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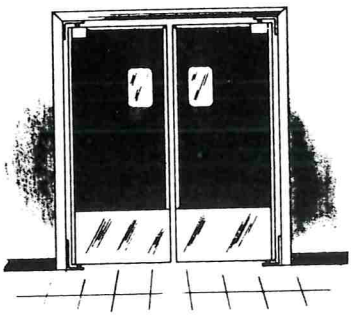

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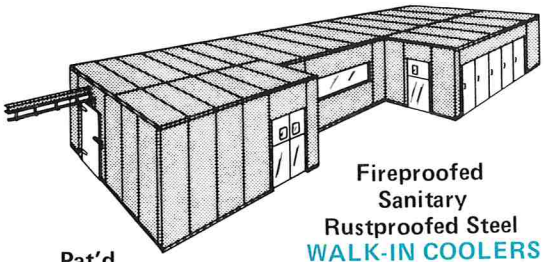

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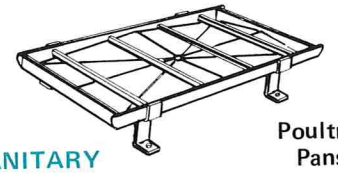

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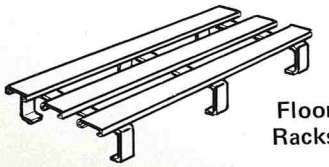

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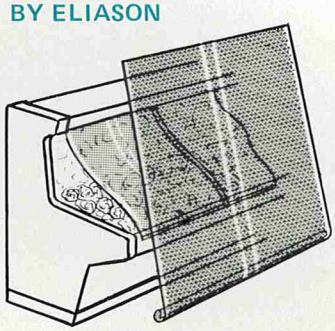

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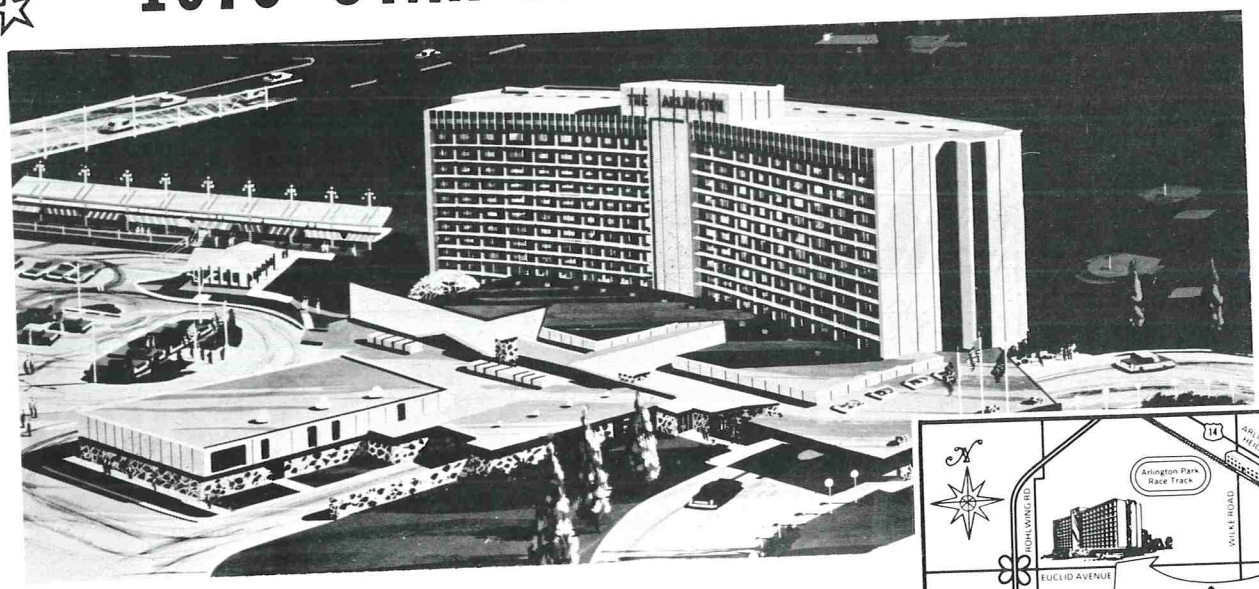

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Vol. 39 July 1976 No. 7

Research Papers

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J. Milk Food Technol. Vol. 39, No. 7, Pages 462-463 (July, 1976)

Freezing Points of Raw and Pasteurized Milks

G. H. WATROUS, Jr., S. E. BARNARD, and W. W. COLEMAN II

Department of Food Science
The Pennsylvania State University
University Park, Pennsylvania 16802

(Received for publication November 28, 1975)

ABSTRACT

Over 2,000 samples of cow milk and 243 samples of processed milk were analyzed for freezing point. Data suggest the need for continuing surveillance of both raw and pasteurized milks. During the course of the study, the incidence of freezing points in processed milks suggestive of added water dropped markedly. It is suggested this may have been due to information gained during the study and consequent response.

The freezing point of cows' milk has long been recognized as one of its most constant values, normally varying between –0.530 and –0.566 C (*7*). More recently (*1*), –0.525 C has been accepted as the upper limit. While feeding and management practices have been shown to effect the freezing point slightly (*2, 4, 5*), values appear to remain within the –0.530 to –0.566 C range. Likewise, while processing milk may affect the value slightly (*3, 6, 8, 9*), the range appears to remain the same.

Rapid increases in the cost of producing milk at the farm level have placed many dairy farmers in an economic squeeze. Furthermore, the price of milk per cwt has increased much more rapidly than the socalled fat differential, or the amount paid for increasing fat levels in milk. This combination has resulted in a situation where watering of milk is economically advantageous to the dishonest milk producer. The incentive for adding water has thus been enhanced.

EXPERIMENTAL

In the present study, 2,019 farm supplies of milk, mostly from Pennsylvania but some from eastern Ohio and southern New York state, were collected by farm tank drivers. Samples were immediately cooled in ice and delivered to the University Creamery laboratory of The Pennsylvania State University. These samples represented the farms supplying seven processors and four large farm cooperatives. In the second portion of this study, 243 processed homogenized milks, representing most of Pennsylvania's commercial milk and many farmer-operated processors, were collected from retail sales cabinets.

In no instance was advance notice given of the intent to collect samples, either at the farm or store. Thus, samples represented a rather accurate cross section of milk as produced and sold in this marketing area. All freezing points were done on a Model 66 or 4L Advanced Milk Cryoscope, following the method as set forth in AOAC (*1*).

After data were collected during the study, results were furnished to the raw milk supply collaborators. Dealers were informed when data on samples of processed milk indicated added water. In addition, regulatory officials of the Division of Milk Sanitation of the Pennsylvania Department of Agriculture were informed of the progress of the study. Trade associations, both producers and processors, were also informed of the study as data were collected. Thus, any changes found during the study may reflect awareness of increased surveillance of producers and processors.

RESULTS AND DISCUSSION

Of 2,019 raw milk samples tested, 5.6% had freezing points above the accepted legal value of –0.525 C (Table 1). A total of 8.57% of the samples had freezing points above –0.530 C. As previously indicated, the literature indicates that few if any normal farm milk supplies have freezing points above –0.525 C, and the vast majority below –0.530 C. It thus appears that water accidentally entered or was deliberately added to some farm supplies. Other than deliberate watering, farmers may unintentionally add water to their milk by rinsing milking equipment and especially by the practice of "chasing" milk from pipeline milkers with water. Whatever the reason, it is obvious that corrective

TABLE 1. *Freezing points of 2019 farm milk samples*

Freezing point (°C)	Milk Processor or cooperative (Number of samples in each range)											Total	%
	A	B	C	D	E	F	G	H	I	J	K		
<-0.450										2	1	3	0.15
-0.451 to -0.474		1							1		4	6	0.30
-0.475 to -0.499	2	1				1			1	1	9	15	0.74
-0.500 to -0.524	6	4	2	5	2	3	1	16	8	12	30	89	4.41
-0.525 to -0.529	7	1	2	2		1	1	7	2	6	31	60	2.97
-0.530 to -0.534	25	3	1	5	1	6	4	29	15	15	101	205	10.25
-0.535 to -0.539	75	4	13	11	6	17	16	48	24	45	184	443	21.94
-0.540 to -0.544	132	11	32	53	8	30	11	51	62	82	162	634	31.54
-0.545 to -0.549	69	6	33	45	5	48	8	23	42	56	74	409	20.26
-0.550 and below	20	5	18	12	1	20	1	3	25	18	32	155	7.68
Total	336	36	101	133	23	126	42	177	180	237	628	2019	100.00

TABLE 2. *Freezing ponts of 243 pasteurized homogenized milk samples collected from June-September 1975*

Freezing point (°C)	June	July	August	Sept.	Oct.	Total
-0.475 to -0.499	1	1	2			4
-0.500 to -0.524	7	6	6	2	1	22
-0.525 to -0.529	8	6	6	1	12	33
-0.530 to -0.534	11	8	17	2	8	46
-0.535 to -0.539	3	13	39	13	25	93
-0.540 to -0.544		6	16	14	7	43
-0.545 to -0.549					1	1
-0.550 and below				1		1
Total	30	40	86	33	54	243
Average	526.5	531.4	534.1	537.2	534.68	
%Above -0.525	26.7	17.5	9.3	6.1	1.9	
% "Legal" or -0.525 and below	73.7	82.5	90.7	93.9	98.1	
%-0.530 and below	46.7	67.5	84.1	90.0	75.9	

measures should be taken where the evidence of added water is found.

A much higher incidence of added water was found in processed milk samples than in the raw supplies, especially during the early phases of the study. As dealers, regulatory people, and trade associations were alerted, the number of infractions dropped greatly. As shown in Table 2, 26.7% of the processed samples had freezing points above –0.525 C in June, 1975, while for October the percentage had decreased to 1.9%. Many opportunities exist for water to enter milk supplies, starting with those suggested at the farm. However, it is common practice to rinse milk tank trucks, raw milk storage tanks, and plant milk lines with water. The reason, of course, is to use water to insure recovery of all milk from these sources. High temperature short time (HTST) pasteurization equipment is usually started using water, and when the proper temperature is reached, milk replaces the water. Timing this operation to prevent mixing of water and milk often is done by observing the color of the product as it leaves the HTST pasteurizer. Thus, some water may easily enter milk at this point. Many plants use hot water sterilization of equipment. In large modern plants with numerous lines, valve clusters, large pasteurized surge vats, etc., many possibilities for entry of water into milk exist. It is common practice to wash interconnected equipment using modern cleaned-in-place (CIP) procedures, relying on automated valves and timing devices to prevent admixture of water, cleaning solutions, and milk. When milk flavor control equipment using steam injection for removal of off-odors is used, great care must be exerted to prevent incomplete removal of this steam. With such equipment, the reverse may also occur, and concentration of milk may result. In practice, close temperature control should avoid either of these conditions. Use of a cryoscope to monitor freezing points before and after use of vacuum flavor control equipment would readily detect improper operation.

The data from this study clearly demonstrate the need for continued surveillance of farm and pasteurized milk to detect addition of water.

ACKNOWLEDGMENT

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J. Milk Food Technol. Vol. 39, No. 7, Pages 464-466 (July, 1976)

Sulfite Compounds as Neutralizers of Spice Toxicity for *Salmonella*

CLYDE R. WILSON and WALLACE H. ANDREWS

Division of Microbiology, Food and Drug Administration
Washington, D.C. 20204

(Received for publication December 10, 1975)

ABSTRACT

The ability of five inorganic chemical salts (K_2SO_3, K_2SO_4, Na_2SO_3, Na_2SO_4, and $CaCO_3$) to neutralize the toxicity of six spices (allspice, cinnamon, clove, garlic powder, onion powder, and oregano) for *Salmonella* was evaluated. Their effect on four spices non-toxic to *Salmonella* (black pepper, white pepper, rosemary, and thyme) was also determined. The inhibitory effects of onion and garlic powders were overcome by addition of 0.5% of K_2SO_3 or Na_2SO_3 to pre-enrichments of lactose broth, nutrient broth, or trypticase soy broth. Allspice, cinnamon, clove, and oregano remained toxic to *Salmonella* in all pre-enrichment broths tested, with or without chemical additives. None of the chemical additives had any effect upon isolation of *Salmonella* from the non-toxic spices. Until a more practical method for analysis of allspice, cinnamon, clove, and oregano is developed, dilution of these spices to non-toxic levels is recommended.

Even though relatively few outbreaks of salmonellosis have been traced to consumption of contaminated spices, numerous isolations of *Salmonella* from a variety of spices, including thyme, paprika, sesame seed, sage, marjoram, oregano, dehydrated green pepper, and black pepper have been made in our laboratories. Black pepper and white pepper have both been implicated as vehicles for the spread of *Salmonella weltevreden* resulting in several cases of salmonellosis (*9*).

Julseth and Deibel demonstrated that many spices, including black and white pepper, exhibited little or no toxicity for *Salmonella* when examined by conventional methods (*4*). They listed only four of 35 spices (allspice, cassia, oregano, and granulated onion) tested as being toxic to *Salmonella* at a 1:10 sample/broth ratio. Wei et al. (*7*) demonstrated that addition of 0.5% K_2SO_3 to an onion-buffer homogenate overcame the bacteriostatic effect of onion and resulted in increased, and presumably more accurate plate counts. Hall (*3*) showed that *Salmonella* could be recovered from artifically contaminated onion powder pre-enriched in lactose broth at the 1:10 sample/broth ratio when 0.5% K_2SO_3 was added to the enrichment broth, but not in the absence of K_2SO_3.

The present study was undertaken to determine: (*a*) the identity and specificity of the cations or anions needed for reversal of *Salmonella* inhibition in selected spices, (*b*) the relative efficiency of various pre-enrichment broths in recovering *Salmonella* from spices, and (*c*) the ability of *Salmonella* to survive when inoculated into these spices.

MATERIALS AND METHODS

Source of spices and herbs

Samples of black pepper, white pepper, cinnamon, clove, thyme, and rosemary were available in the laboratory as reserve portions of samples collected for other examinations. Allspice, oregano, onion powder, and garlic powder were purchased from a local retail grocery.

Salmonella inoculum

S. bredeney, S. glostrup, and *S. thompson,* all previously isolated from spices, were grown for 24 h in brain-heart infusion broth, the cells collected by centrifugation at $3090 \times g$ for 10 min, washed twice, suspended in Butterfield's phosphate buffer, and refrigerated at 4 C until used.

Aerobic plate counts

Serial dilutions of the spices and herbs were made in Butterfield's phosphate buffer, plated in standard plate count agar, incubated 48 h at 35 C, and counted.

Lactose pre-enrichment method

The method of the Association of Official Analytical Chemists (A.O.A.C.), modified only by sample size and volume of pre-enrichment broth, was evaluated by pre-enriching 1-g portions of each spice or herb in 9.0 ml of lactose broth, the pH of the mixture adjusted to 6.8 ± 0.2, and the inoculum of 1.4×10^6 cells of *S. bredeney* added to the pre-enrichment mixture. Subsequent incubation, selective enrichment, and plating were in accordance with the official A.O.A.C. methodology (*1*) with the exception that plating was limited to bismuth sulfite (BS) agar. Since the objective of this experiment was to determine the adequacy or inadequacy of the pre-enrichment and enrichment phases of the method when applied to the examination of spices, only the selective agar with which we usually obtain our best results, BS agar, was used.

Measurement of inhibitory levels of spices

Two-fold decreasing amounts (10.0 - 0.078 g) of the inhibitory spices (allspice, cinnamon, clove, garlic powder, onion powder, and oregano) were added to 90.0 ml of lactose broth, the pH of the mixture was adjusted to 6.8 ± 0.2, and the inoculum of 110-140 cells of *S. bredeney* and *S. thompson* added to the pre-enrichment mixture. Subsequent steps of the examination were as previously described.

Addition of inorganic chemicals

One-gram portions of the inhibitory spices and herbs were pre-enriched in 9.0-ml amounts of lactose broth, nutrient broth, or trypticase soy broth, with and without addition of 0.5% $CaCO_3$, K_2SO_3, K_2SO_4, Na_2SO_3, and Na_2SO_4. The pH of mixtures was adjusted to 6.8 ± 0.2, and mixtures were inoculated with 5-10 cells of *S. bredeney, S. glostrup,* or *S. thompson.* Subsequent steps of the examination were as previously described.

Determination of spices' capacity for self cleansing

Ten grams of black pepper, allspice, cinnamon, clove, garlic powder, onion powder and oregano were inoculated with 1.2×10^7 to 1.6×10^7 cells/g of *S. bredeney, S. glostrup,* or *S. thompson. Salmonella* isolations were attempted immediately after inoculation and at intervals of 24, 48, 72, 96 h, and two weeks. The spices were placed in 90 ml of trypticase soy broth, and the pH of the mixture was adjusted to 6.8 ± 0.2. Onion and garlic powders were examined with and without 0.5% K_2SO_3 in the pre-enrichment broth to verify the ability of K_2SO_3 to overcome the inhibitory effects of these spices. Subsequent incubation, selective enrichment, and plating were in accordance with official A.O.A.C. methods (*1*).

Identification of isolates

Isolates from selective agar plates were picked to triple-sugar-iron agar slants, incubated at 35 C for 18-24 h, and examined serologically to confirm them as being the same somatic group that had been inoculated.

RESULTS AND DISCUSSION

Low aerobic plate counts seemed to indicate that most of the spices had been subjected to some form of treatment. Clove, cinnamon, thyme, rosemary, and white pepper all had counts of <100/g. Per gram figures for black pepper, oregano, and allspice were 170, 5.0×10^3, and 2.6×10^6, respectively. Onion powder counts were 6.3×10^4 without the addition of 0.5% K_2SO_3 to the buffer diluent, and 1.09×10^5 with the added K_2SO_3. Counts of garlic powder were increased from 2.1×10^3 to 6.0×10^3 by the use of 0.5% K_2SO_3 in the buffer diluent.

The modified A.O.A.C. lactose pre-enrichment procedure was evaluated with six spices inhibitory to *Salmonella* (allspice, cinnamon, clove, garlic powder, onion powder, and oregano) plus four non-inhibitory spices (black pepper, white pepper, rosemary, and thyme). Recovery of *Salmonella* was obtained at every level of inoculum, ranging from 14 to 1.4×10^6 cells/g, from the non-inhibitory spices. *Salmonella* was not recovered from the six inhibitory spices, with the exception of onion powder, at the two highest inoculum levels, 1.4×10^5 and 1.4×10^6 cells/g. Results of our own investigations with the above spices, the experiences of various other Food and Drug Administration laboratories in the examination of spices, and results of other investigators (*4*) indicate that most spices may be examined by conventional analytical procedures with no difficulty.

TABLE 1. *Recovery of* S. bredeney *and* S. thompson *from six inhibitory spices at various sample/broth ratios*

Grams of spice per 90 ml of lactose broth	Allspice	Cinnamon	Clove	Garlic	Onion	Oregano
10.00	–[a]	–	–	–	–	–
5.00	–	–	–	–	+[b]	–
2.50	–	–	–	–	+	–
1.25	+	+	–	–	+	+
0.63	+	+	–	–	+	+
0.31	+	+	–	–	+	+
0.16	+	+	+	+	+	+
0.08	+	+	+	+	+	+
0.00	+	+	+	+	+	+

[a]No recovery of *S. bredeney* or *S. thompson*
[b]Recovery of *S. bredeney* and *S. thompson*

The extent of the toxicity of allspice, cinnamon, clove, garlic powder, onion powder, and oregano is shown in Table 1. Onion powder was the least toxic, allowing *Salmonella* recovery at a sample/broth ratio of 5.6%. Clove and garlic powders were most inhibitory, not allowing recovery of *Salmonella* until the spice level had been decreased to 0.18%. Allspice, cinnamon, and oregano suppressed recovery at and above the 2.8% level. These results indicate that dependence upon reduction of the sample/broth ratio to counter inhibition would lead either to examination of very small quantities of the more toxic spices or to the use of large volumes of broth to examine even a single 25-g portion.

Three broths, with and without chemical additives, were comparatively evaluated as pre-enrichment media for recovery of *Salmonella* from spices. Lactose broth was used because of its use in A.O.A.C. methodology for *Salmonella* determinations (*1*). Nutrient broth was selected because of its use in the investigations of Julseth and Deibel (*4*). The inclusion of trypticase soy broth was prompted by its demonstrated superiority to lactose broth as a pre-enrichment broth in recovering *Salmonella* from dried active yeast (*8*).

Inorganic chemical salts used with each pre-enrichment broth were K_2SO_3, Na_2SO_3, K_2SO_4, Na_2SO_4 and $CaCO_3$. The K_2SO_3 was evaluated because of its previously proven usefulness for onion examinations (*3, 7*). Green and Litsky (*2*) found Na_2SO_3 effective as a neutralizer of the bactericidal effects of iodine, and because of its close chemical relationship to K_2SO_3, the effectiveness of Na_2SO_3 as a neutralizer of toxic spices

TABLE 2. *Recovery of* Salmonella *from spices using inorganic chemical salts as additives to the pre-enrichment media*

Inorganic salt (0.5%)	Spice: Allspice	Cinnamon	Clove	Garlic powder	Onion powder	Oregano	Pre-enrichment
K_2SO_3	–[a]	–	–	+[b]	+	–	
K_2SO_4	–	–	–	–	–	–	Lactose broth[c]
Na_2SO_3	–	–	–	+	+	–	or
Na_2SO_4	–	–	–	–	–	–	Nutrient
$CaCO_3$	–	–	–	–	–	–	broth[c]
None	–	–	–	–	–	–	
K_2SO_3	–	–	–	+	+	–	
K_2SO_4	–	–	–	–	+	–	Trypticase
Na_2SO_3	–	–	–	+	+	–	soy
Na_2SO_4	–	–	–	–	+	–	broth[c]
$CaCO_3$	–	–	–	–	+	–	
None	–	–	–	–	+	–	

[a]*Salmonella* not recovered
[b]*Salmonella* recovered
[c]Inoculated with 5-10 *Salmonella* organisms per gram of spice

was evaluated. Na_2SO_4 and K_2SO_4 were included to demonstrate that the basis of the neutralization resided in the sulfite anion rather than the sodium or potassium cation. $CaCO_3$ was added in an attempt to counter the low pH of some spices and possibly neutralize some of the toxic factors.

Results obtained using the three pre-enrichment broths and five additives are shown in Table 2. When onion powder was inoculated with 5-10 *Salmonella*

cells/g, recovery was accomplished in the trypticase soy broth regardless of additive or lack of additive. In nutrient broth and lactose broth, recovery of *Salmonella* was possible only from pre-enrichment broths containing K_2SO_3 or Na_2SO_3. Inoculated garlic powder examined by the same procedure yielded *Salmonella* only from pre-enrichment broths containing K_2SO_3 or Na_2SO_3. Examination of allspice, cinnamon, clove, and oregano by the same procedures recovered no *Salmonella* from any pre-enrichment broth, with or without an additive. These results indicate toxic agents or mechanisms different from those found in onion and garlic. On the basis of its ability to neutralize the inhibitory effect of onion powder without the presence of chemical additives, trypticase soy broth appears to be the superior of the three broths evaluated.

Table 3 illustrates that vapors from onion and garlic

TABLE 3. *Survival of* Salmonella *after various intervals in selected spices having bactericidal properties*

Spice	Period of exposure to spice					
	0[a]	24 h	48 h	72 h	96 h	2 wks
Black pepper[b]	+[c]	+	+	+	+	+
Cinnamon	–[d]	–	–	–	–	–
Clove	–	–	–	–	–	–
Allspice	–	–	–	–	–	–
Oregano	+[e]	–	–	–	–	–
Garlic powder	–	–	–	–	–	–
Garlic powder[g]	+	+	+	+	+	+
Onion powder	+	+	+[f]	+	+	+[f]
Onion powder[g]	+	+	+	+	+	+

[a]Examined immediately after inoculation with 1.2×10^7-1.6×10^7 *Salmonella* cells
[b]Non-inhibitory control
[c]Recovery of *S. bredeney, S. glostrup* and *S. thompson* except as noted
[d]*Salmonella* not recovered
[e]*S. thompson* only
[f]*S. glostrup* and *S. thompson* only
[g]Trypticase soy broth pre-enrichment contained 0.5% K_2SO_3

powders were not able to cleanse these products of relatively large numbers of *Salmonella* (1.2×10^7 to 1.6×10^7/g) over a 2-week period despite reported bactericidal qualities of onion and garlic vapors (*5, 6*). These previous investigations, however, used fresh onion and garlic and only qualitatively showed toxicity by observing the prevention or reduction in amount of bacterial growth. The observations were made with glycerin agar plates which had been exposed to vapors of minced onion or garlic for various intervals of time before inoculation. Recovery of all three *Salmonella* serotypes from garlic powder with trypticase soy broth containing 0.5% K_2SO_3, but not without it, indicates that the organisms survived the garlic vapors, but were inhibited by a substance, capable of being neutralized by K_2SO_3, which had leached into the pre-enrichment medium from the garlic. With oregano, only *S. thompson* was recovered, and only from the examination made immediately after inoculation of the material. No recoveries at all were made from allspice, cinnamon, or clove. It is not known if this lack of recovery was due to the bactericidal properties of these spices or to inadequate methodology. Black pepper was included as a non-toxic spice control, and recovery was obtained with all three *Salmonella* serotypes at each time interval checked.

This study shows that the sulfite anion is responsible for reversal of *Salmonella* inhibition by onion and garlic powders and that trypticase soy broth is the preferred pre-enrichment broth of those tested. The practical implications of the study are that onion and garlic powders may be examined for *Salmonella* using conventional methodology modified only by the addition of 0.5% K_2SO_3 or Na_2SO_3 to the pre-enrichment medium. Allspice, cinnamon, clove, and oregano, however, must be examined by diluting them beyond their toxic levels until a means to neutralize their toxicity can be found.

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Occurrence of *Klebsiella pneumoniae* in Dairy Products

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ABSTRACT

From 165 dairy products with positive coliform counts on violet red bile agar, 410 of 607 colonies (67.5%) were confirmed as lactose fermenters in brilliant green bile broth with 24 h incubation at 35 C. Eighty-seven nonmotile isolates included 43 IMViC type − − + +, of which 38 were confirmed as *Klebsiella pneumoniae.* These 38 isolates originated from 25 products, 15.2% of the total examined. Twelve of the products positive for *K. pneumoniae* were various flavors of gelato, a frozen dessert prepared with pasteurized milk in small retail stores in Metropolitan Toronto, suggesting the importance of human handling in the introduction of *K. pneumoniae.* Thirteen different serotypes, including types previously associated with clinical infections, were represented in 25 isolates from 25 products. Type 13 occurred most frequently with five isolates from two products, cream cheese spread and cottage cheese, originating from two dairies.

There have been an increasing number of reports of the isolation of *Klebsiella pneumoniae* from natural environments (*3, 4, 8, 10, 12, 15*). Special attention and concern has been directed to occurrence of this organism in wastes from certain industries, especially paper and pulp effluents where the organism frequently occurs in large numbers (*2*). This increase in reported isolations of *K. pneumoniae* from the environment is no doubt partly due to clarification of taxonomic classification which formerly identified many of these organisms as *Aerobacter aerogenes.* Nunez and Colmer (*12*), for example, found that more than 86% of 359 isolates of *A. aerogenes* from sugarcane gave biochemical reactions typical of *K. pneumoniae.* Ptak et al. (*13*) reported that 67% of the coliform colonies from treated water samples which, by IMViC tests alone, would have been classified as *A. aerogenes* were, with further biochemical testing, shown to be *K. pneumoniae.*

K. pneumoniae in the environment is of special concern because of its association with a variety of clinical infections, especially those which are nosocomially acquired. Isolates from the natural environment are indistinguishable from those present in clinical infections. Brown and Seidler (*3*) found that 7 of 11 serotypes isolated from vegetables (50% positive for *K. pneumoniae*) had previously been isolated from human urinary tract or other infections. Matsen et al. (*10*) found no dissimilarity in biochemical or serological reactions, or in mouse virulence, between water and human isolates, although water strains demonstrated greater susceptibility to antibiotics.

Eickhoff (*7*) concluded in his review that there is no evidence of human infection from *K. pneumoniae* in recreational waters. Colonization of the intestines by ingestion is suggested, however, by the work of Bergersen and Hipsley (*1*) with guinea pigs fed a diet of sweet potatoes, and Montgomerie et al. (*11*) who implicated milk mixtures as the likely source of the organism in the fecal flora of renal transplant patients. Successful colonization of the intestinal tract with *Klebsiella* is increased by antibiotic therapy (*14*). The significance of intestinal carriage is demonstrated by the work of Selden et al. (*16*) who found in a prospective study that 17 of 31 patients acquiring a *Klebsiella* nosocomial infection had the same serotype isolated from the intestinal tract as was later isolated from the lesion. They also found that the attack rate of *Klebsiella* nosocomial infection was significantly higher for patients carrying hospital-acquired intestinal *Klebsiella* than for noncarriers.

Food, it would therefore seem, can be an important source of *K. pneumoniae,* especially for hospital patients under antibiotic therapy. The study reported here was undertaken to determine the occurrence of *K. pneumoniae* in dairy products by examination of those products submitted to our laboratory which showed positive coliform counts with routine bacteriological examination.

MATERIALS AND METHODS

The dairy products included in this survey were selected at random from those showing positive coliform counts on violet red bile (VRB) agar. These products were delivered to the laboratory as part of normal surveillance sampling from dairy plants and retail outlets for routine bacteriology.

One to four colonies, usually four, were selected from each VRB plate for confirmation in brilliant green lactose bile (BGLB) broth, incubated at 35 C. Negative tubes after 24 h of incubation were discarded while positive tubes were streaked on EMB agar. Single colonies were selected from EMB agar after incubation at 35 C for 18-24 h for gram staining and preparation of a stock culture on a nutrient agar slant.

Semisolid agar was inoculated from the stock culture and incubated at 35 C for 18-24 h. Motile cultures were discarded and nonmotile isolates subjected to IMViC testing. Only those isolates with IMViC pattern − − + + were tested further for urease, lysine decarboxylase,

and ornithine decarboxylase. Slant cultures for urease were considered negative if no reaction was evident after 18-24 h at 35 C. Those isolates which were urease- and lysine-positive but ornithine- and oxidase-negative were accepted as *Klebsiella pneumoniae*. One isolate of *K. pneumoniae* from each positive dairy product was forwarded to the Center for Disease Control, Atlanta, Georgia, for serotyping.

RESULTS

From 165 dairy products with positive coliform counts (Table 1), 607 colonies were fished from violet red bile agar, of which 410 (67.5%) were confirmed as lactose fermenters in BGLB broth with 24 h of incubation. From these 410 lactose-positive, gram-negative bacilli, 87 nonmotile isolates were obtained and subjected to IMViC testing (Table 2). Of 43 isolates with IMViC pattern − − + +, 38 were urease-positive, lysine decarboxylase-positive, ornithine decarboxylase-negative, and oxidase-negative, and, therefore, accepted as *K. pneumoniae*. These 38 isolates originated from 25 dairy products (Table 3), or 15.2% of the total number of products examined.

TABLE 1. *Dairy products with positive coliform counts examined for the presence of* Klebsiella pneumoniae

Type of product	Number examined	Positive for *K. pneumoniae*		Serotypes
		No.	Percent	
Gelato	48	12	25.0	10, 15, 19, 23, 28, 31, 35, 38, 60(2x)
Ice cream	23	2	8.7	31, 64
Milk shake	22	2	9.1	7, 10
Cheese (Ricotta)	9	1	11.1	38
Ice cream mix	8	1	12.5	45
2% milk	7	0	0.0	
Skim milk	7	1	14.3	7
Cottage cheese	7	2	28.6	13(2x)
Chocolate milk	5	1	20.0	64
Soft ice cream	4	0	0.0	
Cream cheese spread	4	3	75.0	13(3x)
Homogenized milk	4	0	0.0	
Buttermilk	4	0	0.0	
Sour cream	3	0	0.0	
14% cream	2	0	0.0	
10% cream	2	0	0.0	
Milk shake mix	1	0	0.0	
18% cream	1	0	0.0	
35% cream	1	0	0.0	
Butter	1	0	0.0	
Whipping cream	1	0	0.0	
Cereal cream	1	0	0.0	
Totals	165	25	15.2	

TABLE 2. *IMViC patters for lactose-positive gram-negative non-motile isolates from dairy products*

IMViC Pattern	Number [1]
− − + +	43
− + + +	10
+ − + +	10
+ + − −	8
− + − +	6
+ + + +	3
+ + − +	3
− − − +	2
+ − + −	2
Total non-motile isolates	87

TABLE 3. *Bacteriology of dairy products containing* Klebsiella pneumoniae

Type of product	SPC/ml or g	Coliforms/ml or g	No. coliform colonies fished	No. lactose positive[b]	No. *K. pneumoniae* isolates
Chocolate gelato	4,800	42	4	3	3
Nocciola gelato	4,100	15	4	1	1
Banana gelato	17,000	>150	4	1	1
Chocolate gelato	1,400	52	4	4	1
Vanilla gelato	33,000	>150	4	3	1
Chocolate gelato	40,000	>150	4	4	1
Chocolate gelato	1,000	14	4	3	2
Banana gelato	10,000	>150	4	3	1
Chocolate gelato	9,000	>150	4	4	2
Nocciola gelato	900	4	4	3	1
Chocolate gelato	34,000	110	4	4	4
Vanilla gelato	8,000	91	4	4	2
Vanilla ice cream	300	6	4	4	1
Ice cream	200	1	1	1	1
Chocolate milk shake	>300,000	>150	4	4	1
Chocolate milk shake	>300,000	>150	4	4	3
Ricotta cheese	ND[a]	>750	4	4	1
Ice cream mix	1,400	7	4	2	2
Skim milk	800	54	4	4	1
Cottage cheese	ND	60	4	4	2
Cottage cheese	ND	>150	4	4	2
Chocolate milk	ND	>150	4	4	1
Cream cheese spread	ND	45	4	4	1
Cream cheese spread	ND	140	4	4	1
Cream cheese spread	ND	1	1	1	1
Totals			92	81	38

[a]ND = Not done.
[b]In brilliant green bile broth within 24 h at 35 C.

One isolate from each positive product was forwarded to the Center for Disease Control, Atlanta, Georgia, for serotyping. All were confirmed as *K. pneumoniae*. The reported serotypes are shown in Table 1.

DISCUSSION

The strictness of the biochemical scheme used to identify *K. pneumoniae* undoubtedly ruled out some additional isolates so that the true incidence was possibly higher than the observed 15.2% of the products examined. For example, of 87 nonmotile isolates, only 43 which were IMViC type − − + + were accepted for further testing while IMViC types − + + + (10 isolates) and + − + + (10 isolates) were discarded. According to Edwards and Ewing (*5*), 6% of *K. pneumoniae* isolates may be indole-positive and 13.3% methyl red-positive. Brown and Seidler (*3*) found about 50% of their environmental and human *K. pneumoniae* isolates were IMViC type − − + +, while 28% produced indol and 36% were methyl red-positive.

The preponderance of manufactured over fluid dairy products yielding *K. pneumoniae* does not reflect any sampling bias since fluid products, in fact, far outnumber manufactured products in samples received at the laboratory. It does reflect, however, a greater incidence in the presence of coliform bacteria in

manufactured products, which constituted the basis for further examination for the presence of *K. pneumoniae.*

Twelve (48%) of the 25 products yielding *K. pneumoniae* were gelatos, a frozen dessert made with pasteurized milk plus flavoring, stabilizers, and food colors. Sometimes raw eggs may be added, and heating may precede freezing. The product is made and served in small retail stores in Metropolitan Toronto whose clientele is primarily of Italian origin. Gelatos do not, therefore, represent a pasteurized product from a dairy plant. The high frequency of coliform bacteria, including *K. pneumoniae,* in these products reflects sanitary practices associated with preparation at the retail level rather than dairy plant conditions, and emphasizes the important factor of human handling in the introduction of these organisms.

Not all products yielding *K. pneumoniae* were, however, manipulated after leaving the dairy plant. Isolations were obtained from intact units of ice cream, ice cream mix, cheese, skim milk, cottage cheese, chocolate milk, and cream cheese spread, representing contamination at the dairy plant before distribution to the market.

Twenty-five isolates, one from each positive product, submitted to the Center for Disease Control were confirmed as *K. pneumoniae,* and included 13 different serotypes. Two isolates were untypable because of insufficient capsule. Some of these serotypes have been reported in clinical infections (*9, 17*), although types 1-6 frequently associated with respiratory disease (*6, 17*) were not represented. Type 13 occurred most frequently with five isolations from two products, cream cheese spread and cottage cheese. The three cream cheese spread samples originated from one dairy and the two cottage cheese samples from a second. The same serotype ocurring in samples of an identical product tested at different times suggests a common plant source. These products require a certain amount of handling during manufacture, therefore, a human source would not be unlikely, emphasizing again this factor in the presence of *K. pneumoniae.*

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Plate Loop Method for Determining Total Viable Count of Orange Juice

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ABSTRACT

The plate loop count as described in *Standard Methods for the Examination of Dairy Products,* 13th Edition, was evaluated as a simplified technique for determining total viable counts in single strength orange juice. This procedure requires use of standardized loops (0.01 and 0.001 ml) for making serial dilutions instead of pipets and dilution bottles. Data show there is a statistically significant difference in results between the plate count and plate loop count procedures for determining total viable population of orange juice. However, since the difference is so small for normal operating conditions, the plate loop method is a reliable alternate to routine plating procedure. It can also be done without flaming the loop between samples. The plate loop count saves time and equipment, but requires analysts to be specially trained to maintain this degree of accuracy.

The total viable microbial populations in citrus juices and concentrates are determined by an agar plate method commonly referred to as the plate count (PC) procedure. This method is used in some citrus plants to determine the microbial population of citrus juices in various stages of the processing operation, while in other plants it is used only to determine the number of viable organisms in finished products. The plate count method is time-consuming in that it requires preparation and use of pipets and dilution bottles.

Donnelly et al. (*2*) found that a 0.001-ml calibrated loop used in connection with the oval tube method gave results that compared closely with those of the standard plate count in examination of raw milk. Thompson et al. (*4*) investigated use of the loop method for determining viable counts of raw milk. Their technique involved use of a 0.001-ml calibrated loop attached to a continuous volume syringe for rinsing the sample into a standard petri dish before pouring with agar. Results of their method compared closely with those of the standard plate count done simultaneously on the same sample. The plate loop method is now recognized as an alternate procedure for determining the total microbial population of milk. As far as is known, it has never been used in the citrus industry for orange juice.

Application of this technique was investigated as a simplified method for determining total viable counts in single strength orange juice.

MATERIALS AND METHODS

The special apparatus required for the plate loop count (PLC) is described in *Standard Methods for the Examination of Dairy products,* 13th Edition (*1*). Briefly, it consists of: (*a*) a 0.01- and a 0.001-ml calibrated loop, (*b*) Luer-Lok hypodermic needle (sawed-off 24-36 mm from point where barrel enters the hub); the wire shank, which has been kinked in several places, is inserted into the sawed-off needle to a point where the bend is about 12-14 mm from the end of the barrel, and (*c*) Cornwall continuous-pipeting outfit (Becton-Dickerson & Co. No. 1251 which consists of a metal pipeting holder, a Cornwall Luer-Lok syringe and a filling outfit, 2 ml capacity, adjusted to deliver 1.0 ml). This apparatus and other parts may be sterilized in the autoclave (121 C for 15 min) or by submerging the completely disassembled unit in boiling water for 10 min.

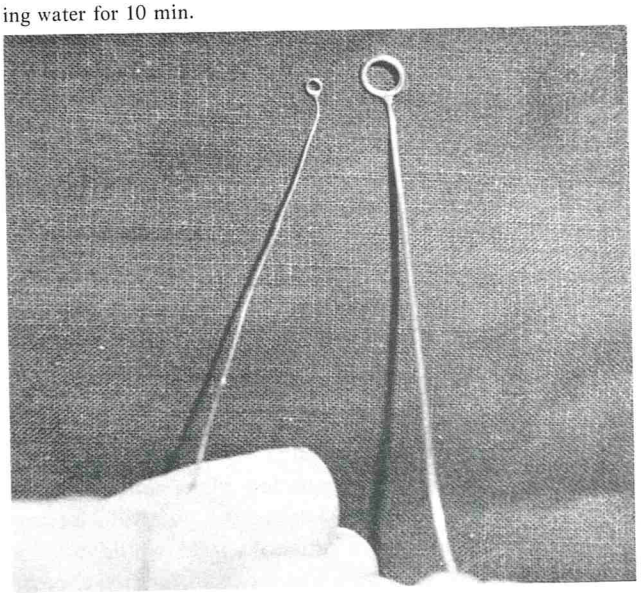

Figure 1. *Calibrated loops 0.001 ml (L) and 0.01 ml (R) used for measuring sample.*

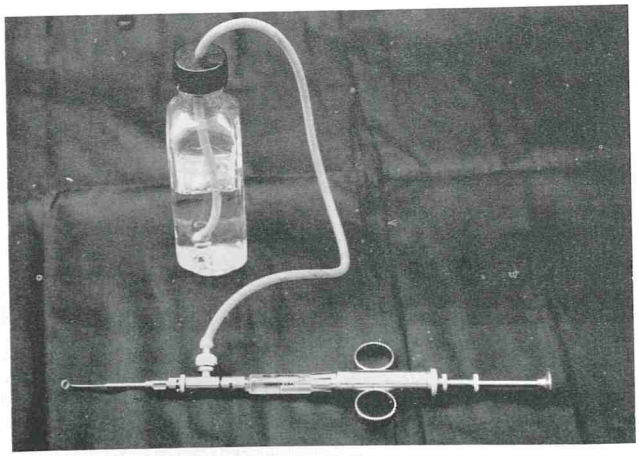

Figure 2. *The 0.01-ml measuring and transfer instrument assembled and ready for use.*

Figure 1 shows the calibrated loop—0.01 and 0.001 ml (notice an approximate 30° bend has been made about 3-4 mm from each loop). Figure 2 shows the assembled transfer and measuring instrument ready for use. The end of the rubber supply tube attached to the syringe is placed in a bottle of sterile distilled water. The syringe plunger is depressed rapidly several times to pump water into the glass syringe (which has previously been adjusted to deliver 1 ml with each depression of the plunger).

In examining a series of samples, the loop is flamed briefly before the initial transfer is made, allowed to cool 15 sec or more, then carefully dipped into the sample which has been gently mixed to avoid formation of foam. The sample is measured by inserting the loop vertically as far as the bend in the shank three times, moving it with a uniform up and down movement over a distance of about an inch (avoid droplets that rinse off the loop.) Each downward movement should be at the rate of 50-60 beats/min. A metronome may be used to establish uniform timing. The speed of removal from the surface of the juice samples affects the accuracy of the measurement. Removing the loop slowly causes less than a calibrated amount to adhere; too rapidly causes more than the desired amount to be removed. After obtaining the sample the cover of a sterile petri dish is raised, the loop inserted, and the plunger depressed, causing sterile water to flow over the charged loop, thus washing the measured 0.01 or 0.001 ml of sample into the dish (Fig. 3). It is very important not to depress the plunger so rapidly that water fails to follow the shank and flow across the loop.

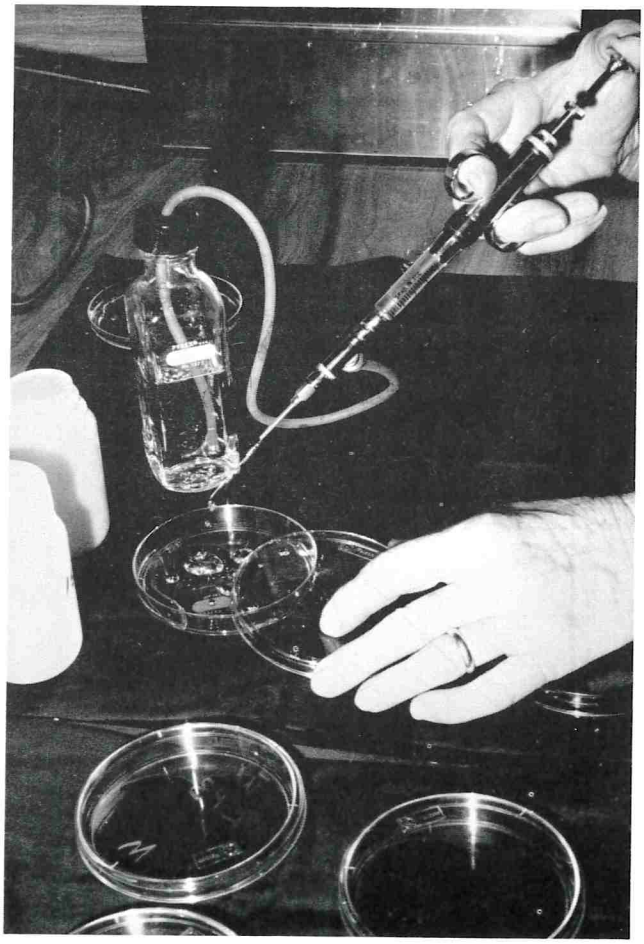

Figure 3. *The plunger is depressed, washing the measured sample of orange juice into petri dish.*

The total viable population in each sample of orange juice was determined using the PC procedure and the PLC method employing the 0.01- and 0.001-ml loops. The PLC using the 0.01-ml loop was determined on a 1:10 dilution of the same sample, thus the 0.01- and 0.001-ml samples both represented a 1:1000 dilution. Orange serum agar (BBL) modified to contain 2% agar and 0.5% sucrose was used as the plating medium. The plates were counted after 48 h of incubation at 30 C (86 F).

RESULTS

Controlled study

To determine if the plate loop method could be used with any degree of accuracy it was necessary to compare this procedure with the standard plating technique. This consisted of collecting 25 different samples of orange juice after the finisher over an 8-h period. Three duplicate analyses were made on each sample, i.e. two each employing the PC, 0.01- and 0.001-ml loops for a total of six platings on each sample. The original data are shown in Table 1. The results were converted to logs and analyzed statistically (Table 2) using Student's "t"

TABLE 1. *Results of plate counts and plate loop counts made in duplicate on samples of orange juice*

Sample no.	PC/ml (× 10^3)		0.01 PLC/ml (× 10^3)		0.001 PLC/ml (× 10^3)	
	Dup. plates		Dup. plates		Dup. plates	
1	110	107	112	120	118	103
2	130	122	118	120	129	110
3	115	102	117	112	88	101
4	117	101	123	118	96	105
5	125	130	114	104	101	92
6	133	125	91	101	97	109
7	126	129	113	121	98	106
8	117	123	117	95	102	96
9	128	115	100	108	99	105
10	134	114	117	102	98	93
11	139	111	115	96	103	98
12	123	115	101	97	101	97
13	117	102	90	123	100	93
14	122	114	104	101	103	100
15	131	124	121	95	106	93
16	53	42	48	45	49	42
17	48	47	42	46	48	40
18	46	57	45	48	45	40
19	35	43	39	37	43	35
20	32	34	36	33	34	29
21	42	35	37	32	39	35
22	32	34	43	33	48	38
23	20	27	25	29	31	27
24	40	36	43	35	48	41
25	34	38	32	34	37	35
Arith. mean	87.52		80.54		76.48	
Geo. mean	4.8775		4.8507		4.8377	

test and Snedecor's "F" ratio (*3*). All mean values compare closely; however, using the statistical "t" test for paired samples there were significant differences between the PC and the PLC at both levels of dilution. Although the differences in results were statistically significant, indicating a slight downward bias with the PLC, the absolute difference in counts were small.

The variance of the duplicate analyses for each procedure was calculated and then compared, i.e., the PC vs. PLC 0.01 and the PC vs.PLC 0.001-ml loop. Comparisons of the variance between the PC and the PLC at both dilution levels were made by using Snedecor's "F" ratio. The "F" test:

$$\frac{Sd^2\ PLC\ 0.01}{Sd^2\ PC}\quad F = 1.24 \text{ (not significant with 24 degrees of freedom)}$$

TABLE 2. *Analysis of paired differences-comparison of plate counts and plate loop counts*

	Controlled study		Plant study (0.001 loop)		
	PLC 0.01	PLC 0.001	Before finisher	After finisher	Evaporator feed
N	25	25	44	52	25
$\bar{d}$	.0279	.0510	.0194	.0062	.0286
Σd^2	.0741	.1392	.2594	.2576	.4175
t	2.92[a]	4.59[b]	1.71[c]	.632[c]	1.11[c]
F	1.24[c]	2.48[d]	—	—	—

[a]Significant at the 0.01 level.
[b]Significant at the 0.001 level.
[c]Not significant at the 0.05 level.
[d]Significant at the 0.05 level.
N—number of samples.
$\bar{d}$—average difference between plate loop and plate counts.
Σd^2—sum of the difference squared.
t—used to test from the significance of differences between sample means.
F—ratio compares precision (variation) of methods.

$$\frac{Sd^2\ PC}{Sd^2\ PLC\ 0.001}$$ F = 2.48 (significant at 0.05 level with 24 degrees of freedom)

For practical purposes, each method has the same precision in test results.

Plant study

A study was made to determine if the PLC procedure could be used with any degree of accuracy under commercial operations. Orange juice was collected over a 3-month period before the finisher, after the finisher, and before entering the evaporator. The juice was checked for total viable population, employing both the PC and the PLC (0.001-ml loop). This study is summarized in Table 3. The range and average of counts show a close relationship between the two methods. A statistical analysis of the data (Table 2) indicates there

TABLE 3. *Summary of results of plate counts and plate loop counts on orange juice sampled from plate operation*

Source of sample	No. samples	Range of counts × 10^3 at 30 C		Average counts × 10^3	
		PC/ml	PLC/ml[a]	PC/ml	PLC/ml[a]
Before finisher	44	11-808	8-860	121	116
After finisher	52	9-904	9-868	114	115
Evaporator feed	25	24-566	19-450	108	106

[a]Counts obtained using 0.001-ml loop.

was no significant difference at the 0.05 level between PC and the PLC mehtods for determining total viable population in orange juice.

A plot of the relationship between the PLC and the PC before the finisher is shown in Fig. 4 and after the finisher in Fig. 5. A theoretical line has been drawn in each figure to represent the point on which a paired PLC and PC value would be plotted if no differences in counts were observed. The plotted circles, on the other hand, are the actual paired results obtained with various samples during the study. Note, for example, the intersection of the dotted lines in Fig. 4 (before finisher) showing the paired PLC value of log 4.833 and the corresponding PC value of log 4.978. Since the PC value is higher than the PLC value the plotted circle is above the diagonal line of

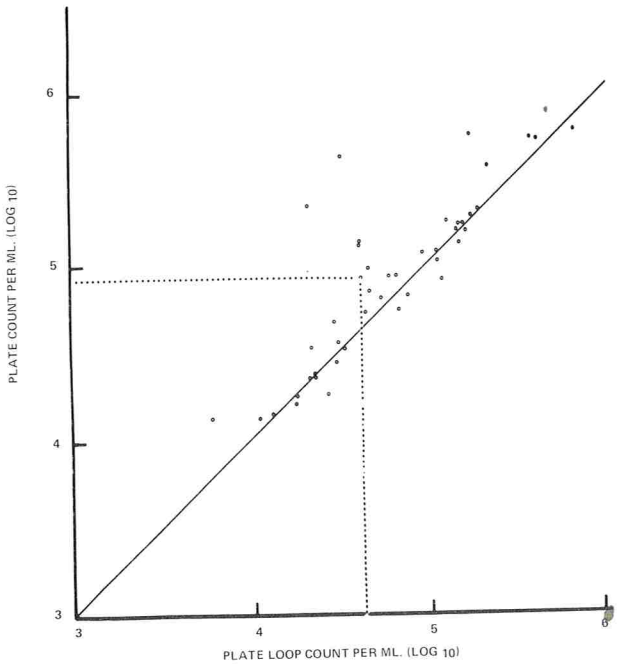

Figure 4. *Relationship between plate counts and plate loop count-sampled before finisher.*

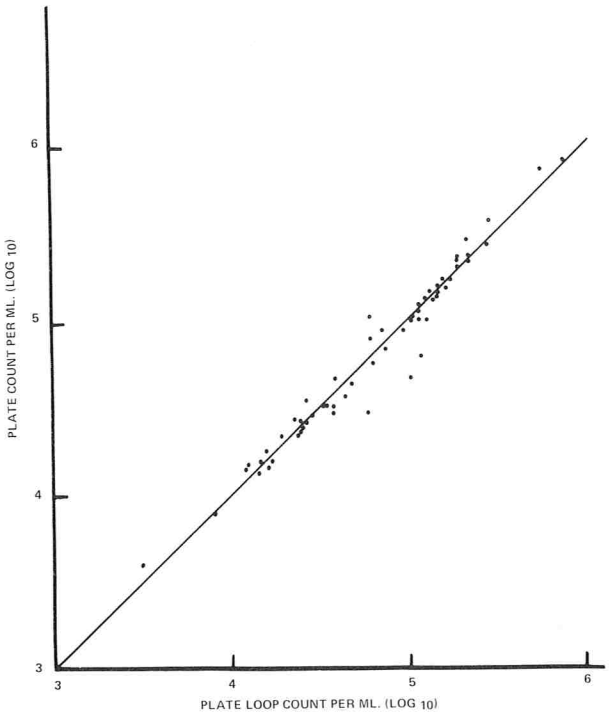

Figure 5. *Relationship between plate count and plate loop count-sampled after finisher.*

no difference.

The plots of Fig. 4 and 5 indicate results of samples taken before the finisher (Fig. 4) are more scattered, suggesting greater differences in results than those from samples taken after the finisher (Fig. 5). Furthermore, samples collected before finisher showed a slight upward bias in that the plate count method tends to indicate

TABLE 4. *Residual microorganisms remaining on 0.01- and 0.001-ml loops after discharging sample*

Sample no.	0.01 PLC/ml (× 10^3)	Colonies on rinse plate[a]	0.001 PLC/ml (× 10^3)	Colonies on rinse plate[a]
1	120	0	110	0
2	120	0	120	0
3	110	1	95	0
4	120	0	100	1
5	110	0	96	1
6	96	0	100	2
7	120	0	100	1
8	110	0	99	0
9	100	0	100	0
10	110	0	95	2
11	110	0	100	1
12	100	0	99	0
13	110	0	97	1
14	100	0	100	0
15	110	0	100	1
Arith. mean	110		101	
Geo. mean	5.0393		5.0024	

[a]Prepared by discharging 1 ml of sterile rinse water across shank and loop afr discharging sample into petri dish.

higher counts than the plate loop procedure. The greater spread in data before the finisher may be due to pulp adhering to the loop.

Residual microorganisms remaining on loop

In this experiment the loop was flamed initially, but not between samples. The charged loop was rinsed in the normal manner. A control plate was then made by flushing the loop and shank into sterile petri dish with 1 ml of sterile rinse water. The results in Table 4 indicate that the residual organisms remaining on the loop after discharging the sample are not significant.

DISCUSSION

Data presented herein indicate under operating conditions there was no significant difference in results at the 0.05 level between the PC and the PLC procedures for determining total viable population of orange juice. Under controlled conditions differences between the plate count and plate loop methods were significant at the 0.01 and 0.001 levels for the 1/100- and 1/1000-ml loops, respectively. Variation in both methods was found to be similar. In conclusion, the PLC can be used as a reliable alternate to the PC method. It can also be done satisfactorily without flaming the loop between samples. However, it is highly important the analyst be specially trained before this method is used.

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Antibiotic-Resistant Bacteria in Raw Meat from Retail Markets

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ABSTRACT

Raw meat samples from retail markets were examined for bacteria resistant to chloramphenicol and neomycin sulfate. Total numbers of bacteria ranged from 17,000 to 30,000,000 per gram and resistant bacteria from < 100 to 450,000 per gram. Twelve isolates resistant to both antibiotics were identified as either *Pseudomonas aeruginosa, Pseudomonas putida,* or *Pseudomonas fluorescens.*

Recently we described media to evaluate the enzymatic capabilities of bacteria and fungi in foods (*1*). Chloramphenicol and neomycin sulfate were added to suppress bacterial growth in the fungal media and these antibiotics suppressed bacteria in all foods tested except raw meat. Some bacteria in the meat were able to grow on media that contained these antibiotics. We report here on the prevalence of bacteria resistant to chloramphenicol and neomycin sulfate in raw meat from retail markets and on identification of some isolates.

MATERIALS AND METHODS

Thirty-one samples, including 22 of ground beef, eight of pork sausage, and one cube steak sample were collected at retail markets. The samples, purchased by inspectors of the Connecticut Department of Consumer Protection but wrapped by a clerk, were refrigerated (ice, insulated container) until delivered to the laboratory on the same day. Upon delivery, a portion was removed aseptically to a sterile, wide-mouth jar and either analyzed immediately or frozen until used. Samples were collected during January, May, and October, 1975.

Appropriate amounts of meat were blended in phosphate solution (*5*), and diluted in the same solution. Total aerobic counts were estimated by spreading 0.1 ml of the sample (or dilution of the sample) on the surface of previously hardened Plate Count Agar (Difco, Detroit, Mich.). These plates were incubated at 30 C. Antibiotic-resistant bacteria were enumerated by the same plating procedure on either a medium used to detect proteolytic or pectolytic bacteria (*4*) to which antibiotics were added. Antibiotics were prepared for test media as previously described (*1, 4*). The final concentrations of chloramphenicol (Chloromycetin, Sigma Chem. Co., St. Louis, Mo.) and of neomycin sulfate (Sigma) in the medium were 100 μg/ml and 50 μg/ml respectively. Total numbers of bacteria were obtained from the plate counts after 48 h of incubation and resistant bacteria after 72-96 h. Isolates resistant to chloramphenicol and neomycin sulfate were tested against other antibiotics by disk assay (*2*).

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RESULTS

The total numbers of bacteria ranged from 24,000 to 30,000,000 per gram in the ground beef and from 17,000 to 15,000,000 in the sausage (Table 1). The number of resistant bacteria ranged up to 450,000 per gram.

On one collection date two samples of beef were obtained from the same market. Also, on another collection date a sample of beef and a sample of sausage were taken from each of two markets. These samples allowed us to determine whether cross contamination between meats prepared at the same market (e.g., through handlers or equipment) was likely. The two ground beef samples from the same market (samples 2 and 4, Table 1) had few resistant bacteria as well as low total numbers of bacteria. One beef and one sausage sample collected on the same day (samples 21 and 31, Table 1) showed that the beef had high total numbers, the sausage relatively low. Yet, the beef contained many resistant bacteria while the sausage did not. The other paired samples, one beef and one sausage collected on the same day from the same market (samples 17 and 30, Table 1), showed the opposite result. The sausage sample contained many more bacteria than the beef, yet both contained very few resistant bacteria.

Calculation of the percentage of resistant bacteria (Table 1) shows that in most samples a small proportion of the total numbers was resistant. Exceptions were found in samples 6, 8, 11, 24, 25. Only eight of the 31 samples contained over 1% resistant bacteria.

Eleven of the 31 samples were also plated on media that contained either 100 μg of chloramphenicol or 50μg of neomycin sulfate/ml, as well as on media that contained both of these antibiotics (Table 1). As expected, more resistant organisms were always found on media with a single antibiotic and the numbers found were usually more than with the media that contained both antibiotics. In most instances the neomycin sulfate alone inhibited more bacteria than did the chloramphenicol alone.

Individual isolates of bacteria resistant to both chloramphenicol and neomycin sulfate were identified (Table 2). Each isolate was obtained from a different

TABLE 1. *Total bacterial count and numbers resistant to chloramphenicol and neomycin sulfate in raw meat from retail outlets*

Sample number	Type of product	Total count (No/g)	Resistant count[1]		Resistant to chloramphenicol[2]		Resistant to neomycin[3]	
			(No/g)	(%)	(No/g)	(%)	(No/g)	(%)
1	ground beef	3,500,000	10,000	0.3				
2[4]	ground beef	70,000	<1,000	—				
3	ground beef	1,100,000	8,000	0.7				
4[4]	ground beef	240,000	<1,000	—				
5	ground beef	770,000	5,000	0.7				
6	ground beef	620,000	86,000	13.9				
7	ground beef	180,000	3,000	1.7				
8	ground beef	1,000,000	82,000	8.2				
9	ground beef	1,600,000	100	<0.1				
10	ground beef	1,100,000	900	<0.1				
11	ground beef	72,000	1,900	2.6				
12	ground beef	30,000,000	120,000	0.4				
13	ground beef	33,000	<100	—				
14	ground beef	100,000	100	0.1	600	0.6	100	0.1
15	ground beef	680,000	1,200	0.2	1,400	0.2	1,600	0.2
16	ground beef	77,000	<100	—	100	0.1	200	0.3
17[6]	ground beef	91,000	100	0.1	3,900	4.3	6,000	6.6
18	ground beef	5,000,000	800	<0.1	33,000	0.7	2,400	<0.1
19	ground beef	60,000	400	0.7	7,900	13.2	1,600	2.7
20	ground beef	24,000	100	0.4	2,900	12.1	1,100	4.6
21[5]	ground beef	14,600,000	18,000	0.1	350,000	2.4	44,000	0.3
22	ground beef	51,000	700	1.4	2,500	4.9	700	1.4
23	cube steak	2,000,000	27,000	1.4				
24	sausage	14,500,000	450,000	3.1				
25	sausage	26,000	3,300	12.7				
26	sausage	280,000	1,500	0.5				
27	sausage	2,900,000	2,000	<0.1				
28	sausage	17,000	200	1.2				
29	sausage	20,000	100	0.5				
30[6]	sausage	840,000	700	<0.1	2,500	0.3	1,600	0.2
31[5]	sausage	70,000	100	0.1	300	0.4	400	0.6

[1]Resistant to combination of chloramphenicol and neomycin sulfate, 100 μg/ml and 50 μg/ml in medium respectively.
[2]Resistant to chloramphenicol alone, 100 μg/ml in medium.
[3]Resistant to neomycin sulfate alone, 50 μg/ml in medium.
[4]Two samples collected from same outlet, same day, but different grades of meat.
[5]Two samples collected from same outlet, same day, one ground beef, one sausage.
[6]Two samples collected from same outlet, same day, one ground beef, one sausage.

TABLE 2. *Identification of antibiotic resistant isolates from raw meat obtained at retail outlets*

Isolate from sample number[1]	Source	Designation
14	ground beef	*Pseudomonas aeruginosa*
15	ground beef	*P. aeruginosa*
31	sausage	*P. aeruginosa*
16	ground beef	*P. putida*
—[2]	sausage	*P. putida*
30	sausage	*P. fluorescens*
17	ground beef	*P. fluorescens*
18	ground beef	*P. fluorescens*
19	ground beef	*P. fluorescens*
20	ground beef	*P. fluorescens*
21	ground beef	*P. fluorescens*
22	ground beef	*P. fluorescens*

[1]Sample number refers to Table 1.
[2]Sample not shown in Table 1.

sample, nine from ground beef and three from sausage. All isolates were pseudomonads; three were *Pseudomonas aeruginosa,* two were *Pseudomonas putida,* and seven were *Pseudomonas fluorescens.* Cross-resistance patterns of these isolates to other common antibiotics showed that all were resistant to penicillin, ampicillin, and cephalothin, and all except one (No. 20) were resistant to carbenecillin. All of the isolates were sensitive to polymyxin B, streptomycin, tetracycline, kanamycin, and gentamicin. Only three of the isolates were sensitive to nalidixic acid; two of the *P. aeruginosa* isolates were resistant and one was sensitive.

DISCUSSION

The reason for the presence of antibiotic-resistant bacteria in the raw meat samples we examined is conjectural. One possibility is that the resistance is due to feeding of antibiotics to meat-producing animals to enhance their growth (*6*). No antibiotic residues are allowed in meat for human consumption, but proposals to add antibiotics to meat to restrict microbial growth have been made and at one time this practice was allowed in a dip for poultry (*3*).

Contamination within each retail market is also possible from containers in which cut-up meat awaits grinding, from food wrapping materials, from the grinding equipment, and even from the handlers themselves. If these possibilities do not exist, then it seems strange that almost every market harbors antibiotic-resistant bacteria and would tend to show that antibiotic resistant organisms are more prevalent than has been supposed or reported. In any event, it is clear that resistant organisms can be isolated in large numbers from raw meat.

Cross-contamination of samples is ruled out (except where noted) since no single person handled every sample. Areas from which samples were taken at any one time were as far as 125 kilometers apart and thus it is unlikely that all samples represent a single original source.

It is interesting that all of the resistant isolates were pseudomonads. Pseudomonads and related species can grow fairly rapidly on properly refrigerated meat. Thus high numbers of bacteria cannot always be attributed to poor sanitation. Nevertheless, the presence of antibiotic resistant bacteria may be of public health importance. Further, that the organisms were pseudomonads and many of this group are psychrotrophs is of concern since they can grow on the refrigerated ground meats. To our knowledge this is the first time that *P. aeruginosa* has been isolated from raw meat.

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Decomposition of Organic Acids During Processing and Storage

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ABSTRACT

Solutions (0.1 *N*) of organic acids as well as tissue concentration levels were processed at several temperatures with varyious F_0 values. Analyses were done using an automatic organic acid analyzer and paper chromatography. Pyrrolidone-carboxylic acid was produced from glutamic acid, fumaric from maleic acid, itaconic and trans-aconitic from cis-aconitic acid, malonic acid decomposed to acetic acid, and oxalacetic to pyruvic acid. At tissue concentration level oxalacetic acid decomposed completely at all process temperatures to pyruvic acid and to a lesser degree during storage without processing. Decomposition of other organic acids increased with increasing process time. The decompositon of malonic to acetic acid was the only reaction which was decreased significantly by use of a High-temperature Short-time process. However, the amount of decomposition of all acids, except oxalacetic, was low both after processing and during storage.

Formation of organic acids during processing and storage is an important parameter in the final quality of a processed food product. The decrease in pH and subsequent degradation of color in green vegetables has been reported to be related to the increase or formation of organic acids (*1, 6, 12*).

One of the major functions of organic acids is to enhance and modify the flavor of products when used as additives (*14*). Equally important is the ability of organic acids to aid in the preservation of foods and in simplifying certain processing operations. In addition, organic acids serve other specific functions such as gelling agents for pectin and as a source of acidity in leavening (*8*).

Thermal behavior and kinetic studies of some organic acids have been reported (*2-5,7*). However, decomposition of organic acids during processing and storage, which could lead to an increase or decrease in organic acid concentration in processed foods, has not been studied extensively.

This investigation was initiated to study the stability of organic acids utilizing different time-temperature parameters during processing and storage. It is realized that the model systems employed are not directly analogous to food systems but nonetheless provide some insight into the pathways involved in the decomposition of these acids.

Alpha-ketoglutaric, cis-aconitic, citric, maleic, malic, malonic, fumaric, glutaric, oxalacetic, pyruvic, succinic, and glutamic acids were investigated. Amoung these, malonic, cis-aconitic, maleic, oxalacetic, and glutamic acids decomposed.

MATERIALS AND METHODS

Preparation of organic acid solutions

Solutions (0.1 *N*) were prepared individually with distilled water from the following acids: *a*-ketoglutaric acid (Sigma Chemical Co.), citric acid (Fisher Scientific Co.), glutaric acid (Calbiochem), maleic acid (Sigma Chemical Co.), malic acid (Eastman Kodak Co.), malonic acid (Calbiochem.), oxalacetic acid (Calbiochem.), pyruvic acid (Eastman Kodak Co.), glutamic acid (Eastman Kodak Co.), and succinic acid (Fisher Scientific Co.). Each of these solutions was processed with an $F_0 = 4.9$ and 49 at temperatures of 240 and 300 F in Thermal Death Time (TDT) tubes as calculated by Gupte and Francis (*9*). After processing, samples were frozen at −20 F until analysis.

Approximate tissue level concentrations for cis-aconitic, malonic, maleic, and oxalacetic acids were obtained by preparing acid solutions with 5μ eq per ml in distilled water. Glutamic acid solution was prepared at a concentration of 66.7μ eq per ml and diluted to 6.67μ eq per ml for processing and storage. Each organic acid solution at these concentrations was processed at temperatures of 240, 270, and 300 F with $F_0 = 4.9$, 14.7, and 24.5, producing nine different treatments. The processing procedure was the same as described previously.

To investigate the effect of storage on decomposition of acids both processed and unprocessed samples were stored at 75 and 38 F and analyzed. It was necessary to use unprocessed samples in some instances since the thermal process totally decomposed certain of the acids and therefore storage effects could not be noted.

Organic acid analysis

Following treatment the acid solutions were quantitatively analyzed by an Automatic Organic Acid Analyzer (AOAA), (Water Associates, Inc., Milford, Mass.) to establish the decomposition products. The principles on which the analyzer is based are described by Kesner and Muntwyler (*11*). The detailed operation, preparation of silica gel, column packing, preparation of indicator, composition of solvent gradient, and calibration were described completely by Lin et al. (*12*).

The number of chambers used in this study depended upon the structure of the particular organic acid. Three chambers were used for the analysis of cis-aconitic and oxalacetic acids. The solvent gradient in the tree chambers were chloroform, 7% (vol/vol) tert-amyl-alcohol/chloroform, and 30% (vol/vol) tert-amyl-alcohol/chloroform respectively. Glutamic, maleic and malonic acids were analyzed using two chambers with chloroform and 30% (vol/vol) tert-amyl-alcohol/chloroform respectively. Alpha-ketoglutaric, citric, glutaric, malic, pyruvic, and succinic acids were analyzed by the original five chamber system.

The error involved in this type of anolysis is less than 1% [Lin et al. (*12*)] and reproducibility of results was found to be within 4%.

Identification

Tentative identification of the decompostion products of acids was based on retention times on the AOAA. A known amount of standard

acid was introduced into the sample which had been analyzed previously. The increased concentration of acid found at the same retention time indicated that the sample acid was identical with the standard acid. Therefore, identical retention times provided tentative identification via the use of this internal standard technique.

Confirmatory identification

Identification of the decomposed products obtained from the 0.1 *N* organic acid solutions was confirmed by comparison with standard acids on paper chromatography using the following solvent systems.

BF: n-Butanol-3 N formic acid (50:50 vol/vol). Samples were developed by the upper phase of the solvent mixture utilizing the lower phase for vapor equilibration. The dried chromatograms were sprayed with a 0.05% solution of bromphenol in 50% ethanol. To achieve a distinct color response between organic acids and background, the sprying reagent was adjusted to pH 12.5 with 0.1 *N* NaOH before spraying. This method is a modification of the work of Markakis et al. (*13*).

PA: n-Propanol-2 N Ammonia (60:40) and (50:50). Samples were developed by each of these solvent mixtures. The dried chromatograms were sprayed with indicator solution which was prepared by mixing 15 ml of Universal Indicator Solution (Fisher Scientific Co.) and 3 ml 0.1 *N* sodium hydroxide. The color resulting from the response of organic acids on the chromatograms was noted immediately after spraying to compensate for color changes on standing (*10*).

RESULTS AND DISCUSSION

As stated previously, paper chromatagraphy was utilized as well as the AOAA for further confirmation of identification. The R_f values of the decomposition products of the acids were determined by BF and PA solvent systems along with standard compounds. The R_f values of the decomposition products were found to be identical to the standard acids in each case (Table 1).

TABLE 1. *R_f values of standard organic acids and acids formed through decomposition in different solvent systems*

	R_f values (×100)					
	BF[a]		A[b]		PA[c]	
Acids	Standard	Heat treated	Standard	Heat treated	Standard	Heat treated
Glutamic	9	9	20	20	51	51
PCA	51	51	47	47	62	62
Cis-aconitic	42	42	21	21	46	46
Trans-aconitic	88	88	22	22	56	56
Itaconic	65	65	23	23		
Pyruvic	69	69	25	25		
Maleic	49	49	31	31		
Fumaric	88	88	33	33		
Malonic	65	65	23	23		

[a]BF:-n-Butanol-3N formic acid (50:50)
[b]PA:-Propanol-2 N Ammonia (60:40)
[c]PA:-Propanol-2 N Ammonia (50:50)

Analysis before and after heat treatment showed that cis-aconitic, glutamic, malonic, maleic, and oxalacetic acids decomposed, whereas other organic acids remained unchanged. Based on analysis of decomposition products and the fact that only distilled water and acid were present before heating, it was concluded that: malonic acid decomposed to acetic acid, oxalacetic to pyruvic, maleic to fumaric, cis-aconitic to trans-aconitic, and itaconic and glutamic to 2-pyrrolidone-5-carboxylic acid, when using 0.1 *N* solutions.

Quantitative analysis

The amount of decomposition and percent conversion for malonic, oxalacetic, cis-aconitic, maleic, and glutamic acids at approximate tissue concentration levels are shown in Tables 2a, 2b, 2c, 2d, and 2e. With malonic acid (Table 2a), a higher conversion rate was shown at 240 F. The decomposition rate increased for each of the temperatures (240, 270, and 300 F) when the process time increased. "High-temperature Short-time" (HTST) processing caused least decomposition.

TABLE 2a. *Decomposition of malonic acid (5μ eq/ml) to acetic acid after processing at temperatures of 240, 270, and 300 F with F_0 values =4.9, 14.7, and 24.5*

	Conc. (μ eq/ml) of acetic acid and % decomposition of malonic acid					
	240 F		270 F		300 F	
F_0	Conc.	% Decomposition	Conc.	% Decomposition	Conc.	% Decomposition
4.9	0.30	12	0.18	7	0.13	5
14.7	0.65	26	0.31	12	0.18	7
24.5	0.75	30	0.41	17	0.40	16

Oxalacetic (Table 2b) showed complete decomposition to pyruvic acid at all processing temperatures. This indicated that oxalacetic acid was the most unstable during processing.

TABLE 2b. *Decomposition of oxalacetic acid (5μ eq/ml) to pyruvic acid after processing at temperatures of 240, 270, and 300 F with F_0 value =4.9.*

	Conc. (μ eq/ml) of pyruvic acid and % decomposition of oxalacetic acid					
	240 F		270 F		300 F	
F_0	Conc.	% Decomposition	Conc.	% Decomposition	Conc.	% Decomposition
4.9	5.25	105	5.11	102	5.02	100

The conversion of maleic acid to fumaric acid was found to be low (Table 2c) at both 240 and 300 F. A longer processing time did not cause much change in conversion. Therefore, samples were not processed at any other temperature.

TABLE 2c. *Conversion of maleic acid (5 μ eq/ml) to fumaric acid after processing at temperatures of 240 and 300 F with F_0 value =4.9 and 24.5*

	Conc. (μ eq/ml) of fumaric acid and % conversion of maleic acid			
	240 F		240 F	
F_0	Conc.	% Conversion	Conc.	% Conversion
4.9	0.20	4.0	0.13	2.6
24.5	0.30	6.0	0.19	3.8

The conversion of glutamic acid to PCA (Table 2d) was

TABLE 2d. *Conversion of glutamic acid (6.67 μ eq/ml) to pyrrolidone-carboxylic acid (PCA) after processing at temperatures of 240, 270, and 300 F with F_0 values =4.9, 9.8, 14.7, and 24.5*

	Conc. (μ eq/ml) of PCA and % conversion of glutamic acid					
	240 F		270 F		300 F	
F_0	Conc.	% Conversion	Conc.	% Conversion	Conc.	% Conversion
4.9	0.26	8	—	—	0.25	8
9.8	0.40	12	0.55	16	0.56	17
14.7	0.60	18	0.90	27	0.72	22
24.5	0.85	25	0.90	27	0.93	28

greater at 270 and 300 F than at 240 F. The percent conversion was increased by increasing the process time at each different temperature.

Decomposition of cis-aconitic acid to itaconic (Table 2e) acid was higher at 240 and 300 F, than at 270 F. This might have been due to a thermal processing error either at 270 F or at the other two temperatures. Itaconic acid concentration increased when the processing time was increased. However, the concentration of trans-aconitic acid varied irregularly for different process times at each processing temperature upon decomposition of cis-aconitic acid.

The decomposition of the acids involved is due to decarboxylation or dehydration. However, whether the decarboxylation and dehydration which occured in this investigation depends upon total heat energy or heating temperature remains unknown.

From these results it is postulated that an increase of some organic acids in the tissues during thermal processing may be due to decompositionof other organic acids. This thermal decomposition might be increased significantly when foods are overprocessed.

All samples, except for oxalacetic acid, were stored both with and without processing. The concentrations of the decomposition products of the acids during storage are shown in Table 3. PCA, which was formed from the decomposition of glutamic acid during processing, completely disappeared during storage at 75 F. However, the concentration of PCA stored at 38 F remained constant. Since it was found previously that oxalacetic acid was completely decomposed to pyruvic acid at all process temperatures, unprocessed samples of oxalacetic acid were stored at 75 F and 38 F to determine stability. Pyruvic acid was produced from unprocessed oxalacetic acid when stored at 75 F and at 38 F. This indicated that oxalacetic acid is unstable both during processing and in storage. Acetic acid increased approximately 16% after processing and storage and approximately 4% after storage alone. The conversion of maleic acid to fumaric acid was irregular. The explanation of this result is difficult since the per cent conversion was very low. Trans-aconitic acid increased about 78% after processing and storage, about 70% after storage at 75 F without processing, and about 40% after storage at 38 F. However, decomposition of cis-aconitic acid to itaconic acid occurred to a lesser extent during storage after processing, and not at all during storage without processing.

In conclusion the effect of storage on the decomposition of organic acids, except for oxalacetic and cis-aconitic, is limited. It may be postulated that decarboxylation or dehydration from organic acids alone in solution requires heat. However, this may not be the case in a complex system, such as a food material.

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TABLE 2e. *Decomposition of cis-aconitic acid (5 μ eq/ml) to trans-aconitic acid and itaconic acid after processing at temperatures of 240, 270, and 300 F with F_0 values =4.9, 14.7, and 24.5*

	Conc. (μ eq/ml) of trans-aconitic and itaconic acids and % decomposition of cis-aconitic acid											
	240 F				270 F				300 F			
	Trans-aconitic acid		Itaconic acid		Trans-aconitic acid		Itaconic acid		Trans-aconitic acid		Itaconic acid	
F_0	Conc.	% Dec.	Conc.	% Dec.	Conc.	% Dec.	Conc.	% Dec.	Conc.	% Dec.	Conc.	% Dec.
4.9	2.90	58	0.35	11	1.60	32	0	0	1.95	39	0.37	12
14.7	3.60	72	0.85	26	3.10	62	0.37	12	2.80	56	0.90	27
24.5	3.35	67	0.85	26	3.50	70	0.50	15	2.35	47	1.25	37

TABLE 3. *Decomposition products from glutaric, malonic, oxalacetic, maleic, and cis-aconitic acids after 10 weeks storage at 75 and 38 F either after processing at 240 F for 19.5 min or without processing*

	Conc. (μ eq/ml) of decomposed compounds and % decomposition							
	Processed				Unprocessed			
	Storage at 75 F		Storage at 38 F		Storage at 75 F		Storage at 38 F	
Decomposed compounds[a]	Conc.	% Decomposition	Conc.	% Decomposition	Conc.	% Decomposition	Conc.	% Decomposition
PCA	0	0	0.28	9	—	—	—	—
Acetic	0.39	16	0.38	15	0.09	3.6	0.10	4
Pyruvic	—	—	—	—	3.85	77	2.00	40
Fumaric	0.28	5.6	0.19	3.8	0.18	3.6	0.13	2.6
Trans-aconitic	3.90	78	3.60	72	3.51	70	2.00	40
Itaconic	0.40	12	0.35	11	0	0	0	0

[a]PCA produced from glutamic acid (6.67 μ eq/ml).
Acetic acid produced from malonic acid (5 μ eq/ml).
Pyruvic acid produced from oxalacetic acid (5 μ eq/ml).
Fumaric acid produced from maleic acid (5 μ eq/ml).
Trans-aconitic and itaconic acids produced from cis-aconitic acid (5 μ eq/ml).

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A Critical Study of the Multiuse Polyethylene Plastic Milk Container System

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ABSTRACT

The absorption of 11 of 16 common chemical substances by multiuse polyethylene plastic milk containers was not detected by the required contaminant detection device. The inability of the detector to respond to significant levels of several potentially hazardous chemical substances suggests that this multiuse milk container system may present a public health problem. Milk stored in five of the 11 "detector-accepted" bottles contained either pesticide residues in excess of legal tolerance limits or had objectionable off-flavors. The contaminant detector will detect volatile hydrocarbons and may not respond to absorbed toxic contaminants which may gain entrance to polyethylene milk containers through misuse by the consumer.

Each of the various milk packaging materials or systems seems to have its own set of limitations or disadvantages. When the evaluation factors for milk packaging are applied: i.e., economics, consumer preference, energy requirements, raw material utilization, environmental impact, nutrient retention, flavor protection, public health, and safety; there is probably no "perfect milk container."

For the last 40 years, packaging of fluid milk and milk products in the United States has traditionally been closely aligned with the relatively high levels of sanitation, public health, convenience, and efficiency practiced by the dairy industry. Generally, the fluid milk processor seeks maximum product protection for this highly perishable product within the limits of optimum economic parameters. The processor or distributor selects the type(s) of milk containers that best meets his conditions of processing, distribution, competition, and pricing strategy. The novelty or potential associated with new forms of milk packaging systems helps determine what new containers are introduced for marketing milk.

In September 1964, the U.S. Public Health Service (*4*) gave approval for limited use of high density polyethylene milk bottles for multiple use in Spokane, Washington. One year later, the USPHS approved unlimited use of returnable plastic containers, provided the following criteria were met: (*a*) the plastic resin complied with FDA specifications and the 3-A Plastics Standard, Section H, (*b*) containers were coded to trace the identity and source of plastic resin, (*c*) an automatic contaminant detector installed in line to the filler to detect volatile organic contaminants with the device sensitivity adjustment sealed and the detector interconnected with the filling equipment, (*d*) use of single-service, non-screw top, closures, and (*e*) plastic bottles should not absorb pesticide residues or chemical contaminants in excess of FDA tolerances.

The developers (*10*) of the linear polyethylene milk bottle recognized that satisfactory multiple use would be dependent upon non-permanent contamination of the interior surface or absorption into the plastic by any chemical substance injurious to health or objectionable to the consumer. A series of tests conducted by the developers (*11*) of this packaging system indicated an uptake and retention by the polyethylene of certain pure chemicals and commercial products or mixtures of compounds. Generally, they claimed that nonpolar substances such as gasoline, turpentine, and fuel oil were highly absorbed, while more polar substances such as wine, detergent, and onion juice were poorly absorbed. Even so, onion odor was detectable by sensory evaluation at concentration levels as low as 0.01% in the plastic. Simulated washing cycles of treated polyethylene showed a high percentage of removal of the more polar chemical contaminants, while the hydrocarbon compounds showed a low percentage of removal. This demonstrated the need for a reliable detection system which would reject and destroy contaminated bottles, rather than attempting to clean such contaminated milk containers.

The rationale employed for selection of a detection device was that most contaminants would be expected to have some degree of volatility hence sampling the air from inside a bottle should indicate the possible absorption of such contaminants. Hydrogen flame ionization detectors (FID) are very sensitive in response to virtually all organic compounds and simultaneously insensitive to water, carbon dioxide, and the permanent gases of the atmosphere (*8*); hence such a unit was selected, but modified for rapid sampling cycles. The relatively rapid rate of bottle passage on the conveyor necessitated a bottle examination approximately every 2 sec (*1, 10*). Therefore, a standard FID and its necessary adjuncts were modified to accomplish more rapid sampling and reduction of response time by reducing the physical size of tubing and other components of the unit. Essentially, a pulse of sample gas had to be withdrawn from the bottle, pass through the flame and be completely flushed from the detector within 2

sec. Likewise, the electronic portion of the detection system would have to respond and recover within 2 sec. Instrument sensitivity to some volatile organic substances was undoubtedly sacrificed to attain the required rapid sampling and cycling capacity.

Wildbrett of Germany studied the role of plastics in the dairy industry (*13*), with emphasis on the response of packaged milk products to the permeability properties of plastic films (*14*), reactions between milk and certain plastics (*15*), and the cleaning, sanitizing and hygienic state of plastic surfaces (*16, 17*). He concluded that it was unlikely that polyolefin containers could be used repeatedly for fat-containing milk products without adverse effects, since polyethylene demonstrated an affinity for lipids (*17*). Wildbrett was also critical of the ease with which thermoplastics are etched and the greater adhesion of fatty impurities on polyolefins than the corresponding adhesion of cleaning solutions (*16*).

Since the introduction of the multiuse polyethylene milk container system in Oregon in 1966, the Oregon Department of Agriculture has received and recorded a significant number of consumer complaints (*3, 9*) related to this method of packaging. For example, in 1970, at least 112 consumer complaints were received by regulatory officials (*4*) in Oregon, Washington, and Idaho concerning multiuse polyethylene milk containers, compared to one complaint regarding multiuse glass (which contained a foreign object). Off-flavor (taste or odor) accounted for 54% of the reported complaints, foreign objects 35%, slack fill 7%, and other 4%. Also, 14 cases of suspected or claimed illness associated with the consumptionof fluid milk from contaminated multiuse polyethylene containers were reported, but unconfirmed. During the 1973 energy crisis, the shortage of gasoline in Oregon resulted in apparent use by consumers of the nonbreakable, accessible polyethylene milk container for automobile fuel storage. Subsequent consumer complaints of "petroleum-flavored" milk (*9*) served to point out that for one reason or another, the hydrocarbon sensing capability of the contaminant detector did not always function.

During the 10 year period the returnable polyethylene milk container has been employed, several other problems have appeared and been reported by consumers or processors (*4,9*). One difficulty was related to incomplete fill, due to distortion and reduction in bottle capacity, especially if bottle washing temperatures exceeded 65.5 C. This problem has been virtually eliminated as a result of an improved bottle annealing process and employing wash temperatures below 65.5 C. The opacity of the container complicates detection of foreign objects in the containers by visual inspection. Presence of money, toys, huckleberries, insects, and mold growth on milk residues in the bottom of containers are typical of reported consumer complaints (*3*). Off-flavored milk frequently results from misuse of this container for home preparation and storage of fruit punch or orange juice. Wildbrett (*17*) stated that absorption of milk fat by reusable plastic containers may promote development of stale or "lack-of-freshness" type off-flavors or catalyze development of an oxidized flavor, especially after a portion of the contents are consumed and oxygen accumulates in the container headspace.

In 1971, Clark (*4*) reviewed problems associated with plastic multiuse milk container systems and evaluated the operation of the packaging system in accordance with prescribed regulations and safeguards; i.e., the "1965 Pasteurized Milk Ordinance and Code" (*12*). Clark indicated that the primary problems were related to off-taste and off-odor of product, slack fill, absorption of objectionable substances by the polyethylene container, and operational limits and malfunctions of the device used to detect contaminants. Lewis (*7*) estimated that approximately 1% of all multiuse plastic milk containers are rejected annually, due to the presence of volatile hydrocarbons.

Suppliers of the multiuse polyethylene milk container system have estimated that each bottle is reused 100 to 200 times (*10*) in a typical dairy wash, fill, and use cycle. Management personnel of several Oregon dairies have stated that, more realistically, each container is used for approximately 20 to 35 trips, before rejection or removal from the container supply.

The typical cycling for the bottle consists of a 65.5-C caustic wash (3.0%NaOH) for 5 min, followed by hot and cold rinses and a final chlorinated cold water rinse. The cleaned and sanitized bottle is then conveyed to the detector and examined for possible presence of volatile organic contaminants, then filled, capped, stored, marketed, and returned by the consumer in approximately 7-12 days.

As a result of the frequency of consumer complaints, the Oregon Department of Agriculture, Dairy, and Consumer Services Division encouraged the authors to conduct an evaluation of the adequacy of the contaminant detector and the tendency for multiuse polyethylene milk containers to absorb chemical contaminants. This publication reports the observations and results of the study.

MATERIALS AND METHODS

Container preparation

A supply of unused one-half gallon size polyethylene milk containers of the multiuse type were secured from an Oregon dairy processor. The containers, constructed of high density linear polyethylene, were subjected to a washing procedure in a commercial milk bottle washer. The milk containers were treated with 16 different commercially available beverage products and household chemicals that could conceivably serve as contaminants. The test chemical products were prepared at typical use concentrations as directed on the product label. The concentration levels for the pesticide products, toxic salts, and the detergent employed in the study are shown in Table 1. The substances were retained in the treated containers for a storage period of 7 days. The bottles were next subjected to a scanning by the contaminant detector, followed by washing through a conventional bottle washer. The sensitivity and reaction of the contaminant detector to the standard test substance, methane, preceded the operation of the unit and the bottle washer. Caustic soak strength was 4.2% as NaOH (pH = 13.0) at 68.5 C for 2 min, 50 sec. Following the final rinse and sanitizing step the treated bottles were conveyed to the contaminant detector for a second scanning to detect the presence of any residual

TABLE 1. *Concentration of certain contaminants used to treat multiuse polyethylene milk containers*[a]

Treatment number	Contaminant[b]	% Aqueous solution	Amount ½ gal. solution
1	DDT emulsifiable liquid (25%)	0.88	16.0 ml
2	DDT wettable powder (50%)	0.77	14.0 g
3	Malathion wettable powder (50%)	0.25	4.5 g
4	2, 4-D Dimethylamine salt (50%)	0.17	3.0 ml
5	Lead arsenate, standard (14.25%)	1.82	33.0 g
6	Copper sulfate, micronized tribasic (92%)	1.65	30.0 g
11	Cleaning compound, "Pine-sol"	1.56	28.4 ml
12	Fruit punch, raspberry flavor	10.14	184.0 g
13	Orange juice concentrate	18.74	340.0 ml

[a]One-half gallon size.
[b]All solutions retained in containers for 7 days, removed, drained, and rinsed with cold water.

volatile contaminants. The bottle punch was disengaged to retain the integrity of the containers, but the response of the detector to each tagged bottle was noted.

Analysis of container contents

Each treated container and the untreated control containers were manually filled with homogenized whole milk and stored for 14 days at 3.5 C. Analysis for pesticide and toxic salt residues in the milk were done after 7 and 14 days. The methods of sample clean up and analysis were those prescribed by AOAC (*2*) and the FDA (*5*) for toxic salts and pesticides, respectively. Sensory evaluation of samples was done by a panel of four experienced milk judges after 7 days of storage; except in the instance of the pesticides and toxic salts, observations were limited to the presence or absence of a characteristic off-odor.

RESULTS AND DISCUSSION

Detector response

Nearly all observations made in this study are summarized in Table 2. The contaminant detector did not respond to the polyethylene containers that had been treated with three of four different pesticides (DDT, malathion, and 2,4-D) and two forms of toxic salt solutions (lead arsenate and copper sulfate). Furthermore, the detector did not respond to washed containers treated with four commercial products commonly available in the home or garage: a pine-scented detergent, antifreeze, brake fluid, and motor oil. The detector responded to an emulsified form of DDT (hexane carrier), kerosene, gasoline, paint thinner, and an outboard motor/power saw fuel mixture. Before bottle washing, the detector signaled the presence of potential contaminants in seven of the 16 treated containers. After the bottles were subjected to washing, the detector mechanism reacted to only five treated containers. Presence of characteristic odors of the given compounds was detected by the panel in 11 of the 16 plastic bottles (Table 2).

The contaminant detector response to treated containers (Table 2) indicates that only those chemical compounds classified as volatile hydrocarbons were detected by the device. This is in complete accord with the generally recognized detection capability and sensitivity of hydrogen flame ionization detectors (*8*). Conversely, the detector device did not respond to inorganic compounds or to non-volatile hydrocarbon compounds.

Sensory evaluation of samples

Following 7 days of storage, the milk in the treated containers was evaluated organoleptically for presence or absence of off-flavors (or off-odors) characteristic of the treatment substance. Seven of the milk samples demonstrated definite taste and/or odor defects,

TABLE 2. *Summary of data for contaminant absorption by multiuse polyethylene milk containers, contaminant detection device response, and sensory observations of stored products*

Sample number	Container contaminant	Detector response: Before washing	Detector response: After washing	Characteristic odor of washed bottle	Milk flavor observations after 7 days	Residue in milk after: 7 days	Residue in milk after: 14 days
						(ppm on whole milk basis)	
Glass control		0[a]	0	None	Feed flavor		
Plastic control		0	0	Paraffin-like odor	Feed flavor		
1	DDT, emuls, liquid	+[b]	+	"Chemical" odor	"Chemical" odor	0.27	0.86
2	DDT, wettable powder	0	0	Sl. chemical odor	Feed flavor	0.28	0.29
3	Malathion, wettable powder	0	0	Typical odor of malathion	Feed flavor	0.08	0.08
4	2, 4-D (Dimethylamine salt)	0	0	V. sl. smell	Feed flavor	<0.01[c]	<0.01[c]
5	Lead arsenate	0	0	None	Not tasted	<0.02[c]	<0.02[c]
6	Copper sulfate	0	0	None	Not tasted	<0.1[c]	<0.1[c]
7	Kerosene	+	+	Kerosene odor	Kerosene odor		
8	Gasoline	+	+	Gasoline odor	Gasoline odor		
9	Paint thinner	+	+	"Solvent" odor	"Turpentine"-like		
10	Outboard motor fuel	+	+	Gas-like odor	Pron. gasoline		
11	"Pine-sol" cleaner	+	0	Def. piney odor	Pron. piney		
12	Fruit punch	0	0	Mod. fruity odor	Pron. fruity		
13	Orange juice	0	0	None	Feed, sl. off-flavor		
14	Antifreeze	0	0	None	Feed, sl. aftertaste		
15	Brake fluid	+	0	None	Feed, sl. aftertaste		
16	Motor oil	0	0	None	Feed flavor		

[a]0 = No detector response for a contaminant.
[b]+ = Positive detector response for a contaminant.
[c]Residue value below detection limits of the method.

indicating that leaching of the treatment substance from the plastic had occurred. Additionally, the milk from three containers exhibited slight off-flavor or aftertastes, compared to that from the control containers (untreated glass and plastic bottles). Quite obviously, several of the test substances (fruit punch and orange juice) represent more of a reduction in flavor quality than a public health question.

Analysis for chemical residues

Results of analyses for pesticide and toxic salt residues in the milk samples after 7 and 14 days are shown in Table 2. The emulsifiable liquid form of DDT was apparently moderately soluble in the polyethylene plastic, as indicated by the more than three-fold increase in the DDT residue in the milk stored an additional week in the container treated with this material (0.27 ppm and 0.86 ppm for 7 and 14 days, respectively). Milk from the container treated with the wettable powder form of DDT had residues of 0.28 ppm and 0.29 ppm after storage for 7 and 14 days, respectively. Malathion was detected at the level of 0.08 ppm for both time periods. The 2,4-D content was less than 0.01 ppm for both examinations. The lead arsenate and copper sulfate treatments showed negative values for all analyses.

CONCLUSIONS

A consideration of the basic operating principles of hydrogen flame ionization detectors indicates that it is not possible to detect the presence of numerous chemical compounds, especially those substances that are not volatile at room temperature or have low vapor pressures (*8*). Many pesticide products and other toxic materials fall into this category. Many commercial pesticide products exist as wettable powders and dusts, and constitute a significant portion of the pesticides used in home gardens. Conceivably, the convenient, shatterproof plastic milk container could be temporarily used for mixing wettable powder pesticides, particulary malathion, perthane and chlordane. In this investigation residues of DDT and malathion above the permissible tolerance levels were found in milk held in polyethylene containers in which solutions of these pesticides had been stored. Milk from five of 11 containers "accepted" by the contaminant detector exhibited excessive amounts of pesticide residue or objectionable off-flavor.

The Grade "A" PMO—1965 Recommendations of the USPHS, Item 7 (*12*) prescribed minimum safeguards and conditions for packaging systems utilizing multiple use plastic milk containers: "the container shall not impart into the product, pesticide residue levels or other chemical contaminants in excess of those considered acceptable by the FDA. If further data become available which would indicate that the use of plastic containers for fluid milk may constitute a public health hazard, such containers will no longer by considered as meeting the applicable provisions of the "Grade A" Pasteurized Milk Ordinance--1965 Recommendations of the USPHS." The FDA has established tolerance limits of 0.05 ppm for DDT, 0.02 ppm for malathion.

The demonstrated inability of the contaminant detector to detect objectionable amounts of several common undesirable chemical substances, raises the question as to the degree of consumer protection actually afforded by this device. In our opinion, the required contaminant detector should have the operational capability, sensitivity, and reliability to readily detect significant amounts of all or most chemical substances that are potentially injurious to human health. Additionally, it would be most desirable to detect those contaminants that tend to impart off-flavors (odor and/or taste) or otherwise affect the purity, wholesomeness, and aesthetic qualities of milk.

In the opinion of the authors, the properties of the material for a multiuse milk container should match or exceed those of the conventional flint glass bottle, as related to ease of cleaning, inert characteristics, porosity, and solubility or absorption of chemical substances. A recently introduced returnable milk container constructed from polycarbonate resin should perhaps be evaluated in the same manner as the polyethylene milk containers. Principal attributes cited for polycarbonate containers include high impact strength, good clarity, high gloss, transparency, high heat resistance, resistance to food stains, odor and taste transfer, and they also meet FDA requirements.

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Use of the Microslide Technique to Measure Staphylococcal Enterotoxin B

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ABSTRACT

A procedure to quantify staphylococcal enterotoxin B by use of the microslide technique was developed. This method consists of matching toxin activity against a series of standard curves obtained with microslides. The procedure was found to be useful in quantifying unheated as well as heated and reactivated staphylococcal enterotoxin B.

The microslide technique of Casman and Bennett (*1*) has been used extensively to detect staphylococcal enterotoxins in food and in bacterial cultures because of its specificity and sensitivity and relative simplicity of operation (*6*). This technique usually gives information on the presence or absence of certain enterotoxins in the samples without providing the quantity of enterotoxins involved. Using a dilution end-point procedure Soo *et al.* (Soo, H.M., S.R. Tatini, and R.W. Bennet, Abs. Ann. Meet. Amer. Soc. Microbiol. 1974:14) were able to quantitate enterotoxins with the microslide technique. The microslide method, however, requires considerable effort in determining the dilution extinction to quantify enterotoxins. In the course of studying heat inactivation and subsequent activities of staphylococcal enterotoxins we observed that the migration distance of the line of identity measured from the center wells of microslides can provide a more detailed quantification of enterotoxin activities. The description of this microslide quantification procedure as well as the usefulness of the procedure to evaluate staphylococcal enterotoxin B activities after heat inactivation and reactivation are presented in this report.

MATERIALS AND METHODS

Enterotoxin and antiserum

Staphylococcal enterotoxin B (SEB) and antiserum B were obtained from Makor Chemical Ltd. (P.O. Box 6570, Jerusalem, Israel). SEB was diluted in phosphate-buffered saline (PBS; 0.02 *M*, pH 7.4; Weirether et al. 7)+ 10% Brain Heart Infusion Broth (BHI; Difco Laboratories, Detroit, Michigan) to make concentrations of 5, 2.5, 1.25, 0.63, 0.31, and 0.15 μg/ml. Antiserum B was diluted in PBS to 1:5, 1:10, 1:30, and 1:50.

Heat inactivation and reactivation procedures

Pyrex ampules (7 cm × 10 mm) were filled with 0.4 ml of 5 μg/ml of SEB in BHI or PBS and heat sealed. The ampules were heated for 5 min at 80 or 100 C. Heat-up times were determined to be 40 and 30 sec respectively for 80 and 100 C. Immediately after heating, ampules were removed and placed in ice water for 30 sec. Samples used for inactivation studies were applied to the appropriate wells of the microslide system. Those samples used to study reactivation were kept in the original heat sealed ampules and placed at 4 and 25 C for 24 h. After this incubation, ampules were opened and samples were applied to the appropriate microslide wells.

Microslide procedure

The microslide method of Casman et al. (*2*) was followed. Basically, a meticulously cleansed and agar-precoated slide was layered with 1.2% agar bordered with two layers of electrical tape. A plastic template with four side wells located at equidistance (4.5 mm) from the center well, was placed on the agar. A suitable dilution of antiserum was introduced to the center well by use of an Eppendorf pipette (25 μl.) Toxin samples (unheated, heated, and reactivated) were introduced in the side wells in a similar manner. The microslide with reactants was allowed to remain at room temperature in a moist chamber for 3 days. After this incubation, the template was removed and the slide was enhanced for a few minutes in 1% acetic acid solution, and observed for line of identity. Measurement of migration distance from the center well was facilitated by use of an ocular micrometer (7×; Bausch and Lomb, Inc., Rochester, New York).

Standard curves

Standard reference toxin slides were prepared by adjusting various concentrations of standard reference SEB with specific antiserum titers to obtain a line of precipitation at ½ to ⅔ the distance from the wells.

When the slide's peripheral wells contained only reference SEB of the same concentration, with the appropriate antiserum dilution in the center well, it was termed the "standard reference slide." "Control slides" were established by placing various concentrations (0.15 to 5 μg/Ml) of enterotoxin (control toxins) in wells flanking the constant reference toxin while holding the appropriate antiserum concentration constant. The control toxins were therefore placed in the side wells and the reference toxins placed in the top and bottom wells. Typically three reference toxins (5, 2.5, 1.25μg/ml) were used for the construction of three standard curves.

Duplicate slides containing reference toxins 5, 2.5, or 1.25 μg/ml in the top and bottom wells were prepared for each toxin sample. Heated SEB and reactivated SEB samples were introduced into the side wells of these six slides. After incubation time (3 days) the lengths of the line of identity measured from the center wells of these slides were obtained. These lengths, after adjustments (to be discussed), were used to match the standard curves to estimate the toxin activities.

Dilution methods

Besides the above quantification method, two dilution methods were also tested. A series of ampules containing 0.4 ml of SEB at 5 μg/ml in BHI were heated at 100 C for 5 min. After heat treatment, the ampules were cooled in an ice bath for 30 sec. Reactivation procedures were carried out as before. Antiserum B titer in the center well was kept constant at 1:50 for all experiments. For dilution method No. 1, samples of identical toxin dilution (1:5, 1:10, 1:50, and 1:100) were applied to all four peripheral wells. Since the antiserium titer was 1:50, only concentrations of SEB between 0.15 − 0.5 μg/ml activities would

form a square. Therefore, the extent the SEB samples could be diluted and still react immunologically to form a square enabled semi-quantitative comparison between SEB samples.

For dilution method No. 2 the top and bottom wells of a series of microslides were filled with control toxin of 0.15 μg/ml while dilutions (1:5, 1:10, 1:20, and 1:50) of toxins (unheated, heated, and reactivated) were introduced to the side wells. When a diluted toxin sample formed a square with the control toxin, the concentration of the toxin sample was calculated by multiplying the dilution factor and the sensitivity factor (0.15 μg/ml). The sensitivity limit of the microslide is usually considred to be 0.05 μg/ml of SEB. We used 0.15 μg/ml as our sensitivity limit to ensure positive results in our controls because occasionally we could not detect 0.05 μg/ml. Using a higher sensitivity limit should not affect our study we were comparing the activities against a high (5 μg/ml) intial concentration of SEB.

RESULTS AND DISCUSSION

The basic assumption of this quantification procedure was that the migration length of the precipitation line measured from the center well was proportional to the concentration of the toxin activities of the side wells while holding the antiserum concentration constant. Thus, as the concentration of the enterotoxin decreased, the line shifted toward the toxin wells and the reverse was true when the toxin concentration increased. To construct a series of standard curves the appropriate antiserum titer must be obtained. They were found to be 1:10, 1:15, 1:30, 1:30, and 1:50 for toxin concentrations of 4, 2.5,1.25, 0.63, 0.31, and 0.15μg/ml, respectively.

On any standard reference microslide (i.e. all four side wells contained the same concentration SEB), the precipitation line formed a square. The average precipitation length was designated the "standard reference length." The lengths of the precipitation lines in control microslides were measured in the same manner. The average reference length (top and bottom wells) and the control toxin band length (side wells) were determined for each slide. Duplicate slides were then averaged resulting in one number for the reference length , and one number for the control length. On the control slide, the length of the reference line of precipitation may differ from its measurement on the standard reference slide. The control toxin length was adjusted proportionally by the following equation:

$$\frac{\text{standard reference length}}{\text{reference length}} \times \text{control toxin length} = \text{adjusted control toxin length}$$

Figure 1 illustrates the basic principle of this quantification procedure. Slide 1 was the standard reference microslide containing 2.5μg/ml of SEB in this example. Slides 2 and 3 were the control slides containing 1.25 and 5 μg/ml in the side wells, respectively. The band length of the control toxin was measured and adjusted by the above formula. These band lengths were then plotted against SER on semi-log paper. For each reference toxin a standard curve was constructed. Figure 2 shows a series of standard curves for SEB suspended in BHI or PBS. Standard curves for

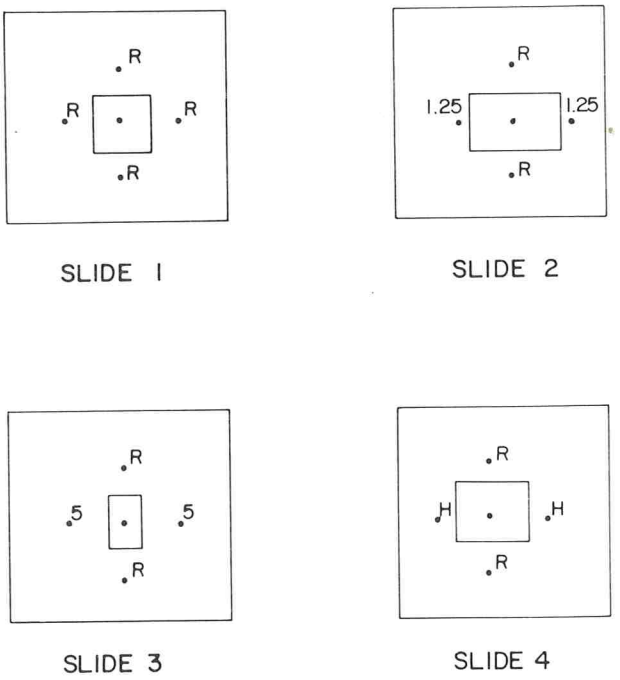

Figure 1. *Illustration of precipitation lines formed in the gel of microslides. Slide 1: Standard reference slide = 2.5 μg/ml (R). Slide 2 and 3 have 1.25 μg/ml and 5 μg/ml, respectively, flanking R. Slide 4: Heat treated (H) flanking R. In this illustration, H is slightly less than 2.5 μg/ml SEB.*

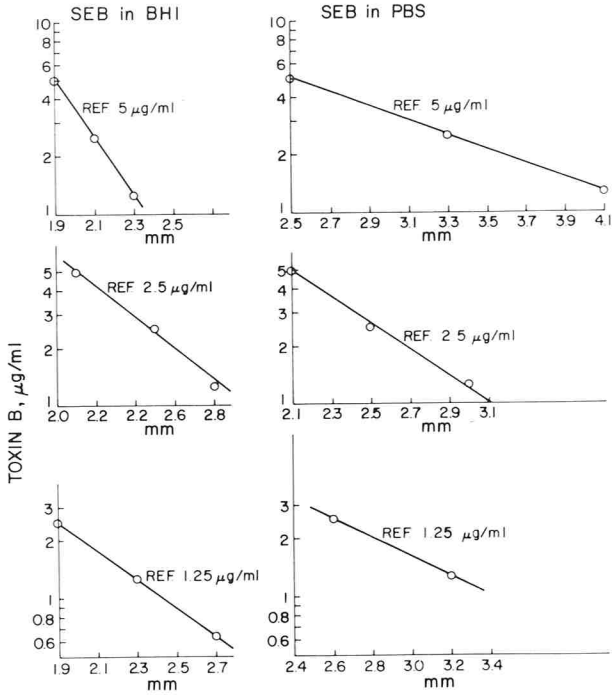

Figure 2. *Typical sets of standard curves for quantification of SEB. Left hand curves are SEB suspended in BHI and right hand curves are SEB suspended in PBS. Reference toxin concentration is labeled on the curve.*

lower reference toxins (0.6 and 0.3 μg/ml) were also obtained but not shown here.

To make use of the standard curves, samples of unknown SEB activities were placed in the side wells of a

series of experiment slides containing reference toxins (fig. 1 Slide 4). After incubation, the toxin concentration of the sample could be estimated for the square or rectangle formed. The formation of a square indicated that the toxin concentration of the sample was identical to the reference toxin concentration of the microslide. When a rectangle was formed, the band lengths were adjusted with the reference length and matched with the appropriate standard curves. The data were then averaged and the concentration of SEB in the sample was established.

Using this method, the activities of SEB heated at 80 and 100 C for 5 min and subsequently stored for 24 h at 4 and 25 C were quantitated. Table 1 shows that after heating the toxin at 80 C, SEB was inactivated more compared to heating at 100 C. This is the phenomenon of aggregate formation observed by Fung et al. (*3*) as well as by Jamlang et al. (*5*) and others. After reactivation time both heat treated toxins regained a certain amount of activity. Slightly better reactivation was observed under 25 C reactivation conditions compared to 4 C reactivation. These data are in general agreement with data collected under similar conditions by Fung et al. (*3*) who used a high initial SEB concentration (100μg/ml) and measured toxin activities with the single gel diffusion method of Fung and Wagner (*4*). A more detailed report of heat inactivation and reactivation profiles measured by this quantification method was presented elsewhere (Reichert, C.A., and D.Y.C. Fung, 1976. J. Milk Technol. 39: in press).

TABLE 1. *Heat inactivation and reactivation of 5μg/ml of SEB in BHI as measured by the standard curve procedure*[a]

	Heat-treatment 5 min		Reactivation after 24 h			
			at 4 C		at 25 C	
SEB sample (5 μg/ml)	80 C	100 C	80 C	100 C	80 C	100 C
Toxin measured (μg/ml)	0.31	1.35	0.38	1.8	0.55	2.2
% of original toxin	6.2	27.0	7.6	36.0	11.0	44.0

[a]Average of triplicate determination

Data concerning heat inactivation and reactivation and SEB at 100 C for 5 min as measured by the two dilution methods were presented in Table 2. Dilution method No. 1 showed loss of activity of SEB after heat treatment since square formation only occured at the 1:5 dilution compared to the 1:10 dilution of the original sample. The exact amount of toxin activity which remained could not be ascertained. After reactivation time, the migration bands of samples were closer to the center well than the heated toxin indicating the activities of these reactivated toxins were greater than immediately after heating but the exact amount also could not be calculated.

Dilution method No. 2 showed that after 5 min of heating at 100 C only 21% of the original activity remained. After reactivation time at 4 C no reactivation appeared to have occurred. However, it could be surmised from the migration length that a small degree of reactivation did occur. Data for 25-C reactivation samples showed reactivation back to 30% of the original toxin activity. Thus, both dilution methods confirmed the heat inactivation patterns of SEB and the reactivation phenomenon.

The advantage of this method is the ability to quantitate small amounts of SEB activity. The disadvantage of this method is the need for a large number of microslides to make standard curves in each set of experiments. Although the procedure was developed for SEB, subsequent work in this laboratory on quantification of small amounts of staphylococcal enterotoxin A showed similar applicability. The usefulness of this procedure to quantitate other antigen-antibody activities clearly exists.

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TABLE 2. *Heat inactivation and reactivation of 5 μg SEB/ml BHI as measured by dilution method No. 1 and dilution method No. 2*[a,b]

	Dilution method #1		Dilution method #2			
SEB sample (5 μg/ml)	Square formation (dilutions)	Band migration (mm)	Square formation (dilutions)	Band migration (mm)	Calculated SEB concentrations[c] (ug/ml)	% Activity
Unheated control	1:10	2.8	1:50	3.1	7.5	100%
			1:50	3.5	7.5	
Heated 5 min at 100 C	1:5	3.0	1:10	3.5	1.5	21%
			1:10	3.5	1.5	
4 C reactivation	1:5	2.6	1:10	3.3	1.5	21%
			1:10	3.5	1.5	
25 C reactivation	1:5	2.8	1:10	2.5	1.5	30%
			1:20	3.4	3.0	

[a]Antiserum titer in center well was 1:50 for all slides

[b]Average of duplicate determinations.

[c]Since the sensitivity limit of this particular series of toxin was 0.15 μg/ml, this concentration was designated as the sensitivity factor. 0.15 μg/ml of SEB was placed in opposite wells and used to estimate square formation of samples (heated or unheated) in the other two wells.

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A Research Note

Inhibitory Substances in the Milk Supply of Southern Ontario

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ABSTRACT

Pasteurized fluid dairy products (998 samples) and raw milk samples from approximately 1200 producers in Southern Ontario (5574 samples) were examined by the disc assay method, having a sensitivity of 0.0125 unit of penicillin per milliliter of milk, for the presence of inhibitory substances during the period April-August, 1975. One pasteurized milk (0.1%) and 50 raw milk samples (0.9%) contained penicillin. No other antibiotics were detected. Results represent a significant decrease in the incidence of antibiotics in milk compared to reports during the 1950's.

Numerous reports appeared in the literature during the 1950's on the presence of inhibitory substances, particularly penicillin, in milk supplies (*3, 6, 9, 10, 12, 15, 18, 20, 21*). Marth and Ellickson (*14*) summarized the results of several surveys on antibiotics in market milk reported before their review in 1959. Cuthbert (*4*) added further information on the incidence of antibiotics in milk for a number of different countries in a review published in 1968. Reductions in the incidence of antibiotic residues were observed over this period with the implementation of enforcement and educational programs (*6, 9*).

The incidence of inhibitory substances in milk reported for any particular survey depends, of course, upon the method used to detect these substances and its sensitivity. Johns (*10*) found, for example, that 7.3% of 344 spring and 5.4% of 298 summer herd milk samples were inhibitory to lactic starter organisms but only 1.4% of the total showed zones of inhibition by the disc assay method, which is the most common procedure employed for detecting inhibitory substances. The accepted sensitivity for this method, using *Bacillus subtilis* (ATCC 6633) as the test organism, is 0.05 unit of penicillin per ml of milk (*1*), which is frequently indicated as the minimum detectible level (*2, 18, 19*). Greater sensitivities, however, have been reported for the disc assay method. Johns (*11*) reported a sensitivity of 0.025 unit per ml. Parks and Doan (*16*) could detect sodium penicillin G at 0.0129 unit per ml but only if the seeded agar was 24-72 h old. The sensitivity of the disc assay method is increased by the use of *Sarcina lutea* (ATCC 9341) as the test organism. Naylor (*15*) reported a sensitivity of 0.005 unit of penicillin per ml of milk with *S. lutea*, and Feagan (*6*) indicated a sensitivity of 0.003-0.004 unit. Read et al. (*17*) found that *B. subtilis* was less sensitive to sulfa drugs and bacitracin than either *S. lutea* or *Bacillus megaterium* (ATCC 9855).

A lower incidence of antibiotic residues would be expected in pasteurized over raw milk if the antibiotic was heat sensitive. This is not true for penicillin at usual time-temperature requirements for pasteurization. Hunter (*8*) found no loss in the potency of penicillin after pasteurization at 145 F for 30 min within the accuracy of the disc assay method, and several workers have made the same observation with respect to the inhibitory activity of penicillin on starter cultures (*5, 7, 13*).

The survey reported here utilized the disc assay method for detecting inhibitory substances in pasteurized fluid dairy products and raw milk samples delivered to our laboratory for routine examinations during the period April-August, 1975.

MATERIALS AND METHODS

Pasteurized fluid dairy products were selected from samples collected at retail outlets and dairy plants and delivered to the laboratory for routine bacteriological examination during the period April 9 to August 13, 1975. Raw milk samples originated from approximately 1200 producers located in Southern Ontario and were delivered to the laboratory for routine examinations. Samples from each producer were tested at least four times during the survey period.

Samples were examined for inhibitory substances by the disc assay method utilizing *B. subtilis* (ATCC 6633) as specified by *Standard Methods* (*1*) for the overnight test, except that whey agar was used as the medium and ¼-inch diameter discs were utilized for sample application. Spore suspensions were prepared in our laboratory by a procedure which included heat-shocking at 70 C for 30 min. The concentration of spores in the medium was adjusted to provide a maximum sensitivity for detection of penicillin, which was usually in the range of $4\text{-}5 \times 10^5$ spores per ml of medium. Seven ml of whey agar containing spores were distributed per petri dish (plastic, square, phage typing, 100 × 15 mm). Prepared plates were stored under refrigeration until use but discarded after 11 days.

Samples showing any detectible zone of inhibition by the overnight

test were confirmed by a rapid test utilizing ½-inch diameter discs on Antibiotic Medium No. 1 (Difco) with the same spore concentration used in the overnight test and incubation at 37 C for 4 h (*1*). A positive result was reported only if the confirmed test gave a clear zone of inhibition around the entire disc.

The sensitivity of the test method for penicillin was confirmed four times during the survey period on fresh and stored plates. Concentrations of 0.00625 and 0.003125 unit per ml could sometimes be detected, but the minimum detectible amount which could be determined with 100% reproducibility was 0.0125 unit per ml.

The sensitivity of the test method for antibiotics other than penicillin which might occur in milk was also determined. Bacitracin at 50 units per ml and colistin at 10 μg per ml could not be detected. Minimum detectible amounts (μg/ml) for other antibiotics were: chloramphenicol, 8.35; neomycin, 3.125; streptomycin, 0.01625; and tetracycline, 0.156.

RESULTS AND DISCUSSION

The numbers of pasteurized fluid dairy products for each of the dairies represented in the survey are shown in Table 1. The total number of products tested was 998 with only one positive for penicillin, an incidence of 0.1%. During the same survey period, 5,574 producer samples of raw milk were tested with 50 positive for penicillin, an incidence of 0.9%. A difference between the incidence of penicillin in pasteurized versus producer raw milk samples may largely be the result of dilution to levels below the sensitivity of the test procedure. No samples showed the presence of any inhibitors other than penicillin.

These low incidences in comparison to reports in the 1950's may reflect actual improvements in producer practices and the effect of educational and enforcement programs. On the other hand, they may also be the consequence of decreased use of penicillin for treatment of mastitis and greater reliance on other antibiotics which are not detectible with the same sensitivity by the disc assay method when *B. subtilis* is used as the test organism.

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TABLE 1. *Pasteurized fluid dairy products included in survey for inhibitory substances*

	Number of samples of dairy products tested for inhibitory substances								
Dairy	Homogenized milk	Chocolate milk	Milk (2% BF)	Skim milk	Cream (10-18%BF)	Whipping cream	Ice cream mix	Milk shake	Totals
A	16	10	12	8	9	4	0	0	59
B	34	24	56	31	53	17	2	1	218
C	37	10	31	14	71	11	0	2	176
D	0	0	0	0	21	0	0	0	21
E	8[a]	1	13	13	9	0	0	0	44
F	1	3	7	4	2	0	0	0	17
G	19	15	12	12	14	7	0	0	79
H	10	9	11	6	19	9	0	0	64
I	16	8	0	8	16	0	0	1	49
J	20	11	12	9	23	9	0	2	86
K	20	7	15	8	24	7	0	0	81
L	20	5	6	6	17	0	0	0	54
M	3	3	1	1	4	0	0	0	12
N	4	0	2	3	6	0	0	0	15
O	5	3	3	5	7	0	0	0	23
Totals	213	109	181	128	295	64	2	6	998

[a]Includes one sample positive for penicillin.

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Sporicidal Properties of Chlorine Compounds: Applicability to Cooling Water for Canned Foods [1,2]

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ABSTRACT

Sporicidal effects of chlorine compounds as measured by many authors are reviewed. Since spore destruction rates and hypochlorous acid concentration appear to be related, the data from the several reports were recalculated in terms of time required for a 90% reduction in spores as a function of hypochlorous acid concentration. From these data a single graph was prepared. Results of the analysis indicate that *Bacillus* spores are more resistant to chlorine than *Clostridium* spores. The sporicidal effect of chlorine solutions increases with (*a*) an increase in free available chlorine, (*b*) a decrease in pH, and (*c*) an increase in temperature. Numbers of *Clostridium botulinum* and other spore-forming organisms in canning plant cooling water will depend on water quality factors such as the quantity of soil and organic matter, pH, temperature, and chlorine level. Control of these variables to desired levels in cooling water will reduce the probability of post-process infection of low-acid canned foods.

Microbial spoilage of canned foods can occur as a result of either underprocessing or the leakage of viable organisms into the container after heat processing. Post-processing infection is commonly called leaker spoilage and represents an economic loss for the processor and a potential public health hazard.

Cooling water is the primary source of microorganisms causing leaker spoilage. There is a direct correlation between high populations of bacteria in cooling waters and the probability of leaker spoilage (*12*). One way of decreasing this probability is to reduce the microbial load in the cooling water. The most widely used method to control the microbial population in water is the application of chlorine compounds. Bacterial spores are more resistant to chlorine than vegetative cells and, therefore, the number of viable spores in cooling water could be used as an indication of the effectiveness of the chlorination programs (*10*).

Put et al. (*12*) carried out studies in eight canning plants. They found chlorine levels from 0.1 to 2 ppm in the cooling water. *Bacillus* spores were found in the cooling water of seven plants and *Clostridium* spores in four of the eight plants. They found larger relative numbers of *Bacillus* spores than *Clostridium* spores. This condition was especially evident when surface water supplies were used for cooling.

In this report we have identified and reviewed the results of research on the sporicidal properties of chlorine compounds. The application of this review is to cooling waters for low acid canned foods; specifically, to insure that there is minimal public health hazard from post-process leakage of organisms into containers.

BACILLUS SPORES

Most of the work on the sporicidal effects of chlorine compounds has been done using aerobic spore-forming organisms. Two of the early studies on spore destruction by chlorine compounds dealt with a spore-forming organism isolated by Charlton and Levine (*3*) and named *Bacillus metiens.* A composite of the results of studies by Charlton and Levine (*3*) and Rudolph and Levine (*13*) are shown in Table 1. These data for calcium hypochlorite indicate the important role of pH on the sporicidal effect of the chlorine. At a pH of 7.3, a 99% reduction in viable spores was obtained in less than 0.33 min with 1000 ppm of chlorine, while at a pH of 11.3 and 1000 ppm of chlorine 70 min was required for a 99% reduction.

Charlton and Levine (*3*) did studies comparing calcium hypochlorite with Chloramine T; their results are also shown in Table 1. The concentration of Chloramine T in Table 1 are those in the solution as initially prepared. They did not determine the amount of free available chlorine (FAC) released from Chloramine T. The FAC concentration was probably low since chloramines release chlorine very slowly (*11*). As is evident from data in Table 1, chloramine T was not effective in killing spores. Even at high pH values (10 to 11.3), calcium hypochlorite was a more effective sporicide than was Cloramine T at low pH values (6.0 to 8.8). Cousins and Allan (*4*) exposed *Bacillus subtilis* spores to 1000 ppm of Chloramine T at pH 6.5 and reported no sporicidal effect in 4 h, while 100 ppm of FAC from sodium hypochlorite at pH 8.0 reduced the spore population by 99% in 60 min. These data indicate that the FAC released from sodium or calcium hypochlorite is more effective in killing spores than the combined available chlorine of Chloramine T. Chlorine in the combined form should not

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[2]*Scientific Journal Series Paper No. 9368, Minnesota Agricultural Experiment Station, St. Paul, Minnesota 55108.*

TABLE 1. *Effect of concentration and pH of chlorine compounds on the destruction of* Bacillus metiens *spores*[a]

Compound	pH	Conc. of chlorine[b] (ppm)		Time to kill 99%[c] (min)
		FAC	FAC and CAC	
Calcium hypochlorite (*13*)	6.0	25		2.5
	7.0	25		3.6
	8.0	25		5.0
	9.0	25		19.5
	10.0	25		80.6
	10.0	100		42.4
	10.0	500		20.6
Calcium hypochlorite (*3*)	7.3	1000		<0.33
	10.4	100		70.0
	11.3	1000		70.0
Chloramine T (*3*)	6.0	—[d]	1000	900.0
	6.0	—	2000	324.0
	6.0	—	4000	156.0
	8.7	—	2000	3840.0
	8.8	—	4000	1404.0

[a]Data adapted from Charlton and Levine (*3*) and Rudolph and Levine (*13*).
[b]FAC = Free available chlorine; CAC = combined available chlorine; FAC plus CAC is the total available chlorine.
[c]Temperature was at 20 C for calcium hypochlorite test and at 25 C for Chloramine T test.
[d]Indicates amount FAC released was not determined.

be considred for chlorination of cooling water as it is an ineffective sporicide.

Several other papers have been published on the chlorine resistance of various species of *Bacillus* spores. Data from these reports are presented in Table 2. To make the results from the several studies comparable, the time for a 90% reduction in numbers of spores and the concentration of hypochlorous acid were calculated from the available data in each report.

The data in Table 2 indicate that for all spores tested the sporicidal activity of the solution increased with increasing amounts of hypochlorous acid (HOCl). The amount of HOCl in a chlorine solution is a function of the solution pH (*11, 15*). At pH values approaching 7 almost all the FAC is present as HOCl, however at pH values near 10 all the FAC is present as hypochlorite ion (OCl^-). Brazis et al. (*1*) reported that HOCl is 100 times more effective in killing spores than OCl^-.

A search was made for an empirical relationship to visually show the effect of HOCl on spore destruction. As a result Fig. 1 was developed, in which the logarithm of the time for a 90% reduction in the number of spores is shown as a function of the logarithm of the HOCl

TABLE 2. *Summary of data for destruction of* Bacillus *spores by chlorine*[a]

Organism	Chlorine compound	Test temp. (C)	pH	FAC[b] (ppm)	Calculated HOCl (ppm)	Time for 90% reduction (min)
B. cereus (*4*)	NaOCl	21	6.5	50	43	1.5
			8.0	100	25	2.5
B. cereus (*15*)	NaOCl	25	7.0	100	75	0.88
			5.2	150	149	0.18
			7.0	150	113	0.40
			8.0	150	38	1.2
B. coagulans (*9*)	NaOCl	20	4.5	20	20	2.5
			6.8	20	17	6.0
			4.5	10	10	8.5
			6.8	10	8.5	12.0
			7.8	10	3.5	23.0
			4.5	5	5.0	21.0
			6.8	5	4.3	28.0
			7.8	5	1.7	59.0
B. macerans (*11*)	NaOCl	21	6.0	15	14	4.3
			6.5	15	12.9	4.6
			7.0	15	11.3	6.4
			7.5	15	7.5	10.3
			8.0	15	4.0	21.0
B. metiens (*13*)	CaOCl	20	6	25	24	1.25
			7	25	19	1.8
			8	25	6	2.5
			10	25	<1	40.3
			10	100	<1	21.2
			10	500	<1	10.3
B. glodbigii (*1*) (*subtilis*)	—	22	6.2	1.8-1.9	1.7	22.8
			6.2	.11-.24	.1-.2	255.0
			7.2	2.5-2.6	1.6	20.5
			7.2	.15-.34	.1-.2	270.0
			8.6	23.8-24.8	1.7	22.5
			8.6	1.8-2.3	.13-.16	285.0
			10.5	454	<1	31.7
			10.5	21.9-23.1	<1	330.0
B. stearothermophilus (*2*)	NaOCl	25	7	1000	750	1.0
			7	2000	1500	.78

[a]References in parentheses after species name
[b]Free available chlorine

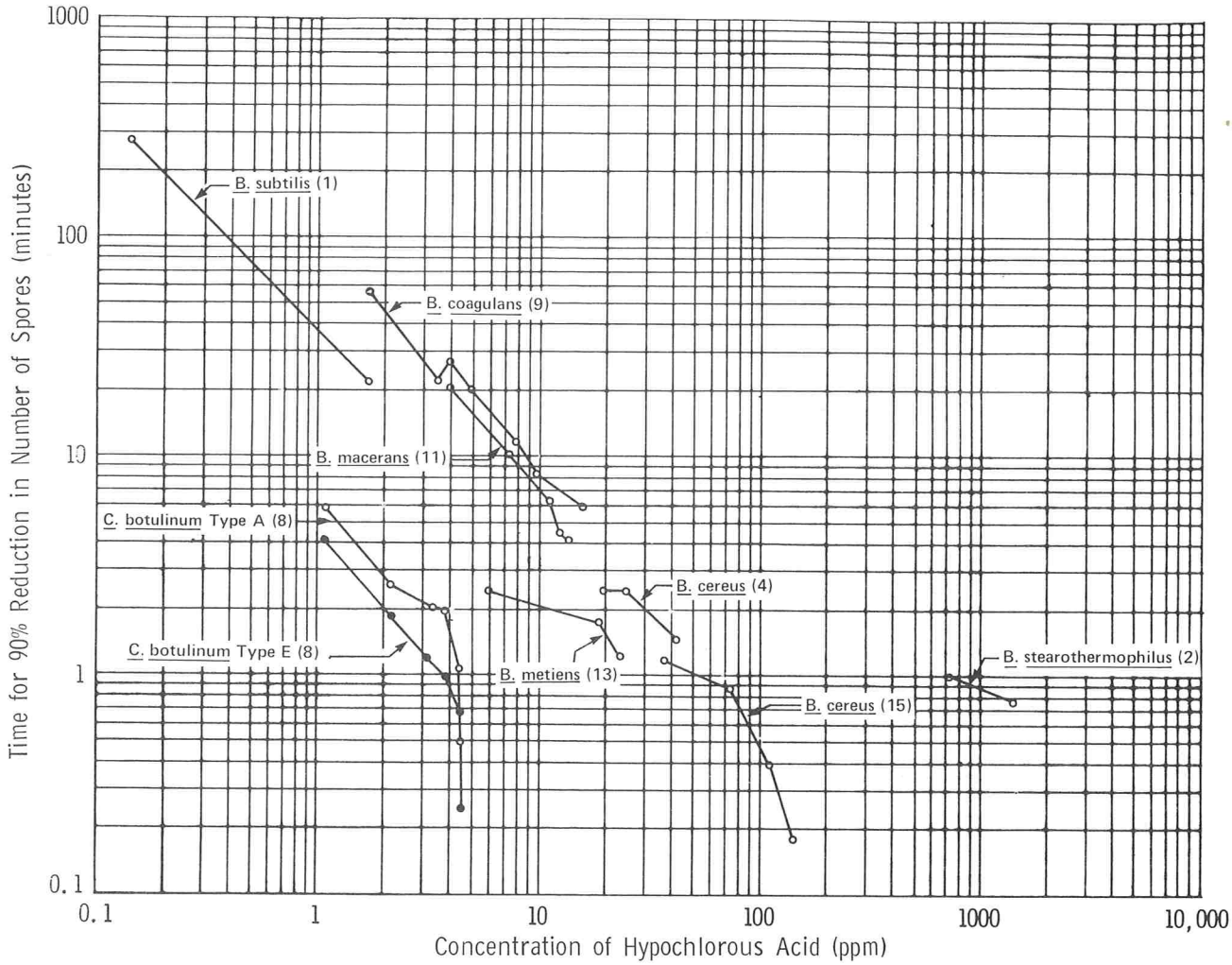

Figure 1. *Summary of data on the sporicidal effect of chlorine solutions expressed as the time to reduce the spore population by 90% as a function of the concentration of hypochlorous acid in parts per million. Numbers in parentheses refer to references.*

concentration. The data in Table 2 are all displayed graphically in Fig. 1.

CLOSTRIDIUM SPORES

Only a few reports have been published on the resistance of *Clostridium* spores to chlorine. Tonney et al. (*14*) found all bacterial spores to be 10 to 1100 times more resistant to chlorine than vegetative cells, and as a group the aerobic spore-formers to be more resistant than the anaerobic spore-formers.

Dye and Mead (*6*) evaluated the effect of chlorine on eight strains of *Clostridium* spores. Their results for spores exposed to 5 ppm of free available chlorine (pH 8.3) at 10 C are shown in Table 3. *Clostridium welchii* (*perfringens*) spores were the most resistant; *Clostridium bifermentans* and *Clostridium caloritolerans* spores were the least resistant. Chloramine T at 200 ppm (pH 9) was not very effective in reducing the number of any of the *Clostridium* spores tested. There was less than a 2-log reduction of any of the eight strains in a 2-h test period. Also *B. subtilis* spores were subjected to 100 ppm chlorine at a pH of 9.8 and found to be considerably more resistant than any of the *Clostridium* spores tested. Results of Dye and Mead confirm the work of Tonney et al. (*14*) that *Clostridium* spores have less resistance to chlorine than *Bacillus* spores (Fig. 1).

TABLE 3. *Effect of 5 ppm free available chlorine (pH 8.3) on destruction of* Clostridium *spores at 10 C*

Strain	Time to kill 99% (min)[a]
C. welchii 6719	>60
C. tertium	12
C. histolyticum 10	60
C. histolyticum 503	10
C. bifermentans	9
C. sporogenes	17
C. caloritolerans	6

[a]Determined from data of Dye and Mead (*6*).

Clostridium botulinum organisms in canning plant cooling waters are a public health hazard. The post-processing entry of a single *C. botulinum* organism into a food container is a deadly problem (*7*). The work of Tonney et al. (*14*) was one of the earliest published reports on the resistance of *C. botulinum* spores to chlorine compounds. They reported the concentration of

free chlorine required to kill the spores in 15-30 sec. Their results for *C. botulinum* Type A, Type B, and a third, unidentified type were 15, 17.5, and 25 ppm of chlorine, respectively. The initial number of spores was not clearly reported in this paper. The temperature at which the test was done and the pH of the chlorine solution were also lacking, so any meaningful comparisons with other studies cannot be made.

Dozier (5) subjected *C. botulinum* spores to sodium hypochlorite solutions at concentrations of 4,500 and 5,000 ppm for 1 h and found it to be ineffective. The tests were done at 20 and 37 C, but no information was given regarding the pH or the amount of free residual chlorine.

Recently, Ito et al. (*8*) conducted a thorough study on the effectiveness of commercial germicides on spores of *C. botulinum* Types A, B, and E. Results of their tests at 25 C are shown in Table 4. These results indicate that calcium hypochlorite, sodium hypochlorite, and gas chlorinated water are approximately of equal

TABLE 4. *Effect of concentration of chlorine compounds on destruction of* Clostridium botulinum *spores (Types A, B, and E) at 25 C*[a]

Compound[b]	Conc. of compound (ppm)	Type	Time for 99.99% kill (min)
Calcium hypochlorite	4.5	A	8.0
Calcium hypochlorite	4.5	B	7.4
Calcium hypochlorite	4.5	E	4.0
Sodium hypochlorite	4.5	B	8.0
Sodium hypochlorite	4.5	E	3.8
Gas chlorinated water	4.5	A	8.2
Gas chlorinated water	4.5	B	8.0
Gas chlorinated water	4.5	E	4.6
Dichloro(s) triazinetrione	10.0	A	15.0
Dichloro(s) triazinetrione	20.0	B	>15.0
Dichloro(s) triazinetrione	10.0	E	7.2
Trichloro cyanuric acid	4.5	A	17.0
Trichloro cyanuric acid	5.0	E	9.0

[a]Data from Ito et al. (*8*).
[b]1×10^4 spores/ml, pH 6.5 phosphate buffer.

effectiveness in causing a 4-log (99.99%) reduction in numbers of viable spores. It can be seen that the resistance of Type A and Type B spores are similar and that Type E spores have approximately one-half the resistance of Type A and Type B spores. This difference in resistance by spore type is also true for the two chlorine-detergent compounds tested, but these compounds were not as effective in reducing the number of viable spores as calcium hypochlorite, sodium hypochlorite, and gas chlorinated water. Ito et al. also evaluated the effect of pH on the germicidal efficiency of calcium hypochlorite. The results are presented in Table 5 and indicate that as pH increases, the rate of destruction decreases. The results are not surprising since chlorine is more effective as a sporicidal agent at acid pH values where hypochlorous acid predominates (*11*).

The effect of temperature of the calcium hypochlorite solution on time for a 99.99% kill of *C. botulinum* Types A, B, and E spores was also investigated by Ito and

TABLE 5. *Resistance of* Clostridium botulinum *spores types A, B, and E to calcium hypochlorite solutions at various pH values at 25 C*[a]

pH[b]	Time for 99.99% kill (min)		
	Type A	Type B	Type E
3.5	2.1	3.7	1.0
5.0	4.3	6.2	2.8
6.5	7.8	7.8	4.0
7.0	8.5	8.7	5.0
7.5	10.6	11.2	7.6
8.0	24.0	26.6	17.0

[a]Data from Ito et al. (*8*).
[b]4.5 ppm free available chlorine, 1×10^4 spores/ml.

co-workers. Their results are presented in Table 6. The time for 99.99% kill decreased with increasing temperature; the kill-time at 25 C was only 0.3 to 0.4 of the kill-time at 15 C.

TABLE 6. *Resistance of* Clostridium botulinum *spores types A, B, and E to calcium hypochlorite solutions at various temperatures*[a]

Temperature[b] (C)	Time for 99.99% kill (min)		
	Type A	Type B	Type E
5	35.0	40.0	24.0
15	15.0	20.0	10.0
25	6.0	6.0	4.0

[a]Data from Ito et al. (*8*).
[b]4.5 ppm free available chlorine, 1×10^4 spores/ml, pH 6.5 phosphate buffer.

Ito et al. (*8*) also reported that organic debris, such as peptone, will combine with the free available chlorine, reducing the amount of free chlorine in the solution, ultimately to the point where it is ineffective. Therefore, in a commercial application such as a cooling canal, chlorine must be added continuously so that the design free available chlorine level is maintained continuously.

Data from Ito et al. for *C. botulinum* Type A and Type E are also shown in Fig. 1. It can be observed in Fig. 1 that 2 ppm of HOCl will reduce a population of *C. botulinum* spores by 90% in 2 to 3 min, whereas 20 to 50 min are required for a 90% reduction in the number of *Bacillus* spores.

DISCUSSION

An obective of this project was to not only review research regarding sporicidal effect of chlorine but to develop data to predict spore destruction as a function of chlorine level. With the exception of the data of Cerf et al. (*2*) the data in Fig. 1 appear to form an overall pattern. If we disregard the data for *B. stearothermophilus* (*2*), then for both *Bacillus* and *Clostridium* spores, at the low effective HOCl concentration, the logarithm of the time for 90% reduction is a linear function of the logarithm of the HOCl concentration. As the HOCl concentration increases to where the time for a 90% reduction is 1 to 2 min, the rate of destruction increases. Figure 2 is a modification of the graph in Fig. 1 to include lines which we believe represent general conditions for destruction of *Bacillus* and *Clostridium* spores as a function of HOCl concentration.

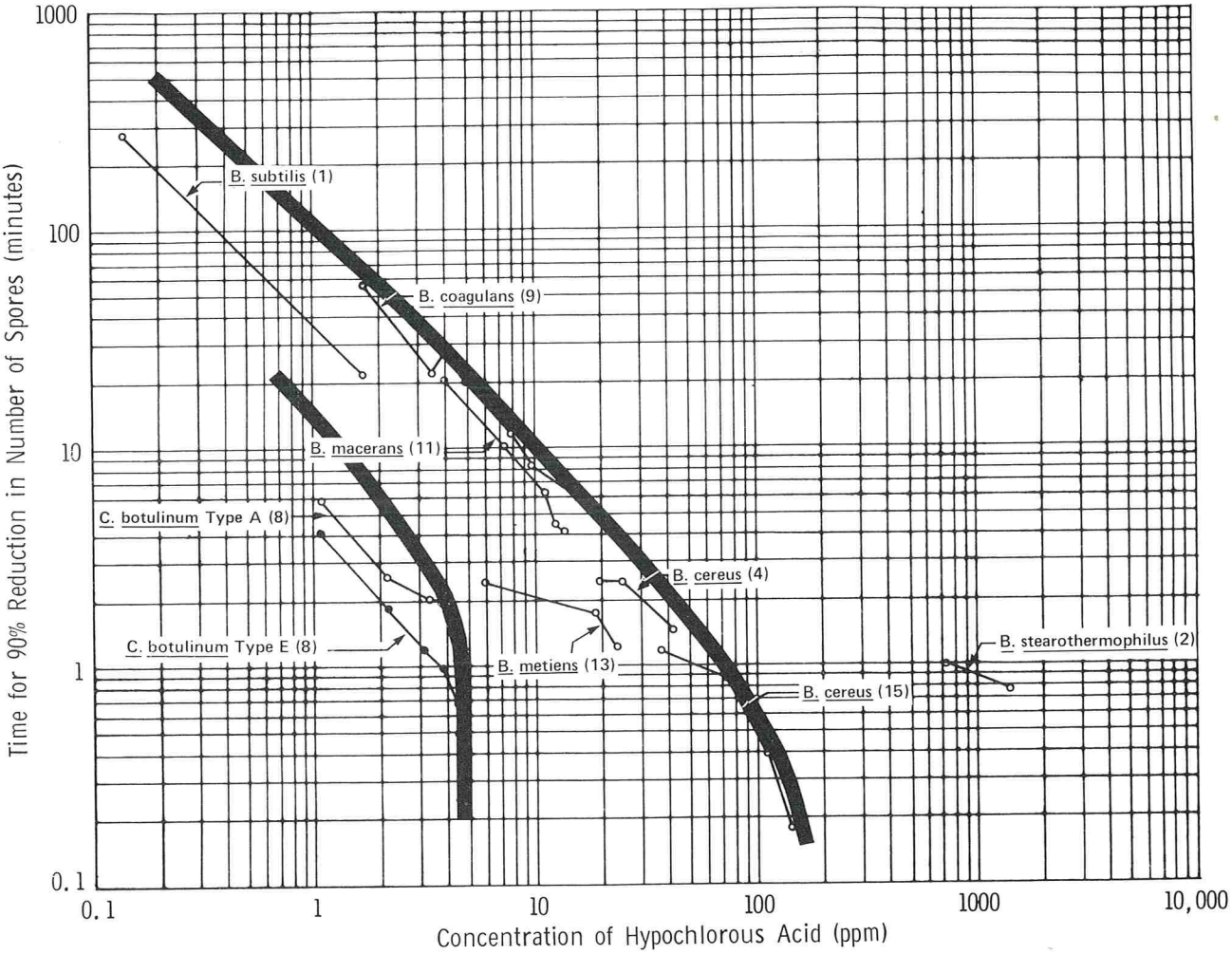

Figure 2. *Suggested relationship between the time to reduce the bacterial spore population by 90% and the concentration of hypochlorous acid (ppm) for* Bacillus *and* Clostridium *spores.*

When chlorine compounds are added to water, so there is free available chlorine present, the solution is both bactericidal and sporicidal. Vegetative bacterial cells are more easily killed than spores; and *Clostridium* spores are more easily killed than *Bacillus* spores. The lethal effect of the chlorine in solution increases with: (*a*) an increase in the free chlorine concentration in the solution, (*b*) a decrease in pH, and (*c*) an increase in temperature.

The relative microbiological quality of the water in the canning plant cooling system will be a function of the quality of the water that is added to the system. Quality factors are: the amount and source of soil and organic matter that are added to the water, pH, temperature, and chlorine level. From the information in Fig. 2 and the literature cited therein it is anticipated that the predominant flora will be resistant *Bacillus* spores when free chlorine levels of 2 to 5 ppm with a pH in the range of 7 to 7.5 are maintained in the cooling water.

The public health hazard from the post-process leakage of *C. botulinum* spores into cans of low acid food should be extremely small if the cooling water is properly chlorinated, the pH level is controlled, and the addition of soil or any other outside source of *C. botulinum* spores is eliminated. Since *C. botulinum* will not likely multiply in cooling water or in a cooling canal containing chlorinated water, only the introduction of large numbers of *C. botulinum* spores into improperly chlorinated cooling water will create a public health hazard.

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Erratum

The Nematodes that Cause Anisakiasis

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This paper appeared on pages 774 to 782 of Volume 38 (December, 1975). The third complete paragraph in the left-hand column on page 780 should read as follows.

Three types of anisakine larvae have been implicated in human disease: *Anisakis* s. l., *Contracaecum* s.l., *Phocanema* s.l. (= "Terranova") (*13*), and possibly *Porrocaecum* s.l. (*4*).

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Staphylococcal Growth and Enterotoxin Production-Factors for Control

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ABSTRACT

Factors controlling growth and enterotoxin production in foods are many and varied. Under optimum conditions, generation times as low as 15 min and maximal cell populations of 10^9 per gram attained within 12 to 18 h have been reported. In naturally contaminated foods, these rates of growth are seldom encountered, because various environmental factors, in combination and individually, influence growth and enterotoxin formation. Resistance to limited moisture conditions is probably the most remarkable feature of the growth of this organism. Other factors controlling growth and toxin formation are the nutritional completeness of the medium, pH, temperature, inoculum size and type, and the effect of competing organisms. In addition, the potential for staphylococcal contamination from various sources must be considered when ascertaining the public health risk presented by this organism in a specific food. Ultimately, it is the sum of these factors which must concern the food hygienist and which determine the wholesomeness of foods in the marketplace.

Despite extensive research on the organism, its growth requirements, epidemiology, and measures for control, *Staphylococcus aureus* continues to be one of the pre-eminent food-borne disease organisms. Although the disease caused by this organism is characterized by low mortality and relatively short duration, the frequency of outbreaks and severity of the symptoms mark staphylococcal food poisoning as an important food-borne hazard in many types of foodstuffs.

The disease is caused by a group of potent, serologically differentiated, heat stable toxins which presently are difficult to detect routinely in food processing plants. For these reasons, measures relating to control of the organism and production of enterotoxin have become especially critical. The basic principles governing control strategy in the food preparation and processing environment are shown in Table 1.

TABLE 1. *General principles of food-borne disease control*

1. Removal of microorganisms
2. Aesepsis
3. Killing microorganisms
4. Prevention or delay of growth

ELIMINATION OF STAPHYLOCOCCI FROM FOODS

The first of these principles, physical removal of the organism cannot, for the most part, be accomplished with the desired degree of effectiveness. Processes employing filtration and centrifugation seldom remove organisms effectively and are limited to liquids. Other means of removal such as washing or trimming may, in some situations, be useful as adjuncts to other control measures.

The second principle, aesepsis or keeping staphylococci out of foods is very important and provides the "first line of defense" against this organism as well as other food-borne pathogens. Here, people are involved. Humans harbor and transfer this organism, directly or indirectly, to foods; and through careless acts, humans often are responsible for allowing conditions to exist in which *S. aureus* may proliferate.

Staphylococci are carried, often asymptomatically, by humans, practically from birth. Although these organisms are found in numerous and anatomically diverse body sites (Table 2), nasal carriage is probably the most important source from the food sanitation stand-

TABLE 2. *Staphylococcal carriage on various skin areas (11)*

Site	% of Flora *S. aureus*
Nose	44
Back of neck	10
Axilla	8
Forearm	20
Hand	40
Chest	12
Leg and ankle	16

point. Estimates for carriage rates in normal, healthy adults range from 20% to 70% (*11*). The proportion of this microbial population represented by enterotoxigenic staphylococci may be from 25% to 75%. Thus, a readily available source of *S. aureus* appears wherever in a process there is people-product contact. This concept can be verified experimentally by sampling for this organism in a process stream. Almost invariably its incidence increases in parts of a process where there is a high level of human contact with the product, such as hand sorting, picking, or product inspection (*8*). In addition to direct contamination, product contact surfaces in these areas may harbor large numbers of staphylococci if conditions for survival and growth are met. Thus, large numbers of vegetative cells and even enterotoxins can be transferred to the product moving over or on equipment surfaces. Control in this situation may be very difficult to achieve;

however, effective sanitization of surfaces and acceptable hygienic practices such as frequent and thorough handwashing, removal of persons with upper respiratory diseases from product contact areas, and shields or transparent covers between the product and persons working on it, may do much to alleviate these problems.

The next principle, killing staphylococci, holds both good and bad news for the food microbiologist. The good news is that *S. aureus* does not produce spores, and thus, complications are fewer when one wishes to destroy it. The bad news is that, once actively growing, it may produce one or more enterotoxins, proteins capable of maintaining their activity under conditions deleterious to the vegetative cell. Thus, we must consider the persistence of both toxin and vegetative cells.

Heat is probably the most commonly used technique for killing staphylococci and for inactivating or reducing toxin potentcy. The z value (degrees required to reduce the thermal death time tenfold) ranges from 5 to 6C, and D_{60} values (time at 60C required to produce a 90% reduction in cell count) are usually 3 to 5 min. An example of the effect of heating on *S. aureus* C-243 is shown in Fig. 1. Several factors influence the rate and extent of thermal inactivation. For example, the

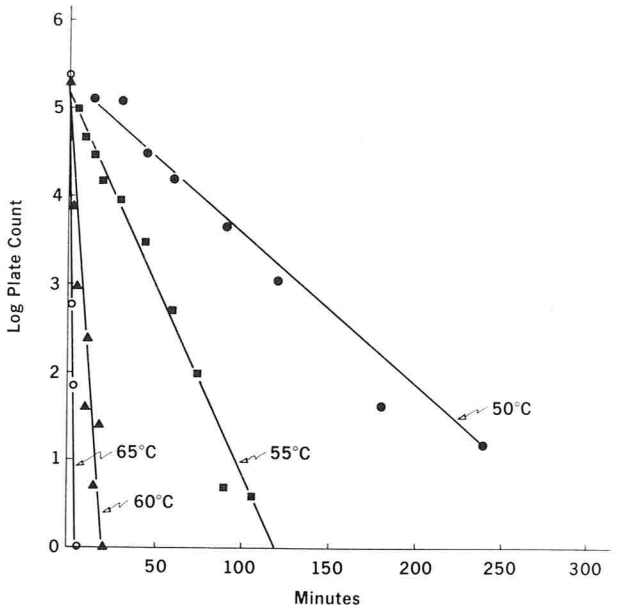

Figure 1. *Thermal inactivation of* S. aureus *C-243 in veronal buffer, pH 7.2.*

composition of the heating menstruum can be very important. If this menstruum is adjusted to a lower water activity or a_w, z values are increased dramatically and in some situations may be doubled. Menstrua containing high levels of proteins, starches, or other carbohydrates also may be protective.

At the other end of the temperature spectrum, freezing brings about an initial kill of 50 to 80% but continued mortality is not observed during prolonged storage at subfreezing temperatures. Damage to staphylococcal cells is even more limited during freeze-drying, and in fact, these organisms and other genera and species, are often preserved by this technique.

Kill of *S. aureus* can also be achieved by gamma irradiation and cells of this organism have been described as moderately resistant. Killing doses of ionizing irradiation for this organism are 0.14 to 0.7 megarads; however, irradiation in the presence of large amounts of protein or high levels of staphylococcal contamination may increase this to 1.0 megarads or greater. Much higher radiation levels are of course, required for food products in which adequate treatment requires the killing of highly resistant *Clostridium botulinum* spores.

Staphylococci are also susceptible to the bactericidal effects of quaternary ammonium compounds. As a result, some applications of these materials in food equipment sanitizing have arisen. It should be remembered, however, that gram negative bacteria that might be present are more resistant to chemical agents than staphylococci and might be expected to survive such treatments.

Moving from procedures and treatments to kill this organism to those limiting its growth, there are a number of ways to accomplish this objective and here, especially, we must consider both growth and enterotoxin production.

CONTROL OF STAPHYLOCOCCAL GROWTH IN FOODS

Product temperature

Just as temperature is described as one of the most effective means to kill this organism, so too does this factor affect growth. Optimal temperatures for toxin production and growth from 35 to 39C are found in the literature. Temperature ranges at which growth has been found to occur are quite broad and vary from 7C to a maximal temperature of approximately 48C. Growth rates, of course, are progressively slowed as one approaches temperature minima and maxima. Enterotoxin production occurs within somewhat narrower temperature limits, normally ceasing at about 10 and 45C. A somewhat smaller temperature range of enterotoxin B production of 15.2 to 43.2C was reported by Marland (*4*) who emphasized that a relatively small amount of this toxin was produced at temperature extremes. Optimal temperature for the enterotoxin production is about the same as that for growth (35 to 39C). It can thus be concluded that temperatures in excess of 45C and less that 5C will severely control or limit growth and enterotoxin production by this organism.

pH of the product

Although strain-to-strain variations exist, the minimal pH for staphylococcal growth initation is about 4.6 to 4.7 depending on the acid used to adjust pH (*6*) and a host of other conditions. The upper pH limit for growth is

probably about 9.5 to 9.8, however, enterotoxin production normally will not occur above pH 9.0 and is reduced by 50% at pH 8.0. At pH levels of 5.0 and below, little or no toxin is produced. The optimal pH value for enterotoxin B and C production is 6.8, whereas, enterotoxin A synthesis optimally occurs over a range from pH 5.3 to 6.8.

The toxin is relatively resistant to pH extremes normally encountered in foods, and, in fact, acid precipitation is one of the methods often used by food microbiologists to separate food proteins from the toxin when analyzing for this substance. Recent work, however, has shown rapid enterotoxin B denaturation at pH 3.5. The combination of high temperatures and very high or low pH values often will accelerate inactivation.

Water activity of the product

One of the more unique aspects of the growth of *S. aureus* is its ability to grow at relatively low water activity (a_W) levels (Fig. 2). Minimal a_W for growth in most

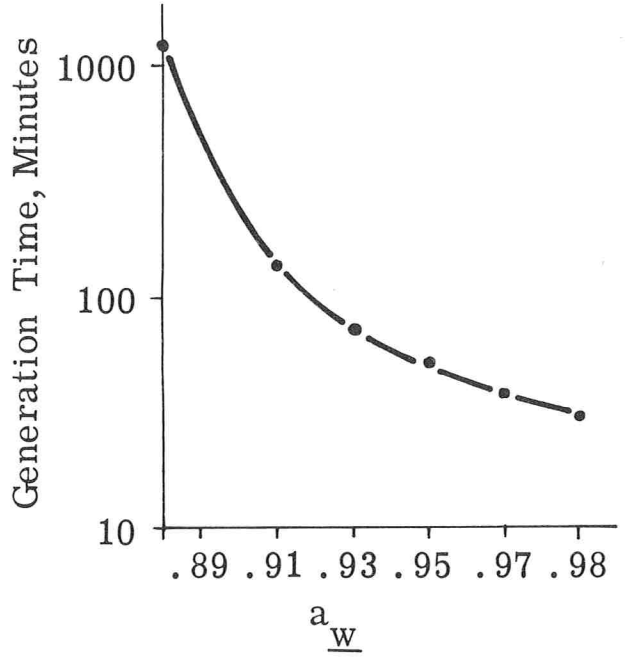

Figure 2. *Effect of water activity on growth rate of* S. aureus *196E in BHI broth incubated at 37 C.*

laboratory media is approximately 0.86 (*7*). Some studies have been reported recently by Hill and his coworkers (*3*) in which a minimal a_W of 0.83 was observed in a pork infusion medium. We have confirmed these results with the C-243 strain of *S. aureus* in a protein hydrolysate medium supplemented with beef extract. Somewhat higher minimal a_W levels were observed if the beef extract was omitted from this medium. In addition, we found that only one strain could grow at 0.83 a_W of the seven tested with the remaining six strains growing minimally at 0.84 to 0.88 a_W. These studies were conducted with NaCl as the a_W-adjusting solute, however, some solute-related differences can be expected.

Enterotoxin production is suppressed markedly by reduced a_W (*9, 10*). Figure 3 shows that toxin production

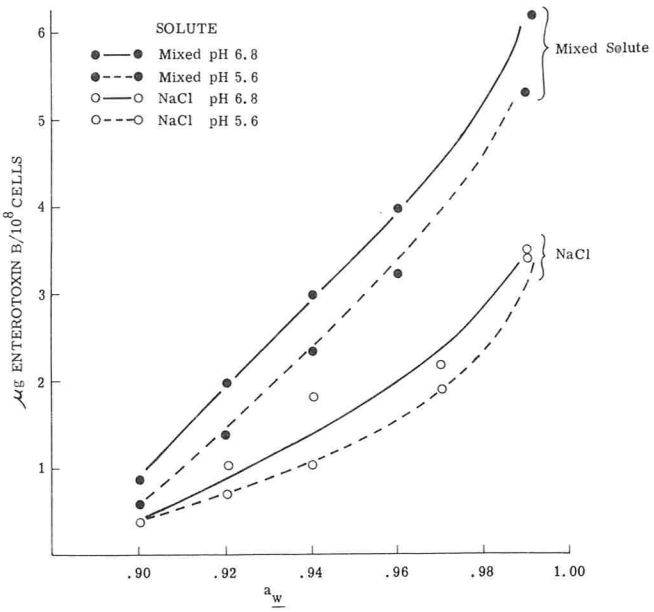

Figure 3. *Effect of a_W, pH, and solute type on enterotoxin production relative to maximal counts* (10).

relative to cell yield virtually ceases at a_W levels ≤ 0.90 however, growth, as noted above, occurs at much lower a_W's. Solute differences also occur as indicated by the differences between NaCl as the sole solute and a mixture of solutes containing NaCl, KCl, and Na_2SO_4 in a 5:3:2 ratio. This figure also illustrates the extent to which pH and a_W interact. Total or absolute toxin levels are also dramatically suppressed as a_W is lowered (Fig. 4). The

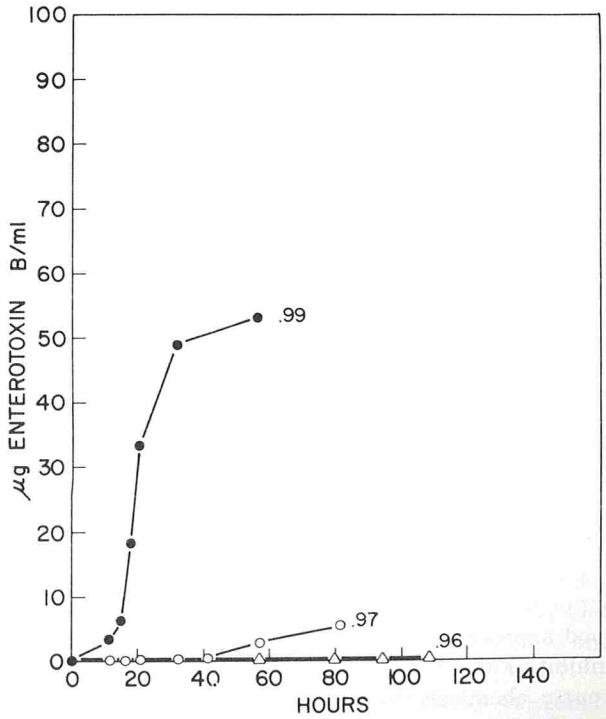

Figure 4. *Effect of a_W on enterotoxin B production by* S. aureus *C-243* (9).

mechanism by which a_w seemingly controls enterotoxin production out of all proportion to growth suppression is not known presently but is under intensive investigation in several laboratories throughout the world.

Associative growth of other microorganisms

Another growth limiting factor which probably occurs commonly in foods, but which is not exploited frequently to obtain active control of staphylococcal growth in foods, is inhibition by other organisms. As a general rule, this organism is not a good competitor and under most circumstances, it tends to be suppressed by the growth of other organisms. The source of these competitive effects may be the results of production of inhibitory substances, nutritional competition or the alteration of various environmental factors to levels that are unfavorable.

Dairy microbiologists have long been aware that good starter activity is essential to prevent growth of *S. aureus* during cheese making. In this situation lactic acid bacteria suppress growth of staphylococci. Similarly, Donnelly et al. (*2*) observed that staphylococci grow poorly in high count raw milk, but prosper and produce enterotoxin in pasteurized milk. Similar suppression of *S. aureus* by bacteria indigenous to a given food has been reported in meats, custards, pot pies, and other foods. In some situations, associative growth by competing bacteria may not affect growth rates or maximal total counts of staphylococci but may inhibit the production of enterotoxin (*5*).

Availability of oxygen

The nature of the atmosphere may also control growth and enterotoxin production. Staphylococci grow well under both aerobic and anaerobic conditions in laboratory media, however, toxin production normally is somewhat reduced under anaerobic conditions. For this reason, scientists interested in producing enterotoxins for research purposes often shake culture flasks or aerate fermenter vessels to maximize production. In foods, a similar situation exists: growth and toxin formation may occur under anaerobic conditions, but the extent of growth and amount of toxin produced is not as great as in the presence of oxygen. Barber and Deibel (*1*) found that in fermented sausages, staphylococcal growth localized at the outer periphery of the sausage where the oxidation-reduction potential was greatest. Enterotoxin synthesis in inoculated sausages was also suppressed when the oxygen content of the incubation atmosphere dropped below 10% although the *S. aureus* counts should have been sufficiently high (10^7 to 10^8/g) to support toxin production.

Chemical additives

Chemical additives presently offer little in the way of suppressing staphylococcal growth. There exists no food-approved "magic bullet" that can be added to inhibit, kill, or otherwise molest this organism. Of course, chemicals inhibitory to *S. aureus* exist, but their use, for a variety of reasons, usually is not permitted in foods. There are, however, a few exceptions. For example, Minor and Marth (*6*) showed that acetic, phosphoric, and lactic acids are relatively effective as growth suppressants; but because these acids are effective primarily in the undissociated form, relatively low pH values are required. Nitrite at concentrations $\geq$ 200 ppm also extends the lag phase and inhibits growth of *S. aureus,* but no information currently exists on the effect of this chemical on enterotoxin production.

Destruction or removal of staphylococci during sanitization of process equipment can usually be achieved by bactericides and bacteriostats such as quaternary ammonium compounds, chlorine, and iodophors. In some situations, these compounds may be obtained as mixtures with detergent cleaners and their use is usually followed by a potable water rinse. The effect of these materials on staphylococcal enterotoxins is not known, but it should be assumed that toxins will be sustained throughout such chemical treatments unless proven otherwise.

Obviously, there are several factors and conditions which can be used to control *S. aureus* in our foods and food processing operations. Seldom should one rely on only one method to obtain adequate control. It should be recognized that this organism grows rapidly and has rather catholic tastes with regard to nutritional, oxygen, pH, and moisture requirements. Add to this its ubiquity, strong connections to humans and the high degree of toxin stability and it is not difficult to understand why this organism has been responsible annually for 20 to 40% of the reported outbreaks of food-borne disease in this country.

ACKNOWLEDGMENT

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Illinois, June 8-11, 1975.

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News and Events

M. W. Jefferson Promoted

M. W. Jefferson, nationally recognized as the "Virginia Gentleman" and a leader in the dairy industry in matters of importance to Virginia and known to the dairymen of Virginia as Chief Inspector and Director of the Bureau of Dairy Services, has been promoted. Commissioner S. Mason Carbaugh recently announced that the Virginia Board of Agriculture and Commerce has unanimously appointed "Jeff" to Director of the Division of Markets in Virginia's Department of Agriculture.

Jefferson has been with Virginia's Department of Agriculture twenty-four years and for the past several years has been serving as Director of Virginia's statewide inspection program as Chief of the Bureau of Dairy Services. He is a graduate of Virginia Tech with a B.S. degree in Dairy Science. He has served as President of the Dairy Division of the National Association of State Departments of Agriculture. Since 1962 he has chaired the National Labeling Committee. He is currently chairman of the Farm Methods Committee of the International Association of Milk, Food, and Environmental Sanitarians and since 1960 he has represented Virginia at the Interstate Milk Shippers Conference. He is a past president of the Virginia Association of Sanitarians.

In making the announcement of Mr. Jefferson's appointment, Commissioner Carbaugh said, "We are fortunate to have someone of Mr. Jefferson's capabilities to promote from within the ranks of the Department. Mr. Jefferson's knowledge of agriculture and the many requirements for marketing agriculture products in today's situation will provide the kind of leadership necessary for the Department's marketing activities in the future.

Marketing, which has always been of prime concern to the farm producers, is even more important in today's world. The farmer must receive a fair return for his commodities if he is to stay in business. At the same time, the farmer, the food processor, and others involved in marketing agriculture commodities must meet a whole host of marketing requirements which have emerged to the last 5-10 years. It is my feeling that Mr. Jefferson has the experience and qualifications necessary to help producers make the best use of the domestic and export markets."

Advance Registration Opens For Food and Dairy Expo '76

Advance registration for Food & Dairy Expo '76, to be held Oct. 10-14, 1976, at Atlantic City Convention Hall, is now open.

Registration information is available from Dairy and Food Industries Supply Association (DFISA) and will also be mailed to thousands of food and dairy processors worldwide. Advance registrants will automatically receive the Expo hotel reservation form and map, transportation and related information. Registration is free to food and dairy processors, public health officials, sanitarians, educators and students, laboratory and testing personnel and international visitors.

More than 245 exhibiting companies have reserved space for the biennial exposition. Exhibits will consist of processing and handling equipment and components, container and packaging machinery and materials, ingredients, merchandising and refrigeration equipment and promotion, transport and delivery, services and supplies, and cleaning and sanitizing systems and materials.

To register, write or call DFISA, 5530 Wisconsin Avenue, Suite 1050, Washington, D.C. 20015. (301/652-4420).

News and Events

3-A Adopts New Standards For Colloid Mills, Blenders

Harold E. Thompson Jr. of the U.S. Public Health Service, left, who has been an active participant in the 3-A Sanitary Standards program for more than 25 years and an official signer of the standards for 10 of them, was presented with 3-A's honor award for extraordinary service to the program. Thompson is head of USPHS's milk sanitation section. His support of the standards, which set the criteria for cleanability of dairy processing equipment and product protection, and the recognition of the standards in the USPHS Pasteurized Milk Ordinance is of salutary importance to industry and regulatory milk control officials. Fred J. Greiner, chairman of the Dairy Industry Committee, presented the award at the 3-A spring meeting at Cincinnati in May 1976.

Two new 3-A sanitary standards, a supplement, and two amendments to existing standards and practices were approved by the 3-A Sanitary Standards Committees at their spring meeting at Cincinnati May 11-13, 1976.

The new standards, for colloid mills and blenders, were approved for signing and publication, subsequent to editorial review, in the Journal of Milk and Food Technology, early next year. The supplement to the fittings standard provides for a boot-type transportation tank outlet valve. The accepted practices for milking systems, first published in 1968, were the subject of several updating amendments to bring these guidelines into conformity with current technology. The standard for silo tanks was amended to provide for uniformity of tank outlet criteria with similar provisions in all tanks.

Standards which were not completed were designated for further revisions and scheduled for action at the next meeting.

More than 75 industry and regulatory representatives considered an agenda which included additional tentative standards and practices for culinary steam, milk drying, pressure sensors, cottage cheese vats, conveyors, milking equipment, wet collectors, fittings, batch processors, storage tanks, powder fillers and sifters.

The 3-A program safeguards the public health through its standards and practices for the cleanability of dairy processing equipment to protect the product against contamination from the equipment itself or foreign elements of dust, dirt or liquids. The program is conducted through the voluntary participation of dairy processors, equippers and suppliers, and public health officials and sanitarians and their trade and professional associations. In general, 3-A standards and practices are accepted in most public health jurisdictions at the federal, state and local level.

1976 ACDPI Conference to Feature International Symposium

Attendance at the 1976 American Cultured Dairy Products Institute Annual Meeting and Conference will be a "record breaker," according to Institute Secretary, Dr. C. Bronson Lane. Well over 200 cultured product processors and allied tradesmen are expected for the September 8 and 9 conclave at the St. Louis Marriott Hotel.

Al Schock, President of Nordica International, Sioux Falls, South Dakota will launch the conference with comments on "Our Collective Image." Presentations by K. M. Shahani, University of Nebraska, Omaha and a Food and Drug Administration spokesman will round out the first half day "mind expansion" series.

An International Symposium featuring cultured product processing and marketing experts from a number of European countries is scheduled for the September 8 afternoon session. Arrangements for this event were made by the Chairman of the ACDPI International Development Committee, Bent Andersen, BEPEX Corp., Santa Rosa, California.

A seminar on effective marketing entitled, "Bringing New Momentum To The Marketing of Cultured Products" is also on tap for the attendees. This one and one-half hour event will be conducted by Michael Paschkes, Stratmar Systems, New York. Additional presentations on whey utilization and successful quality control programs will be given by: Dr. V. Amer,, Gay Lea Foods, Ontario, Canada; Dean Elliott, H. P. Hood, Inc., Boston; Dr. Bob Williams, Kroger Co., Cincinnati.

The conference will conclude with a mini-clinic on manufacturing superior quality cultured products. Consultants Neil Angevine (Angevine-Funke), Dr. H. C. Olson, and Erik Lundstedt (BEPEX Corp.) will participate in this session.

Additional information and/or advance registration materials can be obtained from ACDPI headquarters, 910-17th Street, N.W., Washington, D.C. 20006.

News and Events

Proposals Requested to Study Methods for Analysis of Milk and Milk Products

The Intersociety Council on Standard Methods for the Examination of Dairy Products has limited funds to support studies on methods for analysis of milk and milk products. Such studies should deal with improvements of methods currently in *Standard Methods* or with development of new methods for future use and hence for inclusion in *Standard Methods* beyond the 14th edition.

The studies may be either of a short- or long-term nature, depending on the problem being investigated. Available funds are intended primarily for supplies, although some money for labor may be provided if the proposed study warrants that amount of support. Questions about studies may be directed to Prof. E. H. Marth, Department of Food Science, University of Wisconsin, Madison, Wis. 53706 (telephone: 608-263-2004 or 608-262-3046).

Proposals should be short (at most 5-7 double-spaced typewritten pages) and should include: (*a*) brief review of pertinent literature, (*b*) objectives of the study, (*c*) procedures to be used, (*d*) budget, (*e*) time required for study and when results can be expected, and (*f*) list of references cited in the proposal.

ELEVEN COPIES OF THE COMPLETED PROPOSAL SHOULD BE SENT BY AUGUST 31, 1976 TO: PROF. E. H. MARTH (address above).

E.H.MARTH
Chairman
Intersociety Council on Standard Methods for the Examination of Dairy Products

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Letter to the Editor

New York has no standards for bacteria in raw meats

DEAR SIR:

It has been brought to my attention that in a paper recently published (Vol. 39 pp. 175-178) in the *Journal* the word "standard" was used in error. Dr. E. George, Jr., Director of the New York State Food Laboratory, has correctly pointed out that New York State does not have standards for bacteria in raw meats. They do employ guidelines. I should like to thank Dr. George for bringing this point to my attention and to apologize for inadvertently using the term standard in my discussion of New York State's regulations.

J.M.GOEPFERT
Food Research Institute
University of Wisconsin
Madison, Wisconsin 53706

IAMFES Announces New Staff Member

The appointment of Barbara Lee as Assistant Executive Secretary, International Association of Milk, Food and Environmental Sanitarians (IAMFES), and Associate Editor, the Journal of Food Protection, has been announced by Earl Wright, Exective Secretary of the IAMFES.

Ms. Lee, a native of Blue Ridge, Georgia, attended the University of Georgia and received a B.S. degree from Iowa State University in 1974. She is a candidate for an M.S. degree in Food Technology from Iowa State, where she was a PACE award recipient, a member of the Institute of Food Technologists, and a member of Omicron Nu and Sigma Xi honor societies.

Ms. Lee's responsibilities will include production supervision of the Journal of Food Protection, liaison work with state and national affiliate groups of the IAMFES, coordination of workshops and shortcourses sponsored by the Association, and organization of student affiliate groups, throughout the United States.

Annual Meeting Note

Those attending the 1976 IAMFES Annual Meeting are advised: If you are arriving by airplane at O'Hare International Airport, the Arlington Park Hilton courtesy vans stop only at the lower terminal baggage pick-up level of:

- Eastern Airlines
- United Airlines
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Direct line telephones to the Arlington Park Hilton are located at the baggage pick-up areas of the airlines listed above.

The Arlington Park Hilton staff looks forward to being of service to you.

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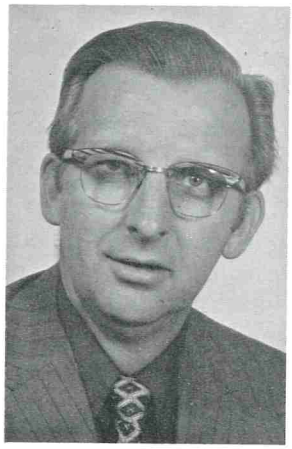
Ross Mickelsen, 1931-1976



Dr. Ross Mickelsen, 45, food science professor in the Dairy and Poultry Science department, Kansas State University, died on May 26 of a massive heart attack. Funeral Services were held on May 29 at the Church of Jesus Christ of Latter Day Saints and interment was at the Sunrise Cemetery, Manhattan. He is survived by his wife, Marilyn, and four children, Sue, Charles, Barbara, and Scott. Other survivors are a brother, Durrell, of Salina, Utah, and a sister, Mrs. Fern Jensen of Richfield, Utah.

Dr. Mickelsen was born January 25, 1931, in Salina, Utah. He received his B.S. (1954) and M.S. (1955) degrees in dairy manufacturing from Utah State University and his Ph.D. (1971) at the University of Wisconsin under the direction of Dr. C. A. Ernstrom.

Dr. Mickelsen joined the faculty of Kansas State University in 1957 as an instructor where he rose to the rank of full professor in 1975. Ross was heavily involved in teaching and research activities and held responsible roles in University committee assignments. He taught courses in cheese and fermented milks, concentrated dairy products, frozen products, processing and chemical analysis of fats and oils, and introductory food science and technology.

His research was wide ranging in studies involving milk, whey, and cheese and the role of these products in other foods. He studied milk clotting enzymes and was the first to outline and explain precautions necessary in mixing pepsin and rennin in cheese making. His extensive research in milk composition as related to yield of cottage cheese presented more complete data than had existed previously. During his career Dr. Mickelsen authored over 40 scientific papers, journal abstracts, and other publications. Several of his papers appeared in the *Journal of Milk and Food Technology.*

At K-State, Ross was chairman of the graduate food science program, a member of the faculty senate, chairman of the senate's faculty affairs committee, chairman of the Kansas Dairy Industry Conference, secretary of the Kansas Dairy Technology Society, chairman of the local arrangements for the American Dairy Science Association annual meeting in 1975, and many other committee assignments.

He was very active in his church having served as chairman of the building committee and superintendent of the Sunday School. His civic activities included Boy Scouts of America, Little League baseball, and Lions Club where he was president-elect for 1976. He was a consultant for All Star Dairies, Inc., Beatrice Foods, and the Midwest Marketing Company.

A Ross Mickelsen Memorial Fund has been established by the family. Contributions may be sent in care of the Dairy and Poultry Science Department, Call Hall, Manhattan, KS 66506.

Ladies Activities
IAMFES 1976 Annual Meeting

MONDAY, AUGUST 9th:

The afternoon is planned for a shopping trip to the Woodfield Mall. Woodfield is the world's largest and most spectacular shopping center.

On a 191 acre site, the multi-level, climate-controlled enclosed Mall offers shoppers four major department stores plus 230 other stores. Woodfield is designed so that the distance from one end to another is no more than three city blocks. Numerous ramps, escalators, elevators and carpeted staircases make access to any shop easy.

Woodfield's focal point is the Grand Court, where three levels provide dramatic views of a three story high sculpture, pools, an exotic aquarium viewed by passing under a waterfall, fountains, and a huge moire whose pattern shifts as the viewer moves. A Greek Amphitheatre provides a stage for special events from a fashion parade to a puppet show for the youngsters. Leafy plants add their touch of color to the scene. Computer programmed lighting changes in color intensity to complement the daylight or darkness sifting in through geometrical shaped skylights.

The dramatic North and South Courts boast sculpture of museum quality, plantings and cushioned and carpeted rest areas that offer the busy shopper a change of pace.

Woodfield offers the widest possible selection of merchandise at every price range. In addition to its top department stores, you will find colorful and unusual boutiques tucked away in corners, elegant shops for special occasion purchases, good restaurants and fun eating places.

TUESDAY, AUGUST 10th:

There is a choice of two tours:

Tour 1: This tour will start with a trip to world famous Sara Lee Bakery. This is the largest and most modern cake bakery in the world. The Sara Lee Bakery uses more fresh dairy products (milk, eggs, butter, cream cheese) than any other bakery in the world. It features the most modern bulk handling system in the food industry. Sara Lee houses the largest automated holding freezer in the world, larger than a football field and four stories high, where products are stored at 10° F until shipped to customers around the country.

From Sara Lee you will go to the Village of Long Grove. We will have lunch here and have the balance of the afternoon to browse, shop, dine and enjoy this unique quaint and colorful village. A trip to Long Grove takes you back to the more leisurely pace of the turn of the century.

Tour 2: This tour will take you into Chicago with the first stop being the Art Institute. The Art Institute of Chicago invites you to enjoy its world famous treasures. On display are paintings, sculptures, prints and drawings, photographs, Oriental art, primitive art and decorative arts.

Especially noteworthy are the Institute's paintings ranging from the fourteenth century to the present. Highlights include the outstanding Japanese Prints, the Chinese sculptures and bronzes and some of the world's finest Impressionist pictures. The unique Thorne Rooms, authentic reproductions in miniature of European and American rooms, offer a fascinating study of interior design from the sixteenth century to the present.

Lunch will be provided for in the Art Institute dining room.

From here you will go to the Museum of Science and Industry.

The Museum features 75 exhibit halls with some 2,000 displays that explain the principles of science and show how they are applied in industry and everyday life. Visitors can trace the evolution of automotive, rail and airline transportation and walk through a full-sized coal mine and a captured German submarine.

WEDNESDAY, AUGUST 11th:

This day will start with a trip to Lee Wards. This is one of their many nation-wide Hobby Crafts Stores. All materials for such things as macrame, decoupage, painting, country carving, quilting and needlepoint are available. There are continuous demonstrations for the different crafts.

From Lee Wards you will go to the Milk Pail for lunch. This is a quaint and unique place to dine.

After lunch you will go to Haeger Potteries where a trained guide will welcome you and conduct you through the fascinating steps that change clay to a work of art. You will see ancient methods blended with today's modern technology. The change from drab clay into rare beauty is by no means a mechanical production. It is a demonstration of skilled hands and talents of the Haeger men and women. The individual skilled craftsman has almost disappeared from the American production scence, but here you will see many of them as beauty takes shape under their talented hands.

THURSDAY, AUGUST 12th:

The morning is planned for another shopping trip to Woodfield Mall.

Association Affairs

Florida Affiliate Meets

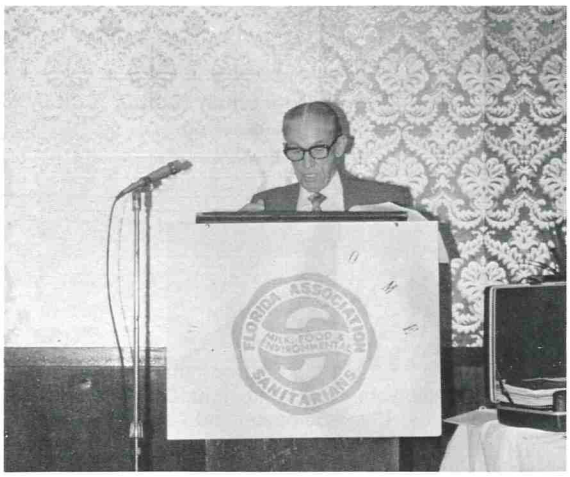
Jay Boosinger, chairman of awards committee, makes presentation of Lifetime membership to Walt Krienke, retired University of Florida faculty member.

1976 Board of Directors (left to right): Tom Hart, President Elect; Jay Boosinger, President; Ron Richter, Past President; Lupe Wiltsey, Chairman Lab section; Chuck Vogelgesgang, Sam Noles, Joe Hayes, Bill Brown, John Miller, Secretary-treas.

The Florida Affiliate held its annual meeting March 16-18 at the Langford Hotel in Winter Park, Florida. More than 90 registrants heard presentations concerning detergents, sanitizers, corrosion of stainless steel, sanitation in water bottling plants, potential submerged inlets, product recall and disaster procedures, and various aspects of food microbiology. Antiobiotic detection methods for milk were discussed in a half day laboratory session.

Lifetime membership awards were presented to John Manning, Walker Stainless Steel and Walt Krienke, University of Florida during the awards banquet which 115 people attended. Ralph Kirkland of Miller Machinery was given an award for outstanding contribution to the Florida Affiliate. A special award was given to Jack Dodd for his long and continued support of the Florida Affiliate. Mr. Dodd resigned his position as Director of the Division of Dairy Industry, Florida Department of Agriculture and Consumer Services to accept the position of Executive Director of The Florida Public Service Commission.

Dr. C. Bronson Lane, Master of Ceremonies at the banquet, conducted the drawing for an all expenses paid trip to the International meeting this year. The trip is made possible by the Florida Affiliate to enable more people to attend the International meeting. Membership in the Florida Affiliate is the only qualification needed to be eligible for the trip. This year's winner was Ivan Wishar of Sealtest Food in Tampa.



John Manning, recipient of lifetime membership, discussing corrosion of stainless steel.

Jay Boosinger (right), chairman of the awards committee recognizing J. Ralph Kirkland for outstanding contribution to FAMFES.

The annual meeting was preceded by a one-day conference held by the Florida Department of Agriculture and Consumer Services Division of Dairy Industries and followed by a meeting of the Florida Quality Milk Council.

Association Affairs

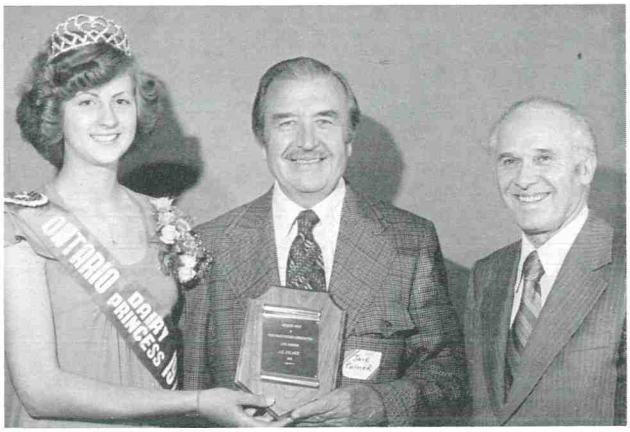
University of Wisconsin-Eau Claire Prepares Annual Meeting Educational Exhibit

Preparing the Exhibit are, from left to right: Dr. Karl Erickson, Tim Morris, Mark Kuba, Diane Sampson and Keith Husby.

Member of the UW-Eau Claire student affiliate of the Wisconsin Association of Milk and Food Sanitarians are helping to prepare an educational exhibit for the Arlington meeting of the International Association of Milk, Food and Environmental Sanitarians. Pictorial development of how a comprehensive educational program was developed by the professional practicing sanitarians so as to: (1) increase the professional image of the Sanitarian; (2) and have available a Bachelor of Science degree in Environmental and Public Health, and (3) to have continuing education with collegiate credit for the professional practicing Sanitarians.

Ontario Affiliate Meets

Dr. C. Sen Gelda (center) receives the Sanitarian of the Year Award from D. J. Varnell, left, on behalf of Klenzade Products. Also pictured are Miss Debbie Rogers, the 1975-76 Ontario Dairy Princess, and J. L. Baker (right), Retired Dairy Commissioner, Ontario Ministry of Agriculture and Food, who read the citation for the award.



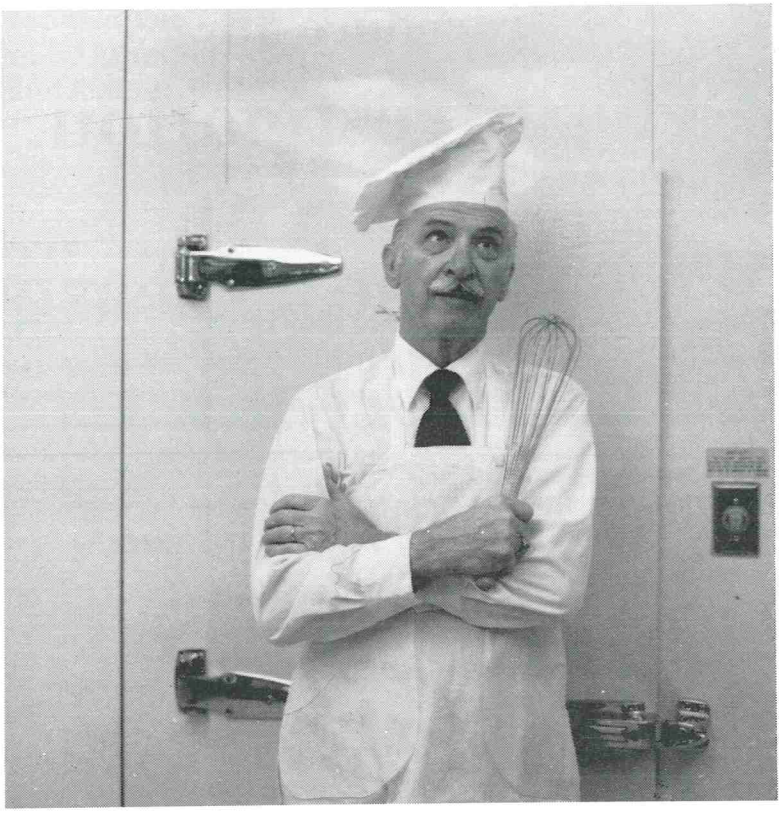
Mr. J. C. Palmer (center) receives Honorary Life Membership from Mr. William Kempa (left), 1975-76 President of the Ontario Milk and Food Sanitarians Association, and Ontario Dairy Princess Miss Debbie Rogers.

The Ontario Milk and Food Sanitarians Association held a very successful meeting at the Holiday Inn in Toronto in March. The affiliate honored two of their members with awards.

The Sanitarian of the Year Award was presented to Dr. C. Sen Gelda. Dr. Gelda received a $200 award on behalf of Klenzade Products Division, Economics Laboratories.

Honorary Life Membership in the Association was presented to Mr. J. C. Palmer. Mr. Palmer, prior to his retirement, was Director of the Milk Industry Branch of the Ontario Ministry of Agriculture and Food, and for many years was in charge of the Milk Quality Program for the Province of Ontario.

Index to Advertisers



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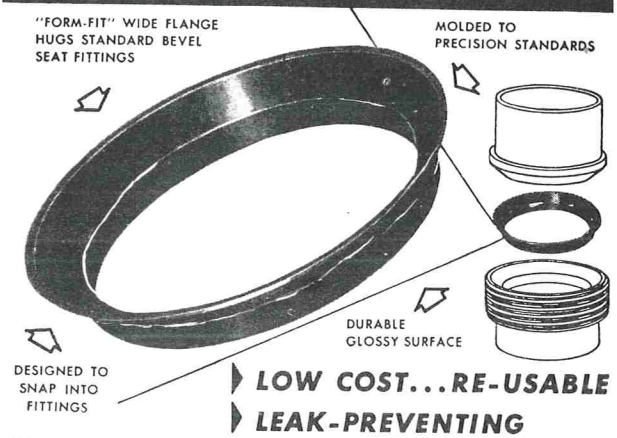

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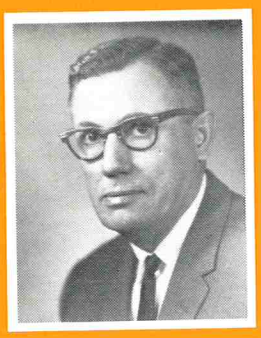
Dairy authorities speak out on better cow milking



Ralph Bonewitz, Extension Specialist
Department of Dairy Science
Kansas State University

What questions should a dairyman ask before expanding or modernizing?

Here are some of the most basic questions I believe dairymen should ask themselves before they begin a program of modernization or expansion:

What about my future in dairying? Will the changes I make be profitable in the long run? Will the changes be feasible in the short run?

Long run profitability refers to a period of eight to ten years or more. It is usually studied through the use of budgets. In the budgeting process, capital expenditures are prorated over the life of the assets by arbitrary depreciation methods.

Short run feasibility refers to the income-generating ability of a business in a short period of time. It is usually studied through the use of a projected cash flow.

Watch your cash flow

Projected cash inflow and outflow during the period are compared, reflecting payment requirements from credit agencies as well as all normal expenditures. Some business changes are capable of being profitable in the long run *but they are not capable of meeting short run demands for cash, particularly when payment requirements for capital expenditures are concentrated in the early years of an investment.*

Management must expand as herds get larger. More failures are due to lack of management than any other one thing. Hours spent thinking and getting facts will pay off and the manager or owner must assume this responsibility. A manager must have the ability to get the right things done at the right time.

Here are some more questions:

Will my expansion or modernization plan improve the chances and ease of producing a higher quality product? Will it increase the ease of the key jobs associated with dairying? Will it increase the efficiency of labor, investment and/or equipment? Will the modernized set-up provide the minimum movement of men, animals and material? Can this modern dairy farm compete with nonfarm occupations for good labor or manpower?

Do you as a manager or owner have a concern for production, people, quality milk and a desire to make the dairy more profitable? Will you develop a good milking program? One that obtains high production per cow, controls mastitis, produces clean excellent-flavored milk and uses a minimum amount of labor?

Check this planning list

Tomorrow's profitable dairy farm can be expected to be a planned operation. Here are some of the things you will want to consider during the planning phase: 1) Size of herd to be accommodated; 2) Dairy breed; 3) Climate conditions; 4) Topography of farmstead, slope, drainage and exposure; 5) Size and productivity of the farm; 6) Feeding program as related to feed storage and equipment for feeding; 7) Existing buildings and equipment that will fit into long range plans; 8) Availability of labor; 9) Capital available for investment; 10) Sanitary regulations or codes or present and potential milk markets; 11) Personal preference of the owner; 12) Water and air pollution regulations.

Planning with pencil and paper prior to the start of construction permits you to analyze combinations of equipment and building arrangements without any expenditure. Visit as many farms as possible and incorporate the good points seen at these farms into your facilities. Use university people and private consulting people.

Unless a dairyman regularly tests production, a pipeline milker can be the best friend that a cull cow ever had. The cull cow strolls in with the good producers, gives only enough milk to color the line, eats almost as much feed as the best cows, and then goes her merry way. A dairyman needs profitable production from every cow. You will call the correct signals and build a profit-winning dairy team if you use production records.

Feeding practices important

Constant attention to feeding practices, breeding for production and herd health during expansion are very important. A well designed, properly installed milking system is essential to proper milking. You can't afford an inadequate or poorly maintained milking system.

Productivity must be the key

The productivity of a resource depends on the amount and kind of other resources with which it is combined. As an example, when capital is substituted for labor, it requires fewer hours of labor per cow. However, unless production increases or more cows can be handled, the machinery and equipment cost will offset the savings in labor. Productivity of a resource then depends on the kind and quality of the resource with which it is combined. Modern technology is closely related then to the substitution of capital for labor and land. It increases the demands on management, emphasizes financial management and increases technical requirements of hired labor.

Finally, all of this modernization should help make dairying more pleasant, as well as more productive for you and your family.