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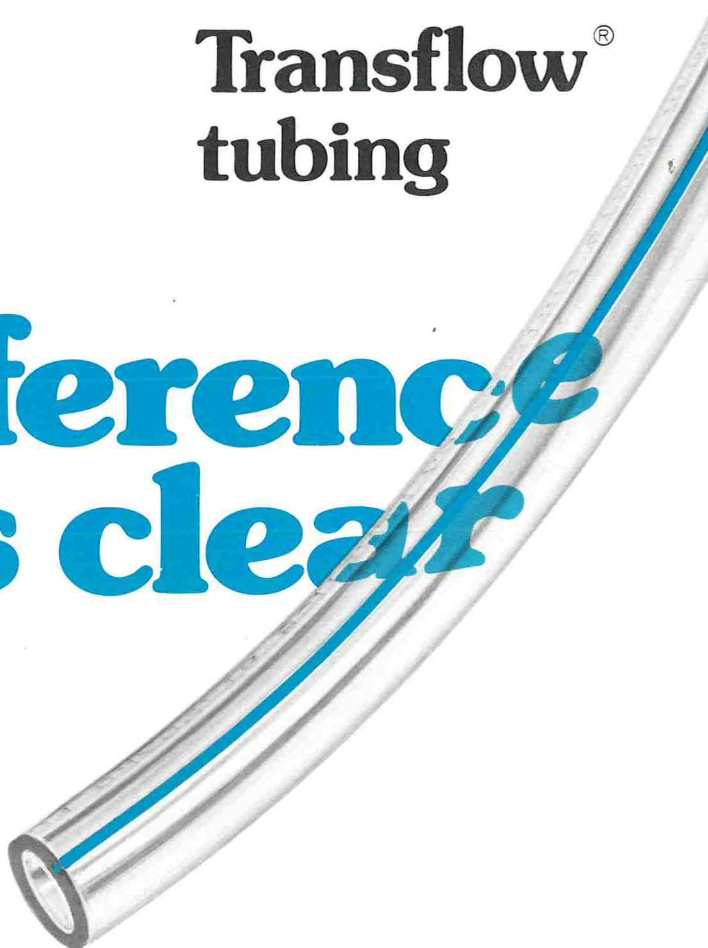

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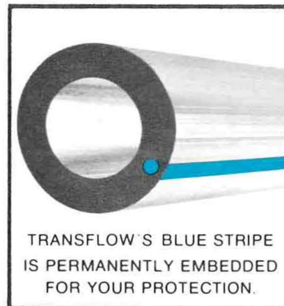

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Stability of Ascorbic Acid in Reconstituted Frozen Orange Juice

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(Received for publication February 14, 1977)

ABSTRACT

The sum of ascorbic acid and dehydroascorbic acid (physiologically available ascorbic acid), in reconstituted orange juice was remarkably stable over a 2-week period, both at 4 C and at room temperature. Stability was not affected by the aerating effect of blendorizing at high speed for 2 min. Stability was only partially due to the pH of orange juice, since available ascorbic acid declined more rapidly in a phosphate buffer solution at the same pH.

Considerable stability of ascorbic acid in fresh orange juice and in that reconstituted from frozen concentrate has been demonstrated in a number of studies (*2, 4-7*). Many consumers reconstitute their frozen orange juice with a blendor which probably introduces a great deal more O_2 into the solution than by hand mixing. Consequently, we compared the initial amounts of ascorbic acid (AsA), dehydroascorbic acid (DHA), and diketogulonic acid (DKG) in fresh and stored solutions which had been prepared by both procedures at room temperature (22 C) with that in the refrigerator (4 C). In addition, we have measured the stability of ascorbic acid at five pH values in phosphate buffer.

MATERIALS AND METHODS

AsA, DHA, and DKG were determined by a method adapted from that of Roe et al. (*7*). Reagent grade chemicals were used.

Frozen orange juice was reconstituted with glass-redistilled water using the proportions on the package. One portion was reconstituted in a Waring blendor, blendorizing at high speed for 2 min. Another portion was reconstituted by stirring. An aliquot from each was diluted immediately with 10% acetic acid-5% metaphosphoric acid. The remainder was divided into two portions, one of which was stored in the refrigerator (ca. 4 C), the other at room temperature (ca. 22 C). The 1-20 dilution was filtered. This was divided into three portions for oxidation, reduction, and untreated, in the same manner as the standard. All reported values are averages of duplicate determinations which agreed to 5%.

RESULTS

Effect of storage temperature

As shown in Fig. 1, in two series of analyses the available AsA (AsA and DHA) content of orange juice showed remarkable stability for 14 days, not only at refrigerator temperature but also at room temperature. The fermentation and scum formation, which occurred

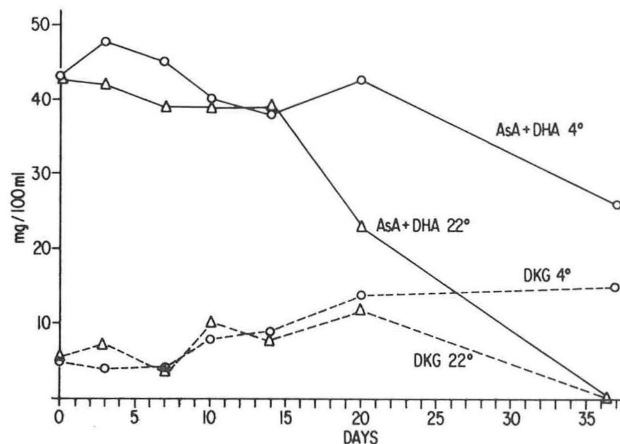

Figure 1. *Stability of ascorbic acid in reconstituted orange juice at 4 and 22 C. Each point is the average of duplicate analyses.*

at room temperature after a few days, made the orange juice unpalatable but did not affect the ascorbic acid content. A similar observation has been mentioned by Evenden and Marsh (*4*). Not unexpectly, conversions of ascorbic acid to DHA and of DHA to DKG proceed much faster at room temperature (22 C). DKG slowly accumulated at 4 C, whereas it is fairly rapidly degraded after 20 days at 22 C. After 14 days the available ascorbic acid decreased rapidly at room temperature and slowly at 4 C. Mills et al. (*7*) measured similar changes in the composition of orange juice stored at 2 C.

These data confirm and extend the long-term studies of Evenden and Marsh (*4*) who measured ascorbic acid by the indophenol dye method. The dye assay is not specific for ascorbic acid and does not measure DHA unless an extra reduction with H_2S is included (*5*). Thus the data in Fig. 1 provide a more detailed view of the stability of available ascorbic acid than those published previously.

Aeration during reconstitution

The oxidation rate of ascorbic acid to DHA might be affected by the amount of dissolved O_2 in the solution. To test this hypothesis, we compared the stability of ascorbic acid in orange juice reconstituted in a Waring blendor (high setting, 2 min) to that reconstituted by gentle

TABLE 1. *Stability of ascorbic acid, dehydroascorbic acid, and diketogulonic acid in phosphate buffer at various pH values*[a]

Days	pH 3.95			pH 5.25			pH 6.35			pH 7.20			pH 8.25		
	AsA	DHA	DKG	AsA	DHA	DKG	AsA	DHA	DKG	AsA	DHA	DKG	AsA	DHA	DKG
Refrigerator temperature (4 C)															
0	95	5	0	96	4	0	84	0	8	96	0	4	85	0	5
3	40	18	38	9	39	33	20	6	49	4	10	42	10	0	24
7	10	15	60	12	8	56	0	14	40	2	13	20	5	0	11
Room temperature (22 C)															
0	95	5	0	96	4	0	84	0	8	96	0	4	85	0	5
3	0	12	58	3	14	42	8	2	13	5	3	0	6	0	6
7	0	4	62	2	0	26	0	0	5						

[a]Ascorbic acid was dissolved in the indicated buffer at a concentration of 100 mg/100 ml. Concentration of each acid is listed as mg/100 ml.

stirring. There was no significant difference in the content of (AA + DHA) in the two solutions, stored at either temperature (data not included).

Effect of pH

The instability of DHA in neutral solutions has been well documented (*5*). Since similar studies have apparently not been carried out, in which AsA, DHA, and DKA have all been measured, we determined the concentrations of the three substances in 0.1 M phosphate buffer at five pH values at 4 and 22 C up to 14 days of storage. The results are in Table 1.

Ascorbic acid in buffer solution at pH 3.95 at 4 C was not nearly as stable as in orange juice at pH 4.0. In 3 days 38% had been converted to DKG, and this increased to 60% in 7 days. At pH 5.25 the oxidation to DKG was somewhat faster and at the higher pH values most of the biologically available ascorbic acid was gone in 3 days. The relative amounts of DHA and DKG decreased even faster at higher pH values and at the higher temperature. These data confirm and extend the study of Mills et al. (*7*) on the stability of DHA in potato slurry at pH 6.7.

DISCUSSION

On the ascorbic acid concentration of fresh and/or reconstituted frozen orange juice

The values of available ascorbic acid reported in this work (44 mg/100 ml) agree, within the limits of error, with those reported by Mills et al. (*7*), Consumers Union (*3*), and Bissett and Berry (*2*). On the other hand, Rohrer and Treadwell (*9*) found an average of 58 mg/100 ml in fresh juice from Valencia and Navel oranges; Evenden and Marsh (*4*) reported 70 mg/100 ml in fresh orange juice; and Horowitz et al. (*6*) 90 mg/100 ml in reconstituted frozen orange juice. The reasons for these discrepancies are not clear.

On the stability of available ascorbic acid in orange juice

Biologically active ascorbic acid, the sum of ascorbic acid and dehydroascorbic acid, remained remarkably constant in reconstituted orange juice for at least 2 weeks at room or refrigerator temperatures, and independently of whether it was prepared in a blendor or by stirring. This conclusion is similar to that of Horowitz et al. (*6*). They found much higher relative amounts of DKG in their stored samples than we. This may be due to their use of New York City tap water as diluent compared to our use of distilled water. Bissett and Berry (*2*) and Evenden and Marsh (*4*) have reported on the long-term stability of ascorbic acid, determined by the indophenol method (*1*), in single strength orange juice and frozen concentrated orange juice. Our results confirm and extend these previous studies.

Effect of buffer and pH on stability of ascorbic acid

Ascorbic acid is much less stable in phosphate buffer than in orange juice at the same pH. Orange juice must contain some factor(s) which protects ascorbic acid against oxidation besides that of pH. Possibly citrate, a known chelator of heavy metal ions, inhibits the catalytic oxidation of AsA. At higher pH values and at room temperature all three reactions, (*a*) oxidation of AsA to DHA, (*b*) hydrolysis of DHA to DKG, and (*c*) the decarboxylation and further degradation of DKG to a mixture of products proceed much more rapidly in phosphate buffer.

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Consumers Can Detect Light-Induced Flavor in Milk

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(Received for publication February 9, 1977)

ABSTRACT

Two-thousand consumers were surveyed at three county fairs in Vermont to determine their taste preference for samples of good milk and milk with light-induced flavor defect. More than 73% of the people surveyed preferred the good milk sample. More females than males could taste a difference between the two samples, had a preference for one sample, and preferred the good sample. The data suggest strongly that it is in the best interests of the dairy industry to prevent light-induced flavor of milk.

In the United States approximately 93% of the dairy products sold are purchased at stores. The dairy cases are attractive and well-lighted with fluorescent tubes. Milk is packaged predominantly in opaque, plastic-coated paper or translucent, blow-molded plastic containers. Dairy scientists have known for many years that when light comes in contact with milk is initiates a series of reactions that lead to a flavor defect, sometimes called light-induced flavor. Researchers have found more recently that a large percentage of milk packaged in blow-molded containers and purchased at stores has a light-induced flavor. This could be one reason why consumers are drinking less pasteurized-homogenized milk.

Light-induced flavor in milk has been studied by many researchers and several good review articles have been written (*1,3,5,6*). However, data have not been published on consumer reactions to this flavor defect.

Last summer R. P. Rogers accepted the assignment for the Vermont Department of Agriculture to organize an exhibit for several County Fairs. As part of the Dairy Division exhibit, we discussed the possiblity of testing consumer reactions to a milk sample with light-induced flavor. Administrators in both The State Department of Agriculture and University of Vermont Agricultural Experiment Station agreed that this was an excellent opportunity to test if consumers considered a light-induced flavor disagreeable.

[1] *University of Vermont.*
[2] *Vermont Department of Agriculture.*

MATERIALS AND METHODS

Milk

Pasteurized-homogenized milk was obtained from the University Dairy Plant. It was pasteurized at 75.5 C for 15 sec, homogenized at 59 C and 2,500 psi, and packaged in 6-gallon bag-in-box dispensers.

Preparation of samples

Blow-molded, translucent, plastic gallon containers were filled with pasteurized-homogenized milk from a 6-gallon bag-in-box dispenser, capped, placed in a walk-in cooler at 4.5 C, and subjected to 400 footcandles of fluorescent light for 40 h. For several years, milk samples with light-induced flavor have been prepared the same way for state and regional Future Farmers of America judging contests. The 8-ft double-tube lamp (Sylvania F96T12-CW tubes) was mounted on its side and the samples placed next to the lamp. Light-intensity was measured with a General Electric color- and angle-corrected light meter with a 10× multiplier attachment. Control samples in 6-gallon bag-in-box containers were taken from the same milk supply and stored in the same walk-in cooler as the light-treated samples.

On the morning of each fair a 6-gallon bag-in-box dispenser was filled with light-treated milk from six blow-molded, plastic, gallon containers. At the fair, the two 6-gallon bag-in-box dispensers of milk samples (control and light-treated) were placed in a milk dispenser cabinet that was cooled to 4.5 C. An experienced flavor judge tasted the two samples at each fair and did not find a flavor defect in the control samples but did find a typical and definite light-induced flavor in the samples treated under fluorescent light.

Design of survey

Responses from a heterogeneous population were collected at three county fairs that represented the southern (Rutland), central (Chittenden), and northern (Orleans) geographical areas of Vermont. Data were collected over a 4-day period, 1 day at the northern and southern fairs and 2 days at the central fair. (The central fair was only 5 miles from the university.)

The dispenser cabinet was placed at the front of the Department of Agriculture exhibit. A poster was attached to the dispenser cabinet where it could be read easily by people as they passed the booth. Printed on the poster was "Free Milk" and "Please Taste These Two Milks."

Approximately 1 ounce of milk sample was poured into coded, 3½-ounce dixie cups. A random letter and two numbers were marked on the bottom of each cup. Sample codes were changed each day.

Ideas for a simple set of questions were found in a publication by Larmond (*4*). Consumers who volunteered to taste the two milk samples were asked the following questions: (*a*) Can you taste a difference between the two samples? (*b*) If you can taste a difference, do you prefer one sample over the other? (*c*) If you prefer a sample, which sample do

you prefer? As part of the Department of Agriculture exhibit, free samples of Vermont cheeses were given away. This promotion helped draw people to the booth.

When a consumer tasted the samples and responded to the questions, data were also recorded for sex and age. Consumers were divided into two groups, young and adult, based upon personal observation and judgment of the age of the tasters by the investigators manning the fair booths. Young people were classified as college age and younger, while adults were over 25 years of age. These subjective categories were selected because they were easiest to judge by observation. Data were statistically analyzed by examination of difference in proportions (2).

RESULTS AND DISCUSSION

Two thousand willing respondents for this survey were composed of a heterogeneous population from three geographical areas of Vermont. We know only their sex, approximate age, and the fact that they drink milk.

Responses to the questions asked each consumer are summarized in Table 1. A comparison of values for each fair show some variation, but the trend is the same for the three fairs. For consumers who could taste a difference between the two samples of milk and had a preference, more than three-fourths of them preferred the good milk sample. Statistical analysis showed a significant preference for the good milk sample ($P < 0.01$).

TABLE 1. *Mean percent responses of consumers at Vermont fairs who tasted good milk and milk with light-induced flavor defect*[a]

Answers to questions	Vermont fairs			
	South[b]	Central[c]	North[d]	All fairs[e]
No difference	8.4	4.0	5.2	4.8
No preference	5.0	1.0	1.3	1.7
Preferred light-induced	20.7	19.6	22.2	20.3
Preferred good	65.9	75.4	71.3	73.2

[a]Three questions were asked to each consumer: (*a*) Can you taste a difference between the 2 samples? (*b*) If you can taste a difference, do you prefer one sample over the other? and (*c*) If you prefer a sample, which sample do you prefer?
[b]299 consumer responses.
[c]1,332 consumer responses.
[d]369 consumer responses.
[e]2,000 consumer responses.

Consumer comments about the milk sample with a light-induced flavor were recorded. Of the 2,000 consumer responses, 20.3% preferred the light-induced sample. Many stated the milk tasted heavier, creamier, or richer, or that it had higher fat percentage. Some said it was sweeter; others said it had more flavor. Several recognized the flavor as one they were familiar with because they purchased milk in translucent, blow-molded plastic containers at stores.

Of the 2,000 people surveyed, 945 were male and 1,055 female. Data in Table 2 show that females are better able to detect flavor differences than are males. More females than males could taste a difference between the two samples, had a preference for one sample, and preferred the good milk sample. When sex was correlated with preference, statistical analysis showed females had a significantly greater preference for the good milk sample ($P < 0.01$).

TABLE 2. *Mean percent responses of male and female consumers at Vermont fairs who tasted good milk and milk with light-induced flavor defect*[a]

Answers to questions	All fairs	
	Male[b]	Female[c]
No difference	6.0	3.8
No preference	2.3	1.0
Preferred light-induced	21.7	19.0
Preferred good	70.0	76.2

[a]Three questions were asked to each consumer: (*a*) Can you taste a difference between the 2 samples? (*b*) If you can taste difference, do you prefer one sample over the other? and (*c*) If you prefer a sample, which sample do you prefer?
[b]945 consumers.
[c]1,055 consumers.

Age distribution of the consumrs surveyed was 47.3% young and 52.7% adult. No significant correlation ($P > 0.01$) was found between age and sample preference for each fair or all fairs combined.

The only information we have on consumer acceptance of milk is data tabulated for milk judging contests. And it must be kept in mind that the contestants in these contests were trained to identify flavor defect. In the Milk Quality and Dairy Foods contest at the Eastern States Exposition in 1975 and 1976, only 29% of the Future Farmers of America contestants from the Northeastern States identified correctly the milk sample that had a definite, light-induced flavor. (Milk samples with light-induced flavor were prepared the same way as those samples for this fair survey.) But all the contestants indicated that the light-induced samples had a flavor defect. These facts indicate that even trained individuals can have difficulty identifying the milk flavor defect correctly, but they do know when a flavor defect exists. They are obviously trained to be more discriminating than the general consumer.

Of the 2,000 people surveyed, 73.2% preferred the good sample. These data suggest strongly that it is in the best interests of the dairy industry to prevent light-induced flavor of milk.

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Effect of Conventional and Microwave Heating on *Pseudomonas putrefaciens, Streptococcus faecalis* and *Lactobacillus plantarum* in Meat Tissue[1]

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ABSTRACT

Conventional cooking in an oven at 176 ± 6 C proved to be more destructive than microwave cooking when individual strains of *Pseudomonas putrefaciens, Lactobacillus plantarum,* and *Streptococcus faecalis* were inoculated and grown in aseptically obtained meat tissue and then heated and compared at similar final internal temperatures. *P. putrefaciens* was the most heat sensitive microorganism in both cooking techniques. *S. faecalis* was the most heat resistant strain when cooked by conventional means but *L. plantarum* proved to be the most resistant when heat was applied by microwave energy.

In a previous study (*9*), when muscle tissue with a general microbial flora was cooked to three different reference internal temperatures, the conventional oven cooking technique proved to be more effective for microbial destruction than was microwave oven cooking. However, due to the mixed microbial contaminants used no information was obtained on the effect of these two heating methods on individual strains of microorganisms in a muscle tissue medium.

The present study was undertaken to follow the dynamics of thermal destruction of selected microbial strains inoculated into sterile beef muscle tissue obtained by the core technique (*6*).

The bacteria used in this study included some of the most common contaminants of meat. *Pseudomonas putrefaciens* is an aerobic psychrotrophic organism normally occurring in meat and poultry plants (*12*). Due to its ability to grow at low temperature, it was often found as the predominant organism associated with meat stored at refrigerated temperatures (*11*). It was implicated as the main spoilage organism in non-vacuum packaged fresh sliced beef stored at 1-7 C for 5 weeks (*15*).

Kleeberger and Busse (*8*) pointed out that *Pseudomonas* and *Lactobacillus* were the most important groups of organisms found in spoiled meat. *Lactobacillaceae* were predominant in the flora of vacuum packaged fresh beef and pork stored at low temperature (*1, 17, 19*). *Lactobacillus plantarum,* used in this study, was found as one of the species of *lactobacilli* most frequently isolated from meat products (*18*). This microorganism inhibited growth of other contaminants that would be the predominant flora in its absence (*20*).

Streptococcus faecalis was observed to be one of the most heat resistant of the common contaminants in meat (*22*). It was reported that *S. faecalis* represents 63% of the enterococci detected in food products (*4*). Recently it was suggested that enterococci may become predominant in the spoilage flora after inhibition of other microorganisms through production of bacteriocins (*10*).

MATERIALS AND METHODS

The specific microorganisms used in this study were obtained from the Department of Microbiology at The Ohio State University. The culture media used for *P. putrefaciens* and *S. faecalis* were tryptone glucose extract agar (TGA, Difco) as plating medium and nutrient broth (Difco) as the liquid medium for inocula preparation. The APT medium, in liquid and solid forms used for plating and cultivation of *Lactobacillus plantarum,* was prepared as described by Sharpe and Fryer (*21*).

The aseptic (aseptic to very low contamination levels) meat samples were obtained by the core technique described by Hone et al. (*6*) from the entire beef rounds of animals slaughtered at the Meat Laboratory of The Ohio State University. The core samples obtained (12 per round) were ground together using an electrical meat grinder with a head previously sterilized by immersion in alcohol for 24 h. Preliminary examination indicated that meat treated in the above manner would have microbial counts of less than 30 organisms per gram of tissue.

The aseptic muscle tissue obtained by this technique (approximately 1000 g/round) was divided into three aliquots and each section was individually inoculated with one of the selected strains of microorganisms recovered in 10 ml of sterile saline (0.9% sodium chloride) solution after separation of a 24-h liquid culture by centrifugation. The inoculated tissue samples were incubated for 48 h at refrigerated temperatures (5 ± 2 C) to allow the bacterial cells to adapt to the meat

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environment. At the end of the incubation period the total microbial population obtained for each species of microorganism and for each trial was rather constant suggesting that the microorganism growth cycle had reached the stationary phase.

Patties (50 g) of inoculated and incubated ground beef were prepared using sterilized aluminum weighing cups as molds, placed on sterile 12-cm paper plates and subjected to different temperature treatments in a conventional electric oven (Toastmaster Model H 540-R) maintained at 176 C and in a microwave oven (Hobart Model 125R) operating at 2450 MHz.

The different experimental internal temperatures covered a range of 30 to 90 C. These temperatures were obtained in the conventional oven by varying the heating period from 5 to 30 min. A similar internal temperature range was obtained in the microwave oven by a combination of four lengths of operation (30, 45, 60, and 90 sec) and five different locations in the oven cavity. Five patties, one in each location, were heated each time in the microwave oven and their positions are illustrated in Fig. 1. Positions 1, 2, and 3 correspond to

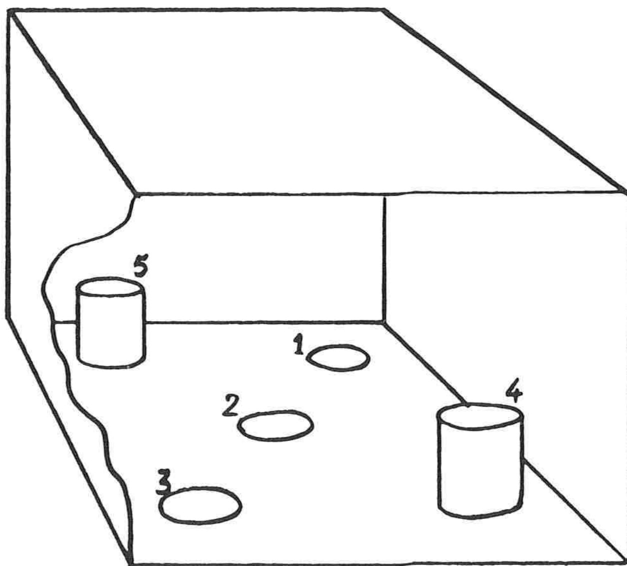

Figure 1. *Diagram of the position of meat samples in the microwave oven cavity.*

samples placed in paper plates directly on the floor of the oven cavity and positions 4 and 5 correspond to samples placed in paper plates and located on the top of inverted 100-ml glass beakers which elevated them 7 cm above the other samples.

After the heating treatment, the internal temperature of each patty was quickly determined by a laboratory thermometer which had been sterilized by immersion in alcohol. The whole patties were used as microbiological samples and appropriate dilutions were made with 0.1% peptone water. The samples were mixed with the plating medium, previously described for each organism, incubated at 25 C for 4 days, and counted to determine the number of survivors. The samples of meat not subjected to heating were used as controls to evaluate the initial counts.

The analysis for the best fitting curve for each strain of microorganism subjected to each heating treatment and statistical evaluation were determined by least squares analysis of variance as described by Harvey (5).

RESULTS

Table 1 gives the internal temperature means (six trials) and standard deviations obtained when 50-g beef

TABLE 1. *Internal temperature of 50-g beef patties heated for 30 seconds at different positions in a microwave oven*

Position[a]	Mean[b] temperature (C)	Standard deviation
1	50	1.7
2	39	0.9
3	54	1.2
4	49	1.2
5	52	1.5

[a]Positions refer to location in Fig. 1.
[b]Values reported are mean of six determinations of the same type of ground meat (same chemical composition).

patties were heated for 30 sec each in the different positions of the microwave oven cavity previously described (Fig. 1).

Figure 2 presents the log of the number of microbial survivors/g for *P. putrefaciens* versus the internal temperatures achieved by the two methods of heating tested. Figures 3 and 4 show similar data for *L.*

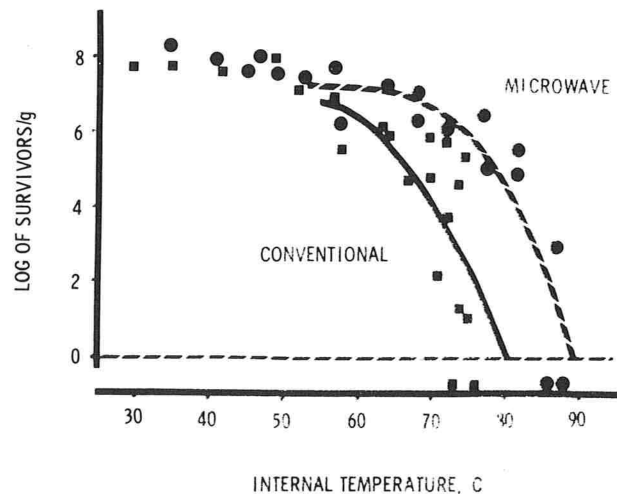

Figure 2. *Log of* Pseudomonas putrefaciens *survivors versus end-point internal temperature after heating in conventional and microwave oven. Points below the 0 line represents no bacterial recovery.*

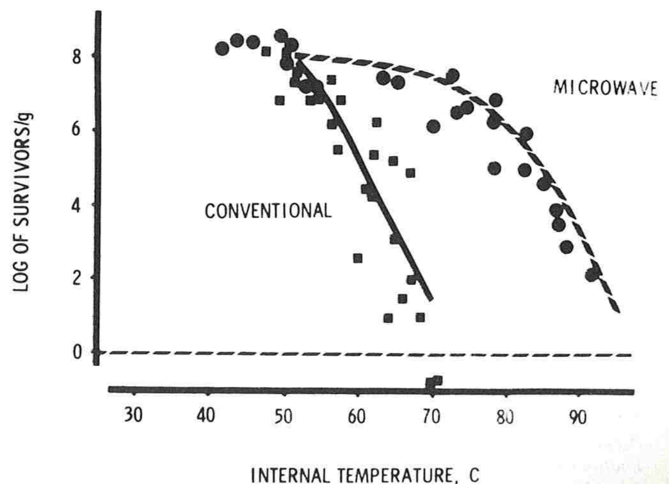

Figure 3. *Log of* Lactobacillus plantarum *survivor versus end-point internal temperature after heating in conventional and microwave oven. Points below the 0 line represent no bacterial recovery.*

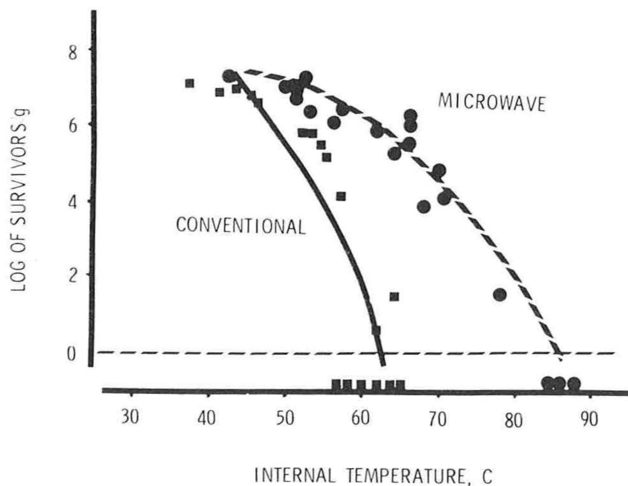

Figure 4. *Log of* Streptococcus faecalis *survivors versus end-point internal temperatures after heating in conventional and microwave oven. Points below the 0 line represent no bacterial recovery.*

plantarum and *S. faecalis,* respectively. The best fitting curves included in these figures correspond to the equations presented in Table 2 and obtained by the technique of Harvey (*5*).

By comparing the graphs it is obvious that the curves are of similar shapes but here are clear differences among the lines as illustrated in Table 3.

P. putrefaciens with a mean initial count of 8.3×10^7 microorganisms/g was very senstive to both conventional and microwave heating treatments. Increasing the temperature to 51 C was required by conventional heating to produce a 2-decimal reduction in the microbial population. To produce a similar effect with *L. plantarum* (initial count of 2.9×10^8/g), a temperature of 57 C was required and a temperature of 62 C was needed to get the same effect with *S. faecalis* (initial count of 1.9×10^8/g) which was the most resistant organism tested by conventional heating.

The temperature required to produce a similar effect (two decimal reductions) with microwave heating was 63 C for *P. putrefaciens,* an internal temperature that produced total microbial destruction in some of the samples when heated in the conventional oven. *L. plantarum* was very resistant to microwave heating. The 78 C required to produce 2-decimal reductions was higher than the 70 C that produced total reduction in the initial population of this microorganism when heated by conventional means. This temperature was also higher than the 74 C that was required to produce 2-decimal reductions in *S. faecalis* organisms using microwave heating.

TABLE 3. *Temperatures (°C) required to produce 2- and 4-decimal reductions in the microorganisms tested by heating with conventional and microwave energy*[a]

Bacterium	Conventional		Microwaves	
	Two reductions	Four reductions	Two reductions	Four reductions
P. putrefaciens	51	57	63	73
L. plantarum	57	62	78	87
S. faecalis	62	70	74	81

[a]Values in this table were calculated from the best-fitting curves equations presented in Table 2.

Although *S. faecalis* was more sensitive to microwave heating than *L. plantarum,* the enterococci were still more resistant to microwave than to conventional heating, following the pattern of the other microorganism tested. The temperature of 74 C required to produce 2-decimal reductions of *S. faecalis* with microwave heating was enough to produce complete destruction in one of the samples subjected to conventional oven heating.

DISCUSSION

The observed variability in the temperature distribution patterns in the microwave oven cavity has been reported in other studies (*2, 23*) and has resulted in vigorous activity in the design area to reduce this temperature variation.

As previously observed (*3, 9*), this study confirms that microwave heating to the same internal endpoint temperatures was less effective (for microbial destruction) than conventional heating. Also observed in this study were differences between microorganisms in the sensitivity to the two heating techniques. *S. faecalis* was the most resistant organism when conventional heating was used, while *L. plantarum* was most resistant to microwave energy. The high resistance of *Streptococcus faecalis* to heating was observed by Stoychew et al. (*22*) who reported that temperatures of 73 C do not assure total destruction of this organism in meat products. Also, Patterson and Gibbs (*16*) found that fecal streptococci were not completely destroyed after cooking chicken carcasses in an electronic oven with circulating air. The high sensitivity of *P. putrefaciens* to heat treatment in a meat medium was not surprising since psychrotrophic microorganisms often are not very heat tolerant (*14*).

TABLE 2. *Equations of the best fitting lines that relate numbers of survivors to end point internal temperatures for the individual species of bacteria and methods of heating*

Species of bacteria	Method of heating	Best fitting equations[a]
Pseudomonas putrefaciens	conventional	$Y = 2.7435 - 0.3827(X-57.9) - 0.0069(X-57.9)^2$
Pseudomonas putrefaciens	microwave	$Y = 6.4135 - 0.1156(X-57.9) - 0.0039(X-57.9)^2$
Lactobacillus plantarum	conventional	$Y = 3.4352 - 0.4141(X-63.65) + 0.0057(X-63.65)^2 + 0.0009(X-63.65)^3$
Lactobacillus plantarum	microwave	$Y = 7.5870 - 0.0302(X-63.65) - 0.0024(X-63.65)^2 + 0.0001(X-63.65)^3$
Streptococcus faecalis	conventional	$Y = 5.8382 - 0.2303(X-64.6) - 0.0100(X-64.6)^2 + 0.0001(X-64.6)^3$
Streptococcus faecalis	microwave	$Y = 7.3641 - 0.0328(X-64.6) - 0.0050(X-64.6)^2 - 0.0002(X-64.6)^3$

[a]Y = Log of the number of survivors; X = end point internal temperature.

The difference in heat sensitivity between conventional and microwave heat was most apparent for *L. plantarum*. This organism was not particularly heat resistant when heated by conventional means, although other lactobacilli responsible for green cores in sausage products have been found to be very resistant to heat treatments (*7, 13*). The observations described in this study on the higher resistance of *L. plantarum* to microwave energy seems to disagree with the results of Watanabe and Tape (*24*); however, these researchers used a two-stage process for applying microwave energy to frankfurter emulsion containing salt and nitrite. Under these conditions they found that lactobacilli and yeast were destroyed by the treatment but streptococci were not appreciably affected.

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Evaluation of Enrichment Broths for Enumerating *Vibrio parahaemolyticus* in Chilled and Frozen Crab Meat

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ABSTRACT

The ability to detect *Vibrio parahaemolyticus* in crab meat which had been held at refrigerated and freezing temperatures was determined using three enrichment broths. Glucose-salt-Teepol (GST), Horie-arabinose-ethyl violet (HAE), and water blue-alizarin yellow (WBAY) broths were equally effective in supporting growth of cells which had been held at −18 C. The HAE medium was superior to GST and WBAY for recovering *V. parahaemolyticus* from crab meat stored at +13 C. Cells were inactivated most rapidly when samples were held at +7 C. Growth of *V. parahaemolyticus* was observed in crab meat stored at +13 C for 3 days.

Vibrio parahaemolyticus has caused numerous large outbreaks of acute gastroenteritis in Japan over the past 25 years. Its presence in coastal waters and on shellfish at various locations around the world has been documented. It was only recently, however, that *V. parahaemolyticus* was incriminated as a causative agent in seafood-borne poisoning in countries outside of Japan. Sixteen outbreaks have been documented in the United States since 1969 (*2*). Two of these occurred on vessels during Caribbean cruises in early 1975 and involved 697 (49%) of 1437 passengers (*15*). Epidemiologic investigation implicated shrimp, crab meat, grouper, and lobster as vehicles of transmission. White crab meat was the food vehicle reported to cause *V. parahaemolyticus* infection on a chartered airflight from Bangkok to London (*16*) and an outbreak of non-cholera *Vibrio* enteritis occurred on a flight from Bahrain to Sydney (*7*). Other reports have confirmed gastroenteritis caused by *V. parahaemolyticus* in Indonesia (*12*), Togo (West Africa) (*5*), and Panama (*13*) and countries throughout the world.

Commercial catches of shellfish and sea fish may be iced and packed in holds aboard vessels for several days before being delivered to port. Upon delivery, catches may require additional icing if they are to be transferred by trucks or other conveyors to processing plants at distant locations. Seafoods are then either preserved by freezing or by refrigerating until offered for purchase in the market place or at eating establishments. Many bacterial genera have been shown to exhibit injury upon exposure to freezing or chilling environments. These bacterial cells are more sensitive than are non-injured cells to selective conditions in most enrichment broths. Nutritional needs and other requirements often change during the repair and recovery of debilitated cells. Recently it was demonstrated that cells of *V. parahaemolyticus* exhibit injury when chilled at 2 C for as little as 30 min (*4*). Others have reported that *V. parahaemolyticus* is readily inactivated at refrigerated and freezing temperatures (*6,11,14,18,19*). However, various inactivation rates have been reported, depending upon the type of seafood examined and the media used for detection and enumeration.

This study was conducted to determine the suitability of three enrichment broths for supporting recovery of *V. parahaemolyticus* from chilled and frozen crab meat. In addition, it was of interest to know the rates of inactivation of the organism during storage at various temperatures used to extend the shelf life of crab meat.

MATERIALS AND METHODS

Organism and culturing conditions

Four strains of *V. parahaemolyticus* were used in this study: 3D3-1, serotype 01:K38; 14D13, serotype 02:K28; T-3765-1, serotype 03:K7; and 8700, serotype 04:K11. Strains 3D3-1 and 14D13 are Kanagawa-negative while T-3751-1 and 8700 are positive. Strain 14D13 was isolated from steamed shrimp and the other three strains were isolated from stool specimens from patients suffering from gastroenteritis.

All strains were grown at 30 C in Tryptic soy broth (Difco, pH 7.2) containing 3.0% NaCl. Equal volumes of 22-h-old cultures of each strain were combined and diluted in 3% NaCl buffered at pH 7.2 with 0.1 M potassium phosphate. These suspensions were used as inocula for all storage experiments.

Preparation and inoculation of crab meat

Meat from blue crabs (*Callinectes sapidus*) was comminuted with a Toledo grinder equipped with an extrusion disk with holes 0.5 cm in diameter. Cell suspensions at two population levels (ca. 1×10^7 and 5×10^4) were added to the comminuted crab meat at a ratio of 1:10 (vol/wt). After thorough mixing, 25-g samples were deposited in sterile glass jars and immediately placed in forced-air chambers at -18, -2, +2, +7, and +13 C.

Enumeration of V. parahaemolyticus

After selected storage periods, duplicate inoculated and uninoculated samples were withdrawn, combined with 225 ml of 3% NaCl, and homogenized in a Waring blender at 8000 rpm for 1 min. The number of viable cells of *V. parahaemolyticus* per gram of crab meat was then determining using the three-tube Most Probable Number (MPN) technique. Three enrichment broths were evaluated for their suitability to support repair and growth of *V. parahaemolyticus*. Glucose-salt-Teepol broth (GST) contained, in grams per liter: peptone, 10; beef extract, 3; NaCl, 30; glucose, 5; methyl violet, .002; Teepol (Shell Chemical Co., Houston, Texas), 4; pH 7.4. Horie-arabinose-ethyl violet broth (HAE) (*10*) contained, in grams per liter: peptone, 5; beef extract, 3; NaCl, 30; bromthymol blue, 0.03; ethyl violet 0.001; arabinose, 5; pH 9.0. Water blue-alizarin yellow broth (WBAY) contained, in grams per liter: peptone, 10; sucrose, 10; NaCl, 30; water blue, 0.02; alizarin yellow 0.02; Teepol, 2; pH 6.9 GST and WBAY were sterlized autoclaving for 15 min at 121 C. Filter sterilized arabinose solution was added to the autoclaved HAE basal medium. Inoculated enrichment broths were incubated at 35 C for 18 h and tubes showing growth were streaked on thiosulfate-citrate-bile salts-sucrose agar (TCBS, BBL). Plates showing typical *V. parahaemolyticus* colonies after 18 to 24 h at 35 C were recorded and the MPN per gram of crab meat was calculated.

Total plate count (TPC) determination

Crab meat was examined for total plate count (TPC) by plating appropriate dilutions on plate count agar (Difco). Colonies were counted after 5 days of incubation at 21 C.

RESULTS

V. parahaemolyticus was not detected in uninoculated crab meat stored at various time/temperature conditions. Survival of *V. parahaemolyticus* in crab meat stored at -18 C for 24 days is shown in Fig. 1. Viable populations

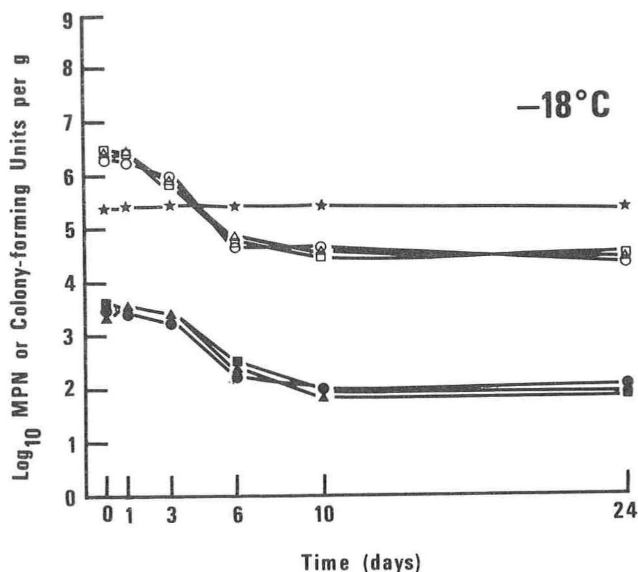

Figure 1. *Survival of* V. parahaemolyticus *in crab meat stored at -18 C. Symbols: circles, MPN of* V. parahaemolyticus *using GST enrichment broth; triangles, HAE; squares, WBAY; stars, total plate count (cfu) per gram of crab meat. Open circles, triangles, and squares indicate the highest initial test inoculum of* V. parahaemolyticus; *closed circles, triangles, and squares indicate a lower initial inoculum.*

dropped 1.5 to 2 logs during the first 6 to 10 days of storage and then remained constant up to 24 days. No overall differences were noted in the abilities of GST, HAE, and WBAY enrichment broths to recover *V. parahaemolyticus* from frozen crab meat. The TPC did not change during the 24-day storage period.

Populations of *V. parahaemolyticus* in crab meat stored at -2 C for various periods up to 15 days are shown in Fig. 2. A steady decline in viable *V. parahaemolyticus*

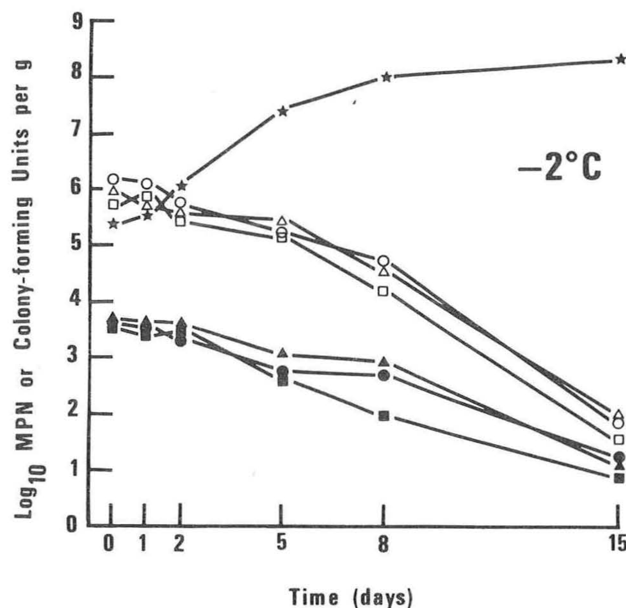

Figure 2. *Survival of* V. parahaemolyticus *in crab meat stored at -2 C. See legend for Fig. 1 for description of symbols.*

cells was observed over the test period. GST and HAE appeared to be superior to WBAY for supporting cell growth. The TPC increased about 1000-fold after 15 days.

Figure 3 shows data from crab meat stored at +2 C. *V. parahaemolyticus* declined more rapidly over the 15-day storage period at +2 C than at -18 C; die-off at +2 C

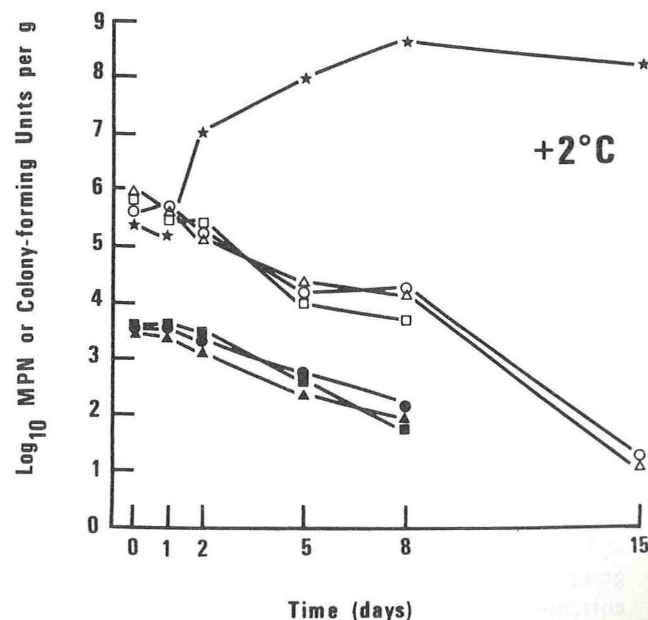

Figure 3. *Survival of* V. parahaemolyticus *in crab meat stored at +2 C. See legend for Fig. 1 for description of symbols.*

appeared to be slightly faster than at -2 C. The organism was not detected in 15-day samples which initially contained a MPN of 5×10^3 per gram, regardless of the enrichment broth used. WBAY was inferior to GST and HAE when used to enumerate cells from crab meat initially containing the higher inoculum level and stored for 8 or 15 days. In fact, *V. parahaemolyticus* was not detected in 15-day samples when WBAY was used as the enrichment broth. The TPC increased to over 10^8 colony-forming units (cfu) per gram during 8 days of storage at +2 C.

V. parahaemolyticus died more rapidly at +7 C (Fig. 4) than it did at -2 or +2 C. HAE was rated slightly better than GST for detecting viable *V. parahaemolyticus* when the initial population was high; WBAY was again poorer

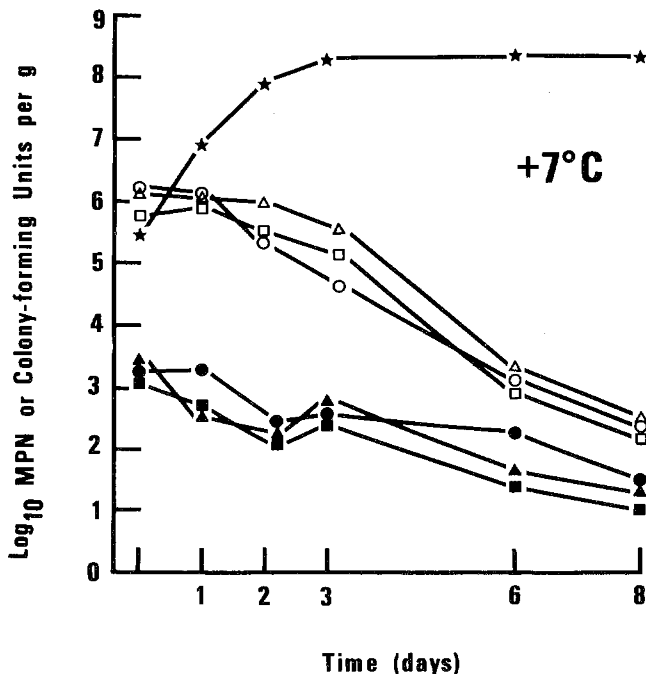

Figure 4. *Survival of* V. parahaemolyticus *in crab meat stored at +7 C. See legend for Fig. 1 for description of symbols.*

than GST and HAE as an enrichment broth for chilled *V. parahaemolyticus.* The TPC increased to 10^8 cfu per gram within 3 days.

Survival and growth curves for *V. parahaemolyticus* in crab meat stored at +13 C are plotted in Fig. 5. Analyses for *V. parahaemolyticus* were not made after 6 days because samples were deteriorated to a state beyond acceptance for human consumption. During the 3-day test period viable *V. parahaemolyticus* declined 10-fold or less in samples containing the highest test inocula. Analyses of the low-population samples for MPN of *V. parahaemolyticus* using GST and HAE revealed a similar trend during the first 2 days of storage. However, at 3 days *V. parahaemolyticus* increased sharply to reach greater numbers than were present initially. The WBAY enrichment broth was clearly inferior to GST and HAE. The latter medium was judged to be best as an enrichment broth for supporting growth of *V. para-*

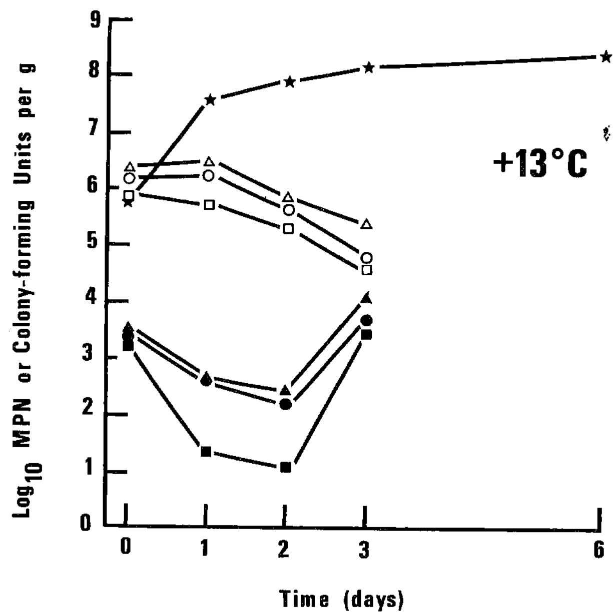

Figure 5. *Survival of* V. parahaemolyticus *in crab meat stored at +13 C. See legend for Fig. 1 for description of symbols.*

haemolyticus which had been stored in crab meat at +13 C. The TPC increased more rapidly at +13 C than at the other test temperatures.

DISCUSSION

In an earlier study it was reported that HAE and WBAY were superior to GST for isolating *V. parahaemolyticus* from non-heated crab meat (*3*). Cells which had been heated in a homogenate of crab meat were recovered in higher numbers from HAE. The superior growth-supporting characteristics of HAE and WBAY inoculated with pure cultures of chill-injured *V. parahaemolyticus* were also demonstrated (*4*). Although GST, HAE, and WBAY were shown in the present study to be equally suitable for recovering *V. parahaemolyticus* from crab meat stored at -18 C, HAE appears to supply the most suitable nutritional and physical environment for supporting repair and growth of the organism when actively growing psychrophiles are also present in the test sample. Many false-positives were noted when WBAY was used as an enrichment borth. These were divided equally between sucrose-fermenting and non-fermenting microflora, and were especially prevalent in crab meat stored at 7 and 13 C.

Certain species of *Pseudomonas* isolated from oysters have been shown to repress the growth of *V. parahaemolyticus* (*9*). Environmental conditions such as pH, NaCl concentration, and temperature of incubation influenced the degree of inhibition. It is probable that the relatively non-selective pH (6.9) of WBAY was conducive to growth of competing microflora which may or may not have produced anti-*Vibrio* metabolites. On the other hand, the alkaline pH (9.0) of HAE most likely had a strong selective effect in favor of *V. parahaemolyticus,*

since its chemical constituents would not particularly restrict the growth of many marine and psychrophilic bacteria.

Stoarage of crab meat at +7 C resulted in the most rapid die-off of *V. parahaemolyticus*. This confirms observation by others that the organism is more sensitive to chilling than to freezing. Faster rates of inactivation at +1 C than at -15 and -30 C were reported to occur in oysters (*11*). Survival of *V. parahaemolyticus* in fish homogenate, however, was observed to be greater at + 0.6 C than at -18 or -35 C (*14*). Rates of inactivation at various refrigerated and freezing temperatures are undoubtedly greatly affected by the nature of the supporting substrate, the rate of freezing, and the types of enrichment media used to resuscitate the organism. In any event, *V. parahaemolyticus* has been shown, in the present study, to remain viable at time-temperature conditions to which freshly caught shellfish would be subjected before processing and during marketing. Moreover, *V. parahaemolyticus* grew at +13 C, suggesting that crab meat with low levels of contamination could represent a health hazard if stored at this temperature for periods as short as 3 days. Growth of *V. parahaemolyticus* has been reported to occur in oysters stored at 10 and 12 C (*18*).

The initial total plate counts observed for crab meat in this study were in agreement with those noted elsewhere. Bacterial counts for fresh crab meat were reported in 1942 (*1*) to be 4×10^5 per gram; in 1976 (*7*) counts ranged from 4.4×10^4 per gram of fresh crab meat to 5.4×10^6 for meat stored at 4 C for 7 days.

Based on data from the experiments reported here, it appears that HAE would be a useful broth for detecting *V. parahaemolyticus* in chilled and frozen crab meat. Results from the present study strengthen observations reported elsewhere (*4*) regarding the superiority of HAE over GST for supporting the repair of chill-injured *V. parahaemolyticus*. Perhaps HAE could be used in parallel with GST, the enrichment broth presently recommended by the U.S. Food and Drug Administration (*8*), for isolating and enumerating *V. parahaemolyticus* in chilled, iced, or frozen seafoods at processing plants and in the market place.

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Accuracy and Speed in Counting Agar Plates[1]

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ABSTRACT

To determine the accuracy of colony counts made by analysts, agar plates were photographed. The agar plates and photographs were compared to obtain a true count (photocount) which was used to determine analyst accuracy over selected count ranges. Analyst accuracy was also determined by comparing analyst's counts to the mean of the counts obtained by several analysts ("established standard"). The "established standard" compared favorably with the photocount. Analysts' counts were within 5% of the photocount and "established standard" on 60 and 68% of plates having 30-300 colonies and 60 and 67% on plates having 20-200 colonies, respectively. Average counting time for plates in the 10-100, 20-200, 30-300, and 40-400 colony count ranges was 18, 30, 41, and 52 sec, respectively. Plates having 20-200 colonies were as suitable for counting as plates having 30-300 colonies and were counted with a time-saving of 27%.

Regulatory agencies and food industry quality control managers are concerned with the microbiological quality of food items. Over the years many methods have been developed to estimate microbial populations. An essential element in the establishment of an accurate estimate of the number of colonies on agar plates with any method is the ability to do an accurate colony count. Current standards (*1,2*) specify that Standard Methods Agar (SMA) plates containing between 30 and 300 colonies must be counted when estimating the microbial population of diary and food products. The colony count range group (CRG) of 30-300 has the advantage of including the counts from only one serial (1:10) dilution. However, Breed and Dotterer (*3*) had originally recognized the CRG of 20-400 to be acceptable. *Standard Methods for the Examination of Dairy Products* (*SMEDP*) (*1*) and Courtney (*4*) suggest the standards for counting colonies on agar plates require individuals to repeat their own counts within 5% and the counts of others within 10%. However, neither *SMEDP* (*1*) nor Courtney (*4*) specifies the methodology that should be used to determine repeatability. In a study specifically designed to test the ability of analysts to reproduce plate counts, Fowler et al. (*5*) found a coefficient of variation of 7.71% for individuals to reproduce their own counts as compared to an 18.19% coefficient of variation for persons to reproduce counts done by others.

The objectives of this study were to determine: (*a*) the counting accuracy of analysts within various CRGs, i.e., 10-100, 20-200, 30-300 and 40-400; (*b*) the feasibility of using the mean of several analysts as a counting accuracy standard; (*c*) the minimum CRG from which reasonably reproducible counts can be obtained; and (*d*) the time required to count plates in the four CRGs.

MATERIALS AND METHODS

A stock culture of *Escherichia coli* was used to prepare SMA pour plates. After incubation at 32 ± 1 C for 48 ± 2 h, each plate was assigned a randomly selected number. Each of the six analysts was assigned a code letter which was used to identify his data throughout the experiment. Except for recognizable colonial morphology on duplicate plates, analysts knew only that the predicted count was between 0 and 400 colonies per plate.

Each plate was photographed and immediately after the photographs were taken, the SMA plates were counted by analysts (*1*). Counts were made using a Quebec Colony Counter (American Optical Corp., Buffalo, NY 14215) and a hand tally. In addition, each analyst, equipped with a stopwatch, recorded the time in seconds required to count each plate. After all analysts had completed counting, the plates were stored in a refrigerator at 4 C. The photographs were processed into 8 × 10 inch black and white prints. The "photocount" or "true count" was determined according to the method previously reported (*6, 7*).

Plate count and counting time data were managed using the Statistical Package for the Social Sciences (SPSS) (*9*) implemented on a Control Data Corporation Model 7600 Computer. SPSS routines were used in the tabulation of data and calculation of percentages. Friedman's 2-Way Analysis of Variance with Multiple Comparisons Based on Rank Sums (Friedman's Test) (*8*) was used to test differences in CRGs. Creation of an "established standard" (mean of the counts on

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each plate for all analysts participating in the study) was accomplished by linking the SPSS file with a Fortran IV program. Statistical significance was based on the probability of 0.95, i.e., 5% level of significance.

Of the six analysts participating in the study, one has the Ph.D. degree, one the M.P.H. degree, two the B.S. degree, and the other two are technicians who have had a year of formal medical laboratory training. All analysts were experienced and were routinely making colony counts on SMA plates. Analysts were required to familiarize themselves with standard counting procedures (*1*). To insure that counting would be done uniformly by all analysts, a formal session to reinforce plate counting procedures was given to all analysts. In addition, before the experiment began a series of trials was conducted to acquaint each analyst with the recording sheets and to relieve analyst's anxiety concerning use of the stopwatch while counting plates.

RESULTS AND DISCUSSION

Analysis of data was undertaken in stages. Initially, the analyst's count for each plate was divided by the photocount and multiplied by 100. By taking the absolute difference between the resulting number and 100, the percent absolute difference (PAD) was calculated. The percentage of plates with a given PAD from the photocount was tabulated by CR. Table 1 displays the percent of plates analysts counted with PADs of ⩽ 5 and ⩽ 10 by CR. CRs of 0-⩽ 5 and 5-⩽ 10 are included in Table 1 for informational purposes.

TABLE 1. *Percent of analysts' counts within 5 and 10% of the photocount by count range*

Count range	No. of plates	Within 5% of photocount	Within 10% of photocount
0-<5	22	59.1	59.1
5-<10	47	52.1	52.1
10-<20	101	34.5	64.8
20-<30	80	61.4	76.8
30-<40	52	46.2	77.6
40-<60	36	61.6	84.7
60<80	34	67.6	92.1
80-<100	49	60.6	90.8
100-<150	36	60.2	88.0
150-<200	52	64.1	90.4
200-<250	69	61.1	89.1
250-<300	33	60.1	94.5
300-<350	40	57.6	90.4
350-<400	13	53.8	91.0

Examination of raw data showing the percentage of plates that different analysts counted with PADs of ⩽5 and ⩽ 10 suggests that analysts did not do plate counts with the precision of the standards suggested by *SMEDP* (*1*). A comparison of raw data from two selected analysts using a PAD of ⩽5 showed that individual analysts varied greatly in their ability to count plates. One analyst counted roughly twice as many plates within 5% of the photocount as another. When comparing the same two analysts using a PAD of ⩽10, both analysts showed marked improvement, but the second analyst still failed to approach the counting accuracy of the first. The data for one analyst, who had a persistent tendency to undercount, demonstrated a progressive loss of ability to count plates with a PAD of ⩽5 in CRs of >80.

The mean PADs by analyst were: 4.2, 4,4, 5.4, 5.5, 7.2, and 8.7. These data are inversely related to accuracy. They are not as precise a measurement of accuracy as a statement such as: "Analyst X counts within 5% of the true count on Y% of the plates; or, Analyst X is within Y% of the true count on 95% of the plates."

The mean deviation from the photocount by all analysts was -2.46%. Thus, analysts counted fewer colonies than were actually on the plate. The mean deviation by individual analyst from the photocount was as follows: -0.117%, -1.88%, -1.99%, -2.36%, -3.58%, and -4.82%.

The number of plates having counts that occurred in each CR was influenced by the coordinator who selected dilution factors designed to provide plates with a predetermined number of colonies. Thus the distribution of counts was not what one would normally see in a food microbiology testing laboratory where no dilution bias existed. When comparing counts ranging from 10-400, produced from samples that have undergone decimal dilution, it was logical to select ranges that represent 1 logarithm to the base 10. For example, each of the CRGs 10-100, 20-200, 30-300, and 40-400 represent 1 logarithm.

Consequently, the CR of 40-< 60, which was included in all four CRGs made up a different proportion of each CRG. In the 10-100 CRG, 40- < 60 represented 22% of total, but only 5.5% of the total in the 40-400 CRG. Thus the percentage of a CR in one CRG could present a sizable discrepancy when compared to another CRG. To compare CRGs, an adjustment for this discrepancy was necessary. It was compensated for by obtaining the difference in the mantissas of the common logarithms of the extremes of each CR. The value obtained for the CRS 10- < 20 was 0.301, 20- < 30 was 0.176, and 30- < 40 was 0.125. The resulting mantissa was multiplied by the percent of the counts the analyst made in the CR for the selected PAD. For example, in Table 1, which uses the PAD of ⩽ 5, the percentages of counts by CR were 10- < 20 = 34.5%, 20- < 30 = 61.4%, and 30- < 40 = 46.2%. Thus $0.301 \times 34.5 = 10.4$, $0.176 \times 61.43 = 10.8$, and $0.125 \times 465.2 = 5.8$. The sum of the mantissas times the percentage of plates in the selected PAD for each CR in the CRG was calculated. This resulted in a realistically weighted overall percent of analyst counts by PAD and was used in comparing the different CRGs.

The percentages of plates that would have PADs of ⩽ 5 and ⩽ 10 by CRG are shown in Table 2. Freidman's test (*8*) was applied to the data from which Table 2 was

TABLE 2. *Percent of analysts' counts within 5% and 10% of the photocount by count range group**

Count range group	Percent of analysts' counts	
	Within 5% of photocount	Within 10% of photocount
10-100	52[a]	78[a]
20-200	60[b]	85[b]
30-300	60[b]	88[bc]
40-400	61[b]	89[c]

For each Count Range Group, mean values followed by the same letter are not significantly different at the 5% level of significance.
*Percentage have been adjusted to reflect normal distribution of counts in different Count Range Groups.

derived and showed that the CRG of 10-100 was different at the 5% level of significance from the CRGs of 20-200, 30-300 and 40-400, which were similar when the PAD was ≤ 5. For a PAD of ≤ 10 the CRG of 10-100 was again different from the others and the CRGs of 20-200 and 40-400 were also different. However, the 20-200 and 30-300 CRGs and the 30-300 and 40-400 CRGs were similar.

Another measure of counting accuracy can be expressed as a statement such as: "X% of the counts analysts made were within Y% of the photocount." Table 3 presents data where X equals 95 and 90% and the Y%'s are listed by CRG. A statement from Table 3 could be "the counts from 95% of the plates were within 17.3% of the photocount in the 10-100 CRG." Friedman's Test applied to the raw data when X = 95% showed the CRG of 10-100 to be significantly different from the other CRGs, and also showed the 20-200 and 40-400 CRG to be different. The 20-200 and 30-300 CRGs were similar as were the 30-300 and 40-400. When X = 90%, the 10-100 CRG was again different from the other CRGs. The 20-200 and 30-300 CRGs were similar and the 40-400 was significantly different from all other CRGs.

TABLE 3. *The counts analysts made were within a certain percentage of the photocount by count range group**

Count range group	95% of Analysts' counts were within Y% of the photocount	90% of Analysts' counts were within Y% of the photocount
10-100	17.3[a]	13.8[a]
20-200	12.8[b]	11.0[b]
30-300	11.8[bc]	10.3[b]
40-400	11.0[c]	9.5[c]

For each Count Range Group, mean values followed by the same letter are not significantly different at the 5% level of significance.
*Percentages have been adjusted to reflect the normal distribution of counts in different Count Range Groups.

The expense and time required to produce photographs from which the photocount standard was obtained cannot be justified under normal circumstances in a food microbiology testing laboratory. Thus, some other standard must be developed. The mean of the counts for each plate obtained by the six analysts participating in the study was used as the "established standard."

The "established standard" was compared by several statistical procedures to the photocount, which was the most accurate count possible. The means of the photocount and the "established standard" were 116.03 and 113.11, respectively. A paired t-test to compare the means of the "established standard" and the photocount showed them to be significantly different. The correlation coefficient of the "established standard" on the photocount was 0.998. The results of these tests indicate that, while the "established standard" deviates slightly from the photocount or true count and is a measurably consistent but low estimate of the photocount over the entire range, it was a practical means of estimating colony counts.

A tabulation of the PADs of ≤ 5 and ≤ 10 for analysts' counts compared to the "established standard" by CR is shown in Table 4. Table 5 shows the PADs of ≤ 5 and ≤ 10 by CRG for analysts when compared to the "established standard." Friedman's Test for PADs of both ≤ 5 and ≤ 10 showed a significant difference between the CRG of 10-100 and CRGs of 20-200, 30-300 and 40-400, which were similar.

The mean PADs by analyst were 3.6,3.6,4.1,5.2,5.5, and 6.6. While the true mean deviation from the "established standard" by analysts was zero, the deviation from the mean by individual analysts was +2.34%, +0.48%, +0.47%, +0.10%, -1.12%, and -2.36%.

TABLE 4. *Percent of analysts' counts within 5 and 10% of the established standard count by count range*

Count range	No. of plates	Within 5% established standard count	Within 10% established standard count
0-<5	24	38.2	48.7
5-<10	66	43.1	71.8
10-<20	94	46.3	77.8
20-<30	80	63.5	89.0
30-<40	46	60.5	84.4
40-<60	31	65.6	92.5
60-<80	39	75.2	96.6
80-<100	43	68.2	94.6
100-<150	35	67.2	86.6
150-<200	55	73.9	93.6
200-<250	68	68.6	91.4
250-<300	39	67.1	93.6
300-<350	36	71.3	94.0
350-<400	8	66.7	83.3

TABLE 5. *Percent of analysts' counts within 5% and 10% of the established standard count by count range group**

Count range group	Percent of analysts' counts	
	Within 5% of established standard count	Within 10% of established standard count
10-100	60[a]	87[a]
20-200	67[b]	91[b]
30-300	68[b]	91[b]
40-400	69[b]	92[b]

For each Count Range Group, mean values followed by the same letter are not significantly different at the 5% level of significance.
*Percentages have been adjusted to reflect the normal distribution of counts in different Count Range Groups.

Table 6 displays the data resulting from the statement "X% of the counts analysts made were within Y% of the 'established standard'." When X = 95%, Friedman's Test showed the 10-100 CRG to be significantly different from the 20-200, 30-300, and 40-400 which were similar. Friedman's Test when X = 90%, also showed 10-100 to be different from the other CRGs and the 20-200 and 40-400 CRGs to be different.

Average analyst counting time was calculated for plates by CRG. Couting times were 18, 30, 41, and 52 sec for the 10-100, 20-200, 30-300, and 40-400 CRGs, respectively. Thus, plates in the 20-200 CRG required 27% less counting time than plates in the 30-300 CRG, whereas plates in the 40-400 CRG required 27% more counting time than those in the 30-300 CRG. There appeared to be no relationship between analyst accuracy

TABLE 6. *The counts analysts made were within a certain percentage of the established standard count by count range group**

Count range group	95% of Analysts' were within Y% of the established standard count	90% of Analysts' were within Y% of the established standard count
10-100	12.5[a]	10.5[a]
20-200	10.8[b]	8.8[b]
30-300	10.3[b]	8.5[bc]
40-400	10.3[b]	8.2[c]

For each Count Range Group, mean values followed by the same letter are not significantly different at the 5% level of significance.
*Percentages have been adjusted to reflect the normal distribution of counts in different Count Range Groups.

and speed in counting. Of the two most accurate analysts, one counted faster than the remaining 5 analysts, and the other was one of the slower counters.

CONCLUSIONS

1. Analysts counted fewer colonies on agar plates than were actually present.

2. Analyst counts of plates with fewer than 10 colonies were erratic and inaccruate.

3. The mean of the counts of several analysts provided a measurably consistent but low estimate of the true colony count.

4. Based on the entire data base, there appeared to be no relationship between the length of time an individual analyst needed to count plates and the accuracy of the analyst's counts.

5. Counts over the 10-100 CRG were considerably less accurate than counts in the CRGs of 20-200, 30-300, and 40-400.

6. The CRG of 20-200 was as suitable as the 30-300 CRG when estimating microbial populations and resulted in a 27% saving in analyst counting time.

RECOMMENDATIONS

Food microbiological testing laboratory managers responsible for large numbers of *SMEDP* plate counts should (*a*) routinely monitor plate count procedures, (*b*) randomly sample analysts' counting accuracy by use of a count established as being true, (*c*) periodically conduct refresher classes in *SMEDP* plate counting procedures, and (*d*) carefullly evaluate any procedural or equipment change that may affect analysts' ability to do plate counts.

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Acid Production and Proteolytic Activity in Milk by Gamma-Irradiation Induced Mutants of Lactobacilli

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ABSTRACT

Biochemical changes in selected gamma-irradiation induced mutants of *Lactobacillus bulgaricus* and *Lactobacillus casei* were examined. Cultures were tested after 24 h of incubation at 37 C for titratable and volatile acidities and proteolytic activity. The gamma-irradiation induced mutants exhibited 50-95% greater proteolytic activity than the unirradiated parent culutres. Some of the mutants produced greater titratable and volatile acidities in milk as compared to parent cultures. Two mutant cultures, Lb/G-1 from *L. bulgaricus* 59 and Lc/G-1 from *L. casei* RTS released significantly greater amounts of soluble nitrogen and amino nitrogen in whole casein and selected fractions than did parent cultures. Combining the mutant cultures with *Streptococcus lactis* C_{10} or *Streptococcus cremoris* C_1 resulted in greater acid producing ability than that of the parent cultures mixed with the streptococci.

Lactobacilli bring about desirable changes such as production of lactic acid and coagulation and partial degradation of casein in milk. Although standard cultures are routinely available and used for these purposes, there is still considerable need for production of genetic variants of lactobacilli having increased acid production and proteolytic activity in milk with a view to utilizing some of them in preparation of cultured milk products. There is a paucity of information on these aspects except for a few recent reports (*4,5,17*).

This paper reports on production of mutants of lactobacilli by gamma-irradiation and on their acid production and proteolytic acitvity in milk.

MATERIALS AND METHODS

Cultures

Lactobacillus bulgaricus 59 and *Lactobacillus casei* RTS from the culture collection of this Institute were used.

Irradiation

Cultures were routinely maintained in reconstituted nonfat dry milk (NFDM). Test cultures were grown in tomato juice broth. Cells were harvested by centrifugation at 2000 × *g*, washed thrice in phosphate buffer (0.02 M; pH 7.0) and resuspended in the same buffer. After adjusting the optical density to 0.55 (7.0×10^8 cells/ml) in Hilger Absorptiometer, 1 ml of the cell suspension was inoculated into 20 ml of tomato juice broth medium in test tubes and incubated at 37 C for 9 h, and exposed to 100 kilorads of ^{60}Co gamma-irradiation. Appropriate dilutions of irradiated and control cell suspensions were plated on tomato juice agar supplemented with 10% sterilized skim milk, in accordance with the procedure described earlier by Singh and Ranganathan (*18*). Petri plates were incubated at 37 C for 48 h.

Selection of mutants

Approximately 10-30 colonies per plate were examined both in control and experimental sets of petri plates. Several hundred control colonies which were examined showed clearance zones of 3-10 mm, while those selected after irradiation exhibited clearance zones of 10-15 mm, thereby ensuring that the observed changes in the exposed culture were not due to variations in the culture. Colonies from control as well as experimental sets of petri plates were individually isolated in NFDM and proteolytic activity was determined by Hull's (*10*) method. The control isolates liberated 0.25-0.35 mg of tyrosine/g, as compared to the mutants which liberated tyrosine in the range of 0.50-0.69 mg/g.

On the basis of enhanced proteolytic activity, five mutants each of *L. bulgaricus* 59 and *L. casei* RTS were selected for further study. Cultures were propagated in sterilized (120 C, 20 min) NFDM and stored in a refrigerator until used.

Acid production and proteolytic activity

Individual cultures were inoculated in NFDM and incubated at 37 C for 24 h. Titratable acidity was determined by titrating 10 g of the sample against 0.1 N NaOH using phenophthalein as indicator and expressed as percent lactic acid.

Volatile acidity was determined by the method of Hempenuis and Liska (*8*). Diacetyl was determined by the method of Owades and Jacovac as modified by Pack et al. (*16*), while acetoin was measured by the method of Anderson and Leesment (*1*).

Proteolytic activity in the cultures was determined by Hull's (*10*) method and expressed as mg of tyrosine liberated per g of sample.

Casein degradation

Two highly proteolytic mutant isolates were used for studies on degradation of casein and its fractions. Whole casein was prepared from cow's milk by isoelectric precipitation, while purified a_s, β- and *k*-casein fractions were prepared according to procedures of Zittle et al. (*19*) and Zittle and Custer (*20*).

Cultures were grown individually in papain-digested milk supplemented with yeast extract (0.2%) and $MnSo_4$ (0.01%). Cells were centrifuged at 9000 × g, washed thrice with phosphate buffer (pH 7.0), and resuspended in the same buffer. Whole casein, and a_s, β-, and *k*-casin fractions were individually dissolved in citrate buffer to bring the final concentration to 1.0% at pH 7.0. Sodium chloride (3%) and merthiolate (0.025 mg/ml) were added to the above solutions and incubated with 10% washed cell suspension. Inoculated tubes were incubated for 0, 5, and 10 days at 37 C. Cells were centrifuged in each case and pH of the supernatant fluid was adjusted to 4.6 and the solu-

ble fraction was used to determine soluble amino nitrogen content according to Association Official Agricultural Chemists (2) by the Micro-kjeldahl method and AOAC (3) by formol titration, respectively.

Horrall-Elliker test

Starter activity was measure by Horrall and Elliker method (9) and carried out with the above ten cultures in combination with *Streptococcus lactis* C_{10} or *Streptococcus cremoris* C_1. The activity of the corresponding combination of parent cultures of lactobacilli with either of the two streptococci was also examined.

RESULTS

Results on titratable and volatile acidities and proteolytic activity in milk produced by 10 gamma-irradiation induced mutants of lactobacilli are in Table 1. The number of organisms at the time of sampling was adjusted to 20×10^8/ml in the parent and mutant cultures.

TABLE 1. *Acid production and proteolytic activity in milk by selected gamma-irradiation induced mutants of lactobacilli*[a]

Culture	Titratable acidity (Percent lactic acid)	Volatile acidity (ml. of 0.01 N NaOH/50 g of curd)	Proteolytic activity (mg. of tyrosine liberated/g of curd)
L. bulgaricus 59			
(Parent)	0.87	8.5	0.35
Lb/G-1	1.10	12.0	0.69
Lb/G-2	0.89	8.6	0.68
Lb/G-3	1.00	10.5	0.68
Lb/G-4	0.90	11.2	0.55
Lb/G-5	0.98	10.1	0.55
L. casei RTS			
(Parent)	0.80	9.6	0.32
Lc/G-1	1.07	11.8	0.65
Lc/G-2	1.00	11.0	0.57
Lc/G-3	0.97	9.9	0.60
Lc/G-4	1.02	10.5	0.50
Lc/G-5	0.85	9.9	0.52

[a]Cultures were grown in reconstituted not fat milk and examined after 24 h. Results represent average of three trials. These cultures did not produce diacetyl or acetone in milk.

Lb/G-1 and Lb/G-3 from *L. bulgaricus* 59 and Lc/G-1 and Lc/G-4 from *L. casei* produced appreciably higher titratable and volatile acidities in milk as compared to the parent cultures. All the 10 mutants exhibited a marked increase in proteolytic activity as compared to the parents. Maximum proteolytic activity was observed with Lb/G-1 and Lb/G-2 from *L. bulgaricus* 59 and with Lc/G-1 from *L. casei* RTS.

Since changes in the nitrogen content of casein substrates can be a measure of proteolysis, soluble and amino nitrogen released by the degradation of whole casein, α_S, β-, and k-casein fractions by two mutants of lactobacilli were also determined. Soluble and amino nitrogen progressively increased with the incubation period, in all the above substrates. As compared to the parent cultures, Lb/G-1 and Lc/G-1 released substantial amounts of soluble and amino nitrogen at the end of 10 days from the casein substrates (Table 2).

Examination of the 10 gamma-irradiation induced mutants of lactobacilli in combination with either of the two streptococci for their activity showed that combination of Lb/G-1, Lc/G-1, Lc/G-4, Lc/G-5 with either *S. lactis* C_{10} or *S. cremoris* C_1 showed appreciable activity (Table 3).

DISCUSSION

The role of lactobacilli in acid and flavor production in cultured milk products is fairly well understood (*6, 11-14*). However, there are only a few reports on improvement in acid production and proteolytic activity by selected induced mutants of lactobacilli. Grinevich and Pantynkhina (*7*) observed increased proteolytic activity in milk by gamma-irradiation induced mutants of lactobacilli. Similar findings have been reported by Dilanyan et al. (*4*). Dilanyan and Sarkisyan (*5*) and Mirganieva (*15*) found that among several gamma-irradiation induced mutants of *Lactobacillus thermophilus,* a few exhibited increased acid production. In the

TABLE 2. *Soluble and amino nitrogen content after degradation of casein substrates by parent and mutant cultures of lactobacilli*[a]

Culture	Period of incubation (day)	Soluble nitrogen (mg of nitrogen/100 ml)				Amino nitrogen (ml of 0.1 N NaOH/100 ml)			
		Whole casein	α_s-Casein	β-Casein	k-Casein	Whole casein	α_s-Casein	β-Casein	k-Casein
L. bulgaricus 59	0	22.4	23.1	23.1	21.0	8.0	8.2	8.2	8.3
(Parent)	5	37.1	40.6	43.4	40.6	8.6	8.9	8.7	8.8
	10	49.1	51.1	55.4	52.0	9.5	9.5	9.5	9.7
Lb/G-1	0	22.8	23.1	21.0	23.1	8.2	8.2	8.0	8.5
(Mutant)	5	43.4	51.0	53.2	53.2	9.0	9.2	9.4	9.5
	10	71.4	72.8	68.6	74.2	16.0	15.6	18.2	18.7
L. casei RTS	0	22.2	21.0	23.1	23.1	8.4	8.2	8.5	8.0
(Parent)	5	35.0	30.8	36.4	39.2	8.6	8.5	9.2	8.8
	10	48.8	50.4	52.0	49.0	9.2	9.4	10.2	10.5
Lc/G-1	0	21.0	22.4	19.6	22.1	8.5	8.5	8.4	8.5
(Mutant)	5	39.2	44.8	37.1	44.8	9.5	9.2	10.5	9.2
	10	68.6	64.4	67.2	65.8	14.4	16.5	18.7	18.2

[a]Cultures were individually grown in casein substrates of pH 7.0 and examined after 0, 5 and 10 days. Results represent average of three trials.

TABLE 3. *Starter activity of lactobacillus mutants used in combination with streptococci*[a]

Culture	Titratable acidity (Percent lactic acid)	
	Mixed with *S. lactis* C_{10}	Mixed with *S. cremoris* C_1
L. bulgaricus 59		
(Parent)	0.35	0.38
Lb/G-1	0.46	0.44
Lb/G-2	0.40	0.40
Lb/G-3	0.38	0.39
Lb/G-4	0.45	0.39
Lb/G-5	0.44	0.39
L. casei RTS		
(Parent)	0.34	0.37
Lc/G-1	0.45	0.44
Lc/G-2	0.42	0.39
Lc/G-3	0.36	0.40
Lc/G-4	0.40	0.43
Lc/G-5	0.42	0.42

[a]*S. lactis* C_{10} and *S. cremoris* C_1 produced 0.31 and 0.32% titratable acidity, respectively. Results represent average of three trials.

light of the above reports, enhanced acid production and proteolytic activity by Lb/G-1 from *L. bulgaricus* 59 and Lc/G-1 from *L. casei* RTS, as compared to the parents, should be regarded with interest. Combination of either of the above mutants with streptococci stimulated acid production, thereby confirming that nitrogen availability limited acid production by lactic streptococci. The possibility of using some of these mutants to manufacture Italian cheese can be explored, if the increased acid production is brought about when the mutants are grown in association with *S. thermophilus.* Greater release of soluble and amino nitrogen by Lb/G-1 and Lc/G-1 as compared to the parent cells, indicates that the mutants may be helpful in shortenting the ripening period of cheese if the increased proteolytic activity by the mutants results in desirable changes in body and flavor characteristics of the product.

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Satellite Food Service System: Time and Temperature and Microbiological and Sensory Quality of Precooked Frozen Hamburger Patties[1]

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ABSTRACT

Use of a precooked frozen entree, hamburger patties, in a satellite foodservice system was assessed as related to time and temperature conditions and microbiological and sensory quality to identify critical phases in utilizing a precooked frozen entree. Time for product storage at the service location was approximately 3 days, and average time at room temperature during assembly was 2 h. Temperature conditions were generally variable with internal temperatures after heating food for service ranging from 152 F (67 C) to 192 F (89 C). Except for some of the low internal temperatures, conditions were acceptable from a food safety standpoint. Mean scores for sensory quality characteristics evaluated ranged from 5.1 to 6.9 (9-point scale) and total plate counts indicated that microbial quality was good. Genera of pathogenic microorganisms were identified, including *Clostridium* and *Staphylococcus;* therefore, the potential exists for public health hazards if precooked food is subsequently mishandled in a system of this type. Critical problems may become apparent in the control of variability within the system, particularly, at the point of heating food for service.

The hazard of foodborne illness associated with the foodservice industry has been recognized and sanitary practices necessary to control such illnesses have been enumerated (*8*). However, few data are available on various systems of operation.

The commissary or satellite type of system uses a central production facility with food transported out of that facility for service in other locations (*14*). This may be a particularly hazardous type of operation because of numerous phases of product flow, multiple service locations, and time span or distance from point of production to ultimate service. Use of commercially precooked entrees, or other totally processed foods, in systems of this type appears to eliminate considerable amounts of the risk in food handling during preparation but would incorporate added risk in eliminating some heat treatment before service.

Definitive data on microbiological and sensory quality of precooked frozen entrees as used in foodservice systems are unavailable. However, data are available on microbiological and sensory quality of food as related to manner in which food is held (chilled or frozen) from time of preparation until service.

Rowley et al. (*15*) investigated use of both chilled and frozen food in regard to establishment of satellite systems for military feeding. Use of food in either the chilled or frozen form was found to be feasible, but chilled food was preferred in terms of microbiological and sensory quality.

Toumi et al. (*18*) considered the microbial flora of ground beef gravy under conditions which simulated those in a satellite system of school foodservice using food in the chilled state. Microorganisms increased to a greater extent during cooling than in holding, and temperatures of food after heating for 35 min in a compartment steamer were no higher than 136 F (58 C). However, in another study of a simulated foodservice system in which previously chilled products were used, Bunch et al. (*5*) demonstrated that beef-soy loaves with excellent microbiological quality could be served.

Glew (*9*) studied a hospital foodservice system using food held frozen until heated for service and found no increase in microorganisms over food prepared within the same operation and served before chilling or freezing.

Cremer and Chipley (*7*) determined time and temperature conditions and microbiological and sensory quality of spaghetti and chili as prepared and held chilled in a satellite system of school foodservice. Average total plate counts per gram of product, at time of service, were 1500 or less and average scores of 5.9 to 7.4 (9-point scale) for the various sensory characteristics evaluated were found.

Since definitive data pertaining to foodservice systems are either limited or unavailable concerning the merit or problems inherent in incorporating precooked frozen entrees, this study was undertaken. Objectives were to determine (*a*) time and temperature conditions during the various phases of product flow of a precooked frozen

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entree through a satellite foodservice system and (*b*) microbiological and sensory quality of food during these various phases.

MATERIALS AND METHODS

Use of a precooked frozen entree in a satellite system was studied in a well-established school foodservice system providing about 18,000 meals per day from a central production facility. This particular system was selected because of its relatively large size and past performance record. Hamburger patties were chosen as the entree through which to study the system because of the relatively high frequency with which this item appeared on the menu and the potential public health hazard associated with mishandling of the product. Beef patties from the same supplier were utilized throughout the study and were comprised of beef, textured vegetable protein, and various additives including flavoring, nutritive, and preservative types.

Through preliminary observation, a flow chart was developed to describe product flow and to provide a basis for recording time and temperature. These phases included: receiving, storage (kitchen), storage (assembly), assembly, storage (kitchen), storage (warehouse), transport, storage (school), heating and service. Meat patties were received as a precooked frozen entree packed in 18-lb fiberboard cartons, placed in freezer storage for a relatively short period, then transferred to the kitchen area and held unrefrigerated during meal assembly. After assembly, food was transferred on dollies to refrigerator storage in the kitchen area, then transferred to a refrigerated warehouse and held until transported to the service locations. Here, food was placed in refrigerated storage until heated for service. In all instances, meals were received at the service locations on Friday for service the following Monday.

Data collection, including microbiological evaluation, time and temperature recording, and sensory evaluation were replicated three times. Statistical analyses included analyses of variance (*17*). Because of the size of the operation, the study was limited in general to morning production (about half the lunches produced), to service in one school, and to transport in one truck carrying food to that school. Preliminary examination of recording potentiometer readings of truck temperatures, kept routinely within the operation, indicated that temperatures in one truck were, in general, representative of the system.

Time recording

Records of time were made through use of a Brenet stop watch and syncronized watches or clocks and rate of pack, by means of a Veeder-Root mechanical counter used in conjunction with other timing instruments. Assembly time was number of meals per minute as calculated from readings taken at 10-min intervals. Time from assembly to storage (kitchen) was for individual dollies sampled at 10-min intervals, and time from storage (kitchen) to storage (warehouse) was for groups of dollies as transferred from kitchen to warehouse storage. Other beginning and end points for time recording are self-explanatory in terms of tabular presentation of data (Table 1).

Temperature recording

Taylor mercury utility thermometers were used to record room or loading dock environmental temperatures. Refrigerator environmental temperatures at the central production facility were read from Marshalltown Manufacturing Company thermometers and Partlow recording potentiometers mounted in the equipment. Environmental temperatures were read at the beginning and end of processes; in addition, temperatures during storage at assembly were read at 1-h intervals, during assembly at 30-min intervals, and at the beginning of storage (kitchen) at 10-min intervals.

Truck environmental temperatures were determined by means of a Partlow recording potentiometer in conjunction with a Thermo-King refrigerator unit mounted on the truck, and refrigerator temperatures at the service location by means of a Traulson thermometer built into the Traulson roll-in refrigerator.

Oven temperatures were read from a Taylor free-standing oven thermometer and internal temperatures of meat in all phases were read through use of Weston dial-type thermometers calibrated for the respective temperatures being determined.

Internal temperatures at receiving were recorded for each dolly of meat. Readings were taken from the top and bottom cases of each stack of cases on each skid with thermometers being inserted through fiberboard containers in the approximate center and 1½ inches from the top. Internal temperature readings for meat during storage at assembly were read according to the same procedure at the beginning of storage, at approximately 1-h intervals, and at the end of the packing period. Internal temperature at assembly was recorded at 30-min intervals by placing a dial-type thermometer in the approximate center of the opened container from which patties were being removed for meal assembly. End of heating internal temperatures were read from 12 positions in the oven (Fig. 1). These positions represented meals on the top, middle, and bottom racks, in the extreme corners, and as nearly as possible in the center back and center front of the oven.

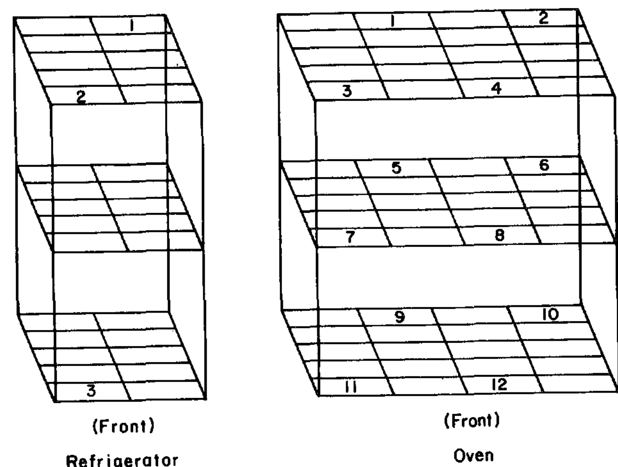

Figure 1. *Diagram of meal arrangement on racks in the refrigerator and oven at the satellite school with blocks numbered to show positions from which samples were taken for either or both temperature reading and microbiological evaluation.*

Sensory evaluation

Sensory evaluation was made by the same eight-member trained taste panel comprised of female graduate-level students. Characteristics of appearance, color, texture, flavor, and general acceptability were judged on a 9-point scale and descriptive terms ranging from 9, "excellent," to 1, "extremely poor" as patterned after Peryam and Shapiro (*11*). Panelists were given meals at random from those being served to students at the school selected for study. Physical conditions under which food was evaluated were those found in the dining area in that school.

Microbiological analysis

Precooked frozen hamburger patties (approximately 75 g), taken for microbiological examination according to a predetermined sampling plan, were aseptically removed and placed in pre-sterilized plastic bags in a refrigerated container for transport to the laboratory. Samples were taken as patties were received, after product assembly, storage, and heating for service. Total time of transport was less than 1 h. Aliquots of 10 g of meat were immediately blended in a sterile laboratory blender for 30 sec with 90 ml of sterile distilled water containing 0.05% (wt/vol) Tween 80 (polyoxyethylene sorbitan monooleate) to emulsify and disperse the blended samples and 0.1% (wt/vol) peptone. Preliminary experiments indicated no adverse effects of this diluent upon several genera of bacteria suspended for periods up to 3 h.

There is currently ample evidence to substantiate the fact that microorganisms subjected to sublethal treatments may remain undetected by procedures normally used for their enumeration. This is primarily due to inhibitory compounds present in selective media which prevent repair and subsequent multiplication of injured cells. With the above results in mind, the following protocol was added to the present

study. Ten ml from each of the blended samples was added to tubes containing 10 ml of Trypticase Soy Broth and incubated 1 h at 77 F (25 C). This is basically the procedure reported by Warseck et al. (*19*) and Ray and Speck (*12*) for repair and enumeration of injured coliforms in frozen foods. Serial dilutions were then immediately prepared in water-Tween 80 diluent and plated onto the appropriate media.

Total microbial counts were obtained using Plate Count Agar, yeast and mold counts with Potato Dextrose Agar, and staphyloccocal counts with Baird-Parker Medium. In addition, coliform counts were obtained using Violet Red Bile Agar with an agar overlay and clostridial counts using Sulfite-Polymyxin-Sulfadiazine Agar with an agar overlay. All media used in this study were purchased from Difco Laboratories, Detroit, Michigan. Isolation and quantitation of staphylococci and clostridia were done by methods of Baird-Parker (*3*) and Angelotti et al. (*2*), respectively. Procedures for incubation and enumeration of all plates were the same as those given in the Bacteriological Analytical Manual, Second Edition (*10*). All subsequent identification of microorganisms was made through procedures outlined by Riemann (*13*) and in Bergey's Manual of Determinative Bacteriology (*4*).

RESULTS AND DISCUSSION

Time and temperature

Data for time involved in various phases of product flow of precooked frozen hamburger patties through a satellite system are in Table 1. Storage time was about 3 days at the service location and at least 1 h during unrefrigerated storage at assembly. Ranges in time indicated variability in all phases, particularly from receiving to storage (freezer) and at assembly. Variations in time from receiving to storage (freezer) may be attributed to problems in materials handling and, during assembly, to mechanical difficulty with the wrapper or container feeder.

Environmental temperatures were variable (Table 2) but in general appeared to represent an area of low risk. Except for one temperature of 47 F (8 C) recorded at the beginning of storage (school), all recorded environmental temperatures in freezer or refrigerated units including the truck were 43 F (6 C) or below, and oven temperatures were 325 F (163 C) or higher (Table 2).

Internal temperatures of meat patties were variable; however, except for some temperatures after heating for service (Table 3), all temperatures recorded might be considered acceptable for control of active microbial

TABLE 1. *Time during the various phases in product flow of precooked frozen hamburger patties through a satellite foodservice system*

Phase of process	Time: Mean	Unit	Range	Observations
Receiving to storage (freezer)	8.5	min	1 to 27	8
Storage at assembly	2.0	h	1.1 to 2.1	4
Assembly	56.1	meals/min	10.3 to 83.3	37
Assembly to storage (kitchen)	3.3	min	1.7 to 8.8	30
Storage (kitchen) to storage (warehouse)	3.9	min	2.4 to 7	11
Transport, total	24	min	13 to 32	3
Transport to storage (school)	2	min	same	6
Storage (school), total	70.5	h	70 to 71.2	4
Storage to heating	5.0	min	same	3
Heating, total	26.6	min	25 to 30	3
Heating to service		less than 1 min		3
Service	11.7	min	10 to 15	3

TABLE 2. *Environmental temperatures during the various phases in product flow of precooked frozen hamburger patties through a satellite foodservice system*

Phase of product	Environmental temperature: Mean F (C)	Range F (C)	Observations
Storage (freezer) beginning	-.3 (-17.9)	-8 to 7 (-22.2 to -13.9)	8
Storage at assembly	71.4 (21.9)	69 to 74 (20.6 to 23.3)	17
Assembly	71.2 (21.8)	68 to 73 (20 to 22.8)	33
Storage (kitchen), beginning	36.7 (2.6)	32 to 40 (0 to 4.4)	29
Storage (kitchen), end	37.1 (2.8)	35 to 39 (1.7 to 3.9)	12
Storage (warehouse), beginning	40.6 (4.8)	38 to 43 (3.3 to 6.1)	11
Storage (warehouse), end	39.5 (4.2)	39 to 40 (3.9 to 4.4)	4
Transport, beginning	35.6 (2.0)	31 to 36 (-0.6 to 2.2)	4
Transport, end	35.3 (1.8)	31 to 39 (-0.6 to 3.9)	3
Storage (school), beginning	40.7 (4.8)	37 to 47 (2.8 to 8.3)	6
Storage (school), end	37.3 (2.9)	34 to 40 (1.1 to 4.4)	4
Heating, beginning	358.3 (181.3)	325 to 400 (162.8 to 204.4)	3
Heating, end	341.0 (171.7)	325 to 349 (162.8 to 176.1)	3

TABLE 3. *Internal temperatures of hamburger patties after heating for service*

Replication		Internal temperature; Sample location: 1	2	3	4	5	6	7	8	9	10	11	12	Mean
1	F	192	156	170	168	180	184	185	166	179	186	180	162	176
	C	(89)	(69)	(77)	(76)	(82)	(84)	(85)	(74)	(81)	(86)	(82)	(72)	(80)
2	F	162	156	178	174	168	176	155	182	160	152	151	180	166
	C	(72)	(69)	(81)	(79)	(76)	(80)	(68)	(83)	(71)	(67)	(66)	(82)	(75)
3	F	192	164	190	178	192	168	180	185	167	182	160	178	178
	C	(89)	(73)	(88)	(81)	(89)	(76)	(82)	(85)	(75)	(83)	(71)	(81)	(81)

growth. Meat temperatures (56 observations) at receiving ranged from -10 to 15 F (-23 to -9 C), at the beginning of storage at assembly (26 observations) from -14 to 24 F (-26 to -4 C), after 1 h at assembly (24 observations) from -9 to 4 F (-23 to -16 C), and after 2 h at assembly (15 observations) from -2 to 4 F (-19 to -16 C). Respective means were -3, 1, -2, and 2 F (-19, -17, -19, and -17 C). The 24 F (-4 C) temperature was observed in one case of product at the beginning of assembly and the relatively high temperature was of unexplained origin. Incongruence in temperatures in the phases of receiving and through storage at assembly was produced by variability in temperatures of individual cases of meat and because the same individual cases could not be followed throughout. Because of temperature variability in individual cases of product, the extent of temperature rise during room storage was not apparent.

Internal temperatures of food after heating for service in a forced convection oven are presented in Table 3. Temperatures of below 176 F (80 C) which might be considered a standard (*15*) were found in every replication of heating. This finding may indicate a critical area for control in food systems using precooked frozen entrees since no heat treatment is likely to occur except at the point of service.

Sensory evaluation

Means for taste panel scores for characteristics evaluated are in Table 4. Except for the factor of general acceptability, scores were similar among replications of study and values corresponded to terms of at least "fair" or "below good, above fair" on the evaluation scale used. The reason for the significantly (P < 0.01) different score for general acceptability in Replication 1 was undetermined.

TABLE 4. *Average scores*[a] *for sensory evaluations of quality characteristics in hamburger patties*[b]

Quality Characteristic	Replication 1	Replication 2	Replication 3	Grand
Appearance	6.1[a]	6.9[a]	6.5[a]	6.5
Color	6.1[a]	6.7[a]	6.0[a]	6.3
Texture	5.5[a]	5.3[a]	5.4[a]	5.4
Flavor	5.7[a]	5.4[a]	5.1[a]	5.4
General Acceptability	6.1[a]	5.4[b]	5.1[b]	5.5

[a]Scale of 9-1 with 9 being optimum.
[b]Means for replication scores on the same line with different letters following are significantly (P < 0.01) different.

Microbiological quality

Results of the microbiological evaluation of precooked hamburger patties are in Table 5. Total plate counts indicated that microbial quality was good. It is of interest to note that either position of the product in the assembly process or in the reheating oven appeared to influence the total count. After reheating, four-fold reductions in total count were observed. Higher values were observed for clostridia than for any other genus detected in the present study. Reductions of 5 to 7-fold in the clostridial population were observed after patties were reheated for service.

TABLE 5. *Microbiological evaluation of precooked hamburger patties*

Phase of process	Total plate count	Coliform	Clostridial	Staphylococcal
As received	5330[a]	223[b]	2650[a]	290[b]
First assembly	4850	190	2140	210
Middle assembly	5200	195	2300	265
Last assembly	5450	225	2460	300
Before reheating	5100	205	2380	220
After reheating[c]	1540	33	480	75
After reheating[d]	1270	20	300	40
After reheating[e]	1340	28	375	60

[a]Expressed as microbial counts/gram of product. Averages of three experiments.
[b]Expressed as microbial counts/10 g of product. Averages of three experiments.
[c]Top back of reheating oven at an internal product temperature of 159 F.
[d]Top front of reheating oven at an internal product temperature of 179 F.
[e]Bottom front of reheating oven at an internal product temperature of 164 F.

Coliform and staphylococcal counts are also in Table 5. Increases in the coliform population apparently due to either position of product in the assembly process or in the reheating oven may again be observed. However, reductions of 7 to 10-fold occurred after patties were reheated for service. Similar results were obtained for staphylococci with reductions of 3 to 5-fold occurring.

Accuracy of coliform counts was confirmed by concurrently using a most probable number procedure since the limit of detection of coliforms by direct plating procedures is generally 10 organisms per gram of sample (*16*). Results from most probable number analyses using lauryl sulfate tryptose broth were almost identical to those obtained by direct plating.

Subsequent identification procedures revealed a wide variety of microorganisms associated with all samples of patties, both as received by the school foodservice system and after reheating. These included yeasts and molds (less than 1/g of patty as received), ***Bacillus*** sp., ***Clostridium sporogenes, Clostridium perfringens,*** micrococci including ***Staphylococcus aureus*** and ***Staphylococcus epidermidis, Escherichia coli, Enterobacter aerogenes, Salmonella typhimurium*** (1 sample of 24 examined as received only), ***Proteus*** sp., ***Pseudomonas*** sp., and ***Alcaligenes*** sp. (***Achromobacter*** sp.). Since cooked meat should have been processed at temperatures sufficiently high to kill nonsporeforming microorganisms, their presence was indicative of either post-processing contamination or underprocessing by the meat supplier. During the phase of reheating for service, wide ranges of end-point temperatures were also noted (Table 5). Thus, while end-point temperatures were sufficient to kill coliforms and staphylococci, actual time of exposure of patties to these temperatures was minimal. All microorganisms identified in the current study have previously been described by Weiser et al. (*20*) as occurring naturally in raw meats and meat products including hamburger. Strains of ***Bacillus*** and ***Staphylococcus*** have also been isolated from several frozen

cooked meat entrees that had been reheated for service (*6*).

General

Time and temperature conditions and microbiological quality during product flow of precooked frozen hamburger patties through a satellite system were variable but generally acceptable at the time of service to a consumer, and sensory quality was at least "fair." However, food temperatures of less than 176 F (80 C) after heating for service indicated need for careful monitoring of product as purchased, as handled within the operating system, and, particularly, as heated for service.

Overall increases, although non-significant, were observed in the microbial population and appeared related to either the position of the product in the assembly process or in the reheating oven. Thus, preventing microbial buildup during assembly and carefully monitoring internal product temperatures during reheating are important keys to the safety of these products. Because of the presence of some known pathogens, the potential exists for public health hazards if precooked food is subsequently mishandled in a system of this type.

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Dry Heat Destruction of Spores on Metal Surfaces and on Potatoes During Baking

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ABSTRACT

Heat destruction characteristics of the normal microflora on potatoes and of *Bacillus subtillis* var. *niger* spores deposited on potatoes were determined during heating in an air oven at 175 C. These results were compared to the heat destruction characteristics of *B. subtilis* var. *niger* deposited in metal cups heated at several temperatures in the same oven. The results of this study indicate that; (*a*) *B. subtilis* var. *niger* spores in tin cups have a D(150 C) of 0.92 min. and a z-value of 21.8 C, (*b*) *B. subtilis* var. *niger* spores on potato surfaces are more resistant to dry-heat destruction than when they are on metal surfaces, and (*c*) the normal microorganisms on potatoes are less heat resistant than *B. subtilis* spores on potato surfaces. Results of this study suggest that the normal flora of a potato are not eliminated during baking and that a spore population inoculated by chance onto a potato also will likely survive the baking process.

Recent environmental microbiological studies indicate that microbial spores under optimum humidity or moisture conditions are very difficult to inactivate. What is the significance of these findings in the food processing or food preparation area? What are the destruction rates of spores on a food product that is prepared by heating in a dry heat environment? Are the spores on the skin of a potato that is prepared by baking in a hot air oven destroyed by the heat treatment? If large numbers of pathogenic microorganisms were accidently deposited on potatoes that were subsequently baked and consumed, would these organisms constitute a hazard? This study was carried out to obtain data that would make it possible to reach some conclusions regarding the sporicidal effect of a dry heat treatment applied to the surface of a potato or organic material containing considerable amounts of water that will diffuse to the surface and evaporate during the heating process. Since many individuals avidly consume the potato skin along with the flesh of the potato, it seemed desirable to determine the microbial destruction on the surface of the potato during the baking process.

Sterilization is the complete removal or killing of all microbial life. It is common practice to divide heat sterilization into "wet-heat" and "dry-heat." In wet-heat sterilization processes, water in the liquid state is present in the system. In dry-heat sterilization processes, the quantity of water is not zero, but the substrate is not wet. Unless heroic measures are employed there will always be some moisture present regardless of the system or item. Therefore, dry-heat sterilization is a heat process in which the quantity of water in the system ranges from a relative humidity value near 0% to near 100% or saturation. (Saturation, or 100% RH, is the condition necessary for wet-heat sterilization.)

The dry-heat death rate of a microorganism or a spore is related to the amount of water in the cell. It is also a function of the heating system. Microorganisms on the surface of a potato heated in an air oven is an "open system." If heating is carried out in an open system (*8*) the quantity of water in the cell or spore depends upon environmental factors such as (*a*) the initial water content of the microorganism or spore, (*b*) the type and density of the microbial or spore population, (*c*) the nature and relative humidity of the gaseous environment in contact with the microorganism, (*d*) the length of the heating period, and (*e*) the cleanliness of the deposit (*7*).

Angelotti et al. (*1*) studied the effect of spore moisture content on the dry-heat resistance of a spore population and found that maximum dry-heat resistance (largest D-value) occurred at a water activity (a_w) of 0.2 to 0.4. The D-value decreased at a_w levels below 0.2 or above 0.4.

Previous investigations in this laboratory (*10*) utilizing the planchet-boat-hotplate dry heat test method showed that the time required to produce a specific reduction in the number of surviving organisms varied with relative humidity. At 90 C, the time for a two-log reduction in the number of survivors was 53 h at 1.5% RH, 300 h at 15% RH, 190 h at 35% RH, 65 h at 55% RH, and 8.5 h at 75% RH. (RH values at 90 C.)

Similar conclusions were reached in studies to determine the effect of conditioning and treatment humidities on the dry-heat resistance of a spore population (*3*). Reducing the humidity level of either the

conditioning or treatment processes lowered the D-value of *Bacillus subtilis* var. *niger* spores on stainless steel surfaces. The suggestion was made that objects to be dry-heat sterilized should be conditioned in a low humidity environment to reduce the dry-heat process requirements.

The objectives of this study were (*a*) to determine the destruction characteristics of microorganisms on the surface of noninoculated heated and unheated potatoes and (*b*) to compare the dry heat destruction characteristics of *B. subtilis* var. *niger* spores deposited on metal surfaces with the results obtained when similar spores were deposited on potato surfaces, the latter were heated under conditions of time and temperature similar to those necessary to bake potatoes in a hot air oven as is done in the American home in preparing the food item "baked potatoes."

MATERIALS AND METHODS

Heating system

A Lab-Line Series 3810A "Reach In" High Temperature Oven with forced air circulation and a Partlow Temperature Control model RFC-15 was used throughout the heating study. The oven temperature control was set at 175 C but the temperature fluctuated between 173 and 175 C during the potato heating studies. A temperature of 175 C (347 F) was chosen arbitrarily as being within the range of temperatures commonly used for preparing homebaked potatoes (*4*). The oven was operated at temperatures of 125, 140, 155, and 175 C in the studies to determine the heat destruction characteristics of *B. subtilis* var *niger* spores in tin cups.

Temperature measurement

The experimental temperatures utilized during the heating studies were sensed by copper-constantan thermocouples. A system of five copper-constantan thermocouples was used to monitor the actual oven temperature (AOT), the external potato surface temperature (EST), internal temperature of the potato (IT), and the temperature of the tin cup (TTC). In potato heating experiments the AOT, EST, and IT were recorded every minute. In the tin cup heating experiments AOT and TTC were recorded every 6 sec.

The AOT was measured using two thermocouples. These two thermocouples were soldered directly onto wires connected to the racks in the oven. One was located above and the other below the test samples.

Thermocouples measured the EST and IT of a representative potato during each heating experiment. The IT measuring thermocouple was placed into a hole made by a 20-gauge hypodermic needle extending half-way through the potato. The EST thermocouple was prepared by removing 5 inches of insulation at the terminal end of the thermocouple wire. An insulated Solderless Butt Connector model #357302 (Vaso Products, Inc., Chicago, Ill.) was positioned on the separated strands of wire *before* the ends were soldered together. The two separated strands were then shaped into a circle and the bare wire ends were soldered together against a flat surface. At time of use the circular wire was placed around the perimeter of the potato and the Solderless Butt Connector was moved down to the potato surface to keep the wire taut around the potato and the thermocouple in contact with the potato surface.

To develop an EST and IT temperature curve for an average potato during a routine heating experiment, the temperatures of eight potatoes were monitored for their individual EST and IT during heating at 175 C AOT. Individual heating curves for each of the eight potatoes were constructed. Temperature values for the eight potatoes at each respective time point were averaged and the values plotted to yield a curve representative of the time-temperature condition on the potato surface during the baking process.

To measure the TTC during heating, a tin cup was soldered to the internal flat bottom surface of a Thermal Death Time (TDT) Can and a thermocouple soldered within the well to the cup bottom. The thermocouple was constructed with long, durable extension wires that made it possible to remove the TDT unit measuring TTC from the oven along with the test TDT unit without interrupting temperature measurement.

Spores

B. subtilis var. *niger* (University of Minnesota Environmental Microbiology Laboratory code AAEF) was used in all spore survival experiments. The spores were grown in our laboratory from spores supplied by the Communicable Disease Center Field Station, Phoenix, Arizona. The spores were grown in Synthetic Sporulation Medium 10 (*5*) in mechanically shaken flasks at 32 C for 48 h. The spore population was washed, transferred to sterile screw cap test tubes, and stored in distilled water at approximately 4 C. The spore suspension titer was 1.1×10^9 spores per ml.

Spore carrier test surfaces

B. subtilis var. *niger* spores were deposited on two types of spore carriers; (*a*) skin of washed potatoes, and (*b*) sterile tin-plated cups (11 mm dia., 8.5 mm deep). All inoculation and recovery procedures were performed in a Class 100 laminar downflow clean room operating at 22 C and 50% relative humidity. The operational regimen offered minimal opportunities for contamination.

Washing of potatoes by insonation before deposition of spores

Potatoes used in spore survival experiments were cleaned using insonation. Each potato was transferred to a sterile 600-ml Pyrex beaker; 200 ml of sterile phosphate (pH 7.2) plating buffer (SPB), was added to the beaker and the beaker containing the potato placed in the ultrasonic tank. Following insonation for 2 min, the potato was removed from the beaker and aseptically hand-rubbed while a 50-ml SPB rinse was poured over the surface. Washed potatoes were placed on sterile stainless steel trays and allowed to equilibrate overnight in the clean room before the spore deposition was made. Washed potatoes were titered for zero time normal flora population numbers.

Spore recovery

A Sonogen-A ultrasonic tank (Branson Instruments, Inc., Stamford, Conn.) operating at a frequency of 25 KHz/sec was used to remove the spores from the test surfaces. The position of the Pyrex container holding either the potato or the tin cup was adjusted so that the level of buffer in the container was the same as the level of fluid in the tank. The aqueous tank fluid contained 0.3% by volume of Tween 80.

Potatoes

Red River Grade "B" potatoes purchased from a local distributor (Kruger, Inc., St. Paul, MN) were used in these studies. All potatoes were from the same crop and had undergone similar harvesting and storage procedures before acquisition. The potatoes were small in size; the diameters ranged from 4 to 5 cm. Potatoes were stored in a walk-in refrigerator held at 4 C (Lab-Line Environeers, Inc., Melrose Park, Ill.) Potatoes were handled aseptically under all circumstances using sterile rubber gloves so as not to contaminate the surface of washed, unwashed, heated, or unheated potatoes.

Deposition of spores

Each spore deposit, regardless of test surface, was made using an Eppendorf Push Button Pipet (Brinkman Instruments, Westbury, NY) having a delivery capacity of 20 μl.

A test tube of spores was removed from the 4-C refrigerator and agitated using a vortex mixer for 15 sec to insure a uniform suspension. Between depositions the suspension was agitated to prevent the spores from settling. The spores were aseptically transferred from the test tube to the test surface using the Eppendorf pipette. Before starting, the Eppendorf was rapidly filled and emptied a few times to prevent bubble formation in the 20-μl tip. In tests involving the tin-plated cups, the 20-μl spore suspension was delivered directly into the well of the cup. In potato studies the spore deposition was made on a relatively flat area of potato. Following spore deposition, both potatoes and cups were equilibrated in the clean room at 22 C and 50% relative humidity for 18

to 24 h. During this period a stainless steel tray was placed over the inoculated surfaces to protect the surface from the direct air currents in the clean room.

Potato testing program

All potatoes involved in a single test were placed on the oven rack at the start of the experiment. The zero time samples were transferred to sterile, foil-covered, 600-ml Pyrex beakers and refrigerated at 4 C awaiting spore recovery. At the end of each heating time the oven doors were opened and two potatoes (in one experiment three potatoes were removed at each sampling time) were aseptically withdrawn, transferred to sterile, foil-covered, 600-ml Pyrex beakers, and refrigerated at 4 C.

Handling and spore recovery procedures for normal flora experiments were identical with those used in inoculated potato experiments. Aseptic precautions were followed in handling all of the potatoes. Potatoes utilized for obtaining the normal flora thermal resistance pattern and those for spore-inoculated thermal resistance patterns were never heated together.

In all analyses, 200 ml of SPB was added to the 600-ml beaker containing the potato. This volume was enough to fully immerse the potato during the 2-min insonation period. Aliquots of this rinse fluid were plated.

Procedures used to recover the normal flora of the potato skin were identical to procedures used to recover organisms from the inoculated potatoes.

Heating spores in tin cups

After 18 to 24 h of drying time the inoculated tin cups were placed in sterile thermal death time (TDT) cans (61 mm in diameter × 9 mm in depth) with removable lids. Two tin cups in a TDT can constituted a TDT unit. An 18-inch rubber-tipped crucible tong was used to insert the TDT unit into and remove it from the oven. Using the tongs, the lid was removed from the can after it had been inserted into the oven. Since heating times were short, TDT units were heated individually.

The TDT unit was rapidly placed in the oven and the lid was removed. The oven doors were closed and remained shut for the aloted heating time and were reopened immediately upon completion of the heating period. The lid was replaced and the TDT unit was removed from the oven with the 18-inch crucible tongs.

Each time the oven doors were opened at the end of a heating time, there was a concommitant depression in the AOT. The oven was allowed to re-equilibrate to the test temperature before starting the next heating interval.

Spore recovery began immediately upon the completion of the heating treatments. TDT units were opened and the individual cups were transferred to sterile 125-ml Erlenmeyer flasks using sterile forceps. A volume of 100 ml of SPB was added to the 125-ml Erlenmeyer flask containing a tin cup. The flask was suspended in the center of the ultrasonic tank for insonation. Each cup stood upright in its buffer (bottom side down) during the 2-min sonication treatment.

Plating procedure

All recovery procedures for spores on potatoes and in TDT units were carried out in the clean room. Trypticase Soy agar (TSA, BBL) was used as the recovery medium. SPB was used in all experimental dilutions. Dilution blanks consisted of 100 ml of SPB. For each sample the appropriate 0.1, 1.0, or 10-ml aliquot of the SPB suspending buffer was plated in duplicate. When 10.0-ml aliquots were plated, 1.5 strength TSA was used. Plates were incubated at 32 C for 48 h and then counted.

Treatment of data

The equivalent heating time (U) assuming instant heating and cooling was determined (9) so a semilogarithmic survivor curve (logarithm of the number of survivors vs. U) could be plotted and the D-value determined.

The D-value (time for a 90% reduction in the microbial population) for each heating experiment was calculated as the negative reciprocal of the slope of the regression line of the semilogarithmic survivor curve. The survivor data for unheated controls (N_0) were not used in the D-value determination. The zero time intercept of the regression line (Y_0) was calculated. The Y_0 and N_0 were used to calculate the intercept ratio:

$$IR = \log Y_0 / \log N_0$$

The z-value was determined analytically by a least squares regression analysis of the logarithm of the D-values as a function of temperature. The z-value is the degrees of temperature change necessary to produce a 10-fold change in the D-value.

RESULTS

Heat resistance of spores in metal cups.

The time-temperature data for the thermal death time cups in open cans were plotted on semilogarithmic paper according to the method of Ball (2). Straight line heat penetration curves were obtained at all temperatures. The average f_h-value was 1.06 min, the average j-value was 1.7.

The D and IR-value data for spores heated in cups are listed in Table 1.

TABLE 1. *Heat resistance of* Bacillus subtillis *var.* niger *spores in tin plated cups heated in an air oven*

Temperature (C)	D-Value (min)	Intercept ratio[a]
125	14.1	1.07
125	10.3	1.05
140	2.9	1.00
155	0.656	0.96
175	0.065	0.79
175	0.048	0.79
175	0.077	0.76

[a]*Intercept Ratio [IR] — Log Y_0/Log N_0*

The D-value data in Table 1 were fitted to the Bigelow thermal death time curve model and the z-value was found to be 21.8 C; the 95% confidence limits were 19.9 and 24.0 C. The D(150 C) value was found to be 0.92 min.

The data in Table 1 show a consistent decrease in the intercept ratio with increasing temperatures. At 125 C the survivor curves were concave downward, however, at 175 C the survivor curves were concave upward.

Heat resistance of microorganisms on potato surfaces

Typical arithmetic temperature curves for potatoes heated in the hot air oven are shown in Fig. 1. The oven temperature varied cyclically over a 2-C range. The potato surface temperature also fluctuated 2 to 3 C. The AOT decreased rapidly when the oven doors were opened to remove potatoes.

The normal flora population values of unheated potatoes are listed in Table 2 and are approximately 10^8 CFU per potato. "Larger" potatoes did not exhibit higher microflora counts compared to "smaller" potatoes. Washed potato surfaces yielded fewer recoverable organisms compared to unwashed potato surfaces.

The numbers of aerobic microorganisms recovered from non-inoculated potatoes heated in a 175 C oven are shown in Table 3; both the heating time (total time in the oven) and the equivalent minutes are shown. A graph of the log of the numbers of survivors as a function of

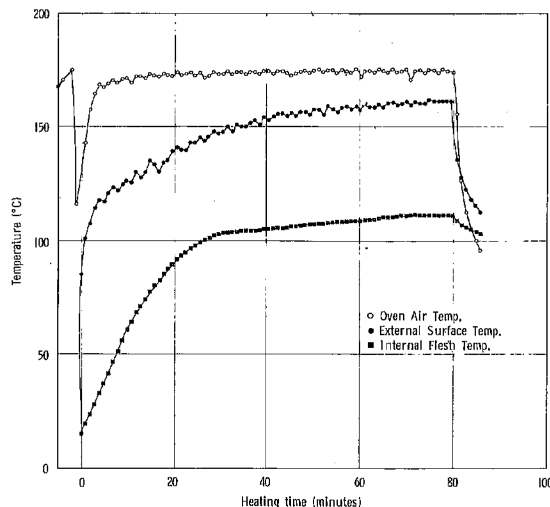

Figure 1. *Internal and surface temperature of potatoes heated in an air oven at 175 C*

TABLE 2. *Aerobic microflora recovered from uninoculated and unheated potato surfaces*

Experiment number	Population numbers (CFU/potato)[a]
Unwashed potatoes	
1	2.5×10^8
2	3.69×10^8
3	7.85×10^7
Washed potatoes	
4	3.16×10^7
5	4.5×10^7

[a]*Colony-forming units per potato*

TABLE 3. *Aerobic microflora recovered from uninoculated potatoes heated at 175 C*

Heating time in 175 C oven (min)	U Equivalent min at 150 C	Population numbers (CFU/potato)[a]	
		A	B
0	0	3.7×10^8	7.8×10^7
5	0.01	6.0×10^7	—
10	0.04	4.2×10^7	4.6×10^7
20	0.26	3.6×10^6	1.2×10^7
30	0.90	3.0×10^5	9.7×10^5

[a]*Each value, colony-forming units per potato, is the log average of duplicate samples.*

clock-heating time (instead of equivalent minutes) is shown in Fig. 2.

The data in Table 3 were subjected to a semilogarithmic curve analysis (logarithm of number of survivors vs. equivalent heating time) and the D- and IR- values determined. In experiment A the D(150 C)-value was 0.40 min, IR was 0.89; in experiment B the D(150 C)-value was 0.53 min, IR was 0.97.

The number of *B. subtilis* var. *niger* spores recovered from inoculated potatoes heated in the air oven at 175 C are shown in Table 4. A graph of the logarithm of the number of survivors as a function of heating time is shown in Fig. 3. The data indicate that at least for the first 60 min of heating the logarithm of the population decreased with heating time. It must be kept in mind that in Fig. 2 and 3 are shown clock-heating time and not

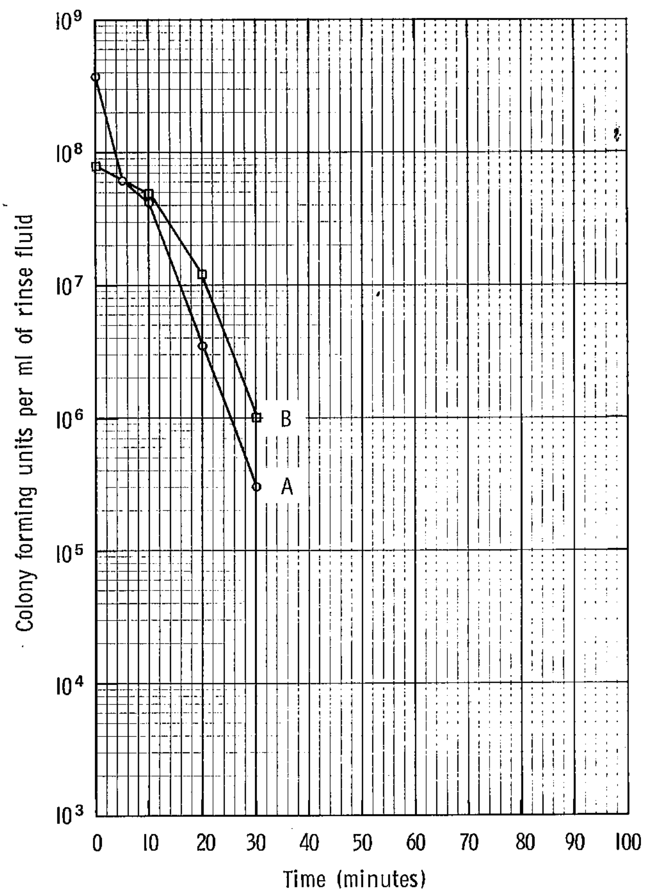

Figure 2. *Semilogarithmic survivor curve for normal flora recovered from potatoes heated at 175 C.*

equivalent-time.

The data in Table 4 were subjected to a semilogarithmic survivor curve analysis and the D- and IR-values determined. The results are shown in Table 5. The average D(150 C)-value, considering only the data for up to 60 min of heating, was 5.6 min.

TABLE 4. Bacillus subtilis *var.* niger *spores recovered from potatoes heated at 175 C*

Heating time in 175 C oven (min)	U Equivalent min at 150 C	Population numbers (CFU/potato)[a]		
		A	B	C
0	0	3.1×10^7	5.5×10^7	2.1×10^7
10	0.04	2.0×10^7	1.8×10^7	1.0×10^7
25	0.50	8.1×10^6	1.2×10^7	3.4×10^6
45	3.33	3.2×10^6	2.3×10^6	4.6×10^5
60	7.53	1.6×10^6	3.5×10^4	4.6×10^5
70	11.20	—	1.7×10^4	—
80	16.80	—	1.8×10^4	—
90	25.10	1.0×10^3	—	—

[a]*Results for experiments A and B are the log averages of duplicate samples; for experiment C the log averages of triplicate samples.*

DISCUSSION

Results of this study indicate that similar populations of *B. subtilis* var. *niger* spores on tin-plate and potato surfaces tested under dry heat conditions have different survivor rates. The D(150 C)-value was of the order of 5.6 min (considering 60 min of heating) for the spores on

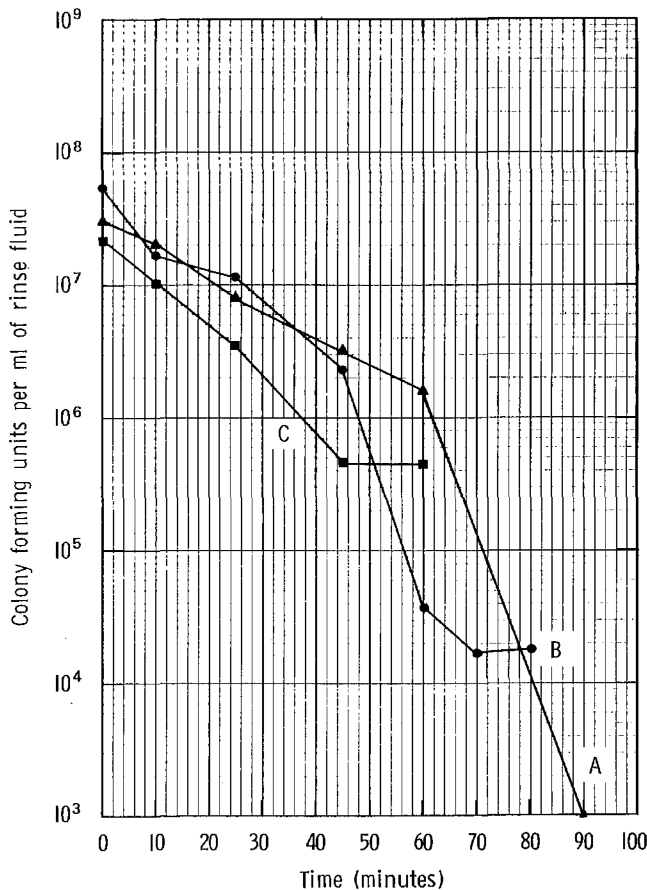

Figure 3. *Semilogarithmic survivor curve for* Bacillus subtilis *var.* niger *spores recovered from potatoes heated at 175 C.*

TABLE 5. *Heat resistance of* Bacillus subtilis *var.* niger *spores on potatoes heated in an air oven at 175 C*[a]

Experiment	Heating time in 175 C oven (min)	Analysis based on total heating time		Analysis based on 60-min heating time	
		D(150 C) (min)	IR	D(150 C) (min)	IR
A	90	6.1	0.96	7.8	0.95
B	80	5.0	0.89	2.8	0.95
C	60			6.1	0.91
				$\bar{x}$ = 5.6	

[a]*Two regression analyses were made in two of the experiments, the first with the data up to and including 60 minutes of heating and the other using all the data.*

the potatoes compared to a calculated D(150 C) value of 0.92 min. for the ***B. subtilis*** spores on a metal surface.

The normal microflora on potatoes decreased more rapidly than ***B. subtilis*** var. ***niger*** spores which were deposited on potato surfaces. The D(150 C)-values of the normal microflora of the potato was found to be 0.40 to 0.53 min. The average D(150 C)-value for ***B. subtilis*** var. ***niger*** spores on potatoes was 5.6 min, based on 60 min of heating (Table 5).

The D-value for the ***B. subtilis*** var. ***niger*** spores on potatoes was about 10 times greater than the D-value of the normal potato microflora. The D-values for the ***B. subtilis*** var. ***niger*** spores on the potato surface were about five times greater than for the ***B. subtilis*** var. ***niger*** spores on tin-plate surfaces.

The results of the comparison of D-values indicate that a potato surface was a much more hospitable environment for spores during a dry-heat treatment than was a tin-plate surface. The nature of the two surfaces was very different, one being a hard metallic surface, the other a rough porous organic surface. Undoubtedly, the characteristics of the surface have an effect on the spore survival rate; however, there are other major differences in these environments. The tin-plate surface was in water vapor equilibrium with the atmosphere in the air oven. In contrast, the spores on the surface of the potato can be assumed to be continuously at a higher vapor pressure than the atmosphere in the air oven since water will be continuously diffusing from the potato. The potato can be assumed to be surrounded by a thin film of gas that has a high relative humidity. The humidity in this film was produced by the moisture diffusing from the potato to the air in the oven.

Heating of the tin-plate surface to the temperature of the air in the oven was rapid and uneventful. In contrast, heating of the potato in the oven was slow and extremely complex. The rate of heat transfer from the air in the oven to the potato was relatively low because of the low surface film heat transfer coefficient. Since the potato has a relatively high heat capacity and also loses water through evaporation during the heating period, the rate of heat gain of the potato was relatively low. During the baking process the surface of the potato on which the microflora was located was actively involved in the heat transfer and moisture transfer processes and at the same time underwent a major change in water content as the baking process proceeded.

At the start of the baking process, the water content of this outside skin of the potato and probably the microorganisms on the surface of the potato were in equilibrium with a relative humidity of about 85% at ambient temperature conditions. At the end of a 60-min baking period, it was probable that the relative humidity below the surface of the potato was still about 85%. During the baking period, the outside layers of cells of the potato will have been reduced in moisture content and at the same time will have become increasingly impervious to the diffusion of water vapor from the potato. During this period the microorganisms on the potato surface were enclosed by a film formed by the water vapor that was diffusing from the potato to the air in the oven. The authors believe that the water diffusing from the potato surfaces effectively raises the ambient relative humidity around the spores on the surface of the potato, which in turn alters the amount of water inside the spore, which in turn greatly changes the D-value of the spore population.

Only bacterial spores will survive moderate dry heat treatments. Therefore, we can assume that the cells of pathogens such as *Salmonella, Shigella,* and *Staphlococcus,* if located on the surface of a potato, should be destroyed during baking.

Perkins (*6*) reports dry heat kill times for spores of a

number of pathogenic organisms, including *Clostridium botulinum, Clostridium tetani, Clostridium perfringens,* as well as *B. subtilis.* The *B. subtilis* spores were more resistant than any of the above-mentioned spores.

We found a D(150 C)-value of 5.6 min for *B. subtilis* var. *niger* spores on potato surfaces during baking for 60 min at 175 C. If we assume, based on the data of Perkins (*6*), that *B. subtilis* spores have two times the dry heat resistance of spores of *C. botulinum* and *C. perfringens,* then the D(150 C) for spores of *C. botulinum* and *C. perfringens* would be on the order of 2.5 to 3 min on potato surfaces. This suggests that for 60 min of heating at 175 C there would be about a 3-log reduction of spores of pathogenic organisms on a potato surface.

These results indicate that under certain conditions it may be possible that viable spores or pathogenic microorganisms are being ingested when baked potato skins are eaten. However, there is no epidemiological evidence that eating potato skins is hazardous.

CONCLUSIONS

The conclusions that can be drawn from this series of studies are: (*a*) The D-value of microorganisms present on the surface of a potato during the baking process will probably be an order of magnitude less than that of organisms on a metal surface. (*b*) The dry heat treatment received by the surface of a potato during baking at 175 C will not destroy all of the dry heat resistant spores on this surface.

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Microbiological Characterization of Human Milk[1]

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ABSTRACT

Milk samples of five breast-feeding mothers were studied for bacterial population, flora, and source. In most instances, samples taken at postfeeding contained higher bacterial populations than prefeeding samples. *Staphylococcus epidermidis* was the predominant organism isolated from 100% of the samples. Increases were noticed in the appearance of *Streptococcus mitis, Gaffkya tetragena, Streptococcus salivarius, Staphylococcus aureus,* as well as *Lactobacillus acidophilus,* in the postfeeding samples. Main sources of bacteria were found to be the infant's mouth and maternal skin.

Human milk is probably the most widely consumed food in the world. For many years physicians have agreed that breast-fed infants are more resistant to respiratory and gastrointestinal infections than bottle-fed children (*4-7,9,19*). Robinson found that both morbidity and mortality rates were reduced in breast-fed infants (*14*). Effects of breast feeding were studied by Mellander et al. (*11*) over different time periods. They found the frequency of acute infections, including diarrhea and upper respiratory infections, was higher in children weaned early than those weaned later. A more recent study has revealed that diarrhea was uncommon in breast-fed infants even though they were constantly exposed to *Salmonella, Shigella,* and *Escherichia coli* (*10*). However, diarrhea reappeared when the children were weaned. Presence of so called protective factors which have been demonstrated in human milk include the bifidus factor, lysozymes, lactoferrin, and specific antibodies (*3,8,18*). These factors, in concert with the microorganisms ingested by the child, are believed to be responsible for development of a protective intestinal microflora.

During the early stages of child development, human milk constitutes the sole source of food and it is usually ingested at 4-h intervals over a 24-h period. Little work has been done on the bacterial content of human milk and consequently little is known of its contribution to the intestinal flora of the child. Therefore, it was the purpose of this study to examine the bacterial content and flora of human milk.

MATERIALS AND METHODS

Volunteer donors for the study were located through the cooperation of the State College chapter of the La Leche League International. Five donors, each approximately in their fifth week of lactation, were selected. The milk samples were obtained once per week for 5 weeks. Each donor was provided with a set of written instructions as to the principles of aseptic collection technique and steps to follow during sampling.

Milk samples were manually expressed by each donor by directly placing the nipple into sterile, 20-ml, screw-cap vials which were cleaned with 70% ethyl alcohol. Approximately 7-10 ml of sample was collected at each sampling time.

For identification purposes, the prefeeding samples taken from the right breast were designated as RB, those from the left breast as LB, those taken at postfeeding from the right breast as RA, and finally those taken from the left breast as LA. Immediately after sampling, donors contacted the laboratory and samples were collected for analysis.

Bacterial counts of each sample were made by using 10^0, 10^{-1}, 10^{-2}, and 10^{-3} dilutions using 0.1% peptone water as diluent. Appropriate dilutions were plated on Brain Heart Infusion agar (BHI; Difco) and incubated at 37 C for 48 to 78 h. Plates with approximately 30-100 colonies were selected and counted. Colonies exhibiting different morphological characteristics, were then isolated and subcultured in BHI broth containing 0.1% of sterile human milk (BHIHM). BHIHM agar was used to examine the colony characteristics, cellular shape, and catalase reaction of each isolate. Using BHIHM agar slants, pure cultures of each isolate were kept at 5 C for further identification.

Classification of isolates into genera and species was done according to the microscopical and biochemical procedures described in *The Manual of Microbiological Methods* (*16*), *The Guide to the Identification of the Genera of Bacteria* (*15*), and *Bergey's Manual of Determinative Bacteriology* (*1*).

RESULTS AND DISCUSSION

Table 1 presents the average bacterial counts per ml of samples and changes in the numbers within the samples as a result of feeding. The total average counts for samples obtained from prefeeding was shown to be 3.8×10^2 and those from postfeeding 1.4×10^3 cells

[1]*Authorized for publication as paper No. 5127 in the Journal Series of the Pennsylvania Agricultural Experiment Station.*

per ml. Changes in the bacterial population within the samples as a result of feeding were calculated by subtracting the prefeeding counts from those of post-feeding. Feeding, resulted in an average increase of 9.8×10^2 bacteria per ml of sample.

Also, as a comparison, one donor (E) who did not wish to use alcohol during sampling was included in this study. Average bacterial counts from prefeeding samples of this donor was found to be 6.1×10^2 cells per ml and those from postfeeding was 3.3×10^4 cells per ml.

These findings would appear to indicate that the child's mouth is responsible for depositing bacteria on the nipples, supporting the conclusions held by other investigators (*2,12,17*). Since, according to Wysham's theory (*19*), a negative pressure may be created as the milk is being removed from the breast, some of the flora of the child's mouth could enter the breast. This could, therefore, account for the increase in total flora of the milk samples obtained at postfeeding.

Bacterial flora isolated from the milk samples were found to belong to five families of microorganisms: *Micrococcaceae, Streptococcaceae, Corynebacteriaceae, Lactobacillaceae,* and *Neisseriaceae. Staphylococcus epidermidis* was predominant microorganism isolated from 100% of the prefeeding and postfeeding samples (Table 2). Postfeeding samples displayed increases in appearance of *Streptococcus mitis, Gaffkya tetragena, Streptococcus salivarius, Staphylococcus aureus,* as well as *Lactobacillus acidophilus.*

The bacterial flora for the donor who did not use any alcohol during sampling is presented in Table 3. Again, *S. epidermidis* was found to be the most common isolate obtained from pre- as well as post-feeding samples. Since this microorganism has been known to be the predominant inhibitant of normal skin (*13*), it probably originates from the maternal skin and it is transferred to milk via the child's mouth during feeding. Increases in percentages of *S. mitis, G. tetragena* and *L. acidophilus* in postfeeding samples could also indicate that bacteria are able to gain entrance to the breast as a result of feeding.

It is of interest to note that in both sets of donors, an increase in percentage of *L. acidophilus* became evident. Reinoculation of lactic acid organisms into infants digestive systems as a result of breast feeding, as well as presence of lysozyme, antibodies, and lactoferrin in human milk contribute to growth inhibition of putrefactive and pathogenic microorganisms in the infant's gut cavity.

TABLE 2. *Percent of bacterial flora isolated from donors who used alcohol prior to sampling*

Source	No. of samples	Isolate	Percent
Pre-Feeding			
	35	*Staphylococcus epidermidis*	100
		Streptococcus mitis	69
		Gaffkya tetragena	19
		Staphylococcus aureus	13
		Micrococcus conglomeratus	9
		Streptococcus salivarius	9
		Corynebacterium pseudo-diptheriticum	6
		Lactobacillus acidophilus	3
		Neisseria subflava	3
Post-Feeding			
	35	*Staphylococcus epidermidis*	100
		Streptococcus mitis	86
		Gaffkya tetragena	29
		Streptococcus salivarius	21
		Staphylococcus aureus	18
		Lactobacillus acidophilus	11
		Micrococcus conglomeratus	3
		Neisseria subflava	2

TABLE 3. *Percent of bacterial flora isolated from the donor who did not use alcohol prior to sampling*

Source	No. of samples	Isolate	Percent
Pre-Feeding			
	10	*Staphylococcus epidermidis*	100
		Streptococcus mitis	86
		Gaffkya tetragena	29
		Streptococcus salivarius	29
		Corynebacterium stratum	14
		Lactobacillus acidophilus	14
		Corynebacterium pseudo-diptheriticum	14
Post-Feeding			
	10	*Staphylococcus epidermidis*	100
		Streptococcus mitis	90
		Gaffkya tetragena	40
		Lactobacillus acidophilus	30
		Micrococcus conglomeratus	10

TABLE 1. *The average bacterial counts per ml of samples and changes in the bacterial population within the samples as a result of feeding*

Donor	No. of times sampled	Counts per ml				Change in population per ml	
		RB[c]	LB[d]	RA[e]	LA[c]	Right breast	Left breast
A	5	4.0×10^1	1.3×10^2	1.2×10^2	1.3×10^2	$+8.0 \times 10^1$	0
B	5	NA[b]	1.2×10^3	NA	3.2×10^3	NA	$+2.0 \times 10^3$
C	5	2.1×10^2	4.4×10^2	1.2×10^2	1.3×10^2	-9.0×10^1	-3.1×10^2
D	5	2.1×10^2	6.6×10^2	3.4×10^3	2.4×10^3	$+3.2 \times 10^3$	$+1.7 \times 10^3$
Average		1.5×10^2	6.1×10^2	1.2×10^3	1.5×10^3	$+1.1 \times 10^3$	$+8.5 \times 10^2$
Total average		3.8×10^2		1.4×10^3		$+9.8 \times 10^2$	
E[a]	5	4.2×10^2	8.0×10^2	2.7×10^4	3.8×10^4	$+2.7 \times 10^4$	$+3.7 \times 10^4$
Average		6.1×10^2		3.3×10^4		$+3.2 \times 10^4$	

[a]70% ethyl alcohol was not used during sampling.
[b]NA-samples were not available.
[c]RB = Right breast before feeding.
[d]LB = Left breast before feeding.
[e]RA = Right breast after feeding.
[f]LA = Left breast after feeding.

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

I. Effects of Growth and Recovery Medium, Temperature of Treatment, Concentration of Peroxide, and Age of Conidia

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ABSTRACT

Effectiveness of hydrogen peroxide was evaluated as a sporicidal agent aginst conidia of *Aspergillus parasiticus* NRRL 2999 and 3315, and *Aspergillus flavus* NRRL 3353. Conidia were harvested from 7-, 10-, and 14-day-old mold cultures grown on modified Moyer's agar, treated with hydrogen peroxide, and then were recovered with mycological agar. An initial spore concentration of 5×10^5 to 1×10^6 per ml was treated with 2, 4, and 6% (wt/vol) peroxide at 40, 30, and 20 C. Survival curves were not straight logarithmic but tended to tail off at the end. Time required for 99.9% reduction of spores ranged from minutes to an hour, and on rare occasions it took hours to achieve the first logarithmic reduction. Conidia of NRRL 3353 consistently were most resistant among the three strains tested. Conidia of NRRL 2999 and 3315 were equally sensitive to peroxide in most instances, although time required for 99.9% destruction of the latter was occasionally greater. Conidia from 14-, and 10-day-old cultures of NRRL 3315 and 3353, respectively, were more resistant than conidia from the other mold cultures. Resistance of conidia from NRRL 2999 was not affected by age of the culture.

The ability of *Aspergillus flavus* and *Aspergillus parasiticus* to produce aflatoxin, one of the most potent hepatocarcinogens known, has been recognized since the early 1960s. Much research has been done to find ways to remove or inactivate the toxin if it is present in food and feed. However, most of the suggested methods either involve excessive labor or alter the organoleptic and nutritive properties of the food or feed (*5,7*). A more feasible approach to combat the hazard of aflatoxin is to control germination of conidiospores from which the toxin-producing mycelia arise rather than to eliminate the metabolites after fungal growth.

Work of Doyle and Marth (*6*) showed that moist heat inactivated spores of *A. flavus* and *A. parasiticus* at 60 C in seconds. Furthermore, their work suggested a positive relationship existed between the degree of heat resistance of conidia and the amount of aflatoxin produced by the different strains of aspergilli.

Data on resistance of *Aspergillus* conidia to chemical treatments are limited. Four percent NaOH, according to the work of Cheng and Levin (*2*), destroyed *Aspergillus niger* conidiospores efficiently at 60 C. Hydrogen peroxide, long known to be an excellent sporicidal and bactericidal agent (*4,9,13,14*), may provide an alternative for inactivating fungal conidia. This investigation was carried out to study the sporicidal activities of diluted hydrogen peroxide on conidiospores of *A. flavus* and *A. parasiticus* under various environmental conditions.

MATERIALS AND METHODS

Cultures

Three *Aspergillus* cultures of different aflatoxin-producing capacity were chosen for the experiments: *A. parasiticus* NRRL 2999 which gave large yields, *A. parasiticus* NRRL 3315 which produced intermediate yields, and *A. flavus* NRRL 3353 which produced poor yields. The same three strains of *Aspergillus* were used by Doyle and Marth (*6*) when they studied the sporicidal effect of moist heat. Cultures were obtained from the Northern Regional Research Laboratory (U.S. Department of Agriculture, Peoria, Illinois), and were transferred bimonthly onto mycological agar slants.

Growth of molds and harvesting of conidiospores

Modified Moyer's agar, a high-glucose (16.5% wt/vol) medium, previously used by Doyle and Marth (*6*) in their experiments, served as the growth medium for the molds. Mycological agar was used for growth of molds only when effects of sporulation medium on survival of spores were tested. Cultures were grown for 7, 10, and 14 days at 28 C in 250-ml prescription bottles. At the end of incubation, sterile water was added to the bottle and conidia were dislodged gently with a sterile stainless steel spatula. Spores were then filtered through several layers of cheese cloth twice to screen out the mycelia and sclerotia. Subsequently conidia were washed with water to remove contaminating nutrients. The suspension was then adjusted with sterile water to a final concentration of 5×10^6 to 1×10^7 spores per ml unless the experiment called for a smaller initial number. The spore suspension was stored at 1-2 C for up to 8 weeks.

Hydrogen peroxide and catalase

Two, 4, and 6% solutions of hydrogen peroxide were used in these experiments. The solutions were freshly prepared for each experiment from 30% hydrogen peroxide (chemically pure; from Fisher Scientific Co., Pittsburg, Pennsylvania). Because of the unstable nature of hydrogen peroxide, the initial concentration of the 30% solution was quantitatively determined monthly by means of iodometric titration.

Catalase C-30 (Sigma Chemical Co., St. Louis, Missouri) was used to decompose residual peroxide after the desired treatment. An excessive amount of catalase was used to ensure complete decomposition of the chemical. The efficiency of peroxide decomposition was tested by addition of potassium iodide and starch solution; a colorless solution indicated absence of peroxide.

Treatment of conidiospores with hydrogen peroxide

Experiments were done at 20, 30, and 40 C, and in the presence of 2, 4, and 6% hydrogen peroxide. A thermostatically controlled water bath equipped with a reciprocal shaker (operated at 80 strokes/min) was used to maintain temperature during treatment and to ensure adequate and equal mixing in all experiments.

Ninety ml of freshly prepared solution was allowed to equilibrate in a thermostatically controlled water bath for 20 min at the temperature chosen. Ten ml of the spore suspension was aseptically added and mixed with the peroxide. At intervals, 1 ml of the reaction mix was withdrawn and discharged into a water blank containing an excessive amount of catalase to decompose residual peroxide. Surviving spores were diluted further and promptly (within 15 min) surface-plated on mycological agar. They also were plated on Moyer's agar when the effect of recovery medium on the spore count was tested. All experiments were done in duplicate, and results reported are averages of two trials. A control experiment was done simultaneously substituting water for hydrogen peroxide.

RESULTS AND DISCUSSION

Appreciable tailing off occurred in most survival curves obtained in this investigation. Consequently, time needed for 99.9% reduction in number of spores was chosen to describe the rate of their inactivation.

Effects of growth and recovery media

Spores of the three selected strains of aspergilli were harvested from and, after peroxide-treatment, recovered with Moyer's and mycological agars. At 30 C and in the presence of 5% peroxide, time for 99.9% reduction in number of 14-day-old conidiospores ranged from 22.3 to 89.0 and 3.0 to 19.0 min when the molds were grown in Moyer's and mycological agar, respectively (Table 1). However, recovery of treated spores was similar with the two media. In all instances, conidia from the three strains behaved consistently in the presence of peroxide; NRRL 3353 was always most resistant, followed in order by NRRL 3315 and 2999 (Fig. 1). The greater resistance

TABLE 1. *Time in minutes required for 99.9% reduction in number of conidia of 14-day-old* A. parasiticus *and* A. flavus *treated with 5% hydrogen peroxide at 30 C*

Strain of aspergillus	Growth medium[a]		Recovery medium[b]	
	Moyer's	Mycological	Moyer's	Mycological
NRRL 2999	22.3	3.0	21.3	22.3
NRRL 3315	15.5	9.8	15.9	15.5
NRRL 3353	89.0	19.0	80.0	89.0

[a]Conidia recovered with mycological agar after treatment.
[b]Conidia produced on Moyer's agar.

to peroxide shown by aspergillus spores harvested from Moyer's rather than from the other medium may have been caused by the large amount of glucose present in the medium. A similar series of experiments by Doyle and Marth (6) showed that thermal resistance of *A. parasiticus* and *A. flavus* spores was proportional to the amount of sugar in the growth medium. The apparent lack of difference in ability of the two media to recover

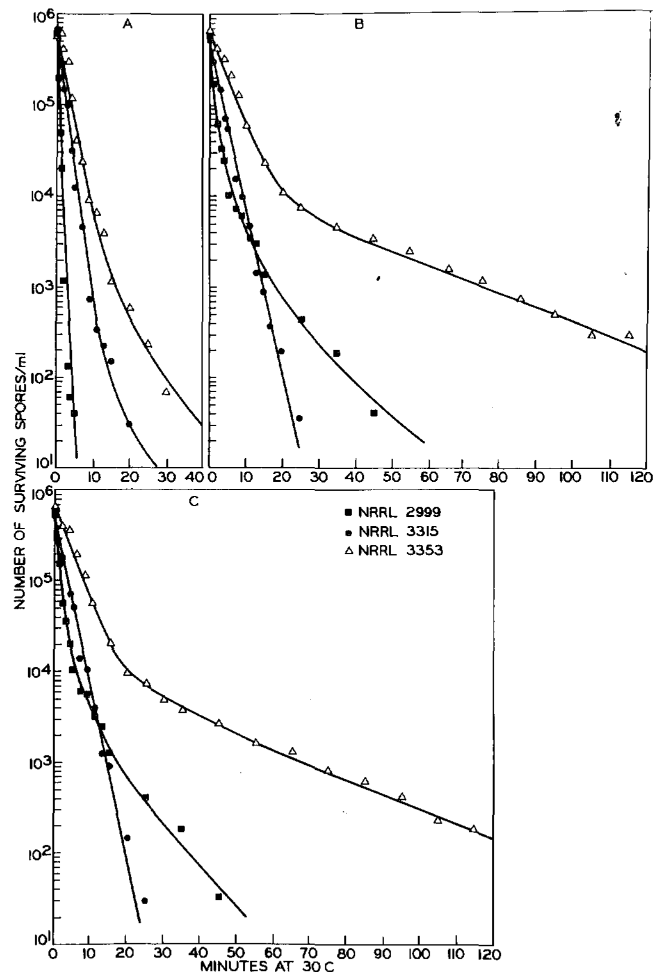

Figure 1. *Inactivation at 30 C of conida from 14-day-old cultures of* A. parasiticus *NRRL 2999 and 3315 and* A. flavus *NRRL 3353 initially grown on and subsequently recovered on mycological agar after treatment with 5% hydrogen peroxide (A), grown on Moyer's agar and recovered on mycological agar (B), and grown and recovered on Moyer's agar (C).*

aspergillus conidia implies that peroxide-treated conidia were not especially exacting in their requirements for germination and growth. Findings in these experiments seem to agree with the general belief that the medium in which an organism is grown before treatment with disinfectants is much more important than the recovery medium in determining survival of the organism.

Effects of temperature and concentration of hydrogen peroxide

Spores from 7-day-old cultures of aspergillus were treated with 2, 4, and 6% hydrogen peroxide at 20, 30, and 40 C. Spores of NRRL 3353 were more resistant to the chemical than were spores of the two other strains. Conidia of NRRL 2999 and 3315 did not differ appreciably in their resistance to peroxide (Fig. 2,3,4). In most instances, it took a few minutes to an hour to attain 99.9% destruction. Rarely were more than 120 min required to reduce the spore population of NRRL 3353 by 90% (Table 2). The Q_{10}, defined as the ratio of the time required to bring about 99.9% reduction in spore population at x C to the time necessary for the same

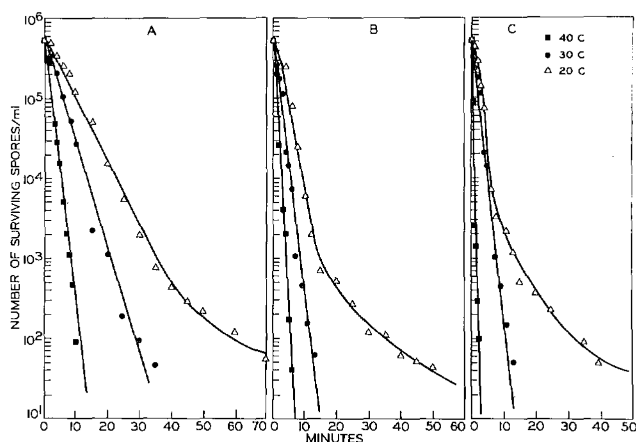

Figure 2. *Inactivation of conidiospores from 7-day-old cultures of* A. parasiticus *NRRL 2999 by 2 (A), 4 (B), and 6% (C) hydrogen peroxide at 20, 30, and 40 C.*

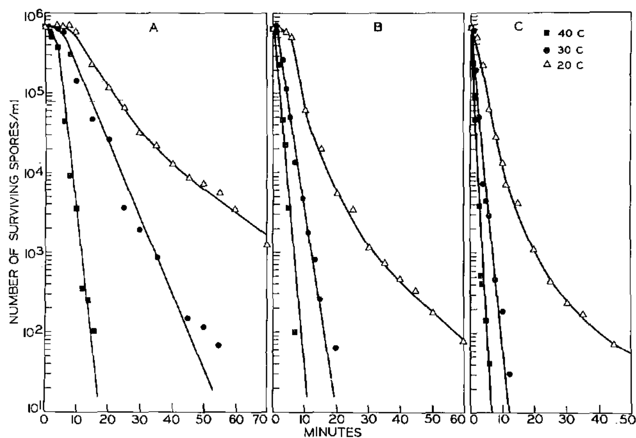

Figure 3. *Inactivation of conidiospores from a 7-day-old culture of* A. parasiticus *NRRL 3315 by 2 (A), 4 (B), and 6% (C) hydrogen peroxide at 20, 30, and 40 C.*

amount of destruction at (x + 10), ranged from 1.30 to 4.71, depending on the concentration of peroxide. Since the sensitivity of the reaction rate to temperature change is indicative of high activation energy (*10*), destruction of spores is likely to be the result of protein denaturation. In addition, the large amount of protein in *Aspergillus* spores [19% in the spore coat and 5% in the spore wall of *Aspergillus oryzae,* as determined by Horikoski and Iida (*8*)] renders spores very susceptible to oxidation by hydrogen peroxide, a potent oxidizing agent. Survival curves obtained, although largely logarithmic, were often characterized by a prolonged tailing off. Often rapid initial inactivation of spores was followed by a phase of slower inactivation. This phenomenon invariably occurred at low temperatures or at a low concentration of peroxide. It might have occurred at all temperatures and all concentrations, but if it did, it was not detectable. Tailing off of the survival curves has been observed in other investigations on germicidal and sporcidal activities of hydrogen peroxide (*15*). It can be explained either by decomposition of peroxide during the course of the experiment or presence of a resistant fraction in the

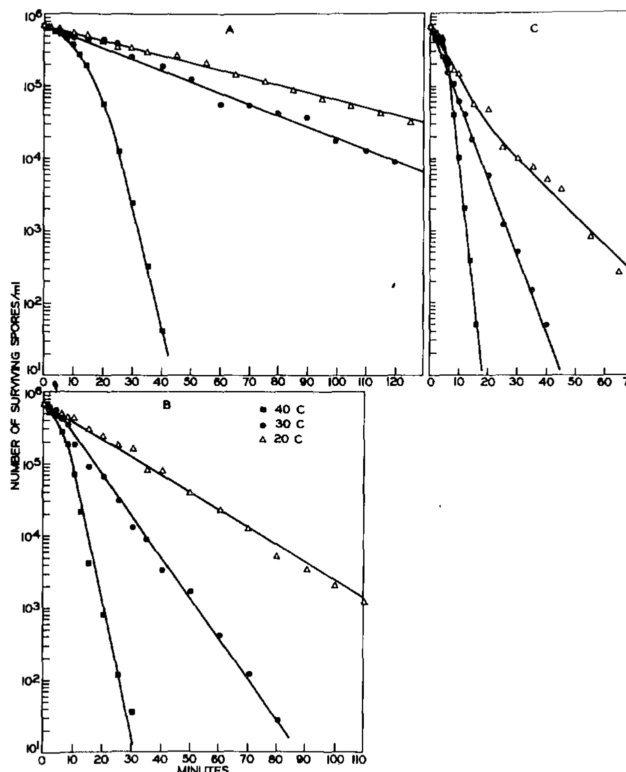

Figure 4. *Inactivation of conidiospores from a 7-day-old culture of* A. flavus *NRRL 3353 by 2 (A), 4 (B), and 6% (C) hydrogen peroxide at 20, 30, and 40 C.*

spore population (*4, 15*). However, the first theory was negated in this instance because quantitative determination of hydrogen peroxide before and after the experiments showed negligible decomposition of the chemical during such a short time. The same prolonged tailing off was observed when Clochard and Guern (*3*) experimented with thermal destruction of peroxidase activity in frozen green peas; this suggests that death of conidiospores could have resulted from enzyme inactivation.

Permeability of the spore, or rate of transport of hydrogen peroxide to the lethal site, were also expected to determine much of the spore's resistance to the chemical. The apparent lack of an initial lag in time in the survival curves suggests that either spores were readily permeable to hydrogen peroxide or the lethal site was located at the outer region of the spore.

TABLE 2. *Time in minutes required for 99.9% reduction in number of conidiospores harvested from 7-day-old cultures of* A. parasiticus *NRRL 2999, 3315, and* A. flavus *NRRL 3353, treated at 20, 30, and 40 C with 2, 4, and 6% hydrogen peroxide*

Strain of aspergillus	Temperature (C)	Hydrogen peroxide (%) 2	4	6
NRRL 2999	20	39.5	18.3	17.0
	30	23.2	8.6	8.8
	40	9.0	4.9	2.0
NRRL 3315	20	70.0	35.9	22.8
	30	36.1	12.7	7.2
	40	12.5	7.2	4.0
NRRL 3353	20	>120.0	>120.0	56.4
	30	>120.0	55.0	28.4
	40	32.9	20.8	13.1

Apparently, hydrogen peroxide was far more effective in reducing the spore count of aspergillus than was heat alone, particularly at lower temperatures. In the absence of peroxide, it took hours and days to obtain a 90% destruction of spores at 45 C (7). In the presence of 6% hydrogen peroxide, a 99.9% reduction was effected in minutes at 40 C (Fig. 2,3,4,). The susceptibility of aspergillus spores to hydrogen peroxide can be accounted for by the absence of microbial catalase. The protective role of catalase during peroxide treatment was well demonstrated in experiments of Amin and Olson (*1*).

Effects of spore age

Conidiospores harvested from 7-, 10-, and 14-day-old cultures of the three strains of aspergillus were treated at 40, 30, and 20 C with 2, 4, and 6% hydrogen peroxide, respectively. The rate of inactivation is graphically represented in Fig. 5, 6, and 7, and the time intervals required for 99.9% destruction are in Table 3. The effect of spore age on inactivation by hydrogen peroxide varied considerably among the strains. Resistance of NRRL 2999 spores, which previously was lowest among the three strains tested, did not seem to be affected appreciably by age. Meanwhile, an appreciable difference was observed with spores of NRRL 3315 and 3353 which were similarly treated. Although it appeared obvious that 14-day-old spores from NRRL 3315 and 10-day-old spores from NRRL 3353 were more resistant than others, a general trend in resistance to peroxide as related to age was not so evident. Other variables like temperature and peroxide concentration have to be taken into consideration. For instance, although 14-day-old spores from NRRL 3353 exhibited greater resistance to 2 and 4% hydrogen peroxide at 40 and 30 C, respectively, than did their 7-day-old counterpart, the relationship was reversed when the spores were treated with 6% hydrogen peroxide at 20 C. It is difficult to explain this observation because information on the

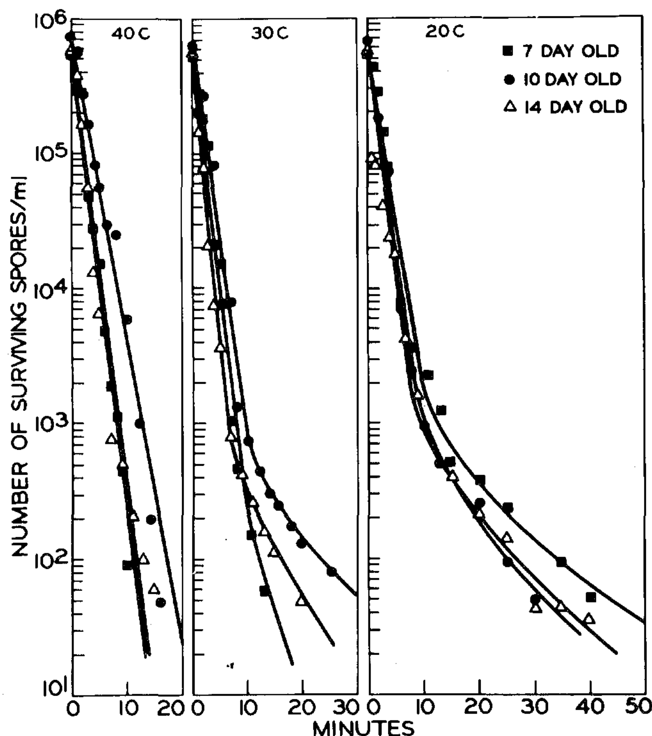

Figure 5. *Inactivation of conidiospores from 7-, 10-, and 14-day-old cultures of* A. parasiticus *NRRL 2999 by 2% hydrogen peroxide at 40 C, 4% at 30 C, and 6% at 20 C.*

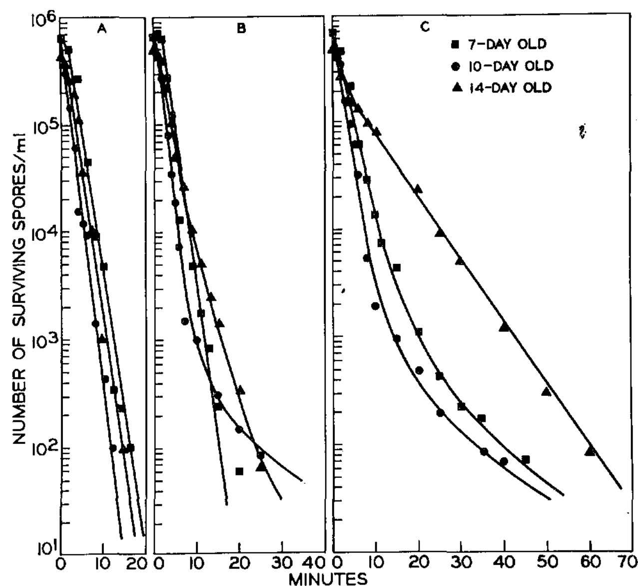

Figure 6. *Inactivation of conidiospores from 7-, 10-, and 14-day-old cultures of* A. parasiticus *NRRL 3315 by 2% hydrogen peroxide at 40 C (A), 4% at 30 C (B), and 6% at 20 C (C).*

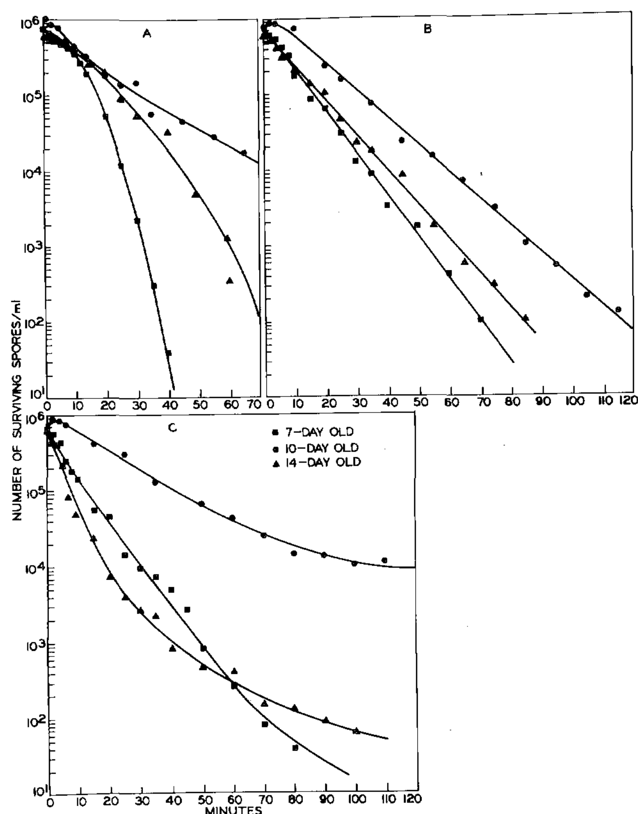

Figure 7. *Inactivation of conidiospores harvested from 7-, 10-, and 14-day-old cultures of* A. flavus *NRRL 3353 by 2% hydrogen peroxide at 40 C (A), 4% at 30 C (B), and 6% at 20 C (C).*

physiology of *A. parasiticus* and *A. flavus* is inadequate. Nevertheless, previous studies showed that spores of *A. niger* increased in thickness and density with age (*11*); this might bring about a change in spore permeability. If the same happened with the test organisms, it might account for the difference in resistance to peroxide exhibited at different ages.

TABLE 3. *Time in minutes required for 99.9% reduction in number of conidiospores from 7-, 10-, and 14-day-old cultures of* A. parasiticus *NRRL 2999 and 3315 and* A. flavus *NRRL 3353 at various temperatures and concentrations of hydrogen peroxide*

Strain of aspergillus	Age of culture (days)	Temperature/Peroxide concentration		
		40 C/2%	30 C/4%	20 C/6%
NRRL 2999	7	9.0	8.6	17.0
	10	13.3	10.5	12.0
	14	8.9	7.3	12.3
NRRL 3315	7	12.5	12.7	22.8
	10	9.1	11.2	16.0
	14	12.0	18.7	47.5
NRRL 3353	7	32.9	55.0	56.4
	10	>120.0	90.5	>120.0
	14	61.9	67.0	46.8

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Changes in the Population of *Clostridium perfringens* Type A Frozen in a Meat Medium[1]

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ABSTRACT

Survival of eight strains of *Clostridium perfringens* type A frozen in a meat medium at -23 C and held under aerobic conditions for 1,7, and 14 days was 17, 12, and 6.2%, respectively. Holding under anaerobic conditions for 1, 7, and 14 days gave survival percentages of 51, 26, and 10%, respectively. Survival was not affected by cooling rates of 0.5, 1.5, and 20 C per min. Addition of 10% sucrose to the meat medium did not affect survival. The meat medium with glycerol concentrations of 0, 0.1, 1.0, and 5.0 M gave survival percentages of 13, 63, 144, and 58, respectively, when frozen at -23 C and held for 22 to 24 h.

Clostridium perfringens was identified as the etiologic agent for 18.8% of all outbreaks which resulted in 25.5% of all cases of foodborne disease in the U.S. from 1970 to 1974 (*4,5*). However, the number of confirmed outbreaks of foodborne disease due to *C. perfringens* is probably disproportionately low because of its sensitivity to freezing, shipping, and storage conditions.

In one early study (*10*), little change was evident in the number of naturally occurring *C. perfringens* surviving at -34 C for up to 6 months; however, no attempt was made to determine if the survivors were spores or vegetative cells. Kemp et al. (*9*) noted that freezing specimens for later microbial examination made recovery of *C. perfringens* extremely difficult. Only 10.8% of the vegetative cells of *C. perfringens* in raw meat survived freezing; after 3 days of storage at -5 C, 2.5% survived, whereas 9.3% survived 3 days at -20 C (*1*). Survival of vegetative cells of *C. perfringens* frozen in chicken gravy was highly variable between duplicate samples (*12*); such a problem has commonly been reported in studies of the effects of freezing on microorganisms (*2*). When 1×10^8 to 2×10^8 cells were subjected to freezing, an adjusted average survival of 12.2% and an adjusted survival range among strains of 8.1 to 17.1% was obtained after 1 day of frozen storage (*12*). Counts after 10 days showed an adjusted average of 6.1% and an adjusted range of 3.9 to 11.3%. Counts after 180 days gave an adjusted average of 0.97% and an adjusted range of <0.1 to 3.7%. Bryan (*3*) reported that a considerable portion of vegetative cells of *C. perfringens* were destroyed by freezing and subsequent storage.

This study was conducted to determine the effect of freezing and holding under different conditions on populations of several strains of *C. perfringens* type A. A meat medium that resembled an actual food item was used.

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MATERIALS AND METHODS

Strains

Eight strains of *C. pergringens* type A were used. Strains T65, NCTC 8239, NCTC 8798, and NCTC 10240 were obtained from C. L. Duncan, Food Research Institute, University of Wisconsin, Madison. Strains H6A and H11A were isolated from fresh lamb and strains T8A and A were isolated from fresh and frozen beef, respectively. The isolated strains were classified type A by the serum neutralization technique in mice (*6*). Stock cultures were maintained in cooked meat medium (Difco) and transferred at approximately 1-month intervals. They were incubated at 37C for approximately 24 h and then held at room temperature.

Test media

Meat test medium (MTM) was prepared by adding 450 ml of water to each 100 g of freshly ground lean lamb. The mixture was steamed for 30 min and allowed to cool slightly. Broth was separated from meat and cooled to solidify the fat; fat was removed and discarded. The equivalent of about 1 g of raw meat and 4.5 ml of broth were placed in each 18 × 125 mm screw cap test tube. Tubes were sterilized in an autoclave at 121 C for 1 h. The final pH was 6.3 ± 0.1. The medium was heated to boiling to drive off oxygen just before use. The appropriate quantities of sucrose and glycerol were added to the MTM tubes just before steaming. Steamed MTM tubes with sucrose and glycerol added were checked for contamination by plating on sulfite polymyxin sulfadiazine agar (SPS).

Inoculation procedure

Test cultures were transferred daily for a minimum of 3 days in fluid thioglycollate medium and incubated at 37 C for 18-24 h. Then a fluid thioglycollate culture, incubated for approximately 18 h, was mixed with an equal volume of sterile peptone water diluent. The desired amount of diluted culture was added to freshly steamed MTM tubes which had been chilled and tubes were stirred on a Vortex Genie mixer. If the tubes were to be frozen and held anaerobically, they were flushed with nitrogen, frozen, and placed in a precooled Torbal anaerobe jar

with the caps loosened. The anaerobe jar was evacuated, flushed with nitrogen, and returned to the freezer. At the time the tubes were inoculated, a portion of the diluted fluid thioglycollate culture was plated on SPS agar to determine the cell population.

Freezing and storage of inoculated media

Inoculated MTM tubes were frozen using three different procedures; uncirculated air at -23 C, uncirculated liquid at -23 C, and circulated liquid at -78 C. Tubes frozen in uncirculated air were placed in a wire test tube rack in a freezer maintained at -23 C. Tubes frozen in uncirculated liquid were placed in a water-alcohol bath precooled in a freezer maintained at -23 C. Tubes frozen in circulated liquid at -78 C were chilled in a dry ice-alcohol bath for 90 sec and then placed in a wire test tube rack in a freezer maintained at -23 C. Sublimation of dry ice provided the circulation. Inoculated tubes were held for 1, 7, and 14 days.

Cooling rates

Cooling rates for the different freezing methods were recorded with a Honeywell multipoint recorder attached to thermocouples placed in tubes of MTM. One thermocouple was placed as nearly as possible at the geometric center, and the other was placed at the edge of the tube. A third thermocouple was placed in the cooling medium to record the actual temperature to which the tubes were being subjected.

The average cooling rate of the tubes containing MTM, in uncirculated air at -23 C was slightly less than 0.5 C/min. The temperature difference recorded at the edge and in the center of the tube was negligible throughout the cooling period. The average cooling rate of the contents of MTM tubes in uncirculated liquid at -23 C was slightly less than 1.5 C/min. The initial difference in cooling rate at the edge and the center of the tubes appeared to be insignificant, and after 9 min both thermocouples read the same temperature. The cooling rate of the contents of MTM tubes in circulated liquid at -78 C averaged 20 C per min. The actual temperature of the circulated liquid was assumed to remain at -78 C throughout the cooling period.

Thawing procedure

After the holding period, the tubes were removed from the freezer, defrosted in a 45 C water bath for 30 sec and agitated at room temperature for 30 sec on a Vortex Genie mixer. This procedure was repeated four times during 4 min. The rate of temperature increase was about 7 C per min, the final temperature being 5 C.

Enumeration procedure

Samples were diluted in peptone water and SPS agar pour plates were prepared using appropriate dilutions. Plates were placed in an anaerobic jar, evacuated, flushed with nitrogen, and incubated at 37 C for 24 h.

Plate counts on SPS agar which failed to provide an accurate estimate of the viable cell number of either the inoculum or the incubated test material were a matter of concern. *C. perfringens* cells existed singly, in pairs, short chains and sometimes in clups in fluid thioglycollate and MTM; each morphological group was a potential colony on agar plates. Thus, if the plating procedure used on the incubated samples resulted in greater physical separation of pairs, chains, or clumps of cells than the plating procedure used on the inoculum, the percent change in colony count would not truly represent the change in viable cell numbers. Attempts to determine the magnitude of the potential error utilized microscopic examinations of preparations made from the inoculum and from the incubated test material. The ratio of cells to potential colonies in the inoculum was compared to the ratio in the test material. By using these ratios, it was determined that the number of cells needed to produce 100 colonies on SPS agar from the inoculum could produce as many as 325 colonies after incubating in MTM. With the same potential for increase in colony-forming units between experiments, comparisons of survival percentages, particularly between methods using paired samples, provided statistically valid differences.

RESULTS AND DISCUSSION

Table 1 shows the percent survival of *C. perfringens* when frozen in uncirculated air in MTM and held for 22 to 24 h. The mean survival rate of all strains was 17% which was comparable to the 12% finding of Strong and Canada (*12*). However, the range among strains was 1.2 to 41.0% which was considerably greater than had been reported previously (*12*). Analysis of variance and Duncan's new multiple range test (*11*) applied to the raw data represented by Table 1 showed that at the 5% level of significance, strain affects survival of *C. perfringens* when frozen and held at -23 C. Of the strains used, T65, NCTC 8239, NCTC 8798, and NCTC 10240 were isolated from previously heated foods and were more susceptible to freezing and frozen storage than the four strains isolated from refrigerated meats. The reason for survival differences between strains may be inherent in the strain and the length of time the strains have been propagated under defined laboratory conditions. Death of microorganisms when frozen is attributed to alteration of the cell membrane lipids during cooling, increased solute concentrations of intra- and extra-cellular liquid when frozen, and damage to the cell permeability barriers during freezing and thawing (*7*).

Table 2 shows the percent survival of *C. perfringens* when frozen in uncirculated air at -23 C and held aerobically and anaerobically in MTM for 1, 7, and 14 days. Analyses of the differences in the means of percent survival (paired t test) suggested that a difference existed at the 5% level for samples held aerobically and anaerobically for 1, 7, and 14 days. It should be noted that as the storage time increased, there was a progressive decrease in survival for both aerobically and anaerobically held cells. The survival ratio of cells held anaerobically to those held aerobically also decreased progressively as storage time increased. The addition of catalase and peroxidases to stored media has been shown to dramatically increase the recovery of *C. perfringens* (*8*). The superior survival of cells held anaerobically to a large extent may be due to the absence of peroxides. The oxygen content of a liquid is inversely proportional to temperature. Consequently, as temperatures are reduced the concentration of oxygen increases, as does the formation of peroxides, which adversely affect the survival of *C. perfringens.*

The survival of *C. perfringens* after freezing in uncirculated air and liquid at -23 C and holding at -23 C for 22 to 24 h is shown in Table 3. Analysis of the difference in the means of percent survival indicates no significant difference between samples frozen slowly in uncirculated air and those frozen rapidly in uncirculated liquid and held at -23 C for 22 to 24 h.

Table 4 shows the percent survival when samples were frozen in uncirculated air and held at -23 C for 22 to 24 h in MTM and MTM plus 10% sucrose. Analysis of the difference in means of survival percentage indicates no difference between samples frozen and held in MTM or MTM plus 10% sucrose.

TABLE 1. *Changes in the population of* Clostridium perfringens *after freezing in uncirculated air at -23 C in meat test medium and holding aerobically for 22 to 24 hours at -23 C*

	Strain								
	T65	NCTC 8798	NCTC 8239	NCTC 10240	T8A	H11A	H6A	A	Grand mean
Mean %	1.2[a]	4.4[a]	5.9[a]	8.1[ab]	20[bc]	27[cd]	29[cd]	41[d]	17

[1]*For each strain mean, values followed by the same letter are not significantly different at the 5% level.*

TABLE 2. *Changes in the population of* Clostridium perfringens *after freezing in uncirculated air at -23 C in meat test medium and holding under aerobic and anaerobic conditions for 1, 7, and 14 days at -23 C*

	Mean percent of original number of cells surviving after:					
	Days under aerobic conditions			Days under anaerobic conditions		
Strain	1	7	14	1	7	14
T8A	25	16	16	47	58	19
H6A	36	13	8.0	119	30	17
H11A	38	31	10	99	50	22
T65	0.31	0.18	0.079	0.63	7.2	0.26
A	45	30	12	119	58	20
NCTC 8239	3.2	0.76	0.12	7.2	1.4	0.24
NCTC 8798	2.3	0.20	0.069	4.8	0.38	0.15
NCTC 10240	5.0	6.3	3.6	16	4.6	2.9
Grand mean	19	12	6.2	51	26	10

TABLE 3. *Changes in the population of* Clostridium perfringens *after freezing aerobically in uncirculated air and liquid at -23 C in meat test medium and holding for 22 to 24 hours at -23 C*

	Mean percent of original number of cells surviving after freezing in:	
Strain	Uncirculated air	Uncirculated liquid
T8A	4.0	8.2
H6A	9.7	7.1
H11A	29	50
T65	2.4	3.3
A	25	48
NCTC 8239	0.76	0.44
NCTC 8798	0.34	0.74
NCTC 10240	22	4.0
Grand mean	12	15

TABLE 4. *Changes in the population of* Clostridium perfringens *after freezing aerobically in uncirculated air at -23 C in meat test medium and meat test medium plus 10% sucrose and holding for 22 to 24 hours at -23 C*

	Mean percent of original number of cells surviving after holding in:	
Strain	MTM	MTM Plus 10% Sucrose
T8A	27	31
H6A	21	35
H11A	18	8.7
T65	0.82	6.8
A	40	35
NCTC 8239	1.4	14
NCTC 8798	7.2	5.3
NCTC 10240	14	23
Grand mean	16	20

The percentages of the original inoculum of *C. perfringens* remaining after being frozen and held in uncirculated air and liquid at -23 C in MTM containing different concentrations of glycerol are presented in Table 5. The analysis of the differences in the means of percent survival suggested that no differences existed between cultures frozen and held in uncirculated air and uncirculated liquid at -23 C. Analysis of variance of the data presented in Table 5 showed the probability to be greater than 0.95 that glycerol affected the survival when frozen and held at -23 C. Application of Duncan's new multiple range test (*11*) to data in Table 5 indicated that the protection provided by 1 M glycerol was significantly greater than that provided by glycerol levels of 0.1 M and 5 M, which in turn were significantly greater than that with no glycerol. The cryoprotective mechanism provided by glycerol is thought to be due to its ability to penetrate the cell and reduce the concentration of intracellular solute reputed to be responsible for protein denaturation and cell death (*7*).

TABLE 5. *Changes in the population of* Clostridium perfingens *after freezing aerobically in uncirculated air and liquid at -23 C in meat test medium tubes containing different concentrations of glycerol and holding for 22 to 24 hours at -23 C*

	Mean percent of original number of cells surviving after holding in:			
Strain	MTM	MTM with 0.1 M glycerol	MTM with 1 M glycerol	MTM with 5 M glycerol
T8A	6.9	29	147	84
H6A	17	72	256	143
H11A	17	114	224	87
T65	5.1	20	41	23
A	50	182	232	79
NCTC 8239	4.5	33	99	19
NCTC 8798	1.9	39	130	15
NCTC 10240	3.0	15	29	18
Grand mean[1]	13[a]	63[b]	145[c]	58[b]

[1]*For each treatment, grand mean values followed by the same letter are not significantly different at the 5% level.*

Data presented in Table 6 show the percent of the original inoculum of *C. perfringens* after freezing in tubes containing MTM and MTM plus 1 M glycerol in circulated liquid at -78 C and in uncirculated air and liquid at -23 C and then holding for 22 to 24 h at -23 C. Analysis of these data suggested that no difference existed between freezing *C. perfringens* in uncirculated air at -23 C or in circulated liquid at -78 C. It also suggested no difference in survival between freezing *C. perfringens* in MTM plus 1 M glycerol in uncirculated liquid at -23 C or in curculated liquid at -78 C. The data

suggest that the MTM plus 1 M glycerol provided a superior survival medium to that of MTM alone at the 5% level of significance.

TABLE 6. *Changes in the population of* Clostridium perfringens *after freezing aerobically in uncirculated air, uncirculated liquid at -23 C and circulated liquid at -78 C and holding in meat test medium and meat test medium with a concentration of 1* M *glycerol for 22 to 24 hours at -23 C*

Strain	Mean percent of original number of cells surviving after freezing in: MTM in uncirculated air at -23 C	MTM containing 1 M glycerol uncirculated liquid at -23 C	MTM in circulated liquid at -78 C	MTM containing 1 M glycerol circulated liquid at -78 C
T8A	3.8	226	14	187
H6A	36	516	44	417
H11A	14	256	19	186
T65	0.13	92	0.34	144
A	56	406	33	376
NCTC 8239	8.2	240	40	224
NCTC 8798	1.9	216	1.4	154
NCTC 10240	0.28	44	0.48	112
Grand mean[1]	15[a]	250[b]	19[a]	225[b]

[1]*For each treatment, grand mean values followed by the same letter are not significantly different at the 5% level.*

CONCLUSIONS

Data presented indicate that the ability of *C. perfringens* to survive freezing is strain-dependent. They also show that the presence of oxygen in the mdium during freezing, storage, and thawing adversely affects viability. Cooling rates of 0.5, 1.5, and 20 degrees per minute did not affect the survival of *C. perfringens*. The addition of glycerol to the freezing medium increased survival significantly. Glycerol at 1 M was superior to other concentrations tested.

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Factors Affecting Quantification of *Clostridium perfringens* Alpha Toxin by the Hemolysin Indicator Plate Test and Other Procedures[1]

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ABSTRACT

Application of the following methods, electroimmunodiffusion on cellulose acetate, radial immunodiffusion, lecithovitellin agar plate, and hemolysin indicator plate, for quantification of alpha toxin of *Clostridium perfringens* revealed that the hemolysin indicator plate method was the most sensitive detecting 0.0003 unit/ml. Sensitivity of the hemolysin indicator plate method was enhanced 67-fold when alpha toxin was diluted with Brewer Thioglycollate medium (Difco, 0236) rather than physiological saline solution. The proteose-peptone ingredient of Thioglycollate medium was the major contributor to increased sensitivity. Less refined agar yielded a larger hemolytic zone. This was attributed, in part, to its higher calcium content. The reducing agent, sodium thioglycollate, diminished the hemolytic zone. In terms of clarity of hemolytic zone, the optimum human red blood cell concentration was 2.1×10^{8}/ml of base agar. Incubation of hemolytic indicator plates at 35 rather than 21 or 45 C yielded greater hemolytic zones. When incubation time was extended, hemolytic zones also increased in size.

A number of microbiological methods have been used to enumerate *Clostridium perfringens* in food systems containing a mixed microbial flora (*5*). There are, however, inherent problems in these procedures. Enumeration of *C. perfringens* in suspected food samples requires use of various agents and techniques some of which will also inhibit the *C. perfringens* organisms or, in some instances permit outgrowth of unrelated organisms (*5,11*). Thus, the ideal medium for isolation and enumeration of *C. perfringens* from food systems has not as yet been devised.

C. perfringens is susceptible to adverse conditions. It will die off rapidly in refrigerated or frozen foods (*1,12*). Consequently, small numbers of organisms in foods may elude detection. Under favorable conditions, even these small numbers could subsequently outgrow to potentially hazardous levels.

Ideally, it would be desirable if we could exploit some unique metabolite of the *C. perfringens* organism which could be detected readily, remain stable under various food processing and storage conditions, and the presence of which would accurately reflect the population level the organisms had attained in the food.

Many of the above requirements could be met by the metabolite, alpha toxin (E.C. 3.1.4.3, phosphoglyceride diglyceride-hydrolase, lecithinase C, or phospholipase C) produced by *C. perfringens*. Various characteristics of this enzyme have been summarized in the literature (*6,8,13*). Some workers (*3,4*) have suggested the possibility that alpha toxin could be used as an indirect measure of *C. perfringens*. It should be emphasized that alpha toxin per se is not involved in food poisoning by *C. perfringens*.

This paper presents some of our experiences in comparing methods for simple, fast, specific, sensitive, and inexpensive detection of *C. perfringens* alpha toxin in model systems. Evaluation was also made of factors affecting sensitivity of the hemolysin indicator plate test.

MATERIALS AND METHODS

Toxin and antiserum

Lyophilized standardized preparations of Type A test toxin (alpha toxin/lecithinase) from *C. perfringens* (*welchii*) and anti-alpha toxin (Type A) serum were purchased from Wellcome Reagents, Ltd., Beckenham, England.

Electroimmunodiffusion (EID) technique

For EID, basically the method of Laurell (*7,9*) was used. The cellulose acetate plates (78 × 98 mm, Helena Laboratory, Beaumont, Texas) were soaked for 10 min in appropriate anti-alpha toxin diluted in 0.0125 M phosphate buffer, pH 7.4. Samples of 0.4 μl were spotted in duplicate on the plate with a one μl Hamilton microsyringe. To remove unreacted proteins, the plate after electrophoresis was washed for 1 h with 0.0125 M phosphate buffer, pH 7.4, containing 0.2 M sodium chloride.

After rinsing with distilled water, the plate was stained with Nigrosin (0.125% in 6% acetic acid). It was then rinsed with distilled water to remove excess dye, washed with 6% acetic acid, and rinsed again with distilled water.

Radial immunodiffusion (RID) technique

One tablet of Panagar (Oxoid, I.D. agar, BR 27) was added to 50 ml of distilled water. After soaking for 15 min, it was placed in a boiling

[1]*Approved as Journal Series Article 23-77, Ohio Agricultural Research and Development Center, Wooster.*

water bath with occasional shaking to dissolve the agar. After it was completely dissolved, the solution was cooled to 45-47 C. The alpha toxin antiserum was added at the concentration of 1% and mixed well. The agar antiserum mixture (10 ml) was poured into a plastic mold (10 × 5 cm) and was allowed to solidify. Just before use, 3-mm test wells were cut into the agar with a sterile thin-walled metal die.

Five microliters of each sample were applied in the test wells of antiserum-agar plates. The plates were incubated for 48 h in a humidified cool (7 C) chamber. Diameters of developed precipitin rings were measured in millimeters with microcalipers. Weak precipitin rings of insufficient contrast were stained with 0.1% thiazine red in 1% acetic acid.

Lecithovitellin agar test

In general, the procedure of Sheldon et al. (*10*) was followed. Lecithovitellin solution was prepared by mixing egg yolk in physiological saline to obtain a 20% suspension in volume, centrifuging at 15,000 RCF for 15 min, and sterilizing the supernatant fluid by Seitz filtration. Agar (1.7%) was prepared by dissolving Agar Agar No. 3 (Oxoid) in physiological saline. The agar (90 ml) was cooled to 45-47 C and 10 ml of the lecithovitellin solution was added and mixed well. Ten milliliters of the lecithovitellin-agar solution was dispensed in Petri dishes (100 × 15 mm) and allowed to air-cool. The test wells were prepared in the same manner as for the RID technique. Samples (5 μl) were then applied in test wells cut into the lecithovitellin-agar. The plates were incubated at 37 C for 24 h. The diameter of reaction zones was then measured as described above for RID.

Hemolysin indicator (HI) plate test

A slight modification of the method described by Harmon and Kautter (*4*) was employed.

Saline agar base. Seventeen grams of Agar Agar No. 3 (Oxoid) and 8.5 g of NaCl were added to 1 liter of distilled water. The pH was adjusted to 7.0. The agar was dissolved by heating and sterlized by autoclaving for 15 min at 121 C.

Washed red blood cells. Packed human red blood cells (rbc) were washed three times by mixing with four volumes of sterile physiological (0.85%) saline and centrifuging for 15 min, at 3,000 RCF. Aseptic techniques were practiced. The rbc were obtained from the American Red Cross Blood Bank, Columbus, Ohio.

Preparation of HI plates. Twelve milliliters of washed rbc were added to 100 ml of saline agar base at 5 C, mixed thoroughly, and dispensed in 10-ml quantities per each 100 × 15 mm Petri dish. The rbc concentration of plates was approximately 16.8×10^{6} rbc/ml. Plates were dried overnight at room temperature and then stored at 4 C. Just before use, test wells were bored. The wells were filled with 5 μl of test samples. Unless otherwise stated, the diameter of hemolytic zones was measured after HI plates had been incubated at 37 C for 24 h.

Treatment of data

Lecithinase/α-toxin activity supplied by Wellcome Reagents was used as the standard. The log of the concentration of lecithinase/α-toxin in units/ml was plotted versus the hemolytic zone or where applicable versus precipitin ring or reaction zone. Linear correlation coefficients were determined by regression analysis.

RESULTS AND DISCUSSION

Comparison of assay methods for alpha toxin

Electroimmunodiffusion. Although the EID procedure with alpha toxin of *C. perfringens* yielded the expected cone shaped precipitin zones, these zones lacked the necessary clarity and sharpness required for accurate quantification. Reasons for failure to obtain clear sharp zones were explored. No improvement in zone clarity was obtained using such variables as antiserum concentration levels of 1.3 and 5%; electrophoresis time of 2 and 4 h; and alpha toxin concentrations ranging from 0.2 to 25 units/ml.

TABLE 1. *Comparison of concentration of alpha toxin required to produce a minimum detectable reaction zone diameter*

Assay method	Minimum detectable reaction zone diameter (mm)	Concentration of alpha toxin (unit/ml)
RID	3.0	3.2
LV	8.0	0.19
HI	3.0	0.0003

Radial immunodiffusion. In the RID procedure, a linear relationship between alpha toxin concentration in the range of 3.2 to 75 units/ml and the diameter of the precipitin ring formed was observed when the data were plotted on a semilogarithmic scale (Fig. 1). The lower limits of detection of alpha toxin appeared to be approximately 3.2 units/ml.

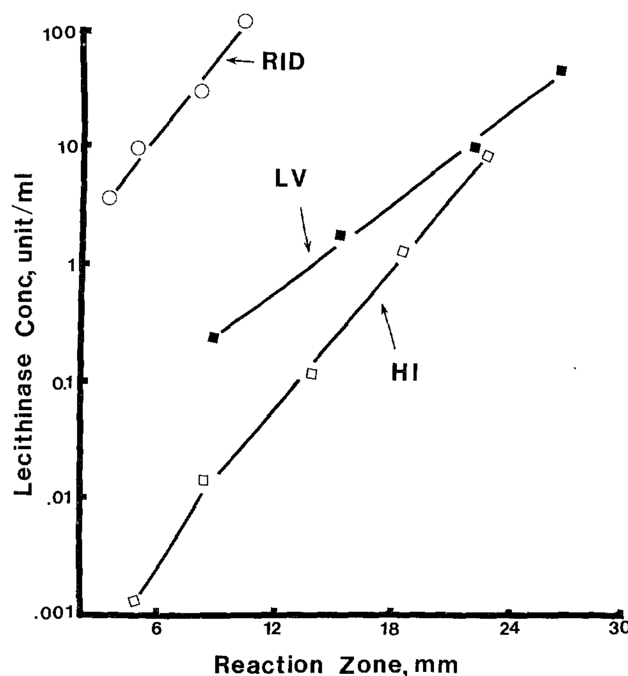

Figure 1. *Sensitivity of the radial immunodiffusion (RID), lecithovitellin agar plate test (LV), and hemolysin indicator plate procedure (HI) for the quantification of the alpha toxin from* C. perfringens. *Linear correlation coefficients (r) for RID, LV, and HI were 0.996, 0.972, and 0.996, respectively.*

Lecithovitellin plate method. A linear relationship was also obtained between the log of the alpha toxin concentration and the reaction zone diameter (Fig. 1) with the LV plate method. Below 0.2 unit of alpha toxin per ml, the zone diameter appeared to vary randomly above and below the predicted value obtained upon a direct extrapolation of the regression line. Lack of sharp contrast between reacted and unreacted lecithovitellin may have been involved. The contrast between areas of reacted and unreacted lecithovitellin was not sufficient to allow accurate measurement of alpha toxin at a concentration of < 0.2 units/ml. An attempt to improve the contrast by manipulating egg yolk concentrations in the range of 0.1 to 2% failed.

Hemolysin indicator plate. The hemolytic zones produced by lecithinase on HI plates were clear and easily differentiated from the red, translucent appearance of the unreacted agar. A linear relationship was obtained between the log of lecithinase concentrations in the range of 0.0003 to 8.0 units/ml and the hemolytic zone diameter. A break point was observed at approximately 0.01 units/ml (Fig. 1).

A comparison was made of the sensitivity of the three different alpha toxin assay methods studied (Table 1). The data reveal that the HI test was 10,000 times more sensitive than the RID and 633 times more sensitive than the LV test.

Apparently, immunological techniques lack the sensitivity necessary for detection of extremely small amounts of alpha toxin. An ideal assay method for *C. perfringens* alpha toxin must be simple, specific, sensitive, quantitative, inexpensive, and rapid. In terms of sensitivity, the HI method was most promising although it may lack the specificity of the RID technique.

The RID procedure is based on formation of an antigen-antibody complex in the presence of specific anti-alpha toxin serum. Immunological techniques are known to be extremely specific and sensitive. However, our findings indicate that the HI procedure, a chemical, non-immunological method, was far more sensitive than RID. The reason(s) for reduced sensitivity of the RID is not known. Antisera potency and/or specificity may be factors.

With respect to specificity of the HI method and possible interference by hemolysins produced by organisms other than *C. perfringens*, there are certain limitations. Many bacteria are known to produce hemolysins (*2*). These include, to name a few, staphylococcal alpha toxin, staphylococcal beta hemolysin, streptolysin O, and *Clostridium hemolyticum* lysin. Therefore, care must be exercised when the HI test is conducted with an unknown food sample. Any positive hemolytic activity observed with a suspected food sample would provide only presumptive evidence that the food product at some time during processing or handling had been heavily contaminated with *C. perfringens*. Confirmatory tests would be necessary before a definite conclusion could be made whether or not the suspected food product had been contaminated with *C. perfringens.* Here the RID test could confirm the specificity of the alpha toxin (hemolysin).

Factors affecting hemolysin indicator test

Because the HI test was found to be the most sensitive assay method for alpha toxin among the techniques studied, attention was directed to a study of factors affecting its sensitivity. These included nature of diluting agents, types of agar used, minerals, reducing agents, red blood cell concentration, and incubation temperature and time.

Diluting agent. Two diluting agents were selected for study: physiological saline (0.85% NaCl), because it is used routinely as a diluent in immunological procedures, and Brewer Thioglycollate medium (Difco, 0236), because this broth is used for propagation of *C. perfringens* in the laboratory.

A comparison of the results obtained with the HI procedure where physiological saline or Thioglycollate medium was used to dilute the alpha toxin is presented in Fig. 2.

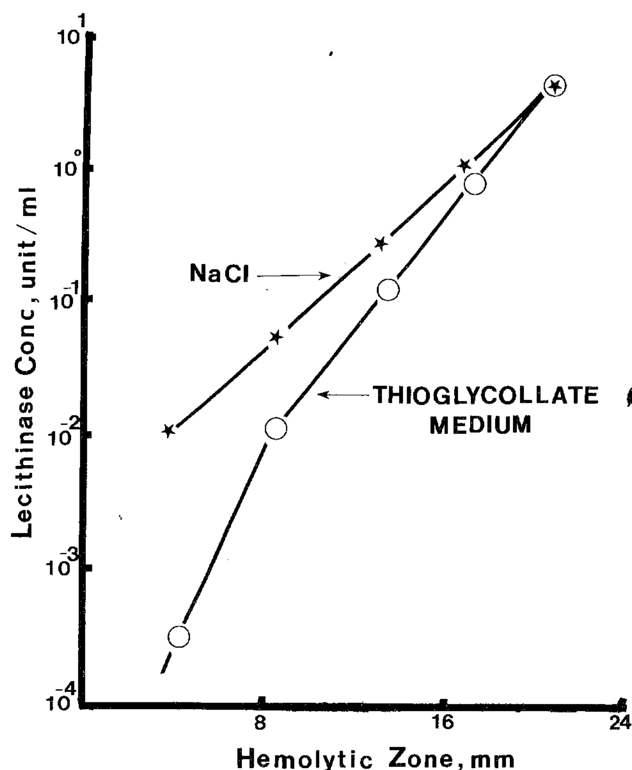

Figure 2. *Effect of the type of diluting agent, physiological saline (NaCl) or thioglycollate medium, used for alpha toxin from* C. perfringens. *Data are for 46 trials with NaCl and 56 trials with thioglycollate medium.*

The concentration of alpha toxin required for the minimum detectable size of hemolytic zone (approximately 3.0 mm) was 0.02 unit per ml when the toxin was diluted in saline. On the other hand, it was 0.0003 unit per ml when the Thioglycollate medium was used as a diluent of alpha toxin. Therefore, sensitivity of the HI test was 67 times greater when alpha toxin was dissolved in a Thioglycollate medium rather than saline. However, at concentrations higher than 3.5 units per ml, the effect of the diluent on the sensitivity of the HI test diminished.

Thioglycollate medium is a complex medium. Many components are used in its formulation. Several of these were tested as diluents for alpha toxin to ascertain their influence on the HI test. Aqueous solutions were prepared of each component at the concentration present in Thioglycollate medium, i.e. 0.5% glucose, 0.2% dipotassium phosphate, 0.6% beef extract, and 1% proteose-peptone.

Among the ingredients, only proteose peptone (Difco, 0120) yielded the same size of hemolytic zone as was observed with Thioglycollate medium at the same alpha

toxin concentration (Fig. 3). It was postulated that the proteose peptone fraction of Thioglycollate medium was the major contributor to the increased sensitivity of the HI procedure, when the medium was used as a diluent for alpha toxin.

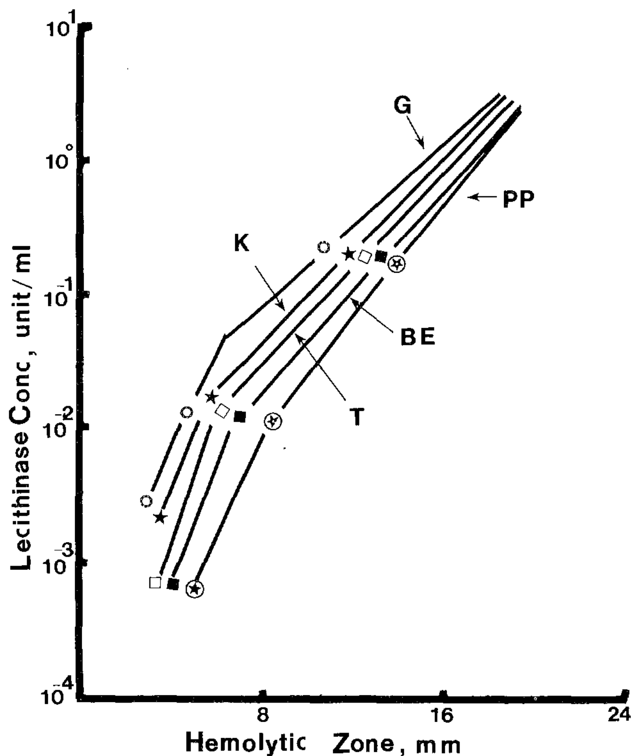

Figure 3. *Effect of glucose (G), K_2HPO_4(K), sodium thioglycollate (T), beef extract (BE), and proteose peptone (PP) as diluents of alpha toxin from* C. perfringens *on the HI test. Data are based on three trials for each agent.*

These findings indicate that the nature of the diluent used for alpha toxin will influence the outcome of the HI test. Furthermore these findings may have application in areas where alpha toxin is prepared and/or diluted with physiological saline as, for example, in animal toxicology studies.

Types of agars. Different commercial agars were compared for their influence on size of the hemolytic zone on HI plates. Agars tested were Epiagar (Oxoid), Panagar (Oxoid), and Agar Agar No. 3 (Oxoid).

Larger hemolytic zones were obtained with Agar Agar No. 3 than with Panagar or Epiagar (Fig. 4). These results point out the importance of the type of agar used in the preparation of the HI plates on the final outcome of the test.

Minerals. Some investigators (*6,8*) have demonstrated that Ca and Mg will influence alpha toxin activity. Thus, the mineral composition of agars was obtained from the manufacturer and is given in Table 3. The agar containing the highest concentration of Ca and/or Mg also yielded the largest hemolytic zones with the HI test (Fig. 4).

TABLE 2. *Mineral composition of the different types of agar*[a]

Mineral	Epiagar	Panagar	Agar Agar No. 3
Ca	0.07%	0.05%	0.45%
Cu	1-4 ppm	—[b]	2 ppm
Fe	30 ppm	—[b]	287 ppm
Mg	—[b]	0.02%	0.24%

[a]Data from Wilson Diagnostics, Inc., Glenwood, Ill.
[b]Value not given.

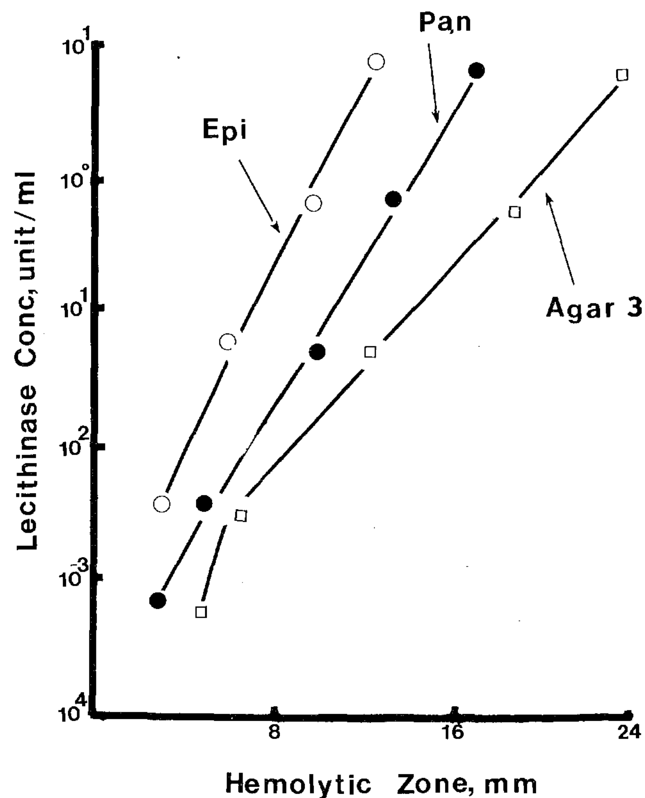

Figure 4. *Effect of the type of agar, Epi (Epiagar), Pan (Panagar), or Agar 3 (Agar Agar No. 3), used in the preparation of HI plates in the HI test. Alpha toxin was diluted in thioglycollate medium. Data are based on three trials.*

Another experiment was done in which calcium chloride was incorporated into Epiagar used for the HI test. Data are presented in Fig. 5.

Addition of 0.3% $CaCl_2$ in the Epi-HI agar sharply increased the size of hemolytic zone. However, a further increase in the concentration of $CaCl_2$ to 1.4% diminished the size of the hemolytic zone. In all instances, however, the zones were larger than those observed with the control without added $CaCl_2$.

Incorporation of 0.3% $MgCl_2$ into the Agar Agar No. 3-HI agar increased the size of the hemolytic zone. Activation of alpha toxin by magnesium has been reported by Zamecnik et al. (*13*).

Reducing agent. Sodium thioglycollate has been reported to inhibit the alpha toxin activity possibly by reducing disulfide linkages of the alpha toxin molecule to sulfhydryl groups (*11*). When 0.5% sodium thiogycollate was incorporated into the HI agar plates prepared with Agar Agar No. 3, the size of the hemolytic zone was decreased in comparison with the control. Sodium

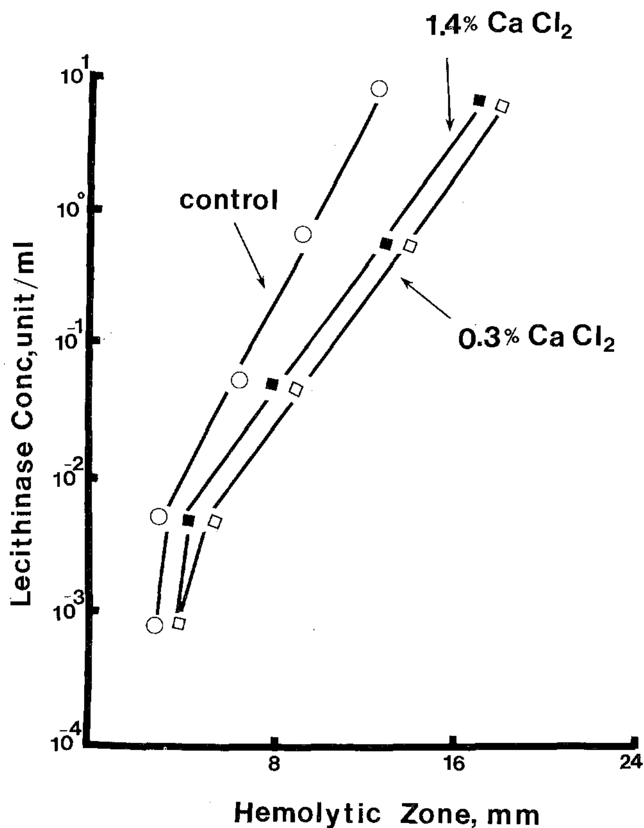

Figure 5. *Effect of 0.3 and 1.4% $CaCl_2$ addition to Epiagar upon the HI test. Thioglycollate medium was used as diluent for alpha toxin. Data are for three trials.*

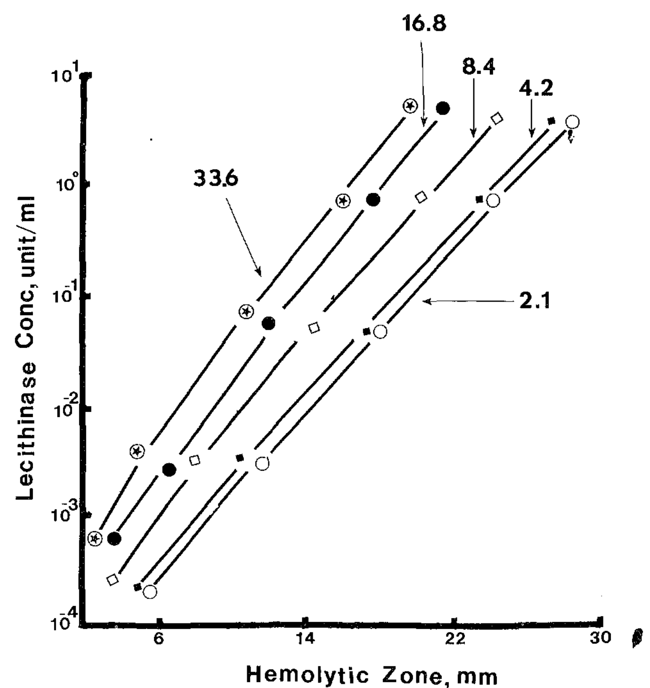

Figure 6. *Effect of red blood cell concentration on the HI test. Numbers refer to the rbc concentration × 10^6/ml of agar. Thioglycollate medium was used as diluent for alpha toxin. Data are for three trials.*

thioglycollate as a diluent of alpha toxin was shown (Fig. 3) to be intermediate in its effect on the HI test.

Red blood cell concentration. The effect of the number of rbc used for preparation of HI plates was also investigated. The red blood cell concentration of the HI plates was manipulated by counting the number of rbc in washed preparations using a hemocytometer chamber and then adjusting the concentration with different volumes of the saline agar base. Results shown in Fig. 6 indicate that the higher the rbc concentration in the HI plate, the smaller the hemolytic zone diameter, and vice versa.

Because phospholipids in the red blood cell membrane may be used as a substrate for alpha toxin in the HI test, it would be reasonable to expect that the size of the hemolytic zone diameter would be inversely proportional to the concentration of rbc in the HI plates. This was observed. However, the higher rbc concentration of HI plates was superior to lower rbc concentration in terms of clarity of hemolytic zone. At lower than 2.1 × 10^6 rbc per ml, the hemolytic zone was too indistinct to be measured precisely.

Incubation temperature and time. Influence on sensitivity the HI test of the incubation temperatures 21, 35, and 45 C, and incubation time of 6, 12, 24, and 48 h was studied.

Hemolytic zones were larger when HI plates were incubated at 35 than at 21 C. With incubation at 45 C, red blood cells deteriorated and readings could not be made. Longer incubation times yielded larger hemolytic zones. The longer the incubation, the farther alpha toxin will diffuse in the HI plate and the longer will be the contact between the substrate (rbc) and the enzyme (alpha toxin). Thus, sensitivity of HI tests will be increased by prolonged incubation. However, shorter incubation time would be more advantageous for a microbiological control laboratory.

The present study furnishes information on a number of factors influencing HI and other tests for *C. perfringens* alpha toxin. It should be emphasized however, that the *C. perfringens* alpha toxin was obtained from a commercial source in a highly concentrated state. The need now is to explore the HI test with native food systems, artificially infected with *C. perfringens* organisms, and to evaluate the reliability and sensitivity of the HI test in detecting lecithinase produced by *C. perfringens* in these food systems.

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HACCP Models for Quality Control of Entree Production in Foodservice Systems

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ABSTRACT

Quality control is a major management function in foodservice systems. Quality is defined as a composite of microbiological, nutritional, and sensory attributes. The Hazard Analysis Critical Control Point (HACCP) concept is a preventive approach to quality control, emphasizing microbiological control and identifying process stages where loss of control could present a food safety risk. Applying this concept, which has been implemented in the frozen food processing industry, HACCP models were developed for quality control of entree production in conventional, cook/chill, and cook/freeze foodservice systems in three phases: identification of control points using flow diagrams, identification of critical control points, and establishment of monitors for control. Time-temperature was a critical control point throughout entree production in each of the models; parameters were established for time-temperature and continuous surveillance of time-temperature was the monitor for control. Equipment and personnel sanitation are critical control points which should be monitored using standards established by the foodservice system. Implementation of the HACCP system is recommended for foodservice operations. However, standards and monitors for control of critical control points must be established for each operation and based on their own system objectives, resources, and constraints.

Quality of meals is a primary objective of foodservice systems; therefore, control of quality is a major management function. Generally, quality assessment in foodservice systems involves inspection at the point of service, a retrospective action which provides little information for quality control.

Quality is a multi-dimensional characteristic of food, defined for this paper as a composite of microbiological, nutritional, and sensory attributes. The literature was reviewed to determine perceptions of quality and emphases of quality control programs in foodservice systems and in the food processing industry. The Hazard Analysis Critical Control Point (HACCP) concept, which has been successfully implemented in the food processing industry, was investigated for its applicability to foodservice systems.

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QUALITY AND QUALITY CONTROL IN THE FOODSERVICE INDUSTRY

In the foodservice literature, quality of food generally referred to sensory attributes such as flavor and appearance. Temperature was also considered a sensory attribute with unique importance as a quality factor for foodservice systems.

Traditionally, quality control in foodservice systems has been interpreted as a threefold process: specify quality ingredients, utilize standardized recipes, and taste the food critically (*20*). Emphasis was usually on tasting the finished product. In fact, Miller (*29*) stated that the only measure of quality was to taste food continuously. This alleged measurement lacks reliability and validity as a tool for quality control.

QUALITY AND QUALITY CONTROL IN THE FOOD PROCESSING INDUSTRY

Quality is usually defined in the food processing literature as a composite of microbiological, nutritional, and sensory attributes. Kauffman (*24*) considered food quality as highly acceptable organoleptic characteristics, nutritional value, and safety. Hawthorn (*21*) defined quality as a composite of positive and negative factors. Positive factors included color, flavor, texture, and nutritional value; whereas, quality, was also freedom from negative factors such as harmful microorganisms and undesirable substances.

Quality of food is related to consumer acceptability. Although quality is frequently confused with excellence, it is only a specified degree of excellence required in the marketplace as determined by the consumer's willingness to pay (*25*).

Quality control and quality assurance are terms which have been used interchangeably in the literature. Recently there has been an apparent differentiation in terminology. Quality assurance is an overall program directed toward compliance with food laws and regulations; whereas, quality control is one aspect of the total quality assurance program (*2,35*).

The purpose of quality control in food processing systems is to insure quality at levels and tolerances acceptable to the consumer and at minimum cost to the processor (*25*). Quality control can be considered in three stages: raw material control, process control, and finished product inspection (*21*). Emphasis may be appropriately placed on any one stage, since the quality control program should be designed to meet the specialized requirements of the processing operation (*30*).

Raw material control is actually a misnomer. The term "control" may imply that all food properties are under the processor's control; this is true only to the degree that control extends to the farm level. Processors can and do specify microbiological standards for raw materials, controlled by testing these raw materials before acceptance. "Control" may also imply improvement in the quality of some raw materials during processing. However, the objective is to conserve the quality of raw materials during processing. Finished product inspection has a confirmatory function in a quality control program, identifying the effectiveness of raw material and process control (*21*).

Quality control is a management function integrated into all subsystems of the food processing organization. Quality control is a dynamic concept which should adapt to changes in materials, machines, and the market.

HACCP CONCEPT

The HACCP (Hazard Analysis Critical Control Point) system is a preventative program for quality control designed to inform management of potential dangers so that corrective action can be taken. Kauffman (*24*) reported that many industry leaders, trade associations, and regulatory agencies consider that the HACCP system is the best quality control program yet devised to assure safe foods in the marketplace.

The objective in developing the HACCP approach was to apply a zero-defects concept to production of food, considering ingredients, processing steps, and potential for consumer abuse (*3*). The analysis of hazards of ingredients was based on a classification of food products according to risk of salmonellosis (*30*). Adequate process control of both biological and physical hazards required: (*a*) delineation of all physical systems, identifying hazards to food safety; (*b*) elimination of hazards that are totally correctable; and (*c*) establishment of a control system for any hazardous condition which must remain part of the process. The potential for consumer abuse required precautions to prevent failure of the product through transportation, distribution, and consumer use (*3*).

The HACCP approach requires examination by qualified personnel of all processing operations to identify hazards, establish controls, select test procedures, and establish sampling schedules (*13*). Ito (*23*) suggested identifying critical control points by questioning and determining consequences of an event occurring during a given process; each processor should determine what is critical to that operation, even though it may not be covered by regulations.

In contrast to canned food processing, a heat treatment that eliminates all viable microorganisms is rarely applied in frozen food processing. The approach to the HACCP system for frozen food processing has implications for the food service industry.

Many critical control points may be identified in frozen food processing, contributing to a "net effect phenomenon". Each critical control point independently contributes to the net microbiological condition of the finished product. If the microbiological limit for any one critical control point is exceeded, control at preceding and subsequent critical control points may be negated. Exceeding the limit at a critical control point may also have an additive effect on the microbiological condition of the finished product (*10*).

Corlett (*11*) presented generalized models for basic processing sequences for frozen foods. Although food items and processing complexities differed, critical control points were similar: microbiology, sanitation, time-temperature, and employee cleanliness. Frequency of monitoring controls varied with the situation; controls were monitored as often as necessary to assure that critical control point limits were satisfied. Development of microbiological limits was discussed, emphasizing the need to use limits to measure specific conditions; limits may be too restrictive or too relaxed if incorrectly applied to different foods or processing circumstances. Microbiological testing of finished products, to provide a basis for ultimate acceptance or rejection of the product, is completed while the product is still under the processor's control.

Generalized models for HACCP systems in frozen food processing operations were also presented by Peterson and Gunnerson (*32*). Critical control points were established at all places in the processing sequence where microbial contamination or proliferation could occur. Critical control points included raw product control and storage, facility sanitation and maintenance, equipment sanitation, employee personal hygiene, microbiological population, and time-temperature. The interdependence of critical control points was discussed; loss of control at one critical control point may result in an additive microbiological effect or loss of control at subsequent critical control points.

Peterson and Gunnerson (*32*) emphasized the feedback characteristics of the HACCP system. Microbiological testing of the equipment and personnel sanitation, in-process food samples, and finished products yielded results too late to be effective for that product. The value of microbiological testing was in identifying problem areas and problem situations in processing, leading to increased preventative activities.

GENERALIZED HACCP MODELS FOR FOODSERVICE SYSTEMS

HACCP models were developed for entree production in conventional, cook/chill, and cook/freeze foodservice systems in three phases: identification of control points using flow diagrams, identification of critical control points, and establishment of monitors for control (*6*).

Control Points

A control point has been defined as a point in a process where a potential food hazard may exist (*12*). Control points are process stages of entree production for the generalized HACCP models.

Table 1 identifies the control points in the flow of entree production for conventional, cook/chill, and cook/freeze foodservice systems. As depicted, there are several identical control points in two or all three of the systems.

TABLE 1. *Control points during entree production in three foodservice systems*

Foodservice systems		
Conventional	Cook/Chill	Cook/Freeze
Procurement	Procurement	Procurement
↓	↓	↓
Preparation	Preparation	Preparation
↓	↓	↓
Heating	Heating	Heating
↓	↓	↓
Hot holding	Chilling and chilled storage	Freezing and frozen storage
		↓
		Thawing
	↓	↓
	Portioning and assembly	Portioning and assembly
↓		
Portioning, assembly and distribution	↓	↓
	Cold holding and distribution	Cold holding and distribution
	↓	↓
	Heating	Heating
↓	↓	↓
Service	Service	Service

These systems may differ in implementation among foodservice operations; however, as described here, entrees are prepared, cooked, and stored in bulk either hot, chilled, or frozen. Entrees are then portioned, and meals are assembled and distributed. In cook/chill and cook/freeze systems, entrees are heated before service.

Critical Control Points

Critical control points are those points in a process that eliminate or reduce a microbiological hazard (*12*). Four critical control points were identified for HACCP models for foodservice systems: ingredient control and storage, equipment sanitation, personnel sanitation, and time-temperature. For each control point identified in Table 1, one or more of the four critical control points were pertinent; see Table 2.

TABLE 2. *Critical control points during entree production in three foodservice systems*

	Critical control points			
Control points	Ingredient control and storage	Equipment sanitation	Personnel sanitation	Time-temperature relationship
Procurement[1,2,3]	X			
Preparation[1,2,3]		X	X	X
Heating[1,2,3]				X
Hot holding[1]				X
Chilling and chilled storage[2]		X		X
Freezing and frozen storage[3]				X
Thawing[3]		X		X
Portioning and assembly[2,3]		X	X	X
Portioning, assembly, and distribution[1]		X	X	X
Cold holding and distribution[2,3]		X		X
Heating[2,3]				X
Service[1,2,3]		X	X	X

[1]Conventional
[2]Cook/Chill
[3]Cook/Freeze

Specific standards for control at the critical control points will have to be determined by each foodservice system. Some factors contributing to microbiological control at critical control points are discussed.

Ingredient control and storage

Ingredient specifications are statements of desired quality. In addition to providing sensory and quantitative descriptions of the food item, specifications should provide for microbiological control of ingredients by identifying the wholesomeness of food at the source, type of packaging, and time-temperature during transit (*27*). Temperature control is necessary to maintain both the microbiological and sensory quality of refrigerated and frozen food. Ingredients should be procured from sources that comply with all laws relating to food and food labeling (*14*).

Inspection of food upon receipt is necessary to ascertain that specifications have been met. In addition to visually checking, counting, and weighing food, temperatures of refrigerated and frozen food should be recorded. Items that do not meet specifications should be rejected.

Time spent in receiving and inspecting food should be minimized by specifying delivery and scheduling adequate personnel for this function. Food, especially refrigerated and frozen items, should be promptly stored under suitable conditions.

An objective of food storage is to prevent or control loss or waste from deterioration or infestation (*38*). Control of ingredients in storage should include removal of items suspected of quality deterioration, especially from microbial spoilage.

The critical control point, ingredient control and storage, includes time-temperature considerations during storage. Three types of ingredient storage based on temperature are provided in foodservice systems: dry, refrigerated, and frozen. Optimal times and tempera-

tures for storage of ingredients should be established to retard deterioration. Some generalizations can be made regarding temperature of storage; time or shelf life of storage should be determined for individual ingredients.

Dry storage is for food requiring protection against invasion of rodents, infestation with insects, and bacterial contamination, but not requiring refrigeration. Storage areas should be at 10-21 C, well-ventilated, and dry with a relative humidity of about 50-60% (*27*). Food should not be stored under exposed sewer or nonpotable water lines or in contact with water or undrained ice (*14*).

The temperature of potentially hazardous food requiring refrigeration should be 7 C or below; food should be stored in facilities equipped with a thermometer accurate to ± 1.6 C (*14*). Longree (*27*) recommended optimal refrigerated storage temperatures by food category: dairy products and eggs, 2-4 C; meat and poultry, -1 to 2 C; fish, -1 to 0 C; and fruits and vegetables, 2-4 C. In addition, cross-contamination of raw and cooked food during refrigerated storage should be avoided. Fresh meat and produce are frequently sources of pathogenic microorganisms (*31*).

Frozen food should be stored at -18 C or below (*14*). Food should be packaged in materials which are impermeable to moisture and oxygen.

Equipment sanitation

The potential for bacterial contamination of entrees from equipment and utensils exists at several control or process stages; equipment sanitation is a critical control point.

Bryan (*8*) reported that two factors contributing to foodborne disease outbreaks were inadequate cleaning of equipment and cross-contamination; specific sources of contamination were not described. Stauffer (*37*) indicated that in many outbreaks common links between contaminated fresh foods and cooked food were sinks, knives, cutting boards, and hands.

The Food and Drug Administration (FDA) has proposed uniform requirements for food service sanitation; one section contains specific requirements for cleaning, sanitization, and storage of equipment and utensils (*14*). Procedures should be established by each foodservice system for cleaning and sanitizing equipment, including methods, materials, and frequency of cleaning. Practical information to assist in developing procedures is in books and trade journals (*19,26,28,33*).

Personnel sanitation

At all control points or process stages in entree production where food is handled, personnel sanitation is a critical control point. Personnel may directly or indirectly contribute to microbiological contamination.

Personnel who are infected with a disease should not be working in the foodservice (*14*). Bryan (*8*) reported that infected persons practicing poor personal hygiene were major contributors to foodborne disease outbreaks; this factor contributed to 151 of 725 outbreaks reported from 1961-1972. Health consciousness should be the responsibility of both management and foodservice personnel. Management should be observant for signs of illness in personnel and should encourage personnel to report all illnesses, especially those of the skin and gastrointestinal and respiratory tracts. Absenteeism for verifiable illnesses should not be penalized; personnel should not return to work until the infectious stage has passed (*36,37*).

Persons who are healthy may also transfer pathogens to food by air or physical contact during food handling activities. Cleanliness, clothing, and practices of personnel are specified by the FDA (*14*); however, interpretation of "good hygienic practices" is left to individual discretion. Personal hygiene and sanitary work practices were discussed by several authors (*1,27,38*). The importance for foodservice personnel of educational programs which include sanitary food handling practices was emphasized in the literature.

Time-temperature

Factors related to abuse of the time-temperature relationship in food have frequently contributed to foodborne disease outbreaks (*8*). Factors included: inadequate refrigeration, preparing foods far in advance of planned service with improper storage during the interval before serving, inadequate heat processing, and holding foods in warming devices at temperatures that favor bacterial growth.

The critical control point "time-temperature" refers to the relationship of time and temperature at control points of entree production. Time-temperature is a critical control point at all of the control points in Table 2 except procurement. The time-temperature parameter is included in the critical control point, ingredient control and storage. Time-temperature parameters for foodservice system control points are based on the zone of growth for microorganisms in food. The FDA has promulgated the regulation that the temperature of potentially hazardous food must be 7 C or below or 60 C or above at all times, except during preparation and heating (*14*).

Time-temperature standards for control points in conventional, cook/chill, and cook/freeze foodservice systems are in Tables 3, 4, and 5. The basis for these standards was to minimize the time that the temperature of entrees was in the zone of growth for microorganism, 7-60 C. Some factors considered in determining time-temperature standards for control points follow.

1. During preparation in all three systems, the temperature of entrees may be within the range of 7-60 C (*14*). Since this temperature zone will permit bacterial growth, time should be minimized. A centralized materials handling unit is one method for reducing the time that food is at ambient temperature by decreasing the time that cooks spend searching for, collecting, and transporting ingredients.

2. Most entrees requiring heating should be cooked to heat all parts of the food to at least 60 C. Poultry, poultry

TABLE 3. *Time-temperature critical control points at control points during entree production in a conventional foodservice system*

Control point	Critical control point	
	Time	Temperature[1]
Preparation	Minimal	7-60 C
Heating	—[2]	≥60 C[3]
Hot holding	—[2]	≥60 C
Portioning, assembly, and distribution	Minimal	≥60 C
Service	Minimal	≥60 C

[1]Internal temperature of entree at completion of control point activity.
[2]Time will vary with entree, equipment, and/or system.
[3]Minimum temperature; will vary with entree.

TABLE 4. *Time-temperature critical control points at control points during entree production in a cook/freeze foodservice system*

Control point	Critical control point	
	Time	Temperature[1]
Preparation	Minimal	7-60 C
Heating	—[2]	≥60 C
Chilling	≤4 h[4]	≥7 C
Chilled storage	≤ 20 h[4]	≤ 7 C
Portioning and assembly	Minimal	≤ 7 C
Cold holding and distribution	—[2]	≤ 7 C
Heating	—[2]	74-77 C
Service	Minimal	≥60 C

[1]Internal temperature of entree at completion of control point activity.
[2]Time will vary with entree, equipment, and/or system.
[3]Minimum temperature; will vary with entree.
[4]Combined time of chilling and chilled storage should be ≤24 h.

TABLE 5. *Time-temperature critical control points at control points during entree production in a cook/freeze foodservice, system*

Control point	Critical control point	
	Time	Temperature[1]
Preparation	Minimal	7-60 C
Heating	—[2]	≥60 C[3]
Freezing	≤1.5 h	≤-20 C
Frozen storage	≤8 weeks	≤-18 C
Thawing	Minimal	≤7 C
Portioning and assembly	Minimal	≤7 C
Cold holding and distribution	—[2]	≤7 C
Heating	—[2]	74-77 C
Service	Minimal	≥60 C

[1]Internal temperature of entree at completion of control point activity.
[2]Time will vary with entree, equipment, and/or system.
[3]Minimum temperature; will vary with entree.

stuffings, and stuffed meat should be heated to 74 C and pork and pork products should be heated to 66 C (*14*).

Longree (*27*) reviewed the literature related to food preparation and bacterial activity at cooking temperatures. The recommendation was made that food should be heated to 74-77 C, thus destroying the vegetative cells of common pathogens that cause foodborne illnesses.

Some food items are commonly given heat treatments to less than 74 C in the conventional system to preserve sensory quality. Entrees in the cook/chill and cook/freeze systems are given a mild heat treatment to preserve sensory quality (*22*). Therefore, heating to 60 C should not be considered an effective control measure for pathogens. Control points or process stages before and after this mild heat treatment should be designed to minimize bacterial contamination and/or growth.

3. The temperature of entrees during hot holding in the conventional system should be at least 60 C; the storage facility for hot holding should be equipped with a thermometer accurate to ± 1.6 C (*14*). This temperature should prevent bacterial growth but may not be sufficient to destroy bacteria. For entrees at 60 C or above, time of hot holding was not a factor for microbiological control.

Temperature of entrees during hot holding should be increased depending on the specific entree and foodservice system. Since the preferred eating temperature for hot entrees is 60-63 C, the cooling rate of the entree and the time lapse between portioning and assembly and service should be considered (*4*). In addition, since hot holding equipment is designed to maintain rather than increase temperature of food, entree temperature should be higher than 60 C at the onset of hot holding. Blaker and Ramsey (*5*) determined that steam tables were able to maintain food at 60 C for at least 1 h if food was 71 C or above when it was placed in the steam table.

4. During chilling in the cook/chill system, hot entrees should be cooled rapidly to 7 C or below to retard bacterial growth. Longree (*27*) recommended that food should reach an internal temperature of 7 C within 4 h and should not remain at 16-49 C for more than 2 h during the chilling process.

Longree (*27*) discussed factors which influence the cooling rate of food: refrigerator temperature, total load, batch size and/or depth, and heat transfer properties of food. A refrigerator temperature of 4 C or below was recommended to achieve rapid cooling; however, temperature should not be so low that food freezes. Circulation of air in a refrigerator must be sufficient to maintain refrigerator temperature at 4 C or below with a capacity hot load; refrigerator temperature should be monitored and adjusted accordingly. Dividing large batches of food into several smaller batches will increase the rate of cooling. Thermal properties of food affect cooling rates; generally, fluid foods cool more rapidly than solid foods.

Chilling and chilled storage time should be limited to 24 h (*18,27*). After chilling entrees to 7 C or below within 4 h, chilled storage should be limited to an additional 20 h. Numbers of aerobic bacteria in beef-soy loaves prepared in a simulated cook/chill foodservice system and held for 24, 48, and 72 h at 5 C were determined by Bunch et al. (*9*). The mean difference in numbers of aerobic bacteria between beef-soy loaves held 24 and 48 h was 10,000/g. Between loaves held 48 and 72 h, the mean difference was 23,000/g. Although differences in APC's among holding times had no practical microbiological significance, bacterial growth was observed with increased chilled holding time.

5. Freezing and frozen storage in the cook/freeze system is designed to preserve food quality. Fennema et al. (*17*) stated that the primary functions of freezing were to inhibit microorganisms and retard chemical changes. Although sensory quality was affected by rate of freezing, frozen storage usually offsets any quality advantages

gained by the freezing method and rate; frozen storage was considered the most detrimental phase of the freezing process.

Freezing methods varied among foodservice systems reported in the literature. The critical control point for freezing given in Table 5 was to reduce the temperature of the entree to -20 C in 1.5 h; this time-temperature parameter was recommended by Dorney and Glew (*15*). The Leeds system achieved this time-temperature using an air blast freezer at -32 C. Temperature of the entree was 80 C before the freezing process. Food was packaged in heavy gauge reusable aluminum pans containing eight portions each (*16*).

Longree (*27*) discussed on-premise freezing of cooked food and cautioned that bacteriological hazards could be formidable if the total production process was not bacteriologically controlled. Recommendations included freezing food in small, shallow batches so that food is solidly frozen within one-half hour of exposure to freezing temperatures; the temperature of freezing equipment was not given.

Storage temperature of -18 C or below is specified for frozen food (*14*). Damage during frozen storage was attributed to recrystallization caused by fluctuating temperatures. Generally, below -15 C, the rate of deterioration decreases as temperature is lowered (*17*).

Time of storage at -18 C or below has not been a consideration for microbiological control because bacterial growth is retarded at subfreezing temperatures. However, Glew (*18*) recommended a maximum shelf life of eight weeks.

6. Thawing frozen entrees in the cook/freeze system should be accomplished in refrigerated units. The temperature of potentially hazardous foods should not exceed 7 C during refrigerated thawing (*14*). Bacterial growth was retarded at temperatures below -18 C; however, growth is resumed when food is thawed. Bacteria can multiply when ice crystals are converted to free water; therefore, food temperature should be maintained at 7 C or below to control growth of microorganisms. Thawed entrees should be used promptly, preferably refrigerated for only a day (*27*).

7. During portioning, assembly, and distribution in the conventional system, entree temperature should be at least 60 C or above. Temperatures should be increased depending on the specific entree and foodservice system; factors to consider in determining the optimal temperature were discussed for hot holding.

In the cook/chill and cook/freeze systems, the temperature of "cold" hot entrees should be maintained at 7 C or below during portioning and assembly.

8. Cold holding and distribution in the cook/chill and cook/freeze systems is that period following portioning and assembly. Meal trays are either stored in refrigerated trucks for distribution to service units or placed on racks which are distributed to service units and stored in refrigerators. Temperature of "cold" hot entrees should be maintained at 7 C or below; holding time will vary with the foodservice system.

9. Immediately before service in the cook/chill and cook/freeze systems, "cold" hot entrees are heated. Longree (*27*) recommended that food should be heated to at least 74-77 C. This temperature is an important control. Entrees in these systems frequently receive a mild heat treatment during initial heating followed by various handling stages which provide opportunites for bacterial contamination and/or growth. Entrees are often one component of a plated meal which is heated by microwave oven; uneven heating of plated meals has been substantiated in the literature and should be considered in determining heating times.

10. During service in all three foodservice systems, the temperature of hot entrees should be maintained at 60 C or above. Service should be supervised to minimize delays.

Monitors for Control

The effectiveness of control measures at critical control points should be determined by establishing monitors for control. HACCP control measures for frozen food processing were evaluated for applicability to foodservice systems.

Microbiological testing of ingredients, critical control points, and finished products is a key criterion for HACCP control in frozen food processing; results of microbiological analyses are compared to established microbiological limits (*11*). However, Peterson and Gunnerson (*32*) emphasized that results of microbiological analyses are obtained too late to be useful for the lot being tested. The importance of anticipating and preventing microbiological problems in the HACCP system was stressed. Peterson and Gunnerson stated that much of in-process control consists of maintaining time-temperature standards and may involve continuous surveillance.

Establishing time-temperature standards is a practical method for monitoring entree production in foodservice systems; standards for conventional, cook/chill, and cook/freeze systems are in Tables 3, 4, and 5 (*6*). If continuous surveillance of time-temperature is implemented, immediate feedback is available for corrective action. With experience in monitoring time-temperature, critical control points, where the frequency of monitoring may be decreased without decreasing control, can be identified.

Monitors for control of equipment sanitation and personnel sanitation are necessary to measure adherence to established policies and procedures for sanitation (*6*).

APPLICATION OF HACCP FOR QUALITY CONTROL IN FOODSERVICE SYSTEMS

The goal of in-process control of entree production is to conserve the inherent quality of the ingredients. The HACCP system is a preventative approach to quality control, emphasizing microbiological control and

identifying process stages where loss of control could present a food safety risk. Conservation of nutritional and sensory quality was incorporated in the HACCP models developed for the three foodservice systems. It was postulated that adherence to the minimal temperature standards for heat processes would not only control microbiological quality but would also conserve the nutritional and sensory quality of the entree (*6*).

Implementation of the HACCP system was recommended for foodservice systems. However, standards and monitors for control of critical control points must be established for the foodservice operation and should be based on system resources and constraints (*6*). Silverman (*34*) indicated that the effectiveness of attempts at quality control in food services are often negated by failure to establish written procedures and effective training and supervision.

Using the HACCP approach, inspection of critical control points could and should be documented. In the future, the foodservice administrator may be required to file reports of self-inspection with State or local regulatory officials. Reports would be monitored by a regulatory official and periodically validated by inspection. The FDA is now in the process of developing a Model Food Service Ordinance that will be available and promoted for State and local adoption to regulate and inspect foodservice establishments (*7*).

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Food Protection in Jails and Prisons

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ABSTRACT

In recent years substantial attention has been given to environmental health and food protection in jails and prisons in the United States. As a result several commissions and task forces, as well as the courts, have studied conditions in the correctional setting which are hazardous to the health and well-being of inmates. This report, based on an investigation of 100 selected jails and prisons, summarizes findings on foodservice operations and their role in penal and correctional institutions.

During the past decade substandard environmental conditions and inadequate health care in penal and correctional systems have been found by the courts to constitute a cruel and unusual punishment in violation of the Constitution of the United States. In its 1971-72 term, the U.S. Supreme Court decided eight cases directly affecting offenders' rights and in each case the contentions of the offenders prevailed. More recently, in its 1976-77 term the highest court ruled that deliberate indifference by prison officials to serious medical needs of an inmate violates the Eighth Amendment's ban against cruel and unusual punishment and gives the inmate grounds to sue the officials in Federal court.

In addition to the judicial system, several governmental and quasi-governmental organizations have been concerned about environmental health services in jails and prisons. Notably, the United States Department of Justice and the National Prison Project separately requested the assistance of the authors in the identification and evaluation of environmental health issues existing within the penal and correctional system. This identification and evaluation required visits to 100 preselected institutions throughout the United States, Puerto Rico and the Virgin Islands in which the Department of Justice and the National Prison Project had reasons to believe that environmental conditions were less than adequate. These visits were made between 1974 and 1976.

It is the purpose of this report to describe only one of the several environmental health issues which were investigated — foodservice and its ramifications.

THE CORRECTIONAL SYSTEM

To summarize intelligently the findings of the investigation, certain dimensions of the correctional system of the United States must be reviewed. Institutionalization as a primary means of enforcing customs, mores, or laws of a people is a relatively modern practice. In earlier times restitution, exile, and a variety of methods of corporal and capital punishment, many of them unspeakably barbarous, were used. Confinement was used only for detention.

Today the backbone of the nation's correctional system is composed of 36 federal prisons, 11 federal community treatment centers, 600 administratively separate correctional facilities operated by state governments, and 3,921 jails operated by local units of government.

State-operated institutions probably embody most of the ideals and characteristics of the early attempts to reform offenders. It is in these facilites that most intensive correctional or rehabilitative efforts are conducted. Here inmates are exposed to a variety of programs intended to help them become productive members of society. But the predominant consideration is still that of security. About half of all state correctional facilities in the United States are located in the South, with the remaining institutions about equally distributed among the other three regions of the country. North Carolina has the largest number (76), followed by Florida (46), Virginia (*38*), and California (35).

Operating expenses of jails and prisons for the latest fiscal year (1975) range from less than $50,000 to more than $3 million. Expenditures made by each institution are a function not only of its type and size, but also of such factors as the proportion of inmates in each confinement status, the amount of labor contributed by inmates toward operating expenses and maintaining the facility, existence of a prison industry, scope of rehabilitative programs, and the extent to which volunteers perform certain functions.

For the most part jails are not places of final deposition. Approximately 2.5 to 5.5 million jail

commitments occur in this country annually. The obvious result is a highly transient jail population. However, pretrial detention can stretch into years through legal maneuvering by law enforcement officials. Local control, multiple functions, and a transient heterogeneous population have shaped the major organizational characteristics of jails. Typically, they are under the jurisdiction of the county government and they retain the dual purposes of custodial confinement and misdemanant punishment. The most conspicuous addition to the jails' functions have been services to the homeless and to alcoholics. Thus jails are, in a sense, one of the catchalls for social and law enforcement problems in a community.

In summary, both jails and prisons are communities within themselves. Their "metabolic" requirements include complex mechanical services, heating and ventilation systems, domestic water supply, sewage disposal facilities, industry and labor progams, recreational services, and foodservice operations. All of these must be managed in such a way as to effectively meet at least four basic human needs: (*a*) fundamental physiological needs, (*b*) fundamental psychological needs; (*c*) protection against contagion, and (*d*) protection against accidents. These needs are common to all human populations and they cut across boundaries and definitions of social behavior, socioeconomic status, criminals and non-criminals. Accordingly, persons confined in penal and correctional institutions are just as susceptible to environmentally-induced diseases and disabilities, including the hazards of food infection and food intoxication, as are law abiding citizens in other institutional settings.

The pattern of foodservice varies among the nation's correctional institutions. In all state operated prisons food is prepared and served within the institution. However, in 70% (2,753) of all jails, meals served to inmates are prepared in the jail, whereas in 1,135 other jails the meals are prepared elsewhere and brought into the institution. In small jails (less than 10 inmates) it is fairly common practice for the sheriff or chief jailer to arrange for meals to be brought in. In at least two small county jails foodservice is provided by a nearby fast-food outlet.

More than two-thirds of the jails and all state prisons serve meals at least three times a day, while the remaining jails serve meals once or twice a day. In 12% of the nation's jails meals are served exclusively in dining halls or in a central foodservice area. However, in 65% of the jails food is served solely in the cells. About 17% of the jails use both dining halls and cells, and 23% have other foodservice arrangements.

For state prisons foodservice follows four general patterns: (*a*) mass feeding and mass cooking in one dining room, (*b*) mass cooking with feeding in several dining rooms or day rooms, (*c*) cottage-type feeding with small kitchens and small dining rooms for each cottage inmate group, or (*d*) one kitchen with feeding in individual cells or rooms. Patterns (*a*) and (*d*) are being followed by an increasing number of institutions because of overcrowded conditions.

FINDINGS

Food supplies

All institutions visited, except two, prepared and served food which either originated from approved sources or which was considered satisfactory by the state and local health authorities. However, two state-operated institutions, each housing more than 2,500 inmates, carried out their own meat processing operation which was not under supervision of appropriate meat control authorities; no antemortem or postmortem inspections were done to eliminate slaughtering of sick or fatigued animals or to detect gross pathology of the carcasses.

Food protection

In all 100 institutions investigated there was substantial evidence of inadequate food protection. This evidence included the following.

1. Food was transported from central kitchen to cell block in unheated and/or uncovered containers, followed by the lapse of an inordinate amount of time (3-4 h) between preparation, delivery to cell blocks, and service to inmates.
2. Raw food ingredients and prepared foods contained animal and insect filth including live insects and insect parts.
3. Raw and cooked products were managed in such a way that the opportunity for cross-contamination was enhanced.
4. Steam tables and similar devices for keeping food hot were defective and in need of major repair.
5. Prepared foods such as salads, hash, and left-overs, were placed in large deep containers which required an extended time interval for the entire mass to chill sufficiently to inhibit bacterial growth, especially in the center portions of the food.
6. Refrigeration space was inadequate to store and maintain perishable food at a proper temperature without packing and crowding and impeding air circulation.
7. Clean food contact surfaces of equipment were not protected from recontamination between uses.

Cleanliness of equipment and utensils

Substandard dishwashing procedures and equipment were prevalent in 85 of the 100 institutions. While mechanical dishwashing was common, problems of inadequate hot water for final rinse cycle, clogged spray jets and inoperable detergent dispensers hampered effective cleaning and sanitizing of eating and drinking utensils; greasy and food-stained tableware was the rule rather than the exception.

In 10 of the institutions dining utensils were not returned from the cells to the central kitchen for washing but were "cleansed" in a utility sink located in the cell block area. The procedure was simply "rinse and dry."

Single-service knives and forks were used and reused

in the maximum security section of four large prisons. Here cleaning of the utensils was the responsiblity of each inmate. This was usually done in individual cells, none of which were provided with warm water or detergent for cleaning purposes.

Vermin control

Regular pest control services were provided in 97 institutions by commercial pest extermination services. Three institutions carried out their own insect and rat control program on an "as needed basis."

However, substantial cockroach infestations were evident in all 100 institutional foodservice operations. Thirty institutions, visited during warm weather, had a significant fly problem.

The missing element in the insect and rodent control service was a comprehensive housekeeping and maintenance program designed to eliminate those conditions which encourage growth and development of flies, cockroaches, and rats.

Personnel

Inmates, under the supervision of civilian personnel, were "employed" as foodservice workers in all of the institutions. None of the instituitons provided food sanitation training and orientation for foodservice personnel.

The warden and foodservice supervisor in all but five institutions gave high priority to "pre-employment" and periodic physical examinations of all inmates who performed duties in the foodservice program.

As is well known among health service personnel, periodic physical examinations are not effective in preventing development and progression of foodborne epidemics because most of the conditions detected in physical examinations are transient and develop and pass away in the interval between such examinations.

On the other hand training of food service workers is one of the most effective approaches in minimizing problems of food hygiene and sanitation at the preparation and service levels of the food distribution system.

However, in the four state-operated institutions where culinary vocational training was established, foodservice sanitation and hygiene were not emphasized in the "curriculum."

DISCUSSION AND CONCLUSIONS

Food and other environmental health issues occupy such an important place in every inmate's life that their effect on morale, and physical and mental health cannot be overestimated. To be sure most incarcerated offenders are of the lower socioeconomic classes which generally have worse nutritional problems than more affluent groups. Thus there is a need for greater attention to the quality and quantity of food served to the confined inmates than on the population at large.

In fact, in many instances food can determine the success or failure of the most carefully designed rehabilitation and correctional programs. This was clearly indicated in the most recent civil disturbance in the Tennessee State Prison in Nashville in September 1975. As described in the *Nashville Tennessean*: "It all began for the lack of pork chops (which ran out during an evening meal and cold bologna was served as a substitute) and when it was over, 39 people had been injured and one inmate was dead" (*1*).

Jail and prison foodservice systems operate under budgetary constraints and under physical limitations which make it difficult to provide the variety of foods which are found in facilities in the "free" community. As such, meals often become monotonous to the inmates who have no choice but to consume food provided by the institution or experience one of the many manifestations of primary malnutrition.

Even the cold gray metal of the food trays detracts from the appetizing appearance of the food. Unless precautions are taken, speedy service often produces an unattractive tray with gravy spilled over the edges and vegetables scattered outside the vegetable compartment.

Unfortunately management of foodservice in jails and prisons is under the supervision of non-professional food service personnel. The workers are inmates, and in such work force there are new and untrained personnel; others who have had limited experience, and very few who have acquired experience which is valuable to the management of mass feeding systems. Among this group of foodservice workers, every attitude from active interest to open antagonism is manifested.

The incarcerated foodservice worker often feels alienated, angry, and isolated in an environment which he does not understand; a situation which frustrates his performance as a member of the foodservice staff.

Compounding this problem is the fact that the correctional officer — who is usually in charge of the "foodservice detail" — sees his primary role as guardian of custody, discipline, and security in the immediate environment and not as that of supervisor of food hygiene and sanitation practices.

This complexity of attitudes of the worker and of the officer in charge is often reflected in the level of sanitation in the food preparation area and quality of food offered to inmates.

In this setting it must be recognized that methods of operating foodservice programs like other subsystems of the correctional institution should never be static. They must be reconciled with changing patterns produced by social and economic characteristics. Changes in the penal and correctional process and the newer aspects of institutional food management demand a continuous evaluation of methodology and the application of resources to promote maximum food protection and reduce the potential of foodborne illness.

Cost must be considered in relation to goals and results; duplication and waste of efforts must be avoided. Economy demands maximum results compatible with the concerns about increased productivity and better acceptability of food service in the institutions.

It would therefore seem appropriate, from a food protection standpoint, to abolish the participation of inmates in the foodservice program and replace them with professional foodservice personnel who could, using modern techniques of foodservice management, plan, prepare and serve all meals required of the institution.

This view is supported by several groups, including the National Sheriff's Association which recently suggested that, "It is time now to think of eliminating inmates entirely from the food service" (*2*). The Federal District Courts have taken a similar position and in one case the judge ordered that "the food served to inmates shall be nutritionally adequate and properly under the supervision of a foodservice supervisor for each institution; each supervisor shall have at least bachelor's level training in dietetics or its equivalent" (*3*). The court also required the institution to employ a qualified nutritionist to assist in menu planning, in food purchasing and preparation, and to monitor foodservice hygiene and sanitation.

Hospitals, academic institutions, and industrial feeding operations have for several years recognized the benefits of putting foodservice management on a sound professional foundation. Such an approach has increased productivity, reduced cost, and improved consumer satisfaction with the quality and quantity of foods which are served (*5*).

The problem of foodservice in jails and prisons is also complicated by the physical environment — the preparation and serving area. Outmoded and archaic food equipment, inadequate ventilation systems, insufficient refrigeration, and totally inadequate working and storage space perpetuate a substandard rather than effective and efficient food management system.

Of the 100 institutions studied in this project 56 were constructed between 1830 and 1900. They were built to be internally and externally secure and reflected concern for complete surveillance of inmates. Evidently in the process of achieving the goals of security, the foodservice plant was given less than a high priority.

Thus some correctional institutions are saddled with the physical remains of last century's concept of foodservice for jails and prisons and with an ideological legacy that poor food and substandard foodservice are part of the penalties which an offender must pay during confinement.

The result has been an inefficient foodservice program, "economical" perhaps in its daily operation, but tragically expensive in its ultimate effect on the overall goal of foodservice hygiene and sanitation.

Contemporary facility planning must recognize the requirement of safe food preparation and storage areas while recognizing the needs for the most efficient expenditure of limited public funds.

Hopefully, reforms in foodservice in jails and prisons will run parallel with current reforms in the entire criminal justice system. Fortunately the Federal government, followed by the states, is beginning to provide important leadership here. In addition, budgetary recognition is being given to the significance of correctional institutions as treatment facilities and correctional administrators are no longer isolating corrections from the general public. For example, statutory provisions now require that at least 20% of Federal funds disbursed by the Law Enforcement Assistance Administration to states to aid crime control be allocated to corrections. The low priority traditionally assigned to budgetary support for the penal system and to prisoners in general is being changed. It is being supplanted by the realization that the quality of life depends in part on creation of a humane, just and efficient correctional system. Coupled with this realization is the knowledge that achievement of such a correctional system must entail substantial reform of each of the subsystems, including the institutional foodservice program.

Unfortunately, we do not know the full extent of malnutrition or the incidence of foodborne illnesses in the correctional system, but problems of adequate nutrition and foodservice sanitation in penal and correctional institutions have ramificatins which are different from other community health problems and therefore require a slightly different approach by the community health specialists. The scope of food management is extensive involving as it does problems of crop production (in which many prison systems engage), farm equipment and manpower, food processing, food preservation, transportation and storage, proper food preparation, as well as both nutrition and food sanitation education, and the diagnosis, prevention and treatment of specific dietary deficiency diseases. In fact the scope is so broad that it cannot be planned, implemented, and evaluated by any one discipline of the health and medical sciences. Close cooperation and intimate working relations among several disciplines are essential (*4*).

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A Field Topic

The Milking Machine as It Relates to Mastitis

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ABSTRACT

Recent studies in the field and in research laboratories indicate pressure changes within the system can cause bacterialaden milk droplets to be introduced into the teat during milking. If this evidence is confirmed, mastitis control will be difficult, if not impossible, until milking systems are modified to prevent this reentry of milk to the teats. Some independent agency should draft safety rules for milking machine design to reduced tissue damage and microbial recontamination of the teat orifice.

There is much evidence that bacteria-laden milk droplets can be introduced into the teat by pressure changes within the teat cup, claw, and pipe system of a milking machine. This was first suggested by Noorlander (*7*) when his research demonstrated that milking machines were a predisposing factor to mastitis. Later the feasibility of this mechanism was studied by jetting bacteria (*2*) and bacterial endotoxin (*11*) against the end of the teat after which signs of penetration into the teat cistern were found. Noorlander later found (*5*), by the use of transparent inflations and shells, that most inflations and many claws became flooded with milk during the milking process and suggested use of milk tube air vents to stabilize the vacuum and to prevent impact of milk droplets against the teat. A recent and complete study (*10*) demonstrated that use of machines with operating characteristics conducive to the impact of milk droplets on the end of the teat (irregular fluctuation of vacuum supply coupled with cyclic fluctuation at each pulsation cycle and fast opening of the teat-cup liner at the start of each milking phase) significantly increased the incidence of infection in teats challenged by dipping in a mixture of *Streptococcus agalactiae* and *Streptococcus dysgalactiae.*

Investigations of dairy herds having serious teat damage, teat errosions, and mastitis have disclosed that the damage to the end of the teat can be associated with liner design, pulsation function, cyclic vacuum drops measured below the teat, vacuum level, and wear or use-life characteristics of the rubber inflation (*4,6*).

Recent unpublished investigation of teat-end damage in Chile where many herds are still milked by hand, by modern pipeline installations, and a non-pulsating constant vacuum milking machine (*8*) demonstrated that vacuum can and does cause tissue damage, but the teat orifice damage reflected by teat errosions appear to be associated with inflation design and improper massage. Whittlestone made similiar observations (*12*).

Experiences with several types of inflations including the Boumatic Triple Collapse, Flat or F1 and F2 liner; The P.V.C. Transparent liners; many of the Hi-Life narrow bore liners; and the Square Vented and Ring Type Square Vented liners sold throughout the United States show that liner or inflation design will influence teat damage, flooding of milk, milking efficiency and contamination of the teat orifice (*9*).

The speed of inflation closure and opening in combination with the type of claw, and inflation, are primarily responsible for the impinging of milk backward against the teat (*10*). The inflation bore size and milk tube outlet are also responsible.

IMPROVEMENTS NEEDED

Although most of the above technical information has been available to dairy farmers and milking machine manufacturers for many years, improvements in milking machine function has been primarily limited to improvements in vacuum pump capacity, air reserve, and lately vacuum controllers. This has helped stabilize the vacuum within the pipes of a milking system and helped prevent milk from impinging backward because of inadequate air reserve. However, little progress has been made to modify or change existing problems associated with the milking unit to prevent the impinging of milk backward against and through the teat orifice. Little has been done to improve inflation design to prevent tissue damage. Little has been done by manufacturers to prevent milk from being impinged backward against the teat because of pulsator, claw, or inflation design.

MANUFACTURING PROBLEMS

It must be remembered that most manufacturers have designed milking machines to solve labor problems. It was not until 1959 that dairymen were even exposed to the possibility that milking machines were a major predisposing cause of mastitis. *Hoard's Dairyman* published a series of articles at that time based on the research done at the University of California. The same articles introduced the California Mastitis Test to dairymen (*1*).

It must also be recognized that manufacturers of patentable products generally do not publish papers on those products that could

reveal to other manufacturers their trade secrets, formulations, or designs that would make their equipment function better than other manufacturers. Patented products are also a legal monopoly.

In understanding the mastitis problem one must also recognize that most dairymen in some parts of the United States still use milking machines that were manufactured or at least function the same as the machines that were manufactured before 1959. This is particularly true of the pulsator and inflation.

WHAT KIND OF MACHINES DO DAIRYMEN USE?

If the recent research results are true, it would appear that mastitis on a national or even international level will not be resolved until the milking machines now sold and used on the market are modified or changed to prevent bacteria from infecting our cows. The following are typical examples:

1. Most milking machines sold and used on the market cannot prevent bacteria laden milk from ejecting backward and into the teat orifice during milking (*5*).
2. Most pulsators used are very snappy in action. This means that most cows are milked with pulsators that cause droplets of milk to impinge backward against the teat (*10*).
3. Most inflations sold in the United States and the world restrict blood outflow at the base of the udder and are made of rubber with a high modulus of elongation. This is the physical force necessary to stretch rubber. Low modulus rubber is necessary for inflations 3/4 inch in diameter for proper teat massage and milk outflow (*4*).
4. Low modulus rubber used at the present time in narrow bore inflations does not maintain its physcial qualities for longer than 1000 individual cow milkings and becomes very open and porous. Such inflations become a perfect place for bacterial growth (*4*).
5. Many claws sold or used in the United States and foreign countries cannot prevent milk from inside the claw from being forced backward against the teats of healthy cows.
6. Most milking machines cannot be sanitized properly between cows (*4, 6*).
7. Some dairymen are using inflations and pulsators that cause the cyclic vacuum to drop so low it is physically impossible for the inflation to close properly because of the use of high modulus rubber (*3, 5*).
8. One company sells vacuum regulators that cannot be adjusted. Cows milked with low lines are milked with vacuum levels that are 3 to 4 inches higher than when the same equipment milks into high pipelines.

RECOMMENDATIONS

In spite of the above observations and published reports from all over the world, there are universities and leaders in the dairy industry who would have one believe that these technological facts are controversial and one should not mention by brand name any component of milking machine equipment that may or may not predispose to mastitis.

New equipment is constantly placed on the market without prior determination of whether or not the equipment will or will not prevent milk from infected cows and equipment from ejecting backward against the teat. This is particularly true with some types of automatic take-off units that do not have automatic shut-off valves.

There is no mystery as to how or why vacuum damages the tissue of a cow. This was first recognized by Ivan Petrovich Pavlov who died in 1931. Louis Pasteur died 36 years before this and taught how to prevent the spread of disease by controlling infection. We have yet to learn to use these two simple principles in the design of our milking machine to prevent the dairy industry's most costly disease.

It is suggested then, that responsible leadership within the dairy industry suggest and draft safety rules concerning the manufacture of milking machines to reduce the amount of tissue damage and bacteriological contamination to our dairy cows. This suggests, however, that funding and research for these safety rules be established by institutions that have no financial involvement with the manufacture of milking machines. These rules could be submitted to the consumer product safety commission under the Consumer Products Safety Acts passed by Congress in 1972. This Federal Act provides specific remedies and damages that the consumer, meaning the dairy farmer or cooperative, may enforce where he is injured or damaged as a result of noncompliance with this act. This act contains a provision for the submission of safety rules to the Public Safety Commission created by the Act.

It can be anticipated that the world's supply of high quality protein will continue to diminish and no nation can afford the luxury of mastitis and it's consequent effect on our milk volume.

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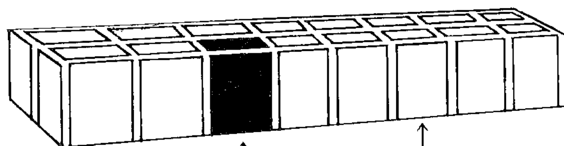

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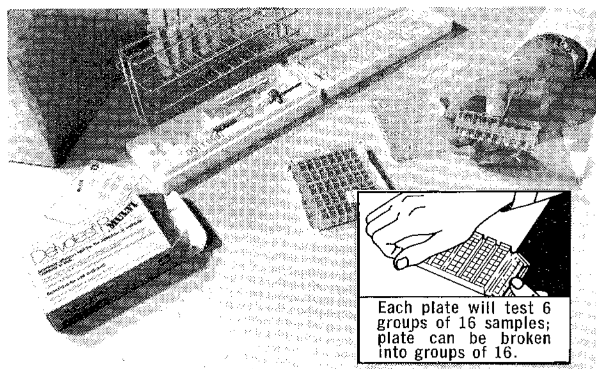

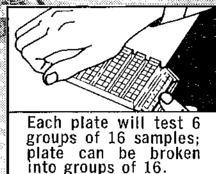

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Holders of 3-A Symbol Council Authorizations on August 20, 1977

Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y.-Treas., P.O. Box 701, Ames, Iowa 50010.

01-06 Storage Tanks for Milk and Milk Products

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St.
Little Falls, New York 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets
Chester, Pennsylvania 19013

2 CREPACO, Inc. (5/ 1/56)
100 C.P. Avenue
Lake Mills, Wisconsin 53551

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

76 Damrow Company (10/31/57)
196 Western Avenue
Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28 59)
113 Park Street South
Peterborough, Ontario, Canada

109 Girton Manufacturing Company (9/30/58)
State Street
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
P.O. Box 2507
City of Industry, California 91746

127 Paul Mueller Company (6/29/60)
P.O. Box 828
Springfield, Missouri 65801

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-06 Pumps for Milk and Milk Products

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wisconsin 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd.
Oak Brook, Illinois 60621

29R Cherry-Burrell Corporation (10/ 3/56)
2400 Sixth St., Southwest
Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 CP Avenue
Lake Mills, Wisconsin 53551

205R Dairy Equipment Company (5/22/69)
1919 South Stoughton Road
Madison, Wisconsin 53716

65R G & H Products, Inc. (5/22/57)
5718 52nd Street
Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way
Costa Mesa, California 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

236 Megator Corporation (5/ 2/72)
125 Gamma Drive
Pittsburgh, Pennsylvania 15238

280 Niro Atomizer (9/10/76)
9165 Rumsey Road
Columbia, Maryland 21045

241 Puriti S. A. (9/12/72)
Alfredo Noble #39, Industrial Pte. de Vigas
Tlalnepantla, Mexico

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Avenue
Springfield, Ohio 45501

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 43rd Street
Kenosha, Wisconsin 53140

219 Tri-Canada Cherry-Burrell Ltd. (2/15/71)
6500 Northwest Drive
Mississauga, Ontario, Canada

175R Universal Milking Machine Div. (10/26/65)
National Cooperatives, Inc.
First Avenue at College
Albert Lea, Minnesota 56007

52R Viking Pump Div. (12/31/56)
Houdaille Industries, Inc.
406 State Street
Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

282 Knudsen Corporation (11/ 8/76)
715 N. Divisadero Street
Visalia, California 93277

04-03 Homogenizers and High Pressure Pumps of the Plunger Type

247 Bran and Lubbe, Inc. (4/14/73)
2508 Gross Point Road
Evanston, Illinois 60201

87 Cherry-Burrell Company (12/20/57)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street
Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
P.O. Box 1441
Minneapolis, Minnesota 55440

256 Hercules, Inc. (1/23/74)
2285 University Avenue
St. Paul, Minnesota 55114

282 Knudsen Corporation (11/ 8/76)
715 N. Divisadero Street
Visalia, California 93277

05-13 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road
Almont, Michigan 48003

70R Brenner Tank, Inc. (8/ 5/57)
450 Arlington,
Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., Southeast
Minneapolis, Minnesota 55114

66 Dairy Equipment Company (5/29/57)
1919 South Stoughton Road
Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street
Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Avenue
Milton, Ontario, Canada L9T 2Y3

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minnesota 56340

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c.p. 758
Drummondville, Quebec, Canada

189 A. & L. Tougas, Ltee (10/ 3/66)
1 Tougas St.
Iberville, Quebec, Canada

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Avenue
North Kansas City, Missouri 64116

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

08-17 Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products

291 Accurate Metering Systems, Inc. (6/22/77)
1731 Carmen Drive
Elk Grove Village, IL 60007

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue
Waukesha, Wisconsin 53186

138R APV-CREPACO of Canada Limited (12/17/62)
1250 Ormont Dr.
Weston, Ontario, Canada M9L 2V4

245 Babson Brothers Company (2/12/73)
2100 South York Road
Oak Brook, Illinois 60521

284 Bristol Engineering Company (11/18/76)
210 Beaver Street
Yorkville, Illinois 60560

82R Cherry-Burrell Company (12/11/57)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52406

266 Condor Manufacturing Company (8/ 1/75)
418 West Magnolia Avenue
Glendale, California 91204

260 CREPACO, Inc. (5/22/74)
100 CP Avenue
Lake Mills, Wisconsin 53551

271 The Foxboro Company (3/ 8/76)
Neponset Street
Foxboro, Massachusetts 02035

67R G & H Products, Inc. (6/10/57)
5718 52nd Street,
Kenosha, Wisconsin 53140

199R Graco, Inc. (12/ 8/67)
P.O. Box 1441
Minneapolis, Minnesota 55440

203R ITT-Grinnell Company (11/ 7/68)
260 W. Exchange St.
Providence, Rhode Island 02901

34R Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

287 Koltek OY (1/14/77)
Kotinummentieiz
SF-00700 Helsinki 70
Finland

239 LUMACO (6/30/72)
Box 688,
Teaneck, New Jersey 07666

200R Paul Mueller Co. (3/ 5/68)
P.O. Box 828
Springfield, Missouri 65801

295 Precision Stainless Products (8/11/77)
5636 Shull St.
Bell Gardens, CA 90201

242 Puriti, S. A. (9/12/72)
Alfredo Nobel #39 Industrial Pte. de Vigas
Tlalnepantla, Mexico

149R Q Controls (5/18/64)
Occidental, California 95465

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street
Kenosha, Wisconsin 53140

191R Tri-Canada Cherry-Burrell, Ltd. (11/23/66)
6500 Northwest Drive
Mississauga, Ontario, Canda L4V 1K4

250 Universal Milking Machine Division (6/11/73)
Universal Cooperatives, Inc.
408 First Ave. South
Albert Lea, Minnesota 56007

278 Valex Products (8/30/76)
9421 Winnetka
Chatsworth, California 91311

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers

09-07 Instrument Fittings and Connections Used on Milk and Milk Products Equipment

269 Babson Bros. Company (1/23/76)
2100 South York Road
Oak Brook, Illinois 60521

206 The Foxboro Company (8/11/69)
Neponset Avenue
Foxboro, Massachusetts 02035

285 Tank Mate Company (12/ 7/76)
1815 Eleanor
St. Paul, Minnesota 55116

32 Taylor Instrument Process Control (10/ 4/56)
Div. Sybron Corporation
95 Ames Street
Rochester, New York 14601

246 United Electric Controls (3/24/73)
85 School Street
Watertown, Massachusetts 02172

10-00 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

11-03 Plate-type Heat Exchangers for Milk and Milk Products

20 A.P.V. Company, Inc. (9/ 4/56)
395 Fillmore Avenue
Tonawanda, New York 14150

30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets
Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53551

267 De Danske Mejeriers Maskinfabrik (10/15/75)
The Danish Dairies' Machine Factory
P.O. Box 66, 6000 Kolding, Denmark

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street
South Peterborough, Ontario, Canada

279 The Schluter Co. (8/29/76)
112 E. Centerway
Janesville, WI 53545

17 The DeLaval Separator Company (8/30/56)
350 Dutchess Turnpike
Poughkeepsie, New York 12602

15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street
Watertown, Wisconsin 53094

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products

248 Allegheny Bradford Corporation (4/16/73)
P.O. Box 264
Bradford, Pennsylvania 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road
Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street
Chester, Pennsylvania 19013

152 The DeLaval Separator Co. (11/18/69)
350 Dutchess Turnpike
Poughkeepsie, New York 12602

217 Girton Manufacturing Co. (1/23/71)
Millville, Pennsylvania 17846

252 Ernest Laffranchi (12/27/73)
P.O. Box 455
Ferndale, California 95536

238 Paul Mueller Company (6/28/72)
P.O. Box 828
Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P.O. Box 188
Mora, Minnesota 55051

13-06 Farm Milk Cooling and Holding Tanks

240 Babson Brothers Company (9/ 5/72)
2100 S. York Road
Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 CP Ave.
Lake Mills, Wisconsin 53551

119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

4R Dairy Equipment Company (6/15/56)
1919 South Stoughton Road
Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street
South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Dutchess Turnpike
Poughkeepsie, New York 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd.
El Monte, California 91732

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road
Preston, Ontario, Canada

12R Paul Mueller Company (7/31/56)
P.O. Box 828
Springfield, Missouri 65801

249 Sunset Equipment Co. (4/16/73)
3765 North Dunlap Street
St. Paul, Minnesota 55112

42R VanVetter, Inc. (10/22/56)
4 South Idaho Street
Seattle, Washington 98134

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

254 Anhydro, Inc. (1/ 7/74)
165 John Dietsch Square
Attleboro Falls, Massachusetts 02763

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street
Buffalo, New York 14207

263 C. E. Howard Corporation (12/21/74)
240 N. Orange Avenue
City of Industry, California 91746

107R C. E. Rogers Company (8/ 1/58)
P.O. Box 118
Mora, Minnesota 55051

277 ConTherm Corp. (8/19/76)
P.O. Box 352
Newbury Port, MA 01950

294 DeLaval Separator Co. (7/19/77)
1600 County Rd. F.
Hudson, WI 54016

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road
Birmingham, Michigan 48010

273 Niro Atomizer Inc. (5/20/76)
9165 Rumsey Road
Columbia, Maryland 21044

17-04 Fillers and Sealers of Single Service Containers For Milk and Milk Products

192 Cherry-Burrell Corporation (1/ 3/67)
a unit of AMCA International Corp.
2400 Sixth St., Southwest
Cedar Rapids, IA 52404

137 Ex-Cell-O Corporation (10/17/62)
2855 Coolidge,
Troy, Michigan 48084

220 Hercules, Inc., Package Equipment Div. (4/24/71)
2285 University Ave.
St. Paul, Minnesota 55114

281 Purity Packaging Corporation (11/ 8/76)
4190 Fisher Road
Columbus, Ohio 43228

211 Steel & Cohen (2/ 4/70)
745 Fifth Avenue
New York, New York 10022

19-02 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

286 Alfa-Hoyer (12/ 8/76)
Soren Nymarksvei 13
DK-8270 Hojbjerb, Denmark

146 Cherry-Burrell Company (12/10/63)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

141 CREPACO, Inc. (4/15/63)
100 CP Avenue
Lake Mills, Wisconsin 53551

22-04 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St.
Little Falls, New York 13365

154 CREPACO, Inc. (2/10/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

181 Damrow Company, Division of DEC (5/18/66)
International, Inc., 196 Western Ave.
Fond du Lac, Wisconsin 54935

262 DeLaval Company Limited (11/11/74)
113 Park Street
South, Peterborough, Ontario, Canada

156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue
South Gate, California 90280

276 Letsch Corporation (8/17/76)
501 N. Belcrest
Springfield, Missouri 65802

155 Paul Mueller Co. (2/10/65)
P.O. Box 828
Springfield, Missouri 65801

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-01 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road
Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc., 869 S. Knowles Ave.
New Richmond, Wisconsin 54017

258 Hercules, Inc. (2/ 8/74)
2285 University Ave.
St. Paul, Minnesota 55114

222 Maryland Cup Corporation (11/15/71)
10100 Reisterstown Road
Owings Mills, Maryland 21117

193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave.
Chicago, Illinois 60635

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St.
Little Falls, New York 13365

158 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P.O. Box 828
Springfield, Missouri 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

275 Bepex Corporation (7/12/76)
150 Todd Road
Santa Rosa, California 95402

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St.
Little Falls, New York 13365

159 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

283 Letsch Corporation (11/10/76)
501 N. Belcrest
Springfield, Missouri 65802

167 Paul Mueller Co. (4/26/65)
Box 828
Springfield, Missouri 65801

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wisconsin 53950

26-00 Sifters for Dry Milk and Dry Milk Products

228 Day Mixing, Div. LeBlond, Inc. (2/28/72)
4932 Beech Street
Cincinnati, Ohio 45202

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard
Mt. Vernon, New York 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P.O. Box 1041
Buffalo, NY 14211

185 Rotex, Inc. (8/10/66)
1230 Knowlton St.
Cincinnati, Ohio 45223

176 Koppers Company, Inc. (1/ 4/66)
Metal Products Division
Sprout-Waldron Operation
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6033 E. Bandini Blvd.
Los Angeles, California 90051

28-00 Flow Meters for Milk and Liquid Milk Products

272 Accurate Metering Systems, Inc. (4/ 2/76)
1731 Carmen Drive
Elk Grove Village, Illinois 60007

253 Badger Meter, Inc. (1/ 2/74)
4545 W. Brown Deer Road
Milwaukee, Wisconsin 53223

223 C-E IN-VAL-CO, Division of Combustion (11/15/71)
Engineering, Inc.
P.O. Box 556, 3102 Charles Page Blvd.
Tulsa, Oklahoma 74101

265 Electronic Flo-Meters, Inc. (3/10/75)
P.O. Box 38269
Dallas, Texas 75238

226 Fischer & Porter Company (12/ 9/71)
County Line Road
Warminster, Pennsylvania 18974

261 Foss America, Inc. (11/ 5/74)
Route 82
Fishkill, New York 12524

224 The Foxboro Company (11/16/71)
Neponset Avenue
Foxboro, Massachusetts 02035

270 Taylor Instrument Process Control (2/ 9/76)
Sybron Corporation, 95 Ames Street
Rochester, New York 14601

29-00 Air Eliminators for Milk and Fluid Milk Products

30-00 Farm Milk Storage Tanks

257 Babson Bros. Co. (2/ 7/74)
2100 S. York Road
Oak Brook, Illinois 60521

31-00 Scraped Surface Heat Exchangers

274 Contherm Corporation (6/25/76)
P.O. Box 352
Newburyport, Massachusetts 01950

290 Crepaco, Inc. (6/15/77)
100 So. CP Ave.
Lake Mills, WI 53551

32-00 Uninsulated Tanks for Milk and Milk Products

264 Cherry-Burrell Company, (1/27/75)
a unit of AMCA International Corp.
575 E. Mill St.
Little Falls, NY 13365

268 Dairy Craft, Inc. (11/21/75)
P.O. Box 1227
St. Cloud, Minnesota 56301

33-00 Polished Metal Tubing for Dairy Products

289 Ladish Co., Tri-Clover Division (1/21/77)
9201 Wilmot Road
Kenosha, Wisconsin 53140

35-00 Continuous Blenders

292 Waukesha Foundry Div. ABEX Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

36-00 Colloid Mills

293 Waukesha Foundry Div., ABEX Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

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Amendment to the 3-A Accepted Practices for the Design, Fabrication and Installation of Milking and Milk Handling Equipment

Number 606-01

(To become 606-02 for reprint distribution)

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

The 3-A Accepted practices for milking and milk handling equipment, number 606-00 are amended in the sections set forth below:*

Add the following to section B. DEFINITIONS and renumber the subsections that follow B.1:

B.2

Solution Contact Surfaces: Shall mean the interior surfaces of the circuit which are used exclusively for supply and recirculation of cleaning and/or sanitizing solutions.

B.3

Change "vacuum line" to "vacuum pulsator line" in two places.

Replace subsection 9 of B. DEFINITIONS with the following three definitions and renumber the subsections that follow B.9.

B.9

Milking Vacuum: The vacuum recommended by the manufacturer for the inside of the inflation or liner.

B.10

Milking Pipeline: A pipe which performs the dual function of conveying milk while supplying milking vacuum.

B.11

Milk Conveying Line: A pipe which performs the single function of conveying milk.

Replace subsection 17 of B. DEFINITIONS with the following two definitions and renumber subsection B.18.

B.17

Main Vacuum Supply Line: The pipe or line from the vacuum supplier through the sanitary trap to the receiver.

B.18

Vacuum Pulsator Line: The pipe or line that supplies vacuum to the pulsator(s).

Add the following to section B. DEFINITIONS:

B.19

Pipeline Milking System: A system utilizing milking pipelines and/or milk conveying lines.

Add the following new subsection 4 to section C. MATERIALS and renumber the C. subsections that follow:

C.4

Solution contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See appendix, Section H), or metal which under conditions of intended use is at least as corrosion-resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent or of heat resistant glass piping. Rubber and rubber-like materials or plastic materials may be used for sealing applications and for short flexible take-down jumpers or connectors.

C.5

Change "Vacuum lines" to "Main vacuum supply lines and/or vacuum pulsator lines."

D.2.6

Change "pipelines" to "milking pipelines and/or milk conveying lines".

Add the following to section E. FABRICATION-SPECIFIC ITEMS:

E.4.4

Pumps, when used, shall be actuated by a sensing device, and if of the probe type, the probes shall be readily demountable for inspection and shall be located so that all of the product contact surfaces are reached by rinse, wash and sanitizing solutions.

E.6

Change *"Milk Pipes"* to *"Milking Pipelines and/or Milk Conveying Lines"*.

E.6.1

Change "milk pipes" to "milking pipelines and/or milk conveying lines".

Replace subsection 6.2 of E. FABRICATION-SPECIFIC ITEMS with the following:

E.6.2

Milking pipelines shall be self-draining except for nor-

**IAMFES, USPHS, and DIC acknowledge the contribution and assistance of the NATIONAL MILK PRODUCERS FEDERATION in the development of the 3A Accepted Practices for the Design, Fabrication and Installation of Milking and Milk Handling Equipment*

mal clingage, and shall have a continuous slope from a high point. (See also APPENDIX L.2).

E.6.3

Change "Milk pipes" to "Milking pipelines" in two places.

E.6.5

Change the first "Milk pipe" to "milking pipeline and/or milk conveying line".

E.6.5

Change the second "milk pipe" to "milking pipeline".

E.6.6

Change "milk pipes" to "milking pipelines and/or milk conveying lines".

E.6.6

Change "Milk pipe" to "Milking pipeline and/or milk conveying line".

E.6.6

Change "milk pipe" to "milking pipeline and/or milk conveying line".

Change subsection 7 of E. FARBICATION-SPECIFIC ITEMS to the following:

E.7

Vacuum Pumps: The exhaust pipe shall not terminate in a milking barn, stable, parlor, milk room or feed room.

E.8.2

Change "milk pipe" to "milking pipeline and/or milk conveying line".

E.9

Change *"Vacuum Lines"* to *"Main Vacuum Supply Lines and/or Vacuum Pulsator Lines".*

E.9.1

Change "Vacuum lines" to "Main vacuum supply lines and/or vacuum pulsator lines".

E.9.2

Change "Vacuum lines" to "Main vacuum supply lines and/or vacuum pulsator lines".

E.9.6

Change "milk pipe" to "milking pipeline".

E.9.6

Change "vacuum line" to "main vacuum supply line".

G.

Change *"PIPELINE SYSTEMS"* to *"PIPELINE MILKING SYSTEMS".*

G.1

Change "milking system" to "pipeline milking system".

Change the title of Section L. of the APPENDIX to the following:

L.

MILKING PIPELINE RECOMMENDATIONS

Change subsection L.1 of the APPENDIX including Notes 1 and 2 to the following:

L.1

The number of milking units that the milking pipeline in a pipeline system can handle satisfactorily depends largely upon the diameter of the milking pipeline. The following table gives the recommended maximum number of milking units that should be used on various installations. The number of units shown in the table is the number per slope.

Size of Milking Pipeline Installations	Recommended Maximum Units Per Slope
1½ inch line	2
2 inch line	4
2½ inch line	6
3 inch line	9

The diameter of the milking pipeline if less than that given in the preceding table should be demonstrated to be adequate by instrumentation. See APPENDIX R.

NOTE 1: When the number of units in use indicate multiple slope lines, a multiple inlet receiver is recommended, or if the receiver has fewer inlets than the number of lines, it should be determined by instrumentation to be adequate.

NOTE 2: Weigh jars and all milk conveying lines should be exempt from these line size provisions. Weigh jars should, however, be connected by means of separate sanitary lines, one to supply vacuum to weigh jars only, the other to carry milk and both should be CIP cleanable.

Change Section M. of the APPENDIX to the following:

M.

VACUUM PUMP CAPACITIES-BUCKET MILKING SYSTEM

The capacity of the vacuum pump(s) used in bucket type milking systems should be at least as large as shown in the table below for the given conditions.

Minimum Vacuum Pump Capacities
Cubic Feet of Air Per Minute (CFM)
(Vacuum Level 15 Inches Mercury)

Number of Units	CFM ASME	CFM NEW Zealand
1	4	8
2	8	16
3	12	24
4	16	32
5	20	40
6	24	48

NOTE 1: For every 2 inches reduction in the operating vacuum level, 0.5 CFM per unit can be subtracted from the above table on the American Standard. (1 CFM on the New Zealand Standard).

Change the table in subsection 4 of Section N of the APPENDIX to the following:

EXAMPLES OF VACUUM REQUIREMENTS,
PIPELINE MILKERS
(VACUUM LEVEL 15 INCHES MERCURY)

Component	ASME Standard	New Zealand Standard
	CFM	CFM
Milker Unit	6.0	12.0
Vacuum-operated Releaser	5.0	10.0
Pulsated Vacuum Line Per 10 ft. of Length	1.0	2.0
Vacuum Bulk Tank	0.0	0.0
Milk Meter	1.0	2.0
Sanitary Couplings Per 20	1.0	2.0
Inlets Per 10	1.0	2.0
Reserve for Regular (ea.)	3.0	6.0
Receiver Group and Milk Pump	0.0	0.0
SAMPLE CALCULATION		
4 Milker Units	24.0	48.0
1 Vacuum-operated Releaser	5.0	10.0
40 Sanitary Couplings	2.0	4.0
4 Milk Meters	4.0	8.0
1 Regulator	3.0	6.0
TOTAL CFM REQUIREMENTS	38.0	76.0

NOTE 1: The 50% reserve capacity recommended in N.1 is included in the table above.

NOTE 2: It is recommended that there be a vacuum system for the pipeline milking system, to operate only the components of the system. If there are vacuum operated accessories such as doors, gates, etc., there should be a separate vacuum system to operate them.

O.2

Change "primary vacuum line" to "main vacuum supply line".

O.2

Change "primary vacuum pipelines" to "main vacuum supply lines".

Change the title of section Q of the APPENDIX to the following:

Q.

MAIN VACUUM SUPPLY LINES AND/OR VACUUM PULSATOR LINES

Q.1

Change "vacuum pipeline" to "main vacuum supply line and/or vacuum pulsator line".

Change subsection 3 of Section Q of the APPENDIX to the following:

Q.3

Adequate vacuum at the milking unit is essential. The inside diameter of a main vacuum supply pipeline should be not less than 1¼inches. The minimum size of line should be that given in the table below, or if smaller, it should be demonstrated to be adequate by instrumentation. See APPENDIX R. The following table gives the recommended minimum size for main vacuum supply pipelines:

MINIMUM SIZES FOR MAIN VACUUM SUPPLY PIPELINES OF PIPELINE MILKING SYSTEMS

Number of Units	Pipe Size (IPS)
2-3	1¼inches
4-5	1½ inches
6-10	2 inches
11-13	2½ inches
14 or more	3 inches

Add the following subsection 4 to section Q of the APPENDIX:

Q.4

Vacuum pulsator lines should be looped to (1) a vacuum balance tank or (2) a vacuum pulsator header line. The opening for a vacuum pulsator line in either a vacuum balance tank or a pulsator header line should be at least as large as the vacuum pulsator line.

MINIMUM SIZE FOR VACUUM PULSATOR LINES OF PIPELINE MILKING SYSTEMS

Number of Units	Pipe Size (IPS)
2-4	1¼inches
5-7	1½ inches
8 or more	2 inches

NOTE: The minimum size of line should be that given in the table above, or if smaller, it should be demonstrated to be adequate by instrumentation.

R.1

Change "milk pipe(line)" to "milking pipe line".

R.1

Change "vacuum pipe line" to "main vacuum supply line".

Delete subsection R.2 of the APPENDIX and add the following to subsection R.1 of the APPENDIX:

To demonstrate this, upon installation of a milking system, a graph of the vacuum fluctuation in the system should be made with all of the milker units in milking operation simultaneously.

Replace the first sentence of subsection 1 of Section S. OPERATION, MAINTENANCE AND SERVICE of the APPENDIX with the following:

It is strongly recommended that a complete service check by an authorized milking machine dealer be performed on an hourly use basis as recommended by the machine manufacturers.

S.2.4

Change "vacuum pipe lines" to "main vacuum supply lines and/or vacuum pulsator lines".

Add the following subsection 6 to Section W of the APPENDIX:

W.6

Milker unit C.I.P. devices when installed outside the milk room, should be so constructed as to prevent in-

sects, rodents, dirt and dust from having access to milk contact surfaces and solution contact surfaces. They should provide complete drainage, except for normal clingage, of milker units, milk hoses and solution contact surfaces. Provisions should be made for adequate warm water under pressure to be available for cleaning the outside or non-milk contact surfaces of the milker unit including hoses. Dismantling for replacing rubber parts and/or manual cleaning of product contact surfaces should be done in the milk room.

X.

Change *"PIPELINE SYSTEMS"* to *" PIPELINE MILKING SYSTEMS"*.

Form 1

Change "MILK PIPELINE" to "PIPELINE MILKING SYSTEM" in two places.

Form 1

in (2) of next to the last paragraph, change "vacuum piping" to "main vacuum supply line and/or vacuum pulsator line".

NOTE: The above subsections will require extensive editorial renumbering in several sections prior to reprinting of the complete accepted practices, which will be number 606-02.

This amendment is effective June 14, 1977; to become 606-02 for reprints.

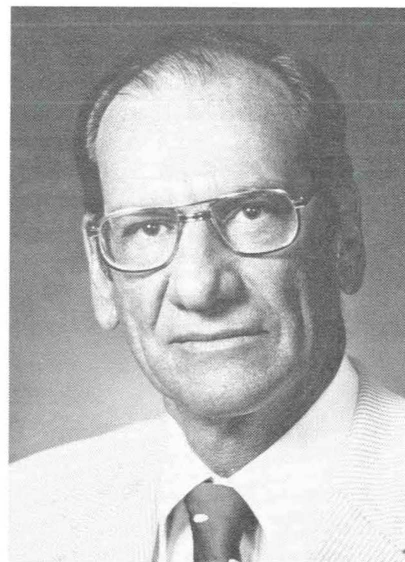
G. W. Reinbold Receives DRINC Award

Criteria for the Dairy Research Foundation Award require that the recipient shall have demonstrated outstanding ability in basic research on milk, milk components, or milk products, the results of which are applicable to solutions of problems of the dairy industry.

This year's recipient, Dr. G. W. Reinbold, has had an outstanding career both in the dairy industry and in the academic field. He is a native of Pennsylvania and received the B.S. degree from Pennsylvania State University and the M.S. and Ph.D. degrees from the University of Illinois. In addition to industrial experience with three large cheese manufacturing organizations he has served as a faculty member of two outstanding state universities. Reinbold's major research interest has been related to various foreign type cheeses. His work has taken him to Norway, Switzerland, France, Finland, and West Germany. His research has concerned the microbiology of foreign type cheeses, their starter cultures, ripening procedures, and flavor development.

The scope of interests, vision, originality, innovativeness, resourcefulness, foresight and creativity, as well as his contributions and dedication to the Dairy Industry are best reflected in his numerous scientific publications, many of which appeared in the *Journal of Milk and Food Technology.* They reflect both applied and basic research, and also the diversity of his research as it relates to the industry.

During the past 10 years he has served as the author or coauthor of approximately 125 scientific papers and of three books. At present Reinbold serves on the Editorial Board of the *Journal of Food Protection* and is a member of the Intersociety Council on Standard Methods for the Examination of Dairy Products. Currently Reinbold is Vice President-Research and Development for the Leprino Cheese Company in Denver, Colorado.



ADSA Awards

Shahani Receives Pfizer Award

Dr. Khem M. Shahani, recipient of the 1977 Pfizer, Incorporated Award, was born in Hyderbad, India. He received the Bachelor of Science Degree with Honors from the University of Bombay in 1943. In 1947, he came to the United States where he obtained the Ph.D. degree from the University of Wisconsin in 1950. For the next two years, he was a postdoctoral fellow at the University of Illinois and conducted research on ultra high heat treatment of milk. In 1953, he joined the Department of Dairy Technology at the Ohio State University as a Research Associate. Since 1957, he has been a member of the Department of Food Science and Technology at the University of Nebraska at Lincoln where he holds the rank of Professor.

Dr. Shahani has taught courses in dairy chemistry, lipids, proteins, and enzymes. He has advised six postdoctoral fellows, seven Ph.D. candidates and 12 M.S. candidates.

Shahani's research activities have been numerous in the areas of dairy and food microbiology and enzymology. His research has emphasized the mode of action of antibiotics, physiology of dairy cultures, nutritional properties of cheese and other cultured dairy products, and use and significance of food and microbial enzymes.

He has published extensively on the B-complex vitamins of cheese, yogurt, buttermilk, and sour cream. He and his co-workers have isolated two broad spectrum antibiotics: Acidophilin from *Lactobacillus acidophilus* and Bulgarican from *Lactobacillus bulgaricus.* He has demonstrated that yogurt inhibits proliferation of Ehrlich ascites tumor in mice. He has also reported on the use of lactase in solution of problems associated with lactose intolerance and lactose degradation. Some of Shahani's research results have appeared in the *Journal of Milk and Food Technology.* His fame is international.

M. A. Cousin Receives Hoyt Memorial Award

A graduate student specializing in either dairy manufacturing or dairy production is selected annually by the American Dairy Science Association to receive the Richard M. Hoyt Memorial Award. This award recognizes excellence in research by the student. Results of the research should apply directly to solving a problem in the dairy industry. The award has been given annually since 1971.

Dr. Maribeth A. Cousin, recipient of this year's award was born in 1949 in Beloit, Wisconsin, grew up on a nearby dairy farm, and attended the University of Wisconsin-Madison. She majored in Food Science and received the B.S. degree in 1971. The M.S. and Ph.D. degrees were earned in the same department in 1972 and December 1976, respectively.

Presence and growth of psychrotrophic bacteria in raw and pasteurized milk and in some milk products is a major problem of the dairy industry. This problem is intensified by extended refrigerated storage of raw milk on the farm, during transport, and in dairy factories. Research completed by the recipient of the Hoyt Memorial Award in 1977 was concerned with this problem.

Cousin's research results demonstrated that milk in which psychrotrophic bacteria had grown had the following characteristics: (1) growth and activity of commonly used lactic acid starter bacteria were enhanced rather than retarded; (2) alpha- and beta-casein were degraded, but not whey proteins; (3) stability of milk to heat and rennet was reduced; (4) a normal manufacturing schedule could not be used to make Cheddar cheese, and Cheddar cheese made from this milk was of inferior quality, particularly after ripening for 6 months; (5) cottage cheese made from such milk immediately was of poor quality; and (6) quality of yogurt, although marginal, was affected less than was the quality of cottage cheese. These results emphasize the need to prevent growth of psychrotrophic bacteria in milk that is to be used for manufacting cultured products.

Reports of research conducted by Dr. Cousin have appeared in or have been accepted for publication in the *Journal of Food Protection, Journal of Dairy Science, Cultured Dairy Products Journal, Milchwissenschaft,* and *Lebensmittel Wissenschaft und Technologie.*

Research completed by the recipient of this year's award was guided by Dr. Elmer H. Marth, Professor of Food Science and Bacteriology at the University of Wisconsin in Madison.

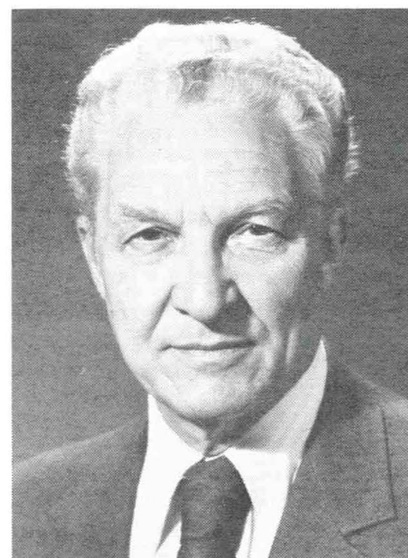
Teaching Award Goes To E. L. Thomas

A study of the nominations for the Milk Industry Foundation Teaching Award reveals that, in our Land-Grant Universities, there are many devoted and competent teachers of dairy technology and science. The life and work of Dr. E. L. Thomas, the man chosen as the 1977 recipient illustrates richly the kind of superior teaching in this broad discipline which deserves our recognition.

Apart from unquestionable command of the subjects he teaches, the traits for which Thomas is praised repeatedly are his excellent rapport with both graduate and undergraduate students; his skill in presenting facts, principles, and hypotheses in clear and understandable terms; his ability to enliven the subject, to relate theory to practice, to stimulate, to inspire, to challenge; and his patience and compassion.

Beyond the classroom and the campus, Thomas is known and appreciated as adviser and consultant to the dairy industry and as a teacher for vocational agriculture instructors and industry personnel. He is exceptionally competent in teaching dairy products judging and has coached 28 judging teams, many of which have captured individual and team awards. For a number of years he served as member of the ADSA Dairy Products Judging Committee, including two terms as its chairman. In this capacity he did much toward maintaining the prestige and significance of the Collegiate Dairy Products Judging Contest.

The man whose exemplary contributions as teacher, adviser, and leader in student activities are recognized grew up on a dairy farm in Ohio, received, in 1941, the B.S. degree in



dairy technology and chemistry from Ohio State University and, later, the Master's degree and the doctorate from the University of Minnesota. He is now a Professor in the Department of Food Science and Nutrition at the University of Minnesota.

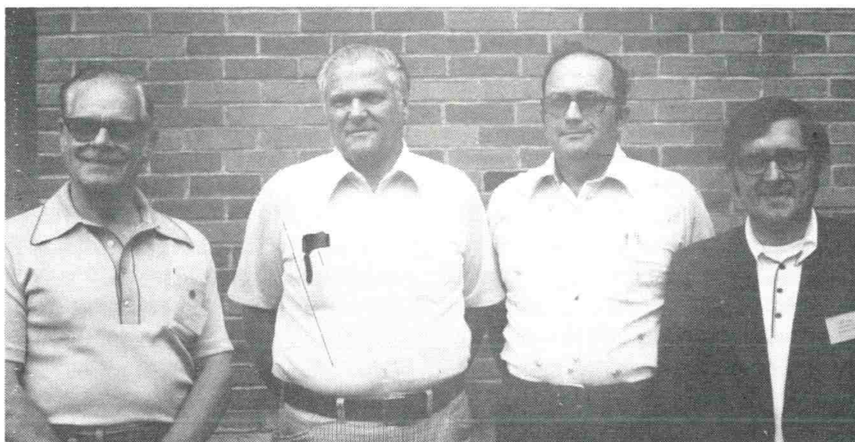
35th Annual Pennsylvania Dairy Fieldman's Conference

More than 250 persons participated in the Pennsylvania Dairy Fieldman's Conference held on June 13-15, 1977 at the Conference Center on the Penn State Campus.

George Mansell, a dairy sanitarian with Lawson Milk Co., was awarded a plaque as the outstanding sanitarian for 1977. He was formerly President of the Pennsylvania Dairy Sanitarians Association and active on a variety of committees.

New officers were elected at the Association Business Meeting. They include Raymond Ackerman, President; Elwood Hench, President Elect; Alfred Gottfried, Vice-President; and William Killough, Secretary-Treasurer.

The Monday evening session centered on automatic controls for bulk tanks and take-off devices for milking machines. Representatives of five manufacturers gave short presentations.



Pennsylvania Dairy Sanitarians Association officers for 1977-78, William Killough, secretary-treasurer; Alfred Gottfried, vice-president; Raymond Ackerman, president; and Elwood Hench, president elect.

The most interesting talks were on somatic cells and their correction in milk. A pilot program of DHI was reviewed along with the successes of reducing herd samples to less than 500,000 per ml. Extension dairy agents in 4 counties are participating in the pilot study.

Recent changes in state and federal regulations were reviewed as they relate to milk product adulteration, hauler responsibilities, waste disposal and farm inspection. Great improvements have been noted in hauler sampling procedures and elimination of added water in milk. Other topics covered were IMS programs, feeding practices and milk quality, future milk prices, calf housing, and northeast dairy guidelines.

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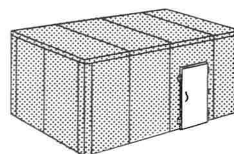

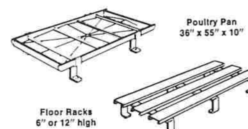

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Study predicts vast growth in diabetic products market

Diabetes afflicts 10 million Americans—50 million if families are included—and the cost to treat the disease amounts to $5.3 billion annually, excluding costs to treat complications. The incidence of diabetes is also increasing at a 6 percent annual rate so that the proportion of the U.S. population suffering from the ailment, at 2.7 percent presently, will climb to 4.1 percent by 1985.

Yet, the diabetic "is probably the most neglected consumer in the American marketplace — neglected by manufacturers of pharmaceuticals, health and beauty aids, food, drink, and candy," according to a new study on the Diabetes Products Market by Frost & Sullivan, Inc. in New York City. "The diabetic is currently offered a bewildering selection of products that are not properly formulated nor clearly labeled as safe to use," the 277-page study says.

The retail pharmacist alone derives three times the revenues from each diabetic customer than he does from the average customer, the study adds. "Diabetes victims will welcome and support products that meet his needs."

Indeed, the market for diabetes products will show tremendous growth:

• *Drugs:* Insulin and oral antidiabetic sales, at $105 million in 1976, will grow to $195 million by 1985, for example. Also, some changes are in store. Upjohn's Orinase comes off patent in 1978, and the study sees "a fairly rapid change" from brand to generic prescription. This will especially be so in the case of hospitals fighting to shave costs and where a patient visiting a diabetes clinic is already charged as much as $35, according to a hospital survey conducted by the Frost & Sullivan researchers.

"Two other branded antidiabetic products will also come off patent prior to 1980," the study notes. And as this occurs, unit dose applications will increase, creating "a good growth area" for the packaging of drugs in this fashion.

• *Supplies and Accessories:* Sales of syringes and needles for insulin injections—largely disposable items —stand at $50 million annually. Diabetics must also test their urine to detect changes in blood sugar, and the market for products that do this job at $46 million currently will climb to $70 million annually by 1985. The diabetes test market will also be abetted by mass screening programs so that test volume will rise by 7 percent annually.

Diabetic accessory items, such as alcohol swabs, travel kits, syringe cases and holders, needle sharpeners, constitute another $5 million market that will climb to $10 million by the end of the decade. Yet, the study finds that "more than 75 percent of the diabetics interviewed were unaware of the availability of most of these accessories and where they could be obtained."

• *Food & Drink:* Specialty diet foods, too, represent an interesting market, with some 11 companies already producing such products. This is especially important since the medical community believes that 90 percent of diabetes cases can be controlled by diet alone. "Thus, diabetics become an important subsector in diet-food markets," the study adds. "Nevertheless, the food industry has missed out on this area of opportunity." The diabetic candy market alone could reach nearly $3 million a year if it were to be pursued. In surveys done by Frost & Sullivan diabetics list "the poor choice of foods that are available" as their foremost problem.

An analysis of cause shows that diabetes victims tend to have a genetic predisposition. "A person's chances of developing the disorder range between 50 percent and 80 percent if both one parent and one sibling have had the disease," the study points out. Obesity also increases risk, For each 20 percent excess weight carried, the liklihood to get diabetes nearly doubles.

Because of the climbing incidence rate of the disease, diabetes research is also receiving stepped-up attention. The National Commission on Diabetes funding of $43 million at the time of the agency's kick-off in 1975, will rise to $126 million by 1979. The ultimate goal of course, is to prevent and cure the disease. However, research into better therapy methods to improve the diabetic's quality of life are also receiving a high priority. New products that await FDA approval, for example, are reputed to be almost ten times as potent as current drugs, while producing fewer side effects. Another drug in a research stage, somagostatin, may eventually turn out to be a good supplement to insulin in treating diabetes.

Two surgical approaches are also under study: transplantation of the pancreas or islet cells and the development of mechanical devices to monitor blood glucose and administer insulin automatically. The techniques, if perfected, would eliminate the daily inconvenience of an insulin injection. Additional areas of opportunity include the use of a computer in diabetic management and educational programs to instruct the new diabetics in the management of their ailment.

The diabetes specialty marketplace could really unfold in a big way were attention paid to the four million persons that are undiagnosed diabetics or that are without medical supervision, according to the study. And it adds, the typical diabetic "is an attentive medicine taker and a prolific reader of most media, such that a well constituted product can win easy acceptance and a lifetime of loyal use."

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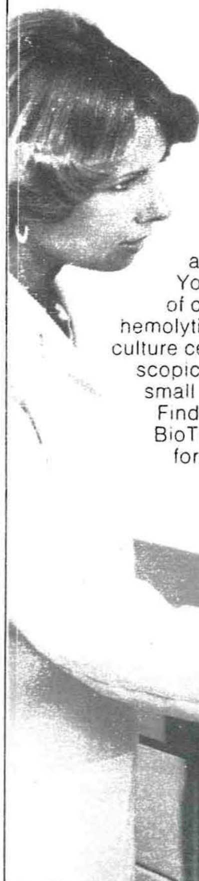

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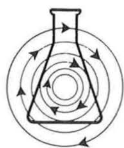

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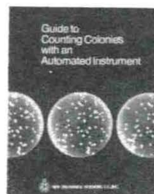

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Polson Co.
625 S. Lander St.
Seattle, Washington 98124

Seiberling Associates, Inc.
417 Eastern Ave.
So. Beloit, Illinois 61080

Southside Milk Producers
Marshall Building
Blackstone, Virginia 23824

Valley of Virginia Co-operative Milk Producers Assn.
41 W. Washington St.
Harrisonburg, Virginia 22801

West Agro-Chemical, Inc.
P.O. Box 1386
Shawnee Mission, Kansas 66222

THE ONLY *Approved*
SANITARY METHOD OF APPLYING A U. S. P. LUBRICANT TO DAIRY & FOOD PROCESSING EQUIPMENT

Haynes-Spray

U. S. P. LIQUID PETROLATUM SPRAY

U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID — CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

SANITARY — PURE

ODORLESS — TASTELESS

NON-TOXIC

This Fine Mist-like **HAYNES-SPRAY** *should be used to lubricate:*

- SANITARY VALVES
- HOMOGENIZER PISTONS — RINGS
- SANITARY SEALS & PARTS
- CAPPER SLIDES & PARTS
- POSITIVE PUMP PARTS
- GLASS & PAPER FILLING MACHINE PARTS
- and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

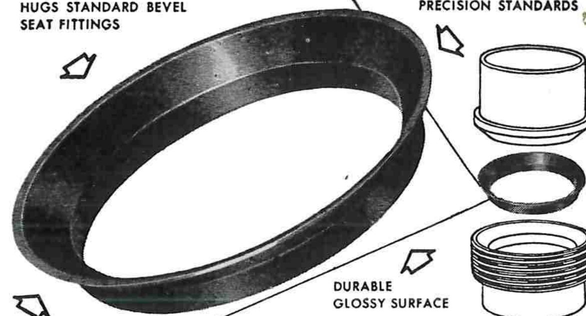
The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT — 7 LBS.

THE HAYNES MANUFACTURING CO.
4180 Lorain Ave. • Cleveland, Ohio 44113

HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS



LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these **SNAP-TITE** *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
- Odorless, polished surfaces, easily cleaned
- Withstand sterilization
- Time-saving, easy to assemble
- Self-centering
- No sticking to fittings
- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES SELF-CENTERING SNAP-TITE® *Gaskets*

*MADE FROM **TEFLON®**

SIZES 1" - 1½"
2" - 2½" - 3" - 4"

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

• *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

* Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

THE HAYNES MANUFACTURING COMPANY
4180 LORAIN AVENUE • CLEVELAND, OHIO 44113

A HEAVY DUTY SANITARY LUBRICANT

Available in both

SPRAY AND TUBE

All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

THE HAYNES MANUFACTURING CO.
CLEVELAND, OHIO 44113

Make Your Cows Worth More.

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

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

DHI Production Records

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

Identify Cows With Hidden (Sub-Clinical) Mastitis

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

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

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

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

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

Routine Milking Machine Service

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

Provide The Environment For Healthy Calves

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

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

Managed Milking Procedure

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

SURGE

Babson Bros. Co.,
2100 South York Road,
Oak Brook, Illinois 60521.

We make your cows worth more.