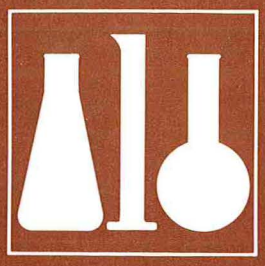

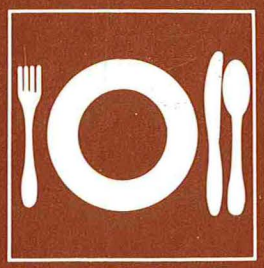

October, 1977
Vol. 40, No. 10
Pages 661-740
CODEN: JFPRDR 40(10):661-740(1977)
ISSN: 0362-028X

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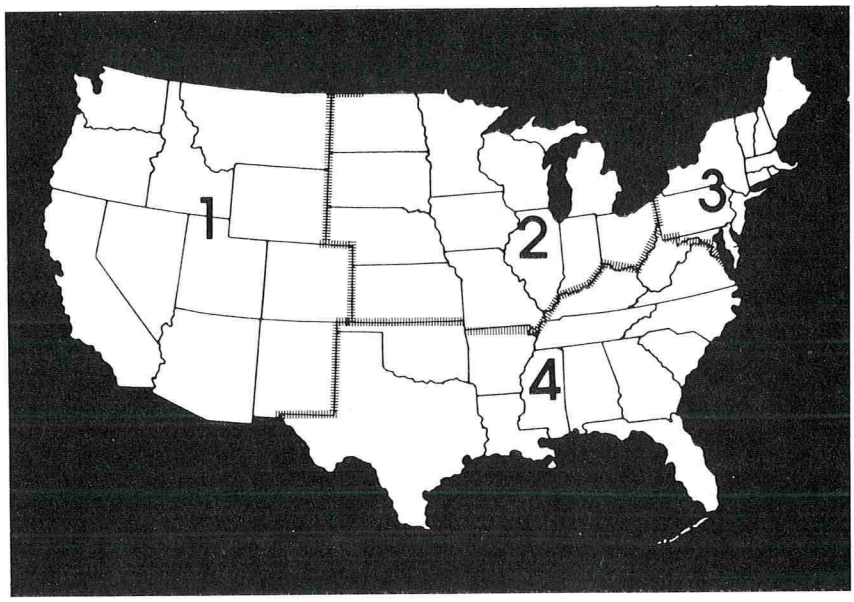


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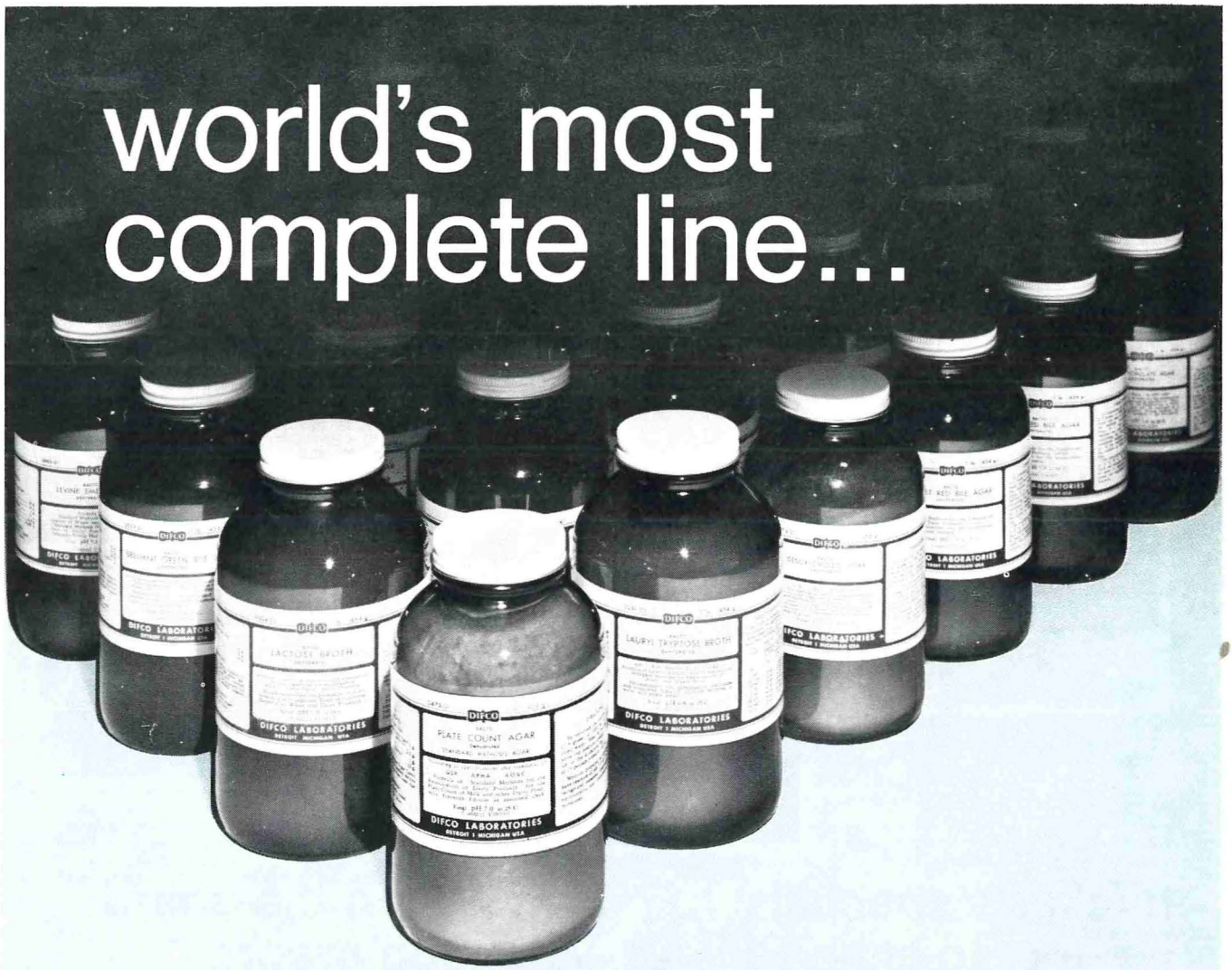


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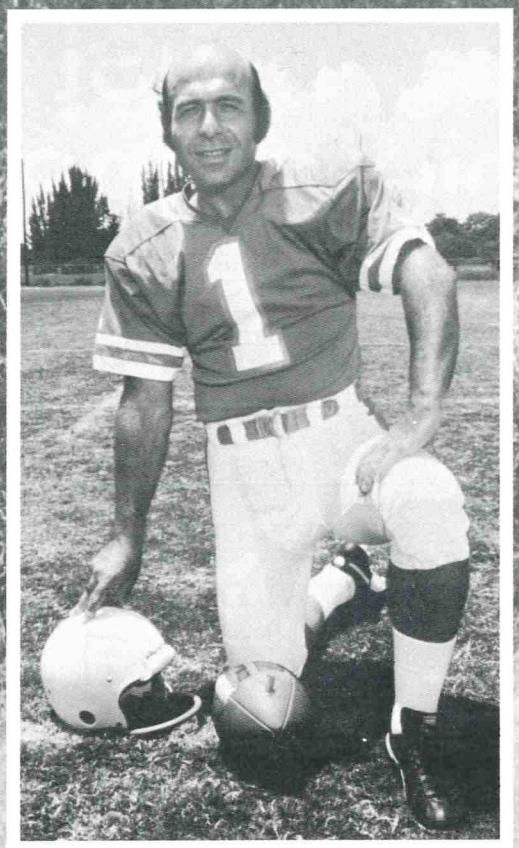

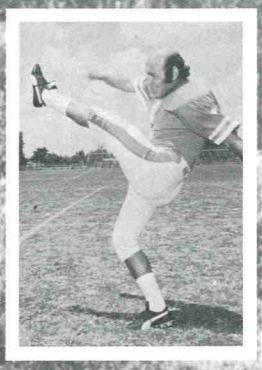

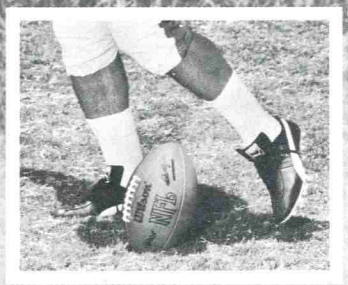

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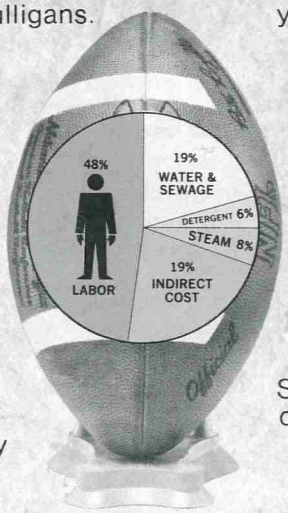


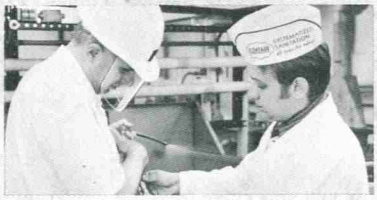

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International Association of Milk, Food, and Environmental Sanitarians, Inc., Reg. U.S. Pat. Off.

Vol. 40 October 1977 No. 10





**The Journal of Food Protection** is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, 413 Kellogg Ave., P.O. Box 701, Ames, Ia. 50010. Printed by Heuss Printing and Signs, Inc., 204 N. Oak, Ames, Iowa 50010.

2nd Class postage paid at Ames, Ia. 50010.

**Editorial Offices:** Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706. Earl O. Wright, P.O. Box 701, Ames, Ia. 50010.

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*Journal of Food Protection Vol. 40, No. 10, Pages 664-667 (October, 1977)*


# Heating Patterns of Products in Crockery Cookers

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(Received for publication April 22, 1977)

ABSTRACT

Heating characteristics at various positions inside two crockery cookers were determined when cookers contained starch gel, beef stew, baked beans (normal amount and overload), or meat loaf (normal amount and overload). Data on heating patterns were compared to growth limits for *Clostridium perfringens* and *Staphylococcus aureus*. Temperatures in the warmest areas of cookers were in the range for growth of *S. aureus* for about 0.4 h and in coolest area for about 3.9 h. The average time products were in the range for growth of *C. perfringens* and *S. aureus* was 1.75 and 1.5 h, respectively. All products, except when cookers were overloaded, were in the range for growth of indicated bacteria for less than or about 2 h. The time difference between when warmest and coolest areas reached 50 C in cooker I ranged from 0.5 to 2.6 h and in cooker II from 0.5 to 3.4 h. Results suggest that growth of the two organisms may occur in certain areas within these cookers if they are overloaded but not when the devices are used according to the manufacturer's directions.

Slow or crockery cookers are among the many types of cooking appliances currently on the market. All of the brands and styles of these devices share the characteristic of cooking food at relatively low temperatures for long periods. Consumer guides (*3*) give the temperature profile for oil or water heated in crockery cookers but these are not necessarily representative of the actual temperature of food in the cookers. This is important when considering the possiblity of bacterial growth and enterotoxin production during the cooking process. *Clostridium perfringens* and *Staphylococcus aureus* are the most likely organisms to be a problem since they can grow at somewhat elevated temperatures. *C. perfringens* grows at 15 to 50 C and *S. aureus* at 6.6 to 46.6 C (*1,2,4,5*). The purpose of this investigation was to determine the heating pattern of various types of foods during cooking in crockery cookers and then to compare those data to growth characteristics of *C. perfringens* and *S. aureus*.

## MATERIALS AND METHODS

Slow cookers used were (*a*) Rival Crock-Pot, Model 3100 (Rival Mfg. Co., Kansas City, MO) (Cooker I), and (*b*) West Bend Bean Pot (West Bend Co., West Bend, WI) (Cooker II). Both cookers were purchased at a local retail outlet.

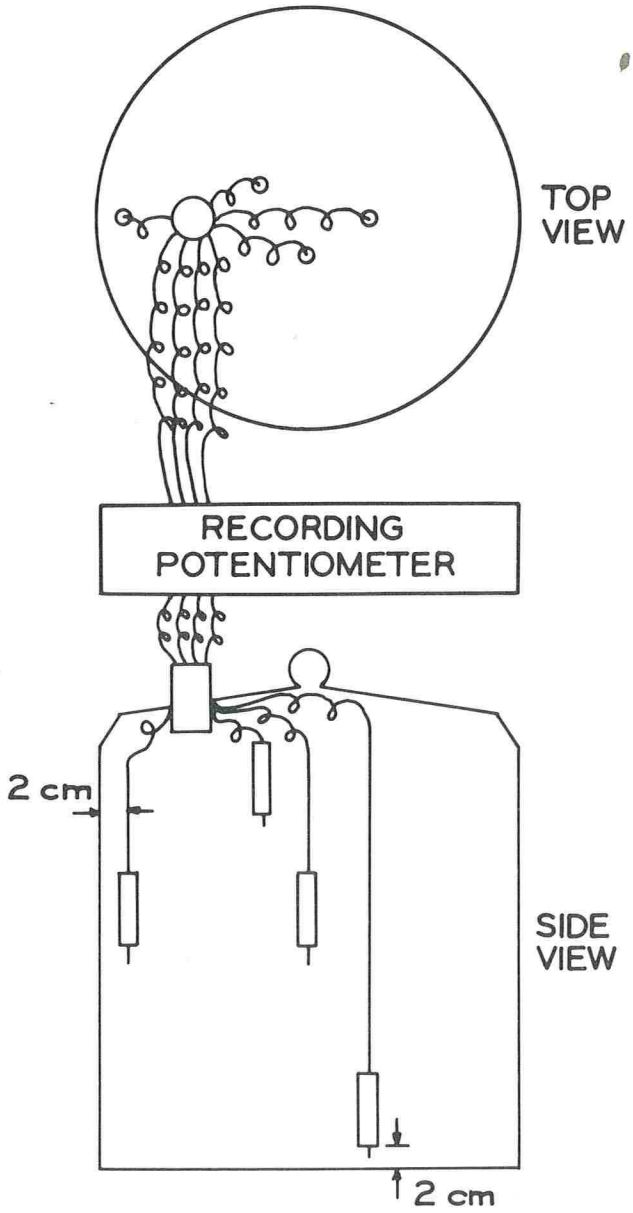


Figure 1. *Arrangement of thermocouples within cookers.*

A recording potentiometer and copper-constantan thermocouples were used for temperature measurements. Thermocouples were positioned at four points (Fig. 1) in cookers and were attached to covers with heat-resistant silicone cement. Thermocouple leads were passed through a 0.75-inch hole near the center of each cover; 00 rubber stoppers were used to seal the holes during tests. This allowed the cookers to operate in a normal manner with no loss of steam.

Measurements of heating patterns were made while simulating normal cooking conditions. All but foods for overload tests and starch gel were prepared following recipes given in owner's manuals for the cookers. Samples tested included 10% starch gel, beef stew, meat loaf, and baked beans. Cookers also were tested when overloaded with baked beans or meat loaf. Materials and amounts used for preparations other than starch gel are listed in Table 1. Products were chosen to allow testing with a viscous, a solid, and a chunky material. Cookers were

TABLE 1. *Ingredients in foods tested in slow cookers*[a]

| Food | Ingredients | Cooker I | Cooker II |
|---|---|---|---|
| Beef stew | ground beef | 2.32 lb. | 1.65 lb. |
| | potatoes | 3 ea. | 2 ea. |
| | carrots | 3 ea. | 2 ea. |
| | dried onion | 3 ea. | 2 ea. |
| | celery | 1 stalk | 1 stalk |
| | water | 1 cup | 2/3 cup |
| Meat loaf | ground beef | 1.5 lb. | 1 lb. |
| | eggs | 2 ea. | 1 ea. |
| | bread crumbs | 2 slices | 1.5 slices |
| | diced onion | 0.5 ea. | 0.5 ea. |
| Baked beans | beans (dry) | 1.5 lb. | 1 lb. |
| | onions | 1 ea. | 1 ea. |
| | brown sugar | 1 cup | ⅔ cup |
| | salt pork | 0.5 lb. | 0.25 lb. |
| | molasses | 1 cup | 2/3 cup |
| | water | 1 cup | 2/3 cup |
| Baked beans (overload) | beans (dry) | 3.5 lb. | 2.5 lb. |
| | onions | 2.5 ea. | 1.5 ea. |
| | brown sugar | 2.5 cups | 1⅔ cups |
| | salt pork | 1 lb. | 0.33 lb. |
| | molasses | 2 cups | 2/3 cup |
| | water | 2 cups | 1.5 cups |
| Meat loaf (overloaded) | ground beef | 4 lb. | 3 lb. |
| | eggs | 3 ea. | 2 ea. |
| | bread crumbs | 5 slices | 3 slices |
| | diced onion | 1 ea. | 1 ea. |

[a]Recipes from Crock-Pot® owner's manual with some alterations to coincide with Bean Pot® owner's manual. Spices not included in preparations.

overloaded to determine behavior of the devices when they were abused. Heating patterns were measured to determine the length of time individual thermocouples, and hence areas within cookers, were at temperatures in the zone of growth for *C. perfringens* and *S. aureus*.

## RESULTS AND DISCUSSION

Heating patterns of individual preparations are shown in Fig. 2-4. In all instances, the heating pattern at various locations within each cooker was different. One of the more noteworthy observations was the erratic temperature profile of starch (Fig. 2), baked beans (Fig. 3), and meat loaf (Fig. 4). When starch gel and baked beans were heated, bubbles moving past the thermocouples caused the erratic results. With meat loaf, variations resulted from changing the temperature setting on Cooker I from "high" to "low". This was done in accord with directions by the manufacturer.

When cookers were overloaded, heating of food was

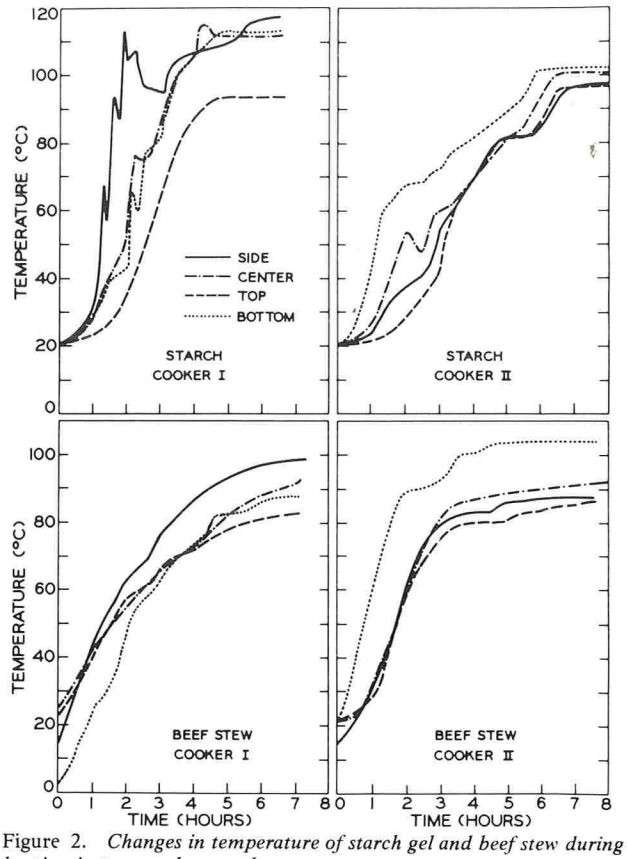


Figure 2. *Changes in temperature of starch gel and beef stew during heating in two crockery cookers.*

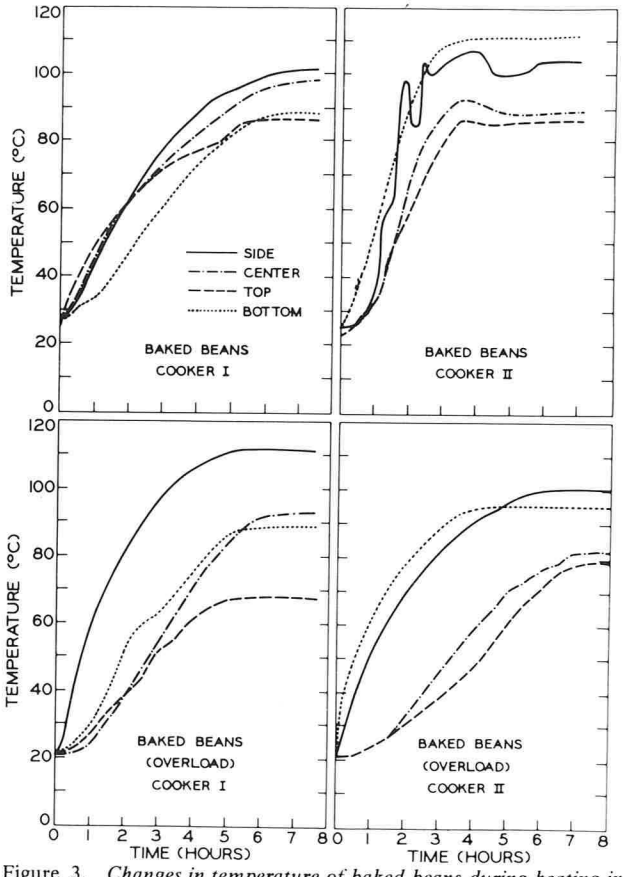


Figure 3. *Changes in temperature of baked beans during heating in crockery cookers. Cookers were operated normally and with an excessive amount of product.*

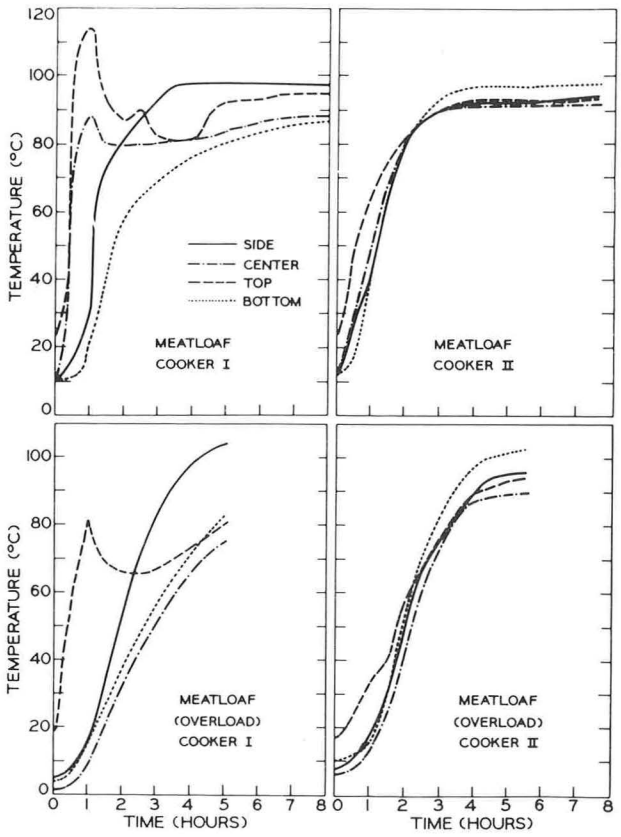


Figure 4. *Changes in temperature of meat loaf during heating in crockery cookers. Cookers were operated normally and with an excessive amount of product.*

generally slower than when the devices were used as recommended by manufacturers. Overloading of cookers except with meat loaf in cooker II resulted in a larger range in temperature within the cooker than occurred when cookers were not overloaded (Fig. 3 and 4).

Table 2 gives times required for individual areas within cookers to reach 46.6 and 50 C. These temperatures were reached fastest in areas closest to the heating elements of the cooker; on sides of cooker I and bottom of cooker II. Exceptions occured with baked beans in cooker I and with meat loaf in both cookers. This happened because of the relatively small amount of product in the cooker so that the top thermocouple was positioned above the sample. The time between when warmest and coolest areas reached 50 C in cooker I ranged from 0.5 to 2.6 h. For cooker II this range was 0.5 to 3.4 h.

The heating pattern in cookers tested seemed to be influenced by several factors. The type of heat transfer is important. Foods which were heated by conduction and convection (e.g., beef stew) heated faster than did products which heated primarily by conduction. Steam was important for supplying heat to the product from the top and/or sides in addition to heat supplied to the food by heating elements of the cooker. This is illustrated by the rapid heating of meat loaf (Table 2). The position of heating elements also had an appreciable effect on the heating pattern of food in cookers. The cooker which

TABLE 2. *Time required for products to reach growth maxima for* S. aureus *(46.6 C) and* C. perfringens *(50 C)*

| Preparation | Cooker | Thermocouple position | | | |
|---|---|---|---|---|---|
| | | Side | Bottom | Center | Top |
| | | (Hours to reach 46.6 C/hours to reach 50 C) | | | |
| Starch Gel | I | 1.2/1.25 | 1.1/2.1 | 1.8/1.9 | 2.4/2.5 |
| | II | 2.75/2.9 | 1.1/1.2 | 1.75/1.8 | 3.1/3.2 |
| Beef Stew | I | 1.1/1.25 | 1.9/2.1 | 1.4/1.6 | 1.4/1.6 |
| | II | 1.6/1.7 | 0.6/0.7 | 1.5/1.7 | 1.6/1.7 |
| Baked Beans | I | 1.1/1.4 | 2.0/2.2 | 1.1/1.2 | 0.9/1.1 |
| | II | 1.2/1.2 | 0.75/0.9 | 1.5/1.6 | 1.4/1.5 |
| Baked Beans (overloaded) | I | 0.7/0.8 | 1.8/2.0 | 2.6/2.75 | 2.7/2.9 |
| | II | 0.8/0.9 | 0.4/0.5 | 3.0/3.1 | 3.9/4.3 |
| Meat loaf | I | 1.0/1.1 | 1.6/1.7 | 0.4/1.0 | 0.4/0.6 |
| | II | 1.1/2.1 | 1.1/2.1 | 0.9/2.0 | 0.45/0.6 |
| Meat loaf (overloaded) | I | 2.8/3.0 | 2.5/2.7 | 1.9/2.0 | 0.3/0.4 |
| | II | 1.9/2.0 | 1.9/1.9 | 2.1/2.2 | 1.7/1.8 |

heated from the sides appeared more efficient than the one that heated from the bottom only. This is evident when comparing results from Cooker I with those from Cooker II (Table 2). The most obvious factor affecting the heating pattern is the amount of food in the cooker. Large amounts heated more slowly than smaller amounts, as is evident from heating patterns of products in overloaded and normally loaded cookers (Table 2).

Sundberg and Carlin (*6*) found that the cooking process in a crockery cooker operating on "low" reduced counts by 3.96 log cycles when a rump roast was inoculated with $6.3 \times 10^6$ *Clostridium perfringens*/g. In their investigation the center of the sample remained in the temperature range allowing growth of *C. perfringens* for about 2 h.

Our results also suggest that there was little danger for appreciable growth of *C. perfringens* or *S. aureus* when foods were prepared in test cookers as suggested in the owner's manuals. In almost all instances different points in the cookers were in the range of temperature for growth of *C. perfringens* less than or about 2 h when the cookers were not overloaded. Thus, appreciable growth and survival of *C. perfringens* would not be expected to occur. When cookers were overloaded with baked beans, there were areas where the temperature remained in the range for growth of *C. perfringens* for up to 4.3 h (Table 2). Furthermore, there were large temperature differences within the same cooker. This suggests that certain areas of a cooker might allow growth of these bacteria if foods were cooked in larger than recommended quantities. Since *S. aureus* grows to 46.6 C (*1*), one would expect the heating patterns to have much the same effect on growth of this organism as on *C. perfringens*.

It must be remembered that these data were obtained with two kinds of cookers. There are many such devices on the market and it is not known whether or not these results would be applicable to other cookers.

ACKNOWLEDGMENTS

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin — Madison. The authors thank Drs. J. H. von Elbe and D. B. Lund for their help in this research.

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*Journal of Food Protection Vol. 40, No. 10, Pages 688-670 (October, 1977)*


# Combined and Individual Effects of Washing and Sanitizing on Bacterial Counts of Meat - A Model System[1,2]

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(Received for publication March 7, 1977)

ABSTRACT

Strips of plate meat were sprayed with acetic acid, sodium hypochlorite, or tap water after they were washed with 0, 12.7, or 25.4 liters of tap water/min. Washing before sanitizing lowered bacteria counts significantly only when the higher volume of water, 1.4 ml/cm², was applied, and this difference existed for samples taken immediately but not 48 h after treatment. Reductions in counts exceeded 99.9% when samples washed with 25.4 liters/min (1.4 ml/cm²) were sanitized with 3% acetic acid. This sanitizer was sprayed at the rate of 6.8 liters/min (1.9 ml/cm²) at a pressure of 14.0 kg/cm² from a distance of 40 cm as the meat moved at 2 cm/sec through the spray. Under comparable conditions of application, both sodium hypochlorite (200 to 250 mg/liter) and tap water reduced counts by about 90%. Acetic acid had a much greater residual effect on numbers of viable bacteria than did hypochlorite. No effect of air drying was observed.

Meat becomes contaminated with microbes during slaughter, processing and preparation for consumption. Growth of organsims on meat is the main cause of discoloration and spoilage that result in loss of quality and value (*13*). Improved methods are needed to more efficiently clean and sanitize carcasses following slaughter.

We previously reported on removal of microbes from meat as influenced by physical factors such as volume of solution, angle of impact, line pressures, force of spray and speed of meat moving through the water sprays (*1*). Also, we reported on sanitizing with hypochlotite (*8*) and several other sanitizers (*2*). Several other researchers (*3,4,7,9,10*) have also studied removal or destruction of microorgansims on meat carcasses by various sanitizing sprays.

Most of the research on carcass disinfection has involved the use of sanitizers only. The purpose of this research was to determine effects of combinations of washing and sanitizing treatments. In decontamination of meat

## MATERIALS AND METHODS

Strips of refrigerated plate meat, about 20 × 30 × 1.5 cm, were obtained from a single source and frozen. Aerobic plate counts of the meat ranged from $10^5$ to $10^9$/cm². Twelve strips were thawed for each experiment and placed individually on holding frames. Four samples (2.54 cm diameter) were removed from the top surface (3 mm depth) of each strip before it was washed and/or sanitized (designated BE in Fig. 1).

Frames with meat were placed on the carriage of the spraying unit and successively moved in a horizontal position past vertically oriented washing, drying, and sanitizing nozzles. Frames and meat were removed and allowed to drain in a vertical position for 1 min. Then four more samples (designated AF in Fig. 1) were taken from each strip.

Meat was removed from the frames, placed on trays, stored at 3.3 C for 48 h, then sampled (designated 48 in Fig. 1).

The experiment was of a randomized, complete block design (3 × 2 × 3 × 2) with two replications. It consisted of three washing conditions—no washing and washing with 12.7 and 25.4 liters of water/min; two drying conditions—drying with 9.3 cm³ of air/cm² of

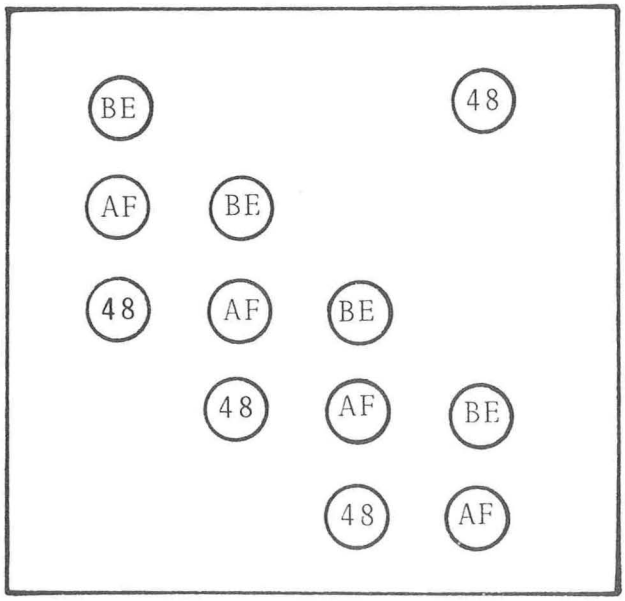


Figure 1. *Pattern for removing samples from meat before (BE), immediately after (AF), and 48 h (48) after treatment.*

[1]*Contribution from the University of Missouri Experiment Station, Journal Series No. 7490.*
[2]*Mention of brand names does not imply endorsement by the United States Government.*
[3]*North Central Region, Agricultural Research Service, United States Department of Agriculture.*
[4]*Department of Food Science and Nutrition.*

meat surface and no drying; three sanitizing conditions—3% acetic acid, 200-250 mg/liter sodium hypochlorite (pH adjusted to 6.0 with acetic acid), and tap water (control); and two speeds of meat travel past the sanitizing nozzles—2 and 10 cm/sec. Conditions of sanitizing were statistically confounded during the first half and conditions of washing were confounded during the second half of the experiment. This type of experimental design allowed us to examine effects of a relatively large number of variables with relatively small numbers of samples. However, the design required the averaging of effects of individual variables over others. This tended to reduce the magnitude of effects of individual variables.

Wash water at 10-15 C was pumped under a pressure of 14.0 kg/cm² through nozzle No. 5015 (12.7 liters/min) or No. 5030 (25.4 liters/min) (Spraying Systems Co., Wheaton, IL) set 40 cm above the meat. Speed of meat travel during washing was 10 cm/sec. Effective width of the spray pattern was 30.5 cm. Therefore, meat was exposed to 0.7 ml/cm² from nozzle 5015 and to 1.4 ml/cm² from nozzle 5030. Air nozzles, fabricated locally, produced elliptical patterns for removing the maximum amount of water from the meat surface.

Sanitizers were sprayed through nozzle No. 5008 (Spraying Systems Co.) at the rate of 6.8 liters/min at a pressure of 14.0 kg/cm² from a distance of 40 cm. Meat traveling at 2 cm/sec through the sanitizing spray received 1.9 ml/cm² and that traveling at 10 cm/sec received 0.4 ml/cm².

Cores of samples were placed in 99 ml of sterile phosphate buffered distilled water in sterile blender jars. Each core weighed approximately 3 g. Blending was at high speed for 1 min. Samples were plated in duplicate, according to standard methods (*6*), and incubated at 28 C for 72 ± 4 h.

Average counts of colonies on duplicate plates were transformed to logarithms. Logarithms of counts made immediately after and 48 h after treatment were subtracted from the logarithms of respective counts made before treatment (termed log difference). Effects of variables on these differences in log counts were determined by analysis of variance and regression analysis (*12*) by the Statistical Analysis System (SAS) in an IBM 370 computer. Differences among means were determined by Duncan's multiple range test (*5*).

## RESULTS AND DISCUSSION

Log differences in counts of viable microorganisms on meat sampled immediately and 48 h after washing and sanitizing (averaged over type of sanitizer, conditions of drying, and speed of meat through the sanitizing spray) are given in Table 1. Washing the meat before sanitizing contributed to decreases in bacteria counts on sanitized surfaces. However, differences were significant only with the highest volume of water, 25.4 liters/min, and for samples taken immediately after sanitizing. No significant effect of washing was observed with samples collected 48 h after treatment.

Drying the meat after washing had no significant effect on counts made after sanitizing. When samples were collected immediately after treatment, differences in log counts on surfaces of dried and undried meat were -1.64 and -1.67, respectively. For samples collected 48 h after treatment respective counts were -0.99 and -0.95. This variable was included because of the possibility that water left on the meat after washing would dilute the sanitizers and make then less effective. These results suggest that the quantity of water remaining was insignificant in relation to sanitizer activity.

Acetic acid was the most effective sanitizer (Table 2).

TABLE 1. *Log differences in counts of viable microorganisms on meat sampled immediately and 48 h after washing and sanitizing*

| Flow rate of wash water | Rate of application | Nozzle no. | Differences in log counts[a,b] Sampled immediately | Samples after 48 h |
|---|---|---|---|---|
| (1 liter/min) | (ml/cm²) | | | |
| 0 | 0 | — | -1.43[b] | -0.70[a] |
| 12.7 | 0.7 | 5015 | -1.59[b] | -1.14[a] |
| 25.4 | 1.4 | 5030 | -1.94[a] | -1.06[a] |

[a]Means of 48 values, averaged over types of sanitizer, conditions of drying, and speeds of meat through the sanitizing spray.
[b]Values in the same column with different superscripts are significantly different ($P < 0.05$).

TABLE 2. *Differences in log counts of microorganisms on meat sampled immediately after sanitizing and 48 h later*

| Sanitizer | Differences in log counts[a,b] Sampled immediately | Sampled after 48 h |
|---|---|---|
| Acetic acid | -2.72[a] | -2.55[a] |
| Hypochlorite | -1.19[b] | -0.19[b] |
| Water (control) | -1.05[b] | -0.15[b] |

[a]Means of 48 values, averaged over volumes of wash water, conditions of drying, and speeds of meat through the sanitizing spray.
[b]Values in the same column with different superscripts are significantly different ($P < 0.05$).

The average difference between log counts taken before and immediately after sanitizing (averaged over other variables) was -2.72 for acetic acid. The average difference for samples taken after 48-h storage was practically the same, -2.55. Hypochlorite was slightly more effective than was water, but mean differences for samples taken immediately after sanitizing averaged less than half those for acetic acid. Counts on meat sanitized with hypochlorite were near the initial values after 48-h refrigerated storage. This indicates that bacteria grew during storage of hypochlorite-treated meat.

Stated in terms of percentage reduction, acetic acid caused average reductions in viable plate counts of 99.6 and 99.5%, respectively, for samples taken immediately after sanitizing and 48 h later. Counts made immediately after sanitizing with hypochlorite and with water showed reductions of slightly more than 90%, but after 48 h of storage, average reductions were 19% for hypochlorite and 14% for water.

Speed of travel of meat through the sanitizing spray proved important. Counts (averaged over all other variables) were significantly lower ($P < 0.05$), both immediately after sanitizing and 48 h later, when samples were sanitized on the slower-moving carriage (2 vs 10 cm/sec) which gave an application rate of 1.9 vs 0.4 ml/cm². Differences in log counts for meat moving at 2 and 10 cm/sec and sampled immediately after sanitizing were -1.80 and -1.51, respectively, whereas values for samples taken 48 h after sanitzing were -1.20 and -0.73.

When data on effect of speed of travel were averaged within sanitizers ($n = 24$), the residual effect of spraying with acetic acid was greater ($P < 0.05$) for samples moved at 2 cm/sec compared with 10 cm/sec. Differences in log counts for these samples were -2.99 (immediate

sampling) and -2.96 (48 h sampling), whereas those for faster moving samples were -2.45 and -2.14, respectively. This greater residual effect probably resulted from the lower pH and higher residual concentration of unionized acid in the slower-moving samples. Hypochlorite had a slightly greater but insignificant residual effect when applied to the slower-moving meat than when applied to the faster-moving meat. The average differences in log counts for all samples treated with hypochlorite at meat travel speeds of 2 and 10 cm/sec and sampled after 48 h were -0.48 and +0.09, respectively. Counts of water-treated samples were slightly lower 48-h after sanitizing than were counts made before washing and sanitizing.

Table 3 gives detailed data showing differences in log counts that are averages of analyses of eight samples each. These data indicate that optimal conditions for treatment were washing at 25.4 liters of water/min at a pressure of 14.0 kg/cm² through nozzle No. 5030 (1.4 ml/cm²) followed by application of 6.8 liters/min of 3% acetic acid from nozzle No. 5008 at a pressure of 14.0 kg/cm² from a distance of 40 cm and at a speed of 2 cm/sec (1.9 ml/cm²). Under these conditions, log differences of -3.52 and -3.27 were obtained for samples taken immediately after sanitizing and 48 h later. Therefore, we can expect more than 99.9% reductions in counts under these conditions.

Based on these data acetic acid (3%) was a better sanitizer than hypochlorite and it had a greater residual effect. Washing with 1.4 ml of water/cm² before sanitizing contributed to decreases in bacteria counts of samples taken immediately after sanitizing but not of samples taken 48-h later.

TABLE 3. *Differences in log counts of microorganisms on meat immediately after sanitizing and 48-hr later as affected by volume of wash water, sanitizer, and speed of meat through the sanitizing spray*

| Flow rate of wash water | Rate of applying wash water | Sanitizer | Speed of meat through sanitizer | Rate of applying sanitizer | Differences in log counts[b,c] | |
|---|---|---|---|---|---|---|
| | | | | | Sampled immediately | Sampled after 48 h |
| (liters/min) | (ml/cm²)[a] | | (cm/sec) | (ml/cm²)[a] | | |
| 0 | 0 | acetic acid | 2 | 1.9 | -2.54$^{bcd}$ | -2.58$^{abc}$ |
| 0 | 0 | acetic acid | 10 | 0.4 | -2.04$^{def}$ | -1.68$^{cdef}$ |
| 12.7 | 0.7 | acetic acid | 2 | 1.9 | -2.91$^{abc}$ | -3.02$^{ab}$ |
| 12.7 | 0.7 | acetic acid | 10 | 0.4 | -2.18$^{cde}$ | -2.40$^{abcd}$ |
| 25.4 | 1.4 | acetic acid | 2 | 1.9 | -3.52$^{a}$ | -3.27$^{a}$ |
| 25.4 | 1.4 | acetic acid | 10 | 0.4 | -3.13$^{ab}$ | -2.35$^{abcde}$ |
| 0 | 0 | hypochlorite | 2 | 1.9 | -1.30$^{fg}$ | -0.25$^{gh}$ |
| 0 | 0 | hypochlorite | 10 | 0.4 | -1.02$^{fg}$ | -1.01$^{gh}$ |
| 12.7 | 0.7 | hypochlorite | 2 | 1.9 | -1.12$^{g}$ | -0.84$^{fg}$ |
| 12.7 | 0.7 | hypochlorite | 10 | 0.4 | -1.38$^{f}$ | -0.08$^{gh}$ |
| 25.4 | 1.4 | hypochlorite | 2 | 1.9 | -1.00$^{g}$ | -0.34$^{gh}$ |
| 25.4 | 1.4 | hypochlorite | 10 | 0.4 | -1.33$^{fg}$ | 0.35$^{gh}$ |
| 0 | 0 | water | 2 | 1.9 | -1.20$^{fg}$ | -0.16$^{gh}$ |
| 0 | 0 | water | 10 | 0.4 | -0.50$^{g}$ | 0.50$^{h}$ |
| 12.7 | 0.7 | water | 2 | 1.9 | -1.20$^{fg}$ | -0.08$^{gh}$ |
| 12.7 | 0.7 | water | 10 | 0.4 | -0.77$^{g}$ | -0.42$^{gh}$ |
| 25.4 | 1.4 | water | 2 | 1.9 | -1.37$^{fg}$ | -0.29$^{gh}$ |
| 25.4 | 1.4 | water | 10 | 0.4 | -1.25$^{fg}$ | -0.47$^{gh}$ |

[a]Rates of applying wash water and sanitizer were not variables as such in the analysis of variance.
[b]Means of 8 values, averaged over conditions of drying.
[c]Values in the same column with different superscripts are significantly different ($P < 0.05$).

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*Journal of Food Protection Vol. 40, No. 10, Pages 671-675 (October, 1977)*


# Optical Somatic Cell Counting and Total Protein Analysis in a Dairy Herd Improvement Program[1]

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(Received for publication October 6, 1976)

ABSTRACT

Milk sample preparation for Optical Somatic Cell Counter II operation was simplified by using a diluter to add fixative, mix, and dilute samples. Potassium dichromate preservative tablets produced a mean increase of 7,000 in somatic cell counts in fresh milk. Samples held at 20-23 C beyond 2 days or at 4-7 C beyond 4 days showed a reduction in somatic cell count. The mean somatic cells in 3 Holstein herds tested over a 6-month period was $3.8 \times 10^5$/ml. A 22-month survey of 52.6 thousand Utah Dairy Herd Improvement samples which were shipped under ambient conditions and then held at 5 C until tested, indicated 75% below 400,000 and 2.7% above 1.6 million somatic cells/ml. Casein, noncasein protein, total protein, fat and milk weight data were also obtained on the three herds. Multiple correlations were obtained. The best correlations suggested that testing for total protein and somatic cells in a central laboratory would estimate casein and noncasein protein. Such tests are most valuable for the cheese industry.

The Optical Somatic Cell Counter I (OSCC I) of Technicon[2,3] has been approved for use at 30 or 60 milk samples per hour by the AOAC (*11,12,21*). After testing an average of 1253 samples per month for 17 months, Ragsdale (*17*) reported that the OSCC eliminated the need for doing both screening and confirmatory tests, allowed the maximum numbers of samples to be tested without additional technical help, and provided a more economical, accurate, and dependable method than manual methods. Heeschen et al. (*10*) operated the OSCC system at 70-80 samples per hour and claimed complete automation with a correlation coefficient of + .97 with the microscopic count method.

Standard electronic methods have demonstrated greater precision than the direct microscopic somatic cell count (*9,18,23*), but they sometimes require extensive preparatory steps, thus discouraging routine use (*12*). New methodology allows simultaneous operation with presently available milkfat test instruments and is completely automated at 180 samples per hour (*2*).

The OSCC II system operates at 120 samples per hour but has not received AOAC approval because of significant changes in the system and lack of collaborative study which is scheduled for complettion in 1977. This paper summarizes the application of the OSCC II in a central Dairy Herd Improvement (DHI) milk test facility, the submission of somatic cell data to a DHI computer service, and modified sample preparation steps to simplify sampling alongside a Milko-Tester Mark III. It also discusses correlation of the somatic cell count with other milk parameters with the intent to establish payment guidelines where casein purchase is involved, primarily for cheesemaking.

The cheese industry could benefit from rapid, accurate assays for casein. Direct assays are tedious (*4,11,19*). Rapid automated indirect casein assay is possible with proper instrumentation (*26,27*). Dye binding tests offer good potential. Indirect methods require assay of total and noncasein protein and obtaining casein by difference (*13,16*). McGann et al. (*15*) developed an assay requiring three amido black dye binding readings. This made it possible to use equal volumes of sample in each test, keep readings in the low-error section of the colorimeter scale, avoid analytical weighing and eliminate time consuming steps of coagulation and centrifugation. Roper and Dolby (*20*) reported a similar technique. This report evaluates the use of this approach using acid orange 12 dye and correlation of fractionation results with the somatic cell data.

## MATERIALS AND METHODS

### *Somatic cell assay*

Fresh raw milk samples were obtained from three local Holstein herds (a mean of 215 animals tested each month) on a monthly schedule for over 6 months. The milk was assayed for somatic cells using the OSCC II (*1*), for milkfat using the Milko-Tester Mark III (*9,11*), for total protein, non-casein protein and casein using a modification of the procedure of McGann et al. (*15*) and for milk weight using DHI approved Milko-Meters (*24*). Subsequently, 52,612 DHI samples were tested for somatic cell count over a 22-month period upon request of DHI patrons.

Somatic cell assay fixation of somatic cells with formalin was required to prevent cell destruction by the solubilizing reagent. Four milliliters of milk were transferred into a tube with a 5-ml syringe (*1*). About .05 ml of formalin fixative [Technicon #T01-0435 (*15*)] was dispensed into each sample. Fixation was carried out at room temperature (20-23 C) for 18 h or in 55-C water bath for 45 min. We modified sample preparation to allow fixation and initial sample

[1]*Contribution No. 2129 of the Utah Agricultural Experiment Station. Approved by the Director.*
[2]*Mention of commercial products implies neither endorsement nor criticism by Utah State University.*
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dilution in a single step through the use of a Fisher Auto Diluter (Model 250)[4]. The diluted was adjusted to provide a milk/diluent ratio of 1/3.6. The fixative/diluent solution was prepared by mixing 8.5 g of NaCl and 5 ml of formalin (37%) in a 10-15% methanol solution in a 1-liter flask and adding distilled water to 1 liter. The diluent reservoir was connected to the Auto Diluter inlet. A freshly mixed milk sample, just tested on a Milko-Tester Mark III, was introduced to the inlet tube of the Auto Diluter. The food switch was activated and 1 ml of milk was drawn into the delivery tip while 3.6 ml of diluent was drawn into the diluter syringe. An empty, clean 12 × 85 mm test tube, compatible with the Technicon Sampler IV tray, was placed under the diluter delivery tip. The foot switch was activated and both milk and diluent were discharged and mixed into the tube. The complete Auto Diluter cycle took only 11 sec. Following fixation, samples were oriented in the Sampler IV tray and tested without further pretreatment.

The OSCC II instrument consists of five modules: Sampler IV, Proportioning Pump II, a Heating Bath, an Optical Cell Counter II, and a Recorder. At 120 samples per hour a 3:1 cam was included which allowed for a 24-sec sample and 6-sec wash time. The heating bath was operated at 65 ± 1 C.

The recorder had a linear presentation from 0 to 100. When standarized with the Technicon standard turkey cell suspension, the recorder tracing was adjusted to 75 for 1.5 million cells per ml. The recorder peak heights were multiplied by $2 \times 10^4$ to obtain the somatic cell count. The range was thus 0-2 million cells per ml. The hydraulic system was simplified as shown in Fig. 1.

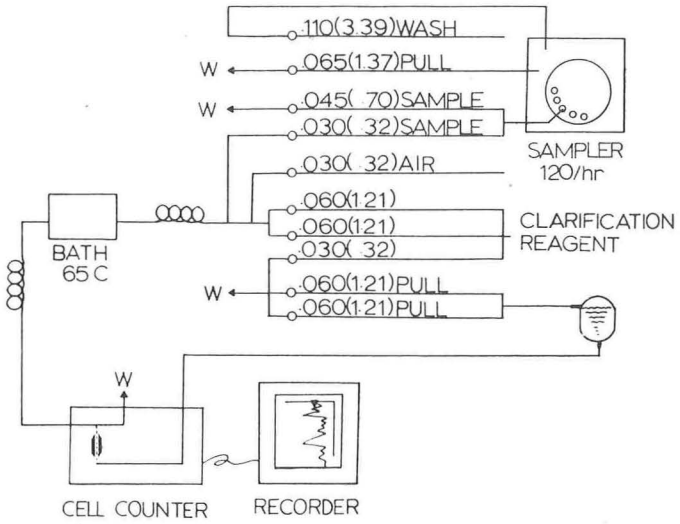


Figure 1. *Diagram of the OSCC II somatic cell counting system following removal of the initial dilution loop. Numbers indicate tubing internal diameters with parenthetical numbers indicating approximate ml/min. The first mixing coil contained 20 turns and the second contained 10 turns.*

Milk samples were stored under ambient and refrigeration conditions with and without preservatives to evaluate the ability to use the OSCC II in connection with a DHI central laboratory testing program. Over 52,000 dichromate-perserved samples were subsequently tested over a 22-month period using the conditions established.

The DHI Computer Service, Provo, Utah, used seven symbols for encoding somatic cell data. The symbols were those routinely used for the California Mastitis Test (CMT). They were assigned values which corresponded with the OSCC II recorder readings. The instrument test data were identified by placing a pillow symbol on the computer and barn sheet records. There was little confusion in interpreting the results once dairymen were informed how to identify the instrumental test data.

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*Protein fraction assays*

Protein fractions were quantitated by replacing acid orange 12 dye in a dye binding procedure (*15*). Noncasein protein (NCP) was obtained by tempering 10 ml of fresh raw milk in a clean, dry test tube in 40-C water bath. Acetic acid, .3 ml of 33% (wt/vol), was added, mixed, and the tube returned to the 40-C bath. After 10 min, .3 ml of 3.33 N sodium acetate was added and mixed. After 3 min, the mixture was filtered through Whatman #1 filter paper. The NCP filtrate was collected and cooled to 20 C. The dye binding assay was followed as outlined in the Udy instruction manual[5]. The syringe pipet was calibrated to deliver 2.24 ml and the cannula bent to a U-shape to increase precision of the volumetric measurements (D. A. Biggs, Personal communication).

Three dye binding readings were obtained. Reading 1 determined total protein after combining 2.24 ml of milk and 40.44 g of acid orange-12 reagent, mixing, and filtering into the colorimeter cuvette. Reading 2 was obtained by adding equal volumes (2.24 ml) of milk and distilled water to the dye reagent. This produced a dilution factor reading. Reading 3 was obtained by adding equal volumes (2.24 ml) of milk and NCP filtrate to the dye reagent. Corrected NCP percentage was determined by subtracting reading 2 from reading 3 and adding a constant value obtained by comparing Kjeldahl NCP and the difference between readings 2 and 3. Casein percentage was then obtained by subtracting the corrected NCP value from reading 1. If reading 1 was over 4.2% protein, reading 3 was over 82% transmittance. When this occurred, reading 3 filtrate was mixed equally with reference dye (.6 g per liter) to allow a more precise estimate of dye concentration.

## RESULTS AND DISCUSSION

*Somatic cell assay*

Sample preparation by the Auto Diluter and the manual syringe were compared using 30 samples of milk containing from $6 \times 10^4$ to $1.9 \times 10^6$ somatic cells per ml. All samples were incubated in a 55-C water bath for 45 min and tested on the OSCC II (*1*). The dilution loop was included on the OSCC II for manual syringe method. There was no difference between the two methods using a t test at the 5% level of significance.

Binding material, present in .lg potassium dichromate preservative tablets (NASCO)[6], has been found to introduce a positive bias in somatic cell counts. (Reported to be 3 to $5 \times 10^5$ cells per ml. Aldo Conetta, Personal communication). When tablets were dissolved in 25 ml of distilled water and pumped through the OSCC II we found no significant reading change. The effect of the preservative on the OSCC II count of milk was determined by testing split aliquots from 60 milk samples. One aliquot had the preservative added at the rate of 1 tablet in 29 ml milk (normally 1 tablet is used per 54 ml milk). The preserved samples showed an average increase of 14,000 cells per ml more than the unpreserved samples. Thus the increase was $7 \times 10^3$ cells per ml in samples with a mean of $3.6 \times 10^5$ cells per ml for a mean increase of 1.9%. This effect would be present as a positive determinate error in all preserved DHI samples.

Thirty dichromate-preserved and nonfixed milk samples were incubated at ambient (20-23 C) and refrigeration (4-7 C) temperatures and tested at different time intervals. The samples ranged from $1.6 \times 10^5$ to $3.7 \times 10^6$ with a mean of $7 \times 10^5$ before storage. The mean values after 1, 2, and 3 days of storage at ambient

temperature were 7, 7.2 and $5.2 \times 10^5$. The mean values after 1 through 5 days at 4-7 C were 6.7, 6.8, 6.5, 6.2, and $5.2 \times 10^5$. Valid estimates should be obtained if samples can be received at central laboratories within 48 h. This period should be extendable by refrigeration. Somatic cell determinations of older samples may be not as accurate but may still give helpful estimates for herd management. This loss of accuracy would have to be accepted in areas where refrigerated sample shipments are impractical. Quantitating such changes and application of correction factors for such losses are not practical. Marschall and Brechbuhler (*14*) reported increased CV values upon more than 36 h of storage of somatic cell samples at 4 C when tested using the Wisconsin Mastitis test. When used under DHI temperature variable conditions, care is required to interpret the data, just as with a screening method such as the CMT (*9*).

Where there is little control on temperature or time of arrival for samples throughout the area served by some laboratories it would be impractical to establish comparative data between herds. Data generated should be of help for within-herd management programs. Delivery time schedules and seasonal temperature changes need to be considered in interpreting the data. When the Wisconsin Mastitis Test was used, dichromate was next to boric acid in preventing storage losses (*7*).

Samples emerging after those with very high cell counts may be covered by the tail of the high sample peak. When this occurs the low sample should be retested (*11*).

Individual Holstein milk samples from three herds were kept refrigerated and then tested for somatic cells within 48 h of collection. The mean cell count of the three herds during October 1974 through March 1975 was $3.8 \times 10^5$ compared to $6.25 \times 10^5$ found by Bodoh et al. (*5*).

The possibility of utilizing the OSCC II routinely in a DHI testing program was evaluated at the Central Milk Testing Laboratory in Logan, Utah. There were 52,614 OSCC II samples processed through this laboratory between March 1973 and April 1975.

Table 1 compares the grading and interpretation of the symbols used in the CMT and OSCC II system. Ideally, a separate symbolology should be used to avoid confusion or continuous cell numbers should be reported directly. However the computer center was programmed to handle only the CMT symbolology. Generally, the OSCC II data selected indicated a lower count than found with the CMT comparable symbol. Samples were composites of four quarters and not individual quarters as with an on-site CMT assay. Equal 400,000 cell intervals between symbols were selected for the OSCC II while intervals for the CMT varied from 200,000 to 4,200,000 due to subjectivity.

TABLE 1. *Grading and interpretation of somatic cell count using the CMT and the OSCC II.*

| | Interpretation | | |
|---|---|---|---|
| | CMT | OSCC II[a] | |
| Symbol | Cells/ml | Recorder reading | Cells/ml |
| — | 0-200,000 | 0-20 | 0-400,000 |
| T | 150,000-500,000 | 20-40 | 400,000-800,000 |
| 1 | 400,000-1,500,000 | 40-60 | 800,000-1,200,000 |
| 2 | 800,000-5,000,000 | 60-80 | 1,200,000-1,600,000 |
| 3 | generally > 5,000,000 | 80-100 | 1,600,000-2,000,000 |
| + | alkaline milk | | |
| Y | acid milk | | |

[a]Identified on DHI Computer records by a pillow symbol (□) to separate it from the CMT test.

Mean percentage results of the samples are presented in Table 2. During the testing period 90% of the animals produced milk with less than 800,000 cells per ml. An average of 2.7% had counts between 1.6 and 2 million per ml. Bodoh et al. (*5*) reported 61.6% of milk samples below 500,000 cells per ml, 20.5% between 500,000 and 1 million per ml, and 17.9% over 1 million. Our survey of 52,614 samples revealed 75.3% below 400,000 per ml, 19.2% between 400,000 and 1.2 million per ml, and 5.5% above 1.2 million per ml. The highest percentage of samples above trace readings occurred during April through September, depending upon weather conditions. During the rest of the year the average above trace reading was below 10%.

TABLE 2. *Percentage of milk samples in each CMT code category on a monthly basis*

| | OSCC II with CMT symbol code (see table 1) | | | | | |
|---|---|---|---|---|---|---|
| Month | — | T | 1 | 2 | 3 | 1 + 2 + 3 |
| | | | (%) | | | |
| July 1973 | 67.4 | 18.3 | 6.3 | 4.9 | 3.1 | 14.3 |
| August | 65.0 | 20.4 | 5.2 | 5.4 | 4.0 | 14.6 |
| September | 73.1 | 16.0 | 3.7 | 4.2 | 3.0 | 10.9 |
| October | 77.6 | 12.7 | 3.2 | 3.7 | 2.8 | 9.7 |
| November | 80.0 | 12.6 | 3.6 | 1.8 | 2.0 | 7.4 |
| December | 80.3 | 10.3 | 4.6 | 1.6 | 3.2 | 9.4 |
| January 1974 | 78.1 | 13.2 | 3.3 | 1.5 | 3.9 | 8.7 |
| February | 75.2 | 16.9 | 2.5 | 1.6 | 3.8 | 7.9 |
| March | 78.5 | 15.7 | 2.2 | 2.2 | 1.4 | 5.8 |
| April | 69.2 | 14.0 | 5.4 | 3.6 | 7.8 | 16.8 |
| May | 63.2 | 22.2 | 5.7 | 4.6 | 4.2 | 14.5 |
| June | 65.9 | 19.5 | 6.5 | 4.6 | 3.4 | 14.5 |
| July | 60.2 | 24.4 | 6.3 | 5.9 | 3.2 | 15.4 |
| August & September | 50.2 | 26.6 | 12.3 | 4.9 | 5.9 | 23.1 |
| October | 86.5 | 10.6 | 3.5 | 0.6 | 0.1 | 4.2 |
| November | 90.7 | 5.7 | 2.6 | 0.8 | 0.2 | 3.6 |
| December | 79.9 | 10.9 | 3.9 | 2.5 | 2.6 | 12.6 |
| January 1975 | 88.9 | 7.6 | 2.2 | 1.0 | 0.5 | 3.7 |
| February | 90.8 | 6.7 | 1.6 | 0.6 | 0.3 | 2.5 |
| March | 85.9 | 9.0 | 2.8 | 1.0 | 1.3 | 5.1 |
| April | 75.6 | 16.7 | 4.4 | 2.0 | 1.3 | 7.7 |
| Average | 75.3 | 14.8 | 4.4 | 2.8 | 2.7 | |

Comparative tests are in order to determine if the OSCC II method correlates with the DNA filter technique (*3,5*). The latter should be a better reference method for instrument calibration than direct microscopic methods. However, there is no probelm in calibrating the OSCC II with turkey cell suspensions. There have been improperly reported counts supplied to users and the supplier has corrected and replaced defective reference suspensions. This has reemphasized the need to compare old suspensions with new, as

suggested by AOAC (*1*), before the new are used for instrument calibration.

The OSCC II should be a valuable addition to the DHI central testing program. The instrument, however, requires careful technical maintenance and is not as simple to operate as modern milkfat testing instruments. It was estimated that a fulltime technician could assay 10,000 samples per month with one instrument. If the instrument were amortized over 4 years, if 2 liters of reagent were adequate for 800 samples, and if the labor costs were based on $3.00 per hour; the per sample cost would be below 7 cents. This is less than half the cost for the filters of the DNA-filter technique (3,14) and less than the cost estimate for the Fossomatic (Herb Gilmore, Personal communication).

The OSCC II in the Utah Central Milk Laboratory has operated continously since the dates indicated in Table 2 at the rate of 4 to 5 thousand samples per month and has been helpful to the dairymen requesting the service. Recently, a study was initiated to monitor 7000 boric acid-preserved samples a month for milkfat (Milko-Tester), protein on the uv detector (*23*), and somatic cells on the OSCC II. The study will take 2 years. There will be casein tests (*14*) conducted also as discussed in the next section.

*Protein fraction-somatic cell count relationships*

Twenty individual cow milk samples from the Utah State University dairy herd were assayed for NCP using semi micro Kjeldahl (*11*) and the McGann et al. (*15*) procedure modified through use of acid orange 12 dye. The percent NCP readings varied from .69 to .98% by Kjeldahl and .56 to .93% by dye binding. The Kjeldahl assays averaged .04% higher which confirmed the differential reported for amido black (*15*). The approach was thus applicable for both dyes. Though the correction factor .04% is used in this report, it may be better eliminated in that the dye binding method gives a better estimate of true protein than the Kjeldahl which includes non-protein nitrogen materials as proteins (*6*).

The dye test for milk fractions was used to assay 1292 fresh raw milk samples and could be conducted at approximately 12 samples per hour. Potassium dichro-

TABLE 3. *The relationships of somatic cell count to fat, protein fractions and milk weight of three herds*

| Variables | | Somatic cells (x $10^5$/ml) 0-3 | 3-5 | 5-10 | 10-40 |
|---|---|---|---|---|---|
| *Herd #1* | | | | | |
| Fat | (%) | 3.9 | 4.1 | 4.0 | 4.0 |
| Protein | (%) | 3.5 | 3.6 | 3.6 | 3.5 |
| Casein | (%) | 2.8 | 2.8 | 2.7 | 2.4 |
| Non Casein Protein | (%) | .7 | .8 | .9 | 1.1 |
| Casein/Protein | (%) | 80 | 78 | 75 | 69 |
| Milk weight | (lb) | 40.9 | 34.3 | 28.9 | 30.5 |
| Total casein | (lb) | 1.12 | 0.96 | 0.78 | 0.73 |
| No. of samples: | | 418 | 134 | 78 | 83 |
| *Herd #2* | | | | | |
| Fat | (%) | 3.9 | 4.2 | 4.2 | 5.4 |
| Protein | (%) | 3.2 | 3.5 | 3.6 | 4.4 |
| Casein | (%) | 2.4 | 2.5 | 2.6 | 3.2 |
| Non Casein Protein | (%) | .8 | 1.0 | 1.0 | 1.2 |
| Casein/Protein | (%) | 76 | 73 | 80 | 72 |
| Milk weight | (lb) | 41.3 | 31.4 | 27.7 | 17.6 |
| Total casein | (lb) | 0.97 | 0.78 | 0.73 | 0.56 |
| No. of samples: | | 204 | 48 | 34 | 1 |
| *Herd #3* | | | | | |
| Fat | (%) | 3.5 | 3.8 | 3.6 | 3.3 |
| Protein | (%) | 3.3 | 3.3 | 3.4 | 3.3 |
| Casein | (%) | 2.6 | 2.5 | 2.5 | 2.3 |
| Non Casein Protein | (%) | .7 | .8 | .9 | 1.0 |
| Casein/Protein | (%) | 79 | 76 | 74 | 70 |
| Milk weight | (lb) | 51.3 | 48.5 | 44.3 | 45.6 |
| Total casein | (lb) | 1.33 | 1.21 | 1.11 | 1.05 |
| No. of samples: | | 146 | 53 | 77 | 16 |

TABLE 4. *Correlation coefficients among somatic cell counts, fat contents, protein fractions, and milk weights*

| Variables | Somatic cells | Fat | Protein | Casein | NCP | Casein/ Protein | Milk weight |
|---|---|---|---|---|---|---|---|
| *Herd #1* | | | | | | | |
| Fat | −.10 | | | | | | |
| Protein | −.09 | +.49 | | | | | |
| Casein | −.18 | +.45 | +.88 | | | | |
| NCP | +.25 | +.11 | +.32 | −.17 | | | |
| Casein/Protein | −.26 | +.14 | +.18 | +.61 | −.86 | | |
| Milk weight | −.30 | −.48 | −.52 | −.35 | −.40 | +13 | |
| Total casein | −.21 | −.31 | −.18 | +.12 | −.62 | +.51 | +.85 |
| *Herd #2* | | | | | | | |
| Fat | −.22 | | | | | | |
| Protein | −.10 | +.58 | | | | | |
| Casein | −.11 | +.53 | +.94 | | | | |
| NCP | +.46 | +.39 | +.59 | −.05 | | | |
| Casein/Protein | −.27 | −.08 | +.09 | +.18 | −.32 | | |
| Milk weight | −.36 | −.34 | −.71 | −.55 | −.70 | +.06 | |
| Total casein | −.38 | −.23 | −.41 | +.17 | −.69 | +.18 | +.91 |
| *Herd #3* | | | | | | | |
| Fat | −.18 | | | | | | |
| Protein | −.05 | +.41 | | | | | |
| Casein | −.08 | +.30 | +.91 | | | | |
| NCP | +.30 | +.25 | +.59 | −.22 | | | |
| Casein/Protein | −.32 | −.08 | −.01 | +.39 | −.81 | | |
| Milk weight | −.25 | −.20 | −.50 | −.37 | −.47 | +.22 | |
| Total casein | −.15 | −.06 | −.14 | +.04 | −.41 | +.40 | +.91 |

mate preservative interferred with the dye binding test results, thus precluding accurate testing of preserved milk. From December 1973 to May 1974 samples were collected from three Holstein herds. Samples were examined for protein fractions and somatic cell count. Somatic cell tests were done within 24 h of milking. Protein fractions were assayed within 48 h. The mean percent casein in milk from individual cows from the three herds tested for 6 months varied from 69 to 80% of the total protein. Data were grouped according to somatic cell count range (Table 3). Non-casein protein increased directly with somatic cell count increase while other parameters varied. Multiple correlation coefficients were obtained as summarized in Table 4. Grappin et al. (*8*) reported a correlation coefficient (r) of -.58 between somatic cell count and percent casein in milk. Our values were much lower. Better r values existed between percent casein and total protein, casein protein ratio and NCP, total casein and NCP, or total casein and milk weight. The data support the observation of Cerbulis and Farrell (*6*) and Mickelsen and Shukri (*16*) regarding the high correlation between true protein via dye binding and casein. The observations of Weaver and Kroger (*25*) confirm our observed lack of correlation between somatic cell count and percent casein. These observations suggest that the cheese industry could use a total protein assay to estimate casein but would need to confirm whether casein or NCP was involved through simultaneous assay for somatic cells. If the casein levels are set genetically (*25*), then milk weights and somatic cell counts could be used for payment formulae after initial total protein and casein estimates are made. Such a two channel system is being evaluated to help establish the above approach. Over 7000 samples will be assayed per month for 2 years and true protein (*27*), somatic cell (*1*), and some casein data (*26*) will be gathered to help establish these relationships. Such data are needed considering the variability of casein content in cheese milk (*22*).

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*Journal of Food Protection Vol. 40, No. 10, Pages 676-677 (October, 1977)*


# Development of *Yersinia enterocolitica*-like Organisms in Pure and Mixed Cultures on Different Bismuth Sulfite Agars

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ABSTRACT

*Yersinia enterocolitica*-like isolates from meats produced black colonies on eight of nine lots of bismuth sulfite agar. Differences were observed in size and black metallic sheen of colonies on different lots of this medium. In addition, inhibition of *Pseudomonas* spp. differed among lots.

In a previous paper Hanna et al. (2) reported the potential use of bismuth sulfite (BS) agar for isolation of *Yersinia enterocolitica* from meats. *Y. enterocolitica* ATCC strains 23715 and 9610 and 19 *Y. enterocolitica*-like isolates from meats produced black surface colonies on BS agar after 3-5 days at 25 C. Many common gram-negative isolates from meats such as *Pseudomonas, Moraxella*, and *Acinetobacter* spp. produced pale and dark green colonies. Some strains of *Erwinia herbicola, Enterobacter liquefaciens, Escherichia coli,* and *Pseudomonas putrefaciens* also produced dark colonies on BS plates but they were not as shiny enamel-black. However, these isolates could be separated easily from *Y. enterocolitica* by a few key biochemical tests. Development of an enamel-black surface color differed somewhat when different brands or different lots of the same brand of BS agar were used. In addition, on some BS agars the *Pseudomonas* spp. were inhibited less effectively and frequently formed somewhat larger colonies than on others which made picking of enamel-black isolates more difficult on crowded plates. Also, with small numbers of *Y. enterocolitica* among large numbers of other microorganisms, *Y. enterocolitica* (black colonies) was detectable at lower dilutions on crowded plates on certain BS agars. This paper reports on counts and differences in colony characteristics of *Y. enterocolitica* in pure culture and in the presence of *Pseudomonas* spp. on nine lots of BS agar.

## MATERIALS AND METHODS

*Y. enterocolitica*-like isolates 1049, 1059, 1157 were obtained from vacuum-packaged beef. Their biochemical and serological characteristics and differences from typical *Y. enterocolitica* strains were described by Hanna et al. (1). *Y. enterocolitica* 23715 and 9610 were purchased from the American Type Culture Collection, Rockville, Md for comparative purposes. *Pseudomonas* spp. 284 and 285 were isolated from vacuum-packaged beef. The cultures were maintained on tryptic soy agar (TSA, Difco) slants. Cultures for plating on BS agar were grown in brain heart infusion (BHI, BBL) incubated overnight at 25 C. Dilution with sterile BHI was used to obtain the desired cell concentration of the *Yersinia* ($10^4$-$10^5$ per ml) and *Pseudomonas* cultures ($10^6$-$10^7$ per ml). Mixing of the *Yersinia* and *Pseudomonas* cultures was carried out just before plating. Decimal dilutions of the cultures were made with BHI. Aliquots (0.1 ml) of the cultures and appropriate dilutions were placed on freshly poured BS plates which were dried for a few hours at room temperature. The inoculum was spread evenly over the surface with a sterile bent glass rod. Plates were incubated for 3-5 days at 25 C. Nine lots of BS agar were used, four from manufacturer A and five from manufacturer B. Manufacturer A supplied two lots upon request, manufacturer B supplied three lots.

## RESULTS AND DISCUSSION

Counts of cultures of *Y. enterocolitica* with or without *Pseudomonas* spp. on eight lots of BS agar were very similar (Table 1). The organism did grow poorly or not at all on lot A-1. Although all *Y. enterocolitica* cultures produced black colonies on the eight BS agars, distinct differences were noted in the colony characteristics (size, metallic sheen). Of brand A, lot 3 was preferred because size of the colonies was fair with a moderate to distinct enamel-black sheen (Table 2). Of brand B, lot 5 and to a lesser extent lot 3 were preferred primarily because the colonies more often had a distinct enamel-black sheen.

In the presence of large numbers of pseudomonads (Table 3, 4), lots A-2, B-5, and B-3 were superior because the *Y. enterocolitica* colonies were easier to pick from crowded plates because of their distinct color and the more extensive inhibition of *Pseudomonas*, particularly on lot A-2. On the basis of colony characteristics of pure cultures, lots A-3, B-5, and B-3 were superior, and with mixed cultures lots A-2, B-5, and B-3 were preferred. If BS agar is tested as a potential medium for isolation of *Y. enterocolitica* from foods with mixed populations, it would be advisable to test several lots with mixtures of *Y. enterocolitica* and organisms known to predominate in

those foods. This approach will (*a*) eliminate use of lots such as A-1 on which little or no growth of *Y. enterocolitica* occurs and (*b*) test the medium for development of distinct colony characteristics in a mixed population. In view of the potential application of BS agar for isolation and enumeration of *Y. enterocolitica* a study of the effect of the medium constituents on the colony characteristics of this organism in mixed microbial populations appears warranted. It is recognized that BS agar is recommended for the isolation of enteric organisms, particularly *Salmonella*. Its effectiveness with respect to the isolation of *Y. enterocolitica* does not reflect upon its usefulness for the detection of *Salmonella*.

TABLE 1. Yersinia enterocolitica *plate counts of cultures of* Y. enterocolitica *with and without added* Pseudomonas *spp. (284, 285) plated on nine bismuth sulfite agars*

| Medium | Log *Y. enterocolitica* count per ml of culture(s) | | | | | |
|---|---|---|---|---|---|---|
| | 23715 | 1049 | 1059 | 1157 | 23715+284 | 2315+284+285 |
| A-1 | —[a] | — | — | — | — | — |
| A-2 | 4.49 | 4.36 | 3.97 | 4.32 | 3.30 | 2.83 |
| A-3 | 4.57 | 4.46 | 4.18 | 4.32 | 3.46 | 3.08 |
| A-4 | 4.53 | 4.51 | 4.18 | 4.32 | 3.28 | 2.95 |
| B-1 | 4.51 | 4.40 | 4.18 | 4.26 | 3.34 | 2.79 |
| B-2 | 4.45 | 4.52 | 4.11 | 4.23 | 3.48 | 2.68 |
| B-3 | 4.53 | 4.53 | 4.30 | 4.26 | 3.51 | 2.92 |
| B-4 | 4.54 | 4.49 | 4.23 | 4.34 | 3.62 | 2.94 |
| B-5 | 4.52 | 4.56 | 4.15 | 4.30 | 3.49 | 2.79 |

[a]No distinct black colonies.

TABLE 2. *Development of* Yersinia enterocolitica *on nine lots of bismuth sulfite agar*

| Bismuth sulfite agar, lot | Size of colony[a] | | | | | Metallic sheen[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 23715 | 9610 | 1049 | 1059 | 1157 | 23715 | 9610 | 1049 | 1059 | 1157 |
| A-1 | 1+ | —[c] | — | — | — | 0 | — | — | — | — |
| A-2 | 3+ | 1+,2+ | 2+ | 2+ | 2+ | 0 | 0 | 1+ | 0 | 0 |
| A-3 | 2+ | 1+,3+ | 2+ | 3+ | 2+ | 3+ | 3+ | 3+ | 2+ | 3+ |
| A-4 | 2+ | 1+,3+ | 1+,2+ | 1+,2+ | 1+,2+ | 2+ | 0 | 2+ | 0 | 2+ |
| B-1 | 3+ | 1+,3+ | 3+ | 3+ | 1+,3+ | 2+ | 0 | 2+ | 0 | 1+ |
| B-2 | 3+ | 2+ | 3+ | 2+,3+ | 1+,3+ | 2+ | 0 | 2+ | 0 | 1+ |
| B-3 | 3+ | 1+,3+ | 2+,3+ | 3+ | 1+,2+ | 0 | 2+ | 2+ | 0 | 2+ |
| B-4 | 3+ | 1+,3+ | 2+ | 3+ | 1+,2+ | 2+ | 0 | 2+ | 0 | 0 |
| B-5 | 3+ | 2+ | 2+,3+ | 3+ | 1+,2+ | 2+ | 2+ | 2+ | 1+ | 1+ |

[a]1+ pinpoint to 0.5 mm; 2+ 0.5 to 1.5 mm; 3+ > 1.5 mm.
[b]0 no sheen; 1+ some sheen; 2+ moderate sheen, 3+ distinct sheen.
[c]No growth of *Y. enterocolitica*.

TABLE 3. *Development of* Yersinia enterocolitica *23715 on nine lots of bismuth sulfite agar in the presence of* Pseudomonas *spp. 284.*

| Bismuth sulfite agar, lot | Color of *Y. enterocolitica* colonies[a] | Degree of inhibition (size) of *Pseudomonas*[b] |
|---|---|---|
| A-1 | —[c] | 2+ |
| A-2 | 3+ | 3+ |
| A-3 | 1+ | 1+ |
| A-4 | 2+ | 2+ |
| B-1 | 2+ | 2+ |
| B-2 | 1+ | 2+ |
| B-3 | 3+ | 2+ |
| B-4 | 2+ | 2+ |
| B-5 | 3+ | 2+ |

[a]1+ dark centered colony, 2+ distinct black, 3+ enamel black.
[b]1+ 1.5-2.0 mm, 2+ 0.5-1.4 mm, 3+ pinpoint to 0.5 mm.
[c]No growth of *Y. enterocolitica*.

TABLE 4. *Development of* Yersinia enterocolitica *23715 on nine lots of bismuth sulfite agar in the presence of two species of* Pseudomonas *(284-285)*

| Bismuth sulfite agar, lot | Color of *Y. enterocolitica* colonies[a] | Degree of inhibition (size) of *Pseudomonas*[b] |
|---|---|---|
| A-1 | —[c] | 3+ |
| A-2 | 3+ | 2+ |
| A-3 | 1+ | 1+ |
| A-4 | 1+ | 1+ |
| B-1 | 2+ | 1+ |
| B-2 | 2+ | 1+ |
| B-3 | 3+ | 1+ |
| B-4 | 2+ | 1+ |
| B-5 | 3+ | 1+ |

[a]1+ dark centered colony, 2+ distinct black, 3+ enamel black.
[b]1+ 1.5-2.0 mm, 2+ 0.5-1.4 mm, 3+ pinpoint to 0.5 mm.
[c]No growth of *Y. enterocolitica*.

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*Journal of Food Protection Vol. 40, No. 10, Pages 678-682 (October, 1977)*


# Nutritional Regime, Post-Slaughter Conditioning Temperature, and Vacuum Packaging Effects on Bacteriology of Beef Carcasses and Retail Meat Cuts [1]

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ABSTRACT

Thirty-eight crossbred steers were used to evaluate effects of nutritional regime (grass-, short-, long-, and forage-fed) and post-slaughter chilling (3 C) and conditioning temperature (13 C) on carcass psychrotrophic and mesophilic bacterial counts. Inside chucks from right halves of carcasses chilled at 3 C for 48 h were used to evaluate effects of nutritional regime and vacuum packaging on total aerobic and anaerobic bacterial counts. Psychrotrophic and mesophilic mean bacterial counts tended to decrease from 1 to 46 h postmortem regardless of temperature treatment. At 46 h postmortem, the forage-fed group mean psychrotrophic count was significantly lower ($P < 0.05$) than that for any of the other feeding regimes. Mesophilic mean counts were significantly different ($P < 0.05$) at 46 h postmortem (grass-fed > short-fed > long-fed > forage-fed). Carcass halves chilled at 3 C for 46 h had lower total psychrotrophic and mesophilic mean bacterial counts than did corresponding halves conditioned at 13 C for 8 h then chilled at 3 C for 38 h. Total aerobic and anaerobic counts tended to remain constant or decrease slightly during vacuum storage for 21 days at 0 to 1 C. Both aerobic and anaerobic counts on vacuum-stored cuts from carcasses of grass-fed steers were significantly higher ($P < 0.05$) than other feeding-regime means. Aerobic and anaerobic counts on vacuum-stored cuts from carcasses of short-, long-, and forage-fed steers were statistically similar. All carcass and inside-chuck bacterial counts were well within acceptable limits. Scalpel-template sampling was considered to be a significant improvement over previously used methods.

Theoretically, microbial growth might differ on carcasses of animals fed different rations, if the rations caused changes in water activity (*2*) or ultimate pH (*6*). However, the effect of animal nutrition on microbiology of carcasses or cuts is not well documented.

Conditioning bovine carcasses at an elevated temperature before conventional chilling has received attention recently because of its possible economic benefits and undesirable effects of cold shortening (*10,19*).

Kotula (*15*) reported that refrigerating meat below 10 C inhibited growth of *Clostridium perfringens, Salmonella,* and *Staphylococcus aureus.* Kastner et al. (*13*) reported that holding carcasses at elevated temperatures could encourage growth of spoilage and potentially pathogenic bacteria. Rey et al. (*24*) found that beef carcasses aged at 16 or 22 C for 2 days, then at 2 C for 2 days, yielded cuts having statistically greater mesophilic and psychrophilic counts than did carcasses held at 2 C for 4 days. Minks and Stringer (*20*) found that cuts taken from conventionally chilled carcasses and aged at 4.4 C for 7 or 15 days had 5.34 times the increase in microbial counts of cuts aged at 0 C for a comparable time.

In contrast, Bouton et al. (*5*) indicated that for mutton carcasses inoculated and held at 0 to 1 C or 7 to 8 C, *Pseudomonas* and *Escherichia coli* counts generally decreased at either temperature range over a 24 h holding period. Fields et al. (*9*), comparing conventionally chilled beef halves with halves conditioned at 14 to 19 C for 12, 16, or 20 h and then chilled at 2 C until 48 h postmortem, found that microbial counts were significantly higher immediately after the high-temperature storage. However, no difference was noted between control and treated halves at 48 h postmortem or between steaks removed from all sides immediately after fabrication or after 5 days of display. Kastner et al. (*13*) found no significant differnence in total aerobic mesophilic or psychrotrophic counts between beef halves held at 16 C for 6, 8, or 10 h and conventionally chilled (2 C) halves.

Vacuum packaging of beef wholesale cuts, is widely practiced in the meat industry. Warnecke et al. (*28*) and Hodges et al. (*11*) reported that vacuum packaging provided a selective environment for bacterial growth. *Lactobacillus* was found to be the predominant microorganism in vacuum-stored meat (*12,22,25*). *Pseudomonas*, the major spoilage microorganism of aerobically packaged or unpackaged fresh meat (*3,14*), was only a small portion of the microflora that developed on vacuum-packaged cuts (*25*).

Ulrich (*27*) stated that vacuum packaging reduced but did not eliminate microbial growth. Many workers have

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reported fewer spoilage bacteria on vacuum-packaged beef than on aerobically packaged beef (*4,11,20,22*). However, both aerobic and anaerobic counts increased during vacuum storage (*4,11,20,22,25*).

Ledward et al. (*18*) and Baran et al. (*4*) indicated that oxygen content of a vacuum package must be drastically reduced to prevent aerobic growth. Hodges et al. (*11*) concluded that degree of vacuum regulates growth of aerobes. Seidman et al. (*25*) found that after cuts had been stored 35 days, total aerobic psychrotrophic and mesophilic counts were lower for those packaged under high vacuum (29.4 inches of Hg) than for those packaged under low (26 inches of Hg) or intermediate vacuum (28.5 inches of Hg). Aerobic counts were low (less than $10^4$ per 6.45 $cm^2$) after 7 and 14 days, storage, regardless of degree of vacuum.

Reagan et al. (*23*) reported that vacuum packaging does not compensate for improper refrigeration during storage of lamb. Jaye et al. (*12*) found that *Lactobacillus* was suppressed at 0 to 1 C but grew rapidly at 3 C or higher. Minks and Stringer (*20*) found a significantly higher increase in microbial counts of vacuum-packaged cuts held at high temperatures.

When animal- or carcass-handling procedures are altered, differences in microbial growth on meat products may occur. Because procedural changes can affect both number and types of microorganisms present, it becomes mandatory to consider the microbial consequences of any proposed changes. New handling procedures or systems, no matter how desirable, will not be implemented if resultant products are microbiologically unsatisfactory.

We evaluated the effects of nutritional regime and post-slaughter conditioning temperature on total aerobic, psychrotrophic, and mesophilic carcass bacterial counts and effects of nutritional regime and vacuum packaging on total aerobic and anaerobic bacterial counts of vacuum-packaged inside chucks.

## EXPERIMENTAL PROCEDURES

Thirty-eight crossbred steers were randomly assigned to four nutritional regimes. All animals were initially fed on a brome and bluestem pasture supplemented with a wintering ration of protein and alfalfa. At the end of summer, 10 grass-fed animals were slaughtered directly off pasture. Ten steers were fed an additional 49 days (short-fed) and eight for 98 days (long-fed) on 80% concentrate and 20% forage ration, and 10 fed 98 days on a 40% concentrate and 60% roughage ration (forage-fed).

Each carcass half was washed for 10 min with cold tap water before initial sampling. Right halves were chilled at 3 C; left halves were conditioned at 13 C for 8 h, then chilled at 3 C until carcass fabrication at 48 h postmortem.

Carcass bacterial samples were taken at 1, 8, and 46 h postmortem. One-hour samples were taken immediately after washing and before placing the carcass in either cooler. The 8-h samples from the left halves were excised before transfer to the 3-C cooler.

Immediately before excising the 1-h samples, six squares of 32.25 $cm^2$ each were outlined anterior to the 13th rib and 6.0 to 8.0 cm dorsal to the ventral midline. The sample areas were outlined by using 2 sterile scalpels (scalpel-template) fixed 5.68 cm apart (Fig. 1) to make three

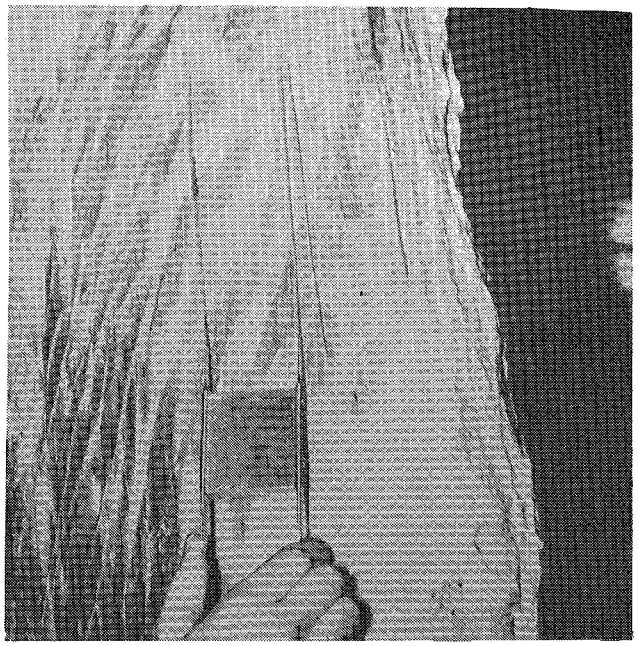

Figure 1. *Scalpel-template used for outlining microbiological sampling areas on carcasses or cuts.*

horizontal and two vertical cuts through the muscle to the underlying fascia. At each sampling period, two squares (64.50 $cm^2$) were aseptically removed using a sterile scalpel and forceps and then placed in sterile phosphate buffer solution, pH 7.2 (*21*). Two sets of duplicate dilutions were made from each sample and plated with Plate Count Agar (Difco). A psychrotrophic bacterial count was obtained by incubating one set for 10 days at 7 C, and a mesophilic bacterial count was obtained by incubating the other set at 32 C for 48 h. This procedure was repeated for each carcass half at each sampling period.

Inside chucks were removed from the right side of each carcass after a 48-h chill at 3 C and prepared for sampling and vacuum packaging. Initial samples were taken immediately after fabrication and before vacuum packaging. The scalpel-template (Fig. 1) was used to outline two sampling areas (32.25 $cm^2$ each) on the subscapular surface. Samples were taken by rolling four sterile cotton swabs, moistened in sterile 0.1% peptone solution, over the outlined area (64.50 $cm^2$). Swabs were placed in sterile peptone solution and two sets of duplicate dilutions were prepared.

Aerobes were counted using one set of duplicates plated on Plate Count Agar (Difco) and incubated at 21 C for 72 h. Anaerobes were counted in the remaining duplicate set by plating with Schaedler Agar (Bio Quest) and incubating at 32 C for 48 h. The incubator was evacuted and flushed with oxygen-free $CO_2$ three times to insure a very low $0_2$ partial pressure.

After initial sampling, cuts were vacuum packaged (25 inches of Hg on machine guage) with heat shrinking in a barrier bag (Cryovac B-620), and stored at 0 to 1 C for 21 days. After storage, counts were repeated from adjacent areas on the inside chucks.

Colonies on each plate were enumerated according to Brazis et al. (*7*) using a Quebec colony counter. The average plate count of each set of duplicates was calculated and converted to its $\log_{10}$. Statistical analyses were done on the logarithmic values. Whenever discrepancies arose between counts of high and low dilutions, the lowest dilutions were considered to be the most accurate. All blank plates were arbitrarily given a count of one.

Data were analyzed by analysis of variance, using a split-plot design. The technique of Duncan (*8*) was employed for mean separation.

## RESULTS AND DISCUSSION

### *Carcass bacterial counts*

Analysis of variance for total psychrotrophs and mesophiles is presented in Table 1. For both classes of micoorganisms feeding regime, chill temperature, sampling time, and the interaction of feeding regime and sampling time were significant.

TABLE 1. *Mean-square values for feeding regime, chill temperature, and sampling time for total psychrotrophic and mesophilic bacterial counts of beef carcasses*

| Source | d.f | Mean squares | |
|---|---|---|---|
| | | Psychrotrophs | Mesophiles |
| Feed (A) | 3 | 7.75* | 26.39* |
| Temp (B) | 1 | 11.45* | 5.69* |
| Feed × temp (B) | 3 | 1.87 | 1.68 |
| Time (C) | 2 | 11.28* | 7.68* |
| Temp × time (C) | 2 | .81 | .40 |
| Feed × time (C) | 6 | 1.57* | 1.23* |
| Feed × temp × time (C) | 6 | .40 | .13 |
| Error for (A) | 34 | 1.23 | .72 |
| Error for (B) | 34 | .65 | .70 |
| Error for (C) | 136 | .39 | .28 |

*Significant at ($P < 0.05$).

Psychrotrophic and mesophilic mean counts tended to decrease with increased time postmortem (from 1 to 46 h), even though animals psychrotrophic means for grass- and long-feed and mesophilic means for grass- and short-fed animals were lowest at 8 h (Table 2 and 3). Assuming that most of the psychrotrophs were *Pseudomonas* (*1,14,26*), that trend would agree with Bouton et al. (*5*), who reported that *Pseudomonas* decreased on the exposed areas of inoculated mutton carcasses chilled at either 0 to 1 C or 7 to 8 C for 24 h. Across all sampling periods, the psychrotrophic and mesophilic mean counts for grass-fed animals were highest, followed in descending order by long-, short- and forage-fed animals for psychrotrophs and short-, long- and forage-fed animals for mesophiles (Table 2 and 3).

TABLE 2. *Psychrotrophic bacterial counts of beef carcasses stratified according to sampling time and feeding regime*[a]

| Feeding regime | Sampling time (h postmortem)[b] | | | $\bar{X}$ |
|---|---|---|---|---|
| | 1 | 8 | 46 | |
| Grass-fed | 2.46[c] | 1.02[c] | 1.28[c] | 1.59 |
| Short-fed | 1.35[de] | 1.07[c] | .93[c] | 1.12 |
| Long-fed | 1.80[d] | 1.27[c] | 1.31[c] | 1.46 |
| Forage-fed | 1.09[e] | .78[c] | .47[d] | .78 |
| $\bar{X}$ | 1.67 | 1.02 | .98 | |

[a]Counts ($\log_{10}$) per 6.45 cm$^2$.
[b]Means in same row underscored by a common line do not differ ($P > 0.05$).
[c,d,e]Means in same column bearing different superscript letters differ ($P < 0.05$).

TABLE 3. *Mesophilic bacterial counts of beef carcasses stratified according to sampling time and feeding regime*[a]

| Feeding regime | Sampling time (h postmortem)[b] | | | $\bar{X}$ |
|---|---|---|---|---|
| | 1 | 8 | 46 | |
| Grass-fed | 3.67[c] | 2.58[c] | 2.72[c] | 2.99 |
| Short-fed | 2.32[d] | 2.14[d] | 2.17[d] | 2.21 |
| Long-fed | 2.39[d] | 1.76[de] | 1.65[e] | 1.93 |
| Forage-fed | 1.63[e] | 1.49[e] | 1.07[f] | 1.40 |
| $\bar{X}$ | 2.51 | 2.01 | 1.92 | |

[a]Counts ($\log_{10}$) per 6.45 cm$^2$.
[b]Means in same row underlined by a common line do not differ ($P > 0.05$).
[c,d,e,f]Means in same column bearing different superscript letters differ ($P < 0.05$).

The means for feeding-regime-by-sampling-time interaction for total psychrotrophic bacterial counts are presented in Table 2. At the 1-h sampling period, the mean count for grass-fed animals was significantly higher than that for any other group, and the mean for forage-fed animals was significantly lower than the mean for long-fed animals. At 8 h, no differences were significant; at 46 h, the mean count for forage-fed animals was significantly lower than the means for any of the other feeding-regimes. Within a feeding regime, there were no significant differences between 8- and 46-h sample means. Grass- and long-fed sample means at 1 h were significantly higher than corresponding means at 8 and 46 h. Sample means from short- and forage-fed groups were significantly higher at 1 h than 46 h.

The means for feeding-regime-by-sampling-time interaction for total mesophilic bacterial counts are presented in Table 3. At the 1-h sampling period, the mean count for grass-fed animals was significantly higher and for forage-fed animals it was significantly lower than for any of the other feeding-regime means. At 8 h, the mean for grass-fed cattle was significantly higher than for any other, and for short-fed cattle it was significantly higher than for forage-fed animals. Means for all feeding-regimes were significantly different at 46 h, with the mean count for grass-fed cattle being highest followed (in descending order) by those for short-, long- and forage-fed animals. Within each feeding regime, means for grass- and long-fed animals at 1 h were significantly higher than corresponding means at 8 or 46 h. No statistical differences were detected among the three sampling periods for short-fed carcasses. The mean count for forage-fed cattle at 46 h was significantly lower than that at either 1 or 8 h.

Conventionally chilled sides had significantly lower total psychrotrophic and mesophilic mean counts except at the initial sampling time than did sides conditioned at 13 C (Table 4). That does not agree with the report of Fields et al. (*9*), who found no ultimate difference in bacterial counts between carcass halves held at 2 C for 48 h postmortem and carcass halves held at 14 to 19 C for 12, 16, or 20 h and subsequently at 2 C until 48 h postmortem. Kastner et al. (*13*) found that carcass halves held at 16 C for 8 h and then at 2 C for 48 h postmortem had lower total psychrotrophic and mesophilic counts than did halves held at 2 C for 48 h.

### *Bacterial counts of vacuum-packaged inside chucks*

Analysis of variance for total aerobes and anaerobes is

TABLE 4. *Psychrotrophic and mesophilic bacterial counts of beef carcass stratified according to sampling time and conditioning temperature*[a]

| Conditioning temperature | Psychrotrophs | | | | Mesophiles | | | |
|---|---|---|---|---|---|---|---|---|
| | Sampling time | | | | Sampling time | | | |
| | 1 | 8 | 46 | $\bar{X}$ | 1 | 8 | 46 | $\bar{X}$ |
| 3 C | 1.56[c] | .72[b] | .72[b] | 1.00[b] | 2.42[c] | 1.84[b] | 1.69[b] | 1.99[b] |
| 16 C | 1.78[c] | 1.33[c] | 1.24[c] | 1.45[c] | 2.59[c] | 2.17[c] | 2.14[c] | 2.30[c] |

[a]Count ($log_{10}$) per 6.45 cm².
[b,c]Means in same column bearing different superscript letters differ ($P < 0.05$).

presented in Table 5. For both aerobes and anaerobes, the effects of feeding regime and feeding-regime-by-vacuum-packaging interaction were significant.

The aerobes tended to decrease slightly (Table 6) during vacuum storage, which disagrees with results of earlier work (*11,20,25*). The tendency for anerobic-mean counts (Table 7) to decrease during vacuum storage, however, agrees with Baran et al. (*4*), who found that anaerobes increased for 3 days, then decreased for 17 days, then increased. However, Hodges et al. (*11*) and Seidman et al. (*25*) found that anaerobes increased throughout vacuum storage. The decline in total aerobic and anaerobic counts or their lack of growth during vacuum storage could be due to storage-holding temperature (0 to1 C). That agrees with Jaye et al. (*12*), who found that *Lactobacillus* growth was suppressed at 0 to 1 C.

TABLE 5. *Mean-square values for feeding regime and vacuum storage for total aerobic and anaerobic bacterial counts of inside chucks*

| Source | d.f. | Mean square | |
|---|---|---|---|
| | | Aerobic | Anaerobic |
| Feed (A) | 3 | 18.85* | 9.61* |
| Vacuum storage (B) | 1 | 1.37 | 7.16 |
| Feed × vacuum storage (B) | 3 | 1.89* | 2.60* |
| Error for (A) | 34 | .63 | .43 |
| Error for (B) | 34 | .47 | .38 |

*Significant at ($P < 0.05$).

TABLE 6. *Mean aerobic bacterial counts for vacuum-packaged inside chucks stratified according to pre- and post-vacuum storage and feeding regime*[a]

| Feeding regime | Vacuum storage[b] | | $\bar{X}$ |
|---|---|---|---|
| | Pre | Post | |
| Grass-fed | 3.51[c] | 2.86[c] | 3.18 |
| Short-fed | 2.22[d] | 1.32[d] | 1.77 |
| Long-fed | 1.31[de] | 1.44[d] | 1.37 |
| Forage-fed | .71[e] | 1.13[d] | .92 |
| $\bar{X}$ | 1.97 | 1.70 | |

[a]Count ($log_{10}$) per 6.45 cm².
[b]Means in same row underscored by a common line do not differ ($P > 0.05$).
[c,d,e]Means in same column bearing different superscript letters differ ($P < 0.05$).

TABLE 7. *Mean anaerobic bacterial counts for vacuum-packaged inside chucks stratified according to pre- and post-vacuum storage and feeding regime.*[a]

| Feeding regime | Vacuum storage[b] | | $\bar{X}$ |
|---|---|---|---|
| | Pre | Post | |
| Grass-fed | 3.05[e] | 1.41[c] | 2.23 |
| Short-fed | .91[d] | .93[cd] | .92 |
| Long-fed | 1.45[d] | .96[cd] | 1.21 |
| Forage-fed | .80[d] | .48[d] | .64 |
| $\bar{X}$ | 1.56 | .95 | |

[a]Counts ($log_{10}$) per 6.45 cm².
[b]Means in same row underlined by a common line do not differ ($P > 0.05$).
[c,d]Means in same column bearing different superscript letters differ ($P < 0.05$).

Means for feeding-regime-by vacuum-storage interaction for total aerobes are presented in Table 6. For pre-vacuum samples, the mean count was significantly higher for grass-fed beef than for any other regime, and the mean count for short-fed cattle was significantly higher than that for forage-fed beef. After vacuum storage the mean count for grass-fed beef was still significantly higher than any other count. Sample means were significantly higher for grass- and short-fed beef before vacuum packaging than after vacuum storage.

The feeding-regime-by-vacuum-storage interaction for anaerobes is presented in Table 7. Grass-fed beef had mean counts that were higher than those for any other feeding regime, both before and after vacuum storage. Mean counts for grass-fed beef decreased significantly during storage. No other differences were detected.

## CONCLUSIONS

Regardless of reported statistical differences, it must be stated that all counts were well known below the limit of $10^7/cm^2$ defined by Kraft and Ayres (*17*) as the surface count at which definite odors can be detected. Changes, which usually involved $log_{10}$ changes of less than 1, had doubtful microbiological significance (*16*).

Some variation in counts could have been due to seasonal effects and carcass-sampling location (*26*). In addition, the relative sample size in relation to total carcass or inside chuck-surface area was small and could have been responsible for some variation. Based on these data, we doubt that any detected differences can be construed as being practical.

These data indicate that carcasses from animals of different nutritional backgrounds can be held at 13 C for 8 h, then conventionally chilled, and still remain within acceptable microbial limits. Also, inside chucks from conventionally chilled carcasses of animals with different nutritional backgrounds can be stored under vacuum for 21 days at 0 to 1 C and remain within acceptable microbial limits.

The scalpel-template technique for outlining sampling areas avoided making templates from metal or paper.

Also, sterilization was simplified, because the blades could be flamed immediately before sampling. This sampling method we consider superior to methods previously used.

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*Journal of Food Protection Vol. 40, No. 10, Pages 683-685 (October, 1977)*


# Consumer Acceptance of Nitrite-Free Bacon

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(Received for publication March 11, 1977)

ABSTRACT

Consumer response to bacon cured with and without nitrite was determined. Bacon slices were scored individually on a 7-point hedonic for "like-dislike" responses and consumers indicated preference between the two treatments. Questionnaires from 704 respondents were analyzed for sex, age, and frequency of consumption of bacon. Bacon was "liked moderately" (5.9-6.1 on the hedonic scale) whether cured with or without nitrite. There were no significant differences as a result of sex, age, or frequency of use. Each bacon was preferred by half of the population tested; there was no significant difference between treatments. An acceptable bacon can be prepared by curing without nitrite; the study did not consider shelf stability or the anticlostridial effect of nitrite.

Sodium nitrite added to the cure either deliberately or as the result of bacterial reduction of nitrate has been used in curing meat products from time immemorial. In addition to its anticlostridial activity, nitrite has been used because a desirable characteristic color and flavor were imparted to the meat product. Recently, however, it has been found that nitrite can react with secondary amines to form nitrosamines, a class of compounds found to produce tumors in a number of animal species (*6*). Nitrosamines, particularly dimethylnitrosamine and nitrosopyrrolidine, have been found consistently in bacon after it has been fried for consumption (*2*). Reduction, or elimination, of the nitrite used in curing bacon could reduce or prevent formation of nitrosamines. Aside from a consideration of the anticlostridial activity there is a question about producing a conventional product, acceptable to the consumer, with low concentrations of, or no, nitrite.

The role of nitrite in development of characteristic bacon flavor has been investigated infrequently since the report by Brooks et al. (*1*) that a satisfactory bacon could be made with sodium chloride and sodium nitrite instead of the conventionally used sodium nitrate. These authors did not present satisfactory sensory evaluation of their samples. Mottram and Rhodes (*7*) investigated the flavor of Wiltshire bacon, demonstrating that salt pork flavor decreased and cured flavor increased as sodium nitrite concentration increased in the cure. However, the product prepared without nitrite was rated as containing identifiable bacon flavor. There are differences between British and American procedures in preparing bacon, as well as in flavor preferences. British pork is, for the most part, leaner than the American, and the Wiltshire cure did not include smoke. The bacon was prepared essentially by cooking in steam. The British perception of bacon flavor, therefore, varies considerably from the American, and results of Mottram and Rhodes may not be directly applicable to studies in the United States. Herring (*4*) also demonstrated that addition of nitrite to the cure resulted in greater acceptance of bacon. In his study, too, there was acceptance of the bacon processed without nitrite, although at a a lower level, particularly in the initial stages of storage. Green and Price (*3*), studied the effects of sodium chloride and sodium nitrite in developing cured flavor in ground fresh pork. They obtained low cured flavor scores in the absence of NaCl. Cured flavor was developed in the presence of NaCl, with only slightly higher panel scores when nitrite was used in addition to the NaCl. Kimoto et al. (*5*) observed a similar effect. On curing the bellies with NaCl alone, they obtained characteristic bacon flavor, which was only slightly improved by addtion of nitrite to the cure.

An opportunity to obtain large-scale consumer reaction to bacon prepared with and without nitrite became available recently at an Open House demonstration at Eastern Regional Research Center. The information presented herein is the result of the data obtained in this study.

## EXPERIMENTAL

### *Bacon*

The bacon for this study was prepared by the processor of a national brand of bacon. Sixteen randomly selected pork bellies in two groups of eight bellies each were pumped to contain either 0 ppm sodium nitrite or 120 ppm sodium nitrite. The basic cure pickle for both groups contained salt, sugar, sodium tripolyphosphate, and ascorbate. The bellies were pumped to 113% of green weight, and smoked and cooked to an internal temperature of 55 C, using a commercial program. Chilled and formed slabs were sliced to 10-11 slices per inch, vacuum packaged in 1-lb. units, and refrigerated until used in the test.

[1]*Agricultural Research Service, U.S. Department of Agriculture.*

*Sample preparation*

The test was conducted 15 days after the bacon was pumped. Packages were removed from the refrigerator 15-20 min before frying to facilitate separation of strips. The bacon was fried in electric fry pans calibrated at 165 C and turned frequently until the adipose tissue of both treatments was golden-brown in color and the lean of the bacon cured with nitrite was dark red, while the lean of the bacon cured without nitrite was reddish-brown. Fried bacon was kept warm under infrared lamps although for the most part the flow of visitors was so great bacon was not held in the warming pan for more than 5 min.

*Test procedure*

Plywood partitions on the laboratory benches made temporary booths for the panelists. Regular fluorescent lighting was used and no attempt was made to equalize color differences in the bacon samples. Visitors to the laboratory were given the form shown in Fig. 1 with brief instructions on the procedure. In the booths, they were supplied with a plate containing the two strips of bacon and a cup of water. The panelists were asked to sample each strip one at a time, indicating their reaction to the first strip on a 7-point hedonic scale before going on the the second strip. They were then requested to indicate their preference between the two samples.

To reduce the possibility of positional effects of the samples on the responses of the consumer panelists, the position of the two bacon treatments on the plate was continously alternated.

*Statistical analysis*

The responses for the two bacon samples were analyzed by an anlysis of variance with bacon sample, sex, age, and frequency of eating bacon as factors. All 3- and 4-way interaction terms were considered negligible and were combined with the error sum of squares (*9*). The preference responses were analyzed by the method of Roessler et al. (*8*) to determine whether there was a significant difference between the number of consumers preferring the nitrate cured sample and the number preferring the sample cured without nitrite.

## RESULTS AND DISCUSSION

Approximately 3,000 people visited the Center during the 8-h Open House demonstration. Of these, 704 participated in the test to evaluate consumer reaction to bacon prepared with and without sodium nitrite in the cure. The 704 responses were from 354 female and 350 male participants. The distribution of the consumer respondants according to age and frequency of eating bacon is shown in Table 1. There were 215 participants under 21 years of age, 121 between 21 and 30, 108 between 31 and 40 and 260 over 41. Frequency of eating bacon was divided into: at least once a week — 277 respondants, at least once a month — 243, and less frequently than once a month — 184. Further distribution of these groups by sex and by age is shown in the table.

TABLE 1. *Distribution of panelists by sex, age, and frequency of eating bacon*

| Sex | Age, years < 21 | < 30 | < 40 | > 41 | Total |
|---|---|---|---|---|---|
| Female | 82 | 70 | 62 | 140 | 354 |
| Male | 133 | 51 | 46 | 120 | 350 |

*Frequency of consumption by sex*

| Sex | Once a week | Once a month | Less frequently | Total |
|---|---|---|---|---|
| Female | 116 | 129 | 109 | 354 |
| Male | 161 | 114 | 75 | 350 |

*Frequency of consumption by age*

| Age | Once a week | Once a month | Less frequently | Total |
|---|---|---|---|---|
| <21 | 84 | 80 | 51 | 215 |
| <30 | 43 | 49 | 29 | 121 |
| <40 | 42 | 38 | 28 | 108 |
| >41 | 108 | 76 | 76 | 260 |

The evaluation of the consumers' responses with respect to their like or dislike of the bacons prepared with and without nitrite are given in Table 2. There was

TABLE 2. *Hedonic evaluation and preference selection of bacon cured with and without nitrite*

| Category | Average rating[a] Nitrite cure (1) | No nitrite cure (2) | Preference[b] (1) | (2) |
|---|---|---|---|---|
| Female | 6.2 | 6.1 | 176 | 178 |
| Male | 6.1 | 6.0 | 176 | 174 |
| <21 | 6.1 | 5.9 | 115 | 100 |
| <21-30 | 5.9 | 5.9 | 60 | 61 |
| <31-40 | 6.2 | 6.1 | 54 | 54 |
| >41 | 6.2 | 6.1 | 123 | 137 |
| Eat once a week | 6.2 | 6.1 | 132 | 145 |
| Eat once a month | 6.1 | 6.1 | 122 | 121 |
| Eat less frequently | 6.0 | 5.8 | 98 | 86 |

[a]Based on 7-point scale: 1 = dislike very much; 7 = like very much.
[b]Number of consumers preferring the particular sample of bacon.

Sex _____ Age: Under 20 _____; 21 to 30 _____; 31 to 40 _____; Over 40 _____.

DO YOU EAT BACON: At Least Once a Week _____; Once a Month _____; Less than Once a Month _____.

| Sample No. | Like Very Much | Like Moderately | Like Slightly | Neither Like Nor Dislike | Dislike Slightly | Dislike Moderately | Dislike Very Much |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |

WHICH SAMPLE DO YOU PREFER? _____

Figure 1. *Questionnaire used for consumer panel.*

no statistically significant difference ($p = .05$) between the consumers' responses to the two bacon preparations due to sex, age, frequency of use, or any of the interaction terms. Both bacons were liked moderately, receiving, on a 7-point hedonic scale, an average rating of 6.1 for the conventionally cured bacon and 6.0 for the bacon cured without nitrite.

The panelists were requested to indicate their preference between the two bacons, although this information could have been obtained from the hedonic ratings assuming the higher rating indicated the preferred sample. The preference information is summarized in Table 3 where the number of consumers in every combination of sex, age, and frequency of consumption is divided into those preferring one sample or the other. The statistical analysis showed a significant difference ($p = .05$) only in the case of 21-30 year old men who consume bacon less than once a month. There was no significant difference in any other category of panelists.

TABLE 3. *Consumer preference for bacon cured with or without sodium nitrite*

| Frequency of eating bacon | Age | | | |
|---|---|---|---|---|
| | < 21 | 21-30 | 31-40 | > 41 |
| | | *Females* | | |
| Eat once a week | 14/9[a] | 8/11 | 12/12 | 20/30 |
| Eat once a month | 22/13 | 12/21 | 12/10 | 17/22 |
| Eat less frequently | 14/10 | 10/8 | 8/8 | 27/24 |
| | | *Males* | | |
| Eat once a week | 30/31 | 9/15 | 9/9 | 30/28 |
| Eat once a month | 22/23 | 11/5 | 8/8 | 18/19 |
| Eat less frequently | 13/14 | 10/1 | 5/7 | 11/14 |

[a]Upper figure is the number of consumers who prefer bacon cured with nitrite; lower figure represents number of consumers preferring bacon cured without nitrite.

Thus, bacon, 15 days after processing without nitrite in the cure, was as acceptable to a large group of consumers as was bacon cured with nitrite in the same manner. This study was not designed to consider the potential risk of the growth of *Clostridium botulinum* or the possible effect on oxidative stability in the elimination of nitrite from the cure.

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*Journal of Food Protection Vol. 40, No. 10, Pages 686-688 (October, 1977)*


# Microbial Growth in Bulk Whole Corn Kernels Subjected to High Pressure Compaction

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(Received for publication March 9, 1977)

ABSTRACT

High pressure compaction of bulk whole kernel was investigated as a means to reduce the volume of corn for storage and transpotation. The effect of compaction resulted in a drastic reduction of viable cell count of bacteria and mold in corn samples during the first 24 h of storage. Thereafter, up to 21 days, the viable cell counts increased gradually. The "compressed shock" observed in this study was similar to injury of microorganisms by other physical and chemical treatments of food systems.

In previous work, high pressure compaction of bulk corn kernels was investigated as a means to reduce the volume of corn for storage and transportation. The physical and chemical aspects of high pressure application on corn were reported by McNulty and Mohsenin (*8,9*). The purposes of these investigations were (*a*) to establish conditions under which major irreversible failure of bulk corn kernals (1.8 - 22.0% mc, w.b.)[4] would occur when subjected to compaction and (*b*) to study their compaction behavior at pressures up to 34.6 MN/m². It was ascertained that low moisture corn (6.6% w.b.) failed due to oil expression at pressures in the range of 140-310 MN/m², whereas high moisture corn (24.2% w.b.) failed due to sample extrusion at a lower pressure range of 40-70 MN/m². Observations noted on samples after corn kernels were compacted under a pressure of 34.6 MN/m² and loading rates of .028, 0.28, 2.8 mm/s, showed that no sample extrusion occurred. However, extensive physical rupture of corn kernels occurred at 1.8% moisture. At 15.9 and 22.0% moisture, samples were plastically deformed but little microscopic kernel damage was evident. Substantial volumes of internal tissue gas were estimated with an average maximum of 21% in corn kernels with 15.9% moisture. Loading rates has no impact on these results.

The purpose of this study was to investigate the microbial growth in bulk whole corn kernels subjected to high pressure.

The effect of hydrostatic pressure on life in the deep oceans and simulated laboratory experiments have been reported by Brauer (*2*), Colwell and Morita (*4*), Kinne (*6*), Sleigh and MacDonald (*11*), and Zimmerman (*12*);. Under certain experimental conditions terrestrial microorganisms have been reported to be killed by high pressure. Mechanisms of death included bubble formation leading to explosive decompression of cell walls, cavitation, and shear (*12*).

Experimental conditions in this study were especially chosen to produce compression conditions to enhance the possibility of destroying microorganisms. Thus a high loading rate of 2.8 mm/s was chosen. After attaining a pressure of 34.6 MN/m² on the sample, compression was released immediately.

## MATERIALS AND METHODS

### *Corn samples*

The yellow dent hybrid corn that was used in this study was conditioned in special chambers until a specified equilibrium moisture content was attained. In one experiment, there was no visible sign of fungus on the corn, while in the other experiment there was noticeable fungal growth on the corn. Moisture levels in the specimens in the first experiment were 12,0, 15.9, and 22.0% (w.b.). They were prepared through equilibration by adsorption over saturated aqueous solution of sodium bromide, potassium chloride, and potassium sulfate, respectively. For contaminated corn in the special experiment, corn of only 22.0% moisture content was used.

### *Sample compaction*

Corn samples (10 g) were compacted by steel punch (94.5 mm long) in steel dies (22.3 mm inner diameter, 23.3 mm outer diameter and 88.1 mm long) at 2.8 mm/sec to a pressure of 34.6 MN/m² using the Amatek universal testing machine. Dies were cleaned and autoclaved

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[4]*List of symbols:*

| *Symbol* | *Defination* |
|---|---|
| *mc* | *Moisture Content* |
| *w.b.* | *Wet Basis* |
| *mm/s* | *Millimeters per second* |
| *MN/m²* | *MagaNewtons per meter²* |
| *CFU/g* | *Colony forming unit of microorganisms per gram of sample* |

between tests, but not before the initial experiment. The load was released at 2.8 mm/sec immediately after the desired pressure had been attained. Samples were then stored aerobically for the first experiment and under sealed conditions for the contaminated corn. The latter experiment was conducted to ascertain whether the combination of high pressure and minimum oxygen availability could destroy high numbers of fungi in corn. For the aerobic study, after compression the series of dies containing compressed corn were left exposed to the air in 25-C enviroment. The compressed corn samples (12.0, 15.9, and 22.0% moisture w.b.) were analyzed after suitable storage intervals up to 21 days. One sample (10 g of corn per die) was analyzed for each moisture level at each sampling time. For the sealed storage study, dies containing compressed corn were sealed with aluminum foil and masking tape to create an environment of lower oxygen tension. Compressed contaminated corn samples (22.0% moisture) were analyzed after suitable intervals up to 21 days of storage. Two samples were analyzed per sampling time. In separate experiments, temperature changes during compression of 6% and 25% moisture corn were monitored (*8*).

*Microbiological examinations*

Corn samples (10 g) were ejected from the dies and suspended in 90 ml of 0.1% aqueous peptone and then homogenized in a Waring Blender. The Plate Count method was used to ascertain the aerobic count, anaerobic count, and mold count of the sample.

Plate Count agar (Difco) with cycloheximide at 1.0 ppm was used for bacterial count and Potato Dextrose agar with 1.0 ppm each of chlortetracycline and chloramphenicol was used for mold count. Plates for bacterial counts were incubated at 35 C for 2 days. For anaerobic count, plates were incubated in anaerobic jars or in anaerobic chambers. Plates for mold count were incubated at 28 C for 5 days.

## RESULTS AND DISCUSSION

The effect of high pressure compression on the aerobic bacteria population in corn found in the first experiment, is shown in Fig. 1. The initial compression resulted in destruction of a portion of bacteria since the non-compressed corn had higher counts than the compressed corn at zero time. The most interesting phenomenon was the drop of bacteria count to a non-detectable level after 24 h of storage. By 72 h (third day) counts increased substantially and then gradually increased to a higher level of about $10^5$ CFU/g for corn with moisture levels at 15.9 and 22.0%. For corn with 12.0% moisture the bacterial number remained at a relatively low level of $10^3$ CFU/g. The combination of the effect of compression and relatively low moisture probably prevented any vigorous growth by the bacteria. The anaerobic count (data not shown) also followed the same general pattern. According to previous work (*8*) there was only a 3 C and 8 C increase in temperature during compression of the corn with 24 and 6% moisture, respectively. Therefore, temperature changes due to compaction probably was not the cause of reduction of microbial count after initial compression and after the first day of storage.

We spectulate that high pressure compression of bacteria resulted in (*a*) killing a portion of bacteria (*b*), injuring another portion, and (*c*) leaving another portion of bacteria uninjured. The delayed death observed after 24 h of storage may be due to the presence of injured cells, although further testing using appropriate methods to detect injured cells must be done to substantiate this

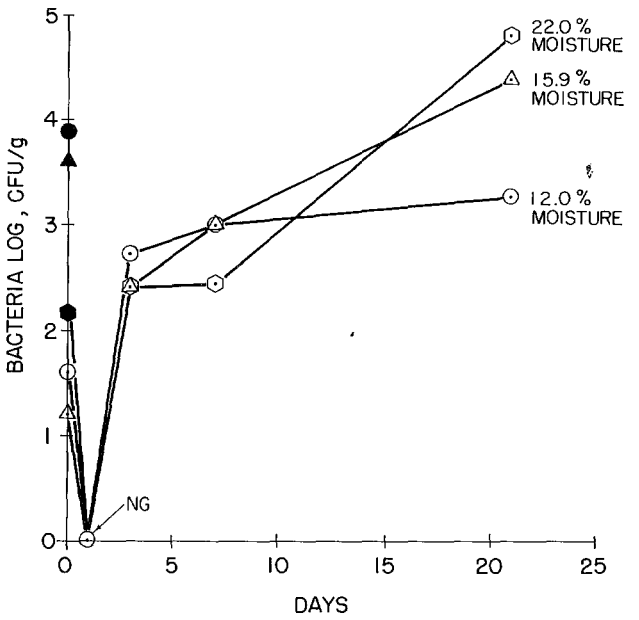


Figure 1. *Aerobic bacterial counts in bulk whole corn kernel in the first experiment subjected to high pressure compaction. Solid symbols indicate that data of corn before compression NG indicates that there was no growth on the plating medium*

hypothesis. The subsequent increase in number of bacteria during prolonged incubation may be the result of growth of uninjured cells as well as of recovered injured cells (assuming that there was a population of injured cells due to compression). This trend is documented for other physical and chemical treatments of microorganisms in laboratory conditions as well as in food systems (*3*). In studying chemical treatment of high moisture corn (27%) Bothast et al. (*1*) also observed an immediate drop of microbial counts with subsequent resuscitation and growth of the microorganisms.

Mold counts of corn in the first experiment also showed a similar "compression shock" phenomenon (Fig. 2). At the first day after compression, the mold count decreased to a non-detectable level. Thereafter the mold count increased substantially. On the 21st day, corn with the greatest moisture content (22.0%) had the highest mold count. This also matched the data of bacterial counts in relation to moisture contents, indicating the importance of moisture in the survival and growth of microorganisms in processed foods.

Data from the contaminated corn study (upper part of Fig. 2) did not show the "compression shock." This may be explained by the fact that compression of contaminated corn resulted in scattering of spores and mycelium of mold such that more viable units occurred and grew into individual colonies thus masking the presence of the injured population of mold. These data also indicate that sealing of the dies by the method used in this study was insufficient to cause detrimental effects on mold growth. It was obvious that preservation of grossly contaminated food by food processing such as this is not likely to be successful.

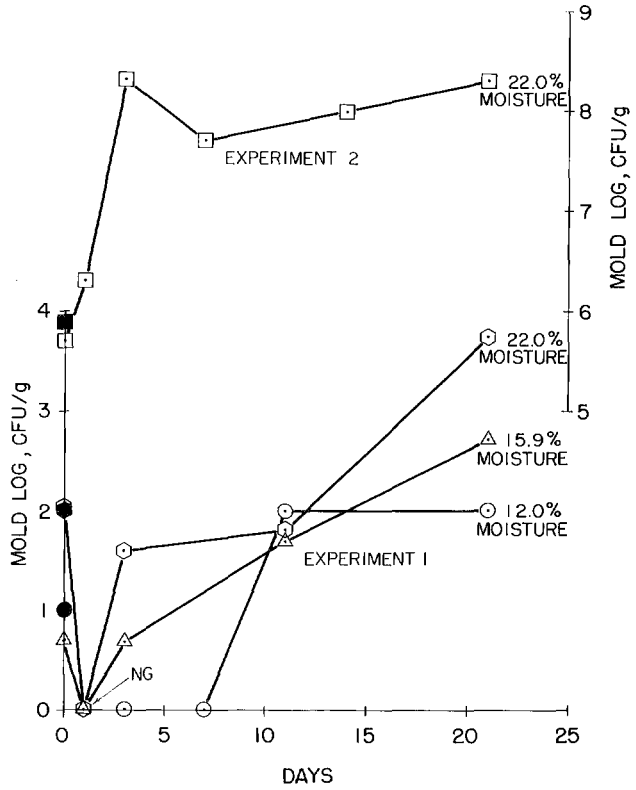


Figure 2. *Effect of high pressure compaction in mold counts in stored corn in the first and second experiments. Solid symbols indicate data of corn before compression NG indicates that there was no growth on the plating medium.*

In conclusion, high pressure compaction of corn may result in savings of space in storage and transportation of corn but the process itself cannot destroy all microorganisms. Surviving organisms will grow at a later time during storage. If an additional stress (e.g. organic acids or heat) is applied together with the compaction process more microorganisms may be destroyed than by compaction alone thus prolonging storage time of corn at a substantial reduction of storage space.

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*Journal of Food Protection Vol. 40, No. 10, Pages 689-692 (October, 1977)*


# Effect of Heating, Freezing, and pH on *Yersinia enterocolitica*-like Organisms From Meat

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ABSTRACT

No survivors of *Yersinia enterocolitica* were detected in beef roasts inoculated at levels as high as 3.1-3.8 × $10^6$ viable cells per g when the final internal temperature in the center was 60 to 62 C. At 51 C some *Y. enterocolitica* survived. Extensive destruction of *Y. enterocolitica* occurred on beef during frozen storage. Counts of surviving organisms were greater on tryptic soy agar than on bismuth sulfite agar plates. Growth of *Y. enterocolitica* in brain heart infusion was better at pH 7 and 8 than at 6 or 9. Little or no growth occurred at pH 5.

*Yersinia enterocolitca* is a gram-negative, facultative anaerobic bacillus of the family *Enterobacteriaceae*. Isolation of this organism often has been reported from feces or lymph nodes of both sick and healthy animals and man (*8,9*). Reported cases of human infection of *Y. enterocolitica* have increased steadily in recent years, particularly in Europe and Canada (*1,12*). In humans, *Y. enterocolitica* produces a variety of clinical symptoms, particularly gastroenteritis and mesenteric lymphadenitis in childhood or adolescence, acute abdominal disorders, diarrhea and arthritis among adults, and erythema nodosum in the higher age classes (*16*). Although the epidemiology of *Y. enterocolitica* is not fully established, contact with infected animals, person to person transmission within an infected family or consumption of contaminated food have been mentioned as modes of transmission (*3,11,13*). *Y. enterocolitica* has been isolated from foods such as beef, pork, ice cream, mussels, oysters, and from drinking water (*2,7*). The first outbreak of illness from *Y. enterocolitica* in which food-borne transmission (chocolate milk) has been documented occurred recently among school children in Oneida County, New York (*14*). Recently, Hanna et al. (*4*) isolated several *Y. enterocolitica*-like organisms from vacuum-packaged beef and lamb. In a subsequent study, large increases in *Y. enterocolitica* count occurred when raw or cooked beef and pork inoculated with these isolates were stored at 7 C (*5*). Further studies (*6*) showed that these *Y. enterocolitica*-like isolates did not survive heating in skim milk ($10^6$-$10^7$ per ml) for 1-3 min at 60 C. The present paper reports data on (*a*) the effect of heating and freezing on survival of these organisms in beef and (*b*) development of this organism in broth cultures at different pH values.

## MATERIALS AND METHODS

### *Cultures*

Test cultures 1157 and 1049 were isolated from vacuum-packaged beef. Their morphological, biochemical, and serological characteristics and differences from "typical" strains of *Y. enterocolitica* were described in a previous paper by Hanna et al. (*4*). Culture 23715 was purchased from the American Type Culture Collection, Rockville, Md for comparative purposes. The cultures were maintained on tryptic soy agar (TSA, Difco) slants. Inocula for the meat samples consisted of cultures grown in brain heart infusion (BHI, BBL) for 6-8 h at 25 C. The desired cell concentration of the BHI inoculum was achieved by dilution of the BHI culture with sterile BHI based on established relations between optical density at 550 nm and viable cell count.

### *Heat resistance studies*

Bottom round (boneless) beef roasts were prepared in the University Meats Laboratory, frozen at -34 C, and subsequently stored for 30 days at -23 C. The roasts (approximately 7.6 × 7.6 × 6.4 cm) weighed about 0.4 kg. They were thawed for 24 h in a refrigerator before inoculation. Roasts were inoculated on opposite sides (top and bottom) by injecting 0.1 ml of a BHI culture to depths of 3.2 and 2.5 cm with 21-gauge needles. Range of viable cells of the BHI cultures was from 8.7 × $10^4$ to 3.3 × $10^8$ per ml. The injections were made in a close geometric pattern with a sterile aluminum foil template (Fig. 1). Thirty-three injections per side or a total of 66 injections per roast were made. A single thermocouple was placed in the geometric center of each roast. The roasts were individually wrapped in heavy gauge aluminum foil and cooked (in an electric oven) at 177 C until the internal temperature reached 38 to 45 C. The roasts were removed from the oven with the thermocouples still inserted to record post-oven increase in temperature. Final internal temperatures ranged from 51 to 62 C. Roasts inoculated with *Y. enterocolitica*, but not heated, were used as controls to determine levels of *Y. enterocolitica*, before heating.

After heating, roasts were sliced vertically down the center with a sterile knife (Fig. 2). A 4 × 4 × ½ cm sample weighing about 10 g was cut from the exposed center of one of the two halves of the roast. This sample was blended for 30 sec with 90 ml of sterile 0.1% peptone water in a sterile Waring Blendor. Appropriate decimal dilutions of the homogenate were made with sterile 0.1% peptone water. Aliquots (0.1 ml) of the dilutions were plated on TSA and bismuth sulfite agar (BS, BBL) plates by the spread-plate method. One milliliter of the 1:10 dilution was plated by the pour plate method. Plates were incubated for

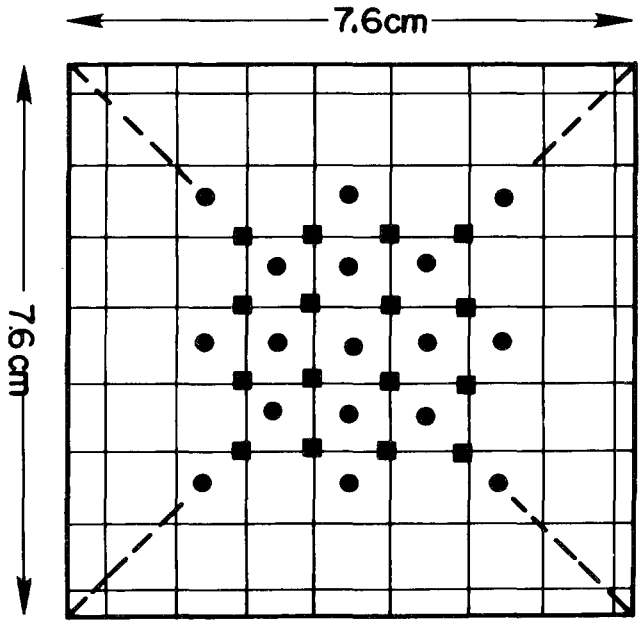


Figure 1. *Aluminum foil template with pattern for inoculation with* Y. enterocolitica *(inoculated to depth of 3.2 cm•, and 2.5 cm ■).*

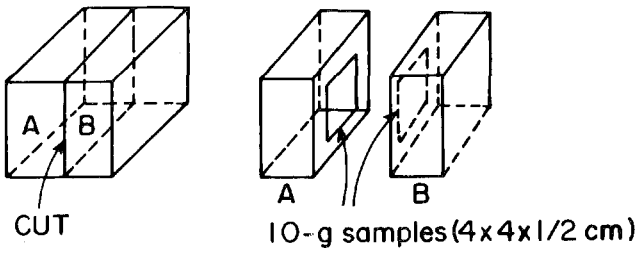


Figure 2. *Slicing and sampling of roasts. One sample was used for analysis at 0 day, one for analysis after 7 days at 5-7 C.*

3 to 4 days at 25 C. The cooked roasts were re-wrapped and held at 5-7 C for 1 week. At this time a second 10-g sample of the other half of the roast was obtained as previously described. The identity of colonies on TSA and BS plates was determined according to diagnostic schemes and procedures published by Vanderzant and Nickelson (*15*). Biochemical tests to confirm isolates tentatively classified as *Y. enterocolitica* are described by Hanna et al. (*4*).

*Freezing studies*

Beef sirloin tip roasts (approx. 3 kg) were prepared in the University Meats Laboratory. They were wrapped in polyethylene coated freezer paper, frozen at -34 C, and stored at -23 C for approximately 1 month before use in the study. Cuts were thawed for 24 h in a refrigerator before cooking.

Beef roasts were covered with aluminum foil, placed on a rack, and cooked in an electric oven pre-heated to and maintained at 177 C. The beef roasts were cooked (approx. 3 h) until the internal temperature, as monitored with an implant thermocouple, was 60 C. The final internal temperature was 70-75 C which included the post-oven temperature increase.

After removal from the oven, meat was allowed to cool for 30 min at room temperature. The outside portion of the meat was removed aseptically with sterile knives. Samples for inoculation with test cultures were prepared from the center portion. Each of these samples (4 × 4 × ½ cm), weighing about 10 g, was placed on a sheet of sterile thin aluminum foil (15 × 15 cm). Meat samples were inoculated by placing 0.1 ml of a BHI culture of *Y. enterocolitica* ($10^5$-$10^6$/ml for low inoculum, $10^8$-$10^9$/ml for high inoculum) on the upper surface. This inoculum yielded in most instances $10^3$-$10^4$ or $10^6$-$10^7$ *Y. enterocolitica* per g of meat. In addition, uninoculated meat samples were used as controls at the beginning and end of the experimental period. After inoculation, meat samples were wrapped in the thin aluminum foil followed by another sterile over-wrap of foil. The samples were stored at -18 to -20 C. Samples were examined periodically for *Y. enterocolitica* up to 4 weeks of storage. At each sampling interval, samples were left at room temperature for 20 min. The over-wrap was removed and discarded. The meat sample was removed from the inner wrap using sterile forceps and placed in 90 ml of sterile 0.1% peptone diluent along with the inner wrap. This was vigorously shaken 25 times. Appropriate decimal dilutions were made with sterile 0.1% peptone water. Aliquots (0.1 ml) of appropriate dilutions were plated in duplicate by the spread plate method on both TSA and BS agar. Plates were incubated for 3-4 days at 25 C. Identification of colonies on TSA and BS plates and confirmation of *Y. enterocolitica* was carried out as described for the isolates of the heating experiment.

*pH studies*

Development of *Y. enterocolitica* at different pH values was tested in BHI broth adjusted to pH values of 5, 6, 7, 8 and 9. Tests were carried out in 250-ml screw cap Erlenmeyer flasks containing 100 ml of BHI broth adjusted to the desired pH value with NaOH or HCl before sterilization. pH values after sterilization were within 0.05 to 0.1 of the desired value. Inocula for the pH tests consisted of cultures grown in BHI for 17-18 h at 25 C. A 2% inoculum was used to yield a viable cell concentration of $10^2$-$10^3$ per ml in the 100-ml test culture. The desired cell concentration of the inoculum was made by dilution with sterile BHI broth based on established relations between optical density at 550 nm and viable cell count. Samples were taken at hourly intervals for 8 h and after 24 h. Samples (0.1 ml) or appropriate decimal dilutions in sterile 0.1% peptone were plated on TSA by the spread-plate method. Plates were incubated at 25 C for 3 to 4 days.

## RESULTS AND DISCUSSION

Data in Table 1 present the effect of heat on survival of *Y. enterocolitica* in center slices of beef roasts with different concentrations of these strains. With a final internal temperature of 55 to 62 C in the center of the roasts, no survivors of *Y. enterocolitica* were present in 0.1 g of beef. This was true for samples plated on both TSA and BS agar plates. When the remainder of these roasts were stored for 7 days at 5-7 C, no *Y. enterocolitica* was isolated from the second of the center slices. In one instance, the final internal temperature was only 51 C and survivors of *Y. enterocolitica* were present both on TSA and BS agar plates.

TABLE 1. *Effect of heat on the survival of* Y. enterocolitica *inoculated into beef roasts at different cell concentrations*

| Strain | *Y. enterocolitica*/g before heating | Final internal temp.[a] (C) | *Y. enterocolitica*/g after heating |
|---|---|---|---|
| 23715 | $2.0 \times 10^3$ | 57 | <10 |
| 23715 | $4.0 \times 10^3$ | 55 | <10 |
| 1157 | $2.0 \times 10^3$ | 59 | <10 |
| 1157 | $6.0 \times 10^3$ | 55 | <10 |
| 1049 | $4.3 \times 10^3$ | 51 | $1.5 \times 10^3$ (TSA)<br>$7.7 \times 10^2$ (BS) |
| 23715 | $1.6 \times 10^4$ | 57 | <10 |
| 1157 | $1.2 \times 10^4$ | 59 | <10 |
| 1049 | $2.5 \times 10^4$ | 58 | <10 |
| 23715 | $3.8 \times 10^6$ | 60 | <10 |
| 1157 | $3.1 \times 10^6$ | 61 | <10 |
| 1049 | $3.3 \times 10^6$ | 62 | <10 |

[a]Final internal temperature included the post-oven warming or temperature increase after removal of meat from the oven.

A final internal temperature between 55 and 60 C was set as a goal because this temperature range most likely represents the lower range of temperature of internal portions of cooked meats which are very rare. The results are consistent with previous data on survival of *Y. enterocolitica* following heating in skim milk (*6*). No survivors of culture 1049 (initial concentration $10^6$ per ml) were detected after heating in skim milk for 7 min at 55 C or for 1 min at 60 C. The reduction in viable count of culture 1049 in skim milk after 10 min at 50 C was about four logs. In skim milk, culture 1049 was more heat sensitive than 1157 or 23715. Hence, at very low final internal temperatures, survivors of cultures 23715 and 1157 can be expected since they are more heat resistant than culture 1049. In general, the data indicate that there is little opportunity for survival of *Y. enterocolitica* in meats with internal portions heated to 60 C or above.

Aerobic plate counts of non-inoculated control roasts before heating ranged from $2.3 \times 10^3$ to $7.9 \times 10^3$ per g. The small number of isolates from heated inoculated roasts consisted of gram-positive cocci and rods.

Extensive reductions in *Y. enterocolitica* counts as measured on TSA and BS agar plates occurred during frozen storage of beef samples inoculated with *Y. enterocolitica* (Fig. 3). Results with culture 1049 were

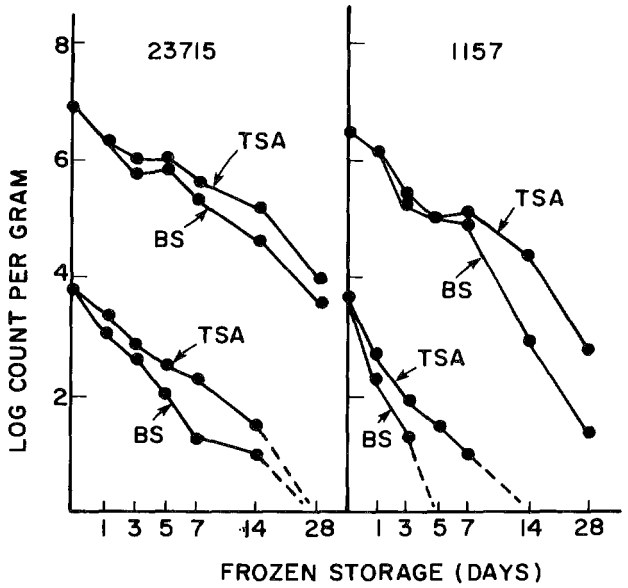


Figure 3. *Effect of frozen storage on* Y. enterocolitica *23715 and 1157 inoculated on beef.*

similar to those obtained with 23715. Four weeks of frozen storage of samples with an initial count of $10^6$-$10^7$ per g reduced the count on TSA 2.9 (strain 23715) to 4.0 logs (strain 1049). With an initial population of about $10^4$ cells per g, no survivors could be detected after 2 (strain 1157) to 4 weeks (strains 23715 and 1049). Decreases in count were consistently greater on BS than on TSA plates, probably because of the deleterious effect of media constituents such as brilliant green on development of cells sublethally injured during freezing. A lower number of survivors on BS agar plates was also noted with the one heated roast in which *Y. enterocolitica* survived (Table 1). It is recognized that enumeration of *Y. enterocolitica* from heated or frozen meats by direct plating on TSA but particularly on BS agar may not recover all potential viable cells. Additional studies are needed to determine appropriate methods for stressed cells of *Y. enterocolitica* to recover or repair themselves before plating, particularly on selective media (*10*). Surface contamination of meat cuts with *Y. enterocolitica* could occur through direct or indirect cross contamination with fecal material, lymph nodes, contaminated tissue, or contact surfaces (*7,13*).

Growth of *Y. enterocolitica* in BHI was better at pH 7.0 and 8.0 than at pH 6.0 and 9.0, with little or no change in viable count over a 24-h period at pH 5.0 (Fig. 4). Results with culture 1049 and 1157 were similar to those obtained with culture 23715. Hence, one would expect a reduced opportunity for growth of *Y. enterocolitica* on beef with low pH values (5.4 or less) than on beef with high pH values (5.8 or higher).

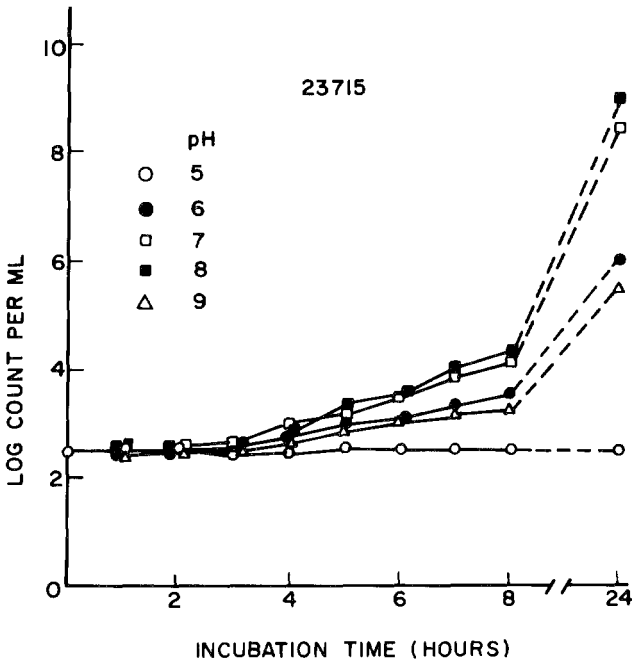


Figure 4. *Effect of pH on the development of* Y. enterocolitica *23715 in brain heart infusion.*

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*Journal of Food Protection Vol. 40, No. 10, Pages 693-697 (October, 1977)*


# Microflora of Retail Fluid Milk Products[1]

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(Received for publication January 7, 1977)

ABSTRACT

Numbers and types of microorganisms in retail pasteurized fluid milk products were determined as well as the effect that type of product, brand, and season of the year had on counts of 13 different microbial types. *Clostridium perfringens* was the only pathogen detected and it averaged less than one organism per milliliter. Chocolate milk samples generally had the highest mean counts, followed by skim milk, low-fat (2%), and whole milk (3.25%). Most brands had means for the various microbial counts which were not significantly different from each other. Only three brands had counts which differed significantly from other brands. Psychrotrophic, coliform, staphylococcal, yeast and mold, and Standard Plate Counts were highest between May and October, while counts for spores, streptococci, and thermophiles were highest between December and March. No seasonal trends were detected for counts of anaerobes, *C. perfringens*, enterococci, or lactobacilli.

The last comprehensive survey of the microflora of retail milk was conducted in 1953 (*1*). Many changes have occurred in the dairy industry since that time. Use of farm bulk tanks, every-other-day pick-up of farm milk, five-day-a-week plant operation, decreased home deliveries, once-a-week purchase by the consumer, as well as other factors, may have affected numbers and types of microorganisms present in retail fluid milk products (*25*). While several researchers have noted that the microflora of retail fluid milk products has shifted toward a largely psychrotrophic population (*14,17*), the exact effect that changes in the dairy industry have had on composition of the microflora of retail milk products has not been fully established.

This study was undertaken to determine the following: (*a*) major groups of microorgansims which comprise the microflora of fluid milk products at time of purchase; (*b*) numbers and types of selected pathogens present in these products, and (*c*) influence of season, brand, and product on microflora.

## MATERIALS AND METHODS

### *Samples*

Half-gallon or quart cartons of whole (3.25% milkfat), low-fat (2%), skim and chocolate milks were purchased from retail outlets in Lexington, Kentucky during the first week of each month for 1 year. Samples were purchased before their expiration dates from outlets with rapid turnover of fluid dairy products. Samples pruchased from these outlets were placed on ice, transported to the laboratory, and held at 4 ± 1 C until analyzed. All samples were analyzed within 8 h of purchase.

### *Microbial analyses*

Coliform, psychrotrophic, spore, thermophilic, yeast and mold, and Standard Plate Counts were done according to *Standard Methods* (*2*). The confirmed test described in *Standard Methods* was used to verify as coliforms colonies obtained from Violet Red Bile Agar. Except as described below, procedures in *Bacteriological Analytical Manual for Foods* (*8*) were used for enumeration of enterococci, staphylococci (surface spread plates of Vogel and Johnson Agar), salmonellae (pre-enrichment in nutrient broth), and *Clostridium perfringens* (overlaid pour plates of Sulfite Polymyxin Sulfadiazine Agar (SPS) incubated anaerobically). The method of Angelotti et al. (*3*) was used to confirm as *C. perfringnes* all black colored colonies on SPS plates. Anaerobic counts were determined using Reinforced Clostridial Agar plates incubated anaerobically in BBL Gas Pak jars at 37 C for 48 h. Lactobacilli were enumerated using LBS agar (BBL) enriched with 20% sterile tomato juice obtained by filtering canned whole tomatoes. Plates with LBS agar were incubated for 48 h at 37 C. Streptosel Agar plates incubated at 37 C for 48 h were used to enumerate streptococci.

### *Statistical analyses*

Counts of less than 30 were used only when obtained on the lowest dilution plated. Data were analysed using the Statistical Analysis System of Barr and Goodnight (*4*). Differences among counts from various products, brands, and months were determined using a Least Significant Difference (LSD) procedure (*23*).

## RESULTS AND DISCUSSION

The 377 fluid milk samples analyzed represented eight brands with 96 samples of whole milk (3.25% milkfat), 92 samples of low-fat milk (2%), 96 samples of skim milk, and 93 samples of chocolate milk.

Neither salmonellae nor coagulase-positive staphylococci were detected in any of the samples examined. *C. perfringens* was the only pathogen detected and it

[1]*Published with the approval of the Director of the Kentucky Agricultural Experiment Station as journal aritcle no. 76-5-93*
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averaged less than one per milliliter. Results obtained for salmonellae were similar to those obtained by other investigators (*13,21*) and were not surprising since no outbreaks of *Salmonella*-related illness have been linked to consumption of pasteurized retail fluid milk products during recent years in the United States (*9*). However, failure to detect coagulase-positive staphylococci differs from results obtained by Foltz et al. (*7*) and Sheikh and Luedecke (*22*) who reported presence of coagulase-postive staphylococci in 3.4% and 4.9% of the retail dairy products they examined, respectively. Differences in results could have been due to differences in sources of samples as well as the medium used for enumeration and isolation of staphylococci.

Mean logarithms, standard deviations, and range of logarithms obtained for the various microbial counts for each of the four milk products are in Table 1. The Least Significant Difference (LSD) procedure was used to determine if the counts between products differed significantly (Table 1). Mean log counts obtained from chocolate milk samples for anaerobes, coliforms, enterococci, lactobacilli, psychrotrophs, spores, Standard Plate, staphylococci and streptococci were higher ($P < .01$) than those obtained from the three non-chocolate milk samples. Skim milk samples tended to have second highest values for most counts, while whole milk samples tended to have lowest values. However, few of the differences in counts among the non-chocolate products were significant. These results are in agreement with those obtained by Langlois et al. (*14*).

*C. perfringens* counts of whole milk samples were higher ($P < .01$) than those of the other three products. However, because of the small numbers detected in this study, it was not possible to determine if the higher counts represented a real difference or were due to random variation in counts because of errors in enumeration and confirmation procedures. Illness should not result from consumption of milk containing the low numbers of *C. perfringens* found in this study, since $5 \times 10^8$ to $5 \times 10^9$ cells are necessary to cause illness (*6,12*).

Spore and thermophilic counts were similar in that highest counts for both were obtained from chocolate milk samples and lowest counts from whole milk samples. The similarities between these two counts suggest that some thermophilic bacteria also were spore-formers. These results agree with those obtained by Hansen (*11*). Spore counts obtained from chocolate milk could be expected, since cocoa powders contain spores which survive pasteurization (*10*).

Staphylococcus counts were higher ($P < .01$) in chocolate milk samples than in non-chocolate milks. No significant differences were detected among staphylococcus counts from non-chocolate milks. In fact, average staphylococcus counts for chocolate milk samples were three times the counts for skim milk samples, almost four times those for whole milk samples, and five times those for low-fat milk samples. All staphylococcus colonies tested were coagulase-negative.

TABLE 1. *Mean logarithms, standard deviation, and range of microbial counts obtained from fluid milk products obtained from retail outlets*

| Products: | Whole | Low-fat | Skim milk | Chocolate |
|---|---|---|---|---|
| No. of samples: | 96 | 92 | 93 | 93 |
| Microbial counts ($log_{10}$/ml) | | | | |
| Anaerobic | $1.92 \pm 0.90^a$ (0.00 – 3.18) | $2.11 \pm 0.91^a$ (0.00 – 2.88) | $2.11 \pm 1.06^a$ (0.00 – 3.04) | $2.77 \pm 1.12^b$ (1.45 – 2.75) |
| *Clostridium perfringens* | $0.06 \pm 0.22^a$ (0.00 – 1.30) | $0.04 \pm 0.17^b$ (0.00 – 1.00) | $0.00 \pm 0.00^b$ (0.00 – 0.00) | $0.02 \pm 0.12^b$ (0.00 – 1.00) |
| Coliform | $0.12 \pm 0.52^a$ (0.00 – 3.67) | $0.16 \pm 0.70^a$ (0.00 – 5.89) | $0.18 \pm 0.54^a$ 0.00 – 2.95) | $0.42 \pm 1.05^b$ (0.00 – 3.65) |
| Enterococci | $0.12 \pm 0.40^a$ (0.00 – 2.15) | $0.05 \pm 0.31^a$ (0.00 – 1.30) | $0.16 \pm 0.53^a$ (0.00 – 1.70) | $0.30 \pm 0.67^b$ (0.00 – 2.79) |
| Lactobacilli | $0.07 \pm 0.27^a$ (0.00 – 1.30) | $0.03 \pm 0.18^a$ (0.00 – 1.00) | $0.02 \pm 0.18^a$ (0.00 – 1.78) | $0.13 \pm 0.52^b$ (0.00 – 3.11) |
| Psychrotrophic | $1.88 \pm 2.10^a$ (0.00 – 7.69) | $1.90 \pm 2.23^a$ (0.00 – 8.86) | $2.74 \pm 2.28^b$ (0.00 – 7.53) | $3.76 \pm 1.97^c$ (0.00 – 7.75) |
| Spore | $1.88 \pm 0.50^a$ (0.60 – 2.60) | $1.93 \pm 0.48^a$ (0.78 – 2.51) | $1.88 \pm 0.47^a$ (0.95 – 2.58) | $2.27 \pm 0.53^b$ (1.60 – 4.76) |
| Standard Plate | $3.22 \pm 1.27^a$ (1.70 – 7.30) | $3.36 \pm 1.55^a$ (1.30 – 8.51) | $3.72 \pm 1.60^a$ (2.00 – 6.46) | $4.07 \pm 1.43^b$ (1.90 – 7.26) |
| Staphylococci | $0.08 \pm 0.39^a$ (0.00 – 3.30) | $0.06 \pm 0.33^a$ (0.00 – 1.70) | $0.10 \pm 0.38^a$ (0.00 – 1.48) | $0.30 \pm 0.62^b$ (0.00 – 2.78) |
| Streptococci | $1.41 \pm 0.97^a$ (0.00 – 3.99) | $1.70 \pm 1.43^a$ 0.00 – 8.90) | $1.44 \pm 1.23^a$ (0.00 – 3.97) | $2.11 \pm 1.46^b$ (0.00 – 6.23) |
| Thermophilic | $1.80 \pm 0.62^a$ (0.00 – 3.34) | $1.99 \pm 0.60^b$ (0.00 – 2.79) | $1.99 \pm 0.72^b$ (1.00 – 5.69) | $2.15 \pm 0.56^b$ (1.00 – 3.15) |
| Yeast and mold | $0.17 \pm 0.49^{ab}$ (0.00 – 2.11) | $0.06 \pm 0.21^c$ (0.00 – 1.11) | $0.10 \pm 0.32^{ab}$ (0.00 – 1.00) | $0.23 \pm 0.75^b$ (0.00 – 6.30) |

$^{a,b,c}$Means with different superscripts are different ($P < .01$) within rows by LSD.

The ability of coagulase-negative staphylococci to produce enterotoxin was not determined. However, based on other studies (*5,16,19,24*) few, if any, coagulase-negative staphylococci would have been expected to produce toxin.

While yeast and mold counts obtained from low-fat samples were lower ($P < .01$) than counts from the other three products, counts obtained from the four milk products averaged less than 2 per milliliter. Thus, any differences in counts could have been due to error inherent in the enumraton procedure.

Flavor evaluations of milk samples were not included in this study. However, if the population levels of psychrotrophic bacteria established by Punch et al. (*18*) as indicating flavor defects are used, then 5.0% of the samples should have had off-flavors. These off-flavors should have been present in 5.2% of whole milk samples, 5.4% of low-fat samples, 5.3% of skim samples, and 4.3% of chocolate samples. Chocolate milk had the greatest number of samples exceeding both the 20,000/ml and 10 coliform/ml limits, but the smallest number of samples exceeding the off-flavor limits of Punch et al. This result is possible because the limits of Punch et al. were extremely high (5 million/ml) and were established using psychrotrophic counts rather than coliform or Standard Plate Counts.

Higher counts were obtained in a study conducted in 1953 (*1*) than were obtained in this study. Streptococcus and yeast and mold counts were more than one log higher, while spore and Standard Plate Counts were about one-half a log higher than corresponding counts obtained in this study. It is impossible to determine the exact reasons for the higher counts obtained in the earlier study. However, when the 1953 survey was done, farm bulk tanks were not yet in widespread use. Thus milk was not refrigerated as quickly after milking as it is today. In addition, in the 1953 study, plates were incubated at 30 C for 5 days which could have accounted for some discrepancy in counts.

The log values obtained for counts from all samples were averaged and plotted against month; results are in Fig. 1. Since no significant differences were observed among the monthly counts obtained for anaerobic, *C. perfringens,* enterococcus, or lactobacillus counts, these counts were omitted from the figure. Standard Plate Counts for the month of April were not determined. Counts obtained for spores and thermophiles were so similar that the two counts were averaged and represented by one curve.

Counts for psychrotrophs, coliforms, Standard Plate, staphylococci, and yeast and molds were highest from May through October, while counts for spores, streptococci, and thermophiles were highest from December through March.

Curves for psychrotrophic and Standard Plate Counts were similar, with highest values occurring in June and October and lowest counts in December and March. However, psychrotrophic counts showed a greater

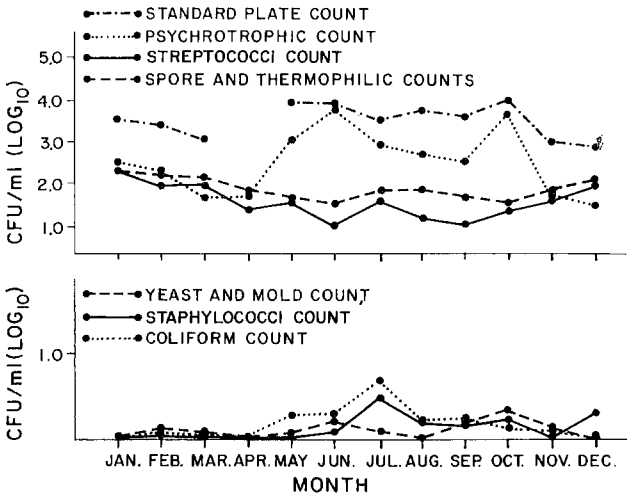


Figure 1. *Selected microbial counts obtained from retail fluid milk products purchased monthly.*

month-to-month variation than did the Standard Plate Counts. These findings disagree with those of Macy (*15*), who reported that season had no effect on Standard Plate Counts.

The curve for spore and thermophilic counts, which was highest during the winter months, roughly paralleled the curve for streptococcus counts. These counts may suggest a greater degree of contamination by dust during the winter months due to the greater amount of feed required to maintain animals during the colder weather. Higher spore counts obtained during the winter months are in agreement with the findings of Ridgeway (*20*).

A seasonal trend for yeast and mold counts is suggested by data in the figure. However, the small numbers obtained make it difficult to establish whether a season trend actually existed or if the differences obtained were due to errors inherent in the plating procedure. Likewise, enumeration errors could have masked any seasonal trends that may have been present for counts of anaerobes, *C. perfringens* enterococci, or lactobacilli.

Mean logarithms, standard deviations, and range of logarithms obtained for the various counts made on each of the eight brands are in Table 2. In addition, the LSD procedure was used to determine if counts between brands differed significantly (Table 2).

Mean log counts from products of brand C were lower ($P < .01$) than those from most other brands for anaerobic, psychrotrophic, spore, Standard Plate, streptococcus, and yeast and mold counts. In addition, this brand had the lowest mean log coliform count; however, only the difference between it and the value obtained for brand D was significant.

Counts obtained from samples of brand D were higher ($P < .01$) than those from most brands for anaerobic, coliform, and psychrotrophic counts, but were lower ($P < .01$) for thermophilic counts. These results suggest that products of this brand were produced under poor sanitary conditions or were improperly processed.

Counts of enterococci, lactobacilli, streptococci, and

thermophiles were higher ($P < .01$) for products of brand A than they were from most other brands. These results could have been obtained if products of brand A were produced under insanitary conditions or were improperly processed.

The only statistically significant difference detected among the remaining brands (B, E, F, G, and H) was that spore counts from brand E were higher ($P < .01$) than those obtained from all but brand H.

In conclusion, retail fluid milk products should present no health hazard. Analysis of Standard Plate Counts showed that 15.6% of whole milk samples, 15.2% of low-fat milk samples, 30.1% of skim milk samples, and 39.7% of chocolate milk samples had counts that exceeded 20,000/ml. The following percentages of samples within each brand had Standard Plate Counts over 20,000/ml: brand A 19.1%; brand B, 18.7%; brand C, 2.3%; brand D, 51.2%, brand E, 13.6%, brand F, 31.7%; brand G, 34.0%; and brand H, 26.1%. The percentage of samples with counts over the 10/ml coliform limit were as follows: brand A, 8.3%; brand B, 10.4%; brand C 0%; brand D 16.6%; brand E, 2.0%; brand F, 8.3%; brand G, 2.0%; and brand H, 6.2%. Examination of coliform counts revealed that 4.1% of whole milk samples, 3.2% of low-fat samples, 6.4% of skim samples, and 12.9% of chocolate samples exceeded 10/ml.

TABLE 2. *Mean logarithms, standard deviation, and range of counts of different brands of fluid milk samples obtained from retail outlets*

| Brand: | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| No. of samples: | 48 | 48 | 47 | 42 | 48 | 48 | 48 | 48 |
| Microbial count ($log_{10}$/ml) | | | | | | | | |
| Anaerobic | $2.44 \pm 1.21^{ab}$ (0.00 – 6.66) | $2.38 \pm 1.11^{ab}$ (0.30 – 5.27) | $1.58 \pm 0.73^{c}$ (0.00 – 3.47) | $2.62 \pm 1.28^{a}$ (0.00 – 5.77) | $2.15 \pm 0.50^{b}$ (1.00 – 4.47) | $2.14 \pm 0.84^{b}$ (0.00 – 3.96) | $2.38 \pm 1.03^{ab}$ (0.00 – 5.17) | $2.08 \pm 1.17^{b}$ (0.00 – 6.17) |
| *C. perfringens* | $0.02 \pm 0.14^{a}$ (0.00 – 1.00) | $0.20 \pm 0.14^{a}$ 0.00 – 1.00) | $0.03 \pm 0.16^{a}$ (0.00 – 1.00) | $0.05 \pm 0.25^{a}$ (0.00 – 1.30) | $0.02 \pm 0.10^{a}$ (0.00 – 0.60) | $0.01 \pm 0.09^{a}$ (0.00 – 0.60) | $0.01 \pm 0.09^{a}$ (0.00 – 0.60) | $0.03 \pm 0.16^{a}$ (0.00 – 1.00) |
| Coliform | $0.28 \pm 0.75^{a}$ (0.00 – 3.65) | $0.25 \pm 0.66^{a}$ (0.00 – 2.94) | $0.01 \pm 0.08^{a}$ (0.00 – 0.60) | $0.58 \pm 1.28^{b}$ (0.00 – 5.88) | $0.07 \pm 0.24^{a}$ 0.00 – 1.27) | $0.23 \pm 0.78^{a}$ (0.00 – 3.39) | $0.13 \pm 0.77^{a}$ (0.00 – 5.32) | $0.19 \pm 0.65^{a}$ (0.00 – 2.80) |
| Enterococci | $0.43 \pm 0.81^{a}$ (0.00 – 3.47) | $0.12 \pm 0.38^{b}$ (0.00 – 1.77) | $0.13 \pm 0.43^{b}$ (0.00 – 2.11) | $0.16 \pm 0.46^{b}$ (0.00 – 1.84) | $0.16 \pm 0.64^{b}$ (0.00 – 3.84) | $0.07 \pm 0.28^{b}$ (0.00 – 1.47) | $0.05 \pm 0.36^{b}$ (0.00 – 2.51) | $0.09 \pm 0.36^{b}$ (0.00 – 1.69) |
| Lactobacilli | $0.21 \pm 0.68^{a}$ (0.00 – 3.11) | $0.06 \pm 0.24^{b}$ (0.00 – 1.00) | $0.04 \pm 0.20^{b}$ (0.00 – 1.00) | $0.06 \pm 0.30^{b}$ (0.00 – 1.69) | $0.09 \pm 0.32^{ab}$ (0.00 – 1.30) | $0.00 \pm 0.00^{b}$ (0.00 – 0.00) | $0.02 \pm 0.14^{b}$ (0.00 – 1.00) | $0.00 \pm 0.00^{b}$ (0.00 – 0.00) |
| Psychrotrophic | $2.96 \pm 2.43^{ab}$ (0.00 – 7.74) | $2.66 \pm 1.84^{ab}$ (0.00 – 6.78) | $1.17 \pm 1.36^{c}$ (0.00 – 5.46) | $3.75 \pm 2.35^{d}$ (0.00 – 8.86) | $1.65 \pm 1.85^{bc}$ (0.00 – 6.53) | $2.82 \pm 2.47^{ab}$ (0.00 – 7.41) | $3.34 \pm 2.54^{ad}$ (0.00 – 7.82) | $2.20 \pm 2.07^{bc}$ (0.00 – 6.59) |
| Spore | $2.01 \pm 0.57^{a}$ (1.00 – 3.44) | $2.00 \pm 0.52^{a}$ (0.94 – 3.20) | $1.82 \pm 0.49^{b}$ (0.60 – 2.91) | $2.00 \pm 0.62^{a}$ (1.00 – 3.00) | $2.18 \pm 0.31^{c}$ (1.38 – 2.58) | $1.88 \pm 0.38^{ab}$ (1.04 – 2.55) | $1.96 \pm 0.48^{ab}$ (1.04 – 3.64) | $2.02 \pm 0.64^{ac}$ (0.00 – 3.20) |
| Standard plate | $3.82 \pm 1.41^{ab}$ (2.27 – 7.25) | $3.73 \pm 1.16^{ab}$ (2.20 – 6.78) | $2.55 \pm 0.80^{c}$ (1.30 – 5.34) | $4.31 \pm 1.71^{a}$ (2.00 – 8.50) | $3.33 \pm 1.22^{b}$ (1.95 – 7.94) | $3.76 \pm 1.61^{ab}$ (1.69 – 7.23) | $3.86 \pm 1.90^{ab}$ (1.30 – 8.27) | $3.37 \pm 1.36^{b}$ (2.04 – 7.30) |
| Staphylococci | $0.26 \pm 0.63^{a}$ (0.00 – 2.77) | $0.17 \pm 0.47^{ab}$ (0.00 – 1.95) | $0.02 \pm 0.15^{b}$ (0.00 – 1.04) | $0.24 \pm 0.67^{a}$ (0.00 – 3.30) | $0.09 \pm 0.35^{ab}$ (0.00 – 1.69) | $0.00 \pm 0.00^{b}$ (0.00 – 0.00) | $0.09 \pm 0.37^{ab}$ (0.00 – 1.95) | $0.15 \pm 0.51^{ab}$ (0.00 – 2.51) |
| Streptococci | $2.24 \pm 1.40^{a}$ (0.00 – 6.23) | $1.44 \pm 1.05^{bc}$ (0.00 – 5.14) | $1.15 \pm 1.07^{c}$ (0.00 – 3.34) | $1.97 \pm 1.83^{ab}$ (0.00 – 8.89) | $1.55 \pm 1.14^{bc}$ (0.00 – 5.04) | $1.50 \pm 1.06^{bc}$ (0.00 – 4.20) | $1.90 \pm 1.24^{ab}$ (0.00 – 5.44) | $1.53 \pm 1.30^{bc}$ (0.00 – 4.85) |
| Thermophilic | $2.18 \pm 0.34^{a}$ (1.47 – 3.14) | $1.94 \pm 0.57^{abc}$ (0.00 – 2.84) | $1.96 \pm 0.91^{abc}$ (0.00 – 5.69) | $1.79 \pm 0.86^{c}$ (0.00 – 3.34) | $2.10 \pm 0.33^{ab}$ (1.39 – 2.77) | $1.88 \pm 0.37^{bc}$ (1.00 – 2.74) | $1.92 \pm 0.56^{bc}$ (0.00 – 3.47) | $2.02 \pm 0.78^{ab}$ (0.00 – 3.50) |
| Yeast and mold | $0.23 \pm 0.95^{ab}$ (0.00 – 6.30) | $0.13 \pm 0.31^{abc}$ (0.00 – 1.11) | $0.01 \pm 0.06^{c}$ (0.00 – 0.30) | $0.29 \pm 0.56^{a}$ (0.00 – 2.11) | $0.22 \pm 0.52^{ab}$ (0.00 – 2.47) | $0.08 \pm 0.31^{abc}$ (0.00 – 1.71) | $0.06 \pm 0.26^{bc}$ (0.00 – 1.34) | $0.06 \pm 0.29^{bc}$ (0.00 – 1.74) |

$^{a,b,c,d}$Means with different superscripts are different ($P < .01$) within rows by LSD.

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*Journal of Food Protection Vol. 40, No. 10, Pages 698-703 (October, 1977)*


# Sporicidal Action of Hydrogen Peroxide on Conidia From Toxigenic Strains of *Aspergillus flavus* and *Aspergillus parasiticus*

## II. Effects of pH, Sucrose, Glucose, and Sodium Chloride

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(Received for publication March 28, 1977)

ABSTRACT

Effects of pH, sucrose, glucose, and sodium chloride on resistance of 14-day-old conidiospores of *Aspergillus parasiticus* NRRL 2999 and 3315, and *Aspergillus flavus* NRRL 3353 to a solution of 6% hydrogen peroxide at 20 C were determined. An increase in time necessary to attain 99.9% destruction of spores resulted when the pH of hydrogen peroxide was adjusted from an initial value of 3.79 to 6.40 and 8.30. However, the amount of the increase was strain-dependent and was directly related to resistance of spores to peroxide. Addition of 10 to 40% sucrose or 3 to 10% sodium chloride to the menstruum caused either an increase or decrease in the time needed to destroy 99.9% of the spore population, depending on the amount of additive that was used. Spores were more difficult to inactivate by peroxide in the presence rather than absence of sucrose or salt, but protection afforded by the additives diminished when their concentration was great. Addition of 10 to 30% glucose elicited a similar response only from spores produced by the most resistant of the three strains tested, whereas resistance of spores from the other strains was not markedly affected.

The sporicidal property of dilute solutions of hydrogen peroxide when used to treat conidiospores of *Aspergillus flavus* and *Aspergillus parasiticus* has been described in a previous paper (*2*). Although hydrogen peroxide proved effective for inactivating *Aspergillus* spores, the instability of the chemical may result in a change in effectiveness when the environment is modified.

Vegetative cells and spores, when produced, of most microorganisms are most resistant to adverse conditions at a neutral or near-neutral pH (*7,10*). Presence of sucrose or glucose in the menstruum during heating of spores or vegetative cells tends to reduce the effectiveness of heat in inactivating the organism, whereas presence of sodium chloride results in less predictable results (*5,9*). Conidia of *A. flavus* and *A. parasiticus*, according to findings of Doyle and Marth (*3,4*), were resistant to inactivation by moist heat in the presence rather than absence of sugar. They noted, however, that the conidia were not necessarily more resistant to heat at a neutral rather than at an acid pH.

Information is lacking on how inactivation of *Aspergillus* conidia by hydrogen peroxide is affected by a change in pH or by the presence of sucrose, glucose, or sodium chloride, all common constituents of many foods. Experiments were done to develop this information which is in this paper.

### MATERIALS AND METHODS

The same strains of aspergilli described in an earlier paper (*2*) were used in this study: *A. parasiticus* NRRL 2999 and 3315, and *A. flavus* NRRL 3353 which produced large, intermediate, and small amounts of aflatoxin, respectively. They were grown and harvested as previously described (*2*). The same procedure as described earlier (*2*) was also adopted for preparation of diluted solutions of hydrogen peroxide and catalase. Furthermore, the same apparatus as used earlier (*2*) was used in these experiments to treat spores with hydrogen peroxide.

Four series of experiments were done: (*a*) effects of pH on sporicidal activities of peroxide, and (*b*), (*c*), and (*d*) dealt with the effects of different amounts of sucrose, glucose, and sodium chloride, respectively, on resistance of conidia to peroxide.

#### *pH*

Use of buffers in this investigation was avoided because the peroxide was acidic. A series of experiments was done to test the effect of pH on the resistance of 14-day-old conidiospores to 6% (wt/vol) peroxide at 20 C. The pH of the peroxide, when measured with a pH meter, was 3.79. However, the pH value seemed to vary slightly with time indicating a constant shift in equilibrium. To achieve uniformity in measurement, the pH reading was always taken 10 min after the electrode was immersed into the peroxide. The peroxide was first adjusted from the initial pH to a near-neutral (6.35) and a basic (8.30) pH by addition of 1 N NaOH, and then the procedure for treatment of spores as previously described (*2*) was followed. A control experiment was done to monitor the pH change, if any, during the course of treatment.

#### *Glucose and sucrose*

Glucose and sucrose were added to the menstruum at concentrations of 10, 20, and 30% (wt/vol). Forty percent (wt/vol) sucrose was also used. A 500-ml Erlenmeyer flask containing the sugar was first autoclaved, and a small amount of 6% (wt/vol) peroxide solution was added to dissolve the sugar. The flask was gently swirled until all the sugar went into solution, and then enough peroxide was added to bring the volume to 90 ml. Fourteen-day-old conidiospores were treated at 20 C with the peroxide as described earlier (*2*).

*Sodium chloride*

Because of the limited solubility of NaCl in aqueous solution and the low concentration of salt in most food products, NaCl at concentrations of 3, 6, and 10% was used in the experiments. Solutions were prepared as previously described for that of sugars, and the conidia were treated similarly.

## RESULTS AND DISCUSSION

The same trend of resistance as described in an earlier paper was shown by conidia of the three strains of *Aspergillus*. Spores of NRRL 3353 were most resistant of the three to peroxide treatment, followed in order by those of NRRL 3315 and 2999. Unlike the straight logarithmic survival curves obtained with thermal inactivation (*3,4*), survival curves obtained in this investigation, though largely logarithmic, were often characterized by a prolonged tailing off. Hence, the time needed for 99.9% reduction is used to describe the rate of spore inactivation.

*Effects of pH*

The possibility that protein oxidation is involved in inactivation of aspergillus spores by hydrogen peroxide has been discussed in an earlier paper. However, the acidic nature of the chemical also may have contributed to the inactivation. The effect of pH on resistance of aspergillus spores to a solution of 6% peroxide at pH 3.79, 6.40, and 8.30 at 20 C was tested. The control showed no appreciable change in pH during the course of the experiment, and use of a buffering system did not seem necessary.

A decrease in inactivation was observed for spores from all three strains of mold when the pH of the menstruum was increased from 3.79 to 6.40. As the pH was further increased to 8.30, resistance of spores of NRRL 3315 decreased slightly (Fig. 1). In spite of the somewhat erratic behavior observed with spores of NRRL 3315, a general trend seemed to exist; an increase in pH was accompanied by reduced inactivation of conidia (Table 1). The amount of the reduction was most pronounced with conidia of NRRL 3353, followed in order by those of NRRL 3315 and 2999.

The phenomenon observed in this investigation can be largely attributed to the sensitivity of hydrogen peroxide to a change in pH. Though a weak acid by itself, presence of additional acid or alkali results in an increase in decomposition rate. Occurrence of the peroxonium ($H_3O_2^+$) and perhydroxyl ($HO_2^-$) ions in acid- and base-catalyzed decomposition, respectively, has been suggested. The decomposition rate of hydrogen peroxide also is at its lowest somewhere between pH 4 and 5 (*8*). Furthermore, the antimicrobial activity of many weak acids, according to Kostenbauder (*7*), is attributed to undissociated acid molecules. The two theories seem to offer a logical explanation for the increase in resistance of conidia when the pH of the menstruum in this experiment was increased from 3.79 to 6.40 and 8.30.

A change in pH, or a change in the proportion of hydrogen to hydroxyl ions, also might have altered resistance of spores. According to Ball and Olson (*1*), death of the bacterial spore is related to the velocity with which molecules and ions move in the environment. They postulated further that the hydrogen ion is more effective than the hydroxyl ion because the former is smaller and has greater speed than the latter. The increased number of hydrogen ions at an acid pH value might have affected the kind of ions adsorbed onto the surface of the spore, and subsequently may have upset the protein structure of the spore wall. Any change in the protein structure is likely to be accompanied by a change in the permeability of the spore which in turn determines the rate at which hydrogen peroxide gains access to the lethal site.

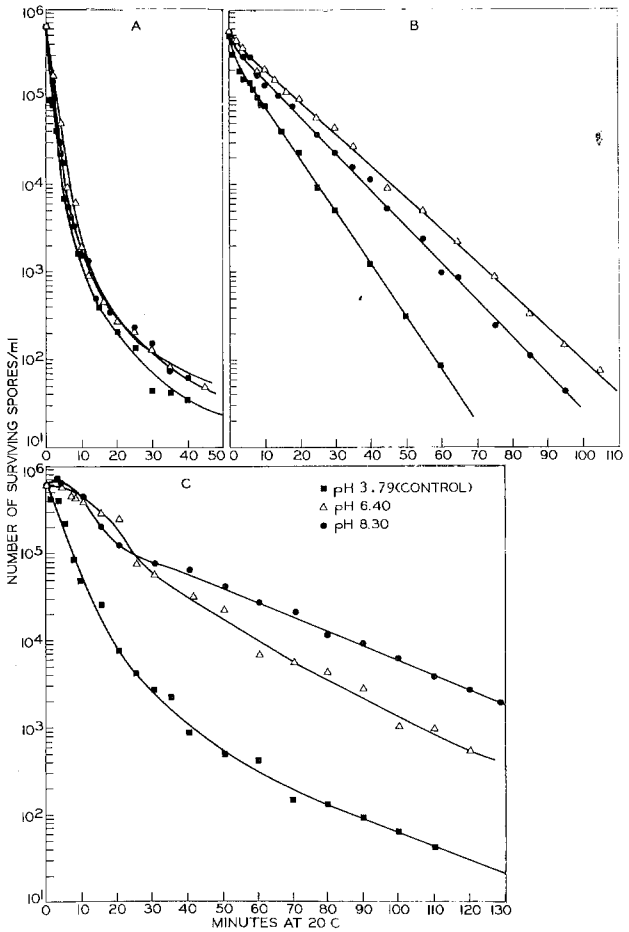


Figure 1. *Inactivation at 20 C of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999 (A), 3315 (B), and* A. flavus *NRRL 3353 (C) and treated with 6% hydrogen peroxide at pH values of 3.79, 6.40, and 8.30.*

TABLE 1. *Time in minutes required for 99.9% reduction in number of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999, 3315, and* A. flavus *NRRL 3353, treated at 20 C with 6% hydrogen peroxide at various pH values*

| pH | Aspergillus strain, NRRL | | |
|---|---|---|---|
| | 2999 | 3315 | 3353 |
| 3.79 | 12.6 | 46.6 | 47.3 |
| 6.40 | 14.8 | 79.9 | >120.0 |
| 8.30 | 15.2 | 68.5 | >120.0 |

However, until there is a better understanding of the physiology of aspergillus spores, an explanation of the effects of pH on resistance to hydrogen peroxide can only be speculative.

*Effect of sucrose*

A series of experiments was done to study the sporicidal activities of 6% hydrogen peroxide at 20 C in the presence of 10, 20, 30, and 40% (wt/vol) sucrose. Unlike the findings of Doyle and Marth (*4*) which showed increasing D values with an increase in sucrose concentration in the heating medium, data from this investigation manifested both the protective and detrimental effects of sucrose on resistance of spores to hydrogen peroxide (Table 2). Resistance of NRRL 2999 spores either remained unchanged or increased in the presence of sucrose, but resistance of NRRL 3353 spores increased at all concentrations of sucrose, when compared to the control. Apparently, spores of NRRL 2999 were insensitive to the presence of sucrose until the concentration was 30%, and their resistance continued to increase as the concentration of sucrose was increased to 40%. The protective effect of the sugar was most pronounced with spores of NRRL 3353, the most resistant strain tested. The time intervals required to inactivate 99.9% of the spores soared from 47.8 min in the absence of sucrose to 98.4 min in the presence of 10% sugar (Fig. 2). Although the protective effect gradually diminished at high sucrose concentrations, time intervals necessary to attain 99.9% reduction, nevertheless, were higher than that of the control. Meanwhile, response of NRRL 3315 spores to hydrogen peroxide in the presence of sucrose was dramatic. Increased sensitivity of spores to hydrogen peroxide was observed in the presence of 10% sucrose. However, a marked increase in the time required to attain 99.9% reduction resulted when 20% sucrose was present. However, the lethality of the peroxide decreased again with a further increase in concentration of sucrose.

Doyle and Marth (*4*) postulated that sucrose protected conidia during inactivation by moist heat by dehydration of the spore and by lowering the water activity in the heating menstruum. However, in the presence of a potent chemical like hydrogen peroxide, effects of the environment on inactivation of the spore are more complicated. Although hydrogen peroxide is a good solvent for organic compounds rich in hydroxyl groups, greater concentration of peroxide is required for equivalent solvent action as compounds increase in molecular weight (*8*). In this experiment no appreciable reaction between sugars in conidiospores and hydrogen peroxide was anticipated because the sugars existed in the form of polysaccharides (*6*) and the concentration of the hydrogen peroxide unless a catalyst, for example, iron is present (*8*). However, since the menstruum was prepared by addition of sucrose to a solution of 6% hydrogen peroxide on a weight/volume basis, the final concentration of the peroxide was no longer 6%, but was in direct proportion to the amount of sucrose added. Hence there are two sets of forces acting in opposite directions; dehydration of spores and lowering of water activity conferred greater resistance, and the concentration of hydrogen peroxide increased and with this the potential to destroy spores. The actual extent of survival observed in the experiments was determined by a balance of the resultant forces. The protective effect of sucrose on

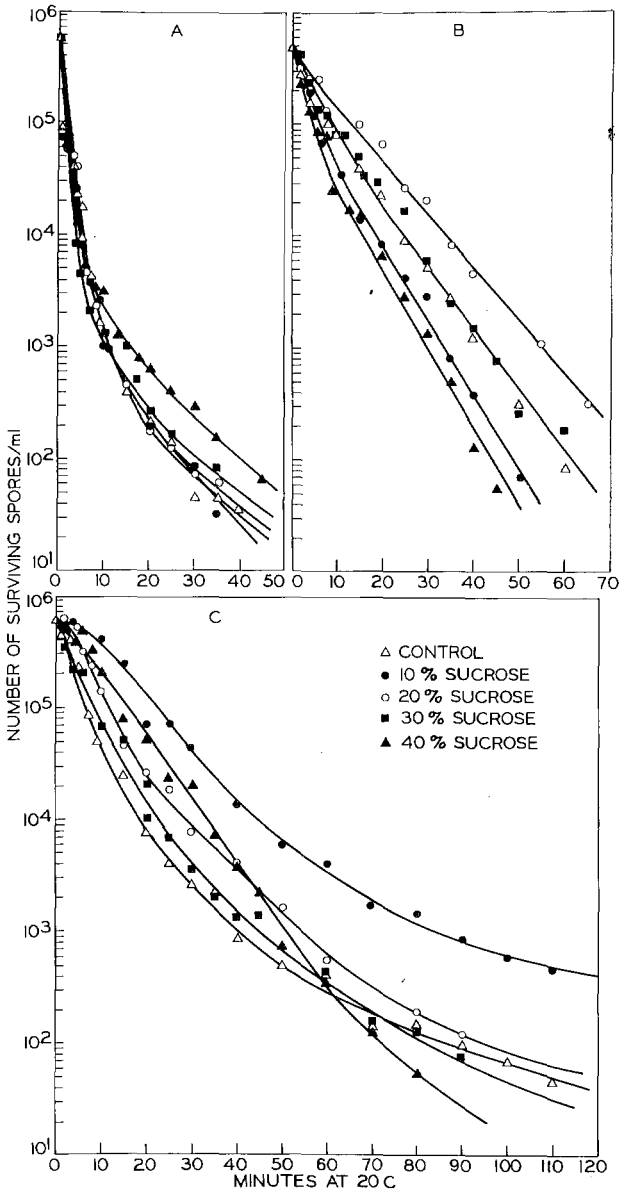


Figure 2. *Inactivation at 20 C of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999 (A), 3315 (B), and* A. flavus *NRRL 3353 (C) and treated with 6% hydrogen peroxide in the presence of 10, 20, 30, and 40% (wt/vol) sucrose.*

TABLE 2. *Time in minutes required for 99.9% reduction in number of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999, 3315, and* A. flavus *NRRL 3353, treated at 20 C with 6% hydrogen peroxide in the presence of different amounts of sucrose*

| Strain of aspergillus | Sucrose concentration, % (wt/vol) | | | | |
|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 |
| 2999 | 13.9 | 13.1 | 13.4 | 15.7 | 21.5 |
| 3315 | 47.3 | 38.5 | 61.5 | 48.8 | 34.4 |
| 3353 | 47.8 | 98.4 | 61.0 | 52.9 | 54.0 |

spores of NRRL 3353, 3315, and 2999 first became evident when 10, 20, and 30% sucrose, respectively was present (Table 2). Spores of NRRL 3353 proved to be the most resistant to peroxide treatment in the absence of sucrose, followed in order by those of NRRL 3315 and 2999. Furthermore, the protective role of sucrose was always evident except for spores of NRRL 3315. Apparently, NRRL 3315 spores were more sensitive to the increased concentration of hydrogen peroxide than to protection afforded by a greater amount of sugar. The different degree of protection provided by sucrose for spores from the three strains of *Aspergillus* also may have been related to structural differences in the spore wall and coat.

*Effects of glucose*

Effects of glucose on resistance of *Aspergillus* spores to hydrogen peroxide were evaluated as described for sucrose. However, 40% (wt/vol) glucose was not used because such a high concentration is only seldom encountered in the food industry. The rate of inactivation in the presence of glucose is represented graphically in Fig. 3, and time intervals for 99.9% destruction are in Table 3. Presence of 10 to 30% (wt/vol) glucose did not elicit any noticeable change in the resistance of NRRL 2999 and 3315 spores to hydrogen peroxide. However, a protective effect was evident when spores of NRRL 3353 were treated. Although time required to attain 99.9% reduction in number of spores increased to a smaller extent than when a comparable amount of sucrose was present (Table 2, 3), the same general trend was evident.

The different amount of protection afforded by sucrose and glucose can be explained in the light of their molecular structures. According to Doyle and Marth (*4*), glucose, a monosaccharide, posses greater water-binding capacity than does a comparable amount of sucrose, a disaccharide. However, the inactivating menstruum in this investigation was prepared with 6% hydrogen peroxide on a weight-volume basis. Hence, the greater the water-binding capacity of the solute, the more hydrogen peroxide would be required to make up the final volume. In other words, the final concentration of the chemical was higher in the presence of glucose than if an equivalent amount of sucrose was added. Like sucrose, glucose was not expected to react with the peroxide (*8*). As long as the two sets of antagonistic forces previously discussed operated when glucose was present, an increase in hydrogen concentration would be followed by an increase in the chemical's potential to inactive the spores. The two forces seemed to balance each other when NRRL 2999 and 3315 were treated. However, when spores of the most resistant strain, NRRL 3353, were treated, protection that resulted could have been caused by spore dehydration and lowering of water activity. This overcame the deleterious effects caused by the increasing concentration of peroxide. It is believed the greater water-binding capacity of glucose was responsible for reduced protection provided the aspergillus spores, as compared to that afforded by sucrose.

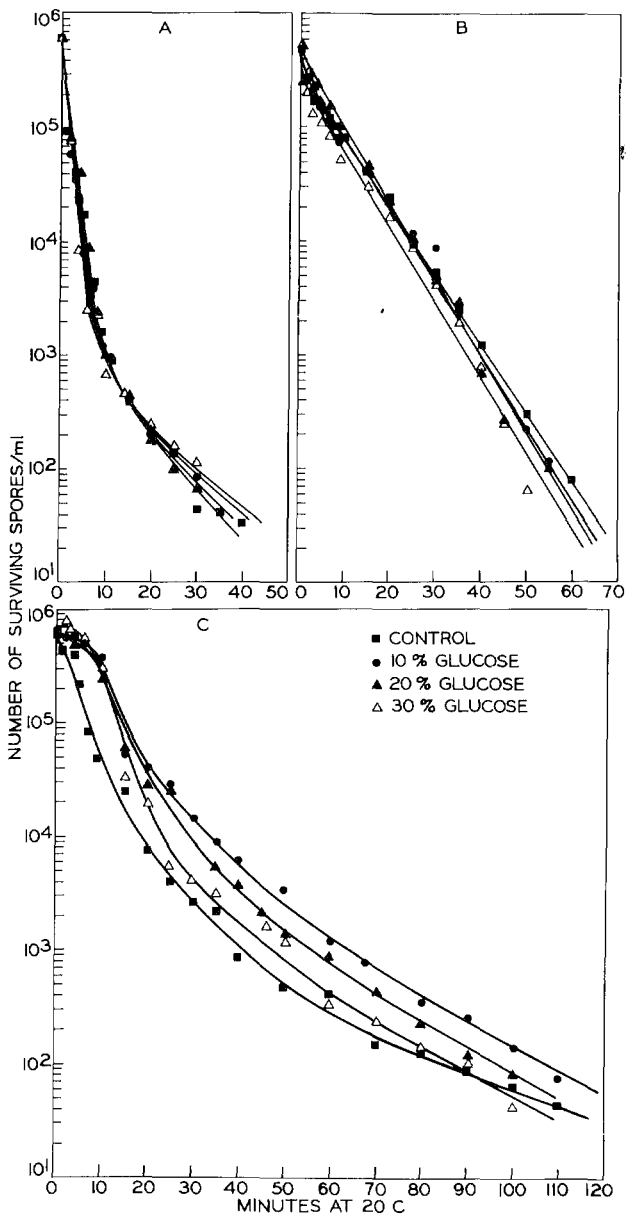


Figure 3. *Inactivation at 20 C of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999 (A), 3315 (B), and* A. flavus *NRRL 3353 (C) and treated with 6% hydrogen peroxide in the presence of 10, 20, and 30% (wt/vol) glucose.*

TABLE 3. *Time in minutes required for 99.9% reduction in number of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999, 3315, and* A. flavus *NRRL 3353, treated with 6% hydrogen peroxide at 20 C and at different glucose concentrations*

| Strain of aspergillus | Glucose concentration % (wt/vol) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| 2999 | 13.9 | 13.9 | 14.3 | 13.8 |
| 3315 | 47.5 | 45.2 | 45.1 | 42.3 |
| 3353 | 47.8 | 72.4 | 63.0 | 53.2 |

*Effects of NaCl*

The influence of NaCl on treatment of aspergillus spores with hydrogen peroxide was evaluated in a manner similar to that described for the sugars. Owing to the relatively small amount of salt in most food, 3, 6, and 10% (wt/vol) salt solutions were prepared with 6%

hydrogen peroxide for treatment of spores.

Data in Fig. 4 demonstrate the protective function of NaCl. Furthermore, NaCl also seemed to be more favorable for survival of spores than was either sucrose or glucose (Table 2,3,4). Once again, protection afforded by the presence of NaCl was most pronounced when spores of NRRL 3353 were treated. Presence of 3% NaCl in the menstruum resulted in a 60% increase in time needed for 99.9% destruction of spores of NRRL 3353. Addition of more NaCl caused a slight further increase in the resistance of spores.

NaCl in aqueous solution exists as an ionic entity. In addition to its osmotic properties, it also exerts an ionic influence on its surrounding. Like sucrose and glucose, it may have rendered the spores less susceptible to inactivation by hydrogen peroxide by dehydrating them and by lowering the water activity of the inactivating menstruum. But unlike the sugars, it dissociates into $Na^+$ and $Cl^-$ ions. Even though the reaction of NaCl and hydrogen peroxide is unknown, there are reasons to believe that the reaction was of no great concern in this investigation. First, dissociation of hydrogen peroxide into $HO_2^-$ and $H^+$ ions is negligible in dilute solutions (*8*). Second, an amino acid tends to have a net charge of zero in a slightly acidic pH and the 6% hydrogen peroxide used in this investigation had a pH value of 3.79. Consequently, neither protein destabilization nor change in ionic balance was likely to play a major role in treatment of spores. However, the two sets of opposing forces previously described for hydrogen peroxide-sugar treatment of spores probably operated when NaCl was used, and they may have been largely responsible for the trends observed in the experiments.

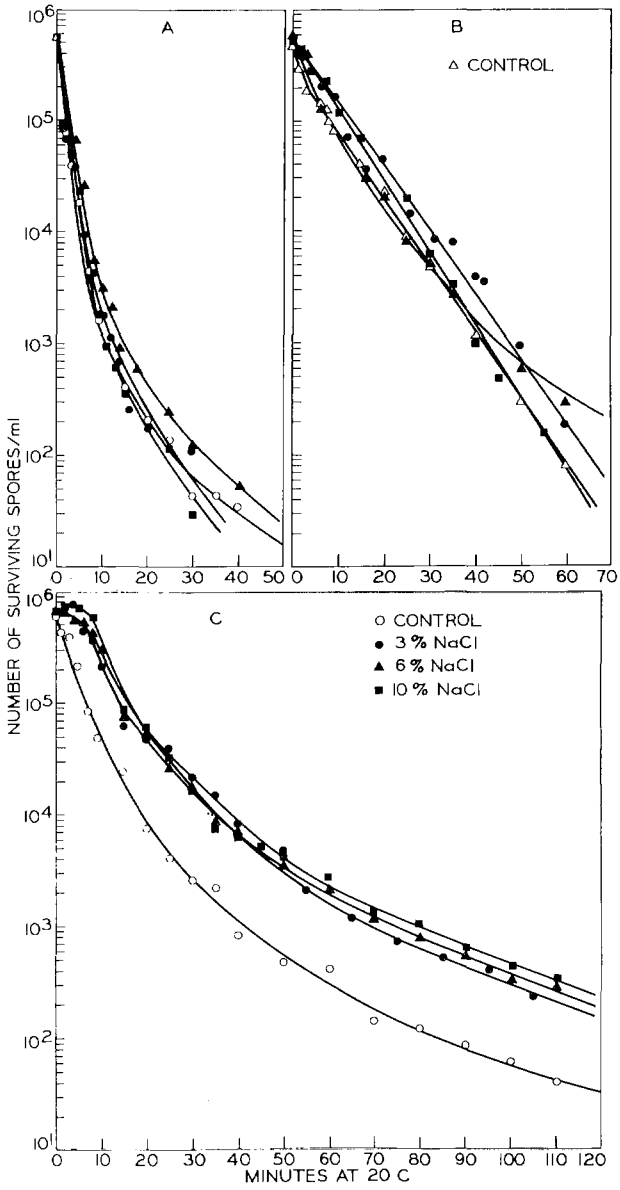


Figure 4. *Inactivation at 20 C of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999 (A), 3315 (b), and* A. flavus *NRRL 3353 (C) and treated with 6% hydrogen peroxide in the presence of 3, 6, and 10% (wt/vol) NaCl.*

TABLE 4. *Time in minutes required for 99.9% reduction of conidiospores harvested from 14-day-old cultures of* A. parasiticus *NRRL 2999, 3315, and* A. flavus *NRRL 3353, treated at 20 C with 6% hydrogen peroxide at different concentrations of sodium chloride.*

| Strain of aspergillus | NaCl concentration % (wt/vol) | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 10 |
| 2999 | 13.9 | 15.7 | 18.9 | 13.6 |
| 3315 | 47.5 | 52.2 | 52.0 | 47.2 |
| 3353 | 47.8 | 78.4 | 85.0 | 90.0 |

As evidenced by data in this and a previous paper (*2*), hydrogen peroxide is an effective sporicidal agent against conidia of toxigenic aspergilli. The low cost of peroxide, and the absence of residual odor make it useful as a chemical sterilant for items, such as packaging materials, that might contain fungal spores. Additional work is needed to determine if peroxide is useful in decontaminating natural products that might contain conidia of aspergilli.

ACKNOWLEDGMENT

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison.

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*Journal of Food Protection Vol. 40, No. 10, Pages 704-708 (October, 1977)*


# A Study of Coagulase-Positive Staphylococci in Salami Before Fermentation

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(Received for publication March 17, 1977)

ABSTRACT

Thirty-six samples of salami formulation were examined for coagulase-positive (CP) staphylococci. A statistical analysis (Analysis of Covariance) was also performed to evaluate six variables and their effects on the dependent variable Y (number of CP staphylococci/g). The six variables evaluated were: (*a*) certification-non-certification, (*b*) daily sample order, (*c*) proportion of meat (pork shoulders, pork jowls, and beef), and (*d*) total plate count. The average coagulase-positive staphylococcal count was determined to be 3400/g (range 225 to 17000). Of the determinable variables, only certification (avg. 500 CP staphylococci/g) versus non-certification (avg. 2100 CP staphylococci/g) was statistically significant. The additional freezing at -10 F (-24 C) for 10 days for trichina certification appeared to reduce the number of viable staphylococci.

Although staphylococcal food poisoning is one of the most commonly reported foodborne illnesses in the United States (*9*), it has rarely been identified as a problem in the manufacture of dry and semi-dry sausage products. However, in recent years a number of staphylococcal food poisoning outbreaks due to consumption of Genoa and Italian dry salami were reported by the Center for Disease Control (*4,5,6*). Analysis of plant samples revealed from 1000 to 100,000 coagulase-positive staphylococci/g and the food poisoning samples contained up to 3,000,000/g. Entertoxin A was recovered from implicated salami samples (*4,5*).

In seeking an explanation for the presence, multiplication, and enterotoxin formation by staphylococci, some facts become apparent. Raw meat used to manufacture sausage may contain large numbers of staphylococci, i.e., pork jowls and cheeks. Also, Genoa and Italian dry salami are fermented meat products dependent on proliferation of lactic acid-type bacteria for flavor and texture. Since lactic acid bacteria are competitiors against staphylococci, it is essential that these fermenting organisms be present in suitable numbers. The combination of a large initial staphylococcal inoculum and reduced numbers of competitive lactic acid bacteria seems to explain these above mentioned outbreaks (*10*).

In the aftermath of the Genoa salami outbreaks, the U. S. Department of Agriculture became interested in the presence of staphylococci in fermented meat products. A letter was sent to manufacturers of dry sausage by the Field Operations Division, Meat and Poultry Inspection Program, APHIS, USDA, requesting information on 10 specific points. One of the 10 questions concerned the usual maximum number of coagulase-positive staphylococci present in the sausage product (*12*).

Requirements for production of staphylococcal enterotoxin lies in one or more factors: (*a*) high initial inoculum of *Staphylococcus aureus,* (*b*) low inoculum of starter culture (lactic acid producers), (*c*) high pH of sausages, (*d*) high water activity ($a_w$), and (*e*) low salt concentration. Since finished Italian dry salami usually has the following composition: pH 4.30-5.15, salt in meat 4.4-8.75%, salt in brine 10.5-21.0%, water 29-41%, and $a_w$ 0.85-0.92 (*10*), the critical time for staphylococcal proliferation and enterotoxin production occurs during the early stages of sausage fermentation when the meat environment is less inhibitory to *S. aureus.*

The sources of contamination for meat products with *S. aureus* are at least four-fold (*a*) environment, (*b*) equipment, (*c*) raw materials, and (*d*) plant personnel. With the approval of a large dry salami manufacturer in San Francisco, it was decided to analyze the raw meat product to answer, in part, one of the 10 questions posed by USDA. The objective was to determine the number of coagulase-positive staphylococci present in sausage production before fermentation and to determine the relationship of several variables on this staphylococcal count.

## MATERIALS AND METHODS

### *Sampling Procedures*

The manufacturer produced 34,200 lb. of salami a day to be fermented. This daily production is usually divided into six distinct lots (5700 lb. per lot).

Each lot is formulated on percentage fat desired in the packaged product, but not to exceed 30% maximum fat, as required by Federal regulations (*19*). Fat content is controlled by the proportion of pork shoulders, pork jowls, and beef necessary to acquire the percentage of fat specified.

The frozen pork jowls and pork picnics (temperature -10 to 0F) are passed through a hydroflaker and then conveyed to a large mixer which holds one full lot. The fresh beef (temperatures 34-36 F) is ground and then added to the mixer which contains the pork jowls and picnics. After thorough mixing of the pork and beef, the non-meat ingredients are added (salt, spices, sugar, finished salami starter culture) and mixed.

The sausage formulation is then carried by a screw-type conveyor to a mechanical tub which transfers the mixture in 250-lb. lots to the chopper. It requires about 24 tubs to empty one lot or mixer full of sausage formulation.

By tubsfull the sausage mixture is thoroughly chopped for about 1 min. It is at this point that sampling was initiated. The temperature of the chopped sausage at the time of sampling was 18-20 F.

The sampling procedure used required the collection aseptically of three sub-samples (usually the first, middle, and last tub of each batch) to be thoroughly mixed by hand. Separate disposable gloves were used at each collection interval. A composite sample weighing 100 g was chosen to represent the one lot. The 100-g sample was then placed in a sterile mason jar, refrigerated in a styrofoam ice chest, and transported with the other five samples to the laboratory for analyses. This research report, therefore, encompasses 36 samples (100 g each) representing six lots for each of six weekly sampling periods.

### *Sample preparation*

Upon arrival at the labortory, sample preparation was immediately begun. To each of the six mason jars containing sausage samples 200 ml of sterile 0.1% Triton X-100 solution (Rohm and Haas, Philadelphia) was added.

A pre-sterilized blender blade was placed in each jar and the sample diluent mixtures were blended for 3 min. The 1:3 dilution was diluted further in three-fold series in saline.

### *Total plate count*

From each dilution, 0.2 ml was pipetted and plated on Plate Count Agar Medium (PCA, Difco, Detroit, Mich.) induplicate. The plates were incubated at 37 C with observations made at 24 and 36 h. A total plate count was made after 24 h of incubation and the average count/g of sausage was estimated for each sample.

### *Staphylococccal counts*

Bacto-TPEY (Difco), prepared as recommended by the manufacturer, was chosen as the selective medium for staphylococci. Plating was similar to that described for the total plate count. After incubation for 48 h at 37 C each plate was examined for coagulase-positive staphylococci as recommended (*2*).

### *Coagulase agar test*

The coagulase agar plating medium consisted of 2.5% bovine fibrinogen, 0.0015% soybean trypsin inhibitor, EDTA-treated rabbit plasma (Difco), 3.7% brain heart infusion broth, and 1.5% agar.

A 4.5% bovine fibrinogen solution was prepared by dissolving bovine fibrogen, citrated, fraction I (Sigma Chemical Co., St. Louis, MO) in saline. The solution was filtered through Whatman #1 paper in a Buchner funnel and sterilized through a Seitz funnel (1.0 $\mu$m). The 25 ml of the sterile fibrinogen solution were added to 460 ml of molten, autoclaved brain heart infusion agar and maintained in a 52 C water bath.

Seven and a half mg of soybean trypsin inhibitor (Schwarz-Mann, Orangeburg, N.Y.) were dissolved in 20 ml of EDTA-treated rabbit plasma (Difco) and sterilized by filtration (Millipore filter, 0.22-$\mu$ pores). The filtrate (approximately 5 ml was absorbed during filtration) was added to molten 52 C brain heart infusion agar already containing sterile bovine fibrinogen. The coagulase plating agar mixture was shaken and dispensed in 12-ml amounts each to Petri plates.

Using sterile toothpicks, colonies fitting the description for coagulase-positive stpahylococcus plus randomly selected colonies were transferred from the Bacto-TPEY plates (after 48 h of incubation) to the coagulase plate. Twenty-five colonies per plate were tested. After incubation for 12-18 h at 37 C, the plate was examined and colonies with an opalescent halo were considered as coagulase-positive.

### *Coagulase tube test*

Bacto-Coagulase Plasma EDTA (Difco) was used following the procedure recommended by the manufacturer.

### *Nuclease*

A colony overlay procedure was used (*14*). Six ml of a molten Toluidine Blue-DNA Agar (TDA) mixture was placed over the surface of the coagulase agar plate. After 3 h of incubation (37 C), the plate was read. Nuclease-positive colonies were identified by a bright pink halo surrounding each colony.

### *pH Determination*

Using a Beckman Expandomatic pH meter (Beckman Instruments, Inc., Fullerton, Calif.), a pH reading was taken on the 1:3 dilution for each of the 36 samples.

### *Statistical analysis*

The BMDO3R computer program (multiple regression with case combinations) was used to analyze the data (*8*). The BMDO3R program was modified to an analysis of covariance (ANOCOVAR) which combines the features of analysis of variance and regression more suitable for the statistical analysis (*17*).

## RESULTS AND DISCUSSION

Ten of the 36 samples did not contain detectable coagulase-positive staphylococci. Since the dilution chosen would detect a minimum of 225 staphylococci/g, it was decided to give these 10 samples values of staphylococcal counts <225. A histogram was plotted on semi-logarithm paper using total coagulase-positive staphylococcal count and total plate count for each of the 36 samples. The histogram indicated a near logarithmic normal distribution for both counts and therefore normal logarithm values for these counts were used in statistical analysis.

Statistical analysis of data (Table 1) was accomplished in three distinct phases. Phase I involved a screening process using the analysis of covariance with four concomitant variables (proportion pork shoulders, pork jowls and beef and total plate count). The two-way analysis of variance evaluated certification versus non-certification and the ordering of each lot per day. The linear regression evaluated the effects of four independent variables (proportion of pork picnics, pork jowls, beef and total plate count). There was a non-significant F value for regression of Y on variables considered ($a = 0.05$).

Phase II involved a restructuring of the data using the variables: non-certification versus certification and proportion of picnics, jowls, and beef. A one-way classification and three auxiliary variables, $X_{1ij}$, $X_{2ij}$, and $X_{3ij}$, all three related to $Y_{ij}$, were used to form the model (17):

$$Y_{ij} = \mu_i + B_1(X_{1ij} - \overline{X}_{1..}) + B_2(X_{2ij} - \overline{X}_{2..}) + B_3(X_{3ij} - \overline{X}_{3..}) + \Sigma_{ij}.$$

TABLE 1. *Coagulase-positive staphylococcal count and several determinable variables*

| Sample No. | Daily order | Certified or non-certified | pH | Total plate count | Total coagulase + staphy |
|---|---|---|---|---|---|
| 1 | 1 | C | 5.75 | $2.7 \times 10^6$ | < 225* |
| 2 | 2 | C | 5.85 | $2.5 \times 10^6$ | 5625 |
| 3 | 3 | NC | 5.80 | $1.5 \times 10^6$ | < 225* |
| 4 | 4 | NC | 5.90 | $1.6 \times 10^6$ | 900 |
| 5 | 5 | NC | 6.00 | $1.2 \times 10^6$ | 1125 |
| 6 | 6 | NC | 5.85 | $1.8 \times 10^6$ | < 225* |
| 7 | 1 | C | 5.90 | $2.1 \times 10^6$ | 5625 |
| 8 | 2 | C | 5.90 | $1.3 \times 10^6$ | 225 |
| 9 | 3 | NC | 5.90 | $3.0 \times 10^6$ | 1125 |
| 10 | 4 | NC | 5.90 | $3.2 \times 10^6$ | 3375 |
| 11 | 5 | NC | 5.85 | $2.1 \times 10^6$ | 1125 |
| 12 | 6 | NC | 5.85 | $2.1 \times 10^6$ | 5625 |
| 13 | 1 | C | 5.85 | $1.5 \times 10^6$ | < 225* |
| 14 | 2 | NC | 5.90 | $1.3 \times 10^6$ | 5625 |
| 15 | 3 | NC | 5.90 | $2.3 \times 10^6$ | 10,125 |
| 16 | 4 | NC | 5.85 | $4.0 \times 10^6$ | 11,250 |
| 17 | 5 | NC | 5.85 | $1.6 \times 10^6$ | 2250 |
| 18 | 6 | NC | 5.80 | $1.4 \times 10^6$ | 1125 |
| 19 | 1 | C | 5.90 | $1.4 \times 10^6$ | < 225* |
| 20 | 2 | NC | 5.90 | $1.3 \times 10^6$ | 1125 |
| 21 | 3 | NC | 5.90 | $2.1 \times 10^6$ | 11,250 |
| 22 | 4 | NC | 5.80 | $1.4 \times 10^6$ | 6750 |
| 23 | 5 | NC | 5.90 | $8.2 \times 10^5$ | 2250 |
| 24 | 6 | NC | 5.95 | $1.7 \times 10^6$ | 4500 |
| 25 | 1 | C | 5.90 | $7.9 \times 10^5$ | < 225* |
| 26 | 2 | C | 5.80 | $1.9 \times 10^6$ | < 225* |
| 27 | 3 | NC | 5.80 | $1.5 \times 10^6$ | < 225* |
| 28 | 4 | NC | 5.80 | $1.5 \times 10^6$ | 5625 |
| 29 | 5 | NC | 5.75 | $1.3 \times 10^6$ | 11,250 |
| 30 | 6 | NC | 5.75 | $1.3 \times 10^6$ | 16,875 |
| 31 | 1 | C | 6.00 | $8.3 \times 10^5$ | < 225* |
| 32 | 2 | C | 5.95 | $7.9 \times 10^5$ | 1125 |
| 33 | 3 | NC | 5.95 | $8.2 \times 10^5$ | 3375 |
| 34 | 4 | NC | 6.00 | $1.2 \times 10^6$ | 1125 |
| 35 | 5 | NC | 5.95 | $1.1 \times 10^6$ | 1125 |
| 36 | 6 | NC | 5.95 | $7.1 \times 10^5$ | < 225* |

* *Denotes 10 samples which had staphylococci counts less than 225/g.*

The one-way analysis of variance portion (certification versus non-certification) provides an F ratio of 5.7507 which is significant at the $a = 0.05$ level of significance ($F_{0.95,1,35} = 4.12$). The linear regression portion ($X_1$ = proportion of picnics, $X_2$ = proportion of jowls, and $X_3$ = proportion of beef) provided an F ratio of 1.4148 which is not significant at $a = 0.05$ ($F_{0.95,3,35}$) =2.88).

Phase III involves a one-way analysis of variance (ANOVA) using certification, non-certification and total coagulase-positive staphylococcal count (Table 2). The calculated F ratio 8.1472 is greater than the tabulated F ratio ($a = 0.05, 1, 34 = 4.13$) which indicates a significant difference in the staphylococcal counts of the certified versus the non-certified. The certified lots (10) averaged 450 coagulase-positive staphylococci/g. Also in six of these 10 samples, no staphylococci were detectable. The non-certified lots (*26*) averaged 2100/g with four samples lacking detectable levels of staphylococci.

TABLE 2. *Coagualse-positive staphylococcal count-certification versus non-certification*

| Certified (10 lots) | | Non-Certified (26 lots) | |
|---|---|---|---|
| No. lots | CP Staph count | No. lots | CP Staph count |
| 7 | < 225 | 3 | < 225 |
| 1 | 1125 | 1 | 225 |
| 2 | 5625 | 1 | 900 |
| | | 7 | 1125 |
| | | 2 | 2250 |
| | | 2 | 3375 |
| | | 1 | 4500 |
| | | 3 | 5625 |
| | | 1 | 6750 |
| | | 1 | 10,125 |
| | | 3 | 11,250 |
| | | 1 | 16,875 |

Because of the anticipated low numbers of coagulase-positive staphylococci, the recommendation for sample preparation by the International Committee on Microbiological Specifications for Foods (ICMSF) had to be modified and three-fold instead of 10-fold dilutions of samples were used for plating (*18*).

Also of importance was the selective medium to be used. The ICMSF recommended use of one of five selective media: (*a*) Baird-Parker medium, (*b*) Egg Yolk Azide Agar, (*c*) Salt Egg Yolk Agar, (*d*) Tellurite-Polymyxin Egg Yolk Agar (TPEY, Difco), and (*e*) Tellurite-glycine medium (*18*). Virtually all these selective media will inhibit to some extent the growth of staphylococci in a debilitated state (*15*). It was

determined that a selective medium like Bacto-TPEY containing egg yolk would be most useful for this study since the clearing of egg yolk as the diagnostic reaction is quite reliable and the medium was designed for optimal recovery of coagulase-positive staphylococci in foods (*7*). Also, this medium can detect small numbers of *S. aureus* when competitive organisms are present and is suitable for analysis of frozen foods (*3*). TPEY has been reported to recover 74% of the staphylococci from mixed cultures (*15*).

Previous reports indicate that up to 95% of coagulase-positive strains produce deoxyribonuclease, whereas 96% of the coagulase-negative strains do not produce nuclease (*1*). Coagulase reaction and nuclease production correlated 100% in this study. On one occasion, nine colonies were coagulase-negative but nuclease-positive. Transfer of these colonies to BHI broth, incubation for 24 h (37 C), and determination by the coagulase tube test revealed that they were indeed coagulase-positive. The colony overlay procedure proved useful in screening for weakly coagulase-positive *S. aureus* and thus indicated that a tube test was required.

The average coagulase-positive staphylococcal count from the 36 samples was 3400/g with a range from less than 225 to 17,000/g. The public health significance of 17,000 staphylococci/g of salami paste cannot be evaluated with certainty at this time. Such an evaluation would require additional data on the minimum number of staphylococci needed to overcome the inhibitory and competitive effect of the salami (pH, salt, nitrite, starter culture, water activity) to initiate growth and toxin production. The number present was much lower than that required to cause food poisoning (> 500,000/g) and indicated that the raw meat products plus ingredients were low in initial staphylococcal contamination.

The total plate count averaged $1.7 \times 10^6$/g (range $7.2 \times 10^5$ to $4.0 \times 10^6$). Since intentional addition of lactic acid bacteria in the salamis starter is less than $3.0 \times 10^5$/g paste, the total bacterial count should be considered rather low.

Certification implied a lower coagulase-positive staphylococcal count. Pork meat products eligible for certification for trichina must be frozen to -10 F (-24 C) for 10 days (*11*). The noncertified pork meat was frozen to 0 F. Upon freezing, staphylococcal counts decrease by one to two logs. After this initial decrease they die slowly with time (*13*). The rate of dying decreases as the temperature of freezing decreases. Thus there is a faster death rate at -11 C than at -30 C storage (*20*). On the basis of these data one may expect better staphylococcal survival in the certified pork, which we did not observe. Possibly lowering the temperature from 0 F to -10 F may explain in the lower numbers of staphylococci observed in the certified pork.

Although it has been stated that pork head meat (jowls, cheeks) may contribute staphylococci, we were unable to find a significant influence upon total staphylococcal count when statistically analyzing the proportion of pork jowls, pork shoulders, and beef. However, the proportions did not vary a great deal; therefore, it was difficult to preclude the contribution of pork jowls without a more extensive study. In future studies it would be imperative to evaluate each of the raw ingredients separately. Such an evaluation of each separate ingredient would substantiate the contribution of each additive (proportion of jowls, shoulders, beef, spices, etc.) to the overall staphylococcal count of the product.

ACKNOWLEDGMENTS

The authors express their gratitude to L.P. Lotter and Bob Tarone for their technical assistance.

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## *Epidemiologic Excerpts*

### Presumed Staphylococcal Food Poisoning Associated With Whipped Butter

Whipped butter produced by a single manufacturing plant in Kentucky has been implicated in a multi-state outbreak of food poisoning which began the last week of July. The illness was characterized by nausea, vomiting, abdominal cramps, diarrhea, and prostration within 4-6 hours of ingestion—symptoms compatible with staphylococcal food poisoning.

Over 100 cases, including several that required hospitalization, were reported to state officials in Illinois, Indiana, Kentucky, Ohio, and Missouri. Most of these cases were associated with restaurants that had received shipments of 16-pound containers of butter produced by the Sugar Creek Division of Beatrice Foods Company on June 28 and June 30. The company also produces consumer-size packages, distributed under a number of different brand names in at least 18 states; in Indiana, Ohio, and West Virginia, 4 small outbreaks have been associated with packages of this size. The other 15 states to which the consumer-size packages were distributed: Arkansas, Florida, Georgia, Illinois, Kentucky, Louisiana, Michigan, Mississippi, Missouri, New Mexico, North Carolina, Oklahoma, Tennessee, Texas, and Virginia. The following labels are involved: Sugar Creek, Prairie Farm, A&P, Armour, Chappel, Mayflower, Blue Valley, Meadowgold, Lucerne, Coleman, and Kountry Fresh.

On August 4 the manufacturer closed the plant, and the following day voluntarily recalled all whipped butter produced by the plant from June 21 through August 4. On August 9 the manufacturer voluntarily recalled all remaining whipped butter produced before June 21. The recalled butter bears lot numbers coded with the first 3 numbers of 216 or below (on the 16-pound containers) or the "pull date" of September 12, 1977, or before (on the consumer-size packages). *Staphylococcus aureus* organisms in counts up to $10^7$/gm have been isolated from lots of whipped butter produced between June 28 and August 3. A sample of butter produced on June 21 showed no growth of the organisms. Enterotoxin studies are pending.

The plant remains closed, and investigations are continuing to determine the source of contamination.

The magnitude of this outbreak is difficult to ascertain because some of the contaminated whipped butter was distributed in consumer-size packages. Illness in persons who ate whipped butter from such packages would appear as isolated incidents that would not be as likely to be reported as restaurant-associated outbreaks.

Staphylococcal contamination of butter is rare because the high lipid concentration in butter is not conducive to growth of the organism. Previous staphylococcal outbreaks attributed to butter have usually involved products to which higher protein foods, such as milk, had been added.

*Journal of Food Protection Vol. 40, No. 10, Pages 709-711 (October, 1977)*


*A Research Note*

# Evaluation of Four ONPG Tests for *Enterobacteriaceae* from Human, Animal and Selected Food Sources

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ABSTRACT

An ortho-nitrophenyl-β-D-galactopyranoside (ONPG) test was done on 250 *Enterobacteriaceae* from human, poultry, and selected food sources by use of four commercially available miniaturized systems and by the conventional test for ONPG. For the 102 cultures from human and poultry sources, all four systems agreed with the conventional test as follows: API (98%), Difco (94.1%) Minitek (98%), and Pathotec (98%). For the 148 food isolates, the percent agreement between the conventional and these four systems was significantly lower, API (87.2%), Difco (85.8%), Minitek (88.5%), and Pathotec (85.8%).

Two enzymes are required for fermentation of lactose by *Enterobacteriaceae,* a permease which enables lactose to enter the cell and β-galactosidase, which attacks the β-galactoside link and hydrolyzes the lactose to glucose and galactose. If an organism lacks either enzyme it will not ferment lactose. If an organism lacks the permease, the presence of the β-galactosidase can be demonstrated by use of ortho-nitrophenyl-β-D-galactopyranoside (ONPG) as the substrate. When ONPG is attacked by β-galactosidase, o-nitrophenol (a yellow substance) is released and indicates the presence of the enzyme (*7*).

The ONPG test was introduced by LeMinor and Ben Hamida (*8*) and by Lowe (*9*) for detecting β-galactosidase in members of the *Enterobacteriaceae* family. Others (*1, 8, 9*) have emphasized the marked sensitivity of the ONPG test, relative to lactose fermentation for detecting β-galactosidase. The ONPG test, however, is not a replacement for the lactose fermentation test, because in some instances in which acid is produced by the lactose fermentation, the ONPG test could be negative because some bacteria oxidize lactose to lactobionic acid instead of splitting the β-galactoside bond. Also some organisms, e.g., certain serotypes of *Shigella dysenteriae,* are ONPG-positive but routinely lactose-negative (*14*).

The ONPG test is important for routine use in the differentiation of many members of the *Enterobacteriaceae* family. The ONPG test and polyvalent fagellar antiserum can be used together in a simplified screening procedure for *Salmonella* (*15*). The ONPG test was of value in differentiating *Salmonella* from *Arizona* cultures (*10*) and in the separation of *Citrobacter* and *Salmonella* (*12*).

The objective of this study was to compare the efficacy of four commercially available tests for ONPG and the conventional ONPG test with *Enterobacteriaceae* isolates from poultry, humans, and selected foods.

## MATERIALS AND METHODS

A total of 250 *Enterobacteriaceae* recently isolated and identified was used in this study: *Arizona* (9 strains), *Citrobacter* (11 strains), *Edwardsiella* (4 strains), *Enterobacter* (56 strains), *Escherichia* (55 strains), *Klebsiella* (21 strains), *Proteus* (18 strains), *Salmonella* (28 strains), *Serratia* (32 strains), *Shigella* (10 strains), and *Yersinia* (6 strains). Of these cultures 36 of poultry origin were from Dr. J. E. Williams, Southeast Poultry Research Laboratory, Athens, GA; 66 were of human origin from the Center for Disease Control, U.S.D.H.E.W., Atlanta, Georgia (Dr. D. J. Brenner and Ms. Alma Murlin); 148 were isolated and identified in this laboratory from retail samples of foods [broiler carcasses, ground beef, frozen chicken comminuted meat, frozen chicken pot pie, and pork sausage, (*2*)]. Each culture was assigned a code number; identity was unknown until the completion of the study. A loopful of each stock culture was inoculated into 5 ml of Brain Heart Infusion (BHI) broth (Difco) and incubated at 37 C for 24 h, then streaked on a BHI slant and incubated at 37 C for 24 h. Then they were stabbed and streaked on slants of Triple Sugar Iron (TSI) agar (Difco) and incubated 24 h at 37 C. Each culture was then tested by five systems for determination of β-galactosidase activity. These systems were (*a*) API-20E, Analytab Products Inc., (*b*) Bacto Differentiation Disks ONPG, Difco Laboratories, (*c*) Minitek ONPG Disc. BBL, Division of Becton, Dickinson and Company, (*d*) Pathotec ONPG, General Diagnostics Division of Warner-Lambert Company, and (*e*) the conventional ONPG test (*4*). Manufacturer's instructions were followed exactly without change or modification. Tests were repeated when results conflicted.

## RESULTS AND DISCUSSION

Results of this comparative study are shown in Table 1. All of the systems showed excellent agreement with the conventional test with the 102 cultures not isolated from food. Agreement was 98% with the API, Minitek, and Pathotec tests, and 94.1% with the Difco test. Other investigators reported similar findings with the ONPG test with clinical isolates: Minitek, 97.5% (*5*) and 100% (*6*) and Pathotec, 97% (*13*) and 100% (*3*). The six discrepancies encountered with the Difco disks all occurred with strains of *Yersinia enterocolitica.* The 24-h conventional tests were positive while the 4-h Difco test was negative with these six strains.

For the 148 food isolates, the percent agreement with the conventional test was 85-88% for the four systems.

TABLE 1. *Percent of agreement of four commercial ONPG tests with conventional procedure for* Enterobacteriaceae *from various sources*

| System | Time of incubation (h) | Isolated from human or animal specimens | Isolated from various foods | Overall |
|---|---|---|---|---|
| API | 24 | 98.0% (100/102)[a] | 87.2% (129/148)[b] | 91.6% (229/250) |
| Difco | 4 | 94.1% (96/102) | 85.8% (127/148) | 89.2% (223/250) |
| Minitek | 24 | 98.0% (100/102) | 88.5% (131/148) | 92.4% (231/250) |
| Pathotec | 4 | 98.0% (100/102) | 85.8% (127/148) | 90.8% (227/250) |

[a]Number in agreement over number tested.

[b]Agreement with the conventional test for all four systems was significantly ($P < 0.05$) lower for food isolates than for isolates from human or animal specimens.

When the proportions obtained with non-food and with food isolates were compared by use of the normal approximation to the binomial distribution (*11*), agreement with the conventional test of food isolates was significantly less than agreement with the isolates of either human or animal origin. The discrepancies observed with the food isolates seemed to follow a pattern: With the Difco and Pathotec tests, most of the disagreements (95% [20/21] and 62% [13/21], respectively) encountered were false negatives, i.e., negative on these two systems, but positive with the conventional test. Differences in incubation time may have contributed to the large number of false negatives if some of the organisms tested were slow to hydrolyze o-nitrophenyl-β-D-galactopyranoside. Both the Difco and Pathotec tests were incubated only 4 h (as per manufacturer's instructions) while the conventional test was incubated 24 h.

For API and Minitek (both incubated 24 h), most of the discrepancies, 95% (18/19) and 59% (10/17), respectively, occurred when these tests indicated a positive reaction while the conventional remained negative. This observation may suggest that these two commercial systems were more sensitive than the conventional test for the detection of β-galactosidase in *Enterobacteriaceae* of food origin.

Most of the disagreements with the conventional ONPG observed with food isolates were for strains of *Escherichia coli* and *Enterobacter agglomerans.* These organisms were involved in 56 of the 78 (72%) discrepancies. More than 50% (24/41) of the "false negatives" were with strains of *E. coli* isolated from food. Three out of every four discrepancies with food isolates were for organisms from ground beef and pork sausage. For the isolates from poultry sources, only 16 out of 66 disagreed with the conventional ONPG test.

The findings of this study indicate that microbiologists working with *Enterobacteriaceae* isolated from food should not assume that the agreement between the conventional ONPG test and the alternative systems is as high as that published for clinical isolates. However, the four systems we evaluated have many advantages over the conventional ONPG test such as ease of performing the test (manipulation), time required for inoculation, cost, and in some instances time of incubation. The agreement of greater than 85% with the conventional test on food isolates may be within the acceptable range for some laboratories. The 24-h system (API and Minitek) may be more sensitive than the conventional for detecting the presence of β-galactosidase in some strains of *Enterobacteriaceae* isolated from foods. The high degree of agreement for animal and clinical isolates observed between commercial systems and conventional ONPG tests indicated that any of these four systems would be an accurate and efficient alternative to the conventional test for ONPG.

ACKNOWLEDGMENT

We thank Michael Carson and Debbie Tanner for their technical assistance in the laboratory.

Mention of specific brand names does not imply endorsement by the authors or institutions at which they are employed to the exclusion of others not mentioned.

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## *Epidemiologic Excerpts*

### Multi-state Outbreak of *Salmonella newport* Transmitted by Precooked Roasts of Beef

A continuing outbreak of salmonellosis in New York, New Jersey, Connecticut, and Pennsylvania during June, July, and August 1977 has been epidemiologically and bacteriologically traced to precooked, ready-to-eat beef served in delicatessens and sold in supermarkets, according to Center for Disease Control in Atlanta (CDC).

The problem was first recognized when 2 outbreaks occurred in 2 upstate New York counties (Erie and Cortland) in late June and early July 1977. In both outbreaks *Salmonella newport* (serogroup $C_2$) was isolated from persons who had eaten precooked roast beef served in delicatessens. Clinical findings in affected cases consisted of diarrhea, cramps, chills, and fever. In the Erie County outbreak, *S. newport* was isolated from the roast beef and from at least 4 patients. In both outbreaks the precooked roasts of beef were from the same source, a meat-processing company in Philadelphia. The U.S. Department of Agriculture (USDA) conducted an investigation of the plant, collecting environmental specimens and whole roasts of beef. Two *Salmonella* serogroups, $E_1$ and $C_1$, were obtained from the environment, and 2 *Salmonella* serogroups, B and $C_2$, were obtained from both the internal and external surfaces of 4 individual roasts of beef. The USDA and the company initiated a recall of all such products distributed up to July 28.

A review of national surveillance data demonstrated 345 *S. newport* isolates in June and July 1977, compared with 222 in the same period in 1976. In this same 2-month period Connecticut, New Jersey, New York, and Pennsylvania experienced marked increases in *S. newport.*

Based on these data, a collaborative study was initiated by the 4 state health departments and CDC. This study has revealed that from June 1 to August 19, 140 *S. newport* cases have been reported by the 4 state health departments. Of the 63 cases interviewed, 32 gave a history of precooked roast beef consumption.

Addditional information is available from investigations in New Jersey, Connecticut, and New York. In New Jersey since June 1, 49 cases of *S. newport* infection have been reported. Ten of 23 interviewed patients consumed precooked roast beef, which had been produced by at least 6 different companies, including the Philadelphia one. The New Jersey State Department of Health obtained unopened roasts from 5 producers and has cultured *S. newport* and *S. waycross* from beef from 1 Jersey City producer. A case-control study conducted by the New Jersey State Department of Health comparing precooked roast beef consumption among cases of *S. newport* salmonellosis and among age-matched cases with other *Salmonella* serotypes demonstrated a statistically significant association with the consumption of roast beef ($p = .012$).

In Connecticut 14 of the 38 cases of *S. newport* infection that have occurred since June 1 have been interviewed by the state health department. Nine have a history of eating precooked roast beef. These cases involve 3 different producers, including the Philadelphia company. Specimens of roast beef are being collected for bacteriologic examination.

In New York 42 *Salmonella* serogroup $C_2$ isolates have been reported since June 1. Of 36 investigated cases 19 had consumed precooked roast beef. A case-control study using hepatitis patients of similar ages demonstrated that salmonellosis was statistically associated with roast beef consumption ($p = .0005$).

Further investigations are in progress in Pennsylvania.

The CDC said, this is the third consecutive year in which precooked roasts of beef have been associated with multistate outbreaks of salmonellosis. This recurrent problem with precooked roasts of beef from different producers emphasizes that this is a continuing problem with significant public health implications.

This is not the first time that salmonellae have been identified in unopened roasts. However, in the 1975 outbreak of *S. saint paul* the beef had been injected with a spice mix in preparation for cooking. In the present situation, no such procedures were used, yet salmonellae were isolated from both external and internal surfaces of the roasts. Whatever the mechanism of such contamination, pre-cooked roasts may continue to pose a risk as long as they are cooked to internal temperatures of less than 130 F. Such temperatures are usual for precooked roast beef but are not high enough to destroy salmonellae.

*Journal of Food Protection Vol. 40, No. 10, Pages 712-717 (October, 1977)*


# Fish, Shellfish, and Human Health

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(Received for publication March 14, 1977)

ABSTRACT

Foodborne illness may result in human beings from ingestion of fish food products containing bacterial or parasitic pathogens. The bacterial pathogens include *Salmonella, Shigella, Streptococcus, Leptospira, Clostridium, Staphylococcus, Mycobacterium, Erysipelotrix, Francisella,* and *Vibrio* species. Parasitic illness may result from *Diphllobothrium latum, Clonorchis sinesis, Opisthorchis felineus, Heterophyes heterophyes, Paragoniums westermani* and *Anisakis* sp. Fishborne intoxication include Ciguatera, Scombroid, and Puffer icthyosarcotoxins, mercury, nitrite-nitrate, pesticides, radionclides, and the toxin of Haff disease. Infections with *Vibrio parahaemolyticus, Salmonella typhi,* and infectious hepatitis virus are associated with shellfish. Toxic substances found in shellfish include paralytic shellfish poison, mercury, pesticides, and toxic hydrocarbons. Prevention of fish- and shellfish-associated illnesses of man is possible by: (*a*) using only fish and shellfish from unpolluted waters, (*b*) use of proper refrigeration facilities, (*c*) practicing strict sanitation in processing plants and storage facilities, (*d*) assuring foodhandlers are free of disease, (*e*) cooking thoroughly all fish and shellfish before eating, and (*f*) not handling aquatic foods when one has wounds or abrasions.

This review has been divided into two parts: (*a*) fish and human health and (*b*) shellfish and human health. The reader is also referred to reviews of aquatic animal biology, production, and management (*10,17,35*), pathology (*32*), toxicology (*16*), hygiene (*3,24,26*), and aquatic food preservation (*6,7,12,28,36*). Fishborne and shellfish-borne illnesses are also described in several monographs of zoonotic disease (*5,11,13,19,31*).

## FISH AND HUMAN HEALTH

Fish and fish products have been associated with several human illnesses (*20*). It has long been known that fish may be a vehicle for foodborne bacterial and parasitic infections in human beings. Foodborne intoxications are another important group of fish-associated human illnesses. The third way fish can affect man's health is by injury through physical attacks or accidents. A fourth miscellaneous category includes the human illnesses associated with envenomization, aflatoxicosis, food allergy reactions, and thiamine deficiency disease. Each of these four general categories of fish-associated illnesses will be presented in greater detail.

### *Fishborne infections and intoxicants (bacterial)*

Most of the fish-related foodborne illnesses in the United States are due to *Salmonella, Staphylococcus, Streptococcus, Clostridium botulinum,* and fish toxins (*2*). Raw whole fish taken from waters not polluted by sewage rarely contain bacteria pathogenic for man, other than *C. botulinum* and *Vibrio parahaemolyticus* (*18*). Spoilage bacteria, however, normally abound in surface slime and in gut contents. Fish may also be passive carriers of human pathogens in water environments polluted by human sewage or diseased animals (*20*). A fish can retain in its digestive tract or on its integument many human pathogens (e.g. *Escherichia coli, Salmonella* sp., *Shigella* sp., *Staphylococcus* sp., and *C. botulinum*) without becoming ill. Fish may also be carriers of water-borne pathogenic bacteria of several genera: *Erysipelas, Leptospira, Pasteurella, Aeromonas, Pseudomonas, Vibrio,* and *Mycobacterium. Aeromonas, Pseudomonas, Vibrio, Mycobacterium,* and *Pasteurella* species have been reported to cause active disease in fish.

*Salmonella, Shigella, Streptococcus, and Staphylococcus.* In *Salmonella* and *Shigella* food poisoning, fish may be contaminated by polluted water or during processing. The reported *Staphylococcus* and *Streptococcus* foodborne illnesses are usually due to contamination of fish on a fishing vessel or in a processing plant.

Fish decompose rapidly enzymatically in comparison to red meat and poultry. Fish also deteriorate much faster than other food products. This has been attributed to the greater free amino acid content of fish tissue. Bacteria found on or in freshly caught fish do not usually present a human health hazard, if fish are promptly chilled on the boat and processed properly in the plant. If allowed to remain on the deck of the boat or if not

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refrigerated, fish may become highly contaminated with organisms or bacterial toxins. Seagulls and other waterfowl flying over or perching on boat masts and piers have been incriminated as a source of *Salmonella* contamination of fish product.

*Clostridium.* Botulism toxin is produced by the bacterium, *C. botulinum.* As a bacterial foodborne intoxication, botulism occurs at a relatively low frequency, but when it does occur, it is often fatal. Between the years 1899-1967, there were 1,669 cases of botulism in the U.S. with 948 deaths, or a case fatality rate of 56.8% (*8*). During the 68-year period covered, fish were involved in 23% of the 640 reported outbreaks.

Type E, *C. botulinum* organisms are widespread in marine and aquatic environments. Fish harvested from most aquatic environment may be contaminated. Vacuum-packed fish may present a potential hazard because the presence of a vacuum tends to encourage growth of anaerobic bacteria like *C. botulinum.* Smoke deposited on the surface of smoked fish lowers the oxidation-reduction potential and this can account for development of the *C. botulinum* toxins in smoked vacuum-packed fish.

*Vibrio.* In the last few years several *Vibrio parahaemolyticus* seafoodborne illnesses have been reported. This organism has been associated with more shellfish-related foodborne outbreaks than fish-related outbreaks, and it will be discussed more thoroughly in the shellfish section.

*Mycobacterium. Mycobacterium ballnei* and *Mycobacterium fortuitum* are pathogens of fish. They have also been isolated from human lesions (*30*). *M. ballnei* causes chronic granulomatous lesions of man's extremities (*21*). The disease is usually traced to swimming pools, aquaria, or tropical fish tanks. Few cases have been recorded. This is probably because the optimal growth temperature for this organism is 25 C and the lethal temperature is 37 C.

*Erysipelothix. Erysipelothix rhusiopathiae* causes swine and avian erysipelas and human erysipeloid. It is commonly found in the slime or mucus covering fresh and salt water fish. This organism has no apparent effect on fish, but it causes a severe cutaneous infection in wounds or abrasions of fish handlers. This condition is commonly called "fish rose" and is considered an occupational disease.

*Leptospirosis.* Another occupational disease of fish workers in certain parts of the world is "Weil's disease" or leptospirosis (*30*). This is a severe febrile illness due to *Leptospira icterohemorrhagiae* and it is contracted in the same way as "fish rose."

*Francisella.* The causative agent of tularemia, *Francisella tularensis,* can infect fish. Contact with catfish has been reported as a cause of human infection. Punctures by the contaminated spines of the fish served to inoculate handlers with the organism.

*Prevention*

Prevention of bacterial fishborne illness in man is dependent upon the following: (*a*) fish in unpolluted waters, (*b*) use proper refrigeration facilities, (*c*) practice strict sanitation in processing plants and storage facilities, (*d*) foodhandlers should be disease-free, (*e*) always cook fish well and never consume raw fish, and (*f*) don't handle fish if you have wounds or abrasions.

*Fishborne infections (parasitic)*

Fish, like other animals, are often parasitized. Internal parasites may be found in the gastrointestional tract or their larva or cysts may be found in muscle tissue or subcutaneously just under the skin. The broad fish tapeworm, *Diphllobothrium latum,* is the only fish parasite of public health importance in the U.S. However, a few other parasitic infections of fish have been reported in humans in this country who have eaten imported fish or who had recently visited a foreign country.

The definitive host of *D. latum* is man. It may also infect dogs and cats. Proglottids containing operculated eggs, leave the body with the feces.

The first stage larvae, or coracidium, develop within the eggs. These larvae have dense cilia, and they are active swimmers when released from the eggs in the water. Each coracidium contains an oncosphere. They are eaten by a copepod in which the procercoid stage develops. Then pike, perch, turbot, salmon, trout, grayling, or eel eat the infected crustaceans. The procercoid migrates through the intestinal wall of the fish and matures into a pleurocercoid in the muscle. Man's ingestion of raw or poorly cooked fresh-water fish completes the cycle. The tapeworm may cause severe anemia in man. *D. latum* presently occurs throughout much of the great Lakes Region of North America. Prevention of diphyllobothriasis is dependent upon abstinence from raw or poorly cooked fish. Researchers have shown that freezing or heating to an internal temperature of 56 C will destroy the pleurocercoids.

In East Asia the cat liver fluke (*Opisthorchis felineus*) and the Chinese liver fluke (*Clonorchis sinensis*) are frequently diagnosed in humans. *Heterophyes heterophyes,* a fluke of fish, and *Paragonimus westermani,* a fluke of crab, are also transmissible to man.

Herring-worm disease has been reported in human beings in the Netherlands (*37*). The etiologic agent has been identified as the larva of *Anisakis* sp. which usually inhabits the viscera of small fish that serve as intermediate hosts. Human infections were contracted by eating raw or improperly cured herring. The syndrome in man was characterized by acute abdominal pain and peritoneal irritation. The parasite apparently penetrates the intestinal wall and migrates in the abdominal cavity.

In the midwest, black and white parasitic grubs, commonly referred to as "black spot" and "white spot," are often found encysted in the muscle of freshwater bass and perch (*34*). They are the larval stage of flukes that have their adult stage in fisheating birds. Based on current knowledge, these larvae present no public health hazard, however, it should be recommended that fish be thoroughly cooked.

*Other fishborne intoxications*

As well as being carriers of bacterial pathogens, fish may contain other toxicants which have caused or have the potential for causing foodborne disease in man. These include Ciguatera, Scombroid, and Puffer ichthyosarcotoxins; mercury; nitrites-nitrates; pesticides; radionuclides; and the toxic substance causing Haff disease.

*Ciguatera-Scombroid-Puffer.* Ciguatera and Scombroid are two different types of fishborne intoxications in man. These fish muscle toxins, or ichthyosarcotoxins, have been recognized for over 200 years.

Ciguatera toxin has been found in more than 300 species of marine fishes. Sea bass, jack, barracuda, snapper, wrasse, parrot fish, and surgeon fish are all renown for carrying this toxin. Gastrointestinal and neurological disturbances, numbness around the lips, and muscle pains are common in poisoned humans. The case fatality rate is less than 10%. This fish species producing this form of poisoning may be toxic in some areas of the world, but safe in others. It is thought that the toxin is produced by blue green algae and transferred to fish through the food chain. The poison is generally not destroyed by cooking procedures.

When Scombroid fishes are kept at room temprature, bacterial spoilage can result in enzymatic decarboxylation of free histidine to produce histamine. Scombrotoxin is thought to be a combination of histamine and other toxic substances. Scombroid poisoning produces symptoms resembling those of severe allergy or histamine intoxication. Symptoms in man are headache, dizziness, abdominal pain, and gastrointestinal upset. Death due to suffocation and shock has been reported. Antihistamine agents may relieve the symptoms.

Puffer fish poisoning is another form of fish poisoning frequently diagnosed in Japan. Gastrointestional upset may be present, but neurological disturbances are more common. Motor paralysis, convulsions and death by respiratory failure within 24 h are common. The case fatality rate is approximately 60%. Puffer poisoning in Japan has accounted for as much as 44% of all fatal food intoxications.

*Mercury.* Fish were first recognized in Japan as being contaminated with and hence a source of mercury in the diet (*27*). Between 1953 and 1956, more than 100 people suffered severe central nervous system illness, and 12 people died after eating mercury-contaminated fish caught in Minamata Bay. A plastics factory on the bay was the source of inorganic mercury contamination in the bay. Chemical analysis of the fish showed 40 ppm of nearly pure organic methyl mercury which is much more toxic than inorganic mercury. In 1956, Japan banned fishing in Minamata Bay. It was later discovered that microorganisms in the bottom muck of the lake had the ability to convert inorganic mercury to methyl mercury. In view of the biological magnification of the mercury through the food chain in the bay and the 200-day half-life of mercury of fish, it is easy to see how fish concentrated mercury 1000 times the amount in water.

In the spring of 1970, fish in Lake St. Clair, a small lake adjacent to Detroit, Michigan, had mercury levels as high as 7 ppm almost 14 times that of the FDA's guideline of 0.5 ppm. Ontario and Michigan subsequently closed the lake to commercial fishing. In December 1970, the FDA recalled from grocers' shelves one million cans of tuna that had mercury levels above FDA guidelines of 0.5 ppm. Danger of mercury poisoning in fish generally increases with the size of the fish; swordfish are usually larger than 100 lb. In May, 1971, FDA issued a notice recommending that the public not eat swordfish. By September 1970, 18 states had closed either a major river or lake to fishing because of the mercury hazard present in the fish. In 1972, the mercury level in the muscle of pike was found to be as high as 20 ppm when the fish came from rivers downstream from sites of pulp or paper industries where phenyl mercury acetate was used as a slimicide. In uncontaminated areas, a level of 0.1 ppm in pike is regarded as natural.

It was later determined that use of mercury fungicides on golf course greens in the U.S. caused high mercury levels in fish located in golf course lakes and ponds (*25*). Some of these impoundments were closed to fishing.

*Nitrite and nitrate.* When nitrites and nitrates are added to meat that has begun to decompose, the meat becomes more red in color thus appearing fresh. This practice of "camouflage" is obviously dangerous because the consumer can not recognize spoiled meat and because excessive amounts of nitrites or nitrates added to food can result in human illness assocaited with methemglobinemia, cynosis, vomiting, and possibly death.

Another possible risk from using nitrites and nitrates as "camouflage" or as curing salts in fish processing is the production of the carcinogen N-nitrosamine; Reducing bacteria on fish convert nitrates to nitrites; these nitrites can interact with secondary amines in gastric fluids to form N-nitrosamine. Whether this occurs when people eat nitrite-containing fish products has not been difinitely proven.

*Pesticides.* DDT, a chlorinted hydrocarbon insecticide, has been reported as the etiologic agent in cases on non foodborne acute and chronic toxicity in man (*33*). Seafood was not involved in any of these cases. Chronic toxicity from seafood has not been reported in man, but the potential hazard is present in food fish because of: (*a*) the phenomenon of biological magnification or concentration of DDT in the food chain, and (*b*) DDT storage in human fat and its slow excretion.

The DDT threat in fish is well illustrated by studies of Coho salmon in the Great Lakes. These salmon were found to contain much more than the permissible 5 ppm. The Cohos rapid weight gain, before migration up tributaries, may be the primary reason the fish accumulated large amounts of pesticide.

In just 2 h, endrin concentration in blood of catfish can reach 1,000 or more times the amount present in

surrounding water. This is a major problem in catfish farming on converted cotton land where pesticides was used as a cotton spray. Consumption of fish containing DDT in concentrations of 5 ppm or less is permitted by FDA provided the menu does not contain fish items for days or weeks at a time. Variety in the diet is recommended.

*Radioactivity.* Another potential threat seafood presents to man is radiation. Oceans covering large portions of the earth are major recipients of man-induced (military and industry) radionuclides. Today most foods have more radioactivity than present before 1945. Studies have shown that aquatic organisms can accumulate considerable quantities of nuclides. Researchers at White Oak Lake and the Columbia River near Hanford, Washington, where plutonium production reactors are located, found that minnows could accumulate $^{32}P$ to levels 150,000 times greater than that of surrounding water. They also found that the total radioactivity of plankton was about 2,000 times that of surrounding water. Clams and oysters collected off the Marshall Islands after the Bikini Atomic Tests had radioactivity 2,000 times greater than that of sea water. The concentrating of radioactivity by clams probably involved their ability to filter large volumes of water. In fish, most radioactive elements are absorbed from water via the gills and not via ingestion and intestinal absorption.

In summary, nuclides with longer half-lifes, ($^{137}Cs$ and $^{90}Sr$) present a potential health hazard (*1*). However, nuclides with a short half-life or extreme insolubility are of relatively little public health concern.

*Haff disease.* In countries bordering the Eastern Baltic Sea, a condition known locally as Haff Disease is caused by consumption of fish containing a factor that inactivates thiamine (Vitamin $B_1$) (*30*). "Haff" means an inland sea. Patients complain of muscular pain in arms, legs, and back. The skin is extremetly sensitive to touch and their urine is brown to black. Haff disease has been associated with Swedish and Russian lakes after luxuriant blooms of blue-green algae. The etiology has been proposed as being either a Ciguatera-like poison or a thiamine-inactivating compound.

*Physical injury.* Piranha and sharks have remarkable teeth. These are well known examples of species which can inflict physical injury on human beings.

*Miscellaneous.* Envenomization of fish stings may be caused by a wide variety of fish species. Over 100 different fish species are known to possess toxin spines. The venoms vary greatly in toxicity; most are merely painful while others are lethal. Stonefish off the coast of East Africa and Australia are the most venomous fish known (*16*). These fish have excellent camouflage; they erect their spines and remain perfectly still when being approached. Most exposures occur when a person steps directly on top of the fish. Several deaths have been reported, usually within 6 h after exposure.

*Allergy.* Fish and shellfish may cause food allergies in certain humans. Fish proteins act as allergens and subsequent meals may initiate anaphylactoid or cutaneous hypersensitivity reactions. Allergic individuals are believed to have inherited a predisposition or capacity to become sensitized. In man, symptoms of allergic reactions are urticaria, angio-neurotic edema, gastro-intestinal distrubances, and migrane headache. Allergy is usually confined to one general class or species of fish. Sensitized people usually react more frequently to sardine and salmon than to cod and halibut.

*Thiamine deficiency.* A number of distinct nutritional problems are related to consumption of fish by animals. The first important nutritional disease of fur-bearing animals was noted on the fox ranch of J. S. Chastek of Glencoe, Minn. in 1932 (*14*). Foxes given a ration of 18% uncooked carp developed paralysis within a few weeks and died. Researchers reported that an enzyme was involved and it seemed to be confined to fresh water fish, with the exception of ocean herring species. Later mink ranchers found they could use fish in their mink diet if fish was cooked at 82-93 C for 15 min to destroy the enzyme.

Fish also contain a high level of unsaturated fatty acids. It has been shown that low dietary level of Vitamin E and a high dietary level of unsaturated fatty acids stimulate development of steatitis in young mink and cats (*23*).

Human beings eating a diet of primarily raw fish might develop thiamine deficiency. A diet of semi-cooked or raw fish would be contraindicated for persons on a low thiamine diet.

*Aflatoxin.* In the early 1960s liver tumors called hepatomas were found in epizootic proportions in rainbow trout in the United States (*38*). California researchers found that a diet containing cotton seed meal was responsible. It was later shown that aflatoxins produced by the mold, *Aspergillus flavus,* were contaminants in the cottonseed meal. This disease is rapidly becoming rare in the U.S. as feed manufacturers improve their feeds and as hatcherymen store their feed under better conditions. Fish farmers and veterinary consultants should develop management procedures that will avoid mold growth in feed for trout, pompano, and catfish commercial operations. Tolerances for aflatoxins in food products have been set at 15-30 ppb. Aflatoxins are partially detoxified by normal cooking temperatures, but even high temperatures over long periods often do not achieve total destruction.

## SHELLFISH AND HUMAN HEALTH

Shellfish, like fish, have been reported to cause a variety of disease conditions in man. Shellfish have been the source of such human infections, as typhoid, *Vibrio* food poisoning, and infectious hepatitis. Shellfish can also be responsible for an intoxication such as paralytic shellfish poisoning. Mercury, pesticides, and toxic hydrocarbons are other toxicants which shellfish may concentrate and pass on to higher tropic levels.

*Shellfishborne infections*

*Vibrio.* A picnic was held in Maryland which was attended by 550 guests (*15*). It was a feast of Chesapeake Bay steamed crabs, a regional delicacy that has been esteemed by Marylanders since colonial days. Approximately 16 h later, 320 of the picnickers became ill with symptoms such as diarrhea, severe abdominal cramps, nausea, vomiting, fever, headache, and chills. A number of the affected people were hospitalized. An organism known as *V. parahaemolyticus* was isolated. This organism has been recognized to be an important cause of foodborne disease in Japan for the last 20 years, but it had never been isolated in the United States before 1968 and never identified with outbreaks until 1971.

*V. parahaemolyticus* is a gram-negative, rod-shaped bacterium; the most favorable condition for its growth is in a medium containing 2 to 4% salt. It particularly favors alkaline conditions and multiplies rapidly at 37 C. It appears to be able to live in marine waters without an animal host. It has been found in coastal and estuarine waters and sediments and on marine fish, crustaceans, and shellfish in many areas of the world, including all coastal waters of the United States. It thrives better in warm weather than in cold and the illness it causes is usually associated with warm weather (*22*).

*V. parahaemolyticus* in food products is associated almost exclusively with seafood, and it has been found in practically all seafood products — fish, shellfish, curstaceans, and others. Fish samples are prepared for bacteriological examination by blending and diluting the sample with saline. The sample is then inoculated into enrichment media and incubated. A plate is then streaked with a portion of the incubated food sample, incubated, and then examined for colonies of *V. parahaemolyticus.*

Some Japanese scientists believe that the Wagatsuma agar plate containing human erythrocytes, can determine the virulence of a strain of *V. parahaemolyticus* (*15*). This hemolytic test, referred to as the Kanagawa phenomenon, is based on the ability of an organism to hemolyze blood cells within 24 h. Cleared zones around colonies is due to hemolysis of the erythrocytes, thus indicating pathogenicity.

*Infectious Hepatitis.* Contamination of shellfish harvested or held in waters polluted with sewage is an important route of transmission of viral agents (*4*). Epidemics of infectious hepatitis in Texas and Georgia were traced to a single Louisiana oyster supplier. Approximately 265 clinical cases of hepatitis A resulted from consumption of raw oysters (*9*). Enteroviruses have also been isolated from oysters taken as far as 4 miles from the nearest outlet of raw sewage into estuary waters.

*Typhoid.* Marine and fresh water vertebrates, crustaceans, and shellfish living in waters not polluted by discharge of crude sewage or effluent are normally free of salmonellae. Oysters and mussels, which feed by filtering organic matter from 20 to 40 liters of sea water hourly from beds in sewage-polluted waters, form a well recognized source of infection with *S. typhi.*

By the process of depuration, placing shellfish in enormous shallow trays and allowing clean estuarine water to flow over them for several days, shellfish cleanse themselves of harmful viruses. In addition to microbiological testing of waters, obviously contaminated areas are designated as "off limits" for shellfish cultivation.

*Shellfishborne intoxications*

*Paralytic shellfish poisoning.* Severe and often fatal human intoxications following ingestion of bivalve mollusks occur sporadically in widely scattered areas throughout the world (*29*). This illness is referred to clinically as paralytic shellfish poisoning (PSP) and is caused by small phytoplankton. This disease in shellfish is similar to Ciguatera poison in fish. Filter-feeding mollusks, mussels, clams, oysters, and scallops accumulate toxin without harm to themselves by ingesting toxic dinoflagellates. PSP in man is manifested by either paralysis or neurotoxic symptoms. Numbness and ataxia usually begin within 30 min after eating toxic shellfish. Patients surviving the first 12 h generally recover. No antidote is known for this toxin. The toxin is not completely destroyed by cooking, but toxicity is reduced by approximately 70%.

PSP does not constitute a major public health concern in the United States, although outbreaks do occur occasionaly. In recent years there have been three confirmed outbreaks of PSP on the west coast. The outbreaks were small and no fatalities resulted. The northern coast of California annually quarantines mussels from May 1 to October 21 because of the PSP threat during the summer months. Most victims of PSP in North America are tourists and picknickers who harvest shellfish for personal comsumption on outings to beaches and estuaries. Visitors and new residents in an endemic area are often unaware of the regions of shellfish toxicity or they may disregard posted warnings against poisonous shellfish.

The greatest number of human PSP poisonings have been traced to various species of mussels, hence the common term, "mussel poisoning." Fortunately, except for butter clams and scallops which may remain toxic throughtout the year, most mollusks lose the toxin rapidly as winter approaches.

Prevention and control lies in surveillance of edible mollusks for toxicity during potential danger periods. The mouse bio-assay is the standard method for monitoring safety of shellfish. Growing areas are quarantined when toxicity is known to reach 400 mouse units/100 g of shellfish.

Extensive blooms of dinoflagellates, "Red Tides," are often accompanied by spectacular displays of luminescense or phosphorescense at night. This feature is reported to have been used by Indians along the Pacific Coast of North America as a warning of shellfish toxicity.

*Mercury.* Some lots of frozen, breaded oysters have been found to contain large mercury residues (*4*). State officials had sampled and analyzed several lots. They

found mercury in one lot in a range of 0.5 to 3.4 ppm while the second lot contained from 3.6 to 10.2 ppm. The amounts were considerably greater than the guidelines of 0.5 ppm.

*Toxic hydrocarbons.* One of the more current problems of concern is the effect of toxic hydrocarbons on the health of shellfish consumers. An estimated 600 oil spills per month have been reported in coastal waters of the United States.

ACKNOWLEDGMENTS

The authors acknowledge resource information received from Dr. Donald L. Hazelwood and Dr. Fred P. Meyer of the University of Missouri, Columbia and Dr. A. W. Childers, Texas A&M University, College Station. We also thank Dr. Milton Bailey for reading and suggesting improvements in the manuscript.

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*Journal of Food Protection Vol. 40, No. 10, Pages 718-727 (October, 1977)*


# *Salmonella* and the Chocolate Industry. A Review

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(Received for publication April 22, 1977)

ABSTRACT

This paper reviews the microbiology of milk and bitter chocolate and of ingredients used in their manufacture with emphasis on the incidence and survival of *Salmonella* in these products. The increased thermal resistance of salmonellae associated with certain physico-chemical properties of chocolate and chocolate ingredients underlines limitations of dry roasting of cocoa beans, heating of cocoa liquor, and conching of chocolate as effective bactericidal treatments. The value of quality control programs, irradiation, fumigation, and bacteriological standards as reliable control measures are also discussed.

Although salmonellosis is of growing concern to the chocolate industry, extension of the present review to include fresh and frozen chocolate pastries and other chocolate-containing foods was not possible owing to insufficient data. The physical and economical aspects of chocolate manufacturing processes (*26*) and desirable physico-chemical attributes of raw and finished materials (*83*) have been reviewed extensively and will not be considered in this paper.

## SALMONELLOSIS: A SERIOUS CONTEMPORARY PROBLEM

Although the ubiquitous distribution of *Salmonella* (*28*) in the environment is currently a point of contention, it is generally agreed that eradication of these zoonotic agents is not scientifically nor economically feasible. Regulatory programs are designed to control recognized reservoirs of infection and to guard against further invasion of salmonellae into the human food chain. The food industry is particularly vulnerable to salmonellae infection because the presence of these deleterious agents in food not only constitutes a public health hazard but may result in regulatory action.

The importance of human salmonellosis should not be underestimated; retrospect analysis of 1963-1973 data has shown an increasing incidence of human *Salmonella* infections in the United States (*45*); international surveillance activities have shown similar trends (139). Clinical symptoms of salmonellosis usually occur between 6 and 48 h after ingestion of contaminated food. Patients may suffer from abdominal cramps, diarrhea, fever, nausea, or vomiting. The disease is usually self-limiting, lasting only a few days; however, serious complications and terminal illnesses have frequently been reported. The average stay for cases requiring hospitalization in Canada is 14 days (*124*). In 1975, the Salmonella Surveillance Activity of the Center for Disease Control reported 23,445 human isolations of typhoid and non-typhoid salmonellae in the United States (*20*); a similar number of cases was reported in 1974 (*17*). Although males and females were equally affected, children less than 5 years of age were particularly susceptible to infection. In the same year, 497 foodborne disease outbreaks were reported in the United States; 38 outbreaks (*19*) compared to 35 in 1974 (*18*) were of *Salmonella* etiology. Beef, poultry and milk products were the principal vehicles of infection.

In 1974, 443 incidents of foodborne diseases were reported in Canada (E. Todd, personal communication) representing a slight increase over that reported the previous year (*132*). *Salmonella* was identified as the etiological agent in 24 incidents involving 622 cases of illness. Food processing establishments, which were responsible for 8.6% of all incidents, were incriminated in three *Salmonella* outbreaks. Poultry was the principal source of infection.

Although the risks of salmonellosis in the chocolate industry are comparatively low, several episodes of salmonellae-contaminated chocolate products have been reported. In 1966-1968, a number of confectionery products consisting mainly of candy coatings and chocolate candy were recalled in the United States (*42,43,53*). In Sweden, cocoa used in confectionery was identified as the vehicle of infection in an epidemic involving 109 cases of illness (*138*). In 1973-1974, chocolate candy was incriminated as the vehicle of infection in a major outbreak involving more than 200 cases of illness in Canada (*30*) and the United States where 38% of the cases were hospitalized (*29*). The contaminated chocolate contained 20-90 salmonellae per 100

g (30); mean standard plate counts (35 C) of $1.2 \times 10^4$/g, coliform counts of 9-90/100 g, and fecal coliform counts of < 4/100 g were also detected (D'Aoust, unpublished data). Subsequent investigations identified *Salmonella* in chocolate items imported to the United States from West Germany (*29*). Epidemiological evidence and retrospect laboratory analysis of contaminated chocolate from the 1973-1974 outbreak (*29,30*) indicated that large numbers of salmonellae may not have been a prerequisite to human infection (*31*). It is conceivable that ingredients present in chocolate protect *Salmonella* against the acidic conditions of the stomach; the few salmonellae present in the finished product (*29,30*) could then colonize the lower gastrointestinal tract and produce clinical symptoms.

## SALMONELLA METHODOLOGY

The following discussion reviews various aspects of conventional and novel methods for isolation and identification of *Salmonella* in foods. The general information included in this section is pertinent to the chocolate industry because analytical schemes and media used for detection of salmonellae do not vary significantly with different classes of food. Many of the new techniques show great potential as means of improving the efficiency and reducing the costs of quality control programs in the industry.

Significant advances have been made in the last decade in developing rapid methods for recovery of small numbers of *Salmonella* in foods. Increasing awareness of the food industry to the hazards of *Salmonella* infection and a concern to produce foods of high bacteriological quality have defined a need for more rapid and efficient detection techniques. Official methods for determination of *Salmonella* in foods should consist of a sampling plan and an analytical scheme. Sampling plans which reflect the stringency of a quality control program are known to differ significantly between manufacturers; however, recommendations by the International Commission on Microbiological Specification for Foods (*69*) and the U.S. Food and Drug Administration (*101*) on representative sampling plans are finding increased application.

Different analytical procedures have been used for isolation of salmonellae in different types of food (*37,84*). Although direct (selective) enrichment of clinical specimens is widely used for recovery of few salmonellae in the presence of large populations of competing microorganisms, the applicability of this procedure to dried and other processed foods is limited owing to the difficulty of stressed *Salmonella* in initiating growth in a selective medium. Components of food samples may also impair the selectivity of enrichment media and adversely affect the recovery of salmonellae. Reports on the increased recovery of *Salmonella* in a variety of foods through pre-enrichment in a non-selective medium (*34,48,100,113*) have resulted in increased recognition of this step as an integral part of *Salmonella* isolation schemes (*4,41,129*). Compositing of dry and high moisture food samples for pre-enrichment reduces the analytical workload without adversely affecting the sensitivity of the isolation procedure (*48,66,119*); the technique is currently advocated by a number of government and international agencies (*41,61,129,133*). Good recovery of salmonellae from pooled pre-enrichment cultures (wet compositing) has also been reported (*106,119*) but the number of samples that can be reliably composited in this manner appears to be limited (*119*). The volume of pre-enrichment culture to be transferred to the enrichment broth does not seem to affect the recovery of *Salmonella* (*33,106*).

Subculturing of pre-enrichment cultures to two different enrichment broths favors increased recovery of *Salmonella* (*4,41,49,120*). Tetrathionate broth with or without added brilliant green dye and selenite cystine broth are used widely as selective enrichment media and their performance under various conditions of use is well documented (*37,84*). Although a consensus on the optimal incubation temperature for enrichment broths has yet to be reached, several studies have shown increased selectivity of different *Salmonella* enrichment media with increasing temperature of incubation (*36,49,114,120*). More salmonellae were recovered from tetrathionate brilliant green enrichment cultures of dried foods and meats incubated at 43 C than at 35 C (*49,120*). Incubation of selenite broth at 43 C was found to be toxic for *Salmonella* (*37*); however, no toxicity could be associated with selenite cystine enrichment of egg products at 43 C (*114*). Current reference methods of analysis recommend incubation of enrichment broths at 35 C and have yet to endorse enrichment at 43 C (*4,41,133*). Conflicting reports on the value of prolonged incubations of enrichment broths for increased recovery of salmonellae have left this problem unresolved (*37,84*). The efficiency of wetting agents such as Tergitol 7 and Tween 80 used in the enrichment of fatty foods may be temperature dependent (*37,93,105,121,126*).

Enrichment broths preferably should be streaked on two or more selective plating media to facilitate recovery of serotypes which may be highly sensitive to one or more of the standard plating media. Brilliant green sulfa (*84*), xylose lysine deoxycholate (*128*), and Hektoen enteric (*74*) agars are widely used for the presumptive identification of *Salmonella* and all respond to lactose utilization; however, the increasing incidence of lactose fermenting salmonellae (*12,38*) places limitations on the value of these media and emphasizes the importance of the hydrogen sulfide dependent bismuth sulfite agar (*25,84*).

Although schemes for the biochemical screening of isolates vary widely between laboratories, reactions on the triple sugar iron, lysine iron, citrate, and urea agars are used extensively for identification of salmonellae. Exhaustive evaluation of API 20E (*16*), Minitek (*59,72*), Enterotube (*70*), R/b (*71*), and PathoTec (*122*) multitest diagnostic kits has led to a wider acceptance of these products as substitutes for conventional tube media. Biochemical reactions obtained with the API, Entero-

tube, and R/b systems are coded into digital profiles which correspond to one or more organisms, each with a computed level of probability.

The need for more rapid screening techniques has in past years led to a number of interesting innovations. The fluorescent antibody (FA) technique (*54*), recently recognized by the Association of Official Analytical Chemists (*39*), is particularly suited to the examination of foods in which the incidence of *Salmonella* is suspected of being low; the method performed satisfactorily in detection of salmonellae in chocolate and chocolate products (*39,68*). Samples found to be negative by the FA technique can be released with good assurance that the product is free of *Salmonella*; however, presumptive positive samples should always be confirmed with conventional cultural methods. Coupling of the direct immunofluorescent technique to the microcolony technique (*130*) has produced a detection method with increased efficiency and sensitivity. Impression smears of microcolonies formed on brilliant green agar plates are fixed on glass slides and stained for immunofluorescence analysis. The technique greatly facilitates detection of salmonellae through the formation of high density loci of stained cells with low background fluorescence. In the enrichment-serology technique (*62,123*), cultures obtained through conventional enrichment procedures are grown for an additional 6-8 h in a nonselective M broth and screened for *Salmonella* with flagellar antisera. The method which has been used to identify salmonellae in a variety of foods including milk chocolate (*123*) compared favorably with conventional methods (*92,123*). An analytical scheme combining the fluorescent antibody and enrichment-serology procedures (*63*) gave excellent agreement with standard cultural methods when challenged with naturally contaminated foods and animal feeds. Comparative studies on the performance of the enrichment-serology and fluorescent antibody techniques (*62,67,92*) also showed that these procedures share similar diagnostic capabilities but differ in the amount of time required to complete each type of analysis. The lysine-iron-cystine-neutral red (LICNR) broth formulated for presumptive identification of *Salmonella* in dairy products was particularly attractive in that it eliminated enrichment steps and provided presumptive evidence of contamination after 24 h of incubation (*60*). However, use of the LICNR as a single step enrichment of foods, food ingredients, and feed materials met with limited success owing to the ability of certain foods to mask or interfere with LICNR color reactions (*64*); the performance of the medium significantly improved when used as a secondary enrichment. Recent evaluation of the LICNR broth in our laboratories has shown that the medium generally fails to recognize *Salmonella* contaminated samples enriched in selenite cystine broth (unpublished data).

## MICROFLORA OF CHOCOLATE INGREDIENTS

In its evaluation of the *Salmonella* problem, the National Academy of Science indicated that raw material inspection constitutes the first line of defense in chocolate manufacturing because processing cannot be expected to effectively eliminate *Salmonella* in contaminated ingredients (*97*). Use of raw products from countries where good hygienic control is generally lacking further indicates the need for stringent bacteriological testing of such materials. Microbiological contamination of primary ingredients must be controlled because it constitutes a potential health hazard to plant personnel and to the consumer; the situation may also lead to serious economic losses to the industry through spoilage or regulatory action. It will become evident from the ensuing discussion that information on the microbiology of several primary ingredients used in chocolate is seriously lacking and needs to be documented.

Fermentation of cocoa beans (*58,65*) frequently carried out under poor hygienic conditions results from a succession of microbial populations. Aerophilic yeasts ferment sugars present in the thick pulp surrounding the cocoa beans to ethanol which is converted to acetic acid through bacterial degradation; these exothermic processes kill cocoa beans as viable seeds. Intermediates of the sugar fermentation are also utilized in lactic acid formation. Fermented beans are frequently dried in the sun to a moisture content of 6% with little or no protection from environmental contamination. The microflora of fermented and dried cocoa beans predominantly consists of members of the genus *Bacillus* (*104*). Bacteria are not restricted to the surface of cocoa beans but can permeate the shell and contaminate the meat of the dicotyledenous seed. Studies on the internal microflora of surface-disinfected cocoa beans showed large populations of molds, yeasts, and bacteria (*58*); total bacterial counts ranged between $10^3$ -$10^8$ cells/g and similar populations of yeasts and fungi, predominantly of the *Penicillium* and *Aspergillus* genera, were enumerated. Dry roasting of raw cocoa beans at 145-150 C for 30-40 min reduced initial levels of contamination by two $\log_{10}$ units (*9,75*). Enterobacteria present in unroasted beans could not be detected in the heat-treated beans where only bacilli, mainly *Bacillus stearothermophilus* and *Bacillus coagulans,* were isolated (*9*); roasting at 180 C for 30 min eliminated all bacterial flora but adversely affected the organoleptic characteristics of cocoa beans. Although literature fails to report the presence of *Salmonella* in raw and roasted beans, it is important to note that cocoa beans were recently incriminated as the probable source of *Salmonella eastbourne* in a major chocolate outbreak (*29,30*).

The increased use of cocoa powder in chocolate, chocolate coatings, and in several chocolate food drinks has stimulated the market for imported cocoa powder. The situation is of some concern because the bacteriological quality of cocoa powder imported from South American and African countries may not always compare favorably with that of domestic products.

*Salmonella* has been recovered from cocoa powder on several occasions (*3,32,137*); monitoring activities of the Health Protection Branch (Health and Welfare Canada) also led to detection of salmonellae in Dutch (*99*) and in English (HPB, unpublished data) products. Contamination in the Dutch product was estimated to be 0.7 cell/100 g by the Most Probable Number technique (D'Aoust, unpublished data). The endogenous flora of 36 lots of cocoa powder with a total plate count ranging between $10^2$ -$10^4$ organisms/g consisted mainly of *Bacillus* species (*47*); although coliforms were not detected, *Bacillus cereus*, a recognized foodborne pathogen (*18,56,94*), accounted for approximately 20% of all isolated bacilli. Study of 547 samples of Dutch cocoa powder showed a median total bacterial count of $2 \times 10^2$ organisms/g (*95*); although *Bacillus subtilis* and *Bacillus licheniformis* predominated as aerobic sporeformers, *B. cereus* was not identified. Similar plate counts were obtained in a separate study where added Tween 80 did not significantly increase recovery (*89*); it was also reported that dry storage of newly manufactured product markedly decreased bacterial populations possibly through an anthocyanin-linked bactericidal effect (*15, 46*).

The microbiology of cocoa butter obtained from pressed or extracted cocoa beans (*83*) is poorly documented owing to the apparent absence of microorganisms in this product (*118*). A recent chocolate outbreak (*29,30*) indicated that the synergistic effect of high holding temperatures and the 2-3% moisture content of chocolate liquor may not be sufficient to destroy bacterial pathogens in this product; momentary use of steam in the roaster provides an additional margin of safety by further decreasing the bacterial flora of cocoa beans by 2-3 $\log_{10}$ units (*26*). Chocolate crumb prepared from a mixture of sugar, milk solids, and cocoa liquor or cocoa powder is dried at low temperatures under vacuum to less than 1% moisture (*65*). Although the product is virtually free of bacteria upon emerging from the drying oven (*65*), faulty processing or cross contamination may result in *Salmonella* contamination (*99*). Confectionery ingredients such as sugar, salt, vanillin, and lecithin are not documented nor suspected as likely sources of salmonellae (*3,75*); however, carmine red which is used as a food additive was previously identified as the cause of a nosocomial outbreak (*79*) and the source of *Salmonella cubana* in candy coatings (*80*).

The potential hazard of milk powder as a source of *Salmonella* infection (*108*) continues to be of concern even though levels of contamination are of the order of 1% or less (*115*). In recent years, numerous lots of milk powder have been subjected to regulatory action (*3,50* and unpublished data) and have been suspected or confirmed as the vehicle of infection in several outbreaks (*50*). Control of cross-contamination of in-line and finished products is one of the major problems confronting milk drying plants. This is particularly true with airborne contamination owing to the extremely large volumes of air used in the drying process where the weight ratio of air to finished product ranges between six and 10 (*50*). Although the predominance of *B. stearothermophilus* in milk powder (*50*) is currently of little public health significance, the ability of salmonellae to survive in milk powder over prolonged periods of storage is disturbing. *Salmonella* populations rapidly decrease during the first weeks of storage (*82,87,109*); however, in spite of this rapid initial death rate, salmonellae have been recovered from milk powder stored for up to 12 months where initial viable counts decreased by approximately 50% (*109*). Survival of *Salmonella* in milk powder varies inversely with storage temperature. Storage of spray dried skim milk powder for up to 8 weeks at 25-35 C reduced populations of *Salmonella typhimurium* and *Salmonella thompson* from $10^4$ to $10^2$ cells per 100 g (*82*); reductions of approximately four $\log_{10}$ units were obtained at 45-55 C with a concomitant decrease in the organoleptic quality of the product. Similar temperature-dependent survival curves were reported for *Salmonella senftenberg* 775W, *S. typhimurium* and *Salmonella new brunswick* in artificially contaminated milk powder stored for up to 15 weeks (*87*).

Inadequate sanitary controls during the harvesting and processing of coconut contribute to *Salmonella* contamination of desiccated coconut (*32,76,88,112*). Meat from the dehusked fruit is blanched in hot water, shredded, and air dried to approximately 2% moisture (*88*); dried coconut spoils rapidly at higher moisture contents (*111*). Storage of freshly grated coconut at moderate (30 C) and elevated (55 C) temperatures resulted in rapid proliferation of the endogenous microflora and the liberation of foul odors (*40*). Plate counts of shredded coconut stored for 24 h at 30 C increased from $10^7$ cells/g to $10^{10}$ cells/g; plate counts at 55 C increased from $10^3$ cells/g to $10^8$ cells/g. Although salmonellae have no measurable effect on the physical and organoleptic quality of coconut meat (*112*), their ability to survive the drying process (*88*) warrants caution in the handling of this commodity, as stipulated by good manufacturing practices (*98*). Decontamination of shredded coconut by roasting was ineffective and led to discoloration of the product (*112*). Gamma radiation doses of 0.13-0.16 megarads were 90% effective in eliminating five *Salmonella* serotypes in artificially contaminated coconut; however, 0.45 megarad was required to sterilize naturally contaminated product (*81*). Salmonellosis outbreaks traced to infected desiccated coconut in home baked (*136*) and commercially prepared (*117*) foods emphasize the importance of testing this ingredient which may be added to foods which may not receive any further bactericidal treatments.

In addition to aflatoxins (*102,118*), the presence of *Salmonella* in nuts is of public health significance (*32,98*) because it is also not unusual for these foods to be added to bakery and confectionery products without prior or subsequent antibacterial treatments. Recent work showed that the microbial populations in processed nuts

ranged between $10^2$ -$10^5$ bacteria/g and included low levels of fungi, yeasts, and streptomycetes (*134*); no enterobacteria were identified in the varieties of nuts tested. Total plate counts of $10^1$-$10^4$ organisms/g were reported for raw almonds (*73*) and cashew nuts (*77,78*). The bacterial flora of almonds consisted of coliforms and *Streptococcus* and *Bacillus* species (*73*); coliforms, including *Escherichia coli* and *Enterobacter aerogenes*, and members of the genus *Bacillus* were detected in cashew nuts (*78*). No attempts were made to isolate *Salmonella* in these studies. Drum and oil roasting effectively reduced the endogenous flora of raw cashew nuts (*77*). Studies on the effectiveness of propylene oxide in controlling the bacterial and fungal microflora of pecans showed that the treatment used in commercial shelling operations was 96% effective in eliminating surface contamination but exerted little antibacterial action on deeply seated microorganisms (*13,21*). Exposure of inshell pecans artificially contaminated with *S. senftenberg* 775W, *Salmonella anatum* and *S. typhimurium* to moist heat treatments at 60-93*C* for 3 min did not eliminate salmonellae (*11*); further studies also showed that storage of infected inshell pecans and pecan halves for up to 8 months at 5 C or lower did not significantly reduce the number of viable cells. Similar survival curves for *S. typhimurium* and *E. coli* were obtained with almond meats stored at 2 C (*73*). Recent work also showed that the ability of *E. coli* to survive at different storage temperatures varied inversely with the moisture content of pecan meats (*10*).

## THERMAL RESISTANCE OF SALMONELLA

Knowledge of the endogenous microflora of primary ingredients used in chocolate confectioneries and awareness of the potential hazards in handling these materials constitute the first line of defense against *Salmonella* contamination of the finished product. An understanding of basic principles in bacterial thermal resistance is equally important because heat is widely used in industry to reduce or destroy microbial populations in raw ingredients. The foregoing discussion deals with relationships between selected characteristics of foods and their effects on the thermal resistance of bacteria. From these considerations, the limitations of dry roasting of cocoa beans, heating of cocoa liquor, and conching as bactericidal treatments will be more fully appreciated.

Chocolate confectioners are faced with a rather unique situation where the low moisture and high sugar content of chocolate do not favor bacterial proliferation, but significantly increase the thermal resistance of bacteria. Bacterial heat resistance is usually expressed as a D value, the amount of time required to kill 90% of a population at a given temperature. The D value is independent of initial cell numbers and is greatly influenced by the chemical composition of foods. A plot of $\log_{10}$ D values against temperature allows one to predict the survival rate of a microorganism at intermediate temperatures; $Z_D$, the temperature interval necessary to effect a tenfold change in D value, can also be derived from the slope of this curve. Water activity ($a_w$) measures the moisture content of foods and is defined as the ratio of the vapor pressure of a solution to the vapor pressure of water at a given temperature (*116*). The vapor pressure of a solution decreases with increasing solute concentration owing to increased binding of water to solute molecules. $a_w$ values vary between zero and unity where $a_w = 1$ is characteristic of distilled water. Water activities of confectionery products and chocolate are low and range between 0.30-0.84 (*96*) and 0.37-0.50 (*1*), respectively. Although $a_w$ values lower than 0.95 are generally bacteriostatic (*1,22,96,107*), growth of some microorganisms has been reported at $a_w = 0.65$ (*116*). Bacteria are also capable of surviving extended periods in environments of low $a_w$ (*8,110,116*).

The heat resistance of salmonellae varies inversely with $a_w$ and directly with the composition of the heating menstruum (*5,27,55*). Extrapolation of thermal characteristics of *Salmonella* in one system to a different system of equal $a_w$ is therefore not justified. Sucrose was more effective than fructose, glycerol, and sorbitol in protecting eight strains of *Salmonella* against heat inactivation in an aqueous environment (*44,55*); it was further demonstrated that heat treatments sufficient to destroy *E. coli* may not inactivate all *Salmonella* serotypes (*55*). Sucrose was significantly more effective than NaCl and glycerol in protecting *Salmonella* suspended at 60 C in solutions of $a_w = 0.85$-$0.98$ (*5*). A more recent study showed that, in order of decreasing effectiveness, sucrose, glucose, sorbitol, fructose, and glycerol conferred thermal resistance to *S. typhimurium* and *S. senftenberg* 775W heated at 65 C in a sugar-supplemented phosphate buffer (*27*). Although rhamnose, mannitol, alanine, and glycine demonstrated limited capacity to protect *S. anatum* against heat inactivation at 55 C, complex media such as trypticase soy broth and whole milk were found to be very effective (*91*).

The low $a_w$ of chocolate and the reported presence of *Salmonella* in this food (*29,30,42,43*) have led to several important studies on heat and its effects on the survival of *Salmonella* in chocolate. Recovery of *S. typhimurium* and *Salmonella enteritidis* from milk and bitter chocolate conched for 30-40 h at 72 C indicate the limited bactericidal action of the conching process; $D_{72}$ values for *S. typhimurium* and *S. enteritidis* in conched milk chocolate were 40 h and 50 h, respectively (*110*). The study also showed that the kinetics of *Salmonella* inactivation during conching were strain specific and that conching at 100 C for up to 30 h did not eliminate infection. The heat resistance of *S. typhimurium* in molten milk chocolate (*52*) held at 70-90 C was approximately two-fold greater than that of *S. senftenberg* 775W, a strain noted for its resistance to moist heat (*55*). The $D_{70}$ values for *S. typhimurium* in this study ranged between 11 and 17 h, whereas comparable values

at 80 C and 90 C were 3.5 h and 1.0 h, respectively (*52*). Survival of *S. anatum* in milk chocolate held at 71 C significantly decreased with increased moisture content (*7,8*); addition of 2% water reduced the $D_{71}$ value from 20 h to 4 h. Although higher moisture levels were more effective, water added in amounts greater than 6% resulted in an irreversible separation of fat from the chocolate mass (*7,8*). Fatty materials in milk chocolate reportedly increase the thermal resistance of salmonellae (*8*); a three-fold increase in cocoa butter increased the heat resistance of *S. anatum* at 100 C by a factor of three. The thermal stability of *Salmonella* in other dried foods is also of interest. Heating of nonfat dry milk for 10 h at 85 C reduced salmonellae to undetectable levels (*87*); a 1-h exposure at 115 C was equally effective but imparted a yellow burnt appearance to milk powder. The bactericidal efficiency of heating powdered milk at 85 C increased 10-fold when the moisture content of normal (4%) powder was increased to 15% (*87*). Thermal resistance of *Salmonella* was also found to be significantly greater in dried than in liquid egg products (*2,6,107*).

The ability of salmonellae to survive extended periods of storage does not support the use of storage as an effective and reliable means of disinfecting contaminated chocolate (*33,125*). *S. typhimurium, S. enteritidis,* and *S. typhimurium* were detected in milk and bitter chocolate stored for 15-18 months at room temperature (*110*); similar survival curves were obtained for *S. anatum* in milk chocolate (*8*). *Salmonella typhi* and *Salmonella paratyphi* B could also be recovered from artificially contaminated chocolate and confectionery products held 4-8 months at ambient temperature (*125*). The mortality rate of the *S. eastbourne* strain involved in a major chocolate outbreak (*29,30*) was significantly lower than that of *S. typhimurium*; comparatively high levels of *S. eastbourne* were detected in artificially contaminated milk and bitter chocolate stored for 6 months at room temperature (*127*). Bitter chocolate was also found to be more inhibitory than milk chocolate to salmonellae (*110,127*) and *S. aureus* (*103*) over prolonged periods of storage. Preliminary results also indicated an inverse relationship between survival and storage temperature (*42*).

The ability of *Salmonella* to survive cold temperatures, extreme pH conditions, and high salinity should not be minimized when evaluating the bacteriological hazards associated with handling and storage of certain foods and food ingredients. Minimal growth temperatures for *Salmonella* range between 5.5 C and 5.9 C (*85*); failure to store refrigerated foodstuffs under adequate conditions could result in growth of salmonellae or other food poisoning microorganisms (*90*). The limited antibacterial action of freezing fails to qualify this process as an effective means for reducing bacterial populations in frozen foods (*107*). Although *Salmonella* can grow at pH values of 4.5-9.0, the optimal pH range reportedly lies between 6.5-7.5 (*107*). Growth at acidic conditions results from a synergistic effect between the nature of the acidulant, the relative oxygen tension, and the incubation temperature, temperature being the dominant factor (*23*). The ability of selected strains of salmonellae to initiate and sustain growth at pH 4.0 in a non-limiting growth medium was found to be acidulant-dependent (*23,51*). The volatile acetic and propionic acids and the long chain dicarboxylic pimelic and adipic acids were more bactericidal at pH 5.1-5.5 than hydrochloric, citric and tartaric acids at pH 4.0-4.1 (*51*). Preliminary work on the relationship between pH and $a_w$ of a medium indicated that the nature of the substrate used to adjust $a_w$ determines the ability of that medium to sustain growth of *Salmonella* (*51*). Although the salt content in confectionery products is low, it is interesting to note that at non-lethal salt concentrations ($< 10\%$), the salt tolerance of *Salmonella* increases with increasing temperature of incubation (*86*).

## PROPECTS FOR CONTROL

We have seen that destruction of *Salmonella* in chocolate is a difficult if not impossible task owing to the low $a_w$ of the product. Heat treatments such as dry roasting of cocoa beans and conching of chocolate are of limited bactericidal activity and do not equal the effectiveness of stringent plant sanitation programs (*3,98*) and bacteriological control of raw and finished products. Raw ingredients should preferably be purchased on specification from reputable suppliers who are conscious of the *Salmonella* problem and who maintain a rigorous in-house surveillance program. Raw materials should always be isolated from other plant operations; failure to comply with this good manufacturing practice was mainly responsible for a recent chocolate outbreak (*29,30*). The importance of maintaining a dry work environment cannot be over-emphasized; water condensates from cold water pipes, refrigerator coils, and cooling tunnels could easily become loci of *Salmonella* infection (*75*). Trimmings and damaged items should not be added to fresh batches of chocolate because such a practice tends to prolong infection and increase the hazards for a massive plant contamination; an operational scheme assuring complete identity of lots during all manufacturing operations would be advantageous.

The value of heat as a bactericidal treatment in chocolate manufacturing is limited owing to the increased bacterial resistance in foods of low $a_w$ and its adverse effects on the organoleptic quality of certain ingredients. Ethylene and propylene oxide fumigation have been used to reduce the endogenous flora of nutmeats and other types of foods (*10,11,13,14*); ethylene oxide has also been used to decontaminate cocoa powder (*89,118*). Disinfection of nutmeats with ethylene oxide is no longer permitted in the United States (*13*) because of the possible formation of toxic chlorohydrins (*135*). Irradiation may prove to be an effective and economical measure for control of infection in raw and finished products. The bactericidal action of irradiation results

from induced changes in the configuration of key cellular components or the formation of lethal, highly reactive compounds. The greater penetration force of gamma irradiation constitutes a net advantage over beta and ultraviolet radiant energies which are only effective in the treatment of foods that can be processed in thin films or layers. Although the D values (radiation dose required to destroy 90% of a bacterial population) for *Salmonella* in a variety of foods range between 0.04-0.07 megarad (*24,35,107*), 0.5-0.75 megarad has been recommended for destruction of salmonellae in foods and feeds (*107,131*). Operational cost estimates for gamma irradiation of foods based on a minimum radiation dose of 0.5 megarad was less than $0.02 per pound (*35*). Irradiation doses of approximately 0.45 megarad were required to disinfect coconut (*81*) and cocoa powder (*57*); however, the technique was found to be of limited application because of adverse effects on the organoleptic characteristics of both products.

The limited effectiveness of heat treatments with dry products and the adverse effects of fumigation and irradiation on the physicochemical characteristics of certain foods supports the use of bacteriological standards as an adjunct to good manufacturing practices in reducing the hazards of foodborne pathogens in raw and finished products. Standards for cocoa powder (*89*) and milk and bitter chocolate (*118*) have been proposed. In addition to its regulations on *Salmonella* in cocoa and chocolate (*61*), the Health Protection Branch (Health and Welfare Canada) recently conducted a survey on the microbiology of chocolate and related products; hopefully, the accumulated data will delineate a representative baseline of endogenous flora which could then be used to draft standards. Although the feasibility of microbiological standards for dried products such as chocolate has been questioned (*140*), recalls of contaminated confectioneries (*42,43,53*) and outbreaks due to chocolate and chocolate products (*29,30,138*) suggest that the problems of foodborne pathogens such as *Salmonella* and *Shigella* (*110*) in chocolate have been underestimated in the past and underline the importance of maintaining stringent controls over all aspects of production.

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## 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., at June 30, 1977, and the related statement of income for the year 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., at June 30, 1977, and the results of its operations for the year then ended, in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year.

Dougherty + Co.

Certified Public Accountants

July 20, 1977

**BALANCE SHEET**
**As of June 30, 1977 and 1976**

**ASSETS**

| | Year Ended June 30, 1977 | June 30, 1976 |
|---|---|---|
| Current Assets: | | |
| Cash on hand, in bank & savings and loan assoc. | $26,319.47 | 22,277.32 |
| Accounts Receivable-trade | 6,391.60 | 4,006.16 |
| Inventory-supplies | 5,208.10 | 3,100.31 |
| Prepaid expenses | 104.25 | 79.92 |
| Total current assets | 38,023.42 | 29,463.71 |
| Fixed Assets: | | |
| Office equipment | 2,601.83 | 2,092.94 |
| Addressing and mailing equipment | 4,138.83 | 2,573.23 |
| | 6,740.66 | 4,666.17 |
| Less: allowance for depreciation | 2,365.49 | 1,864.06 |
| Net fixed assets | 4,375.17 | 2,802.11 |
| Total assets | $42,398.59 | 32,265.82 |

**LIABILITIES AND NET EQUITY**

| | | |
|---|---|---|
| Current Liabilities: | | |
| Accounts payable-trade | $ 655.56 | 676.29 |
| Payroll taxes payable | 2,356.56 | 716.08 |
| Special purpose funds: | | |
| Memorial Fund | 996.55 | 1,031.44 |
| Lab Methods Committee | — | 298.66 |
| Foundation Fund | 2,037.52 | 505.15 |
| Total current liabilities | 6,046.19 | 3,227.62 |
| Equity: | | |
| Balance-beginning of period | 29,038.20 | 21,593.05 |
| Net increase, decrease (-) during period | 7,314.20 | 7,445.15 |
| Balance-end of period | 36,352.40 | 29,038.20 |
| Total liabilities and equity | $42,398.59 | 32,265.82 |

See Notes To Financial Statements—June 30, 1977

## INCOME STATEMENT
**For the Years Ended June 30, 1977 and 1976**

| | Year Ended June 30, 1977 | June 30, 1976 |
|---|---|---|
| Income: | | |
| Affiliate dues | $19,927.73 | 16,894.00 |
| Direct dues | 12,772.89 | 10,796.14 |
| Total dues received | 32,700.62 | 27,690.14 |
| Contributions received for awards | 2,205.66 | 1,710.42 |
| Convention and meeting income | 1,576.52 | 1,503.00 |
| Publications and pamphlets | 14,013.19 | 963.69 |
| Sale of 3-A Standards | 3,970.77 | 3,312.70 |
| Sustaining memberships | 1,500.00 | — |
| Decals, buttons, misc. and expense reimb. | 922.75 | 965.97 |
| Expense reimbursement 3-A | 9,185.92 | 7,766.94 |
| Sale of Equipment | — | 150.00 |
| Interest Income | 532.33 | 265.21 |
| Total Income | 66,607.76 | 44,328.07 |
| Expense: | | |
| Salaries | 30,659.70 | 28,428.02 |
| Payroll tax expense | 2,275.50 | 1,696.86 |
| Travel | 2,756.39 | 3,479.92 |
| Office supplies | 804.41 | 3,025.83 |
| Box rent and postage | 3,185.15 | 2,215.82 |
| Telephone | 914.03 | 842.80 |
| Office rent | 2,250.00 | 2,250.00 |
| Insurance | 234.67 | 158.97 |
| Legal and professional fees | 1,319.84 | 1,175.52 |
| Dues and subscriptions | — | 200.00 |
| Depreciation-office equipment | 186.40 | 227.78 |
| 3-A Standards expense | 3,411.34 | 1,405.76 |
| Citations and awards | 2,000.00 | 2,000.00 |
| Buttons and decals | 7.84 | 3.41 |
| Convention and annual meeting expense | 2,428.74 | 2,205.93 |
| Cost of printing pamphlets | 5,533.80 | 306.11 |
| Miscellaneous | 1,253.42 | 557.53 |
| Total expense | 59,221.23 | 50,180.26 |
| Net income (loss) of Association | 7,386.53 | ( 5,852.19) |
| Add-net income (loss) of Journal-Exhibit B-1 | 1,161.38 | 13,802.49 |
| Total net income (loss) | $ 8,547.91 | 7,950.30 |

See Notes To Financial Statements—June 30, 1977

## INCOME STATEMENT
**For the Years Ended June 30, 1977 and 1976**

| | Year Ended June 30, 1977 | June 30, 1976 |
|---|---|---|
| Income: | | |
| Advertising | $12,785.94 | 15,590.55 |
| Subscriptions | 33,813.01 | 32,626.58 |
| Sale of journals | 1,987.21 | 296.94 |
| Sale of reprints | 12,191.94 | 7,692.12 |
| Page charges | 8,772.46 | 10,475.00 |
| Total income | 69,550.56 | 66,681.19 |
| Expense: | | |
| Editorial salaries | 8,858.38 | — |
| Printing and publishing | 39,507.57 | 36,427.50 |
| Plates, cuts, etc. | 759.00 | 846.00 |
| Mailing and postage | 5,227.09 | 4,215.00 |
| Reprint expense | 4,753.30 | 4,775.74 |
| Advertising and cost-commission and printing | 1,447.60 | 1,678.85 |
| Stationary and supplies | 141.76 | 202.87 |
| Travel expense | 1,849.17 | 385.92 |
| Depreciation-addressing equipment | 315.03 | 149.21 |
| Telephone | 458.54 | 306.94 |
| Consulting | 4,150.00 | 3,600.00 |
| Payroll tax expense | 625.33 | — |
| Miscellaneous | 296.41 | 290.67 |
| Total expense | 68,389.18 | 52,878.70 |
| Net income of journal | $ 1,161.38 | 13,802.49 |

See Notes To Financial Statements—June 30, 1977

## NET EQUITY
**As of June 30, 1977**

| | |
|---|---|
| Balance June 30, 1976 | $29,038.20 |
| Less transfer to Special Purpose Fund (Foundation Fund) | ( 1,532.37) |
| Add-closing of Special Purpose Fund (Reserve For Lab Methods Comm.) | 298.66 |
| | 27,804.49 |
| Add net income for year ended June 30, 1977 | 8,547.91 |
| | $36,352.40 |

See Notes to Financial Statements—June 30, 1977

### Notes to Financial Statements—June 30, 1977

Inventory

Inventory of supplies is recorded at the lower of cost or market.

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. At June 30, 1977, assets fully depreciated and the balance of accumulated depreciation were eliminated from the books.

Recognition of Revenues

Income from dues and subscriptions is recorded on the cash basis. All other income is recorded on the accrual basis.

*Journal of Food Protection Vol. 40, No. 10, Pages 729-733 (October, 1977)*


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

## Sioux City, Iowa, August 14-18, 1977

Abstracts of most papers given at the 64th Annual Meeting of IAMFES appear below. The complete text of many of these papers will appear in future issues of the *Journal of Food Protection.*

### CONTRIBUTED PAPERS

**Heating Patterns of Products in Crockery Cookers.** R. E. Brackett and E. H. Marth. *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

Heating characteristics at various positions inside two crockery cookers were determined when cookers contained starch gel, beef stew, baked beans (normal amount and overload), or meat loaf (normal amount and overload). Data on heating patterns were compared to growth limits for *Clostridium perfringens* and *Staphylococcus aureus.* Temperatures in the warmest areas of cookers were in the growth range of *C. perfringens* for about 0.5 h and in coolest areas for about 4.3 h. Temperatures in the warmest areas of cookers were in the growth range of *S. aureus* for about 0.4 h and in coolest area for about 3.9 h. The average time products were in the growth range for *C. perfringens* and *S. aureus* was 1.75 and 1.5 h, respectively. All products, except when cookers were overloaded, were in the growth ranges of the indicated bacteria for less than or about 2 h. The time difference between when warmest and coolest areas reached 50 C in cooker I ranged from 0.5 to 2.6 h. For cooker II, this range was 0.5 to 3.4 h. Results suggest that growth of the two organisms may occur in certain areas within cookers if they were overloaded but not when they are used according to the manufacturer's directions.

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**A National Uniform Foodservice Manager Sanitation Training and Certification Program.** Charles Dee Clingman. *National Institute for the Foodservice Industry, 120 South Riverside Plaza, Chicago, IL 60606.*

The National Institute for the Foodservice Industry has been chosen by the U.S. Food and Drug Administration to develop a plan for implementing a uniform national foodservice manager sanitation training and certification program. An inventory of all foodservice manager certification programs in the United States was completed. From this inventory a compendium of such training programs was published by NIFI in cooperation with F.D.A. A national committee including representatives from regulatory agencies, industry, education, and trade associations utilized this research compendium and assisted NIFI with development of a uniform national program. The need for uniformity is critical to achieve reciprocity in a high turnover industry. Reciprocity between regulatory agencies at all governmental levels and within industry can be attained if certification programs are based on an agreed national standard uniformly applied.

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**A Sensitive Procedure for Detecting Salmonellae on Whole Broiler Carcasses Without Pre-Enrichment.** N. A. Cox, A. J. Mercuri, J. E. Thomson and J. S. Bailey. *USDA, ARS, Russell Research Center; P.O. Box 5677, Athens, Georgia 30604.*

Four enrichment broths were evaluated to determine their efficiency for detecting low levels of salmonellae on whole broiler carcasses without preenrichment. Each broiler carcass was artificially inoculated with 6-20 cells of a nalidixic acid-resistant strain of *Salmonella typhimurium.* The carcass was then shaken vigorously in a polyethylene bag with 270 ml of sterile water for 1 min, allowed to drain into and then removed from the bag. A concentrated solution of selenite cystine (SC), selenite brilliant green (SBG), selenite brilliant green sulfa (SBGS) or TT broth was added to the rinse water to give a single strength solution before incubation. After 24 h a loopful was streaked onto a plate of MacConkey agar containing 100 ppm nalidixic acid to detect the test organism. The test organism was rarely detected with SBGS or TT; however with SC or SBG it was consistently detected. The marked organism was recovered from 39 of 40 carcasses with SBG and 40 of 40 carcasses with SC. Similar results were obtained when *Salmonella anatum, Salmonella montevideo* and *Salmonella saint-paul* were inoculated on each carcass. This study indicates that direct enrichment of carcass rinse water with SC or SBG is very effective in detecting a low level of salmonellae on broiler carcasses and is non-destructive.

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**Degradation of Aflatoxin by Potassium Bisulfite.** M. P. Doyle and E. H. Marth. *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

Potassium sulfite, in the form of $HSO_3$, can react with both aflatoxin $B_1$ and $G_1$ resulting in their loss of fluorescence. The reaction approximated first order kinetics dependent on the bisulfite concentration. Aflatoxin $G_1$ reacted more rapidly with bisulfite than aflatoxin $B_1$. For example, in the presence of 0.15 M $K_2SO_3$ and at pH 5.5 (0.144 M $HSO_3$), the specific reaction rate constant for degradation of aflatoxin $G_1$ was $2.09 \times 10^{-2}$ h while the specific reaction rate constant for degradation of aflatoxin $B_1$ was $1.58 \times 10^{-2}$ h. No new degradation products were detected when TLC plates containing the bisulfite-treated aflatoxins were observed under long wave U. V. light. However, when TLC plates containing bisulfite-treated aflatoxin $B_1$ were sprayed with 5% $H_2SO_4$ and examined under long wave U. V. light, at least two new spots were noted. When relating the bisulfite-aflatoxin reaction to similar reactions involving bisulfite and conjugated lactones, it appears that the bisulfite reaction involves addition of potassium bisulfite to the lactone part of the aflatoxin molecule.

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**Inhibition of Enteropathogenic *Escherichia coli* by a Lactic Starter Culture.** J. F. Frank and E. H. Marth. *Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706.*

The behavior of enteropathogenic and nonpathogenic *Escherichia coli* was observed when grown in skimmilk with 0.25 and 2.0% lactic starter and incubation at 21 and 32 C for 15 h, and then after refrigeration of the cultured milk at 7 C. At 32 C, *E. coli* was

completely inhibited by both concentrations of starter culture after 6-9 h of incubation and an initial 1-3 log increase in numbers. Complete inhibition of growth by *E. coli* occured sooner at 32 than at 21 C, but smaller numbers were obtained at 21 C, almost no growth having occurred for some strains. Longest survival of *E. coli* in refrigerated fermented milks was about 17 days in milk fermented at 32 C with 0.25% starter. The combination of lower temperature and greater starter concentration was most effective in reducing the number of *E. coli* present in fermented milks. Three methods for enumeration of *E. coli* were compared, including trypicase soy agar (TSA) pour plates, violet red bile agar (VRBA) pour plates, and TSA surface plating with a VRBA overlay. This final method appeared to allow for recovery of stressed *E. coli* cells and gave results similar to the TSA pour plate method.

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**Impedance Changes in Raw Milk as an Alternative Method to the Standard Plate Count.** S. O. Gnan and L. O. Luedecke. *Department of Food Science and Technology, Washington State University, Pullman, Washington 99164.*

Impedance changes in a medium provides a means for monitoring microbial metabolism and growth. The Bactometer 32 was evaluated as an alternative method to the SPC on raw milk. Four treatments were used in the evaluation: raw milk (RM); raw milk plus 1% yeast extract (RM + YE); preliminary-incubated milk (PI); and preliminary-incubated milk plus 1% yeast extract (PI + YE). The number of samples examined in each of the above treatments was: 103, 106, 102, and 107, respectively. Preliminary incubation was at 13 C for 18 h. The SPC was done at the time the samples were placed in the Bactometer. The mean SPC of the RM, RM + YE, PI, and PI + YE samples was 43,000; 25,000; 310,000; and 540,000, respectively. The mean detection time (DT) for each of the above treatments was 10.5, 9.7, 8.3, and 6.1 h, respectively. Of the four treatments used, RM + YE and PI + YE gave the best results. If a SPC of <100,000/ml was considered to be acceptable and a DT of 7.5 h was selected as the cutoff time, 99% of the RM + YE samples were classified correctly. When a 6-h cutoff time was used, 85% of the PI + YE samples were correctly classified when a 200,000/ml SPC was used.

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**Fouling of Heat Transfer Surfaces by Fluids Containing Soluble Proteins.** A. C. Ling and D. B. Lund. *Department of Food Science, University of Wisconsin-Madison, Madison, Wisconsin 53706.*

A major problem which can be encountered when biological fluids are heated is the deposition of components from the fluid on the heating surface. This results in loss of efficiency in the heat transfer process and usually contributes to requirements for more severe cleaning regimes for returning the surface to the original clean condition. Since proteins have been implicated as a major source of the problem in heat exchanger fouling, we studied the effect of process and fluid variables on deposition from protein solutions. An electrically heated stainless steel surface was exposed to egg albumin solutions flowing under a variety of temperatures, fluid flow rates, and concentrations. The system for studying the rate of burn-on is unique and has been described elsewhere. The surface temperature of the stainless steel surface could be monitored and controlled to any desired temperature. Results of the study indicated: (1) fouling rate increased with an increase in protein concentration, (2) increasing the fluid flow rate did not have a significant effect on rate of fouling, (3) increasing the temperature difference increased fouling rate, and (4) surface finish did not influence fouling.

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**Potential Hazards Associated with School Hot Lunch Programs.** Mitsuru J. Nakamura. *Department of Microbiology, University of Montana, Missoula, Montana 59812.*

During 1976 the turkey pot pie served to students in Missoula, Montana in the school district's hot lunch program may have been the cause of an outbreak of food poisoning. Many of the children did not eat the turkey pot pie because of its appearance and odor. However, among those who consumed the food a number of students became ill with classical symptoms of *Clostridium perfringens* food poisoning. A check of the total food preparation, food handling, and food delivery procedures indicated that there was the possibility that the food was not maintained at a sufficiently high temperature to prevent growth of bacteria. Bacteriological examination of the left over food was made. In some of the turkey pot pie samples the following bacteria were present: *Escherichia coli, Enterobacter cloacae,* group D streptococci. Additional studies showed a high population of *C. perfringens.* The efforts of state, county, school district, microbiological diagnostic laboratory, and university scientists produced stringent guidelines to prevent future outbreaks.

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**A Comparison Study of the Antimicrobial Properties of Isolated Human Milk and Egg White Lysozymes.** Douglas Peck and K. Ostovar. *Department of Food Science, 111 Borland Laboratory, University Park, Pennsylvania 16802.*

The susceptibility of several food pathogens to isolated human milk and egg white lysozymes was studied. Spectrophotometric and disc-assay methods of analysis were employed to determine the effectiveness of lysozyme against the organisms. All gram-negative pathogenic and several gram-positive microorganisms tested exhibited vulnerability to both lysozymes. However, the degree of the susceptibility was found to be more pronounced with the human milk lysozyme. Incorporation of sodium chloride had little effect on cell lysis. Susceptibility of the organism to lysozyme was altered by age of the culture.

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**A Study of Coagulase-Positive Staphylococci in Salami Before Fermentation.** M. M. Pullen and C. A. Genigeorgis. *Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, Minnesota 55108, and Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616.*

Thirty-six samples of salami formulation were examined for coagulase-positive (CP) staphylococci. A statistical analysis (Analysis of Covariance) was also done to evaluate variables and their effects on the dependent variable Y (number of CP staphylococci per gram). The variables evaluated were: (1) certification-non-certification, (2) daily sample order, (3) proportion of meat (pork picnics, pork jowls and beef) and (4) total plate count. The average coagulase-positive staphylococcal count was 3400 per gram (range 225 to 17000). Of the determinable variables only certification (avg. 500 CP staph per gram) versus non-certification (avg. 2100 CP staph per gram) was statistically significant. The additional freezing at -10 F (-24 C) for 10 days appeared to reduce the number of viable staphylococci.

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**Assessment of Foodborne Disease Reporting in Canada for 1973-1975.** E. Todd. *Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada.*

Before 1973 the only foodborne diseases reported in Canada were "staphylococcal food poisoning," "botulism" and "*Salmonella* infections with mention of food as vehicle," and not all of these by all provinces. Cases were published weekly and annually by Statistics Canada, but no other data were presented. Starting in 1973 the first systematic collection and collation of foodborne disease data was begun by the Health Protection Branch and information on incidents, cases, foods involved, agents responsible, places of acquiring the food and of mishandling the food, date of onset, clinical data and geographical distribution of incidents were collected. A detailed report was published for 1973 and similar ones are in preparation for 1974 and 1975. Between 378 and 440 separate incidents occurred; microbiologi-

cal agents were the main cause where the etiology was known, and meat and poultry were the foods most frequently involved. Mishandling in foodservice establishments was responsible for most incidents. These reports should stimulate better reporting of foodborne illness, development of improved methodology where information is lacking, and correction by the food industry of problem areas revealed by these reports.

## INVITED PAPERS

**Trouble Shooting a Mastitis Problem.** Sidney H. Beale. *Michigan Milk Producers Association, 24270 W. Seven Mile Road, Detroit, Michigan 48219.*

Mastitis is a very costly problem causing the farmer financial loss as a result of milk lost due to decreased production, discarded milk, medicine cost, animals culled from the herd and veterinarian fees. Mastitis is a luxury the farmer cannot afford yet there are many that feel it is something they must live with. There are three areas of concern when you are confronted with a mastitis problem: (1) management of the herd, (2) adequate milking system properly installed, and (3) sub-clinical or infectious mastitis. To be successful in correcting a mastitis problem requires the use of all resources. A team approach is used consisting of the dairy plant fieldman and laboratory, milking machine serviceman, area extension dairy specialist, veterinarian and, of course, the desire of the farmer to correct the problem. Every person on this team has an important part to play in solving the problem. The result of solving the problem is a financial gain for the farmer.

**Automated Milking Systems.** William G. Bickert. *Agricultural Engineering Department, Michigan State University, East Lansing, Michigan 48824.*

Numerous items of equipment for mechanizing operations in the milking parlor are available to dairymen. Also, a dairyman has several types of milking parlors from which to choose. Equipment and parlors are described and various systems are discussed from the standpoints of performance, initial investments, and annual operating costs. Automatic detaching units are emphasized including the circumstances necessary to justify their installation.

**Laboratory Quality Assurance.** A. Richard Brazis. *Laboratory Evaluation Group, Division of Microbiology, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.*

The effectiveness of Federal, state, municipal and industry laboratory programs for milk, food, water, and shellfish is dependent in part upon quality assurance. Interpretation of laboratory data can be affected by (1) sample collection methods, (2) change in sample composition during transmit to the laboratory, (3) analyst performance, and (4) errors during transmittal of data. Variability of analysts' results within a laboratory can be influenced by facilities, equipment, materials, methods and analyst performance; results of analysts in two or more laboratories can vary significantly due to poor quality control practices. Effective quality assurance programs will identify problems that prevent achieving and maintaining good laboratory practices. Laboratory supervisors in government, commercial and industry laboratories should reassess their quality assurance programs to assure good laboratory practices.

**Rapid Automated Impedance Screening of Milk for Microbial Content.** P. Cady, D. Hardy, S. W. Dufour, and S. J. Kraeger. *Bactomatic, Inc., Palo Alto, California 94303.*

Microorganisms change the electrical impedance of the medium in which they grow when they reach levels between $10^6 - 10^7$ organisms/ml. The time required to reach detection (DT) provides an estimate of initial microbial levels in the sample. DTs were compared to Standard Plate Counts (SPC) for over 500 milk samples including homogenized, low fat, skim, pasteurized, and raw milk. The correlation between DT and SPC allowed screening for various levels of organisms in times of 7-13 h—the higher the level of the screen, the shorter the DT, (e.g., a DT of 7 h screens for $10^4$ organisms/ml and a DT of 13 h screens for $10^3$/ml). Raw milk gave a better correlation than did pasteurized milk. Furthermore, the impedance response of pasteurized milk was smaller as well as slower. Impedance screening provided a far more rapid test than SPC with a savings in labor and comparable costs. It provides an early warning of potential microbial problems and shows promise as an aid in quality assurance for milk processing.

**Training Opportunities for the Sanitarian.** Harry Haverland. *Cincinnati Training Facility, Division of Federal-State Relations, EDRO, Food and Drug Administration, 550 Main Street, Cincinnati, Ohio 45202.*

Short and long term training opportunities are available to the Sanitarian in milk, food and environmental sanitation. Short term technical training courses are presented at training centers or designated locations throughout the United States. In addition, homestudy courses are also offered. Training Bulletins identifying courses and locations can be obtained by contacting the appropriate Federal agency. Long term funded training programs and fellowships are offered through designated colleges, universities or Federal agency.

**Management of Use of Sludge on Cropland.** Charles F. Jelinek and George L. Braude. *Food and Drug Administration, 200 C Street, S.W., Washington, D.C. 20204.*

Passage of the Federal Water Pollution Control Act in 1972 has caused a huge increase in the amount of sewage sludge to be disposed of. The Food and Drug Administration has worked with the Environmental Protection Agency and other agencies to recommend proper management of the application of sludges to food and feed crops to ensure the safety and wholesomeness of the food supply. FDA's concerns about contamination of food for pathogenic microorganisms, heavy metals, persistent pesticides and industrial chemicals such as polychlorinated biphenyls (PCBs) are described. The reasons for especial concern about direct application of sludge onto growing food and feed crops are discussed. The estimated daily intake of lead and cadmium, as compared to the proposed tolerable daily intakes, is presented, together with FDA's program to develop sufficient data on the natural background levels of these metals in raw agricultural products. Limitations recommended to prevent hazardous cadmium, lead, PCB and pathogen contamination of food and feeds by sludge are discussed.

**Fieldmen-Planning their Work.** Edward A. Kaeder. *Mid-America Dairymen, Inc., 2424 Territorial Road, St. Paul, Minnesota 55114.*

Most milk producers, co-op leaders and plant managers feel their fieldmen are doing a good job. The fieldman's basic job is as the milk plant's personal contact with its member producers. In planning their work, the fieldman's basic reasons for contacting member producers include: milk quality, Grade A and Manufacturing Grade milk requirements, flavor control, dairy farm building plans, milking equipment installation, herd health, sales and financial arrangements for purchase of equipment, member relations and dealing with members' problems and complaints. The fieldman also works for his milk plant in the areas of procurement of new members, hauler relations, membership meetings, administration and record keeping, and cooperation in working with sanitarians. Along with the foregoing, fieldmen must maintain proper personal habits in dress, vocabulary, smoking and driving. There have been too few rewards given these industry ambassadors who must fill the varied roles of trouble-shooter, psychologist, sanitarian specialist and salesman. In the interest of energy conservation and driving time, a daily plan of farm calls in a

given area should be worked out by the fieldman. Most fieldmen can best work out their own plan of work, taking into consideration the location of the farm and the urgency of the calls to be made.

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**Lactose Intolerance-The Problem and Solution.** Robert S. Katz. *National Dairy Council, 6300 N. River Road, Rosemont, Illinois 60018.*

Lactose intolerance (LI) is defined as the occurrence of the gastrointestinal symptoms after ingestion of a test dose of lactose, usually 50 g in solution with water. The amount of lactose in an 8 oz. glass of milk is 12 g, and is in a mixture of proteins, fats, salts, vitamins, and water. Unfortunately many people, both in the scientific and health communities, believe LI is synonymous with milk intolerance (MI). The result has been unwarranted recommendations to reduce milk consumption. For the dairy industry this may mean a decrease in sales and to the consumer an unnecessary loss of milk's important nutrients. Current research to accurately measure the prevalence of MI will allow the dairy industry to join with medical and health professionals in identifying individuals with MI and offering them suitable alternatives: (1) cultured dairy products such as some yogurts or cheeses, (2) lactose-hydrolyzed milk, or (3) in some cases the recommendation to consume smaller quantities of milk at one time, more frequently throughout the day. Possible beneficial effects of milk containing bacterial cultures such as *Lactobacillus acidophilus* are also being studied. This research is a positive effort by the dairy industry to maintain milk's important role in a nutritionally balanced diet.

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**The Automated Pyruvate Method as a Quality Test in Grade A Milk.** R. T. Marshall and C. C. Harmon. *Department of Food Science and Nutrition, University of Missouri, Columbia, Missouri 65201.*

Pyruvate (PVA) was recommended to indicate bacterial numbers and activity in milk. Grade A raw and pasteurized milks were analyzed with a Technicon$^{R}$ Autoanalyzer II equipped with dialyzer module for quantitation of PVA reduced to lactate. The method was sensitive and reproducible to 0.1 mg of PVA/liter and differentiated $10^2$ from $10^3$ of certain psychrotrophs in milk held at 20 C for 24 h. Gram-positive bacteria had to be in higher numbrs. With raw milk significant correlations were observed between initial PVA and the log of Wisconsin mastitis test scores and between $\Delta$ PVA (20 C-24 h) and initial Standard Plate count (SPC) of abnormal milk. Psychrotrophic plate counts (PPC) were correlated with $\Delta$ PVA when milk came from normal quarters. In pasteurized milk $\Delta$ PVA was correlated with both initial SPC and PPC. In pasteurized milk held at 5 C for 17 days (PVA increased slowly and steadily in one group which contained mostly pseudomonads. Pure cultures of *Pseudomonas fluorescens* and *Pseudomonas fragi* but not gram-positive bacteria produced PVA profusely than its concentration decreased. (Contribution from the University of Missouri Experiment Station. Journal Series No. 7864.)

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**Antibiotic Residue in Milk Following Dry Cow Treatments.** J. H. Martin, M. E. Johnson, R. R. Baker, and J. G. Parsons. *Department of Dairy Science, South Dakota State University, Brookings, South Dakota 57007.*

Nineteen cows were treated via intramammary infusion at various times before calving with one of two dry cow antibiotic preparations (cloxacillin or penicillin). Milk samples taken from these cows after calving were analyzed for antibiotic residues using the *Sarcina lutea* cylinder plate method. Eight cows were treated with cloxacillin. Cloxacillin was detected in the milk of only two cows after calving and then for only one milking, so no problem should exist with cloxacillin if the manufacturer's recommendations are followed. Penicillin residues were not detected in milk samples taken from the cows treated more than 9 days before calving with the dry cow penicillin preparation. Seven out of nine cows treated with penicillin up to 9 days before calving had detectable penicillin residues in the milk from treated quarters after calving. However, only one cow had detectable penicillin residues longer than the manufacturer's recommended milk withholding time of 96 h, and this cow was treated only 3 days before parturition. Normal routine on a dairy farm and adherence to the manufacturer's recommendations should result in no antibiotic residue problems in milk from cows treated during the dry period for mastitis control with either cloxacillin or penicillin.

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**Pathogenic Non-Sporeforming Anaerobic Bacteria in Convenience Foods.** Paul R. Middaugh and Craig E. Bremmon. *Microbiology Department, South Dakota State University, Brookings, South Dakota 57007 and Department of Chemical Engineering, Michigan Technological University, Houghton, Michigan.*

Thirteen types of commercial convenience foods were examined for pathogenic types of non-sporeforming obligate anaerobic bacteria and for survival at 14 and 42 days of bacteria added to three types of foods. Obligate, non-sporeforming anaerobic bacteria occur in enormous numbers, to $10^{11}$/gram in feces. Since intestinal streptococci and *Escherichia coli* types are found in certain convenience foods, 13 types of commercial convenience foods were examined for anaerobic bacteria. No anaerobic bacteria were found in dilutions of $10^{-4}$ to $10^{-9}$ required to dilute out facultative streptococci. Survival of 12 species of pathogenic types of anaerobic non-sporeforming bacteria was studied after adding to ground meat and storage at +4 C and −18 C in oxygen-free gas. *Bacteroides fragilis* cells after 7 days at −18 C had 46% survival versus 0.05% of *Fusobacterium nucleatum. B. fragilis* cells stored for 42 days at −18 C survived in ground meat, mixed vegetables and chicken pot pie with 48, 46, and 50% survival, respectively. Anaerobic cells stored at +4 C for 14 days had reduced viability with 44 to 1% cell survival. These anaerobic bacteria connot be excluded as potential foodborne pathogens for some of the undiagnosed 56% of food illnesses reported in 1967.

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**Sanitation Training in Retail Food Stores.** Gale Prince. *Eisner Food Stores, Champaign, Illinois 61820.*

During the past year the Food Marketing Institute (FMI) has developed a voluntary sanitation training and certification program for employees in the retail food store industry. The program has been developed with the help of an ad hoc Committee composed of City-County, State and Federal Health Officials and members of the Quality Assurance and Sanitation Committee of FMI. Program content and method of teaching is designed to motivate employees in performing proper sanitation practices during their daily work routine in the interest of consumer protection. The course not only covers basic elements of supermarket sanitation; but, relates the potential for return on investment of an effective sanitation program. Each person undergoing the training will have a pre-class home assignment requiring about 1 h, a classroom session of 5 h, a home study session requiring about 8 h followed by a second classroom session involving review and testing. The program has been field tested with notable success. This Voluntary Sanitation Training and Certification Program covers all the basics for an effective sanitation program with material that is simplified for ease of understanding and job specific to aid an individual in application on the job.

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**Botulism Revisited.** R. B. Read, Jr. *Food and Drug Administration, 200 C Street, S.W., Washington, D.C. 20204.*

Activity concerned with the problems of *Clostridium botulinum* and its toxins in foods has continued with little abatement since the mushroom recalls of 1973-74. *C. botulinum* toxins continue to be a problem in commercial foods with the latest problems involving toxin production in peppers that were improperly acidified. In another sphere, several infants have been hospitalized because *C. botulinum* produced toxin while residing in the digestive tract. The registration

with FDA of the current domestic and foreign canners has been completed and a comprehensive evaluation of the thermal processes used is well underway. Research on *C. botulinum* in foods continues on such things as (1) effect of mold growth on subsequent growth of *C. botulinum,* (2) botulinal toxin production in fresh mushrooms, (3) characterization of *C. botulinum* Type F and G, (4) collaborative study of the mouse test for botulinal toxin, (5) serological tests to replace the mouse test, (6) development of a standardized *C. botulinum* toxin, and (7) outgrowth and toxin production by *C. botulinum* in hermetically sealed cheeses.

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**Mechanically Deboned Meat—Past, Present and Future.** Robert E. Rust. *Department of Animal Science, Iowa State University, Ames, Iowa 50011.*

Mechanically deboned meat is the residual meat removed from bones resulting from normal meat boning operations. Two basic processes are used, grinding and screen separation or pressure separation. The latter tends to yield a superior product for manufacturing purposes. Mechanically deboned meat tends to be high in collagen, moisture and fat and low in muscle protein. Without adequate quality control it could have high microbial counts. The USDA proposal of April 23, 1976 to allow its use in meat products has not been acted upon. Temporary permission for use was withdrawn as a result of court action brought about by some "consumer advocates." Currently, mechanically deboned poultry is allowed in processed meat products with about 150 million pounds produced annually. It is estimated that 1 billion pounds of red meats could be salvaged if this material would be allowed.

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**Automated Milk Plant Operations.** Dale A. Seiberling. *Seiberling Associates, Inc., 441 Clark Street, South Beloit, Illinois 61080.*

The continuing application of increased automation to milk processing operations has included in sequence (*a*) development of recirculation cleaning procedures, (*b*) development of permanently installed spray devices, (*c*) application of automatic control to the CIP (clean-in-place) process, (*d*) application of CIP cleanable air-operated valves, (*e*) installation of the "all-welded" piping systems, and (*f*) application of centralized control of all processing and cleaning operations. As dairy and food processing facilities have expanded with respect to both the physical size of equipment and piping systems, and in processing capacity, the opportunity for product loss has also increased. And, control of systems of ever increasing capacity by fewer people reduces the operator supervision of the process, and accordingly, contributes to less control of product, water usage, and chemical usage. A computer-based process/CIP control system can aid in implementing water and waste reduction programs by (*a*) improving control of the process, and (*b*) generating management information by direct process measurement that may subsequently be used to more effectively control the production process, training and supervision of personnel, and maintenance of processing and control systems. The computer serves a dual purpose of controlling *and* reporting, and represents the next logical addition to the automated dairy process of the future.

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**Microbiological Evaluation of Milk Supplemented with *Lactobacillus acidophilus*.** M. L. Speck. *North Carolina State University, Department of Food Science, Box 5992, Raleigh, North Carolina 27607.*

Pastuerized low fat milk to which is added *Lactobacillus acidophilus* has now become a product of major volume on the U.S. market. The product contains millions of *L. acidophilus* per ml and is considered as being in the general category of cultured milk products for the application of regulatory monitoring. There is a growing interest in developing standards for allowable minimum numbers of *L. acidophilus* in such products at point of sale. This can be accomplished best by use of the medium selective for lactobacilli such as Lactobacillus Selection Agar (LBS); non-selective media, such as APT agar, can also be used in enumerating *L. acidophilus.* The latter would provide less assurance that the counts were due to lactobacilli. The non-selectivity of APT, however, usually results in higher counts of *L. acidophilus.* There is need for application of accurate monitoring of such products to ensure the numbers as well as the identity of the culture used as the milk supplement.

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**The 16th National Conference On Interstate Milk Shipments.** H. H. Vaux. *Indiana State Board of Health, 1330 W. Michigan St., Indianapolis, Indiana 46206.*

The 16th NCIMS took several significant actions affecting essentially all phases of the program. A new Constitution and By-laws were approved, preserving the voting and office holding rights of nonreciprocal states, and prohibiting an individual from being elected to two successive 6-year terms on the Executive Board. The Procedures Manual was amended by reducing the resurvey interval from 90 days to 15 days. The Executive Board referred the Memorandum of Understanding and the comments submitted on it to FDA for consideration and final drafting. We expect it to become a reality in the near future. Significant recommendations to FDA regarding the PMO included retaining the *Bacillus subtilis* disc assay or equivalent method for all antibiotic testing, leaving pesticide testing frequency to the discretion of the health authority, requiring tamper-proof closures on fluid milk products only, and requiring procedural changes relative to bulk milk hauling operations. All Conference recommendations have been forwarded to FDA and, hopefully, will receive favorable consideration when the final draft of the PMO and related documents are drafted.

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## Food Additives and Industrial Flavors to Provide Growing European Market Through 1985

The European food additives and industrial flavors market will continue to grow throughout 1985, according to a new report by Frost & Sullivan, Inc., New York City. The market research firm sees continuing population increases, an improving GNP, the upgrading of real disposable income, and food cost competition as the major factors stimulating slow but steady market expansion.

The 345-page F&S study, covering eight Western European markets, finds total food additive and flavor sales volume reaching the $801-million level by 1985, from a projected $723-million in 1980 and $647-million last year. Volume rose 17-percent between 1974-1976, and is expected to rise another 22-percent by 1985.

West Germany, the U.K. and France, together, account for about 75-percent of the total additive use and consumption, a position expected to remain unchanged. This is attributed mainly to their respective overall food consumption growth, their more advanced food processing technology, and large-scale consumer demands for both exotic and convenience foods.

Flavors account for an estimated 40-percent of the additive market with sales incrementing at a rate of three-percent yearly, all in the form of new sales.

Supply problems affecting availability of gums and exudates used as thickening agents have led directly toward the development of a substantial starch market. Here, the U.K. uses more than double the amount required in other markets due, no doubt, to her large consumption of soups, gravies and custards.

Antioxidants and preservatives are seen undergoing slower development, particularly noticeable after 1980. This is expected due to the emergence of less controversial, more suitable substitutes, and one of this category's main sale inhibitors—its relatively short shelf life.

The coloring sector, the most tangible of food items, is highly vulnerable to consumer criticism, and is forecast to show the least growth. Subject to growing legislative disciplines, scores of coloring agents have been outlawed, especially in France.

The structure of the industry is, according to Frost & Sullivan, "undergoing a clear-cut division between food additive supply companies and food additive specialists purveying supplies services, and joint development. Competition, spurring increased marketing and sales efforts among large companies —IFF, Naarden, Givaudan (Hoffman LaRoche) Albright & Wilson, Bush, Boake, Allen, Firmenich—has cemented closer relationships between manufacturers/formulators and end-users. This makes food manufacturers reliant on a particular source supply.

Constraints, identified by Frost & Sullivan, exist in the form of

significant reductions in food industry stock levels resulting in a short term market view and reduced purchasing commitments (quantity and contract time).

The opportunities, on the other hand, are expected to result from increased use—in most markets—of natural flavors, particularly in the soft drink, protein food and bakery sectors. Acidulants are expected to be in brisk demand, mainly in Italy and Germany. Though lethargic, the antioxidant and preservatives category will find increasing demands for ascorbic acid. On the flavors front, large producers will transfuse their respective sales volume by offering flavors as part of an overall service package.

And where enzymes are concerned, they show promise in view of their ability to simplify and improve production techniques. The expanding use of hydrolized vegetable protein and high protein yeast adds momentum to the thickening/agent sector, with glycerol monostearate the most popular item in the emulsifier & humectants collection. Finally, price and quality will rule as the most critical factors influencing progressive growth of the industry.

For more information, contact Customer Service, Frost & Sullivan, Inc., 106 Fulton Street, New York, N.Y. 10038, (212) 233-1080. Reference Report #E228.

## Book Review

*Staphylococci and Their Significance in Foods.* by Theodore E. Minor and Elmer H. Marth. Published by Elsevier Scientific Publishing Co., Amsterdam and New York. 1976. 297 pp. $33.50.

The authors have demonstrated their ability to gather much detailed information on *Staphylococcus aureus* and associated enterotoxins into a highly readable reference book, based to some extent on their earlier series of papers in the *Journal of Milk and Food Technology.* Probably one of the most noteworthy features of this book compared with other references on the subject is the ease of writing and clarity of presentation. Along with good style, however, a more important attribute is the extensive coverage. Information is given on historical aspects of staphylococcal infections, intoxications, and development of classification, which then leads into taxonomy of the organisms based on the latest edition of *Bergey's Manual.* Among the most useful chapters for laboratory workers are those dealing with isolation and identification and analysis of enterotoxins in media and foods. Although the reader should have no trouble in getting around this book, it might have been more helpful if some cross-referencing were used, e.g., lysis by specific bacteriophages is discussed in one chapter and bacteriophage typing in another with no connection made between the two descriptions. This is only a Minor point, or a Marth point, however, and does not detract from the value of the descriptive material.

Another valuable contribution of the book is the series of chapters on behavior of staphylococci in different foods because the authors very competently point out the characteristics of staphylococci and enterotoxins in direct relation to characteristics of foods, processing and storage conditions. A good description of mode of action of the enterotoxins is also given. One possible discrepancy in organization of the book is the inclusion of a chapter on "sources and transmission of enterotoxigenic staphylococci" as Chapter 9 when it is written as though it could have been introductory material. The sanitarian will be especially interested in Chapter 14 which deals specifically with control of staphyloccal foodborne disease by control of humans, animals, equipment and environmental surfaces and then by treatment of foods and by "people awareness."

A unique feature of the book is the summary at the end of each chapter, in which the authors "put it all together" in a concise manner. The references after each subject are extensive and are evidence of the care the authors have taken in reviewing their material. The book is definitely a worthwhile addition to the library of all personnel concerned with public health, food technologists, and laboratory workers in the food industry as well as a reference book for academic uses.

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## Coming Event

November 9, 1977
University of Maryland 33rd Annual Dairy Technology Conference, Center Adult Education, College Park, MD

# IAMFES Awards

P. J. Skulborstad (left), Senior Past President of the IAMFES, hands the Honorary Life Membership Plaque to Harold Heiskell. →

↑ (Left-right) J. E. Fike, Pennwalt Co.; Harold Bengsch, John Sloan, Diversy Chemicals; James Welch, Klenzade Products. Harold Bengsch stands with representatives of the 3 companies co-sponsoring the $1,000 Sanitarian Award.

## Harold Bengsch Named Sanitarian of the Year

Ensuring the health of over 130,000 people is no easy task. But, it is a task Harold Bengsch, Chief of Environmental Hygiene for the City of Springfield, Missouri, faces daily. For his dedicated service in this respect, Mr. Bengsch received the

Phil D. Bautz (left) hands the Educator Award Plaque to Dr. E. H. Marth. Bautz represents the Farm & Industry Equipment Institute, sponsor of the $1,000 Educator Award. →

## Dr. E. H. Marth Receives Educator Award

Dr. E. H. Marth, food microbiologist at the University of Wisconsin, received the Educator Award from the IAMFES for his outstanding contributions to the advancement of food hygiene.

The Educator Award, which includes a plaque and $1,000, went to Dr. Marth for his prolific research and tremendous educative insight.

The scope of the Educator Award

## Harold Heiskell Given Honorary Life Membership

Harold Y. Heiskell received Honorary Life Membership from the IAMFES for his devoted service. He was awarded the Honorary Life Membership plaque at the 1977 IAMFES Annual Meeting in Sioux City, Iowa.

Now semi-retired, Heiskell served the dairy industry in the Midwest and West Coast. He began his career selling Sharples Cream Separaters in 1934. Shortly after, an opportunity with Babson Brothers Co. led to his appointment as divisional manager of Surge for the Missouri, Kansas and Oklahoma region.

In 1945 Heiskell started his own dairy supply dealership in Arizona. There, he did much to further dairy sanitation. He designed the tri-level milking parlor which developed into the low-line parlor system used today. In 1969 Heiskell left his dealership and moved to California. He worked for Babson Brothers Co., eventually moving to his current position of Management Consultant.

Along with Heiskell's work in the industry he did a great deal to forward the goals of the IAMFES. He served a number of years on the Dairy Farm Methods Committee. Each of those years the IAMFES witnessed a strong gain in membership.

Heiskell was also Chairman of the Local Arrangements Committee for the 1971 IAMFES Annual Meeting in San Diego. Further, he helped found the California Sanitarians Association.

Currently Heiskell is a member of the Food Industry Association, the Arizona Dairy Industry Conference, the California Dairy Industry Association and the National Mastitis Council. Born in Green County Missouri, he now resides in Sacramento, California.

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1977 Sanitarian of the Year Award from the IAMFES.

Since he was enstated to his present position in 1969 Bengsch has done much to further the health and welfare of his community. He developed an epidemiological team for investigation of reported foodborne disease outbreaks. In addition to aiding physicians with case data, the 4-member team follows up foodborne disease outbreaks and prevents recurrences.

Further, Bengsch developed a routine program to protect consumers from diseases transmitted by shellfish. Springfield is now one of the few inland cities with such a program.

As a member of the Missouri State Milk Board, Bengsch has given the Springfield dairy industry a strong voice at the state level. Through his activities the Springfield community is assured a safe, high-quality milk supply. The milk control program under his supervision consistently receives sanitation compliance ratings and enforcement ratings in excess of 90%.

Bengsch's devotion to public health goes much deeper. Under his direction the Springfield Department of Public Health and Welfare (SDPHW) conducted a comprehensive "Community Environmental Health Analysis." The analysis established problem areas needing progressive environmental programming. As a result, the SDPHW gained federal support in a Childhood Lead Poisoning Project which included housing inspection and detoxification. Parents of some 10,000 children under the age of 5, now have the assurance that something is being done to prevent lead poisoning casualties.

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concerns work of the past seven years. In that time Dr. Marth is accredited with extensive research in food quality. By addressing himself to problems of salmonellae in dairy products and staphylococci in the foodservice industry, he provided valuable research information. He also researched contemporary problems of enteropathogenic coliforms, mycotoxins and psychrotrophic bacteria. Results of his works have received international attention.

As a professor at the University of Wisconsin-Madison, Dr. Marth teaches regular courses in foodborne diseases, fermentations, sanitation and scientific report writing. In addition, he teaches numerous short study courses dealing with food safety. His lectures are attended by students, farmers, laboratory personnel, foodservice workers, sanititarians, plant fieldmen, and regulatory and research personnel.

Dr. Marth's contributions to the IAMFES are bounteous. He joined as a graduate student in 1953. In 1967 he began as editor of the *Journal of Milk and Food Technology* and currently edits the *Journal of Food Protection.* He served on the Journal Management Committee, the Applied Laboratory Methods Committee and aided in preparing for publication *Procedures to Investigate Foodborne Illnesses* and *Methods for Production of High-Quality Raw Milk.*

Over his career, Dr. Marth has authored or co-authored over 260 publications. Dr. Robert Bradley Jr., also on the University of Wisconsin staff said, "Dr. Marth's mastery of technical writing certainly has facilitated the translation of technical data to understandable verbage in the shortest time possible. Because of this, industry gets useful information in the shortest possible time."

# Association Affairs

## AFFILIATES OF

## International Assn. of Milk, Food and Environmental Sanitarians

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## Notice-IAMFES Awards-1978

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

The success of this program is dependent not only on those organizations who so generously support the monetary aspects of these Awards Committee with appropriate information and names of potential award winners.

Will you please give serious thought to the following Awards, which will be considered for presentation at our 1978 IAMFES Annual Meeting.

1. *The Sanitarians' Award* of $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* of $1000 to a university or industry employee who has made outstanding contributions of food safety and sanitation. In 1978 the award will be made to an industry worker.
3. *The Citation Award* to a member who has given outstanding service to IAMFES in filling its objectives.
4. *The Shogren Award* to the affiliate organization that has the best statewide or regional program.
5. *Honorary Life Membership* to that member who has given long and outstanding service to IAMFES.

Please contact Harold Thompson, Jr., Chairperson of the IAMFES Recognition and Awards Committee, 5123 Holden Street, Fairfax, Virginia 22030.

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## Coming Event

December 12-16, 1977.
FOOD PROCESSORS ADVANCED MICROBIOLOGY SHORT COURSE. University of California, Davis Campus. $180. For further information, contact: John C. Bruhn, Dept. of Food Science and Technology, University of California, Davis, CA 95616. Phone: (916)752-2192.

## NOTICE to the MEMBERSHIP

In accordance with the IAMFES Constitution and By-laws, which requires a Secretary-Treasurer to be elected by mail ballot, the membership is hereby notified that President David Fry, at the annual meeting in Sioux City, Iowa appointed the following members to the nominating committee for 1978: C. K. Luchterhand, Floyd Bodyfelt, Clair Gotherd, Ben Luce, Ron Richter, and Ray Belknap.

Nominations for the office of Secretary-Treasurer are now open and any member wishing to make a nomination should send a biographical sketch and picture of his nominee to the nominating committee no later than November 1, 1977. To maintain proper balance on the Executive-Board the nominee should be selected this year from the Federal government level (USPHS/FDA).

R. A. Belknap
Chairman, Nominating Committee
International Association of Milk, Food, & Environmental Sanitarians, Inc.
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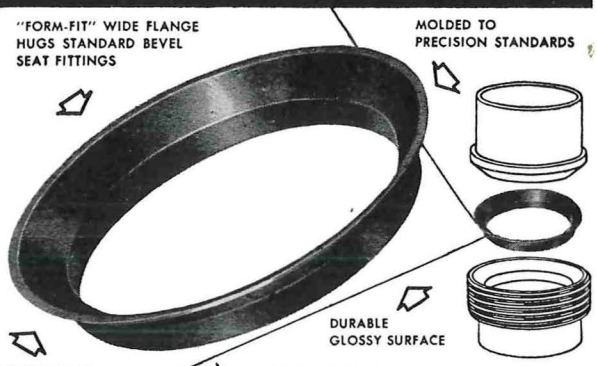


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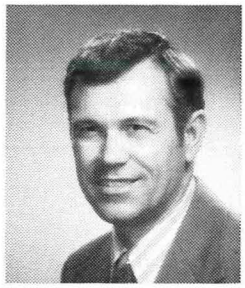
# A Better Milk Harvest Through Good Milking Practices

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



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

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

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

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

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

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