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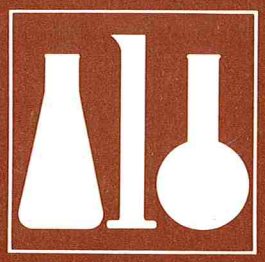

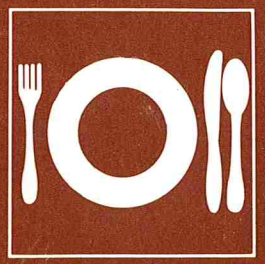

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**Research Papers**



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**General Interest**

*Journal of Food Protection Vol. 41, No. 10, Pages 764-767 (October, 1978)*


# Difference in Injury of Cells of *Vibrio parahaemolyticus* Produced by Heat and Cold Stresses in Liquid and Solid Menstrual [1]

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(Received for publication November 4, 1977)

**ABSTRACT**

Cells of *Vibrio parahaemolyticus* were stressed by heat, cold or heat-cold treatments of 45 C for 12 h, 0 C for 24 h or both. The five menstrua in which cells were stressed were 0.1 M phosphate buffer with 3% NaCl (B + 3%), 0.25 - strength Marine Broth (0.25 × MB) Trypticase Soy Broth with 3% NaCl (TSB + 3%) and Trypticase Soy Agar with 3 or 7% NaCl (TSA + 3% or TSA + 7%). The chill stress produced no cell injury, measured as the difference in colony counts in TSA+3% and TSA+7%, in the three liquid menstrua but caused more than 4 logs of cell injury in the solid menstrua compared to controls. The heat stress caused 2.5- and 1.5-log increases in the number of injured cells from B + 3% or 0.25 × MB, respectively. Cells heat-stressed in TSB + 3% showed no decline in colony counts, but those heat-stressed in TSA+7% produced about a 6-log lower colony count. For cells heated and then chilled in B+3% or 0.25×MB as the liquid menstruum, the percentages of heat-injured cells that apparently regained tolerance to 7% NaCl when shifted to the cold were about 99.7 and 95.0%, respectively. Conversely, cells that were heated and then chilled in the solid menstrua did not regain tolerance to 7% NaCl. Thus, cell injury was greater and apparently less easily reversed for cells stressed in solid than liquid menstrua.

The importance of *Vibrio parahaemolyticus* in seafoods and its ability to withstand physical and chemical stress has been reviewed (*1, 12, 16*). Cell resistance to heat-stress has been studied (*2, 3, 6, 7, 17*) and the need for protein, ribonucleic acid and deoxyribonucleic acid synthesis for repair in heat-stressed cells shown (*7*). Cell stress due to ionic or osmotic changes in media has been postulated for *V. parahaemolyticus* (*2*) and reported for *Pseudomonas fluorescens* (*8*). The sensitivity of this organism to cold-stress in sea water and foods has been widely reported (*1, 4-6,* 9-14). However, a comparison of the reports reviewed by Beuchat (*1*) suggests that some strains of this organism apparently were more cold-tolerant than others.

There have been few reports of the effects of a combination heat-cold treatment on survival and growth of this organism. In this study we report the effects of heat, cold and a combination of heat-cold stresses of stationary phase cells of *V. parahaemolyticus* 8700 in liquid and solid menstrua on the expression of cell injury.

## MATERIALS AND METHODS

*V. parahaemolyticus* strain 8700, serotype 04:K11 was obtained from Dr. J. R. Molenda, Laboratory and Research Administration, Maryland State Department of Health and Mental Hygiene, Baltimore, MD. This organism was isolated in 1971 from a stool specimen of a patient in a food infection outbreak where steamed crab was the vehicle. (*15*).

Stock cultures were prepared by inoculating 9-ml amounts of Fishbein Storage medium (*2*) in 16 × 125 mm screw cap tubes with a loopful of a 30-C, statically grown 24-h-old Trypticase Soy Broth (Bioquest, Cockeyville, MD with a total of 3% NaCl, TSB+3%) culture and incubating the tubes for 24 h at 30 C. These stock cultures were stored at room temperature and transferred every 3 months. An inoculum was prepared by transferring a loopful of stock culture to 50 ml of 0.25-strength Marine Broth (Difco, Detroit, MI, 0.25 × MB) in a 160-ml milk dilution bottle and incubating without shaking for 24 h at 30 C.

Stationary phase cells for stress studies were from 50-ml cultures of 0.25 × MB seeded with 10 ml of inoculum and grown for 8 h at 200 rpm and 30 C in 300-ml flasks in a gyratory water bath shaker (New Brunswick Model G 76). Cells were harvested and washed twice with 0.1 M sodium phosphate buffer with 3.0% NaCl (B+3%) at pH 7.0 by centrifugation at 3000 × *g* for 15 min at room temperature. After stress under static condition in B+3%, 0.25 × MB or TSB+3% cells were enumerated by pour-plating decimal serial dilutions of cells, made in B+3%, with Trypticase Soy Agar (Bioquest) containing a total of 3 or 7% NaCl, w/v, (TSA+3% or TSA+7%). To stress cells in solid menstrua, dilutions of a washed cell suspension were decimally diluted in B+3% to give 30 to 300 colonies per plate and pour-plated with TSA+3% or TSA+7%. Sufficient numbers of replicate plates were prepared so that one set could be removed from each of the stress treatments and incubated at 30 C for 48 h to determine the number of cells showing injury.

The following temperature stress treatments were applied to cells held without stirring or shaking in both solid and liquid menstrua: (a) heating at 45 C for 12 h; (b) chilling at 0 C for 24 h; or (c) treatment (a) followed by treatment (b). As controls, samples of unstressed cells, from the same suspension used for the stress treatment and from the homologous menstruum were held at room temperature for no more than 30 min and plated just before the stress treatment was started. Each dilution of every sample was plated in triplicate. To determine the total

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viable count, i.e., the total number of colony forming units in cell suspensions before and after stress, the appropriate dilutions were plated with TSA + 3%. Plates were incubated at 30 C for 48 h before enumeration.

In contrast to unstressed cells, heat-stressed cells of *V. parahaemolyticus* that became injured are sensitive to 7% NaCl. Thus, a cell population of this organism composed of both injured and uninjured cells will produce a lower colony count on TSA+7% than on TSA+3% (*1, 2, 7*). The difference in colony counts obtained on TSA+3% and TSA+7% indicated the fraction of the cell population that became sensitive to 7% NaCl after the temperature stress and will be referred to as the "injured cells" (*1*). Also, by definition the "total number of viable cells" is defined as the plate count obtained on TSA+3% (*1, 7*).

## RESULTS AND DISCUSSION

Figures 1-4 are histograms comparing the mean colony counts obtained on TSA+3% and TSA+7%. Preliminary experiments (unpublished data) indicated that the TSA + 3% medium gave maximal plate counts of stressed or unstressed cell suspensions as reported by others (*2, 7*). Thus, the 2.5-log lower counts obtained on TSA+3% for cell suspensions that received the heat or heat-cold treatments, bar B versus bar D, compared to the unstressed controls, bar A in Fig. 1 and 2, indicate that this fraction of the original cell suspension had expired due to the treatment. Compared to unstressed controls, the number of survivors determined on TSA+3% was about 2.5 to 3.0 log cycles lower for the heat or heat-cold treatments in any of the three liquid menstrua used for stress, B + 3% (Fig. 1), 0.25 × MB (Fig. 2) or TSB + 3% (Fig. 3).

Heat induced injury, bar B, was about 4 log cycles in B + 3% (Fig. 1), 2.5 logs in 0.25 × MB (Fig. 2) but less than 0.5 log in TSB + 3% (Fig. 3). Cold-induced injury, bar C, was about 2 logs in B + 3% (Fig. 1), but less than 1 log in 0.25 × MB (Fig. 2) and in TSB + 3% (Fig. 3). The heat-cold induced injury, bar D, was about 1.0 log in both B + 3% and 0.25 × MB but less than 0.25 log in TB + 3%. Thus, injury caused by the three stress treatments in liquid

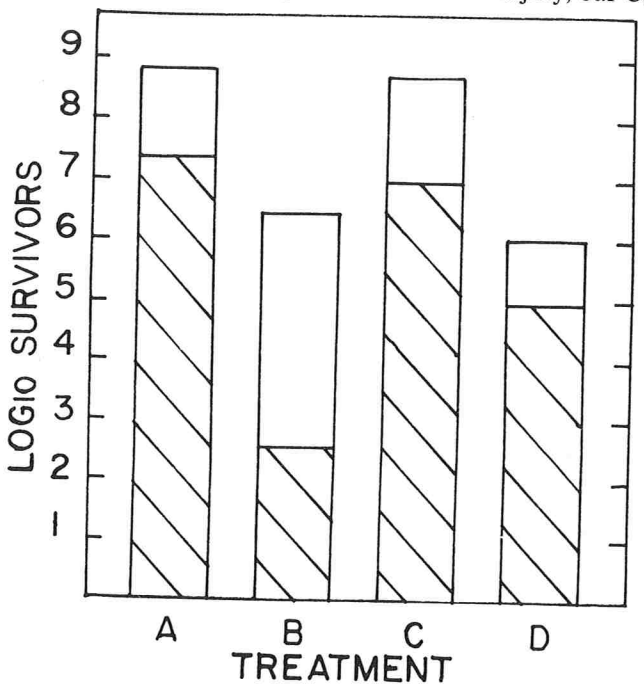


Figure 1. *Plate counts for* V. parahaemolyticus *cells stressed in 0.1 M sodium phosphate buffer with 3% NaCl, pH 7.0. (A), control; (B), heat stress at 45 C for 12 h; (C), cold stress at 0 C for 24 h; and (D), the combination of heat and cold stresses. Counts are means of triplicate plate counts on TSA+3% (□) and TSA+7% (▨).*

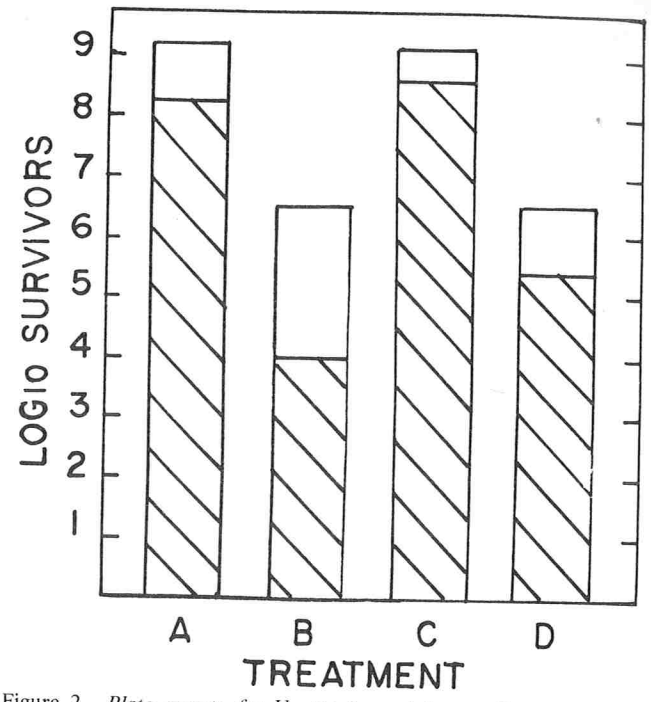


Figure 2. *Plate counts for* V. parahaemolyticus *cells stressed in 0.25 - strength Marine Broth (A), control; (B), heat stress at 45 C for 12 h; (C), cold stress at 0 C for 24; and (D), the combination of heat and cold stresses. Counts are means of triplicate plate counts on TSA+3% (□) and TSA+7% (▨).*

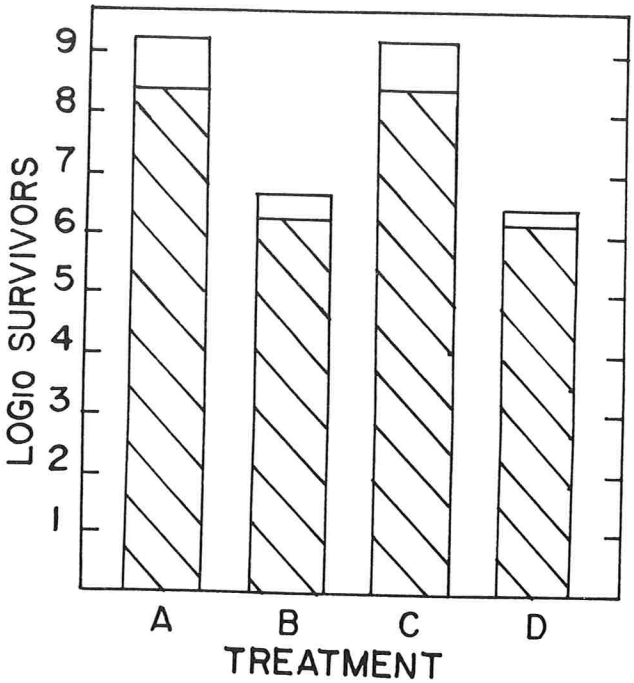


Figure 3. *Plate counts for* V. parahaemolyticus *cells stressed in Trypticase Soy Broth with 3% NaCl. (A), control; (B), heat stress at 45 C for 12 h; (C), cold stress at 0 C for 24 h; and (D), the combination of heat and cold stresses. Counts are means of triplicate plate counts on TSA+3% (□) and TSA+7% (▨).*

menstrua followed a general trend, being greatest in B+3% (no nutrients available, less in 0.25 × MB (intermediate level of nutrients) and least in TSB+3% (rich supply of nutrients).

Concerning the "total viable cell number", there was a 2.5-log cycle decrease in the number of cells tolerant to 3% NaCl when cell suspensions were exposed to the heat of heat-cold stress treatment in the liquid TSB+3% menstruum (Fig. 3). There was virtually no decrease in the number of cells tolerant to 3% salt when cell suspensions were subjected to these same treatments in the corresponding solid TSA+3% medium (Fig. 4). Conversely, concerning enumeration of "injured cells," in the solid menstruum, TSA, cells exposed to any of the three stress treatments showed the same great amount of cell injury, about a 5- to 6-log cycle difference (Fig. 4) versus less than 1 log for any of the cell populations exposed to the same stress treatments in the liquid menstruum, TSB, (Fig. 3) which was of the same composition as TSA except it lacked agar.

Comparing cells in liquid studies that were only heated versus those that were heated then chilled, bar B versus bar D, Fig. 1 and 2, the percentages of cells heat-stressed in B + 3% or 0.25 × MB that apparently regained tolerance to 7% NaCl when held 24 h in the heat shock fluid in the cold were about 99.7 and 95.0%, respectively. There is at least one plausible explanation for the differences observed in the plate counts for bar B and bar D data in Fig. 1 and 2. The bar D cells were stored in the same menstruum in which they were heated. Thus, it is possible there was time for these cells during cold storage to reabsorb some essential cell product(s) lost by cell leakage during heating. Conversely, for the bar B cells there was no time for reabsorption of such cell products lost by leakage during stress in the liquid menstruum since these stressed cells were plated immediately after heating. Thus, the cell products lost by leakage, when given time to be reabsorbed, could confer on the stressed cells more tolerance to 7% NaCl and the ability to produce more colonies on TSA+7%.

Cell suspensions heated in TSB+3% (bar B, Fig. 3) and then plated immediately gave the same counts on TSA + 3% and TSA + 7%, about 6 logs, as cells heated and then stored in the cold in this medium before plating (bar D, Fig. 3.). These data suggest that the relatively rich nutritional composition of the TSB medium was able to counteract any adverse heat-induced effects suffered by cells in a liquid menstruum. There was no apparent heat induced injury in this menstruum, evidenced by there being no difference in plate counts obtained with TSA + 3% and TSA + 7%. Hence, there was no benefit to be gained by the cells from storage in the cold in this rich medium before they were plated.

It is uncertain why more heat-stressed cells recovered tolerance to 7% NaCl during cold storage in the liquid than in the solid menstruum form of the rich medium,

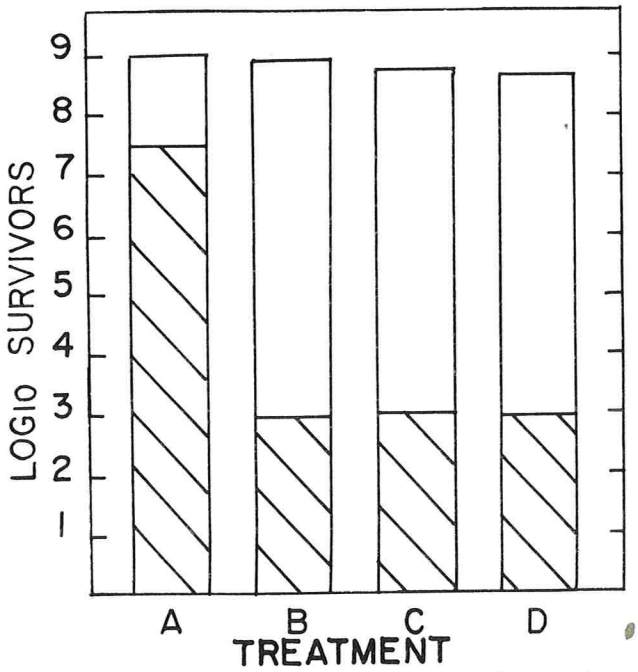


Figure 4. *Plate counts for* V. parahaemolyticus *cells pour-plated immediately in triplicate in TSA + 3% (□) and TSA + 7% (▨) and then subjected to stress. (A), the control; (B), heat stress at 45 C for 12 h; (C), cold stress at 0 C for 24 h; (D), the combination of heat and cold stresses.*

TSB, Fig. 3 versus 4. One difference in this pair of experiments was that during stress there were greater cell densities/ml used in the liquid TSB medium studies (about $10^6$ to $10^8$/ml) than in the solid TSA medium studies (no more than about $10^2$/plate or about 10 cells/ml of agar medium in a plate). Concentrated cell suspensions stressed in the liquid TSB menstruum may be better able to undergo repair than are more dilute cell suspensions stressed in the solid TSA menstruum because of the greater protective effect of the hypothetically larger concentration of cell products released into the menstruum in the former case. These data indicate that the cell products lost by leakage during stress and essential for cell tolerance to 7% NaCl did not easily reenter cells suspended in agar, suggesting the product(s) remained trapped in the agar. Also, the rich TSA medium itself apparently could not supply the factor(s) the cells lost during heat stress and needed to regain tolerance to 7% NaCl.

The observations reported here indicate that more consideration needs to be given to the argument that sublethal stress and recovery studies with bacterial cells of public health significance should be conducted by using low densities per ml or g of $10^1$ to $10^4$ (rather than $10^8$ or more) since these lower densities are the cell concentrations at which these pathogens are likely to naturally occur in foods. The solid menstruum approach suggested here makes possible such studies and provides an independent method to judge more unequivocably the inimical effects of various environmental factors on survival and growth of stressed bacterial cells in model systems and foods.

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**REFERENCES**

1. Beuchat, L. R. 1975. Environmental factors affecting survival and growth of *Vibrio parahaemolyticus.* A review. J. Milk Food Technol. 38:476-480.
2. Beuchat, L. R. 1976. Survey of media for the resuscitation of heat-stressed *Vibrio parahaemolyticus*. J. Appl. Bacteriol. 40:53-60.
3. Beuchat, L. R., and R. E. Worthington. 1976. Relationship between heat resistance and phospholipid-fatty acid composition of *Vibrio parahaemolyticus*. Appl. Environ. Microbiol. 31:389-394.
4. Bradshaw, J. G., D. W. Francis, and R. M. Twedt. 1974. Survival of *Vibrio parahaemolyticus* in cooked seafood at refrigeration temperatures. Appl. Microbiol. 27:657-661.
5. Colwell, R. R., T. E. Lovelace, L. Wan, T. Kaneko, T. Stanley, P. K. Chen, and H. Turblash. 1973. *Vibrio parahaemolyticus:* isolation, identification, classification and ecology. J. Milk Food Technol. 36:202-213.
6. Covert, D., and M. Woodburn. 1972. Relationships of temperature and sodium chloride concentration on the survival of *Vibrio parahaemolyticus* in broth and fish homogenate. Appl. Microbiol. 23:321-325.
7. Emswiller, B. S., M. D. Pierson, and S. P. Shoemaker. 1976. Sublethal heat stress of *Vibrio parahaemolyticus*. Appl. Environ. Microbiol. 32:792-798.
8. Gray, R. J. H., L. D. Witter, and Z. J. Ordal. 1973. Characterization of mild thermal stress in *Pseudomonas fluoreseens* and its repair. Appl. Microbiol. 26:78-85.
9. Johnson, H. C., and J. Liston. 1973. Sensitivity of *Vibrio parahaemolyticus* to cold in oysters, fish fillets, and crab meat. J. Food Sci. 38:437-441.
10. Kaneko, T., and R. R. Colwell. 1975. Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. Appl. Microbiol. 29:269-274.
11. Kaneko, T., and R. R. Colwell. 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. J. Bacteriol. 113:24-32.
12. Lee, J. S. 1973. What seafood processors should know about *Vibrio parahaemolyticus.* J. Milk Food Technol. 36:405-408.
13. Liston, J., and J. Baross. 1973. Distribution of *Vibrio parahaemolyticus* in the natural environment. J. Milk Food Technol. 36:113-117.
14. Matches, J. R., J. Liston, and L. P. Daneault. 1971. Survival of *Vibrio parahaemolyticus* in fish homogenate during storage at low temperatures. Appl. Microbiol. 21:951-952.
15. Molenda, J. R., W. G. Johnson, M. Fishbein, B. Wentz, I. J. Mehlman, and T. A. Dadisman, Jr. 1972. *Vibrio parahaemolyticus* gastroenteritis in Maryland: Laboratory aspects. Appl. Microbiol. 24:444-448.
16. Nickelson, R., and C. Vanderzant. 1971. *Vibrio parahaemolyticus*-a review. J. Milk Food Technol. 34:447-452.
17. Vanderzant, C., R. Nickelson, and R. W. Hazelwood. 1974. Effect of isolation-enumeration procedures on the recovery of normal and stressed cells of *Vibrio parahaemolyticus*. pp. 111-116. In: T. Fujino, G. Sakaguchi, R. Sakazaki and Y. Takeda (eds.) International symposium on *Vibrio parahaemolyticus*. Saikon Publ. Co., Ltd., Toyko.

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*A Research Note*

# Types of *Clostridium perfringens* Isolated from Selected Foods[1,2]

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**ABSTRACT**

To determine the proportion of type A *Clostridium perfringens* occurring among *C. perfringens* isolates obtained from foods, strains were typed by the mouse serum neutralization method. Of 339 isolates from ground beef, ground pork, ground turkey, live crab, cured sausage, and live clams, 320 (94%) were type A. The remaining 19 isolates produced insufficient exotoxin for typing. The predominance of type A strains from the foods surveyed indicates that definitive typing of strains isolated from these foods, when suspected of causing foodborne disease, is not warranted.

Routine laboratory methods (*1, 2, 4*) for enumeration of *Clostridium perfringens* from foods to determine the etiology of suspected foodborne disease or to ascertain incidence of the bacterium in foods do not include typing of the isolates. The possibility exists that strains of *C. perfringens* isolated may be of a type other than type A and, therefore, are not capable of causing classical *C. perfringens* foodborne illness. This study was undertaken to determine the incidence of type A *C. perfringens* isolated from foods.

## MATERIALS AND METHODS

*C. perfringens* strains were isolated in pure culture from foods and confirmed by the method outlined in the *Bacteriological Analytical Manual* (*1*). Before typing, cultures were held in cooked meat medium and transferred at monthly intervals. In preparation for typing, cultures were transferred to fluid thioglycollate medium daily for 3 days and incubated at 37 C. This was followed by three successive 24-h transfers incubated at 37 C in the chopped meat-starch medium prepared in the manner described by Dowell and Hawkins (*3*). Approximately 1 ml of the material from the third chopped meat-starch culture was transferred to a 25 × 200-mm tube containing approximately 20 g of chopped meat-starch medium and incubated at 37 C. After 7 ± 1 h the culture fluid was decanted into a sterile centrifuge tube, which was centrifuged at 3200 rpm for 20 min. Following centrifugation, the mouse serum neutralization typing procedures of Dowell and Hawkins (*3*) were employed. Commercially prepared sera and antisera (Wellcome Research Laboratories, Beckenham, England, BRS 3BS) were used. Strains of *C. perfringens* which failed to produce sufficient exotoxin for typing on two trials were considered to be non-typeable.

## RESULTS AND DISCUSSION

Results of typing 339 strains of *C. perfringens* isolated from six different food items are given in Table 1. A total of 19 isolates were classified as non-typeable. However, 320 definitively typed strains of *C. perfringens* isolated from ground beef, ground pork, ground turkey, live crab, cured sausage and live clams were exclusively type A.

Table 1. *Type A isolates of* Clostridium perfringens *from selected foods.*

| Isolate source | Number of isolates typed | Number of type A isolates | Number of non-typeable isolates | Percent type A isolates |
|---|---|---|---|---|
| Ground beef | 152 | 149 | 3 | 98 |
| Ground pork | 82 | 76 | 6 | 93 |
| Ground turkey | 59 | 56 | 3 | 95 |
| Live crab | 29 | 25 | 4 | 86 |
| Cured sausage | 13 | 10 | 3 | 77 |
| Live clams | 4 | 4 | 0 | 100 |
| TOTAL | 339 | 320 | 19 | 94 |

A higher proportion of non-typeable strains was found in cured sausage than in either ground beef or ground pork. In light of the fact that the major components of cured sausage are ground beef and ground pork, it could be speculated that the natural fermentation process may favor survival and growth of *C perfringens* strains low in exotoxin production. The overall incidence of *C. perfringens* isolated from cured sausage by this laboratory is considerably lower than that found in either ground beef or ground pork.

The effort and expense required in typing strains of *C. perfringens* isolates from these foods, when incriminated in outbreaks of foodborne disease, appears to be unwarranted because of the predominance of type A *C. perfringens*. This is particularly true if epidemiologic information is indicative of *C.perfringens* foodborne disease.

[1] *Reprint requests to: Commander, Letterman Army Institute of Research, 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.*

## REFERENCES

1. Anonymous. 1976. Bacteriological analytical manual for foods. U.S. Department of Health, Education, and Welfare. Public Health Service, Food and Drug Administration, Division of Microbiology, Washington, D.C.
2. A.O.A.C. 1975. Official methods of analysis. 12th Edition. Assoc. Off. Anal. Chem., Washington, D. C. pp. 918-920.
3. Dowell, V. R., Jr., and T. M. Hawkins. 1974. Laboratory methods in anaerobic bacteriology. CDC Laboratory Manual, Department of Health, Education, and Welfare. Publication No. 74-8272.
4. Speck, M. L., ed. 1976. Compendium of methods for the microbiological examination of foods. American Public Health Assoc., Washington, D.C. 701 p.

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# Bacteriological Evaluation of Frankfurters in the Canadian Retail Market

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ABSTRACT

The bacteriological quality of 180 units of frankfurters, obtained from grocery stores in Ontario (Canada) was investigated. About 67% of the samples had aerobic plate counts (APC) in the range of $10^7$ - $10^9$/g; 48% had APCs in the range of $10^8$-$10^9$/g and APCs of four samples exceeded $10^9$/g. One sample was positive for *Staphylococcus aureus* and three samples contained *Escherichia coli*. Enterococci (<1000/g) were recovered from 48 samples. Aerobic plate counts of cooked frankfurters from snackbars did not exceed 500/g. Cooking of frankfurters for 6.5 min at 90 C resulted in APCs of <100/g. Neither salmonellae nor *Clostridium perfringens* were isolated. It was concluded that temperature abuse following manufacture was mainly responsible for the high counts.

A survey was conducted to determine the bacteriological quality of frankfurters (wieners) from seven different manufacturers sold in grocery stores in Ontario. The manufacturing process of these products includes smoking and cooking which is capable of destroying a large number of bacteria present in the raw emulsion (*4, 5*). Surkiewicz et al. (*7*) reported aerobic plate counts (APC) of raw emulsions ranging from $5 \times 10^4$ to $1 \times 10^7$/g and APCs of frankfurters at time of manufacture of about 20,000 or less/g. Generally, the manufacturers recommend a shelf life of about 4 weeks for their products when stored at 4 C or below.

This report presents results of analyses of 180 samples purchased from retail outlets. Frankfurters, cooked and sold in the bun by snackbars or cooked in the laboratory were also analyzed.

## MATERIALS AND METHODS

Retail size packages (180 units) containing 12 frankfurters each and representing seven different manufacturers, were collected at random from 11 grocery stores during the summer months of 1977. The internal temperature of the sausages in the package was determined by inserting a thermometer longitudinally to the center of a sausage at time of purchase. Samples were transported to the laboratory in an iced container and analyzed immediately. Also, a total of 24 cooked frankfurters in the bun, but without condiments added, were purchased from four snackbars. The frankfurters were removed aseptically and placed in sterile plastic bags for transport under refrigerated conditions. For cooking frankfurters, the Ontario Food Council (*6*) recommends a cooking time of 5 to 8 min in a waterbath maintained at 90 C. In this study, frankfurters with total aerobic plate counts of about 1 to $2 \times 10^8$/g were cooked for 6.5 min. A thermister probe was inserted into the center of the sausage and the change in temperature recorded with a YSI model 47 Scanning Telethermometer.

*Sample preparation*

A 30-g sample from each package, taken from diagonal cross-section portions of all sausages in the package, was weighed into a 1-liter glass blender cup and 270 ml of 0.1% peptone water was added to give a 0.1 dilution. The sample was blended at high speed for 2 min and serial dilutions were prepared in 0.1% peptone water.

*Analyses*

Methods for aerobic plate counts (APC 32 C/48 h) coliforms, *Escherichia coli, Staphylococcus aureus*, enterococci, salmonellae and *Clostridium perfringens* have been previously described (*3*).

## RESULTS AND DISCUSSION

### *Frankfurters from grocery stores*

Neither salmonellae nor *C. perfringens* were isolated from any of the samples. Although 57 samples (31.7%) contained coliforms (presumptive, ranging from 2 to 500/g), very few were confirmed and only three samples were positive for *E. coli* with 10, 10 and 7/g for manufacturers A, C, and D respectively. One sample contained *S. aureus* (manufacturer D, 170/g). Table 1 gives a summary of data on the range of total aerobic plate counts and enterococci and the fraction of total samples that contained these organisms for each type of sample. Enterococci were recovered from 48 (26.7%) of 180 samples, but none of the samples contained more than 1000 organisms/g. About half of the samples (48%) had aerobic plate counts (APC) in the range of $10^8$ - $10^9$/g and 118 samples (66.7%) had APCs in the range of $10^7$ - $10^9$/g. Four samples (one from manufacturer C, one from manufacturer F and two from manufacturer G) had APCs of more than $10^9$/g. These samples were purchased 24, 4, 15, and 14 days, respectively, before the expiration date on the package. Thirty-seven samples (20.9%) had APCs of less than $10^6$/g. Data in Table 1 and Fig. 1 indicate considerable variation in APCs among frankfurters of the seven different manufacturers. Only samples of manufacturer A had a mean count (APC) below an arbitrary standard of $10^6$ organisms/g. Internal

TABLE 1. *Arbitrary count ranges (aerobic plate count and enterococci) for 180 units of frankfurter sausages from seven different manufacturers.*

| Manufacturer | Type of sausage | No. of units in APC range/g: No. of units[a] | $10^5$ | $10^5$-$10^6$ | $10^6$-$10^7$ | $10^7$-$10^8$ | $10^8$-$10^9$ | $10^9$ | No. of units in enterococci range/g: No. of units positive[b] | $10^1$-5 $10^1$ | 5 $10^1$-$10^2$ | $10^2$-5 $10^2$ | 5 $10^2$-$10^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Dutch Treat | 7 | 4 | | | 2 | 1 | | 0 | | | | |
| | Red hots | 6 | 3 | 1 | 1 | 1 | | | 0 | | | | |
| | All beef | 11 | 4 | 3 | 2 | | 2 | | 2 | 1 | | 1 | |
| | Regular | 4 | 2 | | 1 | | 1 | | 1 | | | | |
| | Subtotal | 28 | 13 | 4 | 4 | 3 | 4 | | 2 | 1 | | 1 | |
| | % | | 46.4 | 14.3 | 14.3 | 10.7 | 14.3 | | | 50 | | 50 | |
| B | Regular | 15 | 3 | 2 | 3 | | 7 | | 11 | 11 | | | |
| | All beef | 8 | 2 | 1 | 1 | | 4 | | 4 | 1 | 2 | 1 | |
| | Home pack | 2 | | | | 1 | 1 | | | | | | |
| | Subtotal | 25 | 5 | 3 | 4 | 1 | 12 | | 15 | 12 | 2 | 1 | |
| | % | | 20 | 12 | 16 | 4 | 48 | | | 80 | 13.3 | 6.7 | |
| C | Regular | 17 | | 4 | 1 | 6 | 6 | | 10 | 5 | 2 | 2 | 1 |
| | All beef | 7 | | 1 | 2 | 2 | 1 | 1 | 3 | 2 | 1 | | |
| | Subtotal | 24 | | 5 | 3 | 8 | 7 | 1 | 13 | 7 | 3 | 2 | 1 |
| | % | | | 20.8 | 12.5 | 33.3 | 29.2 | 4.6 | | 53.8 | 23.1 | 15.4 | 7.7 |
| D | Regular | 14 | | | 1 | 5 | 8 | | | | | | |
| | All beef | 11 | 3 | | 3 | 1 | 4 | | | | | | |
| | Subtotal | 26 | 3 | | 4 | 6 | 12 | | 0 | | | | |
| | % | | 12 | | 16 | 24 | 48 | | 0 | | | | |
| E | All beef | 26 | 2 | 1 | | 7 | 16 | | 6 | 4 | 2 | | |
| | Subtotal | 26 | 2 | 1 | | 7 | 16 | | 6 | 4 | 2 | | |
| | % | | 7.7 | 3.8 | | 26.9 | 61.6 | | | 66.7 | 33.3 | | |
| F | Regular | 25 | | | 2 | 6 | 16 | 1 | 9 | 5 | 1 | 3 | |
| | Subtotal | 25 | | | 2 | 6 | 16 | 1 | 9 | 5 | 1 | 3 | |
| | % | | | | 8 | 24 | 64 | 4 | | 55.7 | 11.1 | 33.3 | |
| G | Regular | 24 | 1 | | 1 | 2 | 18 | 2 | 3 | 2 | 1 | | |
| | Subtotal | 24 | 1 | | 1 | 2 | 18 | 2 | 3 | 2 | 1 | | |
| | % | | 4.2 | | 4.2 | 8.3 | 75 | 8.3 | | 66.7 | 33.3 | | |
| | TOTAL | 177[c] | 24 | 13 | 18 | 33 | 85 | 4 | 48 | 31 | 9 | 7 | 1 |
| | % | | 13.6 | 7.3 | 10.2 | 18.6 | 48.0 | 2.3 | 26.7 | 64.6 | 18.7 | 14.6 | 2.1 |

[a]Unit: Package containing 12 frankfurters.
[b]Lowest dilution plated was $10^{-1}$.
[c]Results for APC of three samples had to be discarded.

temperatures of frankfurters at time of purchase varied from 4 to 14 C. A summary of temperature ranges and means for samples purchased from the stores is presented in Table 2. The average and range of days between date of purchase and suggested final date for sale for the samples of frankfurters of each manufacturer are given in Table 3. Nine of 180 samples had exceeded the suggested final date of sale by 6 to 22 days. The APCs for these samples ranged from $9.7 \times 10^7$ to $7.8 \times 10^8$/g. Samples of manufacturer A had the highest average number of days (23 days) between date of purchase and date of expiration. Frankfurters of this manufacturer also showed the better overall quality (60.7% of samples had APCs of less than $1 \times 10^6$/g; Table 1). However, 8 of 11 samples with APCs exceeding $1 \times 10^6$/g (Fig. 1) had still more than 20 days remaining until the suggested final date of sale expired. The age of the sample per se at time of purchase seems not to be the most important contributing factor to high counts.

*Frankfurters from snackbars*

APCs for frankfurters purchased in the bun from snackbars, ranged from less than 100 to 120/g. The low APCs and the failure to isolate coliforms, enterococci, *C. perfringens*, *S. aureus* and salmonellae indicated adequate cooking.

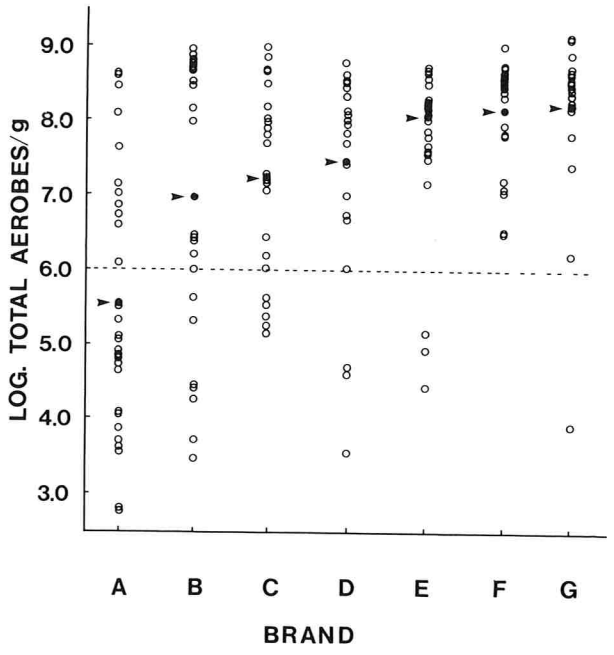


Figure 1. *Total Aerobic Plate Count of frankfurters from seven different manufacturers. •Geometric mean. --- Arbitrary standard.*

TABLE 2. *Number of units of frankfurters purchased from different supermarkets and their internal temperature.*

| Supermarket | No. of units | Temperature (C) Range | Mean |
|---|---|---|---|
| I | | | |
| A[a] | 36 | 5 - 14 | 8.8 |
| B | 30 | 6 - 12 | 8.6 |
| C | 19 | 7 - 12 | 8.9 |
| Sub-total | 85 | | |
| II | 19 | 6 - 12 | 8.4 |
| III | | | |
| A | 17 | 8 - 11 | 9.6 |
| B | 10 | 8 - 10 | 9.2 |
| C | 3 | 8 - 8 | 8.0 |
| Sub-total | 30 | | |
| IV | | | |
| A | 17 | 6 - 10 | 7.9 |
| B | 12 | 6 - 9 | 7.3 |
| C | 11 | 4 - 9 | 6.5 |
| D | 6 | 8 - 8 | 8.0 |
| Sub-total | 46 | | |
| *TOTAL* | *180* | | |

[a]A, B, C, and D refers to different stores from the same supermarket chain.

TABLE 3. *Average and range of days between date of purchase and suggested final date of sale of frankfurters for each manufacturer.*

| Manufacturer | No. of units | Days between purchase and expiration date Range | Average |
|---|---|---|---|
| A | 29 | 14 - 33 | 23 |
| B | 25 | 1 - 23 | 11[a] |
| C | 25 | 6 - 30 | 19[b] |
| D | 25 | 2 - 28 | 14[c] |
| E | 26 | 6 - 23 | 16[d] |
| F | 25 | 1 - 23 | 13[e] |
| G | 25 | 5 - 29 | 17 |

[a]2 samples exceeded the suggested date of sale by 8 and 13 days, respectively, and were not used to calculate the average.
[b]One sample exceeded the suggested date of sale by 6 days.
[c]One sample exceeded the suggested date of sale by 13 days.
[d]Two samples exceeded the suggested date of sale by 8 and 22 days.
[e]Three samples exceeded the suggested date of sale of 9, 8, and 12 days.

### *Boiled frankfurters*

It took 6 to 6.5 min for the frankfurters to reach an internal temperature of 75 C when held in a water bath maintained at 90 C. The gradual increase in internal temperature with time of heating is shown in Fig. 2. After 6.5 min of exposure to heat, the APCs of the frankfurters had decreased from about $10^8$/g to less than 100/g.

The data obtained in this study on the bacteriological quality of frankfurters available to the consumer from grocery stores showed a considerable variation in counts among frankfurters from the different manufacturers. Since at time of manufacture an adequate cooking-smoking process reduces the bacterial flora in the raw emulsion to negligible levels (*7, 8*), the high number of aerobic bacteria/g in the samples obtained at retail strongly suggests temperature abuse during transportation and retail stocking as being the most important contributing factor to high counts.

There was no apparent relationship between total aerobic count and age of the samples; only a knowledge of the history of the product could help to explain the reason for this erratic behavior. Differences in the level and type of viable bacterial populations in the freshly packed product could account for the variations observed between manufacturers. The meat packer can control the number of bacteria in the finished product by using raw material of good quality, adequate cooking and observing sanitary procedures during packaging. He loses much of his direct control, however, once his product enters the merchandising channels. Since the product is in its final package when it leaves the plant, recontamination from subsequent handling is practically non-existent. The only mishandling that can occur is temperature abuse during transportation and storage at the retail level. When fresh frankfurters with initial counts of less than 1000/g were stored at 0-1 C for 30 days, the shelf life recommended by the manufacturers, the population increased to $10^4$-$10^5$ organisms/g (unpublished data). Similar results were reported by Allen and Foster (*1*) for four types of vacuum-packed sliced meat products. Although low temperature control at the retail level may have practical limits, the advantage of such storage temperature in retarding growth of the bacterial flora is obvious. Frankfurturers are not sterile products, they are perishable. There is, therefore, no reason why these products should be "incubated" at temperatures averaging 8 C (ranging from 5 to 14 C) during retailing, even if harmless aciduric lactic acid bacteria would constitute the predominant microflora. Improvement in the present status of bacteriological quality of frankfurters available to the consumer, can be achieved by following two simple procedures: (*a*) application of rules of sanitation during processing and (*b* minimizing bacterial proliferation during retailing by storage at 0-1C. In the absence of

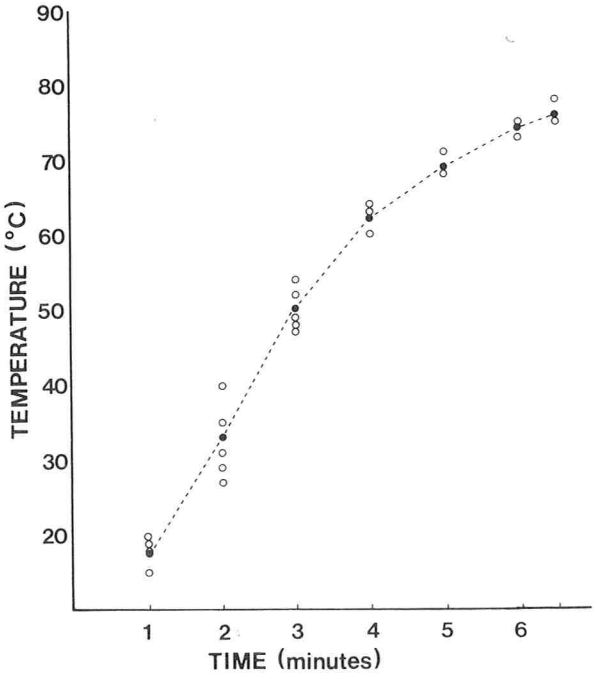


Fig. 2. *Increase in internal temperature of frankfurters exposed to heat for 6.5 min in water bath maintained at 90 C. ∘ mean temperature of 6 replicates.*

opportunities for the consumer to visually confirm that the product was indeed produced and distributed under such conditions, it might be proper to press for a more rigorous enforcement of adequate and uniform national legislation on appropriate temperature for the storage and retailing of chilled foods.

ACKNOWLEDGMENTS

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REFERENCES

1. Allen, J. R., and E. M. Foster. 1960. Spoilage of vacuum-packed sliced processed meats during refrigerated storage. Food Res. 25:19-25.
2. Duitschaever, C. L. 1977. Unpublished data.
3. Duitschaever, C. L., D. H. Bullock and D. R. Arnott. 1977 Bacteriological evaluation of retail ground beef, frozen raw beef patties and cooked hamburger. J. Food Prot. 40:378-381.
4. Kramtlich, W. E., A. W. Pearson and F. W. Tauber. 1973. Processed meats. AVI Publishing Co., Westport, Conn.
5. Mackensie, D. S. 1966. Prepared meat product manufacturing, 2nd. ed. American Meat Institute, Arlington, VA.
6. Ontario Food Council. Ontario Ministry of Agriculture and Food, Parliament Buildings, Ontario, Canada. Personal communication.
7. Surkiewicz, B. F., R. W. Johnston and J. M. Carosella. 1976. Bacteriological survey of frankfurters produced at establishments under federal inspection. J. Milk Food Technol. 39:7-9.
8. Tittiger, F. and P. W. Saschenbrecker. 1973. Studies on the wholesomeness of ready-to-eat meat products. 1. Bacteriological evaluation of comminuted heated products. Can. J. Comp. Med. 37:1-4.

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# Bisulfite Degrades Aflatoxin: Effect of Temperature and Concentration of Bisulfite

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**ABSTRACT**

Bisulfite reacted with aflatoxin $B_1$ and $G_1$ resulting in their loss of fluorescence. The reaction was first order with rate depending on bisulfite (or the bisulfite and sulfite) concentration(s). Aflatoxin $G_1$ reacted more rapidly with bisulfite than did aflatoxin $B_1$. In the presence of 0.035 M potassium acid phthalate-NaOH buffer (pH 5.5) plus 1.3% (vol/vol) methanol at 25 C, the reaction rate constant for degradation of aflatoxin $G_1$ was $2.23 \times 10^{-2}h^{-}$ and that for aflatoxin $B_1$ was $1.87 \times 10^{-2}h^{-}$ when 50 ml of reaction mixture contained 1.60 g of $K_2SO_3$. Besides bisulfite concentrations, temperature influenced reaction rates. The $Q_{10}$ for the bisulfite-aflatoxin reaction was approximately 2 while activation energies for degrading aflatoxin $B_1$ and aflatoxin $G_1$ were 13.1 and 12.6 kcal/mole, respectively. Data suggest that treating foods with 50 to 500 ppm $SO_2$ probably would not effectively degrade appreciable amounts of aflatoxin. Treating foods with 2000 ppm $SO_2$ or more and increasing the temperature might reduce aflatoxin to an acceptable level.

Hazards of aflatoxin have been well documented (*3*, 11, 20). Ingestion of aflatoxin by a variety of experimental animals results in necrosis of their liver and may ultimately lead to death. Furthermore, chronic exposure of susceptible hosts to aflatoxins may cause malignant liver tumors.

Several investigators have studied various chemicals to see if they would degrade and detoxify aflatoxins (*8, 15, 24, 29*). However, many chemicals which effectively detoxify aflatoxin, are not suitable for use in foods. This is true because they may reduce the nutritional quality of the food, produce off-flavors and off-odors in the food, or leave toxic residues in the food. Hence it would be desirable to find a chemical which is an acceptable additive that will also degrade aflatoxin.

Bisulfite is a highly reactive chemical and an acceptable food additive (*9, 13, 19, 21*). It is commonly added to wines, fruit juices, jams, dried fruits, and several other food products where bisulfite inhibits enzymatic and non-enzymatic browning, acts as an antioxidant and reducing agent, and effectively controls growth of microorganisms (*9, 13, 19, 21*).

Since aflatoxin has been detected in various foods including wine (*22, 28*), it would be useful to know what effect, if any, bisulfite has on aflatoxin. This paper provides information on degradation of aflatoxin by bisulfite at several concentrations and at different temperatures. Other aspects and a proposed mechanism for degradation of aflatoxin by bisulfite will be reported in another paper (*6*).

## MATERIALS AND METHODS

*Preparation of reaction mixtures*

A series of 20 sterile, 300-ml Erlenmeyer flasks stoppered with diSPo plastic plugs (Scientific Products, McGraw Park, IL) was used for each temperature or bisulfite concentration studied. Thirty-five milliliters of sterile 0.05 M potassium acid phthalate (Mallinckrodt, St. Louis, MO, Analytical Reagent grade)-NaOH buffer, pH 5.5, were added to each flask.

Two of these flasks were used to determine how much 1.0 N HCl was needed to return the pH of the reaction mixture to 5.5 after the appropriate amount of potassium sulfite was added. These experiments were done at pH 5.5 for two reasons. First, aflatoxins $B_1$ and $G_1$ are sensitive to extremes in pH (*14-17*, personal observations) but are relatively stable in aqueous solution at pH 5.5. Second, the primary reactive form of sulfurous acid salts at pH 5.5 is the bisulfite ion, which is the desired form to be tested in these experiments. Since addition of potassium sulfite raises the pH of the reaction mixture and because it is desirable to immediately convert potassium sulfite to the bisulfite form [sodium sulfite is less stable in aqueous solution than in the bisulfite form (10)], the appropriate amount of 1.0 N HCl needed to return the pH to 5.5 was added to each of the 14 reaction mixtures before addition of the potassium sulfite. Furthermore, these two flasks were used to determine how much sterile, deionized, distilled water was required to bring the reaction mixture to 50 ml after the remaining ingredients were added. This predetermined amount of water was then added to each of the 14 reaction mixtures so all components of the reaction mixture were at their appropriate starting concentrations before aflatoxin was added.

When water alone is used as a solvent for a sulfite solution, passing air through this solution may oxidize up to 50% of the sulfite within 2 min (*21*). Furthermore, small amounts of methanol in sulfite solutions appreciably retard the rate of sulfite oxidation (*21*). Hence, 0.15 ml of methanol (Mallinckrodt, St. Louis, MO, Analytical Reagent grade) was added to each of 14 reaction mixtures. Following this, the desired amount (0.025 to 1.60 g/50 ml) of potassium sulfite (Fisher Scientific Company, Fairlawn, N. J., Certified ACS grade) was added to each of the 14 reaction mixtures. Potassium sulfite was used in lieu of the bisulfite form because the latter contains appreciable amounts of metabisulfite. Although the metabisulfite form has the same properties

as bisulfite, calculation of the molar concentration of bisulfite may result in appreciable error depending on how much metabisulfite is present. In addition, potassium sulfite was used instead of sodium sulfite because the pH of an aqueous solution of $K_2SO_3$ is about 8, whereas that of an aqueous solution of $Na_2SO_3$ is about 9 (*25*). Using potassium sulfite, less 1.0 N HCl was needed to return the pH of the reaction mixture to 5.5.

Finally, purified aflatoxin $B_1$ or $G_1$ was immediately added to 10 of these reaction mixtures. The concentration of aflatoxin $B_1$ or $G_1$ was adjusted so that 200 μg of either was present in 0.5 ml of methanol so this amount could be added to each reaction mixture. Each reaction mixture was checked and, if necessary, more water was added to bring the final volume to 50 ml. The 10 reaction mixtures which contained aflatoxin and potassium sulfite served to indicate how fast aflatoxin was degraded by potassium sulfite at pH 5.5 in a 0.035-M KHP-NaOH buffered solution.

One-half milliliter of methanol was added to each of the four remaining reaction mixtures which were used to determine any change in pH which may have occurred through bisulfite oxidation. These controls were then used to determine how much sodium hydroxide was needed to return the pH of the reaction mixture to 5.5.

The final four flasks each contained 35 ml of 0.05-M KHP-NaOH, pH 5.5, buffer, and to each were added 0.5 ml of aflatoxin dissolved in methanol and sufficient sterile, deionized, distilled water to yield a total of 50 ml. Contents of these flasks served to indicate stability of aflatoxin when incubated under conditions similar to those used for reaction mixtures with added potassium sulfite.

Reaction mixtures were held quiescently in the dark at 25, 35, 45, or 55 C for up to 96 h. To avoid "come-up" times and other problems involving temperature, all solutions used to prepare reaction mixtures, with the exception of aflatoxin in methanol, were adjusted to and maintained at the temperatures at which the mixtures were to be held. The solution of aflatoxin in methanol was adjusted to 25 C.

For each series of treatments, two flasks which contained only aflatoxin and two which contained both aflatoxin and potassium sulfite were extracted immediately after each reaction mixture was brought to the 50-ml volume. The aflatoxin was extracted from each reaction mixture using three aliquots (100, 50, and 50 ml) of chloroform. The remaining eight flasks that contained potassium sulfite and aflatoxin, the four control flasks containing potassium sulfite only, and the two control flasks containing aflatoxin only were immediately incubated at the appropriate temperature. Contents of two flasks from each series of experiments were extracted at periodic intervals for up to 96 h. The pH of controls containing potassium sulfite only was determined periodically, more often for those reaction mixtues incubated at higher temperatures and those containing higher concentrations of potassium sulfite than for the others. When necessary during the incubation, the pH of all reaction mixtures was adjusted to 5.5 using sodium hydroxide. In no instance was the pH allowed to drop more than 0.35 unit below 5.5. Upon completion of each experiment, contents of the two control flasks with only aflatoxin in each series of tests dealing with temperature and bisulfite concentration were extracted.

*Quantitation of aflatoxin*

The principle indicator used to monitor degradation of aflatoxin $B_1$ and $G_1$ was their decrease in or loss of fluorescence on TLC plates. Aflatoxin concentrations were determined by methods of Shih and Marth (*23*). These methods include development of aflatoxins on thin layer chromatographic plates followed by fluorometric measurement (excitation wavelength, 366 nm; emission wavelength, 436 nm) of the aflatoxins. Concentrations of aflatoxins were calculated according to the procedure of Pons et al. (*18*). Values for aflatoxin concentrations used to calculate the various rates of degradation are averages of two determinations made for each sampling interval. In all instances controls which accompanied each series of treatments with potassium sulfite yielded approximately the same amounts of aflatoxin $B_1$ or $G_1$ before and after incubation. This indicates that no nonspecific degradation of aflatoxin $B_1$ or $G_1$ occurred during their storage in an aqueous solution buffered at pH 5.5 with 0.035 M-KHP-NaOH.

## RESULTS AND DISCUSSION

*Primary reactant*

Although potassium sulfite was added to react with the aflatoxins, once the sulfite is in aqueous solution it does not necessarily remain as the sulfite ion ($SO_3^=$). Instead, depending on the pH of the solution, besides remaining in the sulfite ion form, it may be readily converted to the bisulfite ion ($HSO_3^-$) and/or sulfurous acid ($H_2SO_3$, which is actually $SO_2 + H_2O$). By using the two dissociation constants for sulfurous acid, the pH of the sulfite solution, and the molar concentration of potassium sulfite added, one can calculate the molar concentration of each sulfurous acid form with the Henderson-Hasselbach equation. A detailed description of calculations used to determine the equilibrium concentrations of each sulfurous acid form at a specific pH is outlined by Stumm and Morgan (*27*).

Since the pH of the sulfite solution was adjusted to and maintained at 5.5 and the molar concentration of potassium sulfite is known, one only needs to know the dissociation constants for sulfurous acid to predict the molar concentration of its various forms. The literature contains at least three different sets of dissociation constants for sulfurous acid. To make similar calculations, Joslyn and Braverman (*13*) used 1.8 for the first pK and 5.3 for the second pK of sulfurous acid, but *Lange's Handbook of Chemistry* (*5*) gives the values as 1.9 and 7.2 and the *Handbook of Chemistry and Physics* (*30*) reports pK values of 1.81 and 6.91. These last values were used to calculate molar concentrations of each sulfurous acid form when the solution was held at pH 5.5. The amount of potassium sulfite which was present in the form of $HSO_3^-$ and $SO_3^=$ at the beginning of each series of experiments is given in Table 1. Amounts of $H_2SO_3$ ($SO_2 + H_2O$) present at this pH value are relatively small compared to concentrations of $HSO_3^-$ and $SO_3^=$ which are present so these values were not reported. From values in Table 1, it is apparent that approximately 96% of the potassium sulfite was in the $HSO_3^-$ form while only about 4% existed as $SO_3^=$. Therefore, the primary reactant at pH 5.5 was bisulfite.

TABLE 1. *Calculated concentrations of $HSO_3^-$ and $SO_3^=$ present at pH 5.5 for each intial molar concentrations of $K_2SO_3$.*

| g $K_2SO_3$ per 50 ml reaction mixture | Initial molar concentration of $K_2SO_3$ | Actual molar concentration at pH 5.5 | |
|---|---|---|---|
| | | $HSO_3^-$ | $SO_3^=$ |
| 1.60 | 0.201 | 0.193 | 0.008 |
| 1.20 | 0.151 | 0.145 | 0.006 |
| 0.08 | 0.101 | 0.097 | 0.004 |
| 0.40 | 0.0503 | 0.0484 | 0.0019 |
| 0.20 | 0.0252 | 0.0242 | 0.0010 |
| 0.10 | 0.0126 | 0.0121 | 0.0005 |
| 0.050 | 0.00629 | 0.00605 | 0.00024 |
| 0.025 | 0.00314 | 0.00302 | 0.00012 |

*Reaction rates*

When the percentage of aflatoxin $B_1$ and $G_1$ remaining was plotted versus reaction time in tests where different concentrations of bisulfite were used at 25 C, typical first order reaction plots were obtained. To substantiate first

order kinetics, lines from these data should transverse through at least one half-life of the initial aflatoxin concentration. Plots obtained when the three highest concentrations of bisulfite were used pass through at least one half-life of the initial aflatoxin concentration. Therefore, for each aflatoxin tested these three lines serve as a basis to suggest that first order kinetics exist. Figure 1 illustrates the rates at which aflatoxin $B_1$ was degraded by various concentrations of bisulfite. Figure 2 gives similar information for aflatoxin $G_1$. In all instances, straight-line relationships were observed between the log of the percent of aflatoxin $B_1$ or $G_1$ remaining versus reaction time for each specific bisulfite concentration tested. The line of best fit for each set of data was determined by linear regression analysis according to methods of Steel and Torrie (*26*). Assuming

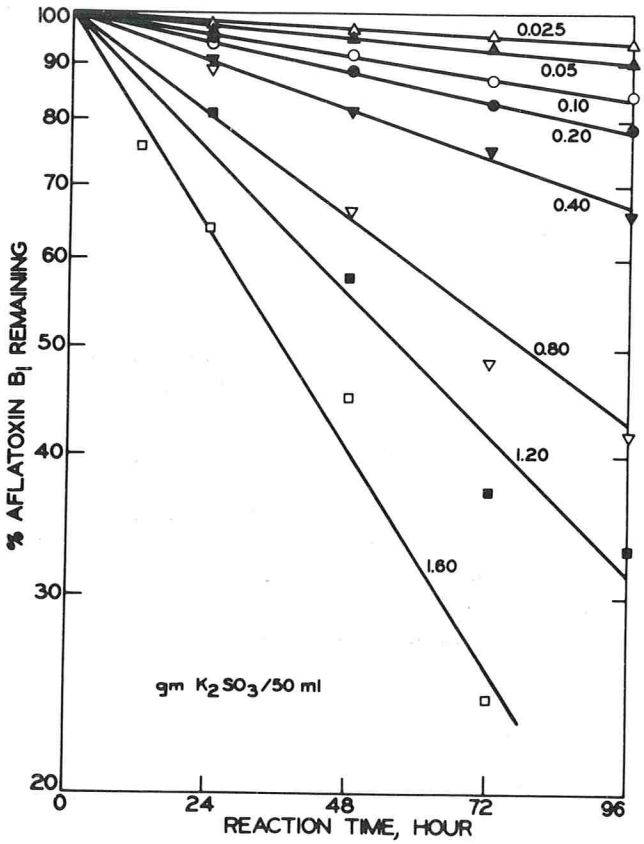


Figure 1. *Degradation (first order reaction) of aflatoxin $B_1$ by different concentrations of potassium sulfite held at pH 5.5 and 25 C. Each reaction mixture contained 0.035 M KPH-NaOH buffer plus 1.3% (v/v) methanol.*

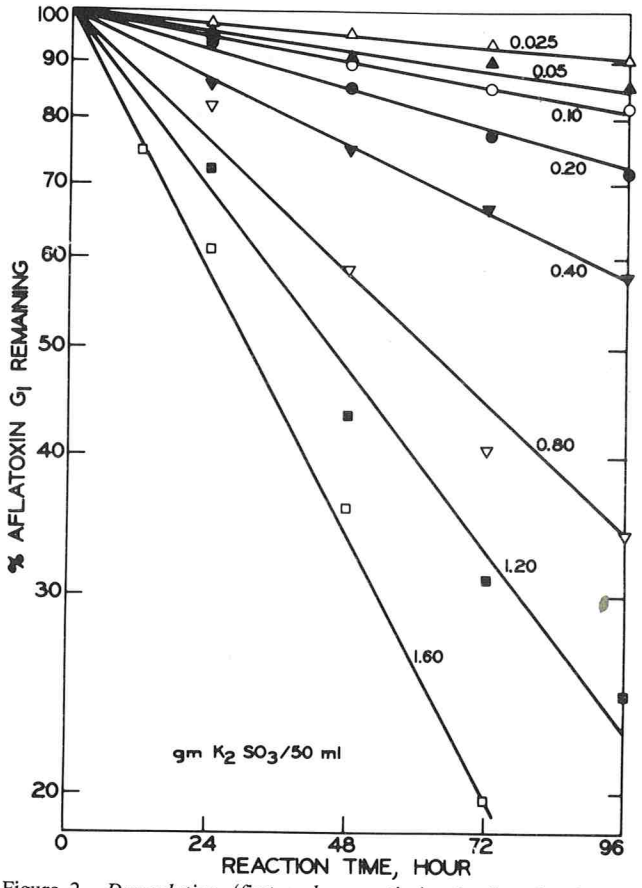


Figure 2. *Degradation (first order reaction) of aflatoxin $G_1$ by different concentrations of potassium sulfite held at pH 5.5 and 25 C. Each reaction mixture contained 0.035 M KHP-NaOH buffer plus 1.3% (v/v) methanol.*

first order kinetics, reaction rate constants (k) can be calculated from slopes of these lines. These values for degradation of aflatoxin $B_1$ and $G_1$ are in Table 2. To compare these reaction rate constants in applied terms, the half-life values of the initial aflatoxin concentrations for degradation of aflatoxin by each bisulfite concentration have been included in Table 2. These values indicate the time required to degrade 50% of the initial aflatoxin concentration by each concentration of potassium sulfite when allowed to react under the conditions previously specified. From the data it is apparent that aflatoxin $G_1$ was degraded at a greater rate than was aflatoxin $B_1$. Compared to aflatoxin $B_1$, the increased reactivity between bisulfite and aflatoxin $G_1$ may be attributed to the terminal lactone ring of

TABLE 2. *Reaction rate constants for degradation of aflatoxin $B_1$ and $G_1$ and half-life values of initial aflatoxin concentrations resulting from aflatoxin degradation by different concentrations of potassium sulfite in the presence of 1.3% (v/v) methanol plus 0.035 M KHP-NaOH at pH 5.5, 25 C.*

| g $K_2SO_3$ per 50 ml reaction mixture | Reaction rate constant, $k^{h^{-1}} \times 10^{-3}$ | | Half-life (h) | |
|---|---|---|---|---|
| | Aflatoxin $B_1$ | Aflatoxin $G_1$ | Aflatoxin $B_1$ | Aflatoxin $G_1$ |
| 1.60 | 18.7 | 22.3 | 37 | 31 |
| 1.20 | 12.4 | 15.1 | 56 | 46 |
| 0.80 | 9.76 | 11.9 | 71 | 58 |
| 0.40 | 4.26 | 5.54 | 163 | 127 |
| 0.20 | 2.54 | 3.53 | 273 | 196 |
| 0.10 | 1.69 | 2.04 | 410 | 340 |
| 0.050 | 1.01 | 1.55 | 686 | 447 |
| 0.025 | 0.572 | 0.961 | 1212 | 721 |

aflatoxin $G_1$. This will be more thoroughly discussed in a later paper (*6*).

To further establish the order of kinetics which predominates in this reaction, a plot of ln k for degradation of aflatoxin $B_1$ and $G_1$ versus ln bisulfite concentration was made and is shown in Fig. 3. The slope of each of these lines is equal to the reaction order at which bisulfite reacts with aflatoxin $B_1$ or $G_1$ (*2*). The slope for aflatoxin $B_1$ is 0.82 while for aflatoxin $G_1$ it is 0.75. If the reaction of bisulfite with aflatoxin $B_1$ or $G_1$ follows first order kinetics, then the slope of these lines should equal 1.0. Since the order of kinetics which most reactions follow is a whole number or integer, i.e., zero-order, first-order, second-order, etc., and because 0.82 and 0.75 are close to 1.0, it appears that these reactions follow first-order kinetics. Therefore Fig. 3 illustrates the first-order dependence that bisulfite concentration had on the rate at which aflatoxins $B_1$ and $G_1$ were degraded.

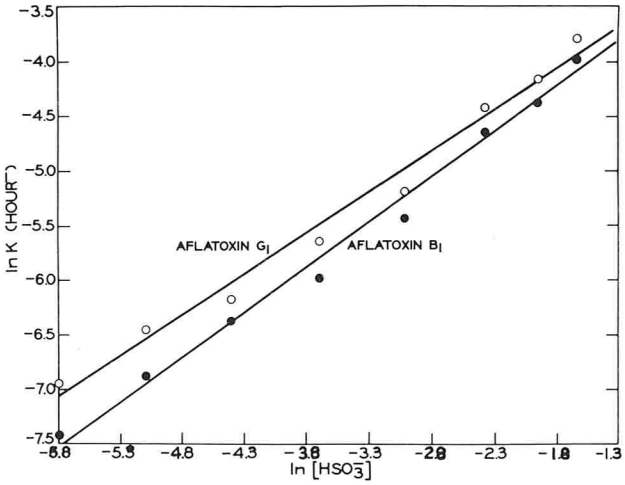


Figure 3. *Plots of ln reaction rate constant for degradation of aflatoxin $B_1$ and $G_1$ versus ln bisulfite concentrations at pH 5.5 and 25 C when 0.035 M KHP-NaOH buffer plus 1.3% (v/v) methanol were present in each reaction mixture.*

It was originally assumed that bisulfite was the only form reacting with aflatoxin; however, as will be described in another paper (*6*), sulfite may also be a participant in the degradation of aflatoxin. Therefore, both the bisulfite and sulfite concentration must be considered. A plot of ln k for degradation of aflatoxin $B_1$ and $G_1$ versus ln of the total bisulfite and sulfite concentration resulted in slopes equal to those previously determined. Hence, if both bisulfite and sulfite are participants in degrading aflatoxin, this reaction would still follow first order kinetics.

*Effect of temperature*

Semilog plots of the percentage of aflatoxin $B_1$ and $G_1$ remaining versus reaction time when reaction mixtures contained a constant concentration of bisulfite (0.40 g of $K_2SO_3$ at pH 5.5) but were held at 25, 35, 45, and 55 C also resulted in typical first order reaction plots. Figures 4 and 5 illustrate the rates at which aflatoxin $B_1$ and $G_1$, respectively, were degraded by bisulfite at four different temperatures. Reaction rate constants for degradation of aflatoxin $B_1$ and $G_1$ at the four temperatures are given in

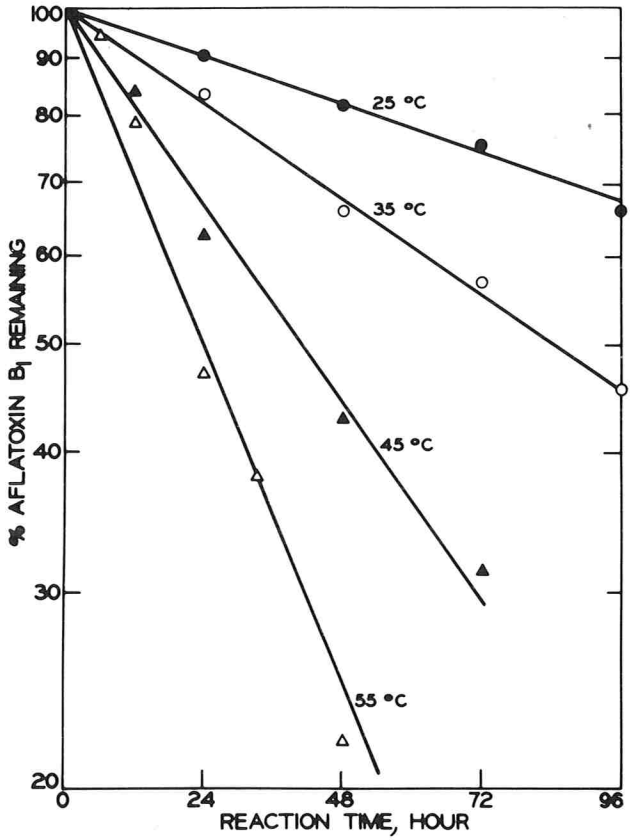


Figure 4. *Degradation of aflatoxin $B_1$ by a specific concentration of potassium sulfite at different temperatures. Each 50-ml reaction mixture contained 0.035 M KHP-NaOH buffered at pH 5.5 plus 1.3% (v/v) methanol and 0.40 g $K_2SO_3$.*

Table 3. The half-life values of the initial aflatoxin concentration for aflatoxin degradation by bisulfite at different temperatures also are in Table 3. As was previously observed, aflatoxin $G_1$ always was degraded more rapidly than was aflatoxin $B_1$.

These reaction rate constants may be used to calculate the $Q_{10}$ values of each reaction. The $Q_{10}$ value is equal to the rate at which the reaction increases for every 10-C increase in temperature. The $Q_{10}$ obtained for aflatoxin $B_1$ was 2.0 and for aflatoxin $G_1$ was 1.9. This means that for every 10-C increase in temperature, the rate at which a specific concentration of bisulfite degrades aflatoxin $B_1$ and $G_1$ approximately doubles.

These reaction rate constants which were obtained from different temperature treatments can also be used to calculate the activation energy needed for bisulfite to degrade aflatoxin $B_1$ or $G_1$. Arrhenius plots were prepared by graphing on a semilog scale these reaction rate constants versus the inverse of absolute temperature. Figure 6 illustrates the Arrhenius plots for these data. Assuming ideal conditions, the activation energies ($E_a$) for degradation of aflatoxin $B_1$ and $G_1$ by bisulfite can be derived by multiplying the slopes of these lines by the molar gas constant, R, (1.987 cal/mole) and the natural logarithm constant (2.303). The activation energies for degradation of aflatoxin $B_1$ and aflatoxin $G_1$ were 13.1 kcal/mole and 12.6 kcal/mole, respectively. These

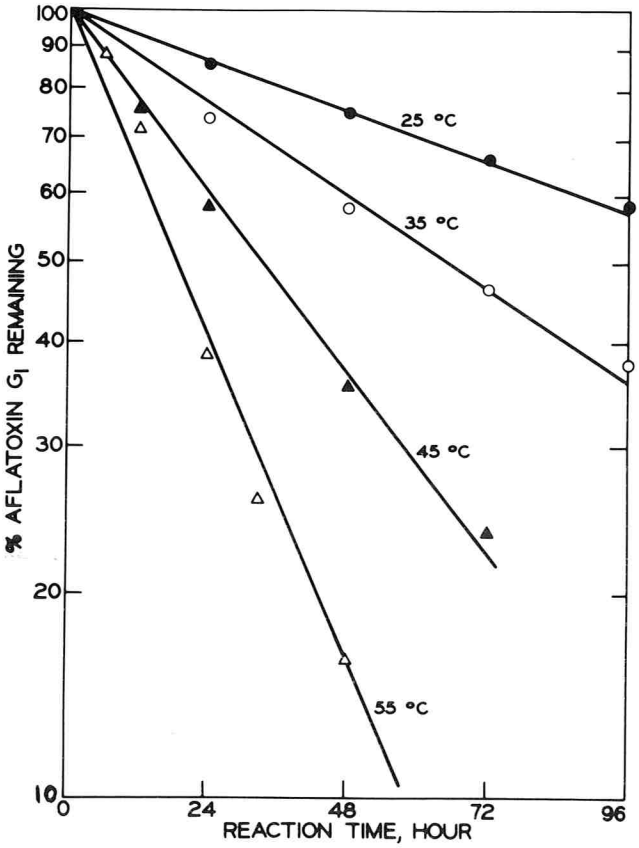


Figure 5. *Degradation of aflatoxin $G_1$ by a specific concentration of potassium sulfite at different temperatures. Each 50-ml reaction mixture contained 0.035 M KHP-NaOH buffered at pH 5.5 plus 1.3% (v/v) methanol and 0.40 g $K_2SO_3$.*

TABLE 3. *Reaction rate constants for degradation of aflatoxin $B_1$ and $G_1$ and half-life values of initial aflatoxin concentrations resulting from aflatoxin degradation by a constant concentration of potassium sulfite (0.40 g $K_2SO_3$) in presence of 0.035 M KHP-NaOH, at pH 5.5, and 1.3% (v/v) methanol at different temperatures.*

| Temperature (C) | Reaction rate constant, $k^{-1} \times 10^{-2}$ | | Half-life (h) | |
|---|---|---|---|---|
| | Aflatoxin $B_1$ | Aflatoxin $G_1$ | Aflatoxin $B_1$ | Aflatoxin $G_1$ |
| 25 | 0.426 | 0.554 | 163 | 127 |
| 35 | 0.816 | 1.01 | 85 | 69 |
| 45 | 1.63 | 2.00 | 43 | 35 |
| 55 | 3.32 | 3.99 | 21 | 17 |

values are relatively low, which would suggest that the major reaction between bisulfite and aflatoxin $B_1$ and $G_1$ occurs at a specific site or bond. This is in marked contrast to the relatively high activation energies of 40 or more than 100 kcal/mole which are required to denature proteins where several bonds are broken (*31*). A possible mechanism for the bisulfite-aflatoxin reaction as well as additional supporting data will be reported in another paper (*6*).

*Practical significance*

What is the practical significance of these data? In foods such as wine, soft drinks, cider, flour, fruit juices, jams, and sausages where only 70 to 450 ppm $SO_2$ are normally added (*1, 19*), some but probably not a substantial amount of aflatoxin would be degraded. Table 4 compares the calculated ppm $SO_2$ present at

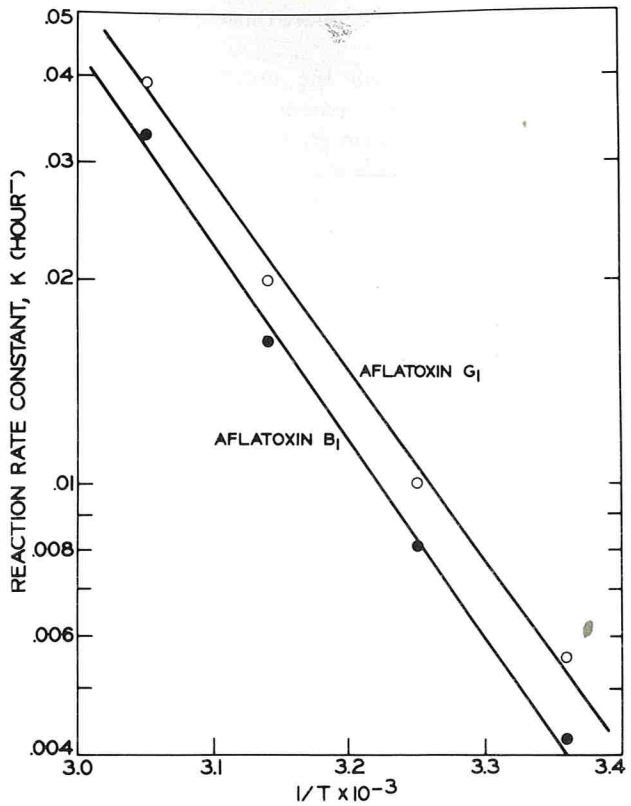


Figure 6. *Rate constants at which aflatoxin $B_1$ and $G_1$ were degraded by bisulfite versus the inverse of absolute temperature at which reactions occurred.*

each molar concentration of $K_2SO_3$ used for these experiments. From these values it is evident that the amount of $SO_2$ normally added to wines and some other foods would be in the range of the two lowest concentrations of potassium sulfite that were tested. The rates at which aflatoxins $B_1$ and $G_1$ were degraded at these initial bisulfite concentrations, serve to suggest that

TABLE 4. *Comparison of molar concentrations of $K_2SO_3$ used in these experiments to the calculated amounts of $SO_2$ (ppm) present.*

| g $K_2SO_3$ added par 50 ml reaction mixture | M $K_2SO_3$ | ppm $SO_2$ |
|---|---|---|
| 1.60 | 0.201 | 12,880 |
| 1.20 | 0.151 | 9,660 |
| 0.80 | 0.101 | 6,440 |
| 0.40 | 0.0503 | 3,220 |
| 0.20 | 0.0252 | 1,610 |
| 0.10 | 0.0126 | 805 |
| 0.050 | 0.00629 | 403 |
| 0.025 | 0.00314 | 201 |

relatively small amounts of either toxin would be degraded in such foods. Furthermore, wine is both fermented and stored at relatively low temperatures (< 25 C). Our data indicate that as temperature is lowered, so is the rate at which aflatoxin is degraded. Therefore, presence of relatively small amounts of bisulfite in wines is of questionable value for degrading aflatoxin.

However, with dried fruits where up to 2000 ppm $SO_2$ may be added, appreciable amounts of aflatoxin, if present, could be degraded. Our data show that

moderate amounts of both aflatoxin $B_1$ and $G_1$ were degraded with bisulfite at a concentration comparable to 2000 ppm $SO_2$. Furthermore, as was illustrated by results of the temperature studies, when temperature is increased, as might occur when fruits are dried, rates of aflatoxin degradation are substantially increased. It should be noted, however, that fruits may contain sugars, organic acids, and other organic compounds which also may react with bisulfite. This could reduce the aflatoxin-degrading effects of bisulfite. Therefore, to determine the effect bisulfite has on degrading aflatoxins in fruits experiments should be done with the products. The wet milling of corn involves steeping corn in a solution of $SO_2$ although no $SO_2$ is added to finished products. Such use of $SO_2$ may inactivate small amounts of aflatoxin if any is present.

*Scientific significance*

The literature appears to contain some misconceptions regarding the stability of aflatoxin in the presence of bisulfite. For example, Trager and Stoloff (*29*) screened many different reagents to determine if any could destroy aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$. They identified a number of chemicals able to degrade aflatoxin and also chemicals which had no apparent effects on aflatoxins when they were exposed to the chemicals for 1 h in an aqueous solution. Included among the chemicals which had no effect on the four aflatoxins were 0.5 M $NaHSO_3$ and 0.5 M $Na_2SO_3$. Since the reaction was allowed to proceed for only 1 h and because the degradation product(s) is (are) water soluble (*6*), which is the fraction in which they would remain after the authors extracted the reaction mixture with chloroform, it is easy to understand why these authors did not observe any effects on the aflatoxins when they were treated with bisulfite.

At about the same time, Davis and Diener (*4*) noted that, depending on concentration, potassium sulfite could be used to inhibit aflatoxin biosynthesis and at the same time allow uninhibited production of mold mycelium. Since the pH of the medium was not monitored or defined, the actual amounts of $SO_2 + H_2O$, $HSO_3^-$ and $SO_3^=$ present in the growth medium could not be determined. Nevertheless, once mold growth begins, metabolities are produced which normally lower the pH of the medium. In addtion, when an aqueous sulfite solution is exposed to oxygen, sulfite oxidation occurs, which results in a decrease in pH (*21*). For these reasons it seems likely that appreciable amounts of $HSO_3^-$ were at some time present in the growth medium. Since it has been established that aflatoxins $B_1$ and $G_1$ are degraded in the presence of potassium sulfite at pH 5.5, it is possible that instead of inhibiting aflatoxin production, aflatoxin was actually produced and degraded. It is noteworthy that Davis and Diener observed no inhibition of aflatoxin biosynthesis when comparable quantities of bisulfite were used in lieu of potassium sulfite.

More recently, Gupta et al. (*12*) observed the effect of different concentrations of sodium bisulfite on the ability of *A. parasiticus* mycelia to incorporate labelled acetate into aflatoxins. When a concentration of 50 mM of sodium bisulfite was present in a "suspension buffer" inoculated with mycelium and incubated for an unstated period at an unstated temperature, they found the specific activities of aflatoxins B and G to be 27% less than that of the control. They attributed these reduced specific activities to inhibition of aflatoxin production; however, this apparent inhibition of aflatoxin synthesis may actually be an illusion. Instead, aflatoxin may have been degraded by bisulfite soon after it was synthesized.

In some preliminary studies on the effect different chemical treatments have on the stability of aflatoxin, Feuell (*7*) noted that the use of sulfur dioxide was an effective treatment for detoxifying aflatoxins but "with reservations." Using a crude aflatoxin preparation dissolved in dilute ethanol, $SO_2$ was added to this solution until it was saturated. This was stored overnight. The excess $SO_2$ was then removed by warming and bubbling air through the mixture. After orally dosing three ducklings with this $SO_2$-treated solution all three ducklings died; however, their death appeared to be due to an anaphylactic type of reaction rather than the typical symptoms of aflatoxicosis. No liver lesions were present in any of the three ducklings. Following this, Feuell used sulfur dioxide to gas thin layers of groundnut meal contaminated with aflatoxin. After overnight exposure to the gas, the groundnut meal was put in a vacuum oven and kept under reduced pressure for 24 h. The groundnut meal was then fed to ducklings. The survival time of the ducklings was not appreciably different than that of controls which received similar but untreated groundnut meal contaminated with aflatoxin. In addition, liver lesions were present in ducklings receiving the $SO_2$-treated groundnut meal. There may be several explanations for the observations made by Feuell. In the preliminary study, a crude methanol-into-chloroform extract of groundnut meal which contained aflatoxin was used. This extract may have contained materials other than just aflatoxin $B_1$ and $G_1$. Therefore other factors present in the reaction mixture which were not affected by $SO_2$ may have killed the ducklings. Since these ducklings did not have liver lesions which are characteristic of aflatoxicosis, the ducklings may have died from some other cause(s). Perhaps the degradation products of $SO_2$-treated aflatoxins are toxic and these killed the ducklings; however, a more controlled experiment must be done to test the biological effects of such degradation products. Feuell's second experiment involving the use of $SO_2$ gas to directly treat groundnuts containing aflatoxins may have proved ineffective because, as will be illustrated in another paper (*6*), $SO_2$ has to be in aqueous solution to be reactive with aflatoxin $B_1$ and $G_1$.

Regardless of what happened in these studies, we found that bisulfite was able to degrade aflatoxin $B_1$ and $G_1$. Aflatoxin $G_1$ was slightly more reactive with bisulfite

than $B_1$. Rates of degradation were dependent on both bisulfite concentration and temperature. Other factors affecting the ability of bisulfite to degrade aflatoxin and a proposed mechanism for this reaction will be discussed in another paper (*6*).

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REFERENCES

1. Amerine, M. A., H. W. Berg, and W. V. Cruess. 1972. Technology of wine making. AVI Publishing Co., Westport, Conn. pp. 151-156, 188-192, 255-258.
2. Capellos, C., and B. H. J. Bielski. 1972. Kinetic systems. John Wiley and Sons, New York. p. 5.
3. Ciegler, A. 1975. Mycotoxins: Occurrence, chemistry, biological activity. Lloydia 38:21-35.
4. Davis, N. D., and U. L. Diener. 1967. Inhibition of aflatoxin synthesis by p-aminobenzoic acid, potassium sulfite, and potassium fluoride. Appl. Microbiol. 15:1517-1518.
5. Dean, J. A. (ed.). 1973. Lange's handbook of chemistry, 11th ed., McGraw-Hill Book Co., Inc., New York. p. 5-15.
6. Doyle, M. P., and E. H. Marth. 1978. Bisulfite degrades aflatoxin: Effect of citrus acid and methanol and possible mechanisms of degradation. J. Food Prot. 41:(In press).
7. Feuell, A. J. 1966. Aflatoxin in ground nuts. IX. Problems of detoxification. Trop. Sci. 8:61-70.
8. Fischbach, H., and A. D. Campbell. 1965. Note on detoxification of the aflatoxins. J. Assoc. Off. Agr. Chem. 48:28.
9. Gehman, H., and E. M. Osman. 1954. The chemistry of the sugar-sulfite reaction and its relationship to food problems. Adv. Food Res. 5:53-96.
10. Gibson, W. B., and F. M. Strong. 1973. Metabolism and elimination of sulphite by rats, mice, and monkeys. Food Cosmet. Toxicol. 11:185-198.
11. Goldblatt, L. A. (ed.). 1969. Aflatoxin. Academic Press, New York and London. 472 p.
12. Gupta, S. R., H. R. Prasanna, L. Viswanathan, and T. A. Venkitasubramanian. 1975. Synthesis of aflatoxins by the non-growing mycelia of *Aspergillus parasiticus* and the effect of inhibitors. J. Gen. Microbiol. 91:417-420.
13. Joslyn, M. A., and J. B. S. Braverman. 1954. The chemistry and technology of the pretreatment and preservation of fruit and vegetable products with sulfur dioxide and sulfites. Adv. Food Res. 5:97-160.
14. Kiermeier, F., and L. Ruffer. 1974. Changes of aflatoxin $B_1$ in alkaline solutions. Z. Lebensm. Unters.-Forsch. 155:129-141.
15. Mann, G. E., L. P. Codifer, Jr., H. K. Gardner, Jr., S. P. Koltun, and F. G. Dollear. 1970. Chemical inactivation of aflatoxins in peanut and cottonseed meals. J. Amer. Oil Chem. Soc. 47:173-176.
16. Parker, W. A., and K. Melnick. 1966. Absence of aflatoxins from refined vegetable oils. J. Amer. Oil Chem. Soc. 43:635-638.
17. Pons, W. A., Jr., A. F. Cucullu, L. S. Lee, H. J. Janssen, and L. A. Goldblatt. 1972. Kinetic study of acid-catalyzed conversion of aflatoxins $B_1$ and $G_1$ to $B_{2a}$ and $G_{2a}$. J. Amer. Oil Chem. Soc. 49:124-128.
18. Pons, W. A., Jr., J. A. Robertson, and L. A. Goldblatt. 1966. Objective fluorometric measurement of aflatoxin on TLC plates. J. Amer. Oil Chem. Soc. 43:665-669.
19. Roberts, A. C., and D. J. McWeeny. 1972. The uses of sulphur dioxide in the food industry. A review. J. Food Technol. 7:221-238.
20. Rodricks, J. V. (ed.). 1976. Mycotoxins and other fungal related food problems. Adv. Chem. 149:1-67.
21. Schroeter, L. C. 1966. Sulfur dioxide. Pergamon Press, Oxford. 342 p.
22. Schuller, V. P. L., T. Ockhuizen, J. Werringloer, and P. Marquardt. 1967. Aflatoxin $B_1$ und Histamin in Wein. Arzneimittel Forschung 17:888-890.
23. Shih, C. N., and E. H. Marth. 1969. Improved procedures for measurement of aflatoxins with thin-layer chromatography and fluorometry. J. Milk Food Technol. 32:213-217.
24. Sreenivasamurthy, V., H. A. B. Parpia, S Srikanta, and A. Shankarmurti. 1967. Detoxification of aflatoxin in peanut meal by hydrogen peroxide. J. Assoc. Off. Agr. Chem. 50:350-354.
25. Stecher, P. G. (ed.). 1968. The Merck index. 8th ed. Merck & Co., Inc., Rahway, N. J. pp. 858, 966.
26. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York. pp. 161-169.
27. Stumm, W., and J. J. Morgan. 1970. Acids and bases. pp. 69-114. In Aquatic chemistry. John Wiley and Sons, Inc., New York.
28. Takahashi, D. M. 1974. Thin layer chromatographic determination of aflatoxins in wine. J. Assoc. Off. Anal. Chem. 57:875-879.
29. Trager, W., and L. Stoloff. 1967. Possible reactions for aflatoxin detoxification. J. Agr. Food Chem. 15:679-681.
30. Weast, R. C. (ed.). 1974. Handbook of chemistry and physics, 55th ed. CRC Press, Cleveland. p. D-130.
31. White, A., P. Handler, E. L. Smith. 1973. Principles of biochemistry. 5th ed. McGraw-Hill Book Co., New York. pp. 232-235.

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# Evidence for a Lactic Streptococcal Role in Nigerian Acidic Cassava (*Manihot esculenta* Crantz) Fermentations[1]

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**ABSTRACT**

The predominant acid-producing organism isolated from Nigerian cassava mixed fermentation cultures was *Streptococcus faecium*. *Corynebacterium manihot* was also abundant in the mixed cultures, but contrary to earlier reports, this organism grew slowly and lacked significant acid-producing capabilities. Cultural characteristics of *S. faecium* indicated that it was the primary fermentation organism in acidic cassava fermentations rather than the earlier indicated *C. manihot*. Diacetyl production in both milk cultures and fermented cassava was demonstrated for *S. faecium*. Detection of diacetyl in fermented cassava products indicated that *S. faecium* may also play an additional role in the flavor development of these products.

Cassava or manioc (*Manihot esculenta* Crantz) is a widely-grown tropical root crop that is consumed in a variety of forms as a food staple in many cultures. This popular carbohydrate source contains the cyanogenic glycoside, linamarin, which degrades to hydrocyanic acid (HCN) and glucose under the influence of the enzyme, linase, and/or low pH conditions (*2*). As a part of the traditional processing or preparation of popular Nigerian food items, such as gari, cassava roots are macerated and allowed to undergo a natural, acidic fermentation process which results in the detoxification of the cassava (*1,3*). This has been reported to occur through the liberation of HCN at low pH (ca 3.9) through the combined activities of *Corynebacterium manihot* and *Geotrichum candidum* (*3*), and substantial amounts of lactic acid that have been found in fermented cassava (*1*). Acid production has been ascribed to *C. manihot* because it was the predominant organism that was isolated in the initial 48 h of fermentation while flavor production was attributed to *G. candidum* which grew abundantly in the latter stages of the 4-day fermentation (*3*). In some instances fermentation occurs as a result of natural microflora associated with cassava roots and utensils, while in more centralized processing situations the juice from previous batches is used as an inoculum for fresh cassava pulp (*1*).

*Corynebacterium* spp. do not consistently ferment sugars, but when they do high acidities are seldom produced. Many species within the genus oxidize glucose completely to carbon dioxide and water (*7*). The fact that high acidity is seldom produced by members of the genus *Corynebacterium* strongly suggests that the organism responsible for lowering the pH of the fermenting cassava pulp is not *C. manihot*. The consistent isolation of this organism as the dominant species in the first stage of the fermentation (*3*) may have resulted from inappropriate selection of the isolation medium. *C. manihot* easily grows on nutrient agar, but lactic-acid bacteria are nutritionally fastidious and were probably selectively excluded from observation. This paper reports the results of a re-investigation of the microbiology of Nigerian cassava fermentation using isolation media suitable for detection of lactic lacid bacteria.

## MATERIALS AND METHODS

### *Isolation and characterization of streptococci*

Mixed cultures from cassava mash fermented locally in Nigeria (Adeladan Amodo, a village on the Ondo Road) were streaked on Plate Count agar (Difco Laboratories, Detroit, Michigan) and Nutrient agar (Difco) to determine microorganisms capable of growing on simple media, and APT agar (Difco) was employed to enhance growth of lactic acid bacteria (*8*). Sodium azide (0.04%) added to APT agar and KF Streptococcus agar (Difco) were used for selective streptococcal growth and presumptive indication of streptococci species (*11*).

Isolated colonies were picked from each of the agar plates, and were transferred into Nutrient, APT and Brain Heart Infusion (BHI, Difco) broths. Cultures were maintained by transferring into appropriate broth every 48 h, and separate cultures were carried at 30 and 37 C. Selected cultures were gram-stained, and checked for morphology.

Isolated colonies from the mixed cultures were inoculated into sterile skimmilk and sterile litmus milk to determine acid production. Suspected streptococci from KF Streptococcus agar were streaked on Low Glucose Agar (LGA, Difco), and were tested for catalase activity by flooding the incubated plates with 3% hydrogen peroxide. The colonies were also tested for presence of iron-porphyrin compounds by flooding incubated LGA plates with benzidine reagent before adding 3% hydrogen peroxide (*5*). Finally, the suspected streptococcus colonies were transferred into pyruvate and sorbitol media (*6*).

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*Characteristics of cassava culture organisms*

Pure cultures of *C. manihot* and *G. candidum* obtained from the Federal Institute of Industrial Research, Oshodi, Nigeria, *G. candidum* ATTC #12784 (American Type Culture Collection, Rockville, MD), and *S. faecium* isolated from naturally fermenting cassava mash were grown at 37 C for 18 h in steamed (1 h at ca 100 C) skimmilk and sterile litmus milk to determine acid-producing capabilities and aroma development in these media.

A limited number of model-system cassava fermentations were carried out using greenhouse-cultured cassava roots (Variety 53101, J. Omeumu, Dept. of Pathology, University of Wisconsin). Cassava roots (ca 200 g each) were washed, peeled, and grated to yield a mash. Fifty-gram lots of mash were inoculated with 1 ml of appropriate seed culture, placed in cheese cloth bags, and placed in 250-ml foil-covered beakers for incubation at 35 C for 1 week. Cultures employed in these trials included natural mixed cultures from fermenting cassava which had been transferred to Nutrient broth before use, and mixtures of pure cultures of *C. manihot* and *G. candidum*.

Laboratory-fermented and naturally-fermented cassava mash, traditionally processed gari, and the previously described milk cultures were analyzed by the gas chromatographic headspace analysis procedure developed by Morgan and Day (*13*). Corresponding unfermented control samples were also analyzed where appropriate to verify production of volatile compounds by fermentation organisms. Analysis conditions included nitrogen-purging (10 ml/min) of volatile compounds from NaCl-saturated aqueous sample systems, and collection of volatile compounds at the head of either a Porapak Q column (12 ft × 1/8 inch stainless steel; Waters Associates, Framingham, Mass.) or a 3% 1,2,3-tris-(2-cyanoethoxy)-propane (Tris) on Chromosorb G column. In each instance 10 g of sample was combined with 5 ml of saturated NaCl solution and 1 g of NaCl to provide a solution for analysis that would contain the same proportions of volatile compounds as the original sample in each instance.

The Porapak Q column was operated isothermally at 155 C, and the Tris column was held initially at 50 C for 5 min, then was programmed at 4 C/min to 175 C and held. Injector and detector temperatures on the Varian Model 1740 gas chromatograph were maintained at 250 C, and the carrier gas was nitrogen at a flow rate of 25 ml/min. Tentative identifications of votalile compounds were assigned on the basis of coincidence of relative retention times of unknowns with those of authentic compounds, and where appropriate occurrence of distinctive aromas were detected through a column-effluent splitter assembly. Peak areas were determined by the triangulation procedure.

## RESULTS AND DISCUSSION

After incubation at 37 C for 48 h, large yellow colonies were observed on Nutrient agar plates streaked with mixed cassava cultures. On similarly incubated plates of Plate Count Agar which were streaked with the same mixed cultures, two different types of colonies were observed. There were many pinpoint colonies which were characteristic of lactic acid bacteria, and an abundance of large yellow colonies which overgrew the pinpoint colonies. The pinpoint colonies probably were not observed on the Nutrient agar plates because Nutrient agar is a less nutritive medium than Plate Count Agar.

The yellow colonies on both the Nutrient agar and Plate Count Agar were picked into tubes of Nutrient broth which were incubated at either 30 or 37 C. No growth was observed in cultures incubated at 30 C, but cultures incubated at 37 C showed heavy growth after 96 h of incubation. Examination of the culture organisms by the usual gram-staining techniques showed that the yellow colonies were composed of gram-positive, club-shaped rods with granules that stained gram-positive. These characteristics agreed closely with those described for *C. manihot* previously isolated and reported in earlier investigations of cassava fermentations (*3*).

Growth of the mixed culture on the APT agar medium was very rapid and nonpigmented colonies were observed after 24 h of incubation at 30 C. Incorporation of manganese ions ($Mn^{++}$) and citrate into the basal nutrient medium results in a unique medium for supporting growth of lactics which do not grow on Nutrient agar or grow poorly on Plate Count Agar (*8*). Colonies from APT agar medium were transferred into APT broth and incubated at 30 C; this resulted in a heavy turbid growth in the tubes after 48 h. These cultures were again streaked on APT agar to which 0.04% sodium azide ($NaN_3$) had been added. Pinpoint colonies appeared on this medium after 48 h at 30 C, and provided strong evidence for the presence of lactic acid bacteria in cassava fermentation mixed cultures.

Pinpoint colonies picked from the APT-sodium azide plates were transferred daily into APT and BHI broths. Heavy growth was observed in all the cultures and each culture was gram-stained and examined under the microscope for gram reaction and morphology. All of the organisms observed were gram-positive cocci in chains which was indicative of *Streptococcus* spp. A further indication that these organisms were *Streptococcus* sp. was their growth as pinkish colonies on KF agar.

Results of the morphological and physiological examinations of primary streptococcal isolate from cassava fermentation mixed cultures are summarized in Table 1. The fact that the organism under investigation gave negative catalase and benzidine tests indicated very strongly that it was a lactic acid bacterium. The morphological characteristics and reaction on the KF Streptococcus agar clearly placed the organism in question in the *Streptococcus* group. Additional tests showed that the organism grew well in skimmilk giving rapid coagulation, and readily changed blue litmus to colorless. The organism also tolerated 6.5% sodium chloride, and showed growth at 45 C. Since the streptococcal isolate from the mixed cassava culture was

TABLE 1. *Physiological and morphological characteristics of primary acid-producing isolate from Nigerian cassava fermentation mixed cultures.*

| Tests | Results |
|---|---|
| Gram reaction | Positive |
| Morphology | Cocci in chains |
| Catalase test | Negative |
| Benzidine test | Negative |
| Growth on APT+sodium azide | Positive |
| Growth on KF Streptococcus agar | Pinkish colonies |
| Growth in pyruvate medium | Negative |
| Growth in sorbitol medium | Negative |
| Growth in a medium containing 6.5% NaCl | Positive |
| Growth at 45 C | Positive |
| Reaction in steamed skimmilk | Coagulation |
| Reaction in litmus milk | Colorless in 12 h and coagulation |

able to grow at 45 C and tolerated 6.5% sodium chloride, it fell in Sherman's enterococcus division that includes *Streptococcus faecalis* and *Streptococcus faecium* (*4*).

Pyruvate is used as an energy source only by *S. faecalis* (*6*) and use of pyruvate as an energy source is employed as an aid in differentiating between *S. faecalis* and *S. faecium*. The inability of the isolate from the cassava fermentation mixed culture to grow in a pyruvate medium confirmed that the organsim was *S. faecium* and not *S. faecalis*. Further, the organism was unable to grow in sorbitol medium. *S. faecalis* can grow in both pyruvate and sorbitol media while *S. faecium* does not grow in these media (*6*).

Inoculation of steamed skimmilk and litmus milk with both single-strain and mixed-strain cultures gave results which supported the hypothesis that *S. faecium* was the principal acid-producing organism in mixed-strain natural cassava cultures (Table 2). Acid production in milk was not observed for pure cultures *C. manihot* or *G. candidum* or for a mixture of these two organisms. These organisms produced only weak fatty acid-like aromas while *S. faecium* gave a pronounced diacetyl aroma similar to that observed for the natural mixed-strain cassava cultures.

TABLE 2. *Behavior of organisms from cassava cultures grown for 18 h at 37 C in steamed skimmilk and litmus milk.*

| Culture | Coagulation of steamed milk | Aroma | Reaction in Litmus milk |
|---|---|---|---|
| Natural mixed cassava culture | + | Strong diacetyl | Coagulation and colorless |
| *S. faceium* (Isolate) | + | Strong diacetyl | Coagulation and colorless |
| *G. candidum* (Isolate[1] & ATTC #12784 | — | Weak, fatty acid-like | No reaction |
| *C. manihot* (Isolate[1]) | — | Indistinct | No reaction |
| Mixed culture of *G. candidum*[1] and *C. manihot*[1] | — | Weak, fatty acid-like | No reaction |

[1]Obtained as pure culture from the Federal Institute of Industrial Research, Oshodi, Nigeria.

Analysis of milk cultures for volatile compounds showed that both the *S. faecium* culture and the natural mixed-strain culture gave substantial diacetyl peaks although the actual levels of diacetyl were not quantified (Table 3). Analyses of laboratory-fermented and naturally-fermented cassava mash (Table 3) also showed the presence of diacetyl in these products. The role of diacetyl in cultured dairy product flavors is well documented (*12*). Data for volatile compounds in traditional gari and sun-dried fermented cassava (Table 4) show that diacetyl is carried through to the finished product, and the presence of diacetyl in these products indicates that *S. faecium* may also play an additional role in development of flavors in fermented cassava products. While the other volatile compounds tentatively identified in the samples are commonly found in lactic fermentations (*9,10*), only acetaldehyde has a sufficiently low flavor threshold to have a possible direct influence on flavors of cassava products. However, ethanol could react with fatty acids to yield esters that could contribute to flavors.

Both laboratory-grated cassava mash and steamed skimmilk samples inoculated with either *C. manihot* or *G. candidum* or combinations thereof showed very little indication of fermentation activity. *S. faecium* apparently was not present in the natural microflora of laboratory-grated cassava samples. As a result acid production did not take place in any of these samples, and molds quickly overgrew the samples.

## SUMMARY AND CONCLUSIONS

*S. faecium* was isolated as the acid-producing organism in Nigerian cassava fermentation mixed cultures. Earlier investigations of the microbial succession in cassava fermentation (*3*) indicated that the acid-producing organism was *C. manihot*. *C. manihot* was abundant in the fermentation cultures, but since this organism grew slowly, and is oxidative rather than

TABLE 3. *Relative abundance of volatile compounds found in gas chromatographic headspace analysis of fermented cassava and skimmilk cultures.*

| Peak no. | Tentative identity | Relative retention time[1] | | Absolute areas of peaks (cm$^2$) | | | |
|---|---|---|---|---|---|---|---|
| | | Observed | Authentic | Lab-fermented cassava with mixed culture | Natural Nigerian fermented cassava mash | *S. faecium* in steamed skimmilk | Natural mixed cassava culture in steamed skimmilk |
| 1 | Acetaldehyde | 0.17 | 0.18 | 33 | 12 | 172 | 22 |
| 2 | Ethanol | 0.29 | 0.34 | 96 | 976 | 512 | 249 |
| 3 | Acetone | 0.42 | 0.48 | 1 | 16 | 10 | 3 |
| 4 | Diacetyl | 1.00 | 100 | 4 | 22 | 10 | 11 |

[1]Relative retention time based on diacetyl equal to 1.00; Packed column: 10 ft × 1/8 inch O.D. stainless steel, Porapak Q.

TABLE 4. *Relative abundance of volatile compounds found in gas chromatographic analysis of headspace volatiles of gari, fermented cassava, and* S. faecium *in skimmilk.*

| Peak no. | Tentative identity | Relative retention Time[1] | | Absolute areas under peaks (cm$^2$) | | | |
|---|---|---|---|---|---|---|---|
| | | Observed | Authentic | Traditionally processed gari from Nigeria | Sun-dried fermented cassava from Nigeria | Lab fermented cassava with mixed culture | *S. faecium* in steamed skimmilk |
| 1 | Acetaldehyde | 0.08 | 0.07 | 5 | 9 | 12 | 96 |
| 2 | Ethanol | 0.21 | 0.19 | —[2] | 15 | 7731 | 164 |
| 3 | Acetone | 0.48 | 0.42 | 3 | 1923 | — | — |
| 4 | Diacetyl | 1.00 | 1.00 | 15 | 256 | 11 | 128 |

[1]Relative retention time based on diacetyl equal to 1.00; Packed column, 12 ft × 1/8 inch O.D. stainless steel, 3% 1,2,3-tris-(cyanoethoxy) propane on Chromosorb G.
[2]Not detected in the product.

fermentative, it is very unlikely that *C. manihot* is responsible for any acid production in cassava fermentations. Based on growth and acid-producing characteristics observed, it is proposed that *S. faecium* is the most important acid-producing organism present in Nigerian cassava mixed-strain fermentations. Diacetyl production by *S. faecium* isolated from cassava mash cultures was also demonstrated, and indicates that this organism may contribute flavors in addition to acidity to fermented cassava products. The role of *C. manihot* and *G. candidum* in the overall fermentation of cassava by natural mixed-strain cultures has not been determined as yet.

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**REFERENCES**

1. Akinrele, I. A. 1964. Fermentation of cassava. J. Sci. Food Agric. 15:589-594.
2. Ayers, J. C. 1972. Manioc: The potential exists for increased use of this tropical plant and its products. Food Technol. 26(8): 128-138.
3. Collard, P., and S. Levi. 1959. A two-stage fermentation of cassava. Nature 183:620-621.
4. Deibel, R. H. 1964. Group D streptococci. Bacteriol. Rev. 28: 331-366.
5. Deibel, R. H., and J. B. Evans. 1960. Modified benzidine test for the detection of cytochrome-containing respiratory systems in microorganisms. J. Bacteriol. 79:356-360.
6. Deibel, R. H., and C. F. Niven, Jr. 1964. Pyruvate fermentation by *Streptococcus faecalis*. J. Bacteriol. 88:4-10.
7. Deibel, R. H., and H. W. Seeley. 1974. Family II. Streptococcaceae. p. 490-501. In: R. E. Buchanan and N. E. Gibbons (eds.) Bergey's manual of determinative microbiology. 8th ed. The Williams and Wilkens Co., Baltimore, Md.
8. Evans, J. B., and C. F. Niven, Jr. 1951. Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products. J. Bacteriol. 62:599.
9. Keenan, T. W., D. D. Bills, and R. C. Lindsay. 1967. Acetone in milk cultures of lactic streptococci and *Leuconostoc citrovorum*. Can. J. Microbiol. 13:1118-1120.
10. Keenan, T. W., R. C. Lindsay, M. E. Morgan, and E. A. Day. 1966. Acetaldehyde production by single-strain lactic streptococci. J. Dairy Sci. 49:10-14.
11. Keener, B. A., J. F. Clark, and P. W. Kabler. 1961. Cultivation and enumeration of streptococci in surface waters. Appl. Microbiol. 9:15.
12. Lindsay, R. C. 1967. Cultured dairy products. p. 315-330. In: H. W. Schultz, et al. (ed.) Symposium on foods: The chemistry and physiology of flavors. AVI Publishing Co., Westport, Conn.,
13. Morgan, M. E., and E. A. Day. 1965. Simple on-column trapping procedure for gas chromatographic analysis of flavor volatiles. J. Dairy Sci. 48:1382-1384.
14. Ramadan, F. M. 1967. Studies on the growth of Group D and other streptococci in different media. J. Appl. Bacteriol. 31:245-252.
15. Sherman, J. M. 1937. The streptococci. Bacteriol. Rev. 1:1-97.

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# Differential Production of Aflatoxin on Natural and Heat-Treated Cocoa Beans

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**ABSTRACT**

Costa Rican-type cocoa beans were tested as a substrate for two known aflatoxigenic fungal species, *Aspergillus flavus* NRRL 3251 and *Aspergillus parasiticus* NRRL 2999. Natural floral growth in both cooked (autoclaved) and raw cultures failed to show aspergilli and aflatoxin as analyzed by thin layer chromatography. Raw, ground cocoa bean medium inoculated with both species of aspergilli had mycelial growth, sporulation, but no detectable levels of aflatoxin. Similarly treated but cooked (autoclaved) cocoa medium also provided for mycelial growth, and sporulation, but total aflatoxin levels were 388.1 μg/g of substrate for the *A. parasiticus* cultures and 2.2 μg/g of substrate for the *A. flavus* cultures. The general results appear to support the low level of a limited number of reports of toxin contamination in natural cocoa. The simplified extraction methods used herein provided results similar to other methods.

Chocolate manufacturers collect and store cocoa beans from many sources. Samples of beans that have been studied in relation to mycotoxins include types from the following countries: Nigeria, Ghana, New Guinea, Trinidad (*14*), and the Philippines (*3*). In 1965 the Tropical Products Institute reported aflatoxin (AFT) positives in cocoa bean (*11*). Campbell (*3*) in 1969 also reported finding up to 17 μg aflatoxin/kg in two of nine samples of cocoa from the Philippines. Following these reports there was an effort to develop better methods for analyzing large numbers of samples for AFTs. In 1971, Scott (*13*) and Scott and Przybylski (*15*) modified the CB method (Contaminants Branch Method, FDA) for use with cocoa beans. Collaborative tests were made and the technique was accepted as the Official AOAC First Action Method (*15*). A modification of the BF Method (Best Foods Method) using 3% silver nitrate solution in the extracting solvent mixture was reported in 1973 (*14*).

There seem to be few reported occurrences of aflatoxin contamination in cocoa and cocoa products. Limited occurrence could be due to the complexity of the test (*10,14*) or limited testing. More recently, Yndestand and Underdal (*19*) surveyed Norwegian foods including cocoa and cocoa products. They found one positive in 40 samples of cocoa products.

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The present study was undertaken to determine the potential of cocoa as a substrate for aflatoxigenic fungi under "near-ideal" laboratory growth conditions. Also, no studies have utilized Costa Rican cocoa beans.

## MATERIALS AND METHODS

A one-kg sample of naturally dried cocoa beans, selected at random as a government inspection sample, from composite stocks, was collected from the Zent Region, Puerto Limon, Costa Rica. This sample served as the source for the experiments completed herein.

*Preparation of cultures and inoculation*

The cocoa beans were minced in a sterile blender for 45 sec. Five g of the blended substrate were placed in each of the culture vials with 7 ml of distilled water required to moisten the ground cocoa beans. Two-ounce, clear glass prescription vials having one flat side were plugged with cotton. They were sterilized (20 psi for 20 min) either with the ground cocoa plus distilled water to provide cooked cocoa or only with distilled water. In the latter case, the cocoa (uncooked) was added to the vials containing sterile water following their return to room temperature after autoclaving.

Eighteen vials were used in the natural flora portion of the study. An equal number of vials contained cooked (sterile, 20 psi for 20 min) or uncooked (non-sterile) cocoa.

Twenty-seven vials were used in the growth and toxin production aspect of the study. Fifteen vials containing water were prepared by sterilizing (cooking) the minced cocoa with the vials. Three of these vials served as controls and were not inoculated. The remaining vials containing sterile water received equal aliquots of uncooked cocoa. Six of these vials having cooked cocoa and six having uncooked cocoa were inoculated with *Aspergillus parasiticus* NRRL 2999 spores or *Aspergillus flavus* NRRL 3251 spores. Spores for both species were taken from potato-dextrose-agar slants maintained in our laboratory. Both of these species used were known producers of aflatoxins (*6, 16*).

*Incubation procedures*

All culture vials were placed in a growth chamber and observed regularly for 30 days. Environmental conditions included relative humidity at 45 ± 5% and temperature at 23 ± 2 C. The chamber was dark except when opened for observations.

*Extraction and analyses*

After 30 days all cultures were attenuated by addition of 30 ml of chloroform. The vials were capped and shaken gently for 1 h at room temperature. All clumps of cocoa were broken. The solids in the culture floated and the chloroform layer was removed with a syringe which penetrated the floating solid layer. Twenty-microliter samples were spotted on silica gel thin-layer plates (205-nm layer of Absorbosil-l Applied Science Lab. Inc., State College, PA). Fluorescent spots were compared visually to reference samples (Applied Science Lab., Inc.,

State College, PA) spotted simultaneously utilizing the AOAC procedure for TLC, (*8*) Dilutions were made as necessary and quantitations were repeated in triplicate. Final aflatoxin concentrations per culture and then per gram of substrate were calculated based on these values and the 30 ml of chloroform first added.

## RESULTS AND DISCUSSION

Natural flora cultures for both raw and cooked medium failed to develop *Aspergillus*-like fungal growth. Aflatoxin analyses were negative for these cultures as well as the cooked, control flasks. Even though in Costa Rica such beans are dried outdoors on movable wheel and rail beds about 10 m in square area, and usually on a small-scale basis by individual farmers, there was no aflatoxin contamination in the sample used in this study. At least for the present, there are no reports indicating that there is a significant aflatoxin problem. These flat-car beds are often located in the front yard of the rural or village farmer. When it rains they are usually pushed under a crude roof and stored in a layer-type fashion. In some instances a local farmer may serve as the dealer who collects and dries beans for further sale. Such small businessmen are often seen during the day rearranging the cocoa beans in the drying beds. Groundnuts are also grown in Costa Rica and they have been found to have the typical AFT problems found in the tropics (*1, 12*). Therefore, the fungal spores apparently are native, but could be regional or possibly Costa Rican cocoa beans are not susceptible to aflatoxin occurrence.

Additional results, as listed in Table 1, may present an interesting aspect of this problem in support of the non-susceptibility of Costa Rican cocoa beans. *A. flavus* NRRL 3251 and *A. parasiticus* NRRL 2999 both produced mycelia and sporulated on raw and cooked moist cocoa bean media. *A. parasiticus* followed its typical growth pattern and sporulated first. Spores were evident after day five and extensive by day 10. For *A. flavus*, spores were evident on day seven and extensive by day 14. Visual evaluations showed similar mycelial growth and sporulation for each individual species on both cooked and raw substrates. The AFT analyses were positive only for the cooked (sterilized) ground cocoa beans. This was true for both species tested. Concurrent tests using the same species on rice and shredded coconut were positive. For example, when *A. parasiticus* was inoculated into sweetened coconut flake cultures (20 g substrate plus 10 ml $H_2O$ and grown for 7 days at room temperature), they were found to contain 0.938 mg/g of substrate for $AFB_1$, 19.715 mg/g of substrate for $AFG_1$, 0.060 mg/g of substrate for $AFG_2$, and no $AFB_2$. It is unlikely that a change from a toxin-producer to a non-producer occurred in the species used. To date, we have no definite explanation for growth and sporulation but lack of detectable levels of AFT on these laboratory cultures of ground raw cocoa beans. Factors under consideration indicate that the cooking that occurred during the autoclaving process may have denatured a natural inhibitory agent, changed the dispersion of the nutrients, or altered favorably the nutritional composition of the ground cocoa beans. Also the absence of microbial competition may have played an important role in this situation.

Other reports have been documented where aflatoxigenic fungi have grown and sporulated but produced little or no toxin. The presence of phytic acid or hydroxy-cinnamic acid derivatives found in uncooked white potatoes or cinnamon seem to act as aflatoxin-inhibitory agents (*2, 5, 17*). Davis and Diener (*4*) reported that *A. parasiticus* produced toxin on various carbon sources. We found that *A. parasiticus* grew poorly on lettuce, cauliflower, celery, and taro root and produced no detectable levels of aflatoxin on the first three products (*9*). In soybeans, different strains of beans seemed to influence the production of aflatoxin apparently due to the nutrients, fats, or acids available but also it was reported that cooking allowed some aflatoxigenic isolates to produce more toxin but other fungal strains produced less toxin than on the raw substrate (*16*).

Some extracts from the non-aflatoxin producing raw cultures in the present study were further concentrated and evaluated again for AFT. No positives were found. The system for quantitation used herein is sensitive to approximately 2 ppb. Although the samples were not extracted following the official procedures the remaining test procedures were official AOAC procedures. To confirm the absence of aflatoxin, several cultures were extracted using the AOAC, IUPAC, and Costa Rican procedures (*1, 8, 10*) and no toxins were found.

In studying the quantities of toxin produced, *A. parasiticus* NRRL 2999, a known producer of AFT did

TABLE 1. *Aflatoxin production on cooked and raw cocoa bean cultures.*

| Inoculation Treatments[a] | Aflatoxin levels (Mean and standard deviations[b]) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $AFB_1$ (μg/g) | % (of total) | AFB (μg/g) | % (of total) | $AFG_1$ (μg/g) | % (of total) | $AFG_2$ (μg/g) | % (of total) | Total (μg/g) |
| *Aspergillus parasiticus* (NRRL 2999, cooked) | 207.3 ±19.6 | 53 | ND[c] | — | 180.1 ±14.9 | 46 | 0.7 ±0.3 | <1 | 388.1 ±27.8 |
| *Aspergillus parasiticus* (NRRL 2999, raw) | ND | — | ND | — | ND | — | ND | — | ND |
| *Aspergillus flavus* (NRRL 3251, cooked) | 1.2 ±0.4 | 55 | ND | — | 1.0 ±0.6 | 45 | ND | — | 2.2 ±0.9 |

[a] *A. flavus* NRRL 3251 with raw cocoa; natural flora; both raw and autoclaved; and autoclaved control cultures contained no detectable levels of aflatoxin.
[b] Mean and standard deviations are for six cultures in triplicate.
[c] None detected. Lower level for detection is approximately 2 ppb.

well on the cooked cocoa having a mean level for $AFB_1$ of 206.3 μg/g of substrate; 180.1 μg/g of substrate for AFG¹; and traces of $AFG_2$. These values and especially the total of 388.1 μg/g are similar to that found for sunflower seed media (*7*). *A. flavus* NRRL 3251 produced significantly less $AFB_1$ and $AFG_1$ than *A. parasiticus* NRRL 2999 and no $AFG_2$ at all. There are indications that there are not only differential AFT levels produced on cooked and raw cocoa but also a preference of toxin production for fungal species or strains. The presence of the mold on cocoa is not an absolute case for aflatoxin occurrence. Due to the number of aflatoxigenic strains, it is suspected that some isolates could produce toxin on raw cocoa or possibly even the strains used herein may be producers under some environmental conditions. Additional aflatoxigenic strains, especially those native to Costa Rica should be evaluated.

Wildman et al. (*18*) appear to have made the only other mention of aflatoxin production under laboratory conditions on cocoa beans. Among the many foods they inoculated with *A. flavus* NRRL A 13794 (a known producer of 1000 μg $AFB_1$ per gram of moistened sterilized wheat biscuits) they report for cocoa beans, 4μg (total aflatoxin) per gram of media. There is no mention of the source of the cocoa bean or the culture medium preparations other than that the beans apparently were not sterilized. This low level of aflatoxin from a high producing strain supports Scott's (*14*) inference and our present study that non-heat-treated cocoa tends to yield little or no aflatoxin.

Further work is planned to evaluate the strain-specific agent as it relates to aflatoxin production as well as the apparent natural resistence of Costa Rican cocoa beans and other types of cocoa beans to aflatoxin occurrence. The rapid and simple extraction technique used herein seemed adequate for aflatoxin analyses and produced results similar to other methods of extraction.

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**REFERENCES**

1. Acuna, D., J. Benavides, and N. Vega. 1977. Analytical G.T. method for aflatoxins in corn samples and other agricultural commodities. In: P. Rosenburg (ed.) 5th International Symposium on Animal, Plant, and Microbiol. Toxins. San Jose, Costa Rica, Aug. 8-13, and 1976 Pergamon Press, In Press
2. Bullerman, L. B. 1974. Inhibition of aflatoxin production by cinnamon. J. Food Sci. 39:1163-1165
3. Campbell, T. C. 1969. 83rd Annual Meeting of the Assoc. Offic. Anal. Chemists report presented at the Joint AOAC-AOCS Committee on Aflatoxins, Oct. 13-16, Washington, D.C.
4. Davis, N. D., and U. L. Diener, 1968. Growth and aflatoxin production by *Aspergillus parasiticus* from various carbon sources. Appl. Microbiol. 16:158-159.
5. Gupta, S. K., and T. A. Venkitasubramanian. 1975. The effect of zinc and phytic acid on the incorporation of 1-$^{14}$C-acetate into aflatoxin by resting mycelia of *Aspergillus parasiticus* Z. Lebensm. Unters. Forsch. 159:107-111.
6. Hilldrup, J. A. L., T. Eadie, and G. C. Llewellyn, 1977. Fungal growth and aflatoxin production on apiarian substrates. J. Assoc. Offic. Anal. Chem. 60:96-99.
7. Llewellyn, G. C., and T. Eadie. 1974. Evaluating the potential for occurrence of aflatoxins on black sunflower seeds. J. Assoc. Offic. Anl. Chem. 60:96-99.
8. Official Methods of Analysis. 1975. 12 ed. Assoc. Offic. Anal. Chem. Soc. Washington, D.C. Sec. 26.
9. Raghu, A., T. Eadie, and G. C. Llewellyn. 1978. Evaluating the potential for aflatoxin occurrence on celery, cauliflower, lettuce, and taro root inoculated with *Aspergillus flavus* and *Aspergillus parasiticus*. J. Assoc. Offic. Anal. Chem. 61:998-1001.
10. Recommended Method for Aflatoxins in Cocoa Beans. 1973. International Union of Pure and Applied Chemistry, Tech. Report No. 8, pp. 1-12.
11. Report of the Tropical Products Institute for 1965. 1966. Her Majesty's Stationery Office, London. p. 6.
12. Sargeant, K., J. O'Kelly, R. B. A. Carnaghan, and R. Allcroft, 1961. The assay of a toxic principle in certain groundnut meals. Vet. Rec. 73:1217.
13. Scott, P. M. 1969. Analysis of cocoa beans for aflatoxins. J. Assoc. Offic. Anal. Chem. 52:72-74.
14. Scott, P. M. 1973. Modified method for the determination of mycotoxins in cocoa beans. J. Assoc. Offic. Anal. Chemists 56:1028-1030.
15. Scott, P. M., and W. Przybylski, 1971. Collaborative study of a method for the analysis of cocoa beans for aflatoxins. J. Assoc. Offic. Anal. Chemists 54:540-544.
16. Sherertz, P. C., T. Eadie, J. W. Young, and G. C. Llewellyn. 1976. Aflatoxin occurrence on raw and cooked soybeans inoculated with three *Aspergillus* isolates. J. Assoc. Offic. Anal. Chem. 59:662-665.
17. Swaminathan, G., and P. E. Koehler. 1976. Isolation of an inhibitor of *Aspergillus parasiticus* from white potatoes (*Solanum tuberosum*). J. Food Sci. 41:313-319.
18. Wildman, J. D., L. Stoloff, and R. Jacobs. 1967. Aflatoxin production by a potent *Aspergillus flavus* Link isolate. Biotechnol. Bioeng. 9:429-437.
19. Yndestand, M., and B. Underdal. 1975. Aflatoxin in foods on the Norwegian market. Nord. Vet - Med. 27:42-48.

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# Cook/Chill Foodservice Systems: Microbiological Quality of Beef Loaf at Five Process Stages

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**ABSTRACT**

Preparation and service of hot entrees in hospital cook/chill foodservice systems require two heat processes. After preparation and mixing, beef loaves composed of ground beef and eggs were heat-processed initially to end-point temperatures of 45, 60, 75, or 90 C in a convection oven operating at 121 ± 6 C; stored 24 h at 6 ± 1 C; portioned into 100-g slices; and single portions were microwave-heated to ≥ 74 C. Four heat treatments of beef loaves were compared to a fifth treatment which excluded initial heating. Quality of beef loaf was evaluated by mesophilic and psychrotrophic aerobic plate counts, coliform counts, streptococcal counts and pH. Microbial reductions caused by handling and processing were: aerobic mesophilic plate count, 88 to 99%; psychrotrophic aerobic plate count and coliform count, ≥ 99%; and streptococcal count, 71-99%. Increasing end-point temperature of initial heat processing consistently ($P \leq 0.05$) decreased mesophilic and psychrotrophic aerobic plate counts. Coliform counts and streptococcal counts did not show a statistical relationship to end-point temperature of initial heat processing. No statistically significant differences existed in any microbiological counts among five treatments of beef loaf portions after microwave-heating. Varying end-point temperature of initial processing had no consistent statistical effect on the pH of beef portions. Temperatures of ≥ 74 C for microwave-heating of beef portions after chilled storage and before service are strongly recommended since chilling 5000 g of beef loaf to ≤ 7 C required 10 to 14 h at 6 C.

New types of foodservice systems, such as cook/chill systems, require more than one heat process for production of entrees (*23*). The cook/chill foodservice system is characterized by initial heating of entrees on the day before service, followed by refrigerated holding and terminal heating of entrees immediately before service. Entrees in cook/chill systems are frequently given a mild initial heat treatment to preserve sensory quality (*14*). Interviews with six foodservice administrators in hospitals that use cook/chill foodservice systems indicated that entrees containing meat are cooked to about 75% of completion during the initial heat process. Temperatures used in initial heat processing of similar entrees varied among foodservice systems. End-point temperature (EPT) of initial processing of beef loaf in simulated cook/chill systems was reported to be 60 and 74 C :*3,6,24*). Bobeng (*4*) recommended EPTs of 60 C for initial heating and 74-77 C for terminal heating of entrees which are served hot in cook/chill systems.

Each process stage in a cook/chill foodservice system, including initial heat processing, contributes cumulatively to the net microbiological condition of the finished product (*8*). Microbiological quality of hot entrees subjected to two heat treatments was evaluated in cook/chill systems under simulated and under actual operating conditions in eight studies (Table 1). Data from these tests indicate that products, when handled according to described procedures, were in good bacteriological condition at point of service. Presence of pathogenic organisms at point of service indicates that a potential hazard exists if food is mishandled or if toxin-producing bacteria are present. With present technology the uneven distribution of power to food in microwave ovens during heat processing results in uneven heating of food (*11,15*). Hence, implications for microbiological quality of food should be considered.

Since range in EPT of initial heat processing is evident under actual and simulated operating conditions (Table 1) and since effects of EPT of initial and terminal heat processing on food safety have not been evaluated throughout processing, this study was initiated. The purpose was to vary EPT of initial heat processing for beef loaves initially cooked in a convection oven, stored 24 h at 6 C and heated for service in a microwave oven to ≥ 74 C. Microbiological quality of beef loaves was observed throughout five process stages in a simulated cook/chill foodservice system.

## MATERIALS AND METHODS

Beef loaf was selected for study because it has a compact consistency which facilitates temperature measurements. Preliminary tests were made to standardize food and equipment handling procedures that were to be controlled during the four trials of the experiment (*10*). Each trial simulating preparation of beef loaf in a cook/chill foodservice system included five process stages (Fig. 1). Four trials were conducted in four successive weeks. Beef loaf was cooked initially to 45, 60, 75 or 90 C; in a fifth treatment for beef loaf (Treatment N), initial heat processing was excluded (Fig. 1).

TABLE 1. *Microbiological quality of hot entrees subjected to two heat processes under actual and simulated operations in cook/chill foodservice systems.*

| Investigator (year) | Product quantity/trial | Initial heat processing | | | Terminal heat processing | | | Indicator of microbial quality | |
|---|---|---|---|---|---|---|---|---|---|
| | | Time (min) | EPT[a] (C) | Equipment and operating temperature[c] | Time | EPT[a] (C) | Equipment operating temperature[c] | Organism or test | Count at point of service (Colonies/g) |
| *Laboratory simulations* | | | | | | | | | |
| 1. Tuomi et al. (*21*) | Ground beef gravy 30 kg | — | > 70 | Steam jacketed kettle | 35 min (31-53)[b] | 43 | Compartment steamer | Aerobic plate count | 830 |
| | | | | | | | | *Clostridium perfringens* | 0 |
| | | | | | | | | Coagulase-positive staphylococci | 0 |
| 2. Tuomi et al. (*22*) | Ground beef gravy 30 kg | — | > 70 | Steam jacketed kettle | 60 min | 74 | Compartment steamer | *Clostridium perfringens* inoculum ($1.4 \times 10^4$ cells/g) | 0 |
| 3. Bunch et al. (*5*) | Beef-soy loaf 5.2 kg | 43 | 60 | Convection oven (121 C) | 55 sec | 80 | Microwave oven[c] | Aerobic bacteria | 4,900 |
| 4. Bunch et al. (*6*) | Beef-soy loaf 5.2 kg | 43 | 60 | Convection oven (121 C) | 55 sec | 80 | Microwave oven[c] | *Staphylococcus aureus* inoculum ($5.0 \times 10^3$ cells/g) | 3 |
| 5. Zallen et al. (*24*) | Beef loaf 10.8 kg | 60 | 74-77 | Electric deck oven (163 C) | 42 min | 74 | Electric deck oven (163 C) | Standard plate count | 0-100 |
| 6. Bobeng (*2*) | Beef loaf 7.2 kg | 35 | 60-63 | Convection Oven (158 C) | 90 sec | 74-77 | Microwave oven[c] | Aerobic plate count | 230 |
| *Actual operations* | | | | | | | | | |
| 7. Cremer and Chipley (*9*) | Spaghetti | 42 (27-54)[b] | 88 (85-93)[b] | Steam jacketed kettle-302 or 379 liters | 23 min (20-25)[b] | 68 (34-96)[b] | Convection oven[d] | Total plate count | 1,480 |
| | | | | | | | | Coliform | 80 |
| | | | | | | | | Clostridia | 365 |
| | | | | | | | | Staphylococci | 20 |
| | Chili | 67 (33-115)[b] | 85 (74-98)[b] | Steam jacketed kettle-302 or 379 liters | 28 min | 73 (38-98)[b] | Convection oven[d] | Total plate count | 1,200 |
| | | | | | | | | Coliform | 75 |
| | | | | | | | | Clostridia | 210 |
| | | | | | | | | Staphylococci | 20 |
| 8. Nicholanco and Matthews (*18*) | Beef stew 5.7 liters | — | — | Steam jacketed kettle | — | 60-63 | Microwave oven[c] | Aerobic bacteria | 66,000 |
| | | | | | | | | coliform | < 10 |

[a]EPT = end point temperature of food at end of initial heat processing.
[b]Range.
[c]Individual portions were heated in a microwave oven.
[d]60 individual meals per oven.

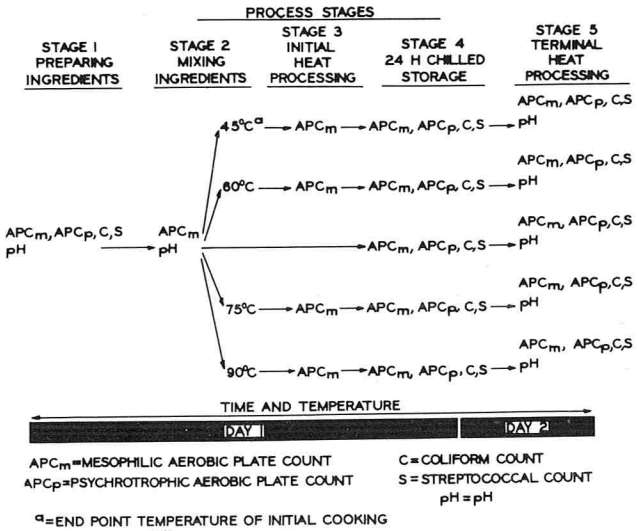


Figure 1. *Tests used to monitor quality of beef loaf during five process stages in a simulated cook/chill foodservice system.*

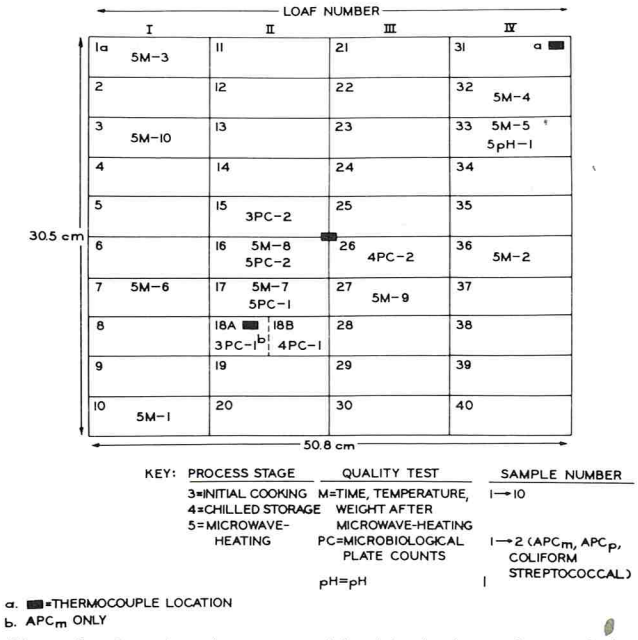


Figure 2. *Locations in a pan of beef loaf of samples used for microbiological tests at process stages 3, 4, and 5 of the cook/chill foodservice system.*

*Preparation of loaves*

Fresh ground beef (29.9 kg) was purchased from a local retail outlet on the first day of each trial. Ground beef was blended mechanically in a mixer (Model S-601, Hobart Corp., Troy, OH) for 15 min in a 30-liter stainless steel bowl at Number 1 (low) speed to form a homogenous mass (Process Stage 1). Three kilograms of raw eggs were added to ground beef; ingredients were mixed for an additional 10 min at Number 1 speed (Process Stage 2). Five thousand grams of the beef mixture were packed into each of five preweighed pans (30.5 × 50.8 × 6.4-cm; Model 7430-2, Vollrath, Sheboygan, WI). Four loaves of approximately equal size were formed from the mixture in each pan.

Each pan of beef loaf was heated initially in a pre-heated oven (Model ROG 1, Vulcan Thermaire, Gas convection oven, Baltimore, MD; single rack in position 6; load control 6) at 121 ± 6 C. When the center, internal temperature of the loaves reached 45, 60, 75 or 90 C, the pan with the loaves was removed from the convection oven. Pans of loaves were covered with aluminum foil and stored in a walk-in refrigerator (6 ± 1 C) for 24 h.

After chilled storage, loaves were sliced into 100-g portions. Portions of beef loaf were heated in a microwave oven (Model 1250L, Hobart Corp., Troy, OH) operating at 2450 MHz on a 3-phase, 208 V circuit. Calculation of wattage output according to the method of Bobeng and David (*3*) showed that the microwave oven operated at 24.3% below the manufacturer's rating for output wattage.

Ten portions of beef loaf (11.5 × 5 × 2.5-cm) were selected randomly from the four loaves in each of five pans of beef loaf (Fig. 2). Temperature measurements of portions were made 30 sec before microwave-heating and 60 sec after heating. Estimated time to heat one portion was based on 10 trials with portions from the same batch of beef loaf. When portions did not reach ⩾ 74 C, they were heated in the microwave oven for five additional seconds. Time was measured with a stopwatch (Galco by Racine, Switzerland).

*Microbiological analyses*

Total mesophilic aerobic plate count ($APC_m$), total psychrotrophic aerobic plate count ($APC_p$), coliform and streptococcal counts were used to determine microbiological quality of beef loaf throughout processing (Fig. 1). Samples were collected aseptically, placed in sterile Whirl Pak bags (Nasco, Fort Atkinson, WI), and refrigerated on ice until tested. $APC_m$ and $APC_p$ were determined on Plate Count agar (Difco) using the pour plate method (*13*). Locations in the pan of beef loaf for test samples are shown in Fig. 2. Coliform counts were made with Violet Red Bile (VRB) agar (Difco); and streptococcal counts with KF Streptococcus agar (Difco) (*13*). Duplicate counts were made at all process stages. Plates having 30-300 colonies were counted using a Quebec Colony Counter.

*Objective measurements*

The pH of beef loaf was determined (pH Meter, Model 10, Corning Scientific Instruments, Corning Glass Work, Corning, NY) according to the recommendations of Bendall (*1*). To each sample (2 g) (Fig. 2) of beef loaf, 40 ml of KCl at 150 mM were added. Samples were mixed 1 min at medium speed in a blender (Model 878, John Oster Mfg., Milwaukee, WI) and allowed to stand at room temperature (26 C) for 15 min.

Temperature measurements in beef loaf during Process Stages 1, 2 and 5 were made using a low-temperature pyrometer and probe-type thermocouple (Type 2300 B, Alnor Instrument Co., Chicago, IL). Temperature measurements during Process Stages 3 and 4 were made using copper-constantan thermocouples plugged into two multi-point recording potentiometers (Model 153 × 64 × P8H-11-111-42, Process Stage 3: Model 153-72P12-26, Process Stage 4; Minneapolis-Honeywell Regulator Co., Minneapolis, MN; Fig. 2).

*Statistical analyses*

Analysis of variance, t-test for significance and trend-t test were calculated on data according to accepted methods (*20*).

## RESULTS AND DISCUSSION

Data in Table 2 are pH and mean percent of initial microbiological counts in processed beef loaf from four simulations of a cook/chill foodservice system. Values for $APC_p$, $APC_m$, coliform and streptococcal counts of raw ground beef were similar to published values (*8,12,19*). Values for pH observed for all treatments of beef loaf at Process Stages 1, 2 and 5 in this study indicated that based on this criterion, portions of beef loaf were near optimal growth conditions for bacteria throughout handling. At Process Stage 3, Treatment N and beef loaf cooked to 45 C appeared to have greater mean percent of initial $APC_m$ than did beef loaf portions initially heat processed to 60, 75 or 90 C. At Process Stage 4, the trend-t test indicated that raising the EPT of initial heat processing had a consistent effect on lowering the $APC_m$ and $APC_p$. At Process Stage 5, no statistically significant

TABLE 2. *Mean percent of initial mesophilic and psychrotrophic aerobic plate counts, coliform and streptococcal counts and pH in beef loaf processed in four simulations of a cook/chill foodservice system.*

| Process stage | pH[a] | Aerobic mesophilic plate count[b] | Aerobic psychrotrophic plate count[c,d] | Coliform count[e,f] | Streptococcal count[g,d] |
|---|---|---|---|---|---|
| | (Mean % of initial counts in heat processed beef loaf)[h] | | | | |
| 3. Initial heat processing (Convection oven at 121 ± 6 C) | | | | | |
| N[i] | —[j] | 100 | — | — | — |
| 45[k] | — | 81 | — | — | — |
| 60[k] | — | 20 | — | — | — |
| 75[k] | — | 13 | — | — | — |
| 90[k] | — | 20 | — | — | — |
| 4. Chilled storage (24 h at 6 ± 1 C) | | | | | |
| N[i] | — | 25 | 21 | ⩽ 1 | 80 |
| 45[k] | — | 31 | 9 | 8 | 37 |
| 60[k] | — | 9 | ⩽ 1 | ⩽ 1 | 46 |
| 75[k] | — | 8 | ⩽ 1 | ⩽ 1 | 24 |
| 90[k] | — | 13 | ⩽ 1 | ⩽ 1 | ⩽ 1 |
| 5. Terminal heat processing (Microwave oven[l]; ⩾ 74 C) | | | | | |
| N[i] | 6.20 | 4 | ⩽ 1 | ⩽ 1 | ⩽ 1 |
| 45[k] | 6.20 | 12 | ⩽ 1 | ⩽ 1 | ⩽ 1 |
| 60[j] | 6.22 | 5 | ⩽ 1 | ⩽ 1 | ⩽ 1 |
| 75[k] | 6.23 | 2 | ⩽ 1 | ⩽ 1 | ⩽ 29 |
| 90[k] | 6.24 | 1 | ⩽ 1 | ⩽ 1 | ⩽ 1 |

[a]Process Stage 1, pH 5.9; Process Stage 2, pH 6.0.
[b]Count of unprocessed beef loaf, Process Stage 2; $2.1 \times 10^5$.
[c]Count of unprocessed beef loaf, Process Stage 1; $2.4 \times 10^4$.
[d]Mean of Simulations 3 and 4.
[e]Count of unprocessed beef loaf, Process Stage 1; $5.1 \times 10^2$.
[f]Mean of Simulation 4.
[g]Count of unprocessed beef loaf, Process Stage 1; $1.9 \times 10^4$.
[h](Count in heat processed beef loaf/count in unprocessed beef loaf) × 100.
[i]N = Treatment of beef loaf which excluded initial heat processing.
[j]No data available.
[k]End point temperature of initial heat processing.
[l]2450 MHz; specified power output by manufacturer, 1250 W.

differences existed among values for $APC_m$, $APC_p$, coliform or streptococcal counts in five treatments of beef loaves after microwave-heating. The accuracy of the four bacterial counts made on portions of beef loaf immediately after heat processing at Process Stages 3 and 5 is questionable because of the possible presence of heat injured cells and/or heat shocked spores.

Viable streptococci (more heat resistant than psychrotrophs or coliforms) remained in five treatments of beef loaf after microwave-heating. Regardless of the EPT of initial heat processing, streptococci present naturally, or because of post-cooking contamination, were not entirely eliminated by microwave-heating to ⩾ 74 C.

Results of this study, and those of Bobeng (*2*), Bunch et al. (*5*) and Zallen et al. (*24*) on microbiological quality of beef loaves cooked to EPTs of 60 or 75 C in simulated cook/chill foodservice systems are given in Table 3. Contrary to the findings of Bunch et al. (*5*) and Bobeng (*2*), populations of all bacteria for which tests were done in this study and which were present at Process Stage 3 decreased in number during 24 h of storage at 6 C (Process Stage 4). Microwave-heating of beef loaves at Process Stage 5 did not change log values for $APC_m$ in results reported in this study or in the study by Bobeng (*2*). However, Bunch et al. (*5*) reported two decimal reductions in $APC_m$ of beef loaves after microwave heating at Process Stage 5. Sampling variation, handling technique, environmental conditions, recipe formulation and type of bacteria present can influence the microbiological quality of beef loaf. Values for $APC_m$ reported by Bobeng (*2*) are probably lower than values reported by this study and by Bunch et al. (*5*) at Process Stages 3, 4 and 5 because Bobeng (*2*) used loaves of smaller dimensions (Table 3). While 5.0 to 5.2 kg of loaf mixture per pan were used in this study and in the study by Bunch et al. (*5*) (Table 3), the $APC_m$ reported by Bunch et al. (*5*) were probably lower than the $APC_m$ of the present study at Stage 5 (Table 3) because 80 C was the EPT of terminal heat processing in their study (Table 1). $APC_m$ reported by Zallen et al. (*24*) (Table 3) decreased after initial heat processing (Process Stage 3) to ⩽ 100/g. The low counts reported by Zallen et al. (*24*) could be related to weight and dimension of the beef loaves (Table 3), EPT of initial heat processing (Table 1), and location for obtaining samples for microbiological analysis (Table 3).

Thirteen hours of cooling time were required for beef loaf initially cooked to 90 C to reach ⩽ 7 C when stored at 6 C. Ten hours of cooling time were required for beef loaf initially cooked to 45 C to reach ⩽ 7 C when stored

TABLE 3. *Mesophilic aerobic plate counts of beef loaves initially heat processed to 60 or 75 C in simulated cook/chill foodservice systems at five processing stages.*

| Process stage | Investigator (Reference) and end point temperature of initial heat processing | | | | |
|---|---|---|---|---|---|
| | 60 C | | | 75 C | |
| | Present study[a] | Bobeng (2)[a] | Bunch et al. (5)[a] | Present study[a] | Zallen et al. (24)[b] |
| (Equivalency) | (cells/g) | | | | |
| 1. Preparation | $2.1 \times 10^5$ | — | $3.8 \times 10^6$ | $2.1 \times 10^5$ | $7.5 \times 10^4$ |
| 2. Mixing | $1.9 \times 10^5$ | $3.1 \times 10^6$ | — | $1.9 \times 10^5$ | $1.3 \times 10^5$ |
| 3. Initial heating | $4.1 \times 10^4$ | $2.7 \times 10^2$ | $6.5 \times 10^4$ | $2.7 \times 10^4$ | $< 100$[g] |
| 4. Chilled storage | $1.9 \times 10^4$[c] | $3.2 \times 10^2$[d] | $1.0 \times 10^5$[e] | $1.7 \times 10^4$[f] | $< 100$[g] |
| 5. Terminal heating | $1.1 \times 10^4$ | $2.3 \times 10^2$ | $4.7 \times 10^3$ | $4.1 \times 10^3$ | $< 100$ |

[a]Sampling location for aerobic plate count, center of loaves.
[b]Sampling location for aerobic plate count, end of loaf and bottom of loaf.
[c]Pan size: 30.5 × 50.8 × 6.4-cm. Weight: 5.0 kg loaf/pan. Temperature of refrigerated storage: 6 ± 1 C. Cooling time from 60 to 7 C; 11 h; from 49 to 16 C: 4-5 h.
[d]Pan size: 20 × 5 × 10-cm. Weight: 0.9 kg loaf/pan. Temperature of refrigerated storage: 3 ± 1.7 C. Cooling time from 60 to 7 C: 5 h; from 49 to 16 C: 2 h.
[e]Pan size: 30.5 × 50.8 × 6.4-cm. Weight: 5.2 kg loaf/pan. Temperature of refrigerated storage: 5 C. Cooling time from 60 to 7 C: 7 h; from 49 to 16 C: 5 h.
[f]Pan size: 30.5 × 50.8 × 6.4-cm. Weight: 5.0 kg loaf/pan. Temperature of refrigerated storage: 6 ± 1 C. Cooling time from 60 to 7 C: 13 h; from 49 to 16 C: 4-5 h.
[g]Pan size: 22 × 11 × 6-cm. Weight: 0.9 kg loaf/pan. Temperature of refrigerated storage: 6 C. Cooling time not reported.

at 6 C. Cooling times for beef loaf heated initially to 60 or 75 C were within these ranges (Table 3). The critical time and temperature range for bacterial growth is from 40 to 16 C for > 2 h and from 60 to 7 C for > 4 h (*17*). The four treatments of beef loaf in this study that were heat processed initially, required > 4 h to reach ≤ 7 C. Regardless of weight of portions of beef loaf or size of cooking container, no researcher of cook/chill foodservice systems met the recommendation of Longree (*17*) to cool food from 60 to 7 C in 4 h.

Results of microbiological investigations in laboratory simulations of foodservice systems may provide data for minimum expected bacterial counts in food. Pans of small size (20 × 5 × 10-cm) and beef loaves of ≤ 1 kg should be considered for use in actual foodservice operations to improve microbiological quality of beef loaf during heat processing and subsequent chilling in cook/chill foodservice systems. Length of heating portioned food in microwave ovens is controlled; this method of heating food results in a wide range of temperature within the food (*11,16*). Microbiological safety of portioned food has been related to EPT (*17*). Findings from this study show that regardless of the extent of initial heat processing (Process Stage 3), terminal heat processing in a microwave oven to ≥ 74 C (Process Stage 5) can be used as a control point to reduce variability in microbiological quality of beef loaf portions.

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## REFERENCES

1. Bendall, J. R. 1971. Postmortem changes in muscle. pp. 244-311. *In* E. H. Bourne (ed.) Structure and function of muscle. Vol. 11., Academic Press, N.Y.
2. Bobeng, B. J. 1976. Hazard analysis critical control point models for quality control of entrees in hospital foodservice systems. Ph. D. Thesis. Dept. of Food Science, Univ. of Wisconsin-Madison. 197 p.
3. Bobeng, B. J., and B. D. David. 1975. Identifying the electric field distributions in a microwave oven: A practical method for food service operators. Microwave Energy Appl. Newsl. 8(6):3-6.
4. Bobeng, B. J., and B. D. David. 1977. HACCP Models for quality control of entree production in foodservice systems. J. Food Prot. 40:632-638.
5. Bunch, W. L., M. E. Matthews, and E. H. Marth. 1976. Hospital chill foodservice systems: Acceptability and microbiological characteristics of beef-soy loaves when processed according to system procedures. J. Food Sci. 41:1273-1276.
6. Bunch, W. L., M. E. Matthews, and E. H. Marth. 1977. Fate of *Staphylococcus aureus* in beef-soy loaves subjected to procedures used in hospital chill foodservice systems. J. Food Sci. 42:565-566.
7. Corlett, D. A. 1973. Freeze processing: prepared foods, seafood, onion, and potato products. Presented at the HACCP Inspector Training Course, FDA, Aug. 9. 19 p.
8. Corlett, D. A. 1974. Setting microbiological limits in the food industry. Food Technol. 28:(10) 34, 36-38, 40.
9. Cremer, M. L., and J. R. Chipley. 1977. Satellite foodservice system assessment in terms of time and temperature conditions and microbiological and sensory quality of spaghetti and chili. J. Food Sci. 42:255-259.
10. Dahl, C. A. 1977. Microbiological and physical qualities of beef loaf after varying end point temperature of initial cooking in a simulated cook/chill foodservice system. M.S. Thesis. Dept. of Food Science, Univ. of Wisconsin-Madison. 300 p.
11. Dahl, C. A., and M. E. Matthews. 1978. Cook/chill foodservice systems: Microwave heating precooked portions of beef loaf. J. Microwave Power. 13(1):87-93.
12. Duitschaever, C. L., D. H. Bullock, and D. R. Arnott. 1977. Bacteriological evaluation of retail ground beef, frozen beef patties, and cooked hamburger. J. Food Prot. 40:378-381.
13. Hausler, W. J., Jr. (ed.) 1972. Standard methods for the examination of dairy products. 13th ed. American Public Health Association, Washington, D.C. 345 p.
14. Hill, M. A., and G. Glew. 1974. Recipe development. Hospitals, J.A.H.A. 48(12):124-126.
15. Leon Crespo, F., and H. W. Ockerman. 1977. Thermal destruction of microorganisms in meat by microwave and conventional cooking. J. Food Prot. 40:422-444.
16. Leon Crespo, F., H. W. Ockerman, and K. M. Irvin. 1977. Effect of conventional and microwave heating on *Pseudomonas putrefaciens, Streptococcus faecalis* and *Lactobacillus plantarum* in meat tissue. J. Food Prot. 40:588-591.
17. Longree, K. 1972. Quantity food sanitation. 2nd ed. Wiley Interscience, John Wiley and Sons, Inc., New York. 422 p.

18. Nicholanco, S., and M. E. Matthews. 1978. Quality of beef stew in a hosptial chill foodservice system. J. Amer. Diet. Assoc. 72: 31-37.
19. Roberts, P., and C. McCleskey. 1957. Bacteriological quality of ground beef in retail markets. Food Technol. 11:318-329.
20. Snedecor, G., and W. Cochran. 1974. Statistical methods. 7th ed. The Iowa State University Press, Ames, Iowa. 593 p.
21. Tuomi, S., M. E. Matthews, and E. H. Marth. 1974. Temperature and microbial flora of refrigerated ground beef gravy subjected to holding and heating as might occur in a school foodservice operation. J. Milk Food Technol. 37:457-462.
22. Tuomi, S., M. E. Matthews, and E. H. Marth. 1974. Behavior of *Clostridium perfringens* in precooked chilled ground beef gravy during cooling, holding and reheating. J. Milk Food Technol. 37:494-498.
23. Unklesbay, N. F., R. B. Maxcy, M. E. Knickrehm, K. E. Stevenson, M. L. Cremer, and M. E. Matthews. 1977. Foodservice systems: product flow and microbial quality and safety of foods. North Central Reg. Res. Bull. 1018. MO Agric. Exper. Sta., Columbia, MO. 36 p.
24. Zallen, E. M., M. J. Hitchcock, and G. E. Goertz. 1975. Chilled food systems. J. Amer. Dietet. Assoc. 67:552-557.

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# Growth and Production of Enterotoxin by *Staphylococcus aureus* S-6 in Soy Proteins and Soy-Supplemented Beef and Pork Sausage

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**ABSTRACT**

The effect of three commercial soy proteins on growth and production of enterotoxin by *Staphylococcus aureus* S-6 was determined. Sterile isolated soy protein (ISP), soy protein concentrate (SPC), and textured soy protein (TSP) were adjusted to 20% protein by diluting with sterile nutrient medium, inoculated with *S. aureus* S-6 and incubated at 37 C. Generation times of *S. aureus* S-6 in ISP, SPC, and TSP were 41, 38, and 33 min, respectively. At 48 h, log 8.4 - 8.5 organisms/g were found in the soy products, and staphylococcal enterotoxin A (SEA) and staphylococcal enterotoxin B (SEB) were produced. Each of three hydrated soy protein products was added to ground beef or pork sausage to attain 20% (wt/wt) and cooked to 71 C. Each product was then inoculated and incubated at 37 C. Generation times of *S. aureus* S-6 did not differ significantly for any beef-soy or pork-soy samples compared to beef or pork sausage controls. Production of SEB (12-72 h) was similar in most beef-soy and pork-soy samples compared to controls, but was significantly lower in the beef-SPC product and beef-TSP and significantly higher in pork-ISP. Small quantities of SEA were produced in beef, beef-soy, pork, and pork-soy samples. Possibly reduction of enterotoxin production in some meat-soy samples was due to outgrowth and competition by spore contaminants of the soy proteins that survived cooking. Production of SEB in all beef-soy and beef control samples was not significantly different when raw samples were autoclaved before inoculation.

Proteins derived from soybeans account for a large portion of the vegetable protein supplements used in recent years. The soybean is processed to yield several forms of protein: isolated soy protein (minimum 90% protein), soy protein concentrate (minimum 70% protein), and soy flour and textured soy protein (40-60% protein). These soy proteins are used in ground meats and sausage-type products as meat extenders or for their functional properties.

Research on the effects of soy proteins on microbial growth has been limited. Judge et al. (*5*) reported no increase in total numbers of spoilage bacteria in ground beef supplemented with either soy protein concentrate or soy flour when stored at 4 C for 7 days. Craven and Mercuri (*3*) found that certain soy proteins stimulated growth of spoilage bacteria when added to ground beef and chicken and stored at 4 C. Other reports have shown that soy proteins stimulate growth of *Clostridium perfringens* in turkey loaf but not in beef loaf (*1, 9*). Kokoczka and Stevenson (*6*) showed that soy flour had no effect, and cottonseed protein a negative effect, on growth of *C. perfringens* when added to beef, chicken, or turkey. Fung (*4*) reported that fish protein concentrate supported production of staphylococcal enterotoxin B (SEB) and, when added to some foods, increased yields of SEB. Tatini et al. (*11*) demonstrated the production of staphylococcal enterotoxins A (SEA) and D (SED) in a liquid medium containing 1.8% protein from fish protein concentrate. Growth but no enterotoxin was found in the medium with 1.8% isolated soy protein. To our knowledge, no published reports describe the effect of soy protein supplements on *S. aureus* growth and toxin production in ground meat products. We undertook this study to determine the effect of isolated soy protein, soy protein concentrate, or textured soy protein on the growth of *S. aureus* S-6 and production of SEA and SEB in cooked ground beef and pork sausage.

## MATERIALS AND METHODS

### *Soy proteins*

Commercial preparations of isolated soy protein (ISP), 90% protein dry weight; soy protein concentrate (SPC), 70% protein dry weight; and textured soy protein (TSP), 56% protein dry weight were used for this study. The TSP contained as additives ferrous sulfate, niacinamide, calcium pantothenate, vitamin $B_6$, riboflavin, vitamin $B_1$ and vitamin $B_{12}$. In the first series of experiments, we determined growth of and enterotoxin production by *S. aureus* S-6 in a medium with soy as the only protein source. The soy proteins were spread as thin layers on aluminum foil and autoclaved at 121 C for 10 min on 2 successive days. A sufficient volume of filter-sterilized solution containing 0.25% dipotassium phosphate, 0.5% sodium chloride, and 0.25% dextrose was used to adjust soy proteins to a protein level of 20% (wt/wt). The medium containing SPC was adjusted from pH 5.0 to 6.8. Portions (25 g) were placed into 40 × 15 mm sterile petri dishes and inoculated.

### *Soy-supplemented ground meat*

In the second series of experiments we determined growth and enterotoxin production of *S. aureus* S-6 in ground beef or in pork sausage supplemented with soy proteins hydrated with distilled water. Beef was ground from boneless round obtained at a local supermarket. Pork sausage was obtained from a local processor and prepared by the addition of 1.75% (wt/wt) NaCl to breakfast sausage mixture containing no NaCl or spices. The dry soy proteins (not sterile) were hydrated 2.5 to 1 with distilled water and without pH adjustment they

were added to ground meats to give a 20% (wt/wt) soy product level. For pork sausage combinations the final mixture was adjusted to 1.75% NaCl. The soy-meat combinations and their corresponding meat controls were mixed in a meat grinder (Sears 400.8260), and 28- to 29-g portions of each placed in 40 × 15 mm petri dishes to form patties, which were removed from the dishes, placed on foil in trays, and cooked to an internal temperature of 71 C in a Despatch rotary oven set at 191 C. The cooked patties were placed in 40 × 15 mm sterile dishes and stored frozen until inoculation. For inoculation the patties were thawed to room temperature, removed from plates, and steamed for 3 min to an internal temperature of 71 C and placed in 40 × 15 mm sterile dishes.

*Inoculum*

*Staphylococcus aureus* S-6, a strain that produces large quantities of SEB and small quantities of SEA, was obtained from J. N. Baldwin, University of Georgia. A loopful was transferred from stock culture on nutrient agar slants to 10 ml of nutrient broth and incubated aerobically at 37 C for 24 h. Samples of 0.1 ml each were transferred to 10-ml tubes of nutrient broth. The cultures were incubated at 37 C for 16 h and centrifuged at 12,100 × *g* for 20 min. The pellets were washed and suspended in sufficient 0.85% saline to yield an absorbance reading of 0.33 at 540 nm on a spectrophotometer (Bausch and Lomb Spectronic 20). For enterotoxin production studies, a surface inoculum of 0.25 ml of this suspension per 25 g of sample gave an initial $5.6 \times 10^5$ colony-forming units (CFU)/g. To determine growth rates of *S. aureus* S-6, 25-g samples were inoculated with 0.25 ml of a 1:10 dilution of the above suspension. Inoculated samples were incubated at 37 C for up to 8 h for the growth studies and up to 72 h for the enterotoxin production studies.

*Growth of S. aureus in soy proteins and soy-supplemented meats*

We measured the colony-forming units of bacteria by preparing a 1:10 slurry of the soy, meat, or meat-soy samples with 0.1% peptone-water, mixing with a Colworth stomacher 400 for 1 min, and spread-plating 0.1-ml amounts of serial dilutions on Vogel-Johnson (V-J) agar and brain heart infusion (BHI) agar plates. The BHI agar plates were incubated at 37 C for 24 h and V-J agar plates for 48 h. Generation times were determined by use of the formula $g=(0.3 \times t)/(\log a - \log b)$, where g = generation time in minutes, t = elapsed time in minutes, a = CFU of *S. aureus* after 8 h of incubation and b = CFU after 4 h of incubation.

*Enterotoxim analysis*

After each of 12, 24, 48, and 72 h of incubation, four patties of each inoculated product were pooled and extracted by the procedure of Reiser et al. (*8*) with some modifications. Each composite sample was combined with 120 ml of distilled water in a pint blender jar and mixed for 3 min on an Oster blender 497 set at stir-mix high. The resulting slurry plus 20 ml of wash from the blender jar was adjusted to pH 4.5 with 5 N HCl and stirred for 15 min. The sample was centrifuged at 16,300 × *g* for 20 min at 4 C. After the supernatant fluid was decanted. the sediment was suspended in 80 ml of distilled water and centrifuged again. The supernatant fluids were combined, adjusted to pH 7.4, extracted with 1:4 volume chloroform, and centrifuged at 4080 × *g* in polypropylene centrifuge bottles for 5 min at 4 C. The aqueous layer was removed and again extracted with chloroform. A 20-ml portion of packed ion-exchange resin (Biorex 70, 200-400 mesh, Bio-Rad Labs) was mixed per 100 ml of extract, adjusted to pH 5.7, and mixed for 45 min with a magnetic stirrer. The resin was filtered with a Buchner funnel and washed with 250 ml of 0.015 M sodium phosphate buffer (pH 5.9) containing 0.09% NaCl. Enterotoxin was eluted from the resin in 40 ml of 0.15 M dibasic sodium phosphate buffer (pH 6.8) containing 0.9% NaCl and stirred for 45 min. The resin was then removed by filtration through a Buchner funnel and washed with 50 ml of the elution buffer (pH 6.8). The filtrate was placed in dialysis tubing and concentrated overnight in 30% Carbowax 20,000 at 4 C. After the tubing was soaked in warm water for 15 min, the contents were washed from the tubing by use of five 2-ml portions of distilled water, for a total of 10 ml of wash, which was lyophilized overnight and rehydrated to 2 ml with distilled water.

To prepare material for the slide test, we incubated a 1:1 mixture of the sample and 1.0% trypsin at room temperature for 30 min. The remaining insoluble material was removed by centrifugation at 27,000 × *g* for 20 min. Subsequent twofold dilutions of the supernatant fluid were made in distilled water for the slide test as described by Casman et al. (*2*). Reference enterotoxins and antisera were obtained from R. W. Bennett, FDA, Washington, D. C. The quantity of enterotoxin produced was determined by multiplication of the reciprocal of the titer by the sensitivity of the slide test (0.05 μg/ml, R. W. Bennett, personal communication).

*Statistical analysis*

Data for growth rates were analyzed by linear regression analysis. Data for enterotoxin production were subjected to analysis of variance and Duncan's multiple range test.

## RESULTS AND DISCUSSION

The generation times and production of SEA and SEB for *S. aureus* S-6 at 37 C in media with soy proteins as their sole source of protein show that, in any of the three forms tested, soy protein was capable of supporting growth and enterotoxin production by *S. aureus* S-6 (Table 1). All tested soy proteins supported production of SEB. Only SPC failed to yield detectable quantities of SEA. Production of less SEA than SEB is a trait typical for strain S-6. In contrast to our results, Tatini et al. (*11*) reported no SEA or SED production for *S. aureus* in isolated soy protein (pH 7.0). However, in addition to using a different strain (Z-88), they used 1.8% whereas we used 20% soy protein in the *S. aureus* growth media.

In other experiments, growth rates for *S. aureus* S-6 in ground beef and pork sausage supplemented with 20% (wt/wt) soy protein products hydrated with distilled water were determined (Table 2). The initial pH of cooked beef differed little from the pH values of the beef-soy combinations. Generation times of *S. aureus* S-6 in these products varied from 36 to 41 min. The initial pH of cooked pork sausage differed from the pH values of pork-soy combinations by no more than 0.4 pH unit. The generation times in pork and pork-soy samples varied from 33 to 35 min. Generation times did not differ significantly ($P<0.05$) in beef-soy or pork-soy combinations compared to their respective controls. Total log viable cells recovered per gram at 12 h were 8.9 for beef, 8.5-8.9 for beef-soy products, 8.7 for pork, and 8.7 - 8.9 for pork-soy products and remained at similar levels through 72 h of incubation. Beef and pork sausage apparently provide a rich nutrient source for growth of *S.*

TABLE 1. *Growth rates and enterotoxin production after 48 h at 37 C of* Staphylococcus aureus *S-6 in soy protein media adjusted to 20% protein.*

| Protein source | Initial pH | Generation time[1] (min) | Log CFU/g[1] (48 h) | SEB[1] (μg/100 g) | SEA[1] (μg/100 g) |
|---|---|---|---|---|---|
| Isolated soy protein | 7.0 | 41 | 8.5 | 4.0 | 0.5 |
| Soy protein concentrate | 6.8 | 38 | 8.4 | 2.4 | <0.2 |
| Textured soy protein | 6.7 | 33 | 8.5 | 6.4 | 0.8 |

[1]Results reported as the mean of two replicate trials

TABLE 2. *Growth rates at 37 C of* Staphylococcus aureus *S-6 in cooked ground meats supplemented with 20% hydrated soy proteins.*

| Meat-soy combination | Initial pH | Mean generation time[1] (min) |
|---|---|---|
| Beef | 5.9 | 36 |
| Beef + isolated soy protein | 6.1 | 36 |
| Beef + soy protein concentrate | 5.6 | 41 |
| Beef + textured soy protein | 6.0 | 39 |
| Pork | 6.5 | 33 |
| Pork + isolated soy protein | 6.7 | 33 |
| Pork + soy protein concentrate | 6.1 | 35 |
| Pork + textured soy protein | 6.6 | 33 |

[1]Results are reported as the mean of generation times from two replicate trials for beef and one trial for pork.

*aureus*, and addition of 20% soy products, which changed the pH only slightly, apparently neither stimulated nor inhibited growth.

Although growth of *S. aureus* was similar in all-meat and meat-soy products, this is not necessarily true for enterotoxin production. Troller (*12*) showed that conditions having little or no effect on *S. aureus* growth may affect production of enterotoxins. In beef and in pork sausage, SEB quantities could be detected after 12 h and increased until they leveled off at 48 h (Table 3). McCoy and Faber (*7*) reported that slurries prepared from cooked pork did not support growth of staphylococci and production of SEA as well as did slurries from cooked ham or beef. However, in our experiments, generation times (Table 2) and production of SEB (Table 3) were comparable in pork sausage and beef.

Addition of 20% soy protein to ground beef and pork sausage influenced production of SEB by *S. aureus* S-6 (Table 3). Beef with added TSP or SPC had significantly lower quantities of SEB than did the control. Pork sausage with added ISP had significantly higher quantities of SEB than did the control or sausage with SPC or TSP. Production of SEA in beef and in pork sausage could not be detected after 12-24 h. In 100 g of sample at 48-72 h only small quantities were produced: beef (1.8 μg), beef-soy products (<0.2-1.1 μg), pork (4.0 μg), and pork-soy products (1.4-2.0 μg). Because SEA quantities were low and data were limited, a statistical comparison was not included.

Even though the meat products were cooked and reheated to 71 C, the possible survival of bacterial spores in these products could not be excluded. The bacterial counts (CFU) on BHI agar for non-inoculated meat and meat-soy patties incubated at 37 C are shown in Table 4. After 6 h of incubation, < log 2.0 CFU/g were detected for any samples. After 48 h of incubation, counts of bacteria ranged from log 8.5 CFU/g to log 9.1 CFU/g for beef-soy products, pork, and pork-soy products. Less than log 3.0 CFU/g were detected for beef controls. Microscopic examination of bacterial colonies revealed

TABLE 4. *Bacterial counts (24 h at 37 C) on brain-heart infusion agar from non-inoculated cooked meat supplemented with 20% hydrated soy protein and incubated at 37 C.*

| Meat-soy combination | Bacteria counts[1] (log CFU/g) | | |
|---|---|---|---|
| | 6 h | 12 h | 48 h |
| Beef | <2.0 | <3.0 | <3.0 |
| Beef + isolated soy protein | <2.0 | 3.6 | 8.5 |
| Beef + soy protein concentrate | <2.0 | 4.3 | 9.0 |
| Beef + textured soy protein | <2.0 | <3.0 | 9.1 |
| Pork | <2.0 | 4.1 | 9.0 |
| Pork + isolated soy protein | <2.0 | 4.8 | 8.5 |
| Pork + soy protein concentrate | <2.0 | 5.9 | 8.8 |
| Pork + textured soy protein | <2.0 | 4.6 | 9.0 |

[1]Results are the mean of two trials; <2.0 indicates no colonies detected on plates of $10^{-2}$ dilution; <3.0 indicates no colonies detected on plates of $10^{-3}$ dilution.

*Bacillus*-like organisms, most of which formed spores. Evidently, the soy protein and pork contained some indigenous spore-forming bacteria that survived cooking.

To determine if these indigenous bacteria might affect the SEB production by *S. aureus* when soy proteins were added to beef, we prepared patties as before and, instead of cooking them to 71 C, autoclaved them at 121 C for 15 min before inoculation with *S. aureus* S-6 (Table 5). Production of SEB after 12 and 48 h of incubation at 37 C was not significantly different between the beef control and beef-soy samples. Thus, when all spore-forming bacteria were eliminated from the beef-soy mixtures, SEB production was similar (Table 5) in samples containing SPC and TSP and not lower (Table 3) compared to the control.

TABLE 3. *Production of enterotoxin B (SEB) at 37 C by* S. aureus *S-6 in cooked ground beef and pork sausage supplemented with 20% hydrated soy proteins.*

| Meat-soy combination | SEB production[1] (μg/100 g) | | | | Overall mean[2] (μg/100 g) |
|---|---|---|---|---|---|
| | 12 h | 24 h | 48 h | 72 h | |
| Beef control | 4.8 | 12.8 | 102.4 | 76.8 | 49.2[a,3] |
| Beef + isolated soy protein | 9.6 | 28.8 | 64.0 | 64.0 | 41.6[a] |
| Beef + soy protein concentrate | <0.8[4] | 4.8 | 16.0 | 13.6 | 8.8[b] |
| Beef + textured soy protein | 6.4 | 8.0 | 8.0 | 16.0 | 9.6[b] |
| Pork sausage | 0.5 | 4.8 | 76.8 | 76.8 | 39.7[a] |
| Pork + isolated soy protein | 0.9 | 12.8 | 307.2 | 307.2 | 157.0[b] |
| Pork + soy protein concentrate | <0.2[5] | 3.2 | 76.8 | 102.4 | 45.7[a] |
| Pork + textured soy protein | 0.6 | 1.2 | 51.2 | 51.2 | 26.1[a] |

[1]Results are reported as the mean of two replicate trials.
[2]The mean of all 12, 24, 48, and 72 h samples for a particular meat-soy combination.
[3]For each meat type, values within a column with the same letter are not significantly different ($p<0.05$).
[4]For the mean of <0.8 and 0.8, 0.8 was used in statistical analysis.
[5]For the mean of <0.2 and 0.2, 0.2 was used in statistical analysis.

TABLE 5. *Production of enterotoxin B (SEB) at 37 C by* S. aureus *S-6 in autoclaved (121 C-15 min) ground beef supplemented with 20% hydrated soy proteins.*

| Beef-soy combination | SEB[1] (μg/100 g) | |
|---|---|---|
| | 12 h | 48 h[2] |
| Beef | 16.0[a] | 38.4[a] |
| Beef + isolated soy protein | ND[3] | 64.0[a] |
| Beef + soy protein concentrate | 4.8[a] | 38.4[a] |
| Beef + textured soy protein | ND | 25.6[a] |

[1]Results are reported as the mean of two replicate trials. Values within a column with the same letter are not significantly different ($p<0.05$).
[2]Mean SEA quantities/100 g after 48 h: beef, 2.4 μg; beef-soy, 1.6-4.0 μg.
[3]Not determined.

Growth of other food bacteria affects staphylococcal growth and enterotoxin production (*7*). Tatini et al (*10*) found that, in raw milk with a very low bacteria count (40 CFU/ml), growth of *S. aureus* can be inhibited.

Conditions of our experiments (cooking and reheating of samples to 71 C, inoculation of large numbers of *S. aureus*, and an incubation temperature optimum for *S. aureus*) probably favored growth of *S. aureus* over spore-forming bacteria. Under less stringent conditions, production of enterotoxin by *S. aureus* might have been affected even more by other bacteria. McCoy and Faber (*7*) showed that inhibition of staphylococcal growth and enterotoxin formation by other food bacteria they tested was more pronounced at 25 C than at 35 C.

The alternative possibility-that substances inhibitory to SEB production may occur in SPC or TSP-cannot be disregarded. Autoclaving could have altered the nutritional properties of the beef-soy mixtures or destroyed any inhibitory substances.

In conclusion, the soy proteins tested (ISP, TSP, SPC) did not alter the growth rates of *S. aureus* S-6 in beef or pork sausage. In the six combinations of beef or pork sausage with the soy proteins, only in the pork sausage-ISP mixture was enterotoxin production increased.


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## REFERENCES


1. Busta, F. F., and D. J. Schroder. 1971. Effect of soy proteins on the growth of *Clostridium perfringens*. Appl. Microbiol. 22: 177-183.
2. 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 enterotoxins. Health Lab. Sci. 6: 185-198.
3. 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.
4. Fung, D. Y. C. 1972. Experimental production of enterotoxin B in fish protein concentrate. J. Milk Food Technol. 35: 577-581.
5. Judge, M. D., C. G. Hough, G. L. Zachariah, C. E. Parmelee, and R. L. Pyle. 1974. Soya additives in beef patties. J. Food Sci. 39: 137-139.
6. Kokoczka, P. J., and K. E. Stevenson. 1976. Effect of cottonseed and soy products on the growth of *Clostridium perfringens*. J. Food Sci. 41: 1360-1362.
7. McCoy, D. W., and J. E. Faber. 1966. Influence of food microorganisms on staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 14: 372-377.
8. Reiser, R., D. Conaway, and M. S. Bergdoll. 1974. Detection of staphylococcal enterotoxin in foods. Appl. Microbiol. 27: 83-85.
9. Schroder, D. J., and F. F. Busta. 1971. Growth of *Clostridium perfringens* in meat loaf with and without added soybean protein. J. Milk Food Technol. 34: 215-217.
10. Tatini, S. R., J. J. Jezeski, J. C. Olson, Jr., and E. P. Casman. 1971. Factors influencing the production of staphylococcal enterotoxin A in milk. J. Dairy Sci. 54:312-320.
11. Tatini, S. R., S. A. Stein, and H. M. Soo. 1976. Influence of protein supplements on growth of *Staphylococcus aureus* and production of enterotoxins. J. Food Sci. 41: 133-135.
12. Troller, J. A. 1976. Staphylococcal growth and enterotoxin production-factors for control. J. Milk Food Technol. 39: 499-503.

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*A Research Note*

# Formation of Hydrogen Peroxide by Meat Starter Cultures

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**ABSTRACT**

The ability of the meat starter cultures, *Pediococcus cerevisiae* ("Accel") and *Lactobacillus plantarum* ("Lactacel DS"), to produce hydrogen peroxide was examined over a temperature range of 10 to 35 C. The meat starter cultures were unable to produce and accumulate hydrogen peroxide under the conditions of the test. The amounts of hydrogen peroxide required to exert a bacteriostatic or a bacteriocidic effect upon both spoilage and pathogenic microorganisms are discussed.

The meat starter cultures *Pediococcus cerevisiae* ("Accel") and *Lactobacillus plantarum* ("Lactacel DS") inhibited growth of *Pseudomonas, Salmonella,* and *Staphylococcus* organisms (*5, 6*). Hydrogen peroxide is a by-product of the metabolism of some lactic acid bacteria (*2*). Its formation varies according to environmental conditions (*1*) and species or strains of the lactic acid bacteria. Some pediococci (*7*) and some lactobacilli (*1*) were found to form hydrogen peroxide.

The advantage of hydrogen peroxide formation is the possible bacteriostatic or bacteriocidic effect on either spoilage or pathogenic microorganisms in association with the lactic acid bacterium. The disadvantage of hydrogen peroxide formation in meat products such as fermented sausage is the discoloration of meat due to the green oxidation products of nitrosylhemochrome (oxidized porphyrines).

The objective of this work was to test the ability of the meat starter cultures to produce hydrogen peroxide.

## EXPERIMENTAL

*P. cerevisiae* was obtained in a lyophilized form, "Accel" (NRRL B5624), while *L. plantarum* was purchased as a frozen concentrate, "Lactacel DS" (NRRL B5632). Both cultures were obtained from Merck & Co., Inc., Rahway, NJ.

The lactobacilli and pediococci were grown in APT broth (18 h at 30 and 35 C, respectively). Each organism was prepared and suspended ($10^9$ cells/ml) according to the method of Dahiya and Speck (*1*) using potassium phosphate buffer (pH 7.0, 0.2 M $PO_4^{\equiv}$) containing 0.25% (w/v) Bacto Dextrose (Difco) and stored at 10, 15, 20, 25, 30 and 35 C.

The enzymatic method (peroxidase - O-dianisidine) of Gilliland (*3*) was used to determine hydrogen peroxide formation. The reaction end point was measured spectrophotometrically (400 nm) using a 1-cm glass cell in a Beckman ACTA III spectrophotometer (Beckman Instruments, Inc., Palo Alto, CA). A standard curve was constructed by plotting the absorbance values against micrograms of hydrogen peroxide. The response was linear up to 12.5 μg of hydrogen peroxide.

## RESULTS AND DISCUSSION

Hydrogen peroxide formation by *L. plantarum* and *P. cerevisiae*, as measured by absorbance values, was in the range of 0.001 to 0.058 for the storage temperatures used. The maximal absorbance value of 0.058 (*L. plantarum* on the third day of storage at 20 and 35 C) is equivalent to about 0.85 μg of hydrogen peroxide per ml of cell suspension. These results show that essentially the meat starter cultures used did not accumulate sufficient hydrogen peroxide for antimicrobial activity under the experimental conditions used.

Much more hydrogen peroxide is needed to repress growth of spoilage and pathogenic microorganisms. Dahiya and Speck (*1*) found that 6 μg of hydrogen peroxide were bacteriostatic to *Staphylococcus aureus* and that a range of 25-35 μg hydrogen peroxide/ml was bacteriocidic to the same organism. Levels lower than 12 μg hydrogen peroxide/ml were bacteriocidic to *Pseudomonas* organisms (*4*). The lack of excessive hydrogen peroxide formation is an advantage (prevention of discoloration) especially when the meat starter cultures are used for fermentation of meat products. Raccach (*5*) did not observe any discoloration of fresh mechanically deboned poultry meat inoculated with a mixture of *P. cerevisiae* and *L. plantarum* ($2 \times 10^9$ cells/g) and stored under refrigeration for 7 days.

This work showed that hydrogen peroxide was not the cause of the inhibition of *Salmonella, Pseudomonas,* and *Staphylococcus* organisms in fresh and cooked mechanically deboned poultry meat or in a culture medium, and of *Salmonella* and *Pseudomonas* organisms in pasteurized liquid whole egg (*5, 6*).

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**REFERENCES**

1. Dahiya, R. S., and M. L. Speck. 1968. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. 51:1568-1572.
2. Doelle, H. W. 1969. Bacterial metabolism. Academic Press, N.Y.
3. Gilliland, S. E. 1969. Enzymatic determination of residual hydrogen peroxide in milk. J. Food Sci. 52:321-324.
4. Price, R. J. and J. S. Lee. 1970. Inhibition of *Pseudomonas* species by hydrogen peroxide producing lactobacilli. J. Milk Food Technol. 33:13-18.
5. Raccach, M. 1977. The control of spoilage and pathogenic microorganisms in poultry meat by lactic acid bacteria. Ph. D. Thesis, Cornell University, Ithaca, New York.
6. Raccach, M., and R. C. Baker. 1978. The role of lactic acid bacteria as an antispoilage and safety factor in cooked mechanically deboned poultry meat. J. Food Prot. 41:703-705.
7. Whittenbury, J. R. 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J. Gen. Microbiol. 35:13-26.

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# Microbial Harboring Characteristics of Dishmachine-Filmed Glassware

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**ABSTRACT**

Alkaline-earth type films, produced by detergent-water interactions, are frequently deposited on eating utensils during mechanical dishwashing. These films are aesthetically unacceptable but their public health significance and food spoilage potential have not been established. Sterile glass petri dishes were washed in an institutional-type dishmachine containing *Bacillus subtilis* spores in the washwater. A film-producing model system was developed to examine film formation in conjunction with spore deposition. The influence of three distinct detergent formulations on this association was also determined. Detergent formulations contained 6.75% phosphorus (P), as sodium tripolyphosphate (STP), 3.0% P as STP, a proprietary phosphate substitute, and the film-producing model formulation devoid of both STP and substitute water conditioning agents. Film deposition was quantified as $\mu$g $Ca^{++}$ per $cm^2$ by an acid rinse-atomic absorption spectrophotometric method. The quantity of *B. subtilis* spores recovered from washed petri dishes in the model system was related to film deposition. The relationship was dependent on the number of consecutive dishmachine cycles and the hardness of the water supply. Above a threshold value of 3 $\mu$g $Ca^{++}$ per $cm^2$, $Ca^{++}$ deposition and *B. subtilis* spore harborage were directly correlated. Both of these conditions were inhibited to varying degrees by the three detergent formulations. These data suggest that dishware filming may be cause for public health and food spoilage concerns.

Contaminated eating utensils have long been recognized as potential vectors in transmittance of disease-causing microorganisms (*4, 10, 11, 14, 16, 20*). This fact demonstrates the need for proper cleaning and sanitizing of foodservice utensils and indicates that dishwashing, either by machine or hand, is definitely a public health concern (*16*). During the past 30 years, automatic dishwashing machines, detergents, and cleaning procedures have been developed to produce maximum cleaning efficiency and consequently to provide sanitary eating utensils. However, the vast differences in water conditions, soil types, detergent formulations, etc. have contributed to conditions where cleaning efficiency and sanitization are reduced. The most apparent manifestation of these conditions is dishware filming.

Although several types of mechanical dishmachine filming have been defined, the most common are alkaline-earth type films produced by detergent-water interactions (*12, 15, 21, 22*). Previous authors have referred to microbial harboring and protection characteristics of these films in relation to food contact surfaces (*4, 6, 17, 18, 19*), but few quantitative data are available which clearly demonstrate this hygienic concern. There is adequate documentation, however, that the use of phosphates, in particular sodium tripolyphosphate (STP) and trisodium phosphate (TSP), in the dishwashing formulation, will minimize this filming tendency (*5, 7, 12, 13, 15, 21*).

A model system was used in this study to investigate this potential public health concern by examining film formation in the presence of bacterial spores. The objectives were two-fold: (*a*) to evaluate the microbial harboring characteristics of glassware filming, and (*b*) to determine the influence of film-inhibiting detergent formulations on this condition.

## MATERIALS AND METHODS

*Detergent formulations*

Three dishmachine detergent formulations and a film-producing formulation were evaluated. Formula A was an in-line product, SCORE ®, Economics Laboratory, Inc., containing 27% STP (yielding ca. 6.75% total P). Formula B was a reduced phosphate modification of formula A. It contained 12% STP (ca. 3.0% total P). Formula C was a proprietary phosphate-substitute product containing 25% water conditioning agents and no phosphates. Formula D was a non-phosphate, non-substituted modification of formula A. It was used as a film producing model and containing no water conditioning agents. The use concentration of formulas A, B, and C was 0.20% (w/v). The use of concentration of formula D was 0.146% (w/v).

Use concentrations of all four formulations were tested for inherent inhibitive effects on *Bacillus subtilis* spore germination and outgrowth. No inhibition was demonstrated by any of the formulas when respective detergent-spore suspensions were heated at 70 C for 1 h.

*Dishwashing machine specifications*

Tests were conducted in a Hobart Model UM-4D automatic dishmachine with manual override controls, a special Luer-Lok syringe port for spore injection, and a mercury-actuated, dial-type thermometer. This type of dishmachine is classified as a single tank,

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stationary rack, single temperature, door-type machine. The automatic cycle consisted of a 10-liter fill, 120-sec. wash, complete drain, 10-liter fill, 30-sec rinse, and complete drain. This sequence is defined as one automatic cycle for future reference.

*Water conditions*

The wash and rinse waters were either St. Paul, MN., city water (86 ppm hardness as $CaCO_3$) or well water (190-230 ppm natural hardness as $CaCO_3$). The temperature range of both water supplies during use was 77-80 C.

*Test organism and inoculum*

The test organism was *B. subtilis* ATCC #6633. Cultures were sporulated on A. K. Agar #2 (BBL) and harvested in 0.85% NaCl according to the method of Arret and Kirshbaum (*1*). Colony forming units (CFU) per ml of inoculum were determined before each usage by heat shocking a 10-ml portion of the inoculum at 70 C for 30 min and plating with Antibiotic Medium #1 (Difco).

*Petri dish washing and inoculation*

Acid-cleaned and steam-sterilized, 90 × 150-mm glass petri dish bottoms (Pyrex) were evenly distributed at predetermined locations in a stainless steel wash rack. The rack was placed in the dishmachine and a pre-measured quantity of the respective test detergent was added to the machine basin. Ten petri dish bottoms were used for each trial in Procedure 1 while 15 dish bottoms were used for each trial in Procedure 2.

*Procedure 1.* Ten ml of a spore suspension containing $7.6 \times 10^8$ to $1.1 \times 10^9$ CFU per ml were injected into the dishmachine with the Luer Lok syringe as the washwater was being added. The resulting spore concentration was $7.6 \times 10^5$ to $1.1 \times 10^6$ CFU per ml of the washwater as determined by samplings at various times during the wash cycle. The petri dishes were washed and rinsed for one complete automatic cycle. Another identical detergent charge was added (no re-inoculation of spores) and the dishes were washed for an additional automatic cycle. The rack was removed from the dishmachine and dishes were dried in an inverted position at ambient temperature (22-25 C) for 1 h. This process was repeated three times for each detergent formulation in each water hardness.

*Procedure 2.* Ten ml of a spore suspension containing $6.8 \times 10^5$ to $1.1 \times 10^6$ CFU per ml were injected into the dishmachine as in Procedure 1. The resultant spore concentration was $6.8 \times 10^2$ to $1.1 \times 10^3$ per ml of washwater. The dishes were washed and rinsed for 1 to 15 cumulative automatic cycles as required. When only one cycle per set of dishes was required, the rack was removed from the dishmachine and dishes were dried at ambient temperature for 1 h. When two or more washings were required, an additional detergent charge and spore inoculum were added to the dishmachine at the beginning at each successive cycle. Upon completion of 2, 3, 4, 5, 6, 8, 10, and 15 cumulative cycles respectively, the dishes were dried in the described manner. This process was repeated in duplicate for each detergent formulation. Sets of 10 dishes each were used to determine CFU per petri dish while sets of five dishes each were used to measure $Ca^{++}$ deposition per petri dish.

*Spore recovery*

A 5-ml portion of sterile, 5% sodium hexametaphosphate solution was aseptically added to the interior of each of 10 washed and dried petri dish bottoms per set. The dish bottoms were covered and left undisturbed at ambient temperature for 1 h. Upon completion of this soaking period the dishes were gently agitated and poured with ca. 20 ml of Antibiotic Medium #1. Solidified agar dishes were inverted and incubated at 37 C for 18-24 h. Where initial counts exceeded 500 CFU per petri dish, repeat trials were made and 1-ml portions of the sodium hexametaphosphate solution were removed from each dish and plated separately. Colonies were counted on a New Brunswick Model C-110 Colony Counter and multiplied by the appropriate dilution factor if applicable. Gram stains were made of any colonies not exhibiting normal *B. subtilis* morphology. Any colonies not presumptively identified as *B. subtilis* were excluded from the total count. Counts were recorded as CFU per petri dish (63 $cm^2$).

*Film recovery apparatus*

The aspirator probe consisted of a disposable 10-ml pipet (Falcon 7751) with the cotton plug removed and a 2-cm section of rubber tubing (Fischer 14-175C) fitted over the drawing end of the pipet (Fig. 1). The delivery tip was severed at the joint and attached to the collection flask hose. A new aspirator probe was used for each set of 5 plates. The collection flask and hose assembly were used repeatedly but thoroughly cleaned between individual determinations. Negative control sampling was done throughout the study to detect possible extraction apparatus contamination.

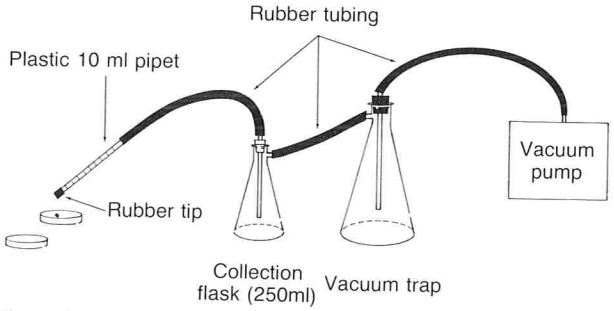


Figure 1. *Film recovery apparatus.*

*Film removal and quantitation*

A 5-ml portion of an aqueous 5% HCl and 1% lanthanum solution (*2, 8*) was carefully added to the interior of each of five washed and dried petri dish bottoms per set in Procedure 2. After a 5-min soak time the entire 5-ml volume was recovered from each plate by aspiration (Fig. 1) and combined in a common reservoir for each set (25-ml total per set). Maximum mineral removal during aspiration was aided by gentle scraping of the test area surfaces with the rubber tip of the aspirator probe.

The 25-ml composite from each separate set was diluted if necessary and then assayed for calcium and magnesium, respectively, using a Perkin Elmer 403, double beam atomic absorption spectrometer. The fuel gas was acetylene and the supporting gas was filtered compressed air. Atomic absorption readouts in ppm (times dilution factor if applicable) were converted to mg of $Ca^{++}$ or $Mg^{++}$ per petri dish unit area by the following calculation:

$$\text{mg } Ca^{++} \text{ (or } Mg^{++}\text{) per 63 cm}^2 = \frac{\text{ppm } Ca^{++} \text{ (or } Mg^{++}\text{)} \times 0.025}{5}$$

Background levels of $Ca^{++}$ and $Mg^{++}$ per unit area of petri dishes before washing were found to be insignificant in comparison to experimental values.

## RESULTS

Use of a detergent formulation containing no water conditioning agents (formula D) provided an ideal model for examination of glassware filming and spore deposition. Initial experimentation (Table 1) using formula D consisted of a single spore inoculum producing an average of $9.3 \times 10^5$ CFU per ml of washwater and two successive wash rinse cycles. Petri dishes washed under these conditions exhibited visibly filmed surfaces from which spores were readily recovered. Both the filming density (visual) and the quantity of spores recovered increased as the hardness of the dishmachine water supply was increased from 85 to 230 ppm. Use of the phosphated and P-substituted detergents (formulas A and C) in this system resulted in petri dish surfaces with no visible filming and significantly reduced spore recovery. Also, in contrast to the model formulation, petri dishes washed in formulas A and C did not demonstrate an increase in either of these conditions as the hardness of the water supply was increased.

TABLE 1. *Colony forming unit (CFU) recovery after one inoculation and two washings with 85 and 230 ppm hard water, respectively.*

| Formula | Water hardness (ppm) | Visible filming | Mean CFU/63 cm$^2$ |
|---|---|---|---|
| A | 85 | — | 16.1 |
| A | 230 | - | 15.9 |
| C | 85 | - | 13.4 |
| C | 230 | - | 10.1 |
| D | 85 | + | 370 |
| D | 230 | + | 1,300 |

The above data using Procedure 1 indicated that spore-harboring films could be developed within the dishmachine, and that these films were accelerated by an increase in water hardness and inhibited by use of well-formulated detergents. Procedure 2 was designed to determine: (*a*) if the filming-spore harboring relationship could be quantified, (*b*) if this relationship could be identified in a cumulative process, and (*c*) if this phenomenon could be prevented by use of a marginally formulated detergent. Subsequently a method was developed to quantitate film density and the spore inoculum was reduced to an average of $8.9 \times 10^2$ CFU per ml of washwater but added to each successive cycle in cumulative series. An additional formulation containing only 3% P was also evaluated. The 230 ppm hard water was used throughout except for the test series in Table 3.

The results using Procedure 2 are listed in Tables 2 and 3. The quantity of CFU recovered per unit petri dish area in the filming model (formula D) increased as the degree of filming (expressed as mg $Ca^{++}$ per unit area) increased upon successive inoculations and washings. This relationship can be expressed by regression analysis as:

$$\log_{10}(\text{CFU}/63\ \text{cm}^2) = 1.49 + 0.7\ (\text{mg Ca}^{++}/63\ \text{cm}^2)$$

This equation was used to predict the level of spore recovery based on $Ca^{++}$ deposition values obtained in an independent experimental series. The hardness of the water supply was decreased to 190 ppm in this experiment but all other variables remained constant. As expected, both filming and spore recovery were reduced as the water hardness was decreased (Table 3). The actual numbers of recovered spores were in most instances greater than the predicted values, but a direct relationship between $Ca^{++}$ deposition and spore harborage was again demonstrated. The differences between the predicted and actual values may reflect the water hardness modification or the inherent variability within the test system. A plot of these values in relation to the regression equation is presented in Fig. 2.

The data using Procedure 2 involving formulas A, B, and C were of relatively low magnitude and consequently did not readily fit the equation (Fig. 2 data below $Ca^{++}$ threshold). These data, however, are not inconsistent with the equation. Despite the fact that filming and spore harboring did not increase significantly upon successive inoculations and washings using these three formulations several performance differences were observed. The mean CFU per petri dish recovered from each cumulative cycle set using the P-substitute formulation (formula C), was greater than the corresponding CFU recovery in either of the phosphated detergent washed sets. This difference was small but consistent. Based on CFU recovery data using Procedure 1, differences in spore recovery with the three detergent formulations would have been greater had the inoculum size been increased.

Variations in the amount of $Ca^{++}$ deposition per cumulative petri dish set were also noted, particularly

TABLE 2. *Amount of calcium [mg $Ca^{++}$) and colony forming units (CFU) recovery after multiple inoculations and washings with 230 ppm hard water.*

| Detergent | Mean CFU and mg $Ca^{++}$/63 cm$^2$ | Cumulative dishmachine cycles 1 | 2 | 3 | 4 | 5 | 6 | 8 | 10 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|
| Formula A | mg $Ca^{++}$ | 0.017 | 0.023 | 0.04 | 0.038 | 0.032 | 0.028 | 0.017 | 0.052 | 0.047 |
| | CFU | 0.9 | 0.5 | 0.7 | 0.9 | 0.2 | 0.1 | 1.3 | 0.3 | 1.0 |
| Formula B | mg $Ca^{++}$ | 0.045 | 0.042 | 0.058 | 0.04 | 0.084 | 0.053 | 0.06 | 0.139 | 0.186 |
| | CFU | 1.0 | 0.9 | 0 | 0.7 | 0.2 | 0.1 | 0.1 | 0.2 | 0.8 |
| Formula C | mg $Ca^{++}$ | 0.012 | 0.015 | 0.024 | 0.032 | 0.044 | 0.044 | 0.044 | 0.096 | 0.120 |
| | CFU | 2.3 | 3.7 | 3.5 | 3.7 | 4.0 | 4.7 | 2.9 | 2.7 | 6.3 |
| Formula D | mg $Ca^{++}$ | 0.04 | 0.295 | 0.635 | 1.05 | 1.225 | 1.63 | 2.1 | 2.27 | 3.05 |
| | CFU | 2.1 | 44 | 96 | 170 | 280 | 500 | 710 | 1,400 | 4,000 |

TABLE 3. *Amount of calcium (mg $Ca^{++}$) predicted and actual colony forming unit (CFU) recovery after multiple inoculations and washings with 190 ppm hard water.*

| Detergent | Mean CFU and mg Ca /63 cm$^2$ | Cumulative dishmachine cycles 1 | 2 | 3 | 4 | 5 | 6[1] | 8 | 10 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|
| Formula D | mg $Ca^{++}$ | 0.045 | 0.38 | 0.535 | 0.69 | 0.975 | 1.42 | 1.56 | 2.155 | 2.66 |
| | Predicted CFU | --- | 57 | 73 | 94 | 150 | 310 | 380 | 1,000 | 2,500 |
| | Actual CFU | 2.5 | 55 | 120 | 180 | 210 | 410 | 560 | 690 | 2,100 |

[1]Petri dishes from this set were used for scanning electron micrographs.

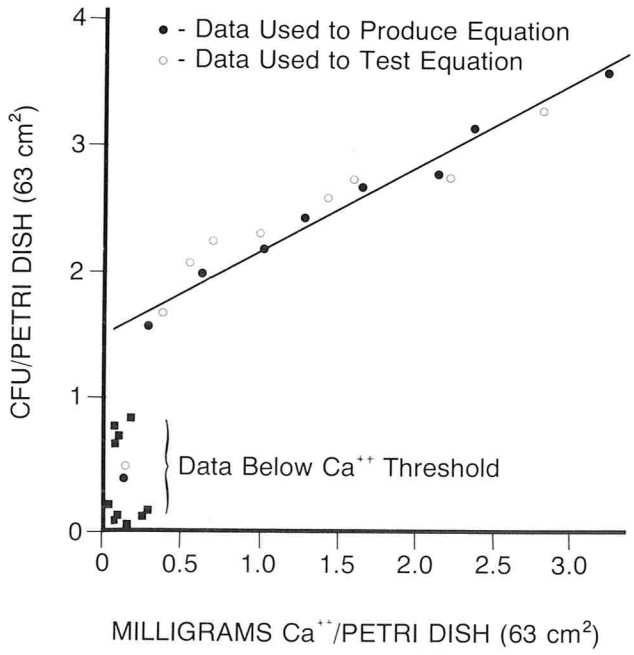


Figure 2. *Relationship of milligrams of* $Ca^{++}$ *and colony forming units [CFU) per petri dish. Line indicates plot of regression analysis equation:* $\log_{10}(CFU/63\ cm^2) = 1.49 + 0.7\ (mg\ Ca^{++}/63\ cm^2)$.

after the fourth successive wash cycle. The least amount of $Ca^{++}$ was recovered from dishes washed with the 6.75% P formulation (formula A) while dishes washed with the 3.0% P formulation (formula B) accumulated the greatest quantities of $Ca^{++}$. Similar analyses made on milk processing equipment surfaces after cleaning suggested that $Ca^{++}$ residuals in excess of 0.08 mg per 100 cm² were indicative of insufficient cleaning (*8*). The data with Procedure 2 correlate well with this referenced value. After 15 consecutive dishmachine cycles, the $Ca^{++}$ deposition per petri dish surface area (calculated to 100 cm²) was 0.075 mg, 0.295 mg, and 0.190 mg for formulas A, B, and C, respectively.

In addition to $Ca^{++}$, $Mg^{++}$ deposition was also measured throughout Procedure 2, though individual values are not listed. Milligrams of $Mg^{++}$ per petri dish were not proportional to the corresponding quantity of recovered spores. In general, $Ca^{++}$ was preferentially deposited in respect to $Mg^{++}$ and subsequently the $Ca^{++}$: $Mg^{++}$ ratio increased as the degree of filming increased. This preferential deposition was most likely due to the greater water solubility of $Mg^{++}$ in relation to $Ca^{++}$ (*22*).

Further analysis of selected film samples (Procedure 2) indicated that the calcium and magnesium components were present as carbonates. These two components accounted for about 85% of the total film composition. Sodium carbonate was present in quantitative amounts while copper, iron, potassium, manganese and zinc were present in trace amounts. Calcium and magnesium phosphates were not detected. The film composition and component ratio remained constant on all plates examined from sets using formulas A, B, and C, but individual quantities varied. Films formed in the model system were consistent with these analyses, but the calcium and magnesium carbonates accounted for about 98% of the total film composition.

## DISCUSSION

The type of filming described in the preceding experiment appears to be of the water mineral type as proposed by Madden (*12*) and others. This type of film consists primarily of calcium carbonate and is prevented by adequate amounts of water conditioning agents, such as STP, in the wash-water. The mechanism of film formation in the model system was probably related to precipitation of calcium and magnesium ions by the sodium carbonate component of formula D, rather than sequestration of these ions as in the other three formulations. It also seems reasonable that the degree of sequestration by the respective water conditioning systems in formulas A, B, and C was related to the observed differences in $Ca^{++}$ deposition.

A second, and more common type of glassware filming was predicted with the use of reduced phosphate formula B, but was not observed. This detergent hard-water precipitate type consists primarily of calcium phosphate and is caused by insufficient use of phosphated detergents necessary to condition hard water (*12, 22*). It was anticipated that the marginal amount of STP in formula B would produce conditions where the degree of filming and spore harboring would be midway between the values obtained with formulas A and D. Apparently the 12% STP in formula B was sufficient to condition the 230 ppm hard washwater without an added challenge such as food soils.

The rate at which the *B. subtilis* spores become integrally associated with the hard water films was directly proportional to $Ca^{++}$ deposition, but only after a threshold level of about 3 μg of $Ca^{++}$ per cm² (0.2 mg per 63 cm²) was achieved. Based on the regression analysis equation described previously, the corresponding CFU threshold would be about 43 CFU per petri dish. The implications of this threshold concept become evident upon examination of the data derived with formulas A, B, and C. The failure to demonstrate a film-spore harboring relationship with these formulations resulted because the $Ca^{++}$ deposition threshold was never reached. However, this threshold value was being approached after 15 consecutive cycles on dishes washed with both the reduced phosphate and P-substitute formulations. In contrast, dishes washed with the 6.75% P formulation did not show a similar increase in $Ca^{++}$ deposition.

The threshold concept also accounts for the accelerated filming-spore harboring observed with an increase in water hardness. The greater availability of calcium and magnesium ions in the harder waters resulted in an increased rate of precipitation and a decrease in the time required to reach the $Ca^{++}$ deposition threshold.

The nature of the film-spore association is only speculative. The actual harboring was probably a physical entrapment on or within film-crystal matrices, since it is unlikely that any charge-associated attractions predominated. Electron micrographs (Fig. 3 and 4) indicate that spore attachment did occur on the crystal surfaces, but as stated by Hess et al. (*9*) in a similar spore-crystal analysis, "It is not unreasonable to assume that spores are also completely occluded within some crystals." Irrespective of the actual mode of harboring, this association confirms the earlier statements of Cox (*4*), Martin et al. (*17*), and Hall and Schwartz (*6*) that glassware films produced during mechanical dishwashing may harbor viable microorganisms. This work, however, does not substantiate their claims that these films also provide an ideal environment for microbial growth.

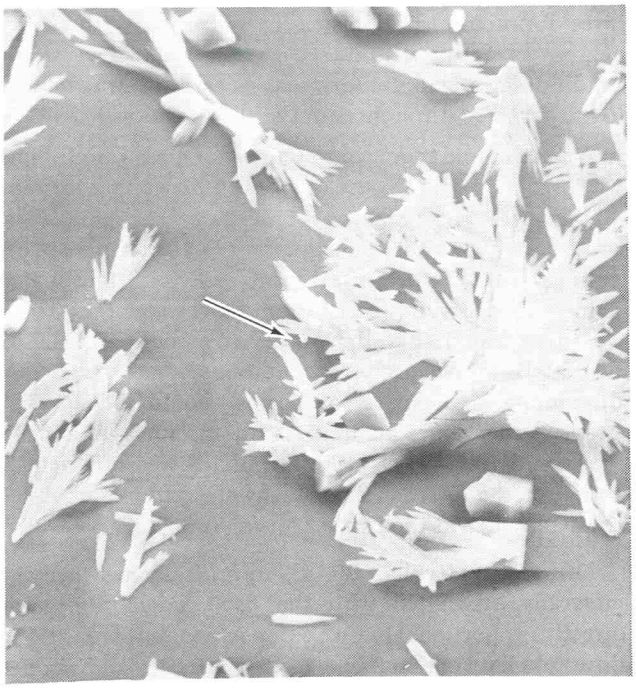

**Figure 3.** ***Scanning electron micrograph of filmed petri dish surface × 2000. Note spore in center of field.***

The significance of viable microorganisms present on washed eating utensils has previously been stated. It is entirely possible that organisms associated with a filmed utensil could contaminate a foodstuff during preparation or consumption. Should food become so inoculated and then mishandled, the potential for microbial growth would be present. Ingestion of the contaminated food would then complete the cycle for a food-associated illness.

The Center for Disease Control listed 438 outbreaks of foodborne disease in 1976 involving 12,463 individual cases (*3*). These figures, however, represent only a small fraction of the actual illnesses related to ingestion of contaminated foods because the public seldom make official complaints or reports. *Clostridium perfringens*, a spore forming microorganism, was implicated in 509 cases of confirmed etiology. *Bacillus cereus*, another spore former, was also involved in reported outbreaks.

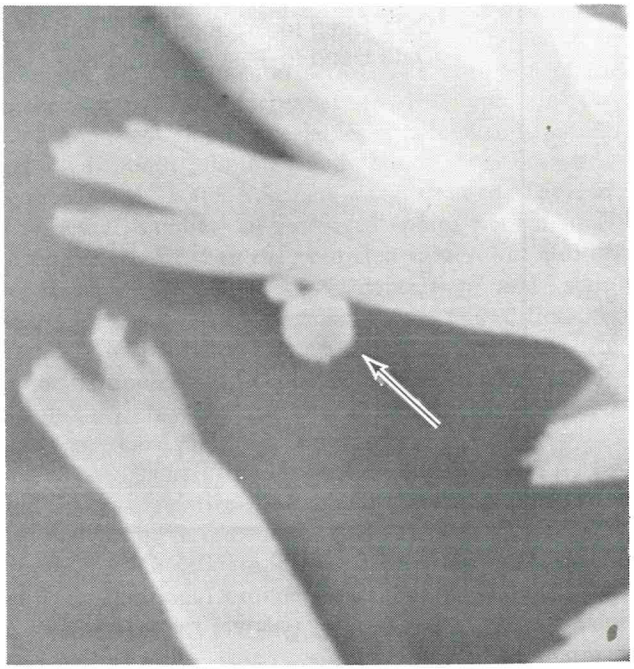

Figure 4. ***Scanning electron micrograph of filmed petri dish surface showing spore - crystal association × 20,000.***

While most other foodborne pathogens and spoilage organisms are vegetative forms that are inactivated at the high temperatures of automatic dishwashing, the potential for spore survival and integral association with utensil films is a realistic concern.

Additional studies must be made to further define the sanitation role of phosphates in cleaning products. The work presented in this report, although brief, constitutes evidence for a recommendation that this public health aspect be thoroughly studied before legislation indiscriminately banning the use of phosphates is considered.

**ACKNOWLEDGMENTS**

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**REFERENCES**

1. Arret, B., and A. Kirshbaum. 1959. A rapid disc assay method for detecting penicillin in milk. J. Milk Food Technol. 22:329-331.
2. American Society for Testing and Materials. 1975. Annual Book of ASTM standards. American Society for Testing and Materials, Philadelphia, PA.
3. Center for Disease Control. 1977. Foodborne and waterborne disease oubreaks. Annual Summary 1976. Center for Disease Control, Atlanta, GA.
4. Cox, W. C. 1937. The use of dishwashing machines: pasteurization of eating utensils. Presented to Lab. Section, American Public Health Assoc., 60th Annual Meeting, A.P.H.A., Washington, D.C.
5. Gray, F. W., V. J. Richter, and R. C. Odioso. 1967. The role of detergent in automatic dishwashing performance. J. Am. Oil Chem. Soc. 44:725-727.
6. Hall, G. O., and C. Schwartz. 1936. Sanitary value of sodium metaphosphate. Ind. Eng. Chem. 29:421-424.
7. Harris, J. C., R. M. Anderson, and J. Satanek. 1961. Removal of fatty soil from glass by surfactants and surfactant-builder compositions. J. Am. Oil Chem. Soc. 38:123-127.
8. Heinz, J. W., R. T. Marshall, and M. E. Anderson. 1967. Determining cleanliness of milk contact surfaces by analyzing for calcium residual: preliminary studies. J. Milk Food Technol. 30:337-339.

9. Hess, H., L. Geller, and X. Bühlman. 1972. Ethylene oxide treatment of naturally contaminated materials. pp. 283-296. In G. B. Phillips and W. S. Miller (eds.) Industrial sterilization. Duke Univ. Press, Durham, NC.
10. Jopke, W. H., S. D. Sorenson, D. R. Hass, and A. C. Donovan. 1972. Microbial contamination on hospital tableware. Hosp. Prog. 53:30-33.
11. Kupchik, G. J., and J. Katz. 1977. Potential health hazards associated with reusable foodservice utensils. J. Food Prot. 40:132-134.
12. Madden, R. E. 1967. Role of dishwasher detergents in the filming of glassware. Soap Chem. Specialties 43:45-49.
13. Madden, R. E., T. G. Edwards, C. B. Kaiser, and R. G. Jaglowski. 1974. Performance of no-phosphate dishwater detergents. Soap Chem. Specialties. 50(9):38-40.
14. Mallmann, W. L. 1955. Hand versus machine dishwashing in the home. J. Home Econ. 47:609-613.
15. Mallmann, W. L. 1950. Sanitation with modern detergents. American Meat Institute, Chicago. Proc. of third conf. on research. pp. 54-58.
16. Mallmann, W. L., L. Zaikowski, D. Kahler, and P. DeKoning. 1947. A study of mechanical dishwashing. National Sanitation Foundation Research Bulletin no. 1. 51 P.
17. Martin, A. T., C. H. Shiflett, and J. L. Wilson. 1952. Use of $P^{32}$, $Sr^{89}$, and $Ca^{45}$ in mechanical dishwashing studies. Minn. Academy of Science, Proc., 20:28-33.
18. Maxcy, R. B. 1975. Fate of bacteria exposed to washing and drying on stainless steel. J. Milk Food Technol. 38:192-194.
19. Maxcy, R. B. 1966. The relationship between residual soil and microbial growth after circulation cleaning. Food Technol. 221:123-126.
20. Ridenour, G. M., and E. H. Armbruster. 1953. Bacterial cleanability of various types of eating surfaces. Am. J. Pub. Health 43:138-149.
21. Schwartz, C., and B. H. Gilmore. 1934. Sodium metaphosphate in mechanical dishwashing. Ind. Eng. Chem. 26:998-1001.
22. Vance, R. F. 1969. Detergent-hard water interactions in machine dishwashing. J. Am. Oil Chem. Soc. 46:639-641.

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# Organoleptic, Chemical and Microbiological Changes in Ultra-High-Temperature Sterilized Milk Stored at Room Temperature[1]

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**ABSTRACT**

The effects of carton materials on flavor of ultra-high-temperature sterilized milks stored 100 days at 22 ± 2 C was investigated. Flavor scores of stored milk decreased concurrent with an increase in stale flavor. At the same time propanal, pentanal, hexanal, and an unidentified compound increased; cooked flavor along with methyl sulfide and another unidentified compound decreased. Differences in browning were observed between ultra-high-temperature sterilized and reference (freshly pasteurized) milks and between 2- and 12-day-old ultra-high-temperature sterilized milks. Thiobarbituric acid values did not increase (indicating no lipid oxidation) until after the milk had been stored 22 days; however, those values were below that which would be detected organoleptically. Abnormally high acetaldehyde concentrations were related to the ethylene oxide sterilizing pretreatment of the carton board. Reference milk was superior in flavor to milk from all other treatments. Aluminum foil-lined cartons were less permeable to gases than were polyethylene-lined cartons. Milk in aluminum foil-lined cartons retained desirable flavor characteristics longer than did that stored in polyethylene-lined cartons. Wrapping cartons with Saran and aluminum foil was detrimental to flavor in all instances. Analysis of variance of microbiological data established that there were no differences in numbers of microorganisms in the different types of cartons during 42 days.

Milk sterilization has been practiced and studied for more than a century. Widespread acceptance of sterile milk in the United States, however, is limited because during storage it develops a stale flavor as cooked flavor disappears. Oxygen (*7, 11*) and light (*7*), thought to contribute to staling, and many other aspects of ultra-high-temperature sterilized (UHT) milk have been reviewed (*5*).

We stored aseptically packaged UHT milks and determined how well different carton materials preserved the milk flavor. Also, by using gas chromatography (GC), we attempted to relate changes in neutral volatile components to off-flavors produced by microbiological and chemical activity.

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## MATERIALS AND METHODS

*Part I. Milk stored 42 days*

*Processing and storage* Between October 1975 and February 1976, nine batches of raw milk were sterilized at a commercial milk plant in Connecticut at 140 C for 3.5 sec by use of a De Laval Auto-Aseptic Processing System that included two-stage homogenization at 52 C. The milk was packaged aseptically with a Pure-Pak NLL machine in either aluminum foil (AL)-lined or plain polyethylene (PE)-lined pint cartons. The AL foil-lined carton board was laminated (from inside out): PE, AL, PE, paperboard, and PE. The PE carton board was laminated PE, paperboard, and PE. Within 2 days the filled cartons were shipped by air to Manhattan, Kansas. On arrival, half of each type (PE and AL foil-lined) cartons was wrapped in Saran[TM], AL and Saran[TM] to provide additional barriers to gases and light; cartons were stored at 22 ± 2 C exposed to 2150 lx (200 ft candles) of cool-white fluorescent light. Each of these three layers overlapped to provide as tight a seal as possible. They were examined at 10-day intervals for 42 days. The code letters used to identify these samples were: WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, NPC = plain polyethylene-lined cartons and R = reference milk.

*Test procedures.* All UHT milks were evaluated by three microbiological tests: (*a*) The Standard Plate Count procedure recommended by the American Public Health Association (*1*) was modified so that 10 ml of milk was distributed among five petri-dishes. After Standard Methods Agar had been poured into the dishes with the milk, the milk-agar mixture was incubated at 32 C for 7 days; colonies then were counted. (*b*) Aerobic and anaerobic tests (*1*) for sterile products were modified in that 10 ml of milk was incubated at 32 C for 7 days; 0.8 ml of this milk, distributed equally among four plates, was mixed with Standard Methods Agar. Two of the plates were incubated aerobically and two anaerobically for 48 h at 32 C; colonies then were counted. (*c*) The litmus test (*6*) was modified in that 10 ml of the milk sample was added to 1 ml of 1% (w/w) sterile aqueous litmus solution. The tubes were incubated at 32 C for 7 days. If the litmus turned pink or white, the test was positive and indicated microbial growth.

Browning was measured by the hydroxymethylfurfural test (*8*) and lipid oxidation by the thiobarbituric acid test (*9*). Neutral volatile compounds were quantified by using the gas chromatographic procedure of Bassette and Ward (*4*).

The milk was judged organoleptically according to the procedure used in the National Collegiate Student Contest. The recommended flavor-score ranges for the presence of slight, definite, and pronounced for the three predominant criticisms were 36, 34, and 31 for oxidized; 37, 34, and 31 for stale; and 39, 37, and 35 for cooked. As a modification of the contest procedures, we asked panelists also to assign an intensity score (on a scale of 0 = none to 9 = very pronounced) to the specific off-flavors observed.

Protein (by Kjeldahl), fat (Babcock procedure), and total solids were determined as described in AOAC (2).

*Statistical analysis.* The experimental design for the analysis of variance (ANOVA) was a randomized complete block design with a 2-factor cross-classified treatment structure; there were five levels for each of the two factors, days, and treatments (types of cartons). This model was used:

$Y_{bdt} = \mu + \beta_b + \gamma_t + (\beta\gamma)_{dt} + E_{bdt}$

$Y_{bdt}$ = response (e.g., stale-flavor intensity, concentration of hexanal).

$\mu$ = mean

$^a\beta_b$ = block effects (blocks 1-9)

$\beta_d$ = day effects (2, 12, 22, 32, and 42 days)

$\gamma_t$ = treatments effects (WAC = Wrapped AL; NAC = non wrapped AL; WPC = wrapped PE; NPC = nonwrapped PE; and R = reference milk, freshly pasteurized at 72 C for 15 sec)

$(\beta\gamma)_{dt}$ = interaction between days and treatments

$E_{bdt}$ = random experimental error, as a deviation from the mean for the $dt^{th}$ treatment combination of the $b^{th}$ block. $E \sim N(0, \sigma^2)$ where $\sigma^2$ is estimated by error mean square.

From the 225 samples analyzed, 15 to 17 observations for most variables were lost because the cartons were not sterile; all results from non-sterile cartons of milk were discarded.

The model was used as presented when we analyzed data for all effects except the day effects ($\beta_d$); to analyze for $\beta_d$ effects, we removed data for the R milks, which were freshly pasteurized and had not been stored 42 days.

After the data had been tested for homogeneity, we obtained regression equation relating the flavor criticisms (scores) to the components observed chromatographically. A program for multiple deletion regression (with a 0.10 alpha level) was used to select the most prominent associated factors.

*Part II. Milks stored 100 days*

Of the nine batches (blocks) used in Part I, the milks of the last six were evaluated again at 100 days. Microbiological, chemical, organoleptic and gas chromatographic analyses were conducted as described previously on milks, except that WPC samples were not included.

The experimental design for the ANOVA of the extended 100-day study was the same as for the 42-day study except for the following changes:

$^a\beta_b$ = block effects (blocks 4-9)

$\beta_d$ = day effects (2, 12, 22, 32, 42, and 100 days)

$\gamma_t$ = treatment effects (when analyzing data at 100 days, we did not include WPC cartons because there were insufficient samples).

## RESULTS

### *Part I. Milk stored 42 days*

ANOVA of the microbiological data (Table 1) established that there were no differences in the number of microorganisms among the various treatments (four types of cartons) and among the days of storage (2-42). Results from litmus test of milk, not presented here, also agreed with results from other microbiological tests. The similarities were expected because obviously spoiled samples had been discarded and were not included in the statistical analysis. Even though statistically there was no difference among the selected cartons, the wrapped cartons (WAC and WPC) had higher average mean bacteria counts than did the nonwrapped.

Flavors (Table 2) deteriorated with time. Reference (R) milk flavor was best, followed by milk in AL and PE cartons. Milk in nonwrapped cartons had higher flavor scores than did that in the wrapped counterpart cartons. Flavor-score trends were similar to those for stale-intensity scores (Table 2), indicating that "stale" was the principal flavor criticism of the panel. Generally, as the stale flavor increased, the cooked flavor decreased (Table 2 and Fig. 3) for the UHT milks. We might have observed, however, a masking effect of the cooked flavor on the stale flavor and vice versa.

TABLE 1. *Probabilities and means for the microbiological analyses of UHT milk stored 42 days at room temperature.*

| Item | Standard plate count | Aerobic count | Anaerobic count |
|---|---|---|---|
| | | *Probabilities* | |
| Days | .2365 | .7964 | .3409 |
| Treatments | .1566 | .2182 | .7319 |
| *Days* | | *Means*[1,2] | |
| 2 | -0.055 | 1.271 | 0.111 |
| 12 | -0.061 | 0.928 | 0.481 |
| 22 | 0.238 | 1.315 | 0.584 |
| 32 | -0.020 | 1.225 | 0.524 |
| 42 | 0.121 | 0.886 | 0.579 |
| *Treatments*[3] | | Means[1,4] | |
| WAC | 0.296 | 1.208 | 0.692 |
| NAC | -0.007 | 0.718 | 0.485 |
| WPC | 0.359 | 1.540 | 0.831 |
| NPC | 0.199 | 1.033 | 0.615 |

[1]Logarithmic values (base 10) of microbiological counts (colonies/ml) are used.

[2]Each mean represents the average of the four UHT milks over 9 replication.

[3]WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, and NPC = plain polyethylene-lined cartons.

[4]Each mean represent the average of the milk over 9 replications during the 42-day trail period.

Statistical differences in the oxidized flavor intensities probably were artifacts because the mean intensities were less than 1 on a 0-to-9 scale. TBA values for UHT milk increased slightly when the milk was stored; however there was no significant difference between TBA values of UHT milks and reference milk.

Browning of milk increased slightly but significantly when stored between 2 and 12 days (Free HMF, Table 2) but none beyond 12 days. That, compared with low HMF levels in the R milk, indicated that some heat damage had occurred in the UHT milks. Total HMF, which measures potential as well as actual browning (*8*), did not change significantly during the 42-day period. Though HMF values of milk stored in AL foil and PE cartons differed, differences were not great enough to be significant. The R milks had significantly lower concentrations of these compounds than did the UHT milks.

Table 3 presents components isolated from steam distillates by use of GC. When concentrations or peak heights were compared with intensity scores of specific criticisms, certain relationships were observed. These are shown in the last column. Peaks designated with "x" were unrelated to either chemical or organoleptic tests studied. Statistical analyses of peaks found to be related to other factors are presented in Table 4.

The abnormally high initial concentrations of acetaldehyde were associated with an ethylene oxide-sterilizing treatment of empty milk cartons. That was confirmed by comparing acetaldehyde levels in milk from aseptically filled cartons that had been treated with ethylene oxide

TABLE 2. *Probabilities and separation of means for the chemical tests and organoleptic analyses of UHTmilk stored 42 days at room temperature.*

| Item | Organoleptic analyses [1] | | | | Chemical tests | | |
|---|---|---|---|---|---|---|---|
| | Flavor score | Stale flavor | Cooked flavor | Oxidized flavor | Free HMF | Total HMF | TBA |
| | | | | *Probabilities* | | | |
| Days | .0000 | .0000 | .0000 | .0000 | .0000 | .0730 | .0021 |
| Treatments | .0000 | .0000 | .0520 | .0000 | .0000 | .0000 | .7192 |
| | | | | *Means*[2,3] | (Micromoles HMF/liter) | | (Absorbance 443 m ) |
| *Days* | | | | | | | |
| 2 | 35.62 | 1.26 | 4.73 | 0.58 a | 0.60 | 4.31 a | .014 ab |
| 12 | 35.00 | 2.69 | 3.49 a | 0.37 ab | 0.88 a | 4.46 a | .016 abc |
| 22 | 34.15 a | 3.68 | 3.17 ab | 0.14 b | 1.00 a | 4.75 a | .013 ab |
| 32 | 33.92 ab | 4.66 a | 3.12 ab | 0.17 b | 0.97 a | 4.73 a | .023 c |
| 42 | 33.49 b | 4.77 a | 2.72 b | 0.17 b | 0.99 a | 4.09 a | .021 bc |
| *Treatments*[4] | | | | *Means*[2,5] | | | |
| WAC | 34.90 a | 2.97 a | 3.93 b | 0.30 b | 0.86 a | 4.26 a | .016 a |
| NAC | 35.14 a | 2.57 a | 3.94 b | 0.25 ab | 0.82 a | 4.30 a | .016 a |
| WPC | 33.23 | 4.71 | 2.64 a | 0.32 b | 0.95 a | 4.70 a | .018 a |
| NPC | 34.50 | 3.47 | 3.28 | 0.27 ab | 0.92 a | 4.62 a | .019 a |
| R | 38.04 | 0.03 | 2.37 a | 0.11 a | 0.57 | 2.09 | .016 a |

[1]Intensity scores 0-9; 0 = none to 9 = very pronounced.
[2]Means not significantly different at 1% level are joined with a common letter of the alphabet.
[3]Each mean represents the average of the four UHT milks over 9 replications.
[4]WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, NPC = plain polyethylene-lined cartons, and R = reference milk.
[5]Each mean represent the average of the milk over 9 replications during the 42-day trial period.

TABLE 3. *Components isolated by steam distillation of UHT milk and analyzed by gas liquid chromatography.*

| | Retention[1] time (min) | Name of compound | Associated with |
|---|---|---|---|
| X | 1.7 | not identified | — |
| | 2.4 | acetaldehyde | ethylene oxide |
| | 3.1 | methyl sulfide | cooked |
| | 3.6 | propanal | stale |
| X | 4.0 | acetone | — |
| X | 4.8 | acrolein | — |
| X | 5.4 | not identified | — |
| | 9.5 | pentanal | stale |
| X | 11.0 | not identified | — |
| | 13.8 | not identified | stale |
| | 16.6 | hexanal | stale |
| X | 25.0 | not identified | — |
| | 28.0 | not identified | stale |

[1]An "X" by the peak retention time indicates that after statistical analysis peak could not be related to chemical or organoleptic changes.

either 24 h or one week before filling. For milk in cartons treated 24 h before filling, the acetaldehyde peak was extremely high; for that in one treated week before, the acetaldehyde peak was insignificant. Milk used to fill both types of cartons was from the same source. Methyl sulfide has been associated with cooked flavor (*10*) and so we observed. Since it decreased as the stale flavor increased, methyl sulfide could be a precursor of some other component that causes staling. Conceivably, however, methyl sulfide has a masking effect. Concentrations of propanal, pentanal, and hexanal increased with time and paralleled increased in intensities of stale flavors; the compounds also have been associated with light-induced and oxidized flavor (*3*). Because the 13.8-min GC peak decreased as the flavor intensity increased, this compound could be a precursor of staling. The compound responsible for the 28-min peak, like pentanal and hexanal, also might be associated with staling.

The mean protein, fat, and total solids contents of the milks in this study were 3.47, 3.50, and 11.80,[2] respectively, and their respective standard deviation were .13, .06, and .12.[2] These values show that the composition in the different blocks varied considerably, however, those differences should not have affected our results.

*Part II. Milk stored 100 days*

Trends we obseved for milk stored 42 days continued for microbiological, organoleptic, chemical, and GC evaluations of milks stored 100 days. Thus details of the statistical analyses are not presented; however, we plotted organoleptic changes to illustrate these trends (Fig. 1-3). Differences in flavor and stale-flavor intensity scores observed among cartons through 42 days were clearer at 100 days; the flavor indices changed almost linearly with time (Fig. 1, 2). Cooked intensity scores leveled off in the UHT milk stored 100 days.

## DISCUSSION

This research indicates that AL foil lining is slightly permeable to gases, probably at the seams but also probably through microscopic pores in the AL foil lining as evidenced by the fact that wrapping AL-lined cartons reduced flavor scores.

However, plain PE-lined cartons were more permeable to gases than the aluminum foil-lined cartons. This is apparent from the more rapid loss of methyl sulfide, and cooked flavor as well as a greater increase in carbonyl compounds and stale flavor in the PE lined than foil lined cartons. These results concur with those of Flückiger (*7*) who compared changes in milk packaged in liter PE-lined cartons with and without foil lining. His aluminum foil-lined cartons did not lose weight during 6

[2]*The total solids means encompass values for only blocks 1-6.*

TABLE 4. *Probabilities and separation of means for the gas-liquid-chromatographic components of UHT milk associated with defects when the milk is stored 42 days at room temperature.*

| Item | Component and/or retention time shown on the chromatogram | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2.4 min[1] acetaldehyde | 3.1 min[1] methyl sulfide | 3.6 min[1] propanal | 9.5 min[1] pentanal | 13.8 min[2] | 16.6 min[1] hexanal | 28.0 min[2] |
| | | | | *Probabilities* | | | |
| Days | .0000 | .0000 | .0000 | .0000 | .1852 | .0000 | .0002 |
| Treatments | .0143 | .0000 | .0000 | .0000 | .0000 | .0000 | .0000 |
| *Days* | | | | | *Means*[3,4] | | |
| 2 | 5237 | 36.11 | 14.2 a | 212 a | 160 a | 68.91 | 38 a |
| 12 | 2088 a | 29.10 a | 15.6 ab | 326 ab | 151 a | 71.80 a | 52 ab |
| 22 | 785 a | 27.40 a | 17.7 bc | 464 bc | 155 a | 73.46 a | 98 bc |
| 32 | 914 a | 27.30 a | 19.6 c | 427 bc | 105 a | 73.95 a | 96 bc |
| 42 | 933 a | 29.11 a | 19.0 c | 517 c | 113 a | 74.03 a | 109 c |
| *Treatments*[5] | | | | | *Means*[3,6] | | |
| WAC | 1384 ab | 31.07 b | 16.2 a | 322 a | 130 a | 70.94 ab | 92 a |
| NAC | 1270 ab | 31.30 b | 15.2 a | 328 a | 104 a | 69.99 a | 77 ab |
| WPC | 2756 b | 28.42 a | 20.6 | 473 b | 211 | 76.74 | 93 a |
| NPC | 2529 b | 28.53 a | 16.7 a | 439 ab | 102 a | 72.06 b | 53 b |
| R | 676 a | 27.58 a | 11.2 | 180 | 104 a | 69.48 a | 6 |

[1]Concentrations in ppb calculated from the regression equation of the standard curve.
[2]Peak heights relative to the peak height of 1 ppm acetone = 1000 units (% full-scale deflection peak height × attenuation).
[3]Means not significantly different at 1% level are joined with a common letter of the alphabet.
[4]Each mean represents the average of the four UHT milks over 9 replications.
[5]WAC = wrapped AL foil-lined cartons, NAC = AL foil-lined cartons, WPC = wrapped plain polyethylene-lined cartons, NFC = plain polyethylene-lined cartons, and R = reference milk.
[6]Each mean represent the average of the milk over 9 replication during the 42-day trial period.

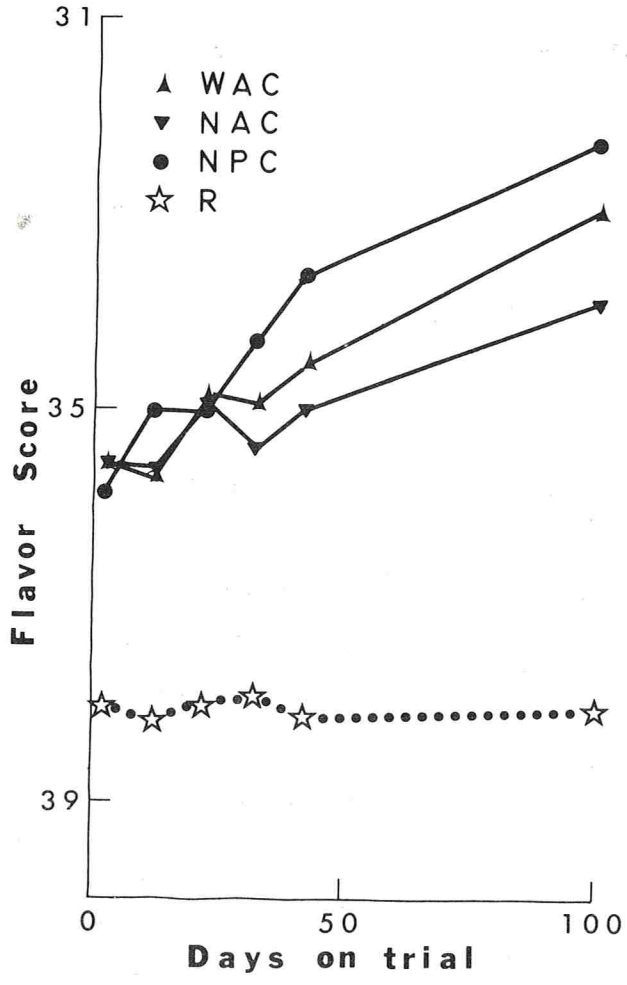


Figure 1. *Changes in flavor scores of UHT milks from cartons exposed to fluorescent light for 100 days. WAC + wrapped AL foil-lined, NAC + AL foil-lined, NPC = PE-lined, and R = reference milk.*

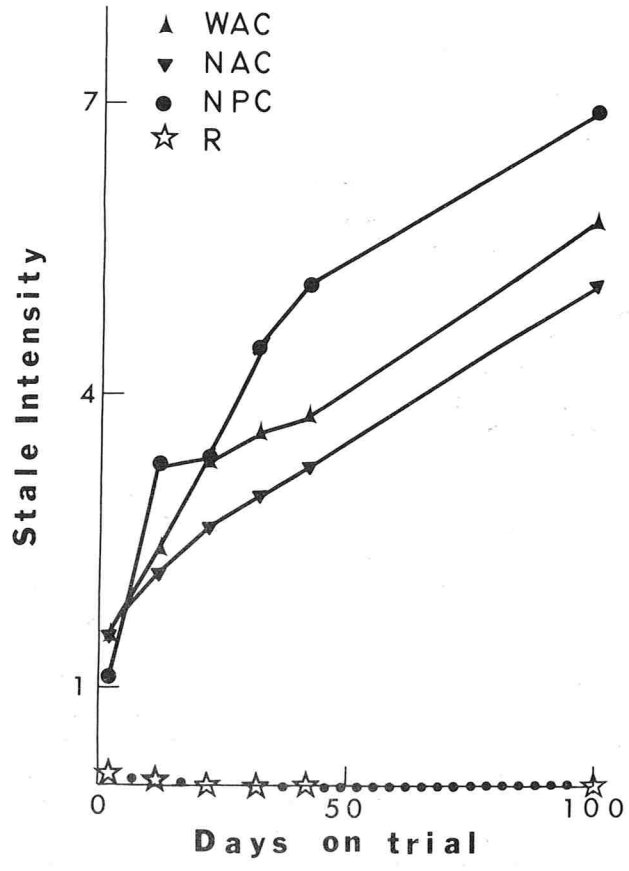


Figure 2. *Changes in stale intensities of UHT milks from cartons exposed to fluorescent light for 100 days. WAC + wrapped AL foil-lined, NAC + Al foil-lined, NPC + PE-lined, and R + reference milk.*

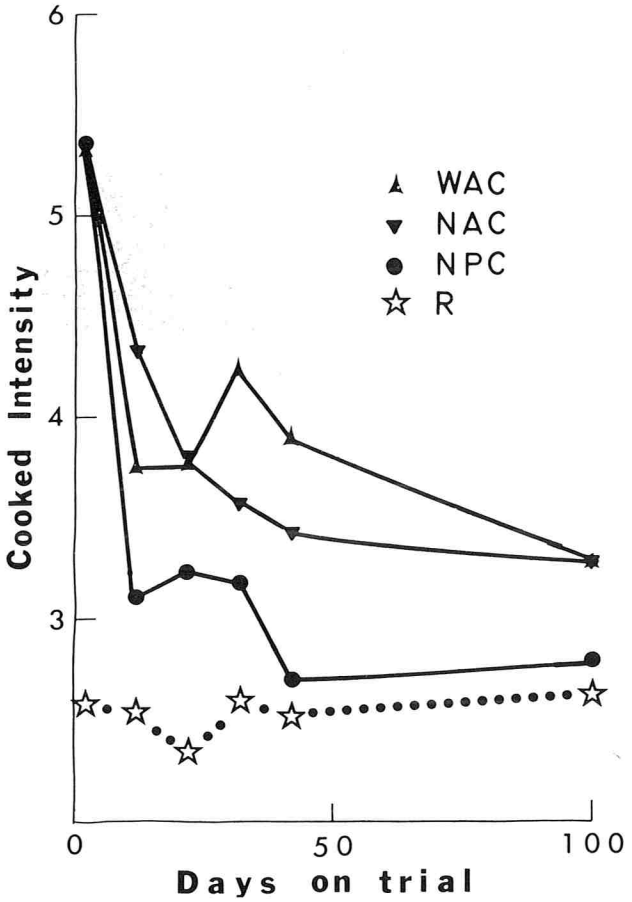


Figure 3. *Changes in cooked intensities of UHT milks from cartons exposed to fluorescent light for 100 days. WAC = wrapped AL foil-lined, NAC = AL foil-lined, NPC = PE-lined, and R = reference milk.*

weeks of storage; oxygen level in the milk remained constant at 1 ppm and milk was organoleptically acceptable for 2 months at room temperature. On the other hand, during the same period the plain PE cartons lost about 0.2% of their original weight at 20 C and 1% at 38 C; the milk was saturated with oxygen (8-9 ppm) after a few days and the milk was organoleptically acceptable only up to 3 weeks at 15 C. Flückiger felt most oxidative changes in the plain PE cartons took place in the first 2-3 days after processing and the reducing substances in the milk offered little protection in cartons without foil lining. The Saran-AL foil-Saran wrapping quite likely not only excluded outside air from the milk but also served to contain volatile compounds exuding from either growth of microorganisms on the surface of the cartons or from the wrapping material itself. Mean bacterial counts were higher in milk from wrapped cartons than from nonwrapped ones, even though they were not found to be statistically significant. One would suspect that the area under the wrapping could be loci for bacteria and perhaps odor. There was no evidence that light contributed to the changes that were observed.

We propose that the UHT milks exhibited two types of off-flavors: (*a*) cooked from volatile sulfhydryl compounds induced by heat, such as methyl sulfide; and (*b*) stale caused by volatile carbonyl compounds produced from the milk, from one or more of the carton layers, from the outer surface of the carton by microorganisms, or from the wrapping materials themselves. Heat damage from the UHT treatment, chemical changes that occur in the milk, and effect of the package all could account for changes in concentration of the off-flavors mentioned. Decreases in these components (as measured by GC) could be caused either by their escape to the atmosphere or by their interaction with other components. A decrease in the oxidation-reduction potential with heat treatment and in the liberation of sulfhydryls are known to produce stability against oxidative deterioration. Whether the mechanism for staling depends on the oxidation-reduction potential has not been established, though development of staling as cooked flavor disappears might suggest that it does. These assumptions, and the assumption that the AL foil carton is slightly permeable (although less so to exchange of gases than is the PE carton), account for our conclusion that AL foil lining helped the milk retain its desirable flavor characteristics.

Wrapping the cartons 2 days after they had been filled with milk proved detrimental to milk flavor. We speculate that that might have been caused by an odor-laden environment at the surface of the carton, confined by the wrappings. Odor could have been emitted by growing microorganisms on the surface of the cartons, by the Saran wrap, or by the carton material itself. Wrapping either the PE-lined or AL foil lined cartons had an adverse effect upon the flavor of the packaged milk during storage. This wrapping effect was less pronounced with AL foil lined cartons than with PE-lined cartons.

## REFERENCES

1. American Public Health Association. 1972. Standard methods for the examination of dairy products. 13th ed. Washington, D.C.
2. Association of Official Analytical Chemists. 1970. Official methods of analysis. 11th ed. Washington, DC.
3. Bassette, R. 1976. Effects of light on concentrations of some volatile materials in milk. J. Milk Food Technol. 39:10-12.
4. Bassette, R., and G. Ward. 1975. Measuring parts per billion of volatile materials in milk. J. Dairy Sci. 58:428-429.
5. Burton, H. 1969. Ultra-high-temperature processed milk. Dairy Sci. Abstr. 31:287-297.
6. Difco Laboratories. 1953. Difco manual. 9th ed. Difco Laboratories, Detroit, MI.
7. Flückiger, E. 1972. Packaging of Uperised milk: a comparison between polyethylene-coated cartons with and without aluminum foil. Milk Ind. 70(4):17-20.
8. Keeney, M. and R. Bassette. 1959. Detection of intermediate compounds in the early stages of browning reaction in milk products. J. Dairy Sci. 42:945-960.
9. King, R. L. 1962. Oxidation of milk fat globule membrane material. I. Thiobarbituric acid reaction as a measure of oxidized flavor in milk and model systems. J. Dairy Sci. 45:1165-1171.
10. Patton, S. 1958. Review of organic chemical effects of heat on milk. J. Agr. Food Chem. 6:132-135.
11. Zadow, J. G. 1970. Studies on the ultra heat treatment of milk. Part 2. Measurement of the products of browning reactions as influenced by processing and storage. Australian J. Dairy Technol. 25:123-126.

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# A Study of Microbial Quality of Vacuum Packaged, Sliced Bologna[1]

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**ABSTRACT**

A study of 113 samples of vacuum packaged sliced bologna offered for sale in the retail marketplace revealed a wide range of total microbial loads. The pH was not closely related with age or microbial load, within the manufacturers' expected shelf-life of the product. Approximately 55% of old samples had pH >6.0, and pH was influenced by manufacturer. Confirmed coliform bacteria were detected in 5% of samples, but *Escherichia coli* was absent (<3/10g). In contrast, group D streptococci were present, sometimes in large numbers, depending on manufacturer. Potentially pathogenic bacteria, including *Clostridium perfringens* (>10/g), coagulase positive *Staphylococcus aureus* (>25/g) and *Salmonella* (in 25 g of sample) were generally not detected. Only one sample contained >25 *S. aureus*/g. No relationship was observed between total microbial load and indicator organisms or pathogenic bacteria.

Bologna is an emulsion-type, non-fermented sausage which is cooked, cured and smoked during manufacture. Frequently sold as sliced, vacuum packaged luncheon meat, bologna is one of the most widely used sausage types in North America. During manufacture, bologna is cooked for 6 to 10 h to an internal temperature near 70 C. Slicing has been shown to be the most important source of contamination by both saprophytic and pathogenic bacteria in these meats (*23,24*). The microflora of vacuum packaged luncheon meat changes during storage. Most bacteria on freshly packaged product die during storage (*23*), whereas lactic acid bacteria tend to increase and often predominate the population within 2 weeks (*1, 2, 23*).

Initial counts in vacuum packaged, sliced, processed meats have been reported as $10^3$ bacteria/g (*1, 30*). These counts increase to $10^8$/g during refrigerated storage (*1, 20, 21, 23*). Although there is usually a marked drop in pH, growth to maximum bacterial population does not necessarily result in product spoilage (*1, 20, 23, 37*). Many factors, including the saprophytic microflora, pH, nitrite and salt concentrations, available water, and oxygen partial pressure influence survival and growth of pathogens (*10, 21, 22, 27*). Storage temperature will also have a marked effect on the microflora. Mesophiles, and therefore many pathogenic bacteria, have minimum growth temperatures of 10 to 15 C, and refrigerated storage of cured meats (below 10 C) allows lactic acid bacteria to develop (*4*).

With many variable factors influencing the microflora of bologna, this study was undertaken to determine the bacterial load of new and old, vacuum packaged, sliced bologna offered for sale in the retail marketplace.

## MATERIALS AND METHODS

*Sampling*

Vacuum packaged, sliced, processed meat, labelled "Bologna", representing the product of six Canadian federally inspected manufacturers was purchased from stores of four different retail chains. Samples were selected to represent "new" bologna (<15 days of manufacturer's shelf life expired) and "old" bologna (>21 days of manufacturer's shelf life expired). "New" samples were also purchased and stored in the laboratory at 4 C for bacterial analysis at the end of designated shelf-life.

*Sample preparation*

An 11-g wedge was cut aseptically through all slices in the package and homogenized with 99 ml of sterile, 0.1% peptone water in a sterile Waring Blendor jar at high speed for 2 min. All bacteriological determinations, except *Salmonella*, were carried out on this sample homogenate. A separate 25-g wedge was cut, weighed and homogenized in 150 ml of sterile nutrient broth (Difco) for *Salmonella* enrichment.

*Bacteriological analyses*

Appropriate dilutions of the homogenized 11-g samples were inoculated in duplicate onto the following media (all media were Difco brand, unless otherwise specified):

*Aerobic plate counts.* Plate count agar incubated at 35 C for 48 h for Standard Plate Count (SPC); incubated at 21 C for 72 h for Total Aerobic Plate Count (TPC); and incubated at 4 C for 10 days for psychrotroph count.

*Group D streptococci.* The presumptive group D *Streptococcus* count was determined on KF Streptococcus agar and incubated at 35 C for 48 and 72 h (*15*).

*Lactic acid bacteria.* Determined on Nitrite-Actidione-Polymyxin (NAP) agar (*8*), incubated at 30 C for 72 h. Randomly picked colonies from NAP were screened using gram stain and catalase activity (*14*).

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*Lactobacilli*. Determined on LBS agar (*28*), adjusted to pH 5.60 ± 0.05 according to Costilow et al. (*6*), and incubated 30 C for 72 h.

*Microbacterium thermosphactum*. Surface streaked on STAA agar (*13*), incubated 21 C for 72 h. Countable STAA plates were flooded with 2-3 ml of oxidase reagent (N,N-Dimethyl-p-phenylene-diamine monohydrochloride, Eastman Chemicals). Oxidase-positive colonies were excluded from the count.

*Clostridium perfringens*. Determined on Tryptose-Sulfite-Cycloserine (TSC) agar (*16*) and on egg-yolk free TSC agar (*17*). Both TSC and egg-yolk free TSC plates were incubated at 35 C, anaerobically in a $H_2/CO_2$ atmosphere (using BBL anaerobic jars and "gas-pak" cartridges) for 24 h.

*Staphylococci*. Determined on Mannitol Salt agar (MSA, presumptive staphylococci) and Baird-Parker (BP) medium and incubated at 35 C for 48 h. Colonies on BP medium were counted according to the Canadian Health Protection Branch acceptable method (*18*), differentiating between type I (shiny, black, smooth colonies causing clearing of the opaque egg-yolk medium, with or without a zone of precipitation around the colony) and type II colonies (Similar colonies without egg-yolk reaction). A selection of type I and II colonies was inoculated into Brain Heart Infusion (BHI) broth, streaked on MSA slants and incubated at 35 C for 24 h. MSA positive cultures were inoculated from BHI broth into EDTA coagulase plasma to test for coagulase positive *Staphylococcus aureus* (*18*).

*Coliforms and fecal coliforms*. The 3-tube method of the Most Probable Number (MPN) technique was adapted from the Canadian Health Protection Branch acceptable method (*19*) and the International Committee on Microbiological Specifications for Foods (ICMSF) procedures (*39*). Appropriate dilutions were inoculated into Lauryl Tryptose (LST) broth. Gas-positive LST tubes at 24 and 48 h were inoculated into Brilliant Green Lactose 2% Bile (BGB) and EC broths. Similarly, gas-positive BGB tubes at 24 and 48 h were streaked onto Levine EMB agar for the completed coliform test. All media, except EC broth, were incubated at 35 C. EC medium was incubated at 44.5 ± 0.05 C for 24 and 48 h. Gas-positive EC tubes were used to determine the MPN of fecal *Escherichia coli*.

*Salmonella*. The 25-g sample was homogenized in non-selective nutrient broth enrichment, incubated at 35 C for 20 h, and an aliquot transferred to Selenite Cystine (SC) broth, selective enrichment, and incubated at 35 C for 24 and 48 h. The SC selective enrichment was streaked onto Brilliant Green (BGA) agar and Bismuth Sulfite (BSA) agar and incubated at 35 C for 24 and 48 h. *Salmonella*-type colonies were screened on MacConkey agar and TSI slants.

Positive controls were done during the study using known cultures of coagulase-positive *S. aureus, S. typhimurium, S. cholerae-suis, E. coli* and *C. perfringens*.

In addition to bacteriological tests, pH was measured on 111 of the bologna samples using a 1:10 blended homogenate of bologna in de-ionized, distilled water (*23, 40*) and for ca. 70% of the samples using a combination (single probe) electrode (Fisher Scientific Co., Cat. No. 13-639-90) directly between the bologna slices (*29, 31, 32, 36*).

*Analyses*

Data were analyzed statistically using Pearson's correlation coefficients (r) as outlined by Nie et al. (*25*). Chi-square analysis for independence (*25*) was carried out to determine the influence of manufacturer and age upon bacteria counts and pH. In addition, using only the results from "new" bologna samples (all brands) and "old" bologna samples (those samples which were purchased "new" and stored at 4 C in the laboratory to pull date) an analysis of variance (*25*) to determine effects of manufacturer and age was carried out.

## RESULTS AND DISCUSSION

A total of 113 samples of bologna were analyzed, representing "new" and "old" samples from six manufacturers. The pH profile of 111 of the bologna samples is shown in Table 1. The pH ranged from 4.9 to 6.7, and almost 50% of samples fell in the range of 6.1 to 6.5. The pH of bologna has been reported as 6.2 to 6.4 (*37*), 5.2 to 5.3 (*26*) and 5.0 to 6.5 (*23*). Results of this study

TABLE 1. *pH of bologna samples*[a] *analyzed in the study.*

| Sample | pH values | | | | |
|---|---|---|---|---|---|
| | 4.6-5.0 | 5.1-5.5 | 5.6-6.0 | 6.1-6.5 | 6.6-7.0 |
| | (Number of samples) | | | | |
| Manufacturer | | | | | |
| A | 0 | 2 | 5 | 11 | 0 |
| B | 0 | 0 | 3 | 9 | 1 |
| C | 3 | 17 | 3 | 8 | 1 |
| D | 0 | 2 | 2 | 9 | 0 |
| E | 0 | 0 | 2 | 7 | 13 |
| F | 0 | 0 | 3 | 10 | 0 |
| New samples | 0 | 2 | 6 | 27 | 1 |
| Old samples | 3 | 19 | 12 | 27 | 14 |
| All samples | 3 | 21 | 18 | 54 | 15 |

[a]Results for 111 samples out of 113 analyzed.

supported the wider range of pH levels reported by Kempton and Bobier (*23*). They noted that the pH drop did not occur until after 4 weeks of refrigerated storage. In this study, however, pH dropped before 4 weeks of storage had expired, possibly as a result of poor storage conditions at retail level. Age had a significant effect on pH ($p<0.002$). New bologna generally fell in the pH range of 6.1 to 6.5, while old product often had pH values below 5.5. However approximately 55% of old samples failed to develop low pH. This poor correlation ($r = -0.2$) was due to manufacturer effect ($p = 0.0001$). The old product of one manufacturer generally had low pH, while that of another manufacturer was generally high pH. This might explain reports by Steinke and Foster (*37*) and Riemann et al. (*26*) that samples had a narrow pH range.

The distribution of saprophytic bacterial counts, including potential indicator organisms, is shown in Table 2. Total aerobic and psychrotroph counts varied over a wide range, which agrees with other literature reports for bologna and frankfurters (*1, 20, 21, 23, 38, 40*). Results for total counts at different temperatures can be used to infer storage history of samples (*38*). Samples stored continuously at 4 C or below should have psychrotroph counts equal to or greater than total counts at 21 or 35 C. For these samples, 44% of counts at 21 C and 29% of counts at 35 C were at least five times higher than at 4 C. In comparison, 40% of counts at both 4 and 21 C were at least five times lower than at 35 C. This suggests widely varying temperature histories of samples, resulting in growth of mesophilic organisms (i.e. storage temperature of 10 C and above). New samples had significantly lower total aerobic counts ($p<0.001$). Manufacturer also had a significant effect on total aerobic counts at 35 and 21 C ($p<0.01$) but not at 4 C, suggesting that differences in handling of product at manufacturing level influenced the mesophile count, but not the psychrotroph count. The psychrotroph count appeared to depend on post-manufacture storage conditions.

Other saprophytic parameters measured in this study included lactic acid bacteria (NAP), lactobacilli (LBS) and *M. thermosphactum* (oxidase-negative count on STAA). As would be expected, the highest lactic acid bacteria and lactobacilli counts were detected in product that developed the lowest pH, correlation coefficients

TABLE 2. *Profiles of saprophytic and indicator organism counts for 113 samples of vacuum-packaged, sliced bologna.*

| Count | Percent of samples with counts/g in the ranges | | | | | Number of samples |
|---|---|---|---|---|---|---|
| | $<10^2$ | $<10^2$ $10^4$ | $<10^4$ $10^6$ | $<10^6$ $10^8$ | $>10^8$ | |
| Total count (35 C) | 1.8 | 24.8 | 23.0 | 30.0 | 20.4 | 113 |
| Total count (21 C) | 0.0 | 21.2 | 19.4 | 31.0 | 28.5 | 113 |
| Psychrotroph count | 11.5 | 18.5 | 23.0 | 37.3 | 9.7 | 113 |
| Lactic acid bacteria (NAP) | 22.7 | 13.6 | 13.6 | 31.8 | 18.2 | 44 |
| Lactobacilli (LBS) | 41.4 | 21.6 | 8.1 | 27.0 | 1.8 | 111 |
| *M. thermosphactum* | 62.8 | 16.8 | 13.3 | 7.1 | 0.9 | 113 |
| Group D streptococci | 38.9 | 13.3 | 15.9 | 26.5 | 5.3 | 113 |

TABLE 3. *Correlation coefficients between saprophytic counts and pH of bologna samples.*

| | Total counts | | | NAP[1] | LBS[2] | KF[3] | STAA[4] |
|---|---|---|---|---|---|---|---|
| | 35C | 21 C | 4 C | | | | |
| pH | -0.60 | -0.55 | -0.36 | -0.66 | -0.62 | -0.60 | 0.16 |
| Total count 35 C | | 0.89 | 0.61 | 0.81 | 0.66 | 0.69 | - 0.13 |
| 21 C | | | 0.73 | 0.82 | 0.65 | 0.63 | 0.04 |
| 4 C | | | | 0.47 | 0.64 | 0.34 | 0.03 |
| NAP[1] | | | | | 0.79 | 0.57 | - 0.42 |
| LBS[2] | | | | | | 0.47 | - 0.15 |
| KF[3] | | | | | | | - 0.15 |

[1]NAP = presumptive lactic acid bacteria count.
[2] LBS = presumptive lactobacilli count.
[3]KF = presumptive Group D Streptococcus count.
[4] STAA = oxidase negative, presumptive *M. thermosphactum* count.

r = - 0.66 and r = - 0.61, respectively, These counts were influenced by manufacturer and age of the product. However, this was not true of *M. thermosphactum*. Contrary to expectation (*14*), *M. thermosphactum* did not appear to be associated with growth of bacteria in this vacuum packaged bologna, and correlations with other saprophytic organisms were extremely low (see Table 3). Furthermore, *M. thermosphactum* was not detected in 71 of the samples, indicating counts <100/g in 63% of samples tested.

The correlation coefficients between pH and different saprophytic bacteria parameters are shown in Table 3. A correlation of >0.7 is necessary to achieve 50% predictability between counts, such relationships were only observed in five of the 28 correlations, but many of the correlations were significant. These data indicate that lactic acid bacteria, lactobacilli and group D streptococci grew in the samples during storage, and their growth was reflected in the total aerobic counts at 35 and 21 C, but not to the same extent at 4 C.

Group D streptococci were detected in 78% of bologna samples analyzed, ranging from 10 to $2.1 \times 10^8$ organisms/g. In 60% of samples, group D *Streptococcus* counts were above 100/g, indicating that many samples had been exposed to undesirable storage temperature conditions (*5, 39*). The group D *Streptococcus* count was significantly affected by age (p = 0.0004) and manufacturer (p = 0.0001). The group D *Streptococcus* count was significantly correlated with total aerobic count at 35 C (r = + 0.685), again suggesting high storage temperatures. In a separate study (Stile, unpublished data), it was shown that 49% of isolates were *Streptococcus faecium* var. *durans*[4], 38% were *S. faecium* and 5% were *Streptococcus faecalis*. *S. faecium* var. *durans* has often been associated with heat processed meats, and in cheese it is not considered to indicate fecal contamination (*12*). However, both *S. faecium* and *S. faecalis* are considered by some to indicate fecal contamination (*5*). In these samples, numbers did not indicate degree of contamination because of the apparent growth opportunities for these organisms. A poor correlation was observed between coliform bacteria and group D streptococci in these samples, confirming the reports of others (*5, 39*). However, the total absence of fecal-type *E. coli* in the samples raised doubts about the fecal implications of *S. faecium* and *S. faecalis*.

For coliform bacteria and fecal *E. coli*, the lowest concentration that could be detected using the MPN technique was 3 organisms/10g. Only 5% of samples had detectable confirmed coliform counts. None of the samples were gas-positive in EC medium at 44.5 C and all samples were salmonella-negative in 25-g enrichments. This contrasted markedly with the group D streptococci, confirming that the group D streptococci were most likely from equipment, implicating sanitation of equipment and storage temperature, rather than poor hygiene as their source.

Potentially pathogenic bacteria were only present at low concentrations in the bologna samples tested. Only 10 (9%) samples had detectable levels of anaerobic spore

[1]S. durans, *originally described by Sherman and Wing in 1937* (35) *is included in the 8th edition of* Bergey's Manual *as* S. faecium (8). *According to Deibel et al.* (33) S. durans *and* S. faecium *only differ in their ability to ferment arabinose and mannitol (the differentiating characteristics used for this study). The similarity between these organisms is widely accepted* (3,9,11) *but for food microbiology the distinction of "durans"-type is important because of different public health implications of this strain* (33,34) *- in particular, its ability to survive and grow outside of the intestinal environment.*

forming bacteria. One sample had coagulase-positive *S. aureus* at $10^2$/g, the remaining samples were <25/g. Only on Mannitol Salt agar (MDA) were appreciable counts, (up to $10^5$/g) obtained. However, 54% of samples had counts <1,000/g, and in 44% of samples there was no detectable count. Counts on MSA represent salt tolerant bacteria that ferment mannitol. Although *S. aureus* would be included in this count, it appeared from the Baird-Parker medium results that potentially pathogenic, coagulase-positive *S. aureus* counts were low. Since the samples had been stored and handled such that many bacteria could grow, it was apparent that for these samples the potentially pathogenic bacteria did not compete well with the saprophytic flora. High saprophytic counts would not necessarily indicate a potentially hazardous product.

## SUMMARY AND CONCLUSIONS

The samples of bologna in the retail marketplace differ widely in bacterial load, both as a function of age and manufacturer. The latter is somewhat difficult to explain, but probably reflects different manufacturing techniques and different bacterial flora predominating in processing plants. Among the bacteria that grow in the bologna samples, the group D streptococci are the only group that might represent some concern. While group D streptococci are generally not accepted as indicators of fecal contamination of foods, they are suspected of having a food poisoning potential (*39*). Results of this study suggest that group D streptococci are not indicating fecal contamination. However, the source of *S. faecium*, in particular, and also *S. faecalis* in these samples should be known before this conclusion can be confirmed.

Low pH is an important protective factor against growth of potentially pathogenic bacteria in vacuum packaged luncheon meats. This study indicated that low pH may not develop in the samples, right up to the end of shelf-life (30 to 35 days from manufacture). Low pH alone, therefore, cannot be relied on for protection against growth of food poisoning bacteria in bologna. The low levels of potential pathogens in the bologna samples analyzed, even in samples with high total bacterial loads, indicate either a low incidence of contamination or failure of the pathogens to survive in the product. Bacterial standards, at least for total bacterial loads, would not appear to be meaningful for vacuum packaged bologna. Inoculation studies using enteropathogenic bacteria would help to clarify the food poisoning potential of bologna.

### REFERENCES


1. Allen, J. R., and E. M. Foster. 1960. Spoilage of vacuum-packed sliced processed meats during refrigerated storage. Food Res. 25:19-25.
2. Alm, F., I. Ericksen, and N. Molin. 1961. The effect of vacuum packaging on some sliced processed meat products as judged by organoleptic and bacteriologic analysis. Food Technol. 15:199-203.
3. Barnes, E. M. 1964. Distribution and properties of serological types of *Streptococcus faecium, Streptococcus durans* and related strains. J. Appl. Bacteriol. 27:461-470.
4. Bartl, V. 1973. Semi-preserved foods: General microbiology and food-poisoning. pp. 89-106. In: B. C. Hobbs, and J. H. B. Christian (eds.) The microbiological safety of food. Academic Press, New York.
5. Buttiaux, R., and D. A. A. Mossel. 1961. The significance of various organisms of faecal origin in foods and drinking water. J. Appl. Bacteriol. 24:353-364.
6. Costilow, R. N., J. L. Etchells, and T. E. Anderson. 1964. Medium for producing cells of lactic acid bacteria. Appl. Microbiol. 12:539-540.
7. Davidson, C. M., and F. Cronin. 1973. Medium for the selective enumeration of lactic acid bacteria from foods. Appl. Microbiol. 26:439-440.
8. Deibel, R. H., and H. W. Seeley Jr. 1974. Family II. *Streptococcaceae* fam. nov. pp. 490-509. R. E. Buchanan, and N. E. Gibbons (eds.) Bergey's manual of determinative bacteriology. 8th ed. Williams and Wilkins Co., Baltimore, U.S.A.
9. Deibel, R. H., D. E. Lake, and C. F. Niven Jr. 1963. Physiology of the enterococci as related to their taxonomy. J. Bacteriol. 86:1275-1282.
10. Dempster, J. F. 1976. Pathogenic microorganisms in cured meats. Food Manuf. 51(1):41-46.
11. Facklam, R. R. 1972. Recognition of Group D *Streptococcus* species of human origin by biochemical and physiological tests. Appl. Microbiol. 23:1131-1139.
12. Frazier, W. C. 1967. Food microbiology. 2nd ed. McGraw-Hill Book Company. New York.
13. Gardner, G. A. 1966. A selective medium for the enumeration of *Microbacterium thermosphactum* in meat and meat products. J. Appl. Bacteriol. 29:455-460.
14. Gardner, G. A., and A. G. Kitchell. 1973. The microbiological examination of cured meats. pp. 11-20. In R. G. Board, and D. W. Lovelock (eds.) Sampling-Microbiological examination of cured meats. Academic Press. New York.
15. Hall, H. E., D. F. Brown, and R. Angelotti. 1963. The recovery of enterococci from food using KF Streptococcus media. J. Food Sci. 28:566-571.
16. Harmon, S. M., D. A. Kautter, and J. T. Peeler. 1971. Improved medium for enumeration of *Clostridium perfringens*. Appl. Microbiol. 22:688-692.
17. Hauschild, A. H. W., and R. Hilsheimer. 1974. Enumeration of food-borne *Clostridium perfringens* in egg yolk-free tryptose-sulfite-cycloserine agar. Appl. Microbiol. 27:521-526.
18. Health Protection Branch. 1974. Determination of coagulase-positive staphylococci in foods. Acceptable Method MFA. Oct., 1974. Bureau of Microbial Hazards, Health Protection Branch, Ottawa, Canada.
19. Health Protection Branch. 1974. Determination of coliforms and faecal coliforms in foods. Acceptable Method MFA-19. May, 1974. Bureau of Microbial Hazards, Health Protection Branch, Ottawa, Canada.
20. Hill, W. M., J. Resume, and J. C. Wilcox. 1976. Total plate count and sensory evaluation as measures of luncheon meat shelf life. J. Milk Food Technol. 39:759-762.
21. Ingram, M. 1962. Microbiological principles in prepackaging meats. J. Appl. Bacteriol. 25:259-281.
22. Ingram, M. 1974. The microbiological effects of nitrite; discussion. pp. 63-75. In B. Krol, and B. J. Tinbergen (eds.) Proceedings of the International Symposium on Nitrite in Meat Products. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
23. Kempton, A. G., and S. R. Bobier. 1970. Bacterial growth in refrigerated, vacuum-packed luncheon meats. Can. J. Microbiol. 16:287-297.
24. Mol, J. H. H., J. E. A. Hietbrink, H. W. M. Mollen, and J. von Tintren. 1971. Observations on the microflora of vacuum packed sliced cooked meat products. J. Appl. Bacteriol. 34:377-397.
25. Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Bent. 1975. Statistical package for the social sciences. 2nd ed. McGraw-Hill Book Company, New York.
26. Riemann, H., W. H. Lee, and C. Genigeorgis. 1972. Control of

*Clostridium botulinum* and *Staphylococcus aureus* in semi-processed meat products. J. Milk Food Technol. 35:514-523.
27. Riha, W. E., and M. Solberg. 1975. *Clostridium perfringens* inhibition by sodium nitrite as a function of pH, inoculum size and heat. J. Food Sci. 40:439-442.
28. Rogosa, M., J. A. Mitchell, and R. F. Wiseman. 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. 62:132-133.
29. Roth, L. A., and D. S. Clark. 1972. Studies on the bacterial flora and vacuum-packaged fresh beef. Can. J. Microbiol. 18:1761-1766.
30. Sakazaki, R., K. Tamura, A. Nakamura, T. Kurata, A. Gohda, and S. Takeuchi. 1974. Enteropathogenicity and enterotoxigenicity of human enteropathogenic *Escherichia coli*. Jap. J. Med. Sci. Biol. 27:19-33.
31. Scheusner, D. L., and L. G. Harmon. 1973. Growth and enterotoxin production by various strains of *Staphylococcus aureus* in selected foods. J. Food Sci. 38:474-476.
32. Scheusner, D. L., L. L. Hood, and L. G. Harmon. 1973. Effect of temperature and pH on growth and enterotoxin production by *Staphylococcus aureus*. J. Milk Food Technol. 36:249-252.
33. Sherman, J. M. 1937. The streptococci. Bacteriol. Rev. 1:3-97.
34. Sherman, J. M., and H. U. Wing. 1935. An unnoted hemolytic *Streptococcus* associated with milk products. J. Dairy Sci. 28:656-660.
35. Sherman, J. M., and H. U. Wing. 1937. *Streptococcus durans* n. sp. J. Dairy Sci. 20:165-167.
36. Smith, J. L., and S. A. Palumbo. 1973. Microbiology of Lebanon bologna. Appl. Microbiol. 26:489-496.
37. Steinke, P. K. W., and E. M. Foster. 1951. Effect of temperature of storage on microbial changes in liver sausage and bologna. Food Res. 16:372-376.
38. Surkiewicz, B. F., R. W. Johnston, and J. M. Carosella. 1976. Bacteriological survey of frankfurters produced at establishments under Federal inspection. J. Milk Food Technol. 39:7-9.
39. Thatcher, F. S., and D. S. Clark. 1968. Microorganisms in foods: Their significance and methods of enumeration. University of Toronto Press, Toronto.
40. Warnecke, M. O., H. W. Ockerman, H. H. Weiser, and V. R. Cahill. 1966. Quality of processed comminuted meat as affected by microbial flora of the raw constituents. Food Technol. 20:686-688.

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# Factors that Contribute to Outbreaks of Foodborne Disease

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**ABSTRACT**

Factors that contributed to foodborne outbreaks that were reported in the U.S. from 1973-1976 are identified and categorized by disease and by locale in which incriminated foods were mishandled. Data from the same years are tallied separately and combined with data from the years 1961-1972. Inadequate cooling was associated with most foodborne outbreaks, with many bacterial foodborne diseases (such as salmonellosis, staphylococcal food poisoning, and *Clostridium perfringens* gastroenteritis), and frequently with outbreaks that originated from foods prepared in foodservice establishments and homes. Inadequate cooling practices were usually either failure to refrigerate cooked foods or the storing of foods in large stock pots or other large containers that were refrigerated. Other important factors that contributed to foodborne outbreaks were the lapse of a day or more between preparing and serving (coupled with inadequate refrigeration or hot-holding during this time), handling of cooked foods by infected persons, inadequate cooking or other thermal processing, inadequately high temperatures during hot storage, inadequately high reheating temperatures, inadequate cleaning of kitchen or processing equipment, ingestion of contaminated raw food or ingredient, and cross contamination. The frequency of involvements of the factors that contributed to outbreaks in England and Wales was quite similar. The principal factors that contributed to staphylococcal food poisoning were inadequate cooling practices, infected person manipulating cooked food, and lapse of a day or more between preparing and serving. The principal factors that contributed to *C. perfringens* gastroenteritis were inadequate cooling practices, lapse of a day or more between preparing and serving, and inadequately high temperatures during hot-holding and reheating. The principal factors that contributed to salmonellosis were inadequate cooling practices, contaminated raw ingredients, inadequate cooking or thermal processing, and cross-contamination.

Epidemiologic and research data collected over the past century have demonstrated that the following sequence of events must occur for persons to get a foodborne disease: (*a*) the etiologic agent must be present either in citizens of a community, in food-source animals, or in the environment in which foods are grown, harvested, processed, or stored; (*b*) the agent itself or the organism that produces it (if it is one of several toxins) must contaminate a food during the growing period or during harvesting, processing, storage, or preparation; (*c*) then, one of the following events must happen; (*i*) the agents must be present on or in the contaminated food in sufficient numbers or concentrations to survive the remainder of the growing period, storage, and processing and still cause illness; (*ii*) bacteria on or in foods in insufficient numbers to cause illness must multiply and reach quantities or produce toxins in sufficient quantities to cause illness; (*iii*) microorganisms, particularly bacteria, enter food preparation areas on or in raw foods, where they are transferred to worker's hands or to equipment surfaces, which if inadequately washed will then contaminate other foods that they subequently touch (and hence, if bacteria, multiply as described in (*3ii*); (*d*) sufficient quantities of the contaminated food that contains enough of the agent to exceed a person's resistance-susceptibility threshold must be ingested. Ingestion of foods contaminated to this level can result in sporadic cases of illness as well as outbreaks. Whether or not outbreaks are detected depends on the number of persons who ingested the contaminated food and on the socio-cultural attitudes of the populace to report illness and the efficiency of a health agency to determine that the illness is foodborne and epidemiologically related to other cases. When numbers of pathogens insufficient to cause illness are ingested, an infected individual may become a carrier and may contaminate other foods that he touches.

Each step in the sequence of events necessary to contribute to outbreaks of foodborne disease is briefly reviewed.

(*a*) Microorganisms that cause foodborne disease are commonly associated with healthy persons or animals and the environment (e.g., soil), as well as with ill persons or animals. Chemicals that cause illness are frequently those used for acceptable agricultural or industrial practices but are applied too close to harvesting, used in a haphazard manner, or added to foods in excessive amounts.

(*b*) There are many sources of contamination of foods. Fruits and vegetables can become contaminated during production on farms, and seafoods and water-grown vegetables can become contaminated in their aquatic

environment. Meat can become contaminated if the meat is taken from an animals that has septicemia, if infectious lesions remain on sites used as meats, if edible organs are infected or inedible infected organs leak on meat surfaces, or if cross-contamination from animal feces occurs. Eggs become contaminated during formation, while in contact with nesting material, or by droppings. Foods are also contaminated by workers and by environmental sources during handling and preparation in food processing plants, food service establishments, and homes. The point at which contamination occurs depends on the natural sources of a pathogen or the source of a poisonous substance and on the opportunities for transfer at each stage of the food production-processing-preparation chain.

(*c*) Pathogenic bacteria on and in the contaminated food will multiply if the food contains sufficient moisture and sufficient quantity and variety of nutrients; if the pH and the redox potential of the food are favorable; if food-holding temperatures are within the growth range (particularly when the temperature is near the optimal growth temperature for the particular kind of pathogenic bacteria for enough time), and if they can successfully compete with the mixed microbial flora on and in the foods. Growth of spoilage bacteria that are usually on raw foods often inhibits growth of pathogenic bacteria. Raw foods, however, become vehicles of foodborne outbreaks when the ratio of pathogen to competitive flora is high or when the contaminating strain of pathogen is particularly virulent or is able to survive the competition of other flora, the effects of processes, and multiply during storage.

For heat-processed foods to become vehicles of foodborne disease outbreaks, pathogens — particularly bacterial spores — survive heat processing or the food becomes contaminated after the heat processing. Pathogenic bacteria that survive processing and preparation usually have to multiply to reach levels that cause disease. Most vegetative bacteria — but not spores — will be killed if during cooking any contaminated portions of food reach 73.9 C (165 F) for a few seconds (or even temperatures as low as 60 C (140 F), if held at such temperatures long enough. All foods, however, are not cooked before being eaten, and the temperatures reached during cooking can be too low or the time too short (at that temperature) to kill pathogens.

Pathogens often get into food preparation environments on contaminated raw foods, particularly raw foods of animal origin. These organisms are killed if food is thoroughly cooked; but, before the food is cooked, pathogens can contaminate hands of workers who touch the raw foods, and they can contaminate equipment that is used in their processing, preparation, or storage. These same foods after heat processing become recontaminated or other foods can become contaminated if they are handled by these workers or processed or prepared with the same equipment which has not been subsequently cleaned and sanitized.

A food contaminated with foodborne disease bacteria can support their growth under certain conditions: the food must contain sufficient moisture and essential nutrients and be within favorable pH and redox ranges to support growth of these pathogens; it must be kept within a temperature range that permits these bacteria to multiply (this is usually near the organism's optimal temperature for growth); and the food must be kept at such temperatures long enough for sufficient organisms (or toxins) to be produced to cause illness in those who ingest the food.

(*d*) Ingestion of contaminated food, however, does not always result in illness. Enough pathogens must be swallowed to exceed a person's threshold of resistance if illness is to result. Adult human volunteer feeding studies have indicated susceptibility resistance threshold levels for various enteric pathogens (*10*). Ten *Shigella dysenteriae* 1, 180 *Shigella flexneri* 2a, or 1,000 *Vibrio cholerae* biotype inaba can, if ingested, cause illness or carrier status. These quantities could conceivably be present on foods that were contaminated in fields or in watercourses by irrigation water containing sewage, or that were contaminated during handling by infected persons. Ten thousand *Salmonella typhi* and *V. cholerae* biotype inaba can cause illness; conceivably this amount could be present on foods recently fertilized with night soil or raw sewage. Most of the other organisms (such as *Salmonella*, *Clostridium perfringens*, and *Escherichia coli*) for which human feeding tests have been carried out usually require time for multiplication before the large number (often 100,000 or more) necessary to cause illness would be generated (*10*). Staphylococcal enterotoxin is produced when enterotoxic strains of *Staphylococcus aureus* multiply; there are usually 500,000 or more staphylococci present per gram before enterotoxin is detectable (*9*). Infants, elderly persons, malnourished persons, and persons with concomitant illness are more susceptible than healthy adults; perhaps a log or more reduction in dosage would cause illness among them.

It is unlikely that an infective dose of salmonellae would be on lettuce or other raw vegetables, for example, but it is possible for the same foods to contain an infective dose of shigellae. Shigellae, however, do not usually survive long in the microbiologically competitive environment of food. Salmonellae, *C. perfringens*, and many other foodborne disease causing bacteria would be problems if contaminated foods were allowed to stand at room temperature, be stored in hot-holding devices at bacterial incubating temperatures, or be stored in large pots or otherwise in bulk in refrigerators for sufficient time to permit them to multiply to sufficient numbers to cause illness.

Each sequence of events described is shown to be an important factor that contributes to foodborne disease outbreaks by critical review of epidemiologic investigations and surveillance reports for the period 1961 through 1976.

## MATERIALS AND METHODS

The present study, as well as those of the past (*5-7*), was done by gathering details on operational or constructional factors that contributed to outbreaks of foodborne illness which were reported in public health literature or national surveillance data. Information was sought from periodic surveillance reports that are published by the Center for Disease Control (CDC); articles in journals; reports of outbreaks that have been sent to CDC from states, local health departments, or Federal agencies; and reports from CDC's Epidemic Intelligence Service. The surveillance reports that were previewed are *Morbidity and Morality Weekly Reports* (*24*) *Salmonella Surveillance Reports* (*25*), *Shigella Surveillance Reports* (*27*), *Hepatitis Surveillance Reports* (*26*), and *Trichinosis Surveillance Reports* (*28*). *Index Medicus* (1961-1977), *Excerpta Medica* (1961-1977), *Abstracts of Hygiene* (Bulletin of Hygiene), (1961-1977) and *Current Bibliography of Epidemiology* (1961-1977) were reviewed for listings of articles concerning foodborne disease outbreaks that occurred in the United States. Only those outbreaks that contained some mention of a food production, processing or preparation history were included in the data. Summary data were only used if there was a narrative to confirm the contributing factor. Outbreaks cited by one source were checked against the other source to avoid duplication. This survey, although pointing out many important factors that contribute to outbreaks, suffers certain shortcomings, which include: inadequate reporting of outbreaks; and incomplete reporting, write-up, or abstracting of these factors and outbreaks.

## RESULTS AND DISCUSSION

In the United States, factors that have been frequently shown to contribute to outbreaks of foodborne disease are (in order of frequency of occurrence): inadequate cooling of foods, lapse of a day or more between preparing and serving, infected persons having touched foods which are not subsequently heat-processed, inadequate time or temperature or both during heat processing of foods, insufficiently high temperature during hot storage of foods, inadequate time or temperature or both during reheating of previously cooked foods, ingesting contaminated raw foods or raw ingredients, and other factors listed in Table 1. This table compares the most frequently reported factors that contributed to the occurrence of foodborne disease outbreaks for three periods, 1961 through 1970 (*5*) 1971 and 1972 (*6, 7*), and 1973 through 1976. A more detailed breakdown of the factors that have been shown to contribute to specific outbreaks of foodborne disease reported from 1973 through 1976 is shown in Table 2.

Inadequate cooling practices are the major contributors to outbreaks of foodborne disease. Data from these outbreaks that were reported in the U.S. from 1973 through 1976 show that the following practices led to inadequate cooling: leaving foods at room temperature for several hours, storing foods in large pots or pans or otherwise in bulk in refrigerators, storing foods in refrigerators that maintain temperatures above recommended levels or permit temperatures above recommended levels because of malfunction, nonrefrigerated transport in trucks or car trunks, and storing in ovens that were turned off (Table 3).

Data from the United States can be compared to a summary of some outbreaks of foodborne disease that have occurred in England and Wales and summarized in the British Medical Journal from 1969 to 1976 (Table 4).

TABLE 1. *The most important factors that contributed to the occurrence of 1,152 outbreaks of foodborne disease (by rank and percent)*[1] *reported in public health literature or surveillance data during 1961 through 1976 and containing information about contributory factors.*

| Contributory factor | 1961-1970[2] (493)[4] | 1971-1972[3] (232) | 1973-1976 (427) | 1961-1976 (1,152) |
|---|---|---|---|---|
| Inadequate cooling | 1 (46) | 1 (48) | 1 (46) | 1 (46) |
| Lapse of a day or more between preparing and serving | 4 (21) | 2 (23) | 2 (20) | 2 (21) |
| Infected person | 3 (22) | 4 (19) | 3 (18) | 3 (20) |
| Inadequate thermal processing, canning, or cooking | 2 (24) | 5 (10) | 6 (11) | 4 (16) |
| Inadequate hot storage | 6 (13) | 3 (21) | 4 (16) | 5 (16) |
| Inadequate reheating | 7 (9) | 6 (9) | 5 (16) | 6 (12) |
| Ingesting contaminated raw food or ingredient | 5 (14) | 7 (6) | 7 (11) | 7 (11) |
| Cross-contamination | 7 (9) | 7 (6) | 10 (4) | 8 (7) |
| Inadequate cleaning of equipment | 9 (8) | 9 (6) | 8 (6) | 9 (7) |
| Obtaining foods from unsafe sources | 10 (6) | 11 (3) | 12 (4)[5,6] | 10 (5) |
| Using leftovers | 11 (3) | 10 (4) | 9 (5) | 11 (4) |

[1]More than 100% shows in percentage figures because multiple factors are usually necessary for outbreaks of foodborne disease to occur.
[2]Reference: Bryan (*5*)
[3]Reference: Bryan (*7*)
[4]Number of outbreaks reviewed in each survey.
[5]Storing high-acid foods in toxic containers ranked eleventh.
[6]Toxic species mistaken for edible varieties also ranked twelfth.

TABLE 2. *Inadequate cooling practices that contributed to 189 foodborne disease outbreaks reported 1973 through 1976.*

| Inadequate practice | Number | Percent |
|---|---|---|
| Left at room temperature | 105 | 56 (68)[1] |
| Stored in large container | 43 | 23 (32)[1] |
| At room temperature in large container | 18 | 10 |
| Refrigerator unit above recommended temperatures | 6 | 3 (6)[1] |
| At room temperature, then in refrigerator at above recommended temperatures | 6 | 3 |
| Transportation unit not refrigerated | 5 | 3 |
| Car trunk | 3 | 2 |
| Oven turned off | 3 | 2 |

[1]Total percent when multiple poor practices occurred

In England and Wales, factors that have been shown to contribute to outbreaks of foodborne disease are (in order of frequency of occurrence): inadequate cooling of foods, lapse of a day or more between preparation and serving, inadequate time or temperature or both during reheating, inadequate time or temperature or both during cooking, infected persons having touched foods which are not subsequently cooked, ingestion of contaminated raw food or ingredient, obtaining foods from unsafe sources, insufficient high temperature during hot-holding, and other factors listed in Table 4. These factors are quite similar to the factors that contribute to foodborne outbreaks in the United States (Tables 1 and 2).

The factors that most often contribute to outbreaks of foodborne disease vary, depending on the type of establishment in which foods are handled. Annual foodborne disease surveillance data show that foods that are implicated in outbreaks are frequently mishandled in foodservice establishments and homes. For instance, from 1973-1976, 67.4% were mishandled in foodservice

TABLE 3. – *Factors that contributed to the occurrence of 427 outbreaks of foodborne disease reported in public health literature and* surveillance data *during 1973 through 1976.*

| Diseases | Factors affecting growth: Inadequate cooling | Inadequate hot storage | Inadequate thawing | Prepared a day or more before serving | Use of leftovers | Factors affecting survival: Growth during germination | Faulty fermentations | Factors affecting contamination: Inadequate cooking, thermal processing, or canning | Inadequate reheating | Infected person | Unsafe source | Contaminated raw ingredient | Toxic species mistaken for edible varieties | Cross-contamination | Inadequate cleaning of equipment | Selling contaminated products or toxic foods | Poor storage practices | Toxic containers | Incidental additives | Intentional additives | Unknown post process contamination | Contamination by fertilizer or soil | Faulty sealing | Improper evisceration | Contaminated water | Mistranding | Number of outbreaks with data on factors that contributed to outbreaks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Bacterial* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Staphylococcal intoxication | 56 (2)[2] | 10 | | 30 | 1 | | 1 | 3 (1) | (10) | 29 (10) | | (1) | | 1 | 4 | | | | | | | | | | | | 69 |
| Salmonellosis | 25 (1) | 8 | 1 | 10 | 3 | | 2 | 6 (1) | 14 | 3 (5) | | 14 (5) | | 11 (1) | 11 | 1 | | | | | | 1 | | | | | 61 |
| Botulism | 2 | 2 | | | | | 8 | 19 (3) | 2 | | | | | | | | | | | | | | 1 | | | | 34 |
| *Clostridium perfringens* gastroenteritis | 18 | 9 (2) | | 13 | 4 | | | 3 (1) | 15 | | | | | (1) | | | | | | | | | | | | | 24 |
| Shigellosis | 1 | | | 2 | 1 | | | | 2 | 5 (2) | | | | | | | | | | | | | | | | | 7 |
| Typhoid fever | | 1 | | | | | | 1 | | 1 (1) | | | | | | | | | | | | | | | | | 4 |
| Brucellosis | | | | | | | | | | | | 3 | | | | 1 | | | | | | | | | | | 4 |
| *Bacillus cereus* gastroenteritis | 2 | | | 1 | 1 | 1 | | | | | | | | | | | | | | | | | | | | | 3 |
| *Vibrio parahaemolyticus* gastroenteritis | 2 | | | | | | | | | | | | | 2 | | | | | | | | | | 1 | | | 3 |
| Group A streptococcal infections | 2 | | | 2 | | | | | | 2 | | | | | | | | | | | | | | | | | 2 |
| Cholera | 1 | | | | | | 1 | | | | 1 | 1 | | | | | | | | | | | | | | | 1 |
| Scombroid poisoning | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| *Yersinia enterocolitica* gastroenteritis | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | 1 |
| Total Bacterial | 110 (3) | 30 (2) | 1 | 58 | 10 | 1 | 12 | 32 (6) | 33 (10) | 40 (18) | 1 | 18 (6) | | 14 (2) | 15 | 2 | | | | | 1 | 1 | 1 | | 1 | | 214 |
| *Viral* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatitis A | | | | | | | | | | 10 (3) | 2 | | | | | | | | | | | | | | | | 15 |
| *Parasitic* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trichinosis | | | | | | | | 6 | | | | 14 (1) | | 1 | 1 | | | | | | | | | | | | 19 |
| Anisakiasis | | | | | | | | | | | | 2 | | | | | | | | | | | | | | | 2 |
| Toxoplasmosis | | | | | | | | | | | | 2 | | | | | | | | | | | | | | | 2 |
| Amebiasis | | | | | 1 | | | | | (1) | | | | | | | | | | | | | | | | | 1 |
| Diphyllobothriasis | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | 1 |
| Total Parasitic | | | | | 1 | | | 7 | | (1) | | 18 (1) | | 1 | 1 | | | | | | | | | | | | 25 |
| *Toxic Plants* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mushroom poisoning | | | | | | | | | | | 15 | | 15 | | | | | | | | | | | | | | 15 |
| Pokeweek poisoning | | | | | | | | | | | | | 2 | | | 1 | | | | | | | | | | | 2 |
| Burdock root poisoning | | | | | | | | | | | | | 1 | | | | | | 1 | | | | | | | | 1 |
| Atropine poisoning | | | | | | | | | | | | | (1) | | | | | | | | | | | | | | 1 |
| Cyanide poisoning | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Total Toxic Plants | | | | | | | | | | | 15 | | 18 (1) | | | 1 | | | 1 | | | | | | | | 20 |
| *Toxic Fish* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Tetradon | | | | | | | | | | | | | 1 | | | | | | | | | | | 1 | | | 1 |
| *Chemical* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Copper poisoning | | | | 1 | | | | | | | | | | | | | | 15 | | | | | | | | | 15 |
| Monosodium glutamate poisoning | | | | | | | | | | | | | | | | | | | | 6 | | | | | | | 6 |
| Detergent (soap) phosphate poisoning | | | | | | | | | | | | | | | | | | | 3 | | | | | | | | 3 |
| Sodium nitrite | | | | | | | | | | | | | | | | | | | 2 | | | | | | | | 2 |
| Niacin | | | | | | | | | | | | | | | | | | | | 2 | | | | | | 2 | 2 |
| Cadmium poisoning | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | 1 |
| Fluoride poisoning | | | | | | | | | | | | | | | | | | 1 | | 1 | | | | | | | 1 |
| Stropine poisoning | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Histamine poisoning | | | | | | | (1) | | | | | | | | | | | | | | | | | | | | 1 |
| Methyl propyl paraben poisoning | | | | | | | | | | | | | | | | | | | 1 | | | | | | | | 1 |
| Soy hypersensitivity | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | 1 |
| Sulfuric acid poisoning | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | 1 |
| Zinc poisoning | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | 1 |
| Total Chemicals | | | | 1 | | | (1) | | | | | | | | | | 1 | 18 | 6 | 10 | | | | | | 2 | 36 |
| Total diseases of known etiology | 110 (3) | 30 (1) | 1 | 59 | 11 | 1 | 12 (1) | 39 (6) | 33 (10) | 50 (22) | 18 | 36 (7) | 19 (1) | 15 (2) | 16 | 3 | 1 | 18 | 7 | 10 | 1 | 1 | | 1 | 1 | 2 | 311 |
| Percent[1] | 35 | 10 | <1 | 19 | 4 | <1 | 4 | 14 | 14 | 23 | 6 | 14 | 6 | 5 | 5 | 1 | <1 | 6 | 2 | 3 | | | | <1 | <1 | 1 | |
| Diseases of unknown etiology but vehicle identified | 86 (1) | 39 | 1 | 27 | 9 | | | 3 (1) | 24 | (6) | | 1 (1) | | (1) | 8 | | | | | | | | | | | | 116 |
| Total | 196 (4) | 69 (1) | 2 | 86 | 20 | 1 | 12 (1) | 42 (7) | 57 (10) | 50 (28) | 18 | 37 (8) | 19 (1) | 15 (3) | 24 | 3 | 1 | 18 | 7 | 10 | 1 | 1 | 1 | 1 | 1 | 2 | 427 |
| Percent[1] | 46 | 16 | <1 | 20 | 5 | <1 | 3 | 11 | 16 | 18 | 4 | 11 | 5 | 4 | 6 | 1 | <1 | 4 | 2 | 2 | <1 | <1 | <1 | <1 | <1 | <1 | |

[1]More than 100% shows in percentage figures because multiple factors are usually necessary for foodborne disease outbreaks to occur.
[2]Data in parentheses represent factors that were suggested but not confirmed.

TABLE 4. *Factors that contributed to the occurrence of 81 outbreaks of foodborne disease, abstracted in the British Medical Journal, 1969 through 1976.*

| Disease | Incubation | | | | Process failure | | | Contamination | | | | | | | Number of outbreaks with data on contributory factors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inadequate cooling | Inadequate hot storage | Prepared a day or more before serving | Improper thawing | Inadequate cooking | Inadequate reheating | Inadequate thawing | Infected person touching cooked food | Unsafe source | Contaminated raw ingredient | Cross-contamination | Inadequate cleaning of equipment | Infected animals | Can seam opening | |
| *Clostridium perfringens* gastroenteritis | 28 (93)[1] | 3 (10) | 24 (80) | | 5 (17) | 15 (50) | 2 (7) | | | | | | | | 30 |
| Salmonellosis | 16 (63) | 4 (15) | 14 (52) | | 11 (41) | 6 (22) | 1 (4) | 1 (4) | 8 (30) | 11 (41) | 7 (26) | 5 (19) | 1 (4) | | 27 |
| Staphylococcal intoxication | 15 (79) | | 12 (63) | 1 (5) | | | | 11 (58) | | | | 1 (5) | | 4 (21) | 19 |
| *Bacillus cereus* gastroenteritis | 2 (100) | | 1 (50) | | | | 1 (50) | | | | | | | | 2 |
| *Escherichia coli* gastroenteritis | | 1 (50) | | | 1 (50) | 1 (50) | | | 1 (50) | 1 (50) | | | 1 (50) | | 2 |
| Group A streptococcal gastroenteritis | 1 (100) | | 1 (100) | | | | | 1 (100) | | | | | | | 1 |
| Total | 62 | 8 | 52 | 1 | 17 | 23 | 3 | 13 | 9 | 12 | 7 | 6 | 2 | 4 | 81 |
| Percent | (77) | (10) | (64) | (1) | (21) | (28) | (4) | (16) | (11) | (15) | (9) | (7) | (2) | (5) | |

[1]More than 100% shows in percentage figures because multiple factors are usually necessary for outbreaks of foodborne disease to occur.

establishments, and 26.6% were mishandled in homes. (*29*). (Percentages were calculated with denominator of number of places where foods were known to be mishandled.)

The factors that occasioned outbreaks of foodborne disease when foods were mishandled in foodservice establishments in the United States during 1973 through 1976 are (in order of frequency of occurrence): inadequate cooling of foods, insufficiently high temperatures during hot-storage of foods, lapse of a day or more between preparing and serving food, infected person having touched foods which are not subsequently heat-processed, inadequate time or temperature or both during reheating of previously cooked foods, and other factors listed in Table 5.

Factors that occasioned outbreaks of foodborne disease when foods were mishandled in homes in the United States during 1973 through 1976 are (in order of frequency of occurrence): inadequate cooling, inadequate time or temperature or both during canning or cooking, mistaking toxic species of mushrooms and other plants for edible varieties, obtaining foods from unsafe sources, lapse of a day or more between preparing and serving, and other factors listed in Table 6.

Factors that have contributed to the occurrence of outbreaks of foodborne disease which resulted from foods mishandled in food processing plants are presented in Table 7 and have been reviewed previously (*6*).

Factors that contribute to outbreaks of foodborne disease form general patterns depending on the classification of the etiologic agent. Table 8 shows that factors that affect growth, survival, and contamination (all) frequently occur in outbreaks of bacterial foodborne illness. Factors that affect survival and contamination are of concern in outbreaks caused by parasites. Factors that affect contamination are of major concern in outbreaks caused by viruses, toxic plants, and chemicals.

Factors that contribute to outbreaks of any specific foodborne disease are unique for that disease. As data are collected over the years, patterns develop as indicated in Table 3-7 and Table 9. These can be studied and methods of prevention and control devised and priorities set.

*Staphyloenterotoxicosis*

Factors that most often contributed to outbreaks of staphylococcal food poisoning are inadequate cooling of foods, infected persons having touched cooked foods, and lapse of a day or more between preparing and serving. These factors and other less important factors are listed in Table 3-7 and Table 9.

Although *S. aureus* is sometimes isolated from raw meat and poultry, food workers or homemakers appear to be the main source of the organisms that contaminate foods which become vehicles of outbreaks of staphyloenterotoxicosis. Many persons carry *S. aureus* in their anterior nares; their hands readily become contaminated. Staphylococci are resident as well as transient on skin (*9*). Most persons who contaminate foods are nasal carriers; only occasionally does the contaminating organism come from cuts, burns, or boils (*2, 9, 19*).

Vehicles are usually foods that are rich in protein. Several particular amino acids and thiamine and niacin

TABLE 5. *Factors that contribute to the occurrence of 235 outbreaks of foodborne disease where mishandling occurred in foodservice establishments, 1973 through 1976.*

| Diseases | Factors affecting growth | | | | | Factors affecting survival | | Factors affecting contamination | | | | | | | | | Number of outbreaks with data on factors that contribute to outbreaks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inadequate cooling | Inadequate hot storage | Inadequate thawing | Prepared a day or more before serving | Use of leftovers | Inadequate cooking | Inadequate reheating | Infected person | Unsafe source | Contaminated raw ingredient | Cross-contamination | Inadequate cleaning of equipment | Contaminated water | Toxic containers | Incidental additives | Intentional additives | |
| *Bacterial* | | | | | | | | | | | | | | | | | |
| Staphylococcal intoxication | 38 (1)[2] | 7 | | 23 | 1 | 2 (1) | (7) | 22 (7) | | (1) | 1 | 3 | | | | | 45 |
| Salmonellosis | 19 (1) | 7 | 1 | 10 | 3 | 1 | 14 | 3 (3) | | 1 (1) | 8 (1) | 9 | | | | | 28 |
| *Clostridium perfringens* gastroenteritis | 12 | 8 (1) | | 9 | 3 | 3 (1) | 11 | | | | (1) | | | | | | 18 |
| Shigellosis | 1 | | | 2 | | | 2 | 5 (1) | | | | | | | | | 6 |
| *Vibrio parahaemolyticus* gastroenteritis | 2 | | | | | | | | | | 2 | | 1 | | | | 3 |
| *Bacillus cereus* gastroenteritis | 2 | | | | | | 1 | | | | | | | | | | 2 |
| Group A *Streptococcus* infections | 2 | | | | | | | 2 | | | | | | | | | 2 |
| Typhoid fever | | 1 | | | | | | 1 | | | | | | | | | 2 |
| Total Bacterial | 76 (2) | 25 (2) | 1 | 47 | 9 | 6 (2) | 28 (7) | 33 (11) | | 1 (2) | 11 (2) | 12 | 1 | | | | 106 |
| *Viral* | | | | | | | | | | | | | | | | | |
| Hepatitis A | | | | | | | | 8 (3) | | 2 | | | | | | | 13 |
| *Parasitic* | | | | | | | | | | | | | | | | | |
| Amebiasis | | | | 1 | | | | (1) | | | | | | | | | 1 |
| Toxoplasmosis | | | | | | | | | | 1 | | | | | | | 1 |
| Trichinosis | | | | | | | | | | 1 | 1 | 1 | | | | | 1 |
| Total Parasitic | | | | 1 | | | | (1) | | 2 | 1 | 1 | | | | | 3 |
| *Chemical* | | | | | | | | | | | | | | | | | |
| Copper poisoning | | | | | | | | | | | | | | 8 | | | 8 |
| Monosodium glutamate poisoning | | | | | | | | | | | | | | | | 4 | 4 |
| Detergent/soap/phosphate poisoning | | | | | | | | | | | | | | | 3 | | 3 |
| Fluoride poisoning | | | | | | | | | | | | | | | | 1 | 1 |
| Zinc poisoning | | | | | | | | | | | | | | 1 | | | 1 |
| Total Chemical | | | | | | | | | | | | | | 9 | 3 | 5 | 17 |
| Total diseases of known etiology | 76 (2) | 25 (2) | 1 | 47 | 10 | 6 (2) | 28 (7) | 41 (15) | 2 | 3 (2) | 12 (2) | 13 | 1 | 9 | 3 | 5 | 139 |
| Percent[1] | 56 | 19 | 1 | 34 | 7 | 6 | 25 | 40 | 1 | 4 | 10 | 9 | 1 | 6 | 2 | 4 | |
| Diseases of unknown etiology but vehicle identified | 72 (1) | 37 | 1 | 21 | 6 | 3 (1) | 24 | (5) | | | (1) | 8 | | | | | 96 |
| Total | 148 (3) | 62 (2) | 2 | 68 | 16 | 9 (3) | 52 (7) | 41 (20) | 2 | 3 (2) | 12 (3) | 21 | 1 | 9 | 3 | 5 | 235 |
| Percent[1] | 63 | 27 | 1 | 29 | 7 | 5 | 25 | 26 | 1 | 2 | 6 | 9 | <1 | 4 | 1 | 2 | |

[1]More than 100% shows in percentage figures because multiple factors are usually necessary for foodborne disease outbreaks to occur.
[2]Data in parentheses represent factors that were suggested but not confirmed.

TABLE 6. *Factors that contributed to the occurrence of 122 outbreaks of foodborne disease where mishandling occurred in homes, 1973 through 1976.*

| Diseases | Factors affecting growth | | | | | Factors affecting survival | | Factors affecting contamination | | | | | | | | | | Number of outbreaks with data on factors that contributed to outbreaks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inadequate cooling | Inadequate hot storage | Prepared a day or more before serving | Use of leftovers | Faulty fermentations | Inadequate cooking or canning | Inadequate reheating | Infected person | Unsafe source | Contaminated raw ingredient | Toxic species mistaken for edible varieties | Cross-contamination | Inadequate cleaning of equipment | Poor storage practices | Faulty sealing | Toxic containers | Improper evisceration of toxic fish | Intentional additives | |
| *Bacterial* | | | | | | | | | | | | | | | | | | | |
| Botulism | 1 | 2 | | | 7 | 18 (1) | 2 | | | | | | | | 1 | | | | 30 |
| Salmonellosis | 6 | 1 | | | | 2 (1) | | (1) | | 7 (4) | | 3 | 1 | | | | | | 20 |
| Staphylococcal intoxication | 12 (1)[2] | 2 | 6 | | | | (2) | 4 (3) | | | | | | | | | | | 15 |
| *Clostridium perfringens* gastroenteritis | 6 | 1 | 4 | 1 | | | 3 | | | | | | | | | | | | 7 |
| Typhoid fever | | | | | | 1 | | (1) | | | | | | | | | | | 2 |
| Brucellosis | | | | | | | | | | 1 | | | | | | | | | 1 |
| Cholera | 1 | | | | 1 | | | | 1 | 1 | | | | | | | | | 1 |
| Shigellosis | | | | | | | | (1) | | | | | | | | | | | 1 |
| Total Bacterial | 26 (1) | 6 | 10 | 1 | 8 | 21 (2) | 5 (2) | 4 (6) | 1 | 9 (4) | | 3 | 1 | | 1 | | | | 72 |
| *Viral* | | | | | | | | | | | | | | | | | | | |
| Hepatitis A | | | | | | | | | | | | | | | | | | | 2 |
| *Parasitic* | | | | | | | | | | | | | | | | | | | |
| Trichinosis | | | | | | 5 | | | | 12 (1) | | | | | | | | | 16 |
| Anisakiasis | | | | | | | | | | 2 | | | | | | | | | 2 |
| Diphyllobothriasis | | | | | | 1 | | | | | | | | | | | | | 1 |
| Toxoplasmosis | | | | | | | | | | 1 | | | | | | | | | 1 |
| Total Parasitic | | | | | | 6 | | | | 15 (1) | | | | | | | | | 20 |
| *Toxic Plants* | | | | | | | | | | | | | | | | | | | |
| Mushroom poisoning | | | | | | | | | 15 | | 15 | | | | | | | | 15 |
| Burdock root poisoning | | | | | | | | | | | 1 | | | | | | | | 1 |
| Cyanide poisoning | | | | | | | | | | | (1) | | | | | | | | 1 |
| Pokeweed poisoning | | | | | | | | | | | 1 | | | | | | | | 1 |
| Total Toxic Plants | | | | | | | | | 15 | | 17 (1) | | | | | | | | 18 |
| *Toxic Fish* | | | | | | | | | | | | | | | | | | | |
| Tetradon | | | | | | | | | | | 1 | | | | | | 1 | | 1 |
| *Chemical* | | | | | | | | | | | | | | | | | | | |
| Copper poisoning | | | | | | | | | | | | | | | | 4 | | | 4 |
| Monosodium glutamate poisoning | | | | | | | | | | | | | | | | | | 2 | 2 |
| Cadmium poisoning | | | | | | | | | | | | | | | | 1 | | | 1 |
| Lead poisoning | | | | | | | | | | | | | | | | 1 | | | 1 |
| Sulfuric acid poisoning | | | | | | | | | | | | | | 1 | | | | | 1 |
| Total chemical | | | | | | | | | | | | | | 1 | | 6 | | 2 | 9 |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total disease of know etiology | 26 (1) | 6 | 10 | 1 | 8 | 27 (2) | 5 (2) | 4 (6) | 16 | 24 (5) | 18 (1) | 3 | 1 | 1 | 1 | 6 | 1 | 2 | 122 |
| Percent[1] | 22 | 5 | 8 | 1 | 6 | 24 | 6 | 8 | 13 | 24 | 16 | 2 | 1 | 1 | 1 | 5 | 1 | 2 | |
| Diseases of unknown etiology but vehicle identified | 15 | 2 | 5 | 4 | | | | (1) | | 2 | | | | | | | | | 19 |
| Total | 41 (1) | 8 | 15 | 5 | 8 | 27 (2) | 5 (2) | 4 (7) | 16 | 26 (5) | 18 (1) | 3 | 1 | 1 | 1 | 6 | 1 | 2 | 141 |
| Percent[1] | 30 | 6 | 11 | 4 | 6 | 21 | 5 | 8 | 11 | 22 | 13 | 2 | 1 | 1 | 1 | 4 | 1 | 1 | |

[1]More than 100% shows in percentage figures because multiple factors are usually necessary for foodborne outbreaks to occur.
[2]Data in parentheses represent factors that were suggested but not confirmed.

TABLE 7. *Factors that contributed to foodborne outbreaks where mishandling occurred in food processing plants.*

| | Factors affecting growth | | | | Factors affecting contamination | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diseases | Inadequate cooling | Growth during germination of seed and development of plant | Faulty fermentations | Inadequate heat processing | Infected person | Contaminated raw ingredient | Inadequate cleaning of equipment | Contamination by fertilizer and soil | Incidental additives | Intentional additives | Unknown post-process contamination | Number of outbreaks with data on factors that contributed to outbreaks |
| *Bacillus cereus* gastroenteritis | | 1 | | | | | | | | | | 1 |
| Botulism | 1 | | 1 | 1(2) | | | | | | | | 4 |
| Brucellosis | | | | | | 2 | | | | | | 2 |
| Salmonellosis | | | 2 | 3 | (1) | 6 | 1 | 1 | | | | 12 |
| Staphylococcal intoxication | 4 | | 1 | 1 | 2 | | 1 | | | | | 7 |
| *Yersinia enterocolitica* gastroenteritis | | | | | | | | | | | 1 | 1 |
| Trichinosis | | | | 1 | | | | | | | | 1 |
| Chemical | | | (1) | | | | | | 2 | 1 | | 4 |
| Total | 5 | 1 | 4 (1) | 6(2) | 2(1) | 8 | 2 | 1 | 2 | 1 | 1 | 32 |
| Percent | 16 | 3 | 16 | 25 | 9 | 25 | 6 | 3 | 6 | 3 | 3 | |

TABLE 8. *Percent[1] of factors that contributed to the occurrence of outbreaks of several classes of foodborne diseases reported in public health literature or surveillance data during 1961 through 1976.*

| Factor | Diseases | | | | |
|---|---|---|---|---|---|
| | Bacterial (707)[2] | Viral (44) | Parasitic (72) | Toxic plants (44) | Chemical poisonings (79) |
| *Factors affecting growth* | | | | | |
| Inadequate cooling | 55 | | | | |
| Inadequate hot storage | 16 | | | | |
| Inadequate thawing | <1 | | | | |
| Growth during germination | <1 | | | | |
| Faulty fermentation | 2 | | | | 1 |
| Lapse of day or more between preparing and serving[3] | 26 | | | | |
| Use of leftovers[3] | 4 | | 1 | | 1 |
| *Factors affecting survival* | | | | | |
| Inadequate cooking, thermal processing, or canning | 20 | 2 | 59 | | |
| Inadequate reheating | 14 | | | | 1 |
| *Factors affecting contamination* | | | | | |
| Infected persons | 25 | 66 | 1 | | |
| Unsafe source | 1 | 36 | | 52 | |
| Contaminated raw ingredient | 13 | | 64 | | |
| Toxic species mistaken for edible varieties | | | | 66 | |
| Cross contamination | 9 | | 8 | | |
| Inadequate cleaning of equipment | 8 | | 8 | | |
| Toxic containers | | | | | 47 |
| Incidental additives | | | | 9 | 15 |
| Intentional additives | | | | | 34 |
| Selling contaminated products or toxic foods | <1 | | | 2 | |
| Poor storage practices | | | | | 6 |
| Contamination of washed dishes | | 2 | | | |
| Unknown post-processing contamination | <1 | | | | |
| Contamination by fertilizer or soil | 1 | | | | |
| Faulty sealing of cans or jars | <1 | | | | |
| Improper evisceration | <1 | | | | |
| Contaminated water | <1 | | | | |
| Misbranding | | | | | 3 |

[1]Percentages tabulated for each class of diseases separately. More than 100% show in percentage figures because multiple factors are usually necessary for outbreaks of foodborne disease to occur.
[2] Number of outbreaks on which data are based.
[3]Indirect factors that influence situation only when other factors occur.

are necessary for the growth of staphylococci and production of enterotoxin. Many of the vehicles (such as (custard or ham) contain relatively high concentrations of sugar or salt; thus, they have a lower water activity than is optimum for most bacteria. Such substances inhibit the growth of many bacteria, but not staphylococci (*8, 9*). Foods that have been implicated in outbreaks of staphylococcal food poisoning have usually been cooked and then cut, sliced, mixed, grated, ground, or otherwise handled by persons who are carriers of enterotoxigenic strains of *S. aureus* (*9, 16*). The cooking is itself often an important factor that indirectly contributes to outbreaks because *S. aureus* does not compete successfully with the bacteria that are commonly found in raw foods. Cooking reduces or eliminates the nonsporeforming bacterial flora, including staphylococci. After cooked foods are subsequently handled and thus contaminated, they must remain at room temperature or be refrigerated in such bulk as to retain heat for a sufficient time for *S. aureus* to multiply and produce enterotoxin in the absence of numerous competitive organisms. Staphyloenterotoxins are not destroyed by the usual reheating in routine food processing and service operations. (*9*).

*Salmonellosis*

Factors that have most often contributed to outbreaks of salmonellosis are inadequate cooling of foods, ingesting contaminated raw foods or ingredients, inadequate time or temperature or both during heat processing of foods, cross-contamination from raw foods (frequently raw meat, raw poultry, eggshells, and unpasteurized egg products) to cooked foods, lapse of a day or more between preparing and serving, inadequate cleaning of equipment, insufficiently high temperatures during hot storage, infected person having touched foods which are not subsequently heat processed, and inadequate time or temperature or both during reheating of previously cooked foods. These factors and other less frequently reported factors are listed in Tables 3-7 and Table 9.

Current epidemiologic evidence indicates that meat-

TABLE 9. *Percent*[1] *of factors that contributed to the occurrence of outbreaks of several bacterial foodborne diseases reported in public health literature or surveillance data during 1961 through 1976.*

| Factor | Bacterial Diseases | | | | | | |
|---|---|---|---|---|---|---|---|
| | Salmonellosis (238)[2] | Staphylococcal intoxication (214) | *Clostridium perfringens* gastroenteritis (93) | Botulism (85) | Shigellosis (27) | Typhoid fever (14) | *Vibrio parahaemolyticus* gastroenteritis (12 |
| *Factors affecting growth* | | | | | | | |
| Inadequate cooling | 47 | 78 | 76 | 13 | 56 | 7 | 67 |
| Inadequate hot-holding | 14 | 18 | 46 | 2 | | 7 | |
| Lapse of day or more between preparing and serving | 17 | 44 | 51 | | 22 | | |
| Use of leftovers | 4 | 3 | 12 | | 4 | | |
| Faulty fermentations | 1 | | | 9 | | | |
| *Factors affecting survival* | | | | | | | |
| Inadequate cooking, heat processing, canning | 21 | 3 | (9)[3] | 80 | | 7 | |
| Inadequate reheating | 13 | (7)[3] | 45 | 2 | 7 | | |
| *Factors affecting contamination* | | | | | | | |
| Infected person | 13 | 53 | | | 89 | 79 | |
| Contaminated raw ingredients | 32 | | | | | | 42 |
| Cross-contamination | 21 | 3 | 2 | | | | 33 |
| Inadequate cleaning of equipment | 15 | 9 | 1 | | | | |
| Unsafe source | 1 | | | | | 14 | 17 |
| Contaminated water | | | | | | | 8 |

[1]Percentages tabulated for each group of diseases separately. More than 100% shows in percentage figures because multiple factors are usually necessary for outbreaks of foodborne disease to occur. Factors occurring in less than 1% of outbreaks omitted.
[2]Number of outbreaks in which data are based.
[3]Poor practices reported but probably would not have altered outcome of outbreak.

source animals are major reservoirs of salmonellae. Animals become infected either from feed which frequently contains salmonellae or from their environment which has been contaminated by previous flocks or herds. The infection rate increases when swine or cattle are transferred to slaughter and held in pens. A few infected (or superficially contaminated) animals are the source of salmonellae that are spread from fecal matter to many carcasses. Considerable contamination occurs early in processing, during defeathering of fowl or dehairing of swine. Washing reduces the level of contamination, but subsequent handling enhances the possibility of cross-contamination, which increases the number of contaminated carcasses. The primary sources of salmonellae in retail stores, food processing establishments, and homes are poultry carcasses, cuts of meat, and unwashed eggshells. Consequently, poultry, meat, and eggs have been more commonly associated with outbreaks than have other foods (*2, 12, 23*).

Foods that have been vehicles in outbreaks of salmonellosis usually become contaminated by salmonellae in one of the following ways: (*a*) animal and fowl carcasses are contaminated with fecal matter during processing; (*b*) foods that receive no further heat treatment or that are inadequately heated are contaminated by raw ingredients, such as checked or cracked raw eggs that already contain salmonellae or have them on their shells; (*c*) cooked foods become contaminated by touching unwashed or inadequately cleaned equipment that has been used to process contaminated raw foods of animal origin or by persons who have previously handled such contaminated foods (cross-contamination); and, to a lesser extent (*d*) human carriers who practice poor personal hygiene. It has been difficult to determine during outbreak investigations whether infected food workers were carriers and the source of contamination or whether they had eaten the same foods as did the other persons who had become ill or had handled the contaminated food.

To become dangerous, foods contaminated by salmonellae usually must be held long enough at suitable temperatures for salmonellae to multiply to sufficient numbers to cause infection. In most episodes of salmonellosis, the ill have ingested millions of salmonellae. But smaller numbers of some serotypes can apparently cause illness in susceptible persons (*1, 10, 13, 17*).

*Clostridium perfringens gastroenteritis*

Factors that most often contributed to outbreaks of *C. perfringens* gastroenteritis are inadequate cooling of foods (meat, poultry, gravy, stock), lapse of a day or more between preparing and serving, inadequate time or temperature or both during hot storage of foods, and inadequate time or temperature or both during reheating of previously cooked foods. These and other less important factors are listed in Tables 3-7 and Table 9.

*C. perfringens* is commonly found in the intestinal tract of man and animals and in dust and soil (*15*). During processing, meat and poultry frequently become contaminated by vegetative cells or spores of *C. perfringens* from one or more of these sources. Spores of some strains of *C. perfringens* survive boiling for 4 to 6 h; so, they survive the time-temperature effects of most cooking operations. Furthermore, cooking drives off oxygen; this can reduce the redox potential of the food to a level at which *C. perfringens* can initiate growth. Cooking also kills competitive organisms and heat-shocks any *C. perfringens* spores (that are present so that a greater number of *C. perfringens* spores) germinate than would occur without heating. Because this organism is commonly found in the environment of or on equipment in foodservice establishments, as well as in the intestinal tracts and on the hands of workers, foods can easily become contaminated after cooking (*11*). Spores that survive cooking must germinate and vegetative cells that evolve or that have contaminated cooked foods must multiply to sufficient numbers in the food to cause illness when ingested.

*C. perfringens* can grow only if several particular amino acids and vitamins are available to it. Few foods, other than meat and poultry (or their stock or gravy) contain the necessary nutrients. *C. perfringens* will multiply in a protein-rich substrate when such foods are stored for several hours at room temperature, stored in large pots in a refrigerator, or stored in a warming device that holds the food at 50 C or below (*4, 14, 15*).

*Botulism*

Outbreaks of botulism result when *Clostridium botulinum* survives the effects of time-temperature exposure during thermal processing, and when this organism multiplies and produces neurotoxin in the anaerobic environment of cans, jars, plastic bags, or bulk food (Table 3, 6, and 8). Inadequate time or temperature or both during cooking (as the failure to process hot-packed jars in pressure cookers) of home-preserved foods still contributes to most outbreaks. Other outbreaks, however, have been traced to fermented, smoked, and dried foods which are inadequately processed. Sources of *C. botulinum* are soil, mud, or water from which foods are grown and harvested (*21, 22*).

*Vibrio parahaemolyticus gastroenteritis*

*Vibrio parahaemolyticus* is found in high numbers in warm sea waters, and is frequently isolated from raw marine seafoods (*18, 20*). Several factors usually contribute to outbreaks (Tables 3, 5 and 9). Inadequate refrigeration has contributed to multiplication of *V. parahaemolyticus*. Inadequate time or temperature or both during cooking has allowed these organisms to survive in seafoods. Handling raw seafoods and then cooked foods and processing them on the same equipment have recontaminated the cooked foods.

*Other foodborne diseases*

In outbreaks of shigellosis, typhoid fever, and streptococcal pharyngitis, foods are usually contaminated by infected workers who practice poor personal hygiene (Table 3-7, and Table 9). The contaminated

food frequently is either not refrigerated or inadequately refrigerated. Occasionally the practice of obtaining foods from unsafe sources (e.g., shellfish from contaminated bays) has led to outbreaks of typhoid fever (*3*). Foodborne hepatitis A occurs either after sewage contaminates waters from which oysters or clams (which are eaten raw) are harvested or after infected persons touch foods which are not subsequently heat-processed. Factors that contribute to outbreaks of trichinosis are either inadequate time or temperature or both during cooking or ingestion of raw pork (and sometimes other meat such as bear meat and rarely beef or mutton if cross-contamination from infested pork occurred in grinding machines).

## CONCLUSIONS

Factors that contribute to the occurrence of foodborne disease which are derived from epidemiologic data are compatible with information that is derived from the study of the ecology, biology, and toxicology of etiologic agents of foodborne disease. This information should be used to plan and direct food protection programs and to set priorities based on prevention of contemporary disease problems.

### REFERENCES


1. Armstrong, R. W., T. Fodor, G. T. Curtis, A. B. Cohen, W. T. Martin and J. Feldman. 1970. Endemic *Salmonella* gastroenteritis due to contaminated imitation ice cream. Amer. J. Epidemiol. 91:300-307.
2. Bryan, F. L. 1968. What the sanitarian should know about salmonellae and staphylococci in non-dairy foods. Part I, Staphylococci; Part II, Salmonellae. J. Milk Food Technol. 31: 110-116; 131-140.
3. Bryan, F. L. 1969. Infections due to miscellaneous microorganisms. *In*: H. Riemann (ed.) Foodborne infections and intoxications. Academic Press, New York.
4. Bryan, F. L. 1969. What the sanitarian should know about *Clostridium perfringens* foodborne illness. J. Milk Food Technol. 32:381-389.
5. Bryan, F. L. 1972. Emerging foodborne diseases. II. Factors that contribute to outbreaks and their control. J. Milk Food Technol. 35:632-638.
6. Bryan, F. L. 1974. Microbiological food hazards today--based on epidemiological information. Food Technol. 28:52-66, 84.
7. Bryan, F. L. 1975. Status of foodborne disease in the United States. J. Environ. Health 38:74-83.
8. Bryan, F. L. 1976. Public health aspects of cream-filled pastries. A. review. J. Milk Food Technol. 39:289-296.
9. Bryan, F. L. 1976. *Staphylococcus aureus*. *In* M. deFigueriredo and D. F. Splittstoesser. (eds.) Food microbiology: Public health and spoilage aspects. AVI, Westport, Conn.
10. Bryan, F. L. 1977. Diseases transmitted by foods contaminated by wastewater. J. Food Prot. 40:45-56.
11. Bryan, F. L., and E. G. Kilpatrick. 1971. *Clostridium perfringens* related to roast beef cooking, storage, and contamination in a fast food service restaurant. Amer. J. Public Health. 61:1869-1885.
12. Cohen, M., and P. Blake. 1977. Trends in foodborne salmonellosis outbreaks 1963-1975. J. Food Prot. 40:798-800.
13. Craven, P. C., D. C. Mackel, W. B. Baine, W. H. Barnes, E. J. Gangerosa, M. Goldfield, H. Rosenfeld, R. Altman, G. Lachapelle, J. W. Raines, and R. C. Swanson. 1975. International outbreak of *Salmonella eastbourne* infection traced to contaminated chocolate. Lancet 1:788-793.
14. Duncan, C. L. 1976. *Clostridium perfringens*. *In*: M. deFigueriredo and D. F. Splittstoesser, (eds.) Food microbiology: Public health and spoilage aspects. AVI Westport, Conn.
15. Hobbs, B. C. 1969. *Clostridium perfringens* and *Bacillus cereus* infections. *In*: H. Riemann, (ed.) Food-borne infections and intoxications. Academic Press, New York.
16. Hodge, B. E. 1960. Control of staphylococcal food poisoning. Public Health Rep. (U.S.) 75:355-361.
17. Lang, D. J., L. S. Kunz, A. R. Martin, S. A. Schroeder and A. Thompson. 1967. Carmine as a source of nosocomial salmonellosis. New Engl. J. Med. 276:829-832.
18. Liston, J. 1976. *Vibrio parahaemolyticus*. *In*: M. deFigueriredo and D. F. Splittstoesser (eds.) Food microbiology-Public health and spoilage aspects. AVI, Westport, Conn.
19. Minor, T. E., and E. H. Marth. 1976. Staphylococci and their significance in foods. Elsevier Scientific, New York.
20. Miwatani, T., and Y. Takeda. 1976. *Vibrio parahaemolyticus*. A causative bacterium of food poisoning. Saikon, Tokyo.
21. Reimann, H. 1969. Botulism types A, B, and F. In: H. Riemann (ed.) Foodborne infections and intoxications. Academic Press, New York.
22. Sakaguchi, G. 1969. Botulism-type E. In: H. Riemann (ed.) Foodborne infections and intoxications. Academic Press, New York.
23. Troller, J. A. 1976. Salmonella and Shigella. In: M. deFigueiredo and D. F. Splittstoesser (eds.) Food microbiology: Public health and spoilage aspects. AVI, Westport, Conn.
24. U.S. Department of Health, Education, and Welfare. 1961-1977. Morbidity and Mortality Weekly Report, Center for Disease Control, Atlanta.
25. U.S. Department of Health, Education, and Welfare. 1962-1977. Salmonella Surveillance Report. Center for Disease Control, Atlanta.
26. U.S. Department of Health, Education, and Welfare. 1960-1977. Hepatitis Surveillance Report. Center for Disease Control, Atlanta.
27. U.S. Department of Health, Education, and Welfare. 1964-1977. Shigella Surveillance Report. Center for Disease Control, Atlanta.
28. U.S. Department of Health, Education, and Welfare. 1967-1977. Trichinosis Surveillance Annual Summary. Center for Disease Control, Atlanta.
29. U.S. Department of Health, Education, and Welfare. 1974-1976. Foodborne and Waterborne Disease Outbreaks. Center for Disease Control, Atlanta.

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# Abstracts of Papers Presented at the Sixty-fifth Annual Meeting of IAMFES

## Kansas City, Missouri, August 13-17, 1978

Abstracts of all papers given at the 65th Annual Meeting appear on this and the following pages. The complete text of many of these papers will appear in future issues of the *Journal of Food Protection*.

### CONTRIBUTED PAPERS

**The Environmental Health Professional - Can He Communicate and Motivate?** C. Dee Clingman, *National Institute for the Foodservice Industry, 120 S. Riverside Plaza, Chicago, Illinois 60606.*

The environmental health professional is a public personality. He or she must be well trained, educationally competent and possess a sound "sense" of effective public relations. Communicating successfully among and between fellow professionals, industry and the public is a must. Similarly, motivating staff workers, industry and especially oneself is an essential key in becoming a respected environmental health professional. These necessary capabilities are not inherent, but are learned and refined through review and practice. Their mastery is the culmination of professional dedication.

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**Development of a Reciprocity System for Foodservice Management Training and Certification Programs.** Patricia Franks, *National Institute for the Foodservice Industry, 120 S. Riverside Plaza, Chicago, Illinois 60606.*

The National Institute for the Foodservice Industry, under a contract with the U.S. Food and Drug Administration, is developing a reciprocity system for foodservice manager certification programs, in cooperation with several participating states. This project will establish minimum levels of course content and program administration among and between the participating states. Such uniformity will result in similar and equivalent programs and will lead to the establishment of a national reciprocity mechanism.

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**Cost of Quality in a Food Plant.** C. S. Gelda, *The Borden Company, Limited, Technical Center, P.O. Box 7, 95 Townline Road, Tillsonburg, Ontario, Canada N4G 4H3.*

The paper deals with identification and control of factors such as waste, use of substandard products, warranty services, and inspection and laboratory costs in food operations. It provides a guideline to monitor such costs and relate them to production efficiency.

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**Toxicity of Chlorinated Compounds Found in Drinking Water Assessed by a Fertile Egg Injection Technique.** M. Hekmati, R. L. Bradley, Jr. and M. L. Sunde, *Departments of Food Science and Poultry Science, University of Wisconsin, Madison, Wisconsin 53706.*

Organohalides occur in some drinking waters after chlorination because of the haloform reaction. Public concern has been focused on the presence of these organohalides because some are suspected carcinogens. Apparently contamination with these chemicals can occur at the time of chlorination when water from highly polluted sources is used. Some research evidence shows as many as 46 chlorine-containing compounds in the primary stage of sewage treatment where chlorination is practiced. Chlorinated and/or brominated methane, ethane, and benzene and methyl iodide were used in this study. Dilutions were made using 1:1 (v/v) propylene glycol and ethanol. Injections were made into fertile chicken eggs using the method of Duthachie and Fletcher. Mortality assessed each week during incubation and hatchability were used as indices of toxicity. Toxicity was calculated on the basis of $LD_{50}$ using a designation of extremely toxic when the amount to cause $LD_{50}$ was <1 mg/kg of body weight to practically nontoxic when $LD_{50}$ was >5 g/kg. Results showed that 1,3,5-trichlorobenzene and chloroform were extremely toxic, while the remaining chemicals were rated highly toxic; 1,2-dichloroethane, *o* and *p*-dichlorobenzene, dichlorobromomethane and methyl iodide. The results of this screening test indicate the need for further study with other animal species.

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**Extract-Release Volume Method as a Test of Microbial Quality of Chicken.** Y-W Huang, and J. C. Ayres, *Department of Food Science, University of Georgia, Athens, Georgia 30602.*

The extract-release volume (ERV) of ground lean chicken meat decreases directly as the meat undergoes spoilage. Decreases in ERV of chicken meat were shown to be accompanied by an increase in bacterial numbers and in pH values. Distilled water or .5% peptone solution at pH 5.8 are suggested as the extractives of choice and 37 C as the temperature to be employed. Since the ERV of chicken meat was less than that of other kinds of meat such as pork, fish, shellfish or beef, it is suggested that ERV's be collected for 30 min rather than 15. Under these conditions, chicken meat that produces ERV values less than 20 ml can be considered spoiled. ERV responds to pH changes and, regardless of the microbial quality of the meat, maximum ERV values occur at pH 5.4.

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**Stability of Polyvinylchloride Tubing in Dairy Processing Operations.** Jane S. Mueller and R. L. Bradley, Jr., *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

The dairy industry uses long lengths of polyvinylchloride (PVC) tubing to convey products otherwise sent through a maze of stainless steel fittings and pipes. PVC tubing is flexible because of external plasticizers used in its manufacture. Since these plasticizers, esters of phthalic acid (PAE), are not bound chemically in the physical matrix of PVC, removal is facilitated with a resulting loss in tubing flexibility. Microbial invasion can occur when the surface becomes brittle enough to check and crack. Tygon tubing, formulation B-44-4x approved for processed milk and milk products, cut in 10-cm squares was soaked in water at 16, 44, and 71 C; milk at 38 C; chlorinated alkaline cleaners at 62 C; sodium hypochlorite at 24 C; iodophor at 24 C; and "tamed" chlorine at 24 C. Timed intervals of immersion in the laboratory were calculated to be equivalent to an exposure of 1 day under average plant use conditions. Solutions used for soaking were extracted using the method for organochlorine chemicals in industrial effluents and assayed by gas-liquid chromatography with an electron capture detector. Calculations were made from standard curves of three PAEs, dibutylphthalate, diethylhexylphthalate, and diethylhexylisophthalate. Results showed leaching of PAEs by all solutions used. Curves showing amounts of PAE extracted were approximately similar in shape but differed markedly in the magnitude of extractant showing an initial large amount of extractant followed by a slope asymptotic with time. Milk leached the least.

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**Ochratoxin Production by a Penicillium Species Isolated from Cheese.** Mary M. Pohlmeier* and L. B. Bullerman, *Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68483.*

A *Penicillium* sp., isolated from Cheddar cheese and identified as *Penicillium commune*, was found to be a strong ochratoxin producing organism. Toxin production was verified using thin layer chromatography, ultraviolet and infrared absorption, and mass spectrophotometry. Biological activity and toxicity were verified using chicken embryos and 1-day old white Leghorn cockerels. The $LD_{50}$ to chick embryos was found to be 10-20 μg/egg. Ochratoxin production was studied using a basal salts broth, yeast-extract sucrose broth and several foods. The mold grew well over a range of temperatures, but ochratoxin production was best at 25°C on YES broth, and did not occur at 5°C in any of the broth substrates. Toxin production at 12°C was greatly reduced in amounts when compared with toxin production at 25° C, indicating temperature to be an important variable to toxin production by the mold. The mold grew extremely well on substrates containing lactate and a cheese extract but produced little or no ochratoxin. This indicates that conditions which favor heavy growth do not necessarily also favor ochratoxin production. Although the mold grew extensively and had heavy sporulation on the foods at all temperatures studied, it produced varying amounts of ochratoxin.

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**Inhibition of *Vibrio parahaemolyticus* by Sorbic Acid in Crab Meat and Flounder Homogenates.** M. C. Robach and C. S. Hickey, *Monsanto Company, 800 N. Lindbergh Blvd. Saint Louis, Missouri 63166.*

The effect of sorbic acid on growth of three strains of *Vibrio parahaemolyticus* was studied using both a crab meat and flounder homogenate (pH 6.2) incubated at 35 C. Addition of 0.05% sorbic acid resulted in delayed growth of all three strains of *V. parahaemolyticus* in both crab meat and flounder homogenates. When 0.1% sorbic acid was incorporated into the homogenates, no increase in numbers of the three strains was observed in the crab meat homogenate, and only slight increases in cell numbers were noted in the flounder homogenate. Results of this study indicate that sorbic acid is an effective growth inhibitor of *V. parahaemolyticus* in a model seafood system under conditions favorable for rapid growth of the organism.

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**Inhibition of *Clostridium botulinum* Type A and B Spores by Phenolic antioxidants.** M. C. Robach and M. D. Pierson, *Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, Missouri 63166, and Dept. of Food Science and Technology, Virginia Polytechnic Institute and State Univ., Blacksburg, Virginia 24061.*

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) were tested against three strains of *Clostridium botulinum* spores in prereduced thiotone yeast-extract glucose medium (TYG; pH 7.0) at 37 C. There was outgrowth and toxin production from strain 10755A spores when 25 ppm of BHA were present in the medium, while growth was inhibited by 50 ppm of BHA. Strains 62A and 213B were inhibited by 25 ppm of BHA throughout an incubation period of 7 days. Spore outgrowth and toxin production for all the strains were inhibited in the presence of 200 ppm of BHT in the TYG. None of the levels of PG tested delayed outgrowth or toxin formation of the test spores for more than 24 h.

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**Effects of Foam-Reducing Methods on Recovery of Indicator Organisms from Foods.** A. K. Stersky and C. Thacker, *Food Directorate, Health Protection Branch, Department of National Health and Welfare, Canada, Tunney's Pasture, Ottawa, Ontario, K1A OL2, Canada.*

Two antifoam agents (Dow Corning Antifoam AF and C), vacuum (23 inches of mercury) and the Stomacher 400 were used to reduce foam during processing of pudding (P), skim milk powder (SMP), and whole egg powder (WEP) for determination of bacterial counts. One minute blending of 99 ml 0.1% peptone plus 11 g of P, WEP, SMP in commercial blenders at liquefying velocity, resulted in up to 100, 60 and 50 ml of foam, over the food homogenates, respectively; these values were reduced to 4, 20 and 10 ml when Antifoam AF was used at 200, 500 and 1000 ppm (Antifoam C was less effective). Twenty-three inches of mercury of vacuum during blending resulted in 13, 10 and 6 ml of foam, and use of the Stomacher produced 4, 8 and 8 ml of foam with P, WEP, and SMP, respectively. For foam-reduction, the Stomacher is the method of choice. However, no detrimental effects were observed in recovery of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus faecalis* when Antifoam AF at 200 ppm, vacuum (23 inches of mercury), or the Stomacher were used to suppress foaming of the three foods.

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**Foodborne Disease - Data From Six Countries.** E. Todd, *Food-borne Diseases Reporting Centre, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario.*

Data on foodborne disease outbreaks from Canada, the United States, Japan, Australia, England and Wales are compared. Japan had the greatest number of outbreaks reported (6109) and Australia the least (48), both over 5-year periods. Canada recorded the most outbreaks per 100,000 population (2.18), Australia the least (0.07). The percentage of outbreaks of unknown etiology varied from 0.6% for England and Wales to 61.7% for the United States and 84% for Canada. For all countries, however, where etiology was known, microbiological agents were the most important, particularly *Staphylococcus aureus, Salmonella* sp. and *Clostridium perfringens. Vibrio parahaemolyticus* was responsible for most illnesses in Japan. Meat and poultry were the main foods involved, with fish also being important in Japan and the United States. Mishandling that led to illness took place mainly in foodservice establishments. To permit a more complete comparison in the future, surveillance systems of different countries need to be developed on common criteria.

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**Inhibition of *Salmonella typhimurium* and *Staphylococcus aureus* by Butylated Hydroxyanisole and the Propyl Ester of p-Hydroxybenzoic Acid.** K. R. Vantassell, L. A. Smoot and M. D. Pierson, *Virginia Polytechnic Institute and State University, Dept. of Food Science and Technology, Blacksburg, Virginia 24061.*

Inhibition of *Salmonella typhimurium* and *Staphylococcus aureus* in Trypticase Soy Broth by the propyl ester of p-hydroxybenzoic acid (propyl paraben; 0 to 500 ppm) and butylated hydroxyanisole (BHA; 0 to 400 ppm) alone and in combination was studied. A concentration of 250 ppm of propyl paraben delayed growth of *S. aureus* for 36 h. There was a decrease in cell numbers over 48 h of incubation in the presence of 500 ppm of propyl paraben. BHA at 100 ppm delayed growth of *S. aureus* for 36 h while viable cell numbers decreased to less than 1 per ml within 3 h in the presence of 200 ppm of BHA. A 100 ppm BHA-100 ppm propyl paraben combination was bacteriostatic and 150 ppm BHA-150 ppm propyl paraben combination was bactericidal. Growth of *S. typhimurium* was progressively restricted as the concentration of propyl paraben was increased to 250 ppm, BHA to 200 ppm or the BHA-propyl paraben combination to 75 ppm - 75 ppm. In the presence of either 300 ppm of propyl paraben, 400 ppm of BHA or a combination of 100 ppm BHA-100 ppm propyl paraben, a chemical injury recovery phenomenon was observed.

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## INVITED PAPERS

**Ultrafiltration and Reverse Osmosis.** Neil C. Beaton, *Dorr-Oliver Inc., 77 Havemeyer La., Stamford, Connecticut 06905.*

The general applicability of ultrafiltration and reverse osmosis in the dairy industry is reviewed. Consideration is given to the problems of hygienic design and operation of membrane systems, particularly from the point of view of cleanability and sanitation. Recent technological advances which may accelerate the acceptance of ultrafiltration and reverse osmosis as unit operations in the dairy industry are also discussed.

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**Sanitary Processing of Egg Products.** Dwight H. Bergquist, *Henningsen Foods, Inc., 14334 Industrial Road, Omaha, Nebraska 68144.*

The egg products industry has gone through dramatic changes in recent years as influenced by regulations requiring pasteurization of egg products, mandatory USDA inspection, establishment of E-3-A Standards for equipment, and tight customer specifications. Quality control for egg products include: (*a*) procurement of good raw materials; (*b*) use of adequate pasteurization, processing, and sanitary procedures; (*c*) microbiological testing, including salmonella; (*d*) safeguards against recontamination. Pasteurization methods have been developed which substantially reduce the hazard of potential pathogens and still retain the heat-sensitive properties of the raw egg. Present egg products have very good functional and microbiological quality.

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**Food Protection for the 80's.** F. F. Busta, *Department of Food Science and Nutrition, University of Minnesota, 225 Food Science & Nutrition Bldg, 1334 Eckles Avenue, St. Paul, Minnesota 55108.*

New social, economic, and political demands for conservation of energy, water, and consumable products coupled with changes in lifestyle including increased meals away from home will require new approaches to food handling. These modifications may increase or uncover new hazards and potential opportunities for foodborne illness. Microbial hazards will remain a major problem but will be only one of the many concerns of the consumer about food. Increase in awareness of newly identified pathogens, carcinogens, mutagens, and the like will demand greater efforts but will also increase costs. Acute sensitivity to escalating costs will, in turn, bring about more objective evaluations of benefit/risk ratios on all programs. Education of producers, handlers, processors, and consumers will be required so that they may monitor and serve as protectors of the food system minimizing regulatory costs and placing responsibility at the point of action. This process will be successful only with the appropriate educational and research support to evaluate and implement modified programs.

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**Psychrotrophs in Relation to Keeping Quality of Milk Products.** Maribeth A. Cousin, *Department of Animal Sciences, Smith Hall, Purdue University, West Lafayette, Indiana 47907.*

The introduction and use of alternate-day collection of refrigerated bulk milk, five-day-per-week plant operations, shipping of milk long distances, and sale of packaged milk have resulted in increased age of milk and dairy products on consumption. Consequently, the most important aspect affecting quality of milk and dairy products is growth of psychrotrophic microorganisms. Much attention has focused on methods to enumerate and identify the psychrotrophic bacteria; correlation of the presence of these bacteria in milk with sanitation on the farm, in transport, and at the dairy plant; and sources of contamination by these bacteria. These microorganisms can cause physical, chemical, and organoleptic changes in milk and dairy products. The major changes include lipolysis, proteolysis, and over-all product spoilage. In recent years research on psychrotrophs has centered around their effect on starter cultures, cultured product manufacture, heat stability of the enzymes produced by these microorganisms, consumer acceptance of products made from milk that had previous psychrotrophic growth, and more rapid methods to detect growth of these organisms in milk. This research has indicated that the dairy industry must reevaluate the practice of extended holding of milk before processing.

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**Current Concepts in Brucellosis.** Francis J. Drazek, *Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.*

Brucellosis continues to be a very devastating disease in the United States and throughout the world. In dairy herds, spread of the disease often results in severe milk losses due to the necessary slaughter of infected animals. Many herds have been depopulated because herd owners have not followed our management recommendations to control the spread of infection. Undulant fever is a frequent occurrence in farm families where herds are infected. Increased movement of cattle, including importation of animals, has contributed to its spread. Prevention, control and eradication have been impeded by present housing and management practices. The general apathy on the part of the owner by not having herd additions blood-tested for brucellosis before entry into the herd is also an important contributing factor.

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**Designing Tomorrow's Foods.** J. E. Edmondson, *Department of Food Science and Nutrition, University of Missouri-Columbia, Columbia, Missouri 65211.*

Most individuals consume new food products without concern for composition, nutrition, processing, packaging and/or safety. In recent months, there has been a developing awareness of new food products. Such awareness has been exhibited by both the consumer and the food industry. One of the areas of greatest interest is development of new food sources, many utilizing waste. Changes in packaging materials, increased shelf life and nutritional composition by the food

industry become alarming factors to many consumer groups. These and other future topics will be discussed which will govern what we will be eating tomorrow.

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**Energy Converters.** Darl L. Evans, *Babson Bros. Co., 2100 South York Road, Oak Brook, Illinois 60521.*

The energy shortage is real! Public awareness started with the Arab oil embargo and the resulting gasoline shortage. Temporary? Political? Imaginary? Perhaps, but the fact remains - our supply of fossil fuels is diminishing and the day is coming, in the forseeable future, when the world supply is exhausted. The United States now consumes 35% of the world's total energy, and although dairying uses only 2 to 4% of the nation's supply, dairymen will be victimized by spiralling energy costs; as much as 15% a year or double in the next 5 years, according to one estimate. The cost of operating tractors and field equipment is beyond our control, but we can do something about the cost of cooling milk. New milk cooling equipment available today is designed to offset the increased cost through improved energy efficiency ratio and energy conservation. Thirty million BTUs of heat are removed annually cooling the milk from a 50-cow herd with a 12,000-lb. average. Improved condensing units producing 10 BTUs per watt can recover this heat and convert it to usable energy in the form of hot water. Solar collectors can provide adequate space heating for milking centers with condensing units furnishing the back-up system. Cooling systems that are energy-efficient will provide adequate cooling and preserve the natural quality of milk.

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**Sanitation Problem of Soft Drink Mixing Machines.** George R. Gregory, *Springfield-Greene County Public Health Center, Springfield, Missouri.*

Samples of rootbeer, which is manufactured in each store, were checked for coliform, standard plate count, and yeast and mold. Results have shown that it is difficult for rootbeer to meet the standards for bottled beverages. By education of store personnel, high quality rootbeer can be achieved. Laboratory results have been the best means to show the operator that aseptic techniques are necessary for production of a quality product.

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**Quality Standards for Retail Meats.** Gail C. Holland, *Meat Packers Council of Canada, 5233 Dundas St. W., Islington, Ontario, Canada M9B 1A6.*

To be of public benefit a quality standard for meat at retail must do as it purports to do--i.e. to reduce the public health hazard or prevent consumer deception. Among other factors, it also must be technically workable for both the industry and the administrative branches of a regulatory agency. With processed meats and ground beef at retail it has not been demonstrated that these foods present a potential health hazard. In addition, if a health hazard were demonstrated, it would not be reduced by use of microbiological quality standards such as APC and *Escherichia coli*. Use of the bacterial criterion, APC of $10^7$/g, in place of organoleptic standards could reduce processed meats' retail shelf-life by 20-66%. An APC of $10^7$/g could remove in excess of 33% of the ground beef sold at retail level. In both instances a substantial quantity of wholesome meat would be prematurely removed from the retail market. Chemical standards such as protein, fat, moisture, cereal are relatively static and do not substantially change over the retail shelf-life of meat. However the bacterial populations in meats demonstrate a dynamic growth pattern for APC. Hence, APC may indicate product age. However, it does not reflect product deterioration as determined by microbiological quality. *E. coli*, although an intestinal bacterium, is capable of growing outside the host intestine; thrives on a variety of substrates; and thrives for long periods. Thus presence of *E. coli* cannot be correlated with the extent of initial fecal contamination, nor with presence of pathogens. A program of increased awareness of personal hygiene, temperature control, stock rotation, elimination of areas for potential cross-contamination and a regular sanitation program throughout the meat cycle, will guarantee the microbiological quality of meats at retail.

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**Functions of Cleaners.** James J. Jezeski, *H. B. Fuller Company, Monarch Chemicals Division, Minneapolis, Minnesota.*

A basic discussion of the specific activities of various base materials in cleaning agents whether they are blended together in a proprietary formulation or used separately as bulk chemicals. The operational or use factors relating to each function and/or class of chemical will also be covered as will the coordination and scheduling of certain classes of chemicals in the overall cleaning process. Various individual functions include water treatment-softening, emulsification - suspension, saponification - peptizing, prevention of redeposition, and demineralization. Various operational activities cover prerinsing, the several means of application of cleaning solutions, disposal of used solution, and final rinse. Interactions between various functions, operations, and individual chemicals will be evaluated with emphasis on practical operational considerations. Some regulatory aspects and energy conservation opportunities will be discussed.

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**Goat Milk Production and Problems.** Judy Kapture, *Countryside Publications, Hwy. 19 East, Waterloo, Wisconsin 53594.*

There are 30 goat dairies which distribute fluid milk in 21 states. Three are large, they supply the Los Angeles, Chicago and New York areas with up to 1,000 gallons of milk weekly. The others are producer/distributors which sell from 30 to 250 gallons weekly. Most distribute raw milk. Potential market is about one quart per 1,000 population weekly. Necessary dairy building and equipment to market milk may cost $5,000 to $15,000, pasteurization may add $3,000 to cost. High leucocyte counts in fall and low summer milkfat tests are common. Goat milk is a valuable food, but fluid goat milk is not available to perhaps 80% of the urban population. Limited market and high costs discourage production. Inexpensive equipment to process small volumes is needed. Evaporated and powdered goat milk, produced in California and Arkansas, are widely distributed.

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**Innovative Water Saving Techniques in Canneries.** Allen M. Katsuyama, *National Food Processors Association, 1950 Sixth Street, Berkeley, California 94710.*

The food processing industry annually uses 130 billion gallons of water to prepare and preserve canned, cured, and frozen foods and discharges about 120 billion gallons of wastewater. Research designed to minimize fresh water requirements while protecting the safety of prepared foods has been conducted over several decades. However, the relative widespread availability of fresh water and the relative low costs for wastewater disposal did not provide sufficient incentive for general industrial adoption of newly-devised water saving techniques. The drought experienced in several areas of the country during 1976-1977, coupled with sky-rocketing costs for wastewater treatment and disposal, provided the impetus required for the food industry to begin serious implementation of water conservation programs. By avoiding wasteful practices, by reusing water in a manner demonstrated to protect product integrity, through process alterations, and with process

modifications and new operational techniques, food processors have achieved substantial reductions in water consumption. These measures will contribute significantly to the conservation of one of our precious resources, water.

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**Opportunities to Improve Milk Quality.** W. S. LaGrange, *Department of Food Technology, Iowa State University, Ames, Iowa 50011.*

Opportunities to improve the bacterial quality of manufacturing-grade milk both on the farm and in the processing plant still exist. Data gathered from dairy laboratories in Iowa indicate a gradual increase in the percent (70-80%) of farm milk samples that qualify for Class I (<500,000 bacteria/ml) in recent years. Contrast this improvement with the lack of improvement, according to the USDA data, of processing plant's commingled milk. Recent results from Iowa plants classify only 5.8 to 13.3% of the samples in Class I. Flavor evaluation of American cheese and butter samples entered in Iowa exhibits reveal a significant number of these samples have flavor defects associated with poor quality raw milk. Milk quality problems involve bacterial contamination of milk during milking and storage, too much time between milking and processing, storage temperatures exceeding 40 F and large-volume inflexible milk storage facilities in processing plants.

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**Kids, Cartons, and Quality.** C. Bronson Lane, *Dairy and Food Nutrition Council of Florida, P.O. Box 7813, Orlando, Florida 32854.*

Two years ago, Dairy Farmers, Inc. with cooperation from the Florida Department of Health, Department of Education, Department of Agriculture and Consumer Services, University of Florida Dairy Science Department, and Florida Dairy Products Association, conducted a statewide school milk survey. Purpose of the study - done in four phases - was to organoleptically evaluate taste and flavors of milks from half the state's schools, determine product shelf-life, analyze storage and serving temperatures, and gather information on frequency of study complaints about milks offered in the school feeding programs. Of the 877 milk samples evaluated for flavor, 80% scored good or excellent, 11% obtained a fair rating, and 9% were classified as poor. Serving temperature data were mildly surprising - and indicated that for the most part - school foodservice personnel were dispensing the product at appropriate temperatures. Data obtained from approximately 1000 school foodservice managers, however, showed that 71.4% of these individuals received five gripes or less per month, 13.4% got 6-10 complaints per month, and 15% received more than 10 "bad-mouthings" per month about milk quality from cafeteria patrons. "Bad taste" and "too warm" were culprits in 27% of the complaint cases. However, dirty cartons, hard to open packages, and empty or partially filled containers got blamed for 73% of the criticisms. The dairy industry can't afford to subject its school patrons to a quality and container gamble. The stakes are too high. Basic eating patterns are established during the school years. Kids who are "turned-off" by inferior quality and poorly packaged milks in the school cafeterias may opt to boycott our products during adulthood.

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**Bacterial Numbers in Milk: What Do They Tell Us?** R. T. Marshall and B. L. O'Brien, *Department of Food Science and Nutrition, University of Missouri, Columbia, Missouri 65211.*

Because bacteria vary widely in their rates of growth and in metabolic activities and because sources of contamination of milk differ in importance, high total aerobic bacterial counts cannot be interpreted to indicate specific causative factors or remedies. Data gathered by in-depth studies of the microflora of milk of a large fluid milk plant and in evaluation of the automated pyruvate test with grade A pasteurized milk were used to illustrate problems of quality evaluation in the fluid milk industry. Usually bacterial growth was logarithmic when counts reached $10^3$/ml. Lag times varied from <1 to 7 days. Generation times of dominant bacteria ranged from 3.2 to 15.1 h. Rates of pyruvate production generally paralleled growth of psychrotrophs for 7 days at 7 C. Neither psychrotrophic plate count nor pyruvate produced in 24 h at 20 C were good predictors of the day a sensory panel of 10 by triangle test differentiated milk stored at 7 C from the same milk stored at 1 C. Variations inherent in microbiological tests and sensory analyses, and differences in abilities of microorganisms to spoil milk make prediction of keeping quality extremely difficult.

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**Communications the Key to Public Relations.** Arthur W. Nesbitt, *Nasco International, Inc., Fort Atkinson, Wisconsin 53538.*

Communication is to share. Public relations is the degree of understanding and good will achieved. Feeding the increased world population has brought new demands on all sanitarians yet the consumer has little appreciation for the day-to-day protection which is provided to us all by this dedicated group of people. In the conduct of their normal duties how can sanitarians, the "Quiet Professionals," share with the public the services they render routinely to provide us with safe products and the world's best standard of living?

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**The International Dairy Federation.** Kenneth G. Savage, *International Dairy Federation, 6661 Tamany Drive, Victoria, B.C., Canada.*

The International Dairy Federation (I.D.F.), a non-governmental, non-political international organization was established in 1903. The I.D.F. derives its finances from the annual membership fee (current membership, 28 countries). Membership is accorded to countries through their National Committees representing the country's various dairy interests. These Committees are the link between I.D.F. and the member country. The I.D.F. Executive Committee deals with policy matters delegated by the supreme body of I.D.F., the General Assembly, and develops proposals for General Assembly consideration. A permanent secretariat under the direction of Mr. P. Staal, Secretary-General, coordinates I.D.F. activities and carries out the current business at the I.D.F. House, Brussels, Belgium. The technical and scientific work of I.D.F., under guidance of the Commission of Studies, is carried out in six special Commissions whose subject matter covers all aspects of interest to the dairy industry. The objective of I.D.F., which is to promote, through international cooperation, the solution of scientific, technical and economic problems in the international dairy field, is achieved through the voluntary work of hundreds of the world's dairy industry leaders. This work is done by Groups of Experts or at Annual Sessions, I.D.F.-sponsored Seminars or Symposia or at Congresses.

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**Retortable Food Pouches for Military Rations.** G. L. Schulz, and H. A. Hollender, *U.S. Army Natick Research and Development Command, Natick, Massachusetts 01760.*

This paper will summarize the retort pouch work done at the US Army Natick Research and Development Command (USA NARADCOM) and will focus on testing conducted to assure sufficient durability and reliability to withstand military transportation, handling, and storage requirements. Recognizing the potential advantages of retortable pouches for operational rations, USA NARADCOM began work in this area over 15 years ago. Working closely with industry, we have conducted extensive testing to assure that this new package would meet the requirements for military use. Although some requirements are peculiar to the military, most of the advantages which make the retort pouch attractive to the military also apply to commercial markets.

Lightweight, energy efficiency, and the potential for improved quality of processed foods are major factors which created and maintained interest in the retort pouch by the food industry. Utilizing the advantages of the retort pouch as well as other advances in food and packaging technology, USA NARADCOM has developed a new operational ration, the Meal, Ready-to-Eat, Individual. Procurement of 24 million of the new combat meals in currently under way.

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**A Justifiable Food-Energy-Legislative Triangle?** Nan Unklesbay, *Department of Food Science and Nutrition, College of Agriculture, University of Missouri-Columbia, Columbia, Missouri 65211.*

Recent regulations to protect the consumer and assure public health and safety have impacted upon energy usage throughout the food industry. Thus, effects of the food-energy-legislative triangle are pervasive. Triangles have always been included in folklore; the Bermuda Triangle is one of today's most perplexing phenomena. This presentation includes an analogy between issues surrounding the Bermuda and food-energy-legislative triangles. Although different in scope, both triangles are increasing public awareness that something is wrong. Human resources are being lost in the former; legislation in the latter ensures food safety for humans but depletes energy resources. Reasons for bizarre disappearances have been partially explained scientifically. However, completely reliable methods to identify all cost/benefit factors and risks to inflation, energy, competition, productivity, and other forces, associated with legislation, do not exist. Unraveling dimensions of both triangles is hampered by limited scientific time and resources to comprehend complex issues. Thus, cataclysmic forces are used to explain the Bermuda Triangle and food/energy regulations are based upon philosophies and biases of estimators. Can either triangle be solved?

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**PBB-The Michigan Program.** Kenneth M. Van Patten, *P.B.B. Project Unit, Michigan Department of Agriculture, Lewis Cass Building, Box 30017, Lansing, Michigan 48909.*

The PBB Law in Michigan requires testing of a composite sample of milk from each dairy farm once a year and testing of each adult dairy cow that is culled for slaughter. Milk found to contain more than 5 parts per billion of PBB, on a sample basis, is excluded from the market. Any cow found to contain more than 20 parts per billion of PBB on a fat basis in a tissue sample is quarantined, branded by the Michigan Dept. of Agriculture and disposed of in disposal facilities selected by the Dept. of Natural Resources. The owner of each dairy cow tested is paid $30.00 for the holding and maintenance of that dairy cow during the testing period. The owner of each violative cow is indemnified the fair market value (meat price) of the animal on the date of appraisal plus a payment of $2.50 per day for each day the cow is held on the farm before pickup for disposal. Tissue samples are taken by contract veterinarians, which number more than 350. All sample analysis work is done in one main contract laboratory with another contract laboratory doing limited analytical work. The program is designed for fast processing of samples after they are taken. The entire record system has been computerized. Turn around time from sampling to reporting to the farmer is 10 to 12 days. Emergency slaughter samples have a 3 to 4-day turn around time. The legislature may exempt a category of dairy cattle from the law by concurrent resolution when 0.5% or less of a category of dairy cattle are found to be nonviolative.

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**Beyond the One Shot Clean-Up.** Charles F. Vogt, *City of Kansas City, Missouri, 414 E. 12th St., Kansas City, Missouri 64106.*

Most people think of spring and summer as clean-up, fix-up and beautification times. Whether it be public or private property, no results of a clean up on a vacant lot or street remain very long. Debris and litter clutter the environment as before. The Clean City Commission of Kansas City, Missouri, in conjunction with Keep America Beautiful, Inc., New York, has devised a four-front approach to sustaining litter reduction through changing behaviors and attitudes that fail to properly contain litter and trash. These measures consist of devising ordinances that assign responsibilities for pick-up and containment of trash; introducing new technology and waste handling methods to improve solid waste clean-up and disposal; increasing educational awareness of the environment and teaching persons new habits which can be learned and sustained; and finally, providing a fail-safe measure by which proper enforcement of litter ordinances can be maintained. To do this, the Commission is getting the facts about littering, involving the people, focusing in on results of successful anti-litter projects, planning systematically through goals and objectives, and providing positive reinforcement (recognition) for jobs well done.

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**3A Sanitation Criteria and their Development.** D. H. Williams, *Dairy and Food Industries Supply Association, 5530 Wisconsin Avenue, Washington, D.C. 20015.*

3-A sanitation criteria provide for cleanability and product protection in dairy processing equipment. The elements of construction that assure these criteria are the critical selection of safe materials for product contact surfaces and their ultimate surface finish, and the arrangement into a configuration that will permit cleaning and sanitizing so that all surfaces are restored to their original character for repeated use. A means for evaluating safety of materials is considered, and a bench mark for surface finish is described. All 3A Standards are organized into four basic sections dealing respectively with Scope, Definition, Materials, and Fabrication. Appendices are frequently provided to amplify understanding of the basic criteria as well as to provide appropriate collateral information on the equipment. 3A Standards do not deal with criteria for performance, with minor exceptions for insulation, agitation, and the cooling capability for farm tanks. Otherwise, the efficiency for equipment is not within the purview of 3A Standards. 3A Standards are limited to equipment having product contact surfaces, thus do not include such equipment as refrigerated cabinets, cases, etc. In addition to Standards, the 3A Committees also promulgate a category of document entitled, Accepted Practice, which is comprised of sanitary quidelines for accomplishing a specific function, such as production of air under pressure, installation of welded pipelines, production of culinary steam, milking systems milk drying, and the monumental Practices for HTST Pasteurization. The latter is a fail-safe procedure for milk pasteurization, widely adopted in most regulatory milk control jurisdictions. Its impact on fluid milk pasteurization is virtually absolute; there is no other way of doing it. Sanitation criteria developed in 3A Standards have been extended to corresponding equipment that is used in the egg products industry. Much of the equipment used by the dairy processing industry is also found in the egg products industry, which has the applicable criteria for handling liquid and dry eggs, including special E-3A Standards for egg washers and egg breakers. Regulatory sanitarians are urged to adopt the applicable classical criteria for cleanability and product protection that have been developed for dairy processing equipment for extension to all food processing equipment.

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# Report of the Committee on Food Equipment Sanitary Standards

The IAMFES Committee on Food Equipment Sanitary Standards, known hereafter as the Committee, is charged with the responsibility of carrying out the following objectives:

1. To cooperate with other interested health organizations and related industries in the formulation of sanitary standards and educational materials for the fabrication, installation, and operation of food equipment and food vending machines.
2. To aid the food and vending industry in improving the design, construction and installation of food equipment so that it will lead to easy cleaning and proper functioning when it is placed into service.
3. To cooperate with the food industry in the preparation of standards or guidelines which public health agencies will accept, thereby securing uniformity in the manufacture and nation-wide acceptance of such equipment.
4. To present to the IAMFES membership those standards and educational materials which the Committee recommends be endorsed by the Association.

The following report will outline the Committee's activities during the past year in working with two health and industry organizations (National Sanitation Foundation's Joint Committee on Food Equipment Standards and the National Automatic Merchandising Association's Automatic Merchandising Health Industry Council) and progress in meeting its purposes and objectives. It is expected these organizations will be the two groups that the Committee will work with during the coming year.

## *NATIONAL SANITATION FOUNDATION (NSF)*

The Committee was represented at the 1978 meeting of the National Sanitation Foundation's Joint Committee on Food Equipment Standards, where action was taken on several proposals; and prior to the meeting, the Committee reviewed and submitted comments on each draft of these proposals.

### *NSF Food Equipment Standards 1,2,12,18*

NSF has developed a "Guide to Requirements for the Protection of Potable Ice". According to this Guide, no appurtenances are accepted in the ice bin, such as syrup lines, carbonators, drop-in cold plates, drain lines, or bottle holders. Furthermore, all typical ice bins are to be protected by some means so as to prevent water, syrup, and other materials from splashing, dripping or leaking into the bins and contaminating the ice.

### *NSF Standards 1, 18, 25*

A complaint has been raised by the Surgeon General of the Navy concerning the present construction of cup-stops and dispensing equipment activating mechanisms for dispensing beverages. According to the Complainant, the presently approved equipment at self-service locations would permit contamination of the mouth contact surfaces of beverage containers. The Joint Committee has asked the Foundation to review this matter and/or to submit it to a Task Committee for Recommendations.

### *NSF Standard 2*

A question has been raised as to the adequacy of the NSF Guidelines concerning the shielding of food at self-service counters from droplet infection and mishandling by the customer. The present Guidelines are based on the customers average mouth height of 4 ft. 6 in. to 5 ft. with a tray rail between the customer and the food. It was decided that the food guards should be designed to provide the same degree of protection with or without a tray rail.

Furthermore, the Joint Committee felt that these Guidelines would be acceptable against droplet infection but had some serious reservations as to the ability of such guards to protect the food on display from contamination due to mishandling by the customer. Consequently the Committee recommended that the Foundation appoint a committee to study the need for developing additional requirements to protect food on display from contamination due to mishandling by the customers.

### *NSF Standards 3, 29*

The proposed revision of Standard 29 concerning chemical feeders would incorporate chemical sanitizer feeders in this Standard with specific requirements therein to cover these new chemical feeders. It is anticipated by the Foundation staff that appropriate provisions will be incorporated in Standards 3 and 29 designating that each dishwashing machine and feeder will be so labeled as to inform the purchaser and user of the specific type of feeder which is needed for a specific chemical sanitizing/dishwashing machine. The Committee also recommended that the chemical feeder be attached to each dishwashing machine prior to testing machines by the Foundation.

### *NSF Standard 3*

Standard 3 as revised in 1977 has been reprinted. Each new dishwashing machine shall be equipped with a pressure gauge, and a gate valve shall be installed immediately ahead of the pressure gauge to help minimize damage to the gauge due to water hammer. The Foundation staff said there was not enough field experience to know whether or not gauge failure has been alleviated by the gauge installation procedure in the revised Standard.

### *NSF Standard 4*

The Foundation, as well as many regulatory agencies, has received requests to evaluate slow cookers to determine their acceptability. Consequently, the following points are considered by the Foundation and should be considered by regulatory agencies in evaluating slow cookers under NSF Standard 4:

1. Most of these units exhibit a glazed pottery cooking well and a glass cover. Such material would not meet food zone material requirements as indicated in item 3.1 of Standard 4.
2. The cooking well would need to be readily removable from the unit for cleaning, as generally these units are not submersible.
3. The on-off high-low temperature switch is not calibrated in degrees Fahrenheit. The units would need to have capability of attaining 165°F food temperature and be capable of maintaining a food temperature of 140°F or above
4. Since most of these units are designed for domestic use, there will probably be numerous other problems revealed when evaluated against Standard 4, such as handles that do not fit tightly, feet that are not closed on the bottom, seams on the side wall that are not closed, etc.

### *NSF Standard 12*

Some regulatory agencies have requested the Foundation to consider requiring a tight closure around or on dispensing mechanisms of ice-maker dispensing machines so as to exclude insects, dust, and so forth from reaching the ice stored in the machine. The Foundation staff said there was a closure machanism on each machine to prevent the free flowing of ice. The Committee felt for the present this would be sufficient and more could be accomplished by public health and industry by concentrating on restaurants, institutions, and motel/hotel operations to eliminate open ice bins, which have been found to be in many cases very unsanitary and unsatisfactory devices for producing a safe product, and to permit only the use of ice-maker dispensing machines.

### *Future Proposals*

The NSF Staff reported that steps are being taken to combine Standards 1, 2, 4, and 7, similar to the combination of Standards related to swimming pools. This is being planned to avoid duplication of requirements and to bring related equipment under one standard.

A question has been raised as to the problem of maintaining a uniform temperature in hot and cold food holding carts and cabinets. Apparently, the temperature of the food in some of these cabinets has been found to vary from 165°F to 205°F depending on the location of the food tray and the design of the cabinet. In the past, the Committee has limited its work on Standards to the public

health aspects and has not included the quality of the food. However, it was felt by the Joint Committee that food must be acceptable to the consumer; and if it were not, it could create a public health problem. Therefore, it was recommended that a NSF task committee be appointed to study this matter in detail and to report its recommendations at the 1979 meeting as to the need for assuring a greater degree of uniformity as to temperatures in these cabinets and broadening the scope of the standards to include performance.

Two additional Standards have been recommended for adoption by the Joint Committee during the past year "Plastic Component on Food Equipment" and "Proposed Standard on Supplemental Flooring". It is anticipated that these Standards will be approved by the Council of Public Health consultants and be implemented by the Foundation within the next year.

Some regulatory and industry personnel have been concerned that the NSF Standards for the most part are not designed as performance standards. Consequently, some of the surfaces of food equipment have been found to readily corrode and deteriorate without effecting the more critical portions of equipment. Never-the less, the appearance of equipment has a decided effect on the opinion of the customer, user and regulatory people as to the acceptability of food service equipment. Consequently, the Foundation has made plans to conduct a seminar within the next year to study this matter in detail, particularly concerning the use of noncorrosive materials on splash and non-food contact surfaces, and to make recommendations for appropriate action.

## *NATIONAL AUTOMATIC MERCHANDISING ASSOCIATION*

The National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council (AMHIC) held its twenty-second annual meeting during October, 1977, and this Association and other public health organizations and the affected industries were represented and participated in AMHIC's discussion.

### *Evaluation Manual Revision — Water Supply Protection*

A number of proposed amendments to the Evaluation Manual were discussed; and the following motion was approved by the Council: the proposed September 26, 1977 Manual revision draft, as amended at this meeting, be incorporated into appropriate sections of the Evaluation Manual. Some of the substantive changes made by the Council at this meeting to the Manual included: (a) the insertion of "spring-loaded" to check valve references; (b) the substitution of "4 inches" for the "12 inch" critical elevation of vacuum breakers; (c) the substitution in section 605 of "overflow level" for "flood level" (as applicable to tubing connections in waterbath compartments); and (d) a new definition of "liquid waste".

A copy of all of the Amendments to the Evaluation Manual as made by the Council at its 1977 meeting may be obtained from NAMA.

### *Remanufactured Machines*

According to an industry representative of the Council, steps are being taken to increase participation in the remanufactured machine program (two new applicants were received in 1977). The Secretary pointed out NAMA's concern for product liability in remanufactured machines and the present requirements of the Administrative Policies governing supplemental nameplates.

### *Food Shelf Life Study*

A representative of one of the NAMA Evaluation agencies reported highlights of the most recent bacteriological studies of vended and manually served sandwiches. Although his findings indicated acceptable plate counts during a four-day period, it was agreed that too many variables were present to permit NAMA publication of recommended shelf life guidelines. There was a concensus that NAMA should survey the industry for present shelf life practices and prepare a generalized operator bulletin on "how to assure maximum shelf life".

### *Handicapped Access to Machines — Energy Conservation*

Reporting on NAMA activities in these two areas, the Secretary was asked to keep AMHIC informed of developments in both areas, particularly those relating to standards for machines used by the handicapped.

### *Ice-maker Research*

An industry representative on the Council, reported progress in the development of a new automated system for ice-maker cleaning and his plan to begin tests in installations in one or more machine models within a few months. The Secretary stated that NAMA has not arranged for effectiveness studies of the two presently available automated cleaning systems for two reasons: (1) automated systems, as designed, are clearly superior to manual cleaning; and, (2) the fact that 90% of the two models sold are ordered with auto-systems is proof of their effectiveness in reducing "algae" growth problems. On the basis of this information, the Council adopted the following motion: "NAMA should prepare a research protocol for determining the effectiveness of automated ice-maker cleaning systems in controlling microorganisms within the system for discussion by AMHIC not later than the 1978 annual meeting."

### *Water Vending Machines*

Addressing the one water vender presently NAMA-Listed, the NAMA Machine Evaluation Agency personnel stated that: (a) independent laboratory tests showed excellent bacterial counts (of vended water); (b) the Letter of Compliance covers all machine features; and (c) more detailed standards in the Evaluation Manual would be of value. Although the present vender is marked "For Municipal Water Supply Connnection Only", it was agreed that his qualification should also appear on the Letter of Compliance and in the NAMA Listing.

Questions and cautions expressed by various Council members included these for future activity in this field:

1. Does NAMA certification of a water vending machine cover all components (including UV systems, if used)?
2. Is water vending a "rip-off" to which NAMA should not lend its name or can water "improvement" be demonstrated?
3. What machine labeling and advertising claims/statements are justified and proper (pure, purified, sanitized, safe, sparkling, etc.)?
4. What is the potential for degrading the water when (if) filters (pressure gauges, cut-off controls, etc.) become excessively clogged?

### *Provisional Certification Policy*

A proposal to amend NAMA's Evaluation Program "Administrative Policies" to include a "Provisional Letter of Compliance" under specified circumstances was discussed; and the following paragraph was incorporated in Part I,E, of the Policies:

5. The evaluation agency may issue a provisional letter of compliance whenever, in the opinion of the agency, new vending technology or new products associated with the applicant machine require field use and servicing experience to confirm the machine's safety and acceptability.

### *Recommendations*

1. The Association reaffirms its support of the National Sanitation Foundation and the National Automatic Merchandising Association and continues to work with these two organizations in developing acceptable standards and educational materials for the food industry and public health.

2. The Association urges all sanitarians to obtain a complete set of the National Sanitation Foundation's Food Equipment Standards and Criteria and a copy of the National Automatic Merchandising Association — Automatic Merchandising Health-Industry Council's Vending Machine Evaluation Manual and related educational materials; to evaluate each piece of food equipment and vending machine in the field to determine compliance with the applicable sanitation guidelines (construction and installation specifications); and to let this Committee and the appropriate evaluation agency know of any listed manufacturer or fabricator failing to comply with these guidelines.

3. The Association urges all sanitarians and regulatory agencies to support the work of the Association's Committee, submit suggestions for developing new guidelines and for admending same, and subscribe, by law or administrative policy, to the principles represented by the Standards, Criteria, and

...con't. on page 844

# Financial Report IAMFES

Members of the Board of
International Association of Milk, Food
and Environmental Sanitarians, Inc.

We have examined the accompanying balance sheet of the International Association of Milk, Food and Environmental Sanitarians, Inc., as of June 30, 1978 and 1977, and the related statements of the Association income, journal income, and changes in fund balance for the years then ended. Our examination was made in accordance with generally accepted auditing standards and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying statements present fairly the financial position of International Association of Milk, Food and Environmental Sanitarians, Inc., as of June 30, 1978 and 1977 and the results of its operations for the years then ended, in conformity with generally accepted accounting principles applied on a consistent basis after giving retroactive effect to the change (with which we concur) in the method of recording income from dues and subscriptions as explained in Note (2) to the financial statements.

Dougherty & Co.

Certified Public Accountants

July 21, 1978
Ames, Iowa

**BALANCE SHEET**
**As of June 30, 1978 and 1977**

ASSETS

| | Year Ended June 30, 1978 | Year Ended June 30, 1977 |
|---|---|---|
| Current Assets: | | |
| Cash on hand, in bank & savings & loan assoc. | $ 30,721 | $ 26,319 |
| Accounts receivable - trade | 4,405 | 6,392 |
| Inventory - supplies, lower of cost or market | 3,875 | 5,208 |
| Prepaid expenses | 163 | 104 |
| Total current assets | 39,164 | 38,023 |
| Fixed Assets: (Note 1) | | |
| Office equipment | 2,852 | 2,602 |
| Addressing & mailing equipment | 4,138 | 4,138 |
| | 6,990 | 6,740 |
| Less: allowance for depreciation | 2,982 | 2,365 |
| Net fixed assets | 4,008 | 4,375 |
| Total assets | $ 43,172 | $ 42,398 |

**LIABILITIES AND FUND BALANCE**

| | | |
|---|---|---|
| Current Liabilities: | | |
| Accounts payable - trade | $ -- | $ 655 |
| Payroll taxes payable | 821 | 2,356 |
| Unearned dues and subscriptions income | 30,559 | 28,276 |
| Special purpose funds: | | |
| Memorial Fund | 961 | 997 |
| Foundation Fund | 4,153 | 2,038 |
| Total current liabilities | 36,494 | 34,322 |
| Fund Balance | 6,678 | 8,076 |
| Total liabilities and fund balance | $ 43,172 | $ 42,398 |

See Notes to Financial Statements - June 30, 1978

**STATEMENT OF ASSOCIATION INCOME**
**For Years Ended June 30, 1978 and 1977**

| | Year Ended June 30, 1978 | Year Ended June 30, 1977 |
|---|---|---|
| Income: | | |
| Affiliate dues | $ 19,071 | $ 19,170 |
| Direct dues | 13,619 | 11,784 |
| Total dues received | 32,690 | 30,954 |
| Contributions received for awards | 1,095 | 2,206 |
| Convention and meeting income | 2,449 | 1,577 |
| Publications and pamphlets | 6,897 | 14,013 |
| Sale of 3-A Standards | 3,640 | 3,971 |
| Sustaining memberships | 3,250 | 1,500 |
| Misc. and expense reimbursement | 1,143 | 923 |
| Expense reimbursement 3-A | 9,213 | 9,186 |
| Interest income | 696 | 531 |
| Total income | 61,073 | 64,861 |
| Expense: | | |
| Salaries | 31,644 | 30,660 |
| Payroll tax expense | 2,059 | 2,276 |
| Travel | 4,273 | 2,756 |
| Office supplies | 2,611 | 804 |
| Box rent and postage | 3,596 | 3,185 |
| Telephone | 1,008 | 914 |
| Office rent | 2,250 | 2,250 |
| Insurance | 244 | 235 |
| Legal and professional fees | 1,408 | 1,320 |
| Dues and subscriptions | 216 | -- |
| Depreciation - office equipment | 244 | 186 |
| 3-A Standards expense | 2,864 | 3,411 |
| Citations and awards | 1,000 | 2,000 |
| Buttons and decals | 43 | 8 |
| Convention and annual meeting expense | 37 | 2,429 |
| Cost of printing pamphlets | -- | 5,534 |
| Miscellaneous | 651 | 1,253 |
| Total expense | 54,148 | 59,221 |
| Net income (loss) of Association | $ 6,925 | $ 5,640 |

See Notes to Financial Statements - June 30, 1978

**STATEMENT OF JOURNAL INCOME**
**For Years Ended June 30, 1978 and 1977**

| | Year Ended June 30, 1978 | Year Ended June 30, 1977 |
|---|---|---|
| Income: | | |
| Advertising | $ 15,244 | $ 12,786 |
| Subscriptions | 35,536 | 33,220 |
| Sales of journals | 208 | 1,987 |
| Sale of reprints | 10,594 | 12,192 |
| Page charges | 12,975 | 8,773 |
| Miscellaneous | 288 | -- |
| Total income | 74,845 | 68,958 |
| Expense: | | |
| Editorial salaries | 9,567 | 8,858 |
| Printing and publishing | 46,926 | 39,508 |
| Plates, cuts, etc. | 1,181 | 759 |
| Mailing and postage | 6,397 | 5,227 |
| Reprint expense | 5,684 | 4,753 |
| Advertising cost - commission and printing | 1,244 | 1,448 |
| Stationery and supplies | 312 | 142 |
| Travel expense | 1,842 | 1,849 |
| Depreciation - addressing equipment | 373 | 315 |
| Telephone | 277 | 459 |
| Consulting | 4,750 | 4,150 |
| Payroll tax expense | 1,105 | 625 |
| Computer service costs | 1,054 | -- |
| Miscellaneous | 341 | 297 |
| Total expense | 81,053 | 68,390 |
| Net income (loss) of Journal | ($ 6,208) | $ 568 |

See Notes to Financial Statements - June 30, 1978

**STATEMENT OF CHANGES IN FUND BALANCE**
**For Years Ended June 30, 1978 and 1977**

| | Year Ended June 30, 1978 | Year Ended June 30, 1977 |
|---|---|---|
| Balance beginning of period | $ 36,352 | $ 29,038 |
| Less prior period adjustment - change in accounting method for dues and subscriptions - Note (2) | ( 28,276) | ( 25,938) |
| | 8,076 | 3,100 |
| Add closing of Special Purpose Fund (Reserve for Lab Methods Committee) | -- | 299 |
| Less transfer to Special Purpose Fund (Foundation Fund) | ( 2,115) | ( 1,531) |
| Add net income (loss) for the year: | | |
| Association | 6,925 | 5,640 |
| Journal | (6,208) | 568 |
| Balance end of period | $ 6,678 | $ 8,076 |

**Notes to Financial Statements - June 30, 1978**

1) *Summary of Significant Accounting Policies*

*Fixed Assets*

Office equipment and addressing and mailing equipment are recorded at cost. Depreciation is computed on the straight-line method over the estimated useful life.

*Income Taxes*

The Organization has received a letter dated April 3, 1953 indicating they are exempt from income taxes under Code Section 501 (c) (3).

2) *Change in Accounting Method used for Dues and Subscription Income:*

The method of accounting for revenue received from dues and subscriptions has been changed from the cash basis to the accrual basis, retroactive to the year ended June 30, 1976. The effect of these changes on amounts previously reported is as follows:

| | Year Ended June 30, 1977 | Year Ended June 30, 1976 |
|---|---|---|
| Effect on net income increase (decrease) | ($ 2,340) | ($ 7,863) |
| Effect on fund balance increase (decrease) | ( 28,276) | ( 25,936) |

# Amendment to 3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Number 08-17

NUMBER 08-17B

*Formulated by*
*International Association of Milk, Food and Environmental Sanitarians*
*United States Public Health Service*
*The Dairy Industry Committee*

The "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Number 08-17" are amended by the following changes: (underscoring indicates new language):

Part One

I. Rewrite paragraph E.8 to read as follows:

E.8

Air-operated or electrically-operated automatic positive displacement samplers shall comply with the applicable provisions of this standard and the following (reference revised 3-A drawings, in Part Two numbers 3A-100-36, 3-A-100-37, and 3A-100-38):

---

II. Rewrite second line of E.8 1 to read as follows:

E.8 1----------

"head, O-rings, seals, and an air or electrically-operated mechanism."

These corrections have been made in Number 08-17 Rev Part One and Two.

# Amendment to the 3-A Sanitary Standards for Fillers and Sealers of Single Service Containers for Milk and Fluid Milk Products, Number 17-04

NUMBER 17-05

*Formulated by*
*International Association of Milk, Food and Environmental Sanitarians*
*United States Public Health Service*
*The Dairy Industry Committee*

The 3-A standards for fillers and sealers, Number 17-04, are hereby amended by the following:

I. Add a new sub-section C.1.2.1, as follows:

C.1.2.1

Integral stainless steel pumps may be covered by an engineering plating of electroless nickel alloy conforming to the applicable provisions of Military Specifications MIL-C-26074B, as amended[4]. (See also Appendix Section L.)

II. Add a new subsection D.3.1, as follows:

D.3.1

The minimum thickness of an engineering plating of electroless nickel alloy, as specified in C.1.2.1, shall be 0.002 — inch.

III. Add Section L. to the appendix to read as follows:

L.

Electroless Nickel Alloy

L.1

An electroless nickel alloy coating having the following composition is deemed to be in compliance with C.1.2.1 herein:

Nickel — 90% minimum

Phosphorous — 6% minimum and 10% maximum, as a supersaturated solution of nickel phosphide in nickel.

Traces of carbon, oxygen, hydrogen, and nitrogen.

No other elements.

[4] *MIL-C-26074B, 26 March 1969 — Military Specifications; coatings, electroless nickel, requirements for; as amended by Amendment One, 14 May 1971. Supt. Documents, U.S. Printing Office, Washington, D.C. 20402*

*This Amendment will be included in Standard Number 17-06.*

# Amendment To 3-A Sanitary Standards for Batch and Continuous Freezers for Ice Cream, Ices, and Similarly-Frozen Foods, Number 19-02.

NUMBER 19-02-B

*Formulated by*
*International Association of Milk, Food and Environmental Sanitarians*
*United States Public Health Service*
*The Dairy Industry Committee*

The 3-A Sanitary Standards for Batch and Continuous Freezers for Ice Cream, Ices, and Similarly-Frozen Dairy Foods, Number 19-02, are hereby further amended as indicated in the following:

I. Add a new sub-section to C.2, as follows (and re-number accordingly): Optional metal alloy may be used but only in applications requiring disassembly and manual cleaning, such as scraper blades, shaft and shaft bearings, discharge gate, front head, and bearings. (See Appendix, Section I. for the composition of an acceptable optional metal alloy.)

II. Add a new section to the Appendix, as follows, and re-letter accordingly:

I.

*OPTIONAL METAL ALLOY*

An optional metal alloy having the following minimum and maximum composition is deemed to be in compliance with C.2 herein.

| | |
|---|---|
| Zinc | -8% maximum |
| Nickel | -19½% minimum |
| Tin | -3½% minimum |
| Lead | -5% maximum |
| Iron | -1½% maximum |
| Copper | -the balance |

An alloy of the composition given above is properly designated "nickel silver," or according to ASTM[4] Specification B149-70 may be entitled "leaded nickel bronze."

This Amendment will be included in 19-03 when reprinted.

# Amendments to 3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-08

NUMBER 20-08A

*Formulated by*
*International Association of Milk, Food and Environmental Sanitarians*
*United States Public Health Service*
*The Dairy Industry Committee*

The "3-A Sanitary Standards for Multiple Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Number 20-08", are hereby further amended as indicated in the following:

Section I. Standards for Acceptability, Sub-paragraph (2): Add the following materials to the list of Generic Classes of Plastics:

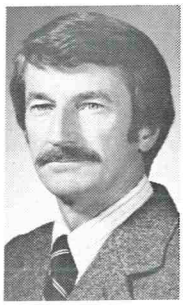
| | Maximum Percent of Weight Gain | | |
|---|---|---|---|
| | Cleanability Response (Section F. Regimen) | Product Treatment (Section G Regimen) | |
| | | Solution I | Solution J |
| Polyoxmethylene copolymer* | 0.25 | 0.60 | 1.00 |
| Ethylene-vinyl acetate copolymers* * | 0.25 | 0.55 | 0.10 |

* *covered by CFR 177.2470*
* * *covered by CFR 177.1350*

# ADSA Award Winners Announced


Prof. E. W. Custer


Dr. G.H. Richardson


Dr. N. F. Olson

E. W. Custer, professor of Dairy Science at Mississippi State University, was named winner of the 1978 Kraftco Teaching Award of the American Dairy Science Association. Dr. G. H. Richardson, professor of Nutrition and Food Sciences at Utah State University, received the 1978 Pfizer, Inc. Award, and Dr. N. F. Olson of the University of Wisconsin won the ADSA's Dairy Research Foundation Award.

Prof. Custer has taught, supervised dairy plant operations, researched, and counseled in the dairy industry for 31 years. He teaches six courses and helped develop others. Custer helped design and now directs operations of a modern dairy processing facility and its program to give practical experience to students and to help them finance their college expenses. Custer is also an active force in continuing education of dairy professionals in his state and nationally. He is an official U.S.D.A. Grader of Dairy Products and Plant Inspector and is Executive Director of the Mississippi Dairy Products Association. He served the American Guernsey Cattle Club as Research Committee Chairman and was Senior Quality Consultant for All Jersey, Inc. Custer is a long-time IAMFES member.

Dr. Richardson's contributions to the dairy industry include the development of a low-cost bacteriophage-resistant culture medium that can be prepared from whey in a cheese factory. When used with proper controls, the starter can be produced at constant pH with much higher activity than can be developed in conventional bulk starters and, as a result, much less starter is needed.

Richardson is the author or co-author of over fifty scientific publications related to milk and cheese. He is also an active member of several professional organizations and societies, including the ADSA, Association of Official and Analytical Chemists, Institute of Food Technologists, and the Intersociety Council on Standard Methods for the Examination of Dairy Products. He is a member of the Editorial Board of the *Journal of Food Protection.*

Dr. N. F. Olson, recipient of the Dairy Research Foundation Award, has concentrated much of his research on cheeses, including solving the cause of pink discoloration in Italian cheese, development of a rapid method for determining suitability of various lactic cultures on mozzarella cheese, and informing the dairy equipment industry of cheese curd flow during processing. Dr. Olson is serving on the Editorial Boards of the *Journal of Food Protection* and *Journal of Dairy Science.* He also participated in the preparation of the 14th edition of *Standard Methods for the Examination of Dairy Products.*

# Coming Events

Oct. 18-IOWA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC., Fall meeting. Waterloo, IA.

Nov. 15-17- FUNDAMENTALS OF THERMAL PROCESSING. Pick Congress Hotel, Chicago, IL. Contact: Dan Weber, Institute of Food Technologists, 221 N. LaSalle St., Chicago, IL 60601.

Nov. 28-29-NATIONAL CONFERENCE ON WATER CONSERVATION AND MUNICIPAL WASTEWATER FLOW REDUCTION. Ramada-O'Hare Inn, Chicago, IL. Contact: U.S. Environmental Protection Agency, c/o Enviro Control, Inc. P.O. Box 1687, Rockville, MD 20850.

Nov. 28-30-FOODSERVICE EQUIPMENT MATERIALS AND FINISHES SEMINAR. Ann Arbor, MI. Contact: Education Service, National Sanitation Foundation, NSF Building, P.O. Box 1468, Ann Arbor, MI 48106.

April 22-25, 1979-1979 CONFERENCE ON INDUSTRIAL ENERGY CONSERVATION TECHNOLOGY. Hyatt Regency, Houston, TX. Co-sponsored by Dept. of Energy and Texas Industrial Commission. Prospective authors send abstracts (300-350 words) to: Dr. Philip Schmidt, Technical Program Chairman, University of Texas at Austin, Austin, TX. 78712.

# Committee Seeks Awards Candidates

Each year IAMFES recognizes outstanding contributions and performance by its members.

The success of the awards is dependent not only on the organizations which generously support the monetary aspects of the awards program, but also on you, for providing the Awards Committee with the names and background information of potential award winners.

Please give serious thought to candidates for the following awards to be considered for presentation at the 1979 IAMFES Annual Meeting.

1. *The Sanitarians' Award* — $1000 to a state or federal sanitarian who, during the past seven years, has made outstanding contributions to the health and welfare of his community.

2. *Educator/Industry Award* — $1000 to a university or industry employee who has made outstanding contributions to food safety and sanitation. In 1979 the award will be made to an educator.

3. *The Citation Award* — Presented to a member who has given outstanding service to IAMFES in fulfilling its objectives.

4. *The Shogren Award* — Presented to the affiliate organization which has the best statewide or regional program.

5. *Honorary Life Membership* — Awarded to a member who has given long and outstanding service to IAMFES.

Contact Henry Atherton, Chairperson of the IAMFES Recognition and Awards Committee, Dept. of Animal Science, Dairy Building, Univ. of Vermont, Burlington, VT 05401; 802-656-2070.

# 1981 IAMFES Meeting Set for Seattle

The Executive Board of IAMFES decided during its meetings at the 1978 Annual Meeting in Kansas City to pursue sites for the 1981 Annual Meeting in Seattle, WA.

The Board's decision followed the news earlier this summer that the National Environmental Health Association's (NEHA) best possibilities for the 1981 meeting centered around Arizona and Nevada. IAMFES does not have affiliates in either location. Richard March, IAMFES President-Elect said, "Our decision has been that during this conference we'll need to make a decision for the location of the 1981 meeting," when he announced the Board action to NEHA representatives Paul Taloff, President, and Larry Krone, Executive Secretary.

"We owe it to our affiliates to respond in the traditional way because we feel strongly that the success of our conference depends on a strong affiliate in the area of the annual meeting," March explained.

"This doesn't preclude plans for a joint meeting with NEHA in 1982," March added.

IAMFES and NEHA will hold a joint meeting in 1980 in Milwaukee, WI.

# AIB offers New Manual

A new book, "Basic Food Plant Sanitation," has been released by the American Institute of Baking.

Prepared by the Department of Sanitation Education, this updated manual contains current information and new laws governing proper sanitation practices for food processing operations.

The cost of the manual is $35.00 and includes postage and handling.

To order, contact: Department of Sanitation Education, American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502.

## Secretary-Treasurer Nominations open

The constitution and by-laws of IAMFES require that a Secretary-Treasurer be elected by mail ballot each year. President Howard Hutchings at the Annual Meeting in Kansas City appointed the following members to the Nominating Committee for 1979: Roy Ginn, Jerry Brown, C. M. Russell, Roger Wray and Harold Barnum.

Nominations for the office of Secretary-Treasurer are now open. Any member wishing to make a nomination for this position should send a biographical sketch and picture of his nominee to the Nominating Committee no later than November 1, 1978. To maintain proper balance on the Executive Board, the nominee this year should be selected from Education.

Harold J. Barnum, Chairman
Nominating Committee
International Association of
Milk, Food and Environmental
Sanitarians
736 Cloute Street
Fort Atkinson, WI 53538

## Pennsylvania Sanitarians Meet

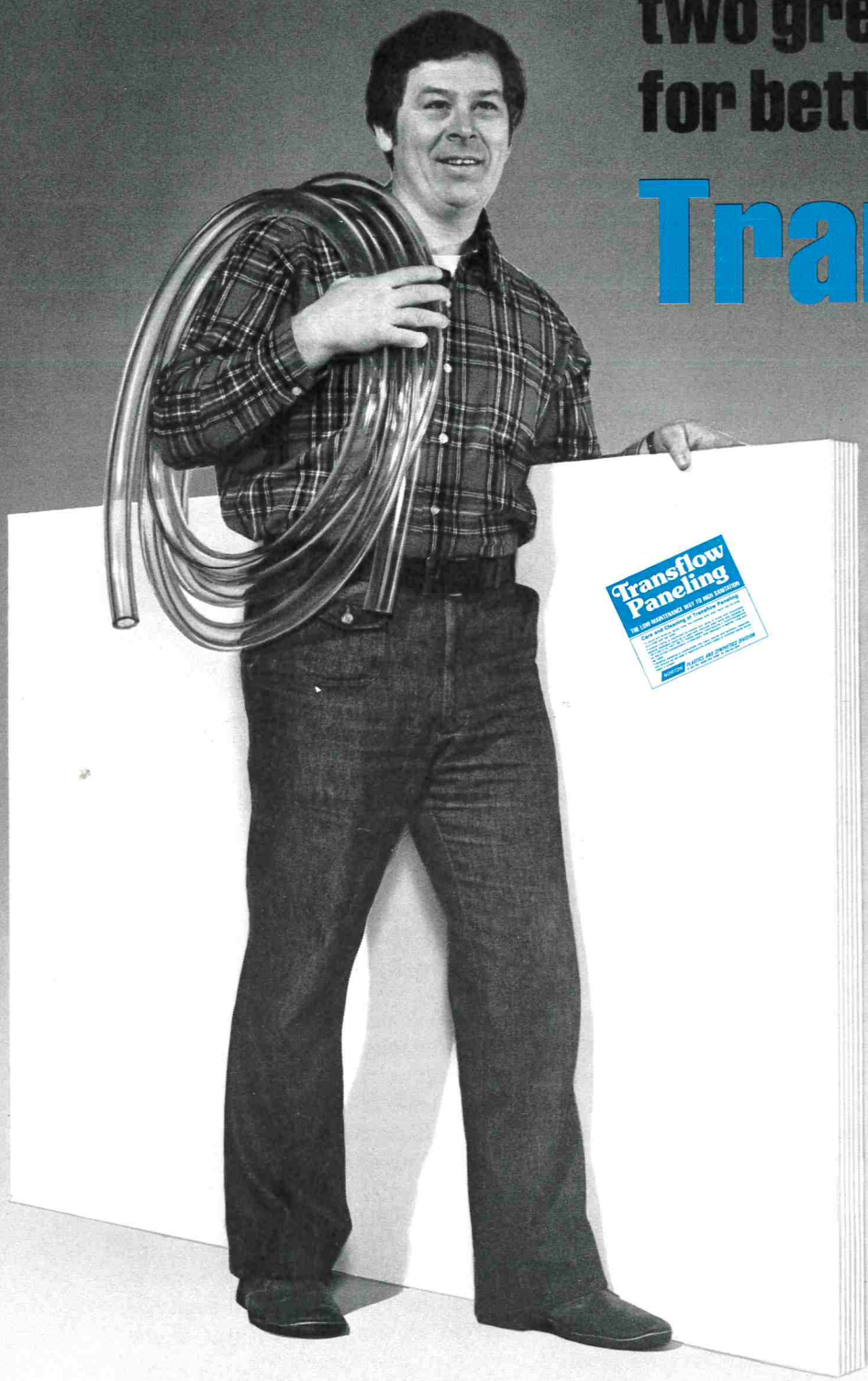
The Pennsylvania Dairy Sanitarians Association held their annual meeting as part of the 36th Dairy Fieldmen's Conference on the University Park campus of Pennsylvania State University. More than 250 persons participated in the three-day conference held June 5-7, 1978.

William Killough, fieldman for Penn Dairies, was given the Sanitarian of the Year Award. Killough has served as secretary-treasurer of the Pennsylvania Association.

Officers elected for 1978-79 were: Elwood Hench, President; Al Gottfried, President-Elect; Allen Murray, Vice-President; and William Killough, Secretary-Treasurer. Association advisors are Sidney Barnard, Stephen Spencer and George Fouse.

# One name, two great ideas for better sanitation:

# Transflow®




## Transflow® tubing

Dairymen across the country rely on Transflow raw milk tubing to protect the wholesomeness and flavor of their product. That's because Transflow tubing is specially designed for the rigid sanitation requirements of the dairy industry.

Transflow tubing cleans quickly and thoroughly. Its inner bore — smoother than rubber tubing and stainless steel pipe — leaves no place for butterfat or milkstone to accumulate. And because Transflow tubing is clear, you can **see** that it's clean and residue-free.

Insist on genuine Transflow M-34R tubing, which complies with FDA and 3-A standards. Just look for its distinctive blue strip.

## Transflow® paneling

The Transflow paneling system is fast gaining a reputation as the premier wall and ceiling covering for dairy operations.

The system — panels, moldings and connectors — installs easily with no special tools. Transflow panels are built tough to withstand repeated cleaning, yet stay bright and new-looking for years. They inhibit mold growth, require little maintenance, and never need painting. Like Transflow tubing, Transflow paneling meets FDA and 3-A standards.

Don't accept substitutes — choose Transflow paneling for your operation. When Transflow paneling goes up, maintenance goes down.

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## Food Protection Conference Proceedings Available

The National Institute for the Foodservice Industry has announced that the *Proceedings* of the National Conference on Food Protection in Foodservice are now available.

The conference addressed many of the nation's concerns and responsibilities regarding food protection in the foodservice industry. This document provides concerned comments and recommendations from food protection leaders in regulatory agencies, industry, and education.

Convened in Chicago, the four-day conference was attended by 136 representatives from 33 states and Canada.

A copy of the *Proceedings* can be obtained from: Mr. C. Dee Clingman, Director, Food Protection Programs, National Institute for the Foodservice Industry, 120 S. Riverside Plaza, Chicago, Illinois 60606.

# Make Your Cows Worth More.

*Dr. Allan Bringe*
*Professor, Dairy Science*
*University of Wisconsin*
*Madison, Wisconsin*

Efficient production of clean, natural-tasting milk which will be in demand by consumers, should be every dairyman's goal. The milking operation and care of your herd should have the highest priority because a full harvest of quality milk will mean more income to you. Dairymen can take advantage of current knowledge and technology to achieve this goal, and make better use of their time while earning more profit.

**DHI Production Records**

These tools are essential for measurement of production to make feeding, breeding, and culling decisions. Use records to detect and correct weaknesses in herd management. You won't know which cows are worth more unless production is measured. Ideally, your milking equipment should have provisions for obtaining DHI milk weights and samples.

**Identify Cows With Hidden (Sub-Clinical) Mastitis**

The invisible loss of milk for each infected quarter is more than a thousand pounds per year. You need some routine method of identifying infected cows early —before you can see clinical mastitis. Each cow can be monitored for mammary infection by:

1. Somatic cell report in DHI programs.
2. California mastitis test.
3. Bacteriological culturing.

Once infected cows are identified, you and your veterinarian can make management decisions regarding proper handling and treatment. When cows become infected with sub-clinical mastitis you should also play the role of a detective to determine the cause and correct the situation that caused the new infection. Mastitis can be kept under control with the following measures:

1. A strict sanitation program.
2. Proper installation, maintenance, and use of milking equipment.
3. Using recommended procedures including teat dipping.
4. Proper treatment of infected quarters. (Select antibiotics for treatment of infected quarters based on previous culturing and sensitivity testing.)
5. Culling.

Dairymen attempting to control mastitis by treatment alone will always be in trouble.

**Routine Milking Machine Service**

You are milking 1977 model cows, bred for high milk production. That means your milking equipment needs to be up to 1977 operating standards. Just because your milking machine starts running when you hit the switch, doesn't mean that it's operating properly. Schedule your equipment for routine service by a competent milking machine serviceman. Make sure that pulsation, vacuum control, vacuum pump, inflations, and other essential parts are functioning correctly. Don't guess. Check equipment performance when all units are milking the highest producing cows. Remember, your milking equipment operates more hours than any other piece of farm equipment, and it's the only equipment that operates on living tissue.

**Provide The Environment For Healthy Calves**

Proper environment, care and attention is essential to raising healthy calves. Poor calf care allows scours and pneumonia to pre-cull many genetic assets from your herd and its future productivity. Genetically superior calves, raised in a healthy manner, give the dairyman an opportunity to cull more selectively and eliminate mastitis problem cows.

This, in turn, helps prevent the spread of pathogenic organisms throughout the herd.

**Managed Milking Procedure**

Plan the best sequence for proper cow milking. Even the best milking system cannot achieve maximum production and avoid udder irritations unless proper milking procedures are followed. The milker's attitude and desire to consistently milk properly is essential. Handle cows gently so they associate milking with a pleasant experience. The preparation and stimulation of the udder is important to saving milking time, obtaining more milk, and reducing teat and udder irritation. Attention to the important routine of sequencing stimulation, time of machine attachment, and proper machine removal will pay big dividends. The challenge is to control procedures so they are properly performed when milking each cow in the herd, regardless of her characteristics. This can be accomplished equally well in a stanchion barn or fully automated milking parlor. The rewards will be better use of your time, better herd health and more profit. You can make your cows worth more!!