. Strip Foremilk: Stripping acts to further stimulate the cow and, at the same time, eliminates much of the bacteria-laden first milk.
4. Timely Application of Milking Machine: This should be done one minute after starting stimulation to take advantage of maximal letdown.
5. Adjust Machine: Proper forward/downward adjustment is important for complete milking.
6. Remove Teat Cups as Quarters Milk Out: Avoid over-milking which can lead to tissue irritation and mastitis.
7. Dip Teats: Teat dipping gives the teat end the protection it needs against mastitis-causing bacteria while the streak canal regains its full constriction.
8. Maintain Equipment Properly: Properly adjusted and maintained equipment is an essential step toward better milking.

The amount of milk a cow produces in a lactation from the time she freshens until she stops lactating is determined by a number of factors; some can be controlled—others cannot. However, good milking practices will go a long way toward helping you achieve more nearly the full potential of your cows' production. In other words, good milking will give a more complete harvest of your most important crop—and that means more profit.